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A Colorimetric Sensor Enabled with Heterogeneous Nanozymes with Phosphatase-like Activity for the Residue Analysis of Methyl Parathion

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Abstract: In this study, a colorimetric sensor was developed for the detection of organophosphorus pesticides (OPs) using a heterogeneous nanozyme with phosphatase-like activity. Herein, this heterogeneous nanozyme (Au-pCeO₂) was obtained by the modification of gold nanoparticles on porous cerium oxide nanorods, resulting in synergistic hydrolysis performance for OPs. Taking methyl parathion (MP) as the target pesticide, the catalytic performance and mechanism of Au-pCeO₂ were investigated. Based on the phosphatase-like Au-pCeO₂, a dual-mode colorimetric sensor for MP was put forward by the analysis of the hydrolysis product via a UV-visible spectrophotometer and a smartphone. Under optimum conditions, this dual-mode strategy can be used for the on-site analysis of MP with concentrations of 5 to 200 µM. Additionally, it can be applied for MP detection in pear and lettuce samples with recoveries ranging from 85.27% to 115.87% and relative standard deviations (RSDs) not exceeding 6.20%, which can provide a simple and convenient method for OP detection in agricultural products.

Keywords: heterogeneous nanozyme; cerium oxide; gold nanoparticles; phosphatase-like activity; methyl parathion



Citation: Zhao, F.; Li, M.; Wang, L.; Wang, M. A Colorimetric Sensor Enabled with Heterogeneous Nanozymes with Phosphatase-like Activity for the Residue Analysis of Methyl Parathion. *Foods* **2023**, *12*, 2980. <https://doi.org/10.3390/foods12152980>

Academic Editor: Soledad Cerutti

Received: 20 June 2023

Revised: 28 July 2023

Accepted: 31 July 2023

Published: 7 August 2023



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1. Introduction

Pesticides are a group of chemicals used to regulate plant growth and prevent pests, diseases, and weeds, which are the main chemical hazards to the quality and safety of agricultural products [1–4]. One of the main categories is organophosphorus pesticides (OPs), which are organic compounds containing phosphorus, mainly phosphate or thiophosphate, and are widely used in the growth of crops because of their broad spectrum and high efficiency [5–7]. When OPs are applied to crops, they will inevitably accumulate in the environment and in organisms. Studies show that these residues can accumulate through the food chain and inhibit the activity of cholinesterase in the body, thus causing symptoms in the central nervous system and posing a threat to human health [8–10]. Up until now, OPs have been reported as the main pollutants in water and plants [11,12]. Therefore, it is of great significance to detect OP residues for human safety and environmental protection.

In recent years, various methods based on gas chromatography [13], chromatography–mass spectrometry, and other confirmatory techniques have been applied for OP detection [14,15]. Although accurate and sensitive, the above methods are time-consuming and require specialist personnel to operate, which cannot meet the demand for rapid detection. As an important complement, biosensors have the advantage of being fast, field detectable, and easy to miniaturize, making rapid and on-site analysis for OPs possible [16–18]. Currently, various biosensors, including colorimetric [19], fluorescent immunoassay [20], and electrochemical biosensors [18,21], have demonstrated excellent analytical performance in

the detection of OPs. However, most biosensors are based on the reaction between biological enzymes and OPs, such as acetylcholinesterase or organophosphorus hydrolases [22,23]. Despite their high specificity, these biological enzymes are expensive, difficult to prepare, and have poor stability [24–26]. Therefore, it is necessary to develop biomimetic recognition elements to broaden the practical application of biosensors.

Recently, with the development of nanotechnology, nanomaterials have opened new possibilities for wide application and marketization [27]. Due to their high stability, ease of synthesis, low cost, and high enzyme-like activity, nanozymes have a wide range of applications in environmental and food safety. In recent years, nanozymes have been used in electrochemical or optical sensors to analyze OP residues by exploiting their unique phosphatase [28] or peroxidase [29,30] activity. Although they have high sensitivity, peroxidase-like nanozymes commonly require the involvement of a substrate, such as H_2O_2 , which may have autolysis and high toxicity [31]. Meanwhile, phosphatase-like nanozymes can hydrolyze OPs into small molecules with low toxicity directly, making catalysis simpler and more convenient. Recently, various phosphatase-like nanozymes, such as cerium oxide (CeO_2) [32], porous hydroxy zirconium oxide (ZrOH-OH) [33], and zeolitic imidazolate frameworks (ZIFs) [34], have been explored. Among them, CeO_2 nanozymes, including nanospheres, nanorods, and nanocubes, are inexpensive and environmentally friendly and have been regarded as prospective artificial phosphatases owing to the presence of Ce^{3+} and Ce^{4+} species on their surface [32,33]. However, the low specific surface area and weak adsorption ability may limit their catalytic performance. To address these issues, researchers have adopted the hybridization strategy to obtain higher catalytic activity [35–37]. Meanwhile, noble metals such as gold, platinum, and palladium are getting a lot of attention. As favorable catalysts, they can exhibit multienzyme activity, such as peroxidase-like activity and oxidase-like activity [26,29,38]. Therefore, the modification of noble metals on the CeO_2 nanozyme may have a synergistic catalytic effect on the hydrolysis of OPs.

Inspired by this, we developed a heterogeneous nanozyme with phosphatase-like activity. Based on the hydrothermal method, the CeO_2 nanorods (denoted as CeO_2) were synthesized. After the calcination treatment, the porous CeO_2 (denoted as pCeO_2) nanozyme was obtained with higher phosphatase-like activity. In order to further improve the catalytic performance, gold nanoparticles (AuNPs) were modified on the substrate of pCeO_2 to form the Au- pCeO_2 nanozyme. Taking methyl parathion (MP) as the model pesticide, the catalytic performance and mechanism of Au- pCeO_2 were studied by measuring the hydrolysis product (i.e., *p*-nitrophenol, *p*-NP). Due to the high hydrolysis performance, we constructed a Au- pCeO_2 nanozyme-enabled colorimetric sensor. This dual-mode colorimetric sensor could be applied for the on-site detection of MP residues via a UV-visible spectrophotometer (UV-Vis) and a smartphone, which can provide a rapid and reliable approach for the detection of OP residues in agricultural products (Figure 1).

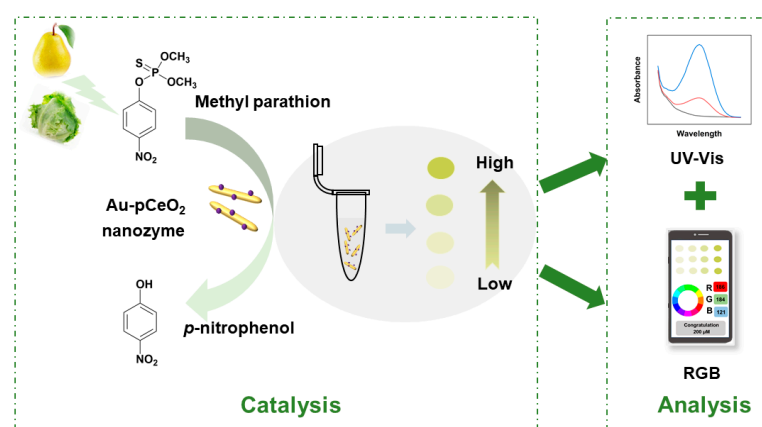


Figure 1. Schematic illustration of the dual-mode colorimetric sensor for MP detection based on Au- pCeO_2 nanozyme in agricultural products.

2. Materials and Methods

2.1. Reagents and Instruments

Cerium nitrate hexahydrate ($\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$) and carbendazim were obtained from Shanghai Aladdin Bio-Chem Technology Co., Ltd. (Shanghai, China). Sodium hydroxide, ethanol, and ethylene glycol (EG) were obtained from Beijing MREDA Technology Co., Ltd. Tris-HCl solution was obtained from Shanghai Sangon Biotech Co., Ltd. (Shanghai, China). MP, paraoxon, phosphoramidite, and monocrotophos were obtained from Alta Scientific Co., Ltd. (Tianjin, China). All other chemicals and reagents used were of analytical grade.

X-ray diffraction (XRD) patterns were obtained using an X'Pert Pro XRD device from XRD-6100 (SHIMADZU, Kyoto, Japan). High-resolution transmission electron microscopy (HRTEM) images were recorded on a Tecnai G2 F30 S-TWIN (FEI, Hillsboro, OR, USA). Raman spectroscopy was obtained with a laser Raman spectrometer (Horiba Jobin Yvon, Kyoto, Japan). To describe the surface electronic states and compositions, a Thermo Fisher Scientific K-Alpha+ X-ray photoelectron spectrometer (XPS) was used.

2.2. Synthesis of Nanozymes

A total of 1.736 g of $\text{Ce}(\text{NO}_3)_3 \cdot 6\text{H}_2\text{O}$ and 19.2 g of NaOH were dissolved in 10 and 70 mL of deionized water, respectively, and then mixed and stirred in a glass vial for 30 min. After that, the mixture was transferred to an automatic temperature-controlled electric furnace and heated in a reaction kettle at 100 °C for 24 h. The obtained materials were cooled naturally to room temperature and then washed with 30 mL water and ethanol alternately by centrifugation until the pH was near neutral. After that, the prepared CeO_2 was calcined at 400 °C for 1 h to obtain p CeO_2 [35]. A total of 50 mg of p CeO_2 powder was then dispersed in 25 mL of EG and sonicated for 30 min to make it homogeneous. A total of 250 μL of NaOH (1 M) in EG and 0.3 mL of chloroauric acid were added and then heated and stored in an oil bath at 140 °C for 3 h [36]. The reaction solution was cooled naturally to room temperature, and the precipitate was washed three times by centrifugation with ultrapure water. After drying in a vacuum oven at 75 °C and grinding to powder with a mortar and pestle, the Au-p CeO_2 nanozymes were obtained and stored at room temperature.

2.3. Detection of MP

A total of 10 mg of Au-p CeO_2 was first added into a 0.8 mL Tris buffer solution (pH 9.0, 10 mM). After the addition of 200 μL of MP solution with the known concentration, the mixture was reacted at 75 °C for 5 h in a metal bath. Then, the tube was centrifuged for 8 min at 13,523 rcf. The supernatant was then filtered through a 0.22 μm filter membrane and added to the centrifuge tube. Then, 200 μL of the supernatant was transferred into a 96-well plate for UV-Vis detection in a wavelength range of 300–500 nm. The absorbance was recorded at a wavelength of 400 nm. To realize the OP detection by a smartphone, the imaging of the samples was analyzed with the image analysis app “Color Picker” by reading the color information (RGB value) within each well.

2.4. Real Sample Analysis

Pears and lettuces were selected for the real sample analysis. They were purchased from a local supermarket in Beijing. Firstly, these samples were crushed in a blender and then weighed at 1.00 g (accurate to 0.01 g) into a 50 mL centrifuge tube. After that, the known concentration of MP was added to the samples for 0.5 h at room temperature. To extract MP from samples, 5 mL of acetonitrile and 1 g of NaCl were added and vortexed for 5 min. After that, the tube was centrifuged at 2348 rcf for 10 min. To reduce the matrix interference, 25 mg of primary secondary amine (PSA) and 25 mg of octadecyl silane (C_{18}) were added to 1 mL of the supernatant and then vortexed for 3 min. After centrifugation for 10 min (2348 rcf), the obtained supernatant was filtered through a 0.22 μm filter membrane and collected as the extraction solution. The above solution was then blown dry with nitrogen and redissolved with Tris buffer (pH 9.0, 10 mM) for further analysis.

3. Results and Discussions

3.1. Comparison of CeO_2 , pCeO_2 , and Au-CeO_2 Nanozymes

First of all, the morphology and chemical structure of three nanozymes were compared in this study. As shown in the insert of Figure 2a, the color of CeO_2 is dark yellow, and pCeO_2 is light yellow. After the addition of the precursor solution and the reduction process, the obtained nanozyme appears black-purple, which is due to the formation of AuNPs on the surface of pCeO_2 . From the TEM images in Figure S1a, our CeO_2 nanozyme appeared to have a rod-like structure. After the high-temperature calcination, the nanozyme had a porous rod-like morphology, indicating the successful preparation of pCeO_2 (Figure S1b,c). As shown in Figure S2, the length and width of pCeO_2 were 42.91 ± 2.82 nm and 7.04 ± 0.17 nm, respectively.

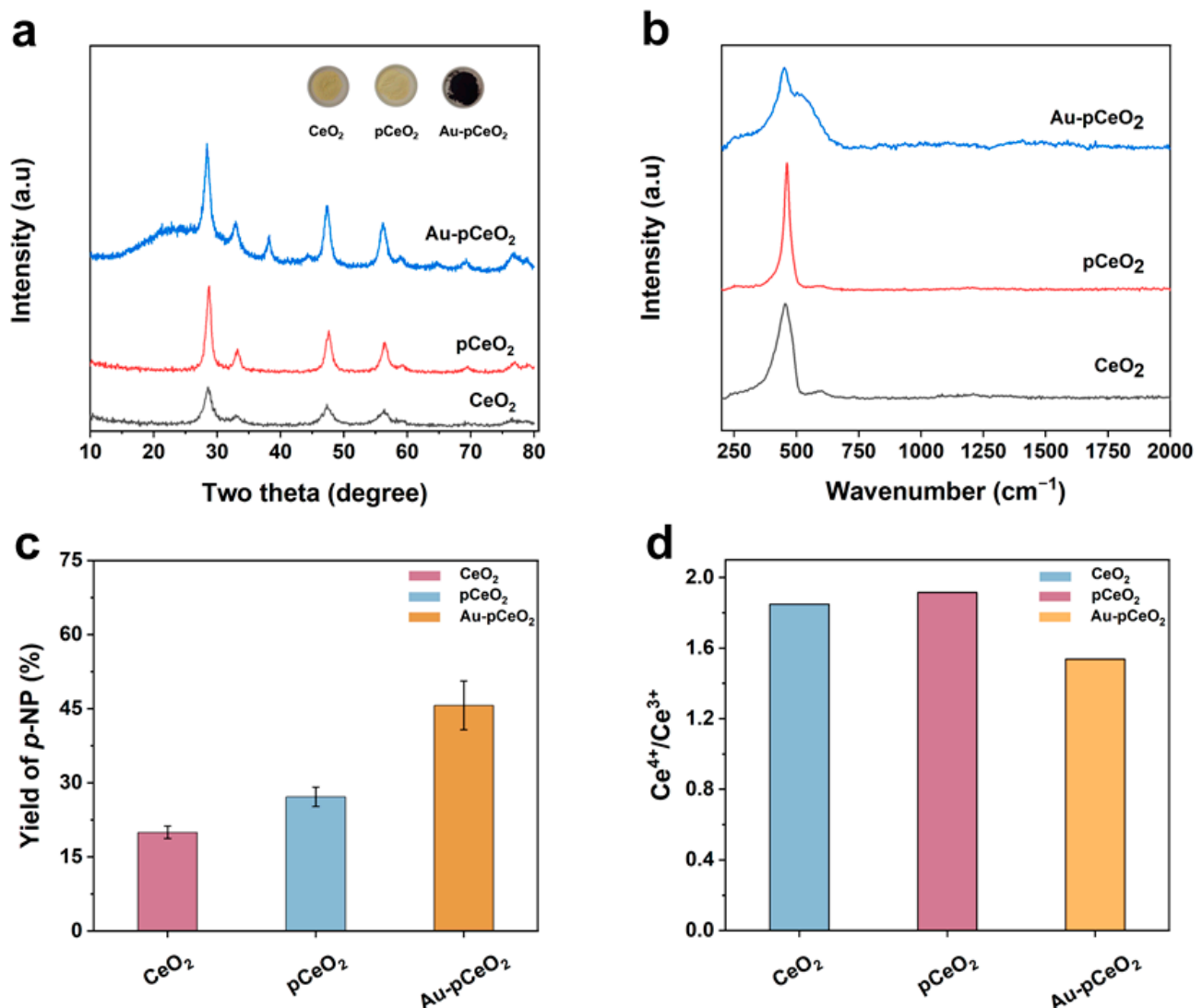


Figure 2. (a) XRD patterns of CeO_2 , pCeO_2 , and Au-pCeO_2 ; the insert shows the digital photos of the prepared nanozymes. (b) Raman spectrogram of CeO_2 , pCeO_2 , and Au-pCeO_2 . (c) Yield of *p*-NP of CeO_2 , pCeO_2 , and Au-pCeO_2 for MP (200 mM). (d) Proportions of Ce^{3+} and Ce^{4+} species simulated from XPS data.

After that, we analyzed the chemical structure of the nanozymes. The XRD patterns of the CeO_2 , pCeO_2 , and Au-pCeO_2 nanozymes are shown in Figure 2a. The diffraction peaks of CeO_2 were present at 2θ of 28.7° , 32.9° , 47.5° , and 54.5° , which is consistent with the reported literature [37]. After calcination, the peak pattern of pCeO_2 was sharper than that

of the CeO₂ nanorods. After the modification of AuNPs, a weak diffraction peak appeared at 38.4°, indicating the successful preparation of the Au-pCeO₂ nanozyme [39]. Moreover, our Au-pCeO₂ nanozyme still had a similar crystal structure compared to pCeO₂, so our nanozymes have the two features of AuNPs and pCeO₂, which could provide the chance for the synergistic effect of heterogeneous nanozymes.

The Raman spectra were used to analyze the structural arrangement of the nanozymes. As shown in Figure 2b, these three nanozymes exhibited a prominent vibration at 455 cm⁻¹, which was caused by the strong Raman-active F_{2g} vibrational mode typical of the CeO₂ fluorite structure. Moreover, the peaks at this position were relatively similar for all three nanozymes, indicating the integrality of a rod-like structure. Due to the existence of a defect-induced (D) mode [40], CeO₂ and pCeO₂ showed a weak peak at 600 cm⁻¹. After the loading with AuNPs, there was a significant change in the peak here, which also indicated AuNPs doping into the CeO₂ lattice. It could be further concluded that the AuNPs were present on our prepared Au-pCeO₂ nanozyme.

Using MP as the model substrate, the phosphatase-like catalytical performance of CeO₂, pCeO₂, and Au-pCeO₂ for OPs was assessed by the yield of *p*-NP. A nanozyme with phosphatase-like activity can catalyze the generation of *p*-NP from MP. According to the *p*-NP standard curve (Figure S3), we can calculate the yield of *p*-NP. Here, three parallel experiments were set up. As shown in Figure 2c, the yields obtained for CeO₂-, pCeO₂-, and Au-pCeO₂-catalyzed MP were 19.98% ± 1.25%, 27.16% ± 1.96%, and 45.65% ± 4.93%, respectively. Compared to CeO₂, the catalytic performance of pCeO₂ was higher, which was due to the surface defects and higher Ce⁴⁺ species [41,42]. After the hybridization of AuNPs, the activity of the nanozyme effectively increased, reaching levels twice as high as those of pCeO₂. To reveal the catalytic mechanism, the distribution of Ce species on CeO₂, pCeO₂, and Au-pCeO₂ was investigated by chemical valence state analysis (Figure S4a–d). According to Figure S4h and Figure 2d, the proportion of Ce⁴⁺ species increased after the calcination treatment for CeO₂. This indicates that Ce³⁺ was shifted to Ce⁴⁺ in pCeO₂ due to high-temperature (400 °C) calcination, which can increase the hydrolysis ability of the nanozyme [30]. In the Au 4f XPS spectra of Au-pCeO₂, double peaks appeared and were assigned to Au 4f_{7/2} and 4f_{5/2}. The peaks at 83.46 and 87.13 eV could be assigned to Au (Figure S4g). Although the modification of AuNPs can lead to a decrease in Ce³⁺/Ce⁴⁺, the higher catalytic performance of Au-pCeO₂ for MP may be attributed to the synergistic catalytic ability of two heterogeneous catalysts.

3.2. Optimization and Characterization of Au-CeO₂ Nanozymes

Based on the above, we designed a series of Au-CeO₂ nanozymes with different proportions of AuNPs by modulating the addition amount of the precursor solution (50 mM HAuCl₄). In three parallel sets of experiments (Figure 3a), with the addition of HAuCl₄ solution, the yield of *p*-NP of the obtained nanozyme increased. However, the catalytic performance of the obtained Au-CeO₂ nanozyme became lower as the volume continued to increase. We inferred that was because more AuNPs could aggregate on the surface of pCeO₂, which could occupy the active sites of the nanozymes. When the addition volume of HAuCl₄ was 0.3 mL, the obtained Au-pCeO₂ nanozyme showed higher and more stable catalytic performance with a 44.24% ± 2.62% yield of *p*-NP. Thus, the optimal addition volume of HAuCl₄ was set at 0.3 mL.

After that, the morphology of the optimal Au-pCeO₂ nanozyme was characterized using TEM and HRTEM (Figure 3b,c). It is obvious that AuNPs with a size of 10.52 ± 0.30 nm (Figure S2c) had a favorable distribution on the surface of the Au-pCeO₂ nanozyme, and pCeO₂ still retained a rod-like structure with a clearly visible porous structure. Subsequently, the elemental composition of the Au-CeO₂ nanozyme was further confirmed using EDS mapping analysis. As shown in Figure 3d, Au, Ce, and O elements are clearly visible, corresponding to the composition of the Au-CeO₂ nanozyme and indicating the successful modification of AuNPs on the surface of pCeO₂.

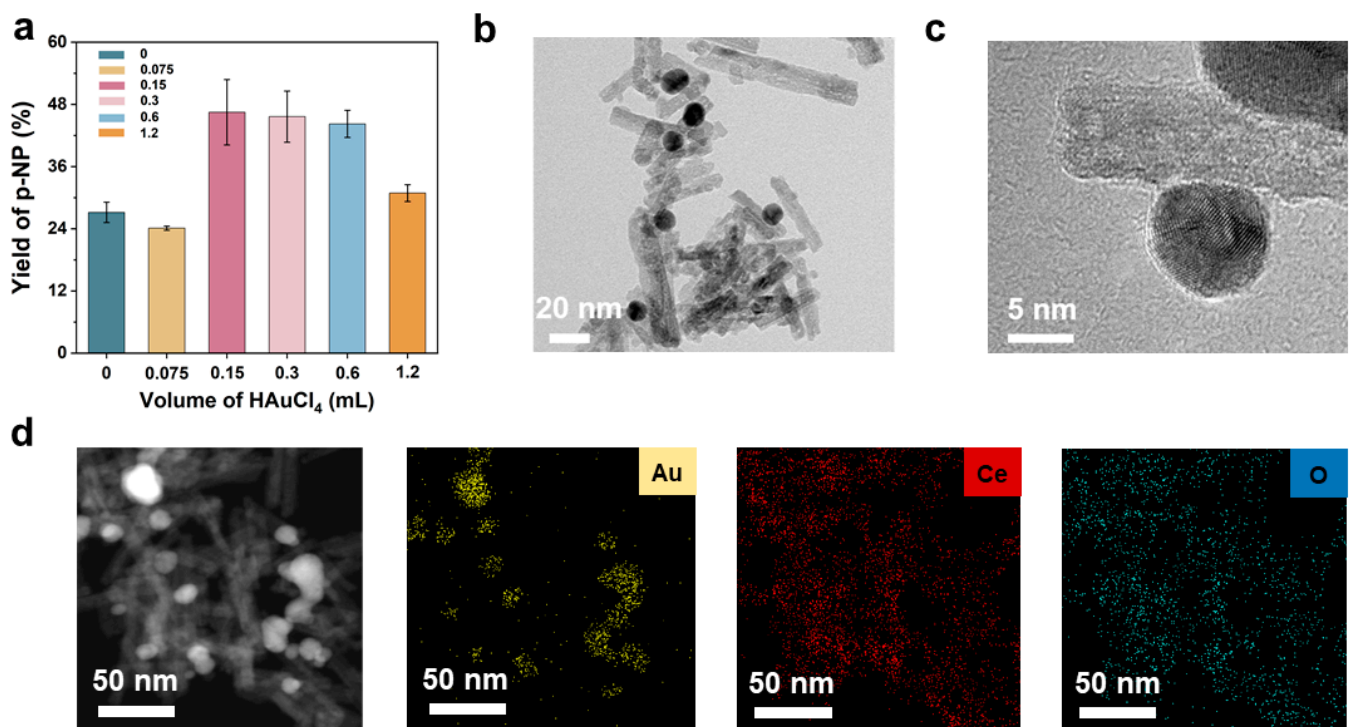


Figure 3. Optimization and characterization of Au-pCeO₂ nanozyme. (a) Various *p*-NP yields of nanozymes at different HAuCl_4 addition levels. (b) TEM and (c) HRTEM images of the optimal Au-pCeO₂ nanozyme. (d) EDS spectra of the Au-pCeO₂ nanozyme.

3.3. Catalytic Conditions of Au-pCeO₂ Nanozyme

To improve the performance of the assay, the influence of various factors on catalytic activity was studied, including reaction temperature, reaction time, buffer pH, and the amount of Au-pCeO₂. The optimum catalytic conditions were investigated by calculating the *p*-NP yields of MP (200 μM) under different conditions. Firstly, we assessed the influence of reaction temperatures (35, 45, 55, 65, 75, and 85 $^{\circ}\text{C}$) on the hydrolysis performance of the Au-CeO₂ nanozyme (10 mg) with a reaction time of 1 h. As shown in Figure 4a, the reaction temperature had a significant effect on the production of *p*-NP. With the increase in temperature, the yield of *p*-NP increased. After the temperature reached 75 $^{\circ}\text{C}$, the yield of *p*-NP increased the most. Therefore, we selected 75 $^{\circ}\text{C}$ as the optimum reaction temperature. Then, the yields of *p*-NP under different reaction time (0.5, 1, 2, 3, 4, 5, and 6 h) were compared at 75 $^{\circ}\text{C}$. As shown in Figure 4b, a higher *p*-NP yield was obtained with a reaction time of 5 h. Thus, the optimal reaction time was set to 5 h.

After that, we investigated the effect of pH (6, 7, 8, 9, and 10) on the activity of Au-pCeO₂ (10 mg) under the reaction condition of 75 $^{\circ}\text{C}$ for 5 h. From Figure 4c, the absorbance at 400 nm increased when the pH value of Tris buffer tended to be alkaline. This takes place because the absorption peak of *p*-NP would shift to 310 nm under acid conditions, leading to a weak absorption peak [43]. When the pH value of the buffer solution was 9, the peak of *p*-NP was the highest. Thus, the optimum pH value of the buffer solution was 9. Finally, the amount of Au-pCeO₂ nanozyme was studied under the above optimized conditions. As shown in Figure 4d, upon increasing the amount of Au-pCeO₂ from 0 to 10 mg, more *p*-NP was produced by the hydrolysis action. While the yield of *p*-NP tends to decrease keeping increasing the amount of Au-pCeO₂. We inferred that too many nanozymes had poor dispersion in the reaction system, so the optimal amount of Au-pCeO₂ was 10 mg.

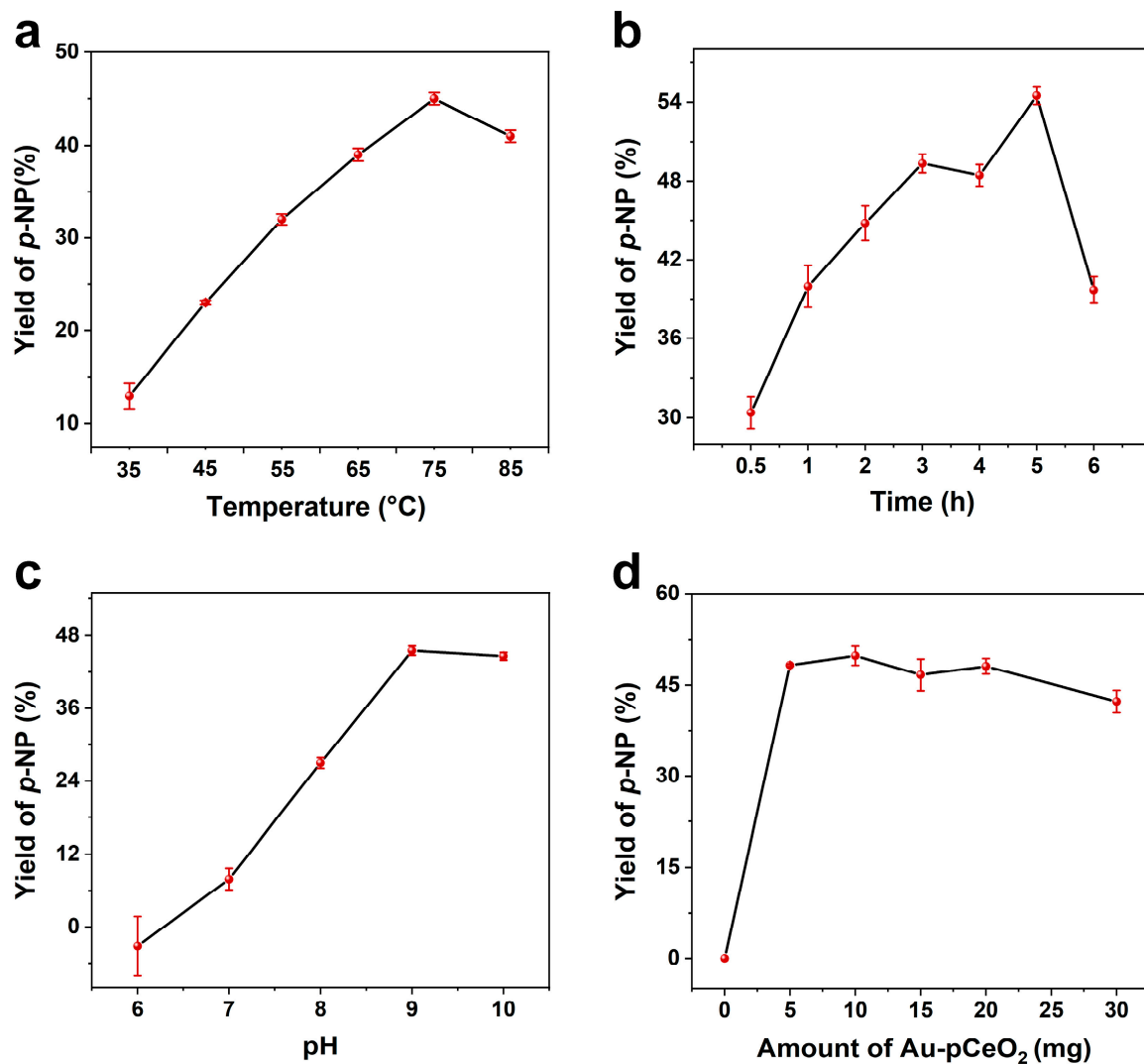


Figure 4. Optimization of reaction conditions of Au-pCeO₂ nanozyme for the hydrolysis of 200 μ M MP. (a) Reaction temperature, (b) reaction time, (c) pH value of Tris buffer, and (d) amount of Au-pCeO₂ for the catalytic performance.

3.4. Detection Performance for MP

Under optimal conditions, we developed a Au-pCeO₂ nanozyme-based colorimetric sensor and applied it for MP analysis via UV-Vis and a smartphone. First of all, the UV-Vis method was used to investigate the detection performance of our sensor. When the MP concentration was in the range of 5 to 200 μ M, the absorbance at 400 nm ($A_{400\text{ nm}}$) had a linear relationship with the concentration of MP (Figure 5a). As shown in Figure 5b, the standard curve was fitted with the MP concentration as the horizontal coordinate and A_{400} as the vertical coordinate. The linear equation was $y = 0.0029x + 0.0821$, with a high correlation coefficient ($R^2 = 0.9984$), where y represented A_{400} and x represented the MP concentration. On the basis of the equation of detection limit ($\text{LOD} = 3 \text{ Sb}/m$ (Sb is the standard deviation of UV-Vis in the blank experiment and m is the slope of the calibration curve) [33], the LOD of this method is 0.5 μ M, which exhibits comparable analytical performance compared to the reported phosphatase-like nanozyme-enabled sensors (Table S1).

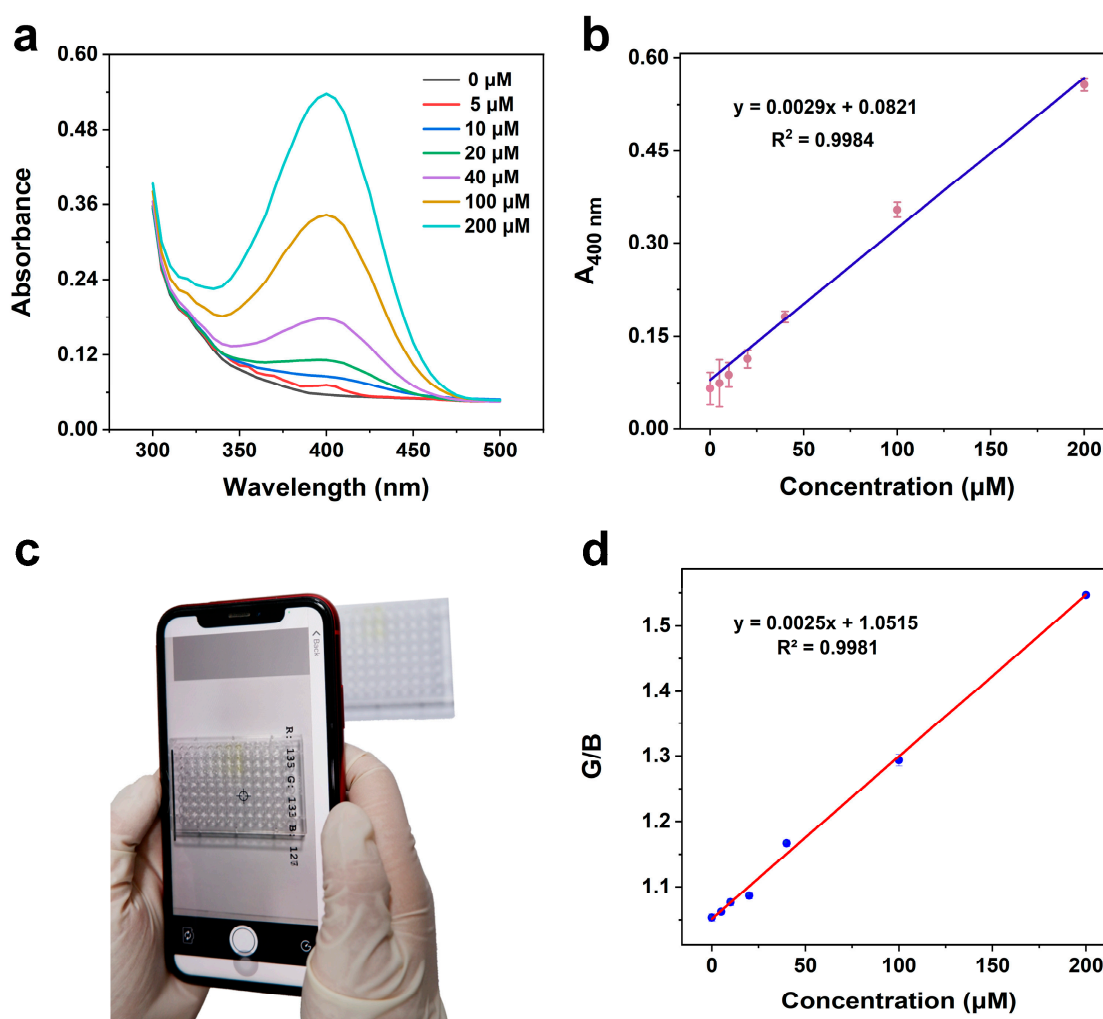


Figure 5. Au-pCeO₂ nanozyme-based colorimetric sensor for MP detection. (a) UV-Vis spectra of various concentrations of MP (0–200 μM). (b) The corresponding calibration curve at 400 nm with UV-Vis spectra. (c) Photograph of the smartphone analysis of various concentrations of MP (0–200 μM). (d) The corresponding calibration curve with the G/B value on a smartphone.

In addition, the analytical performance of this sensor was also evaluated on a smartphone, which can be used for the on-site analysis of MP without complex equipment or instruments. By taking a picture of the reaction solution in a 96-well plate, the R/B value was recorded by the “Color Picker” app (Figures 5c and S5). As shown in Figure 5d, the G/B value was also linearly related to the concentration of MP, with a linear equation of $y = 0.0025x + 1.0515$ (where y is the G/B value and x is the MP concentration) and a correlation coefficient of $R^2 = 0.9981$. Based on these two analytical methods, the dual-model strategy proposed in this study can provide an accurate and reliable method for the on-site detection of MP.

3.5. Selectivity, Interference, Stability, and Recoverability

To investigate the selectivity of the prepared sensor, we compared the hydrolysis ability of Au-pCeO₂ for methyl paraoxon, carbendazim, phosphamidon, and monocrotophos with the same concentration of MP (100 μM). As shown in Figure 6a, our nanozyme exhibited high *p*-NP yields for MP ($24.16\% \pm 4.06\%$) and methyl paraoxon ($65.46\% \pm 4.07\%$), while other pesticides produced almost no *p*-NP, indicating its excellent selectivity for these two OPs. The higher yield of *p*-NP for catalytic paraoxon may be due to its P=O bond, resulting in its higher toxicity, which is more easily hydrolyzed by the phosphatase [44].

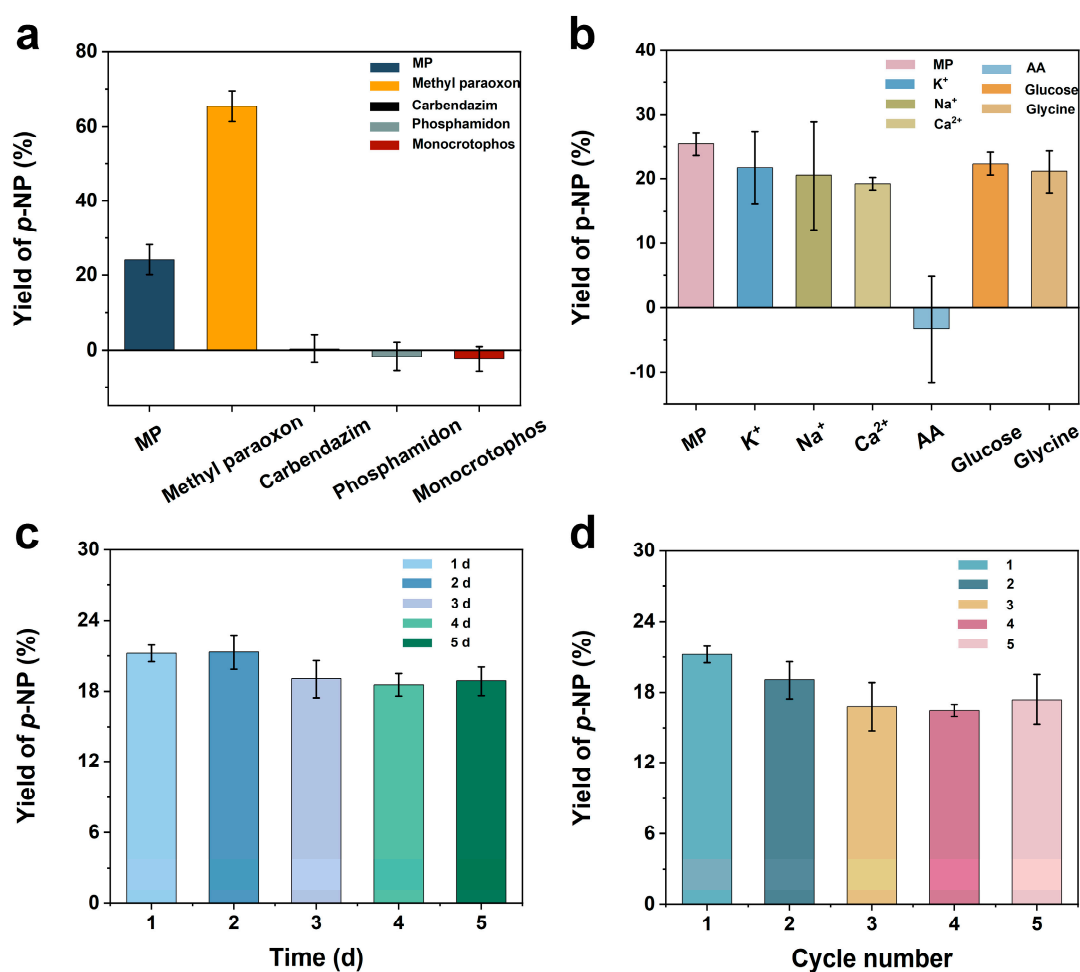


Figure 6. (a) Selectivity for various pesticides with the same concentration (100 μ M); (b) interference resistance in the presence of coexistence substances (K^+ , Na^+ , and Ca^{2+} at 20 mM and ascorbic acid, glucose, and glycine at 10 mM); (c) storage stability in five days; (d) reproducibility study under five cycles of the proposed colorimetric sensor.

In addition, the anti-interference study of our sensor was evaluated by testing the hydrolysis product (i.e., *p*-NP) of MP (100 μ M) along with different coexisting substances (K^+ , Na^+ , and Ca^{2+} at 20 mM and ascorbic acid, glucose, and glycine at 10 mM). As shown in Figure 6b, the interference was negligible for most of the co-existing substances, except for ascorbic acid. The addition of ascorbic acid may change the original pH of the buffer, which is significantly different from the optimized conditions, thus affecting the yield of *p*-NP. It is worth noting that the amount of ascorbic acid in most agricultural products is less than 10 mM. Hence, our sensor has good anti-interference performance, making real sample analysis possible.

After that, the storage stability of our Au-pCeO₂ nanozyme was investigated by testing the yield of *p*-NP for MP (100 μ M) in five days at room temperature. As can be seen from Figure 6c, Au-pCeO₂ retained 89% of the activity after five days compared to the original nanozyme, which demonstrates the satisfactory storage stability of the synthesized Au-pCeO₂.

Recoverability is also an important indicator for catalysts in practical applications. For the recoverability study, our nanozyme was used for the hydrolysis of MP (100 μ M) under optimal conditions, and then the used Au-pCeO₂ was washed three times and dried. The recyclable Au-pCeO₂ was then sonicated and dispersed into the buffer for the next five rounds of reaction. As shown in Figure 6d, the yield of *p*-NP was not less than

17.3% $\pm$ 2.08%, indicating our Au-pCeO₂ nanozyme can be reused many times, offering economic benefits and convenience.

3.6. Real Sample Analysis

In this study, pears and lettuces were selected as the real samples. Before the analysis, no MP was detected in these samples, allowing them to be further used as blank samples for the recovery experiment. Herein, three spiking levels (25, 50, and 100 μ M) were set in the blank samples. The sample pretreatment method was performed in Section 2.4. According to the UV-Vis analysis, the recoveries in the pear samples ranged from 87.47% to 104.83%, with relative standard deviations (RSDs) ranging from 1.69% to 2.69% ($n = 3$), while the recoveries in the lettuce samples ranged from 93.33% to 103.12%, with RSDs of 1.21% to 2.15% ($n = 3$) (Table 1). To verify the availability of our dual-mode strategy, the recovery experiment was also assessed by the RGB analysis, which also exhibited similar recoveries ranging from 85.27% to 115.87%, with RSDs of 1.96% to 6.20% for these two samples, demonstrating the favorable availability of our dual-mode strategy for MP detection in agricultural products.

Table 1. Recovery study of MP in pear and lettuce samples by UV-Vis and RGB analysis ($n = 3$).

Method	Samples	Added (μ M)	Found (μ M)	Recovery (%)	RSD (%; $n = 3$)
UV-Vis	Pear	25	26.21 $\pm$ 0.71	104.83	2.69
		50	49.22 $\pm$ 0.83	98.45	1.69
		100	87.47 $\pm$ 1.76	87.47	2.01
	Lettuce	25	25.78 $\pm$ 0.56	103.12	2.15
		50	46.67 $\pm$ 0.80	93.33	1.72
		100	95.92 $\pm$ 1.16	95.92	1.21
RGB	Pear	25	28.97 $\pm$ 0.57	115.87	1.96
		50	42.63 $\pm$ 1.60	85.27	3.76
		100	94.40 $\pm$ 4.85	94.40	5.14
	Lettuce	25	23.67 $\pm$ 0.85	94.67	3.59
		50	44.07 $\pm$ 2.73	88.13	6.20
		100	93.23 $\pm$ 2.31	93.23	2.47

4. Conclusions

In this study, a heterogeneous nanozyme with phosphatase-like activity was developed through the modification of AuNPs on the surface of a pCeO₂ structure. Owing to the synergistic catalytic mechanism of these two kinds of nanostructures, our Au-pCeO₂ nanozyme appeared to have favorable phosphatase-like activity, which can be used as a biomimetic hydrolase for the degradation of OPs. Based on this nanozyme, we proposed a dual-mode colorimetric sensor for the on-site detection of MP via UV-Vis and a smartphone. Under optimum conditions, this sensor showed a favorable linear relationship with the concentration of MP ranging from 5 μ M to 200 μ M. In addition, this dual-mode analytical strategy was successfully applied for MP detection in pear and lettuce samples with satisfying recoveries and low RSDs. This dual-mode assay has excellent recoverability, high stability, and good selectivity and does not need complicated equipment or professionals, which can provide a reliable and simple analytical method for OP residue determination in agricultural products.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/foods12152980/s1>, Figure S1: TEM images of (a) CeO₂, (b,c) pCeO₂ at different magnifications; Figure S2: (a) Length and (b) width of pCeO₂. (c) Size of AuNPs of Au-pCeO₂ nanozyme; Figure S3: A standard curve of *p*-NP concentration versus absorbance change. Figure S4: XPS spectra of the (a) Ce and (b) O of CeO₂, (c) Ce and (d) O of pCeO₂, and (e) Ce, (f) O, and (g) Au of Au-pCeO₂. (h) Proportions of Ce³⁺ and Ce⁴⁺ species in three nanozymes, which are simulated from XPS data; Figure S5: Digital photo of the hydrolysis product of different

concentrations of MP under the action of Au-pCeO₂ nanozyme; Table S1: Comparison of various phosphatase-like nanozymes for OP determination [28,32–34].

Author Contributions: F.Z.: Conceptualization, supervision, funding acquisition, project administration, and writing—review and editing. M.L.: Methodology, validation, formal analysis, investigation, and writing of the original draft. L.W.: Methodology, validation, and formal analysis. M.W.: Supervision and writing—review and editing. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the National Natural Science Foundation of China (grant number 32202160) and the Research Foundation for Youth Scholars of Beijing Technology and Business University (grant number QNJJ2022-27).

Data Availability Statement: The data used to support the findings of this study can be made available by the corresponding author upon request.

Conflicts of Interest: The authors declare no conflict of interest.

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