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Article

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Dual-Mode Colorimetric-SERS Detection of Fenthion Pesticide with γ -Cyclodextrin-Capped Nanozymes

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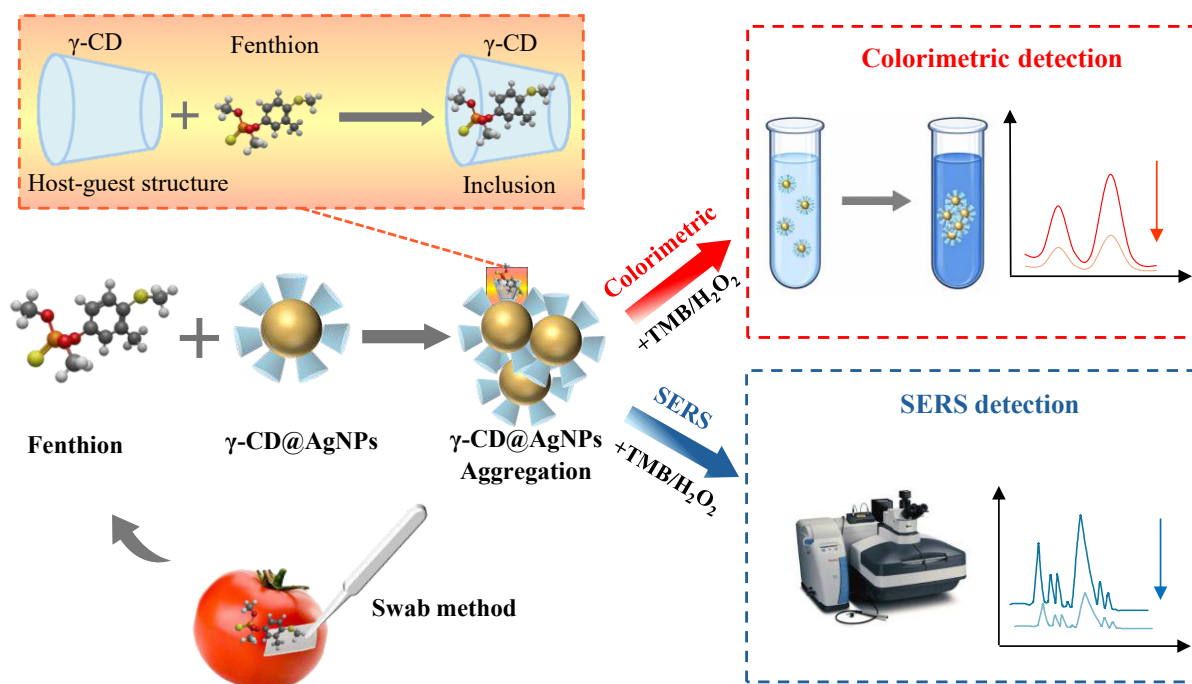
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Abstract

This study reports a multifunctional sensing platform that integrates molecular recognition by γ -cyclodextrin (γ -CD), nanozyme catalytic activity, and Raman enhancement within a single nanoparticle system for the detection of fenthion, a widely used organophosphate pesticide in agriculture. Unlike conventional single-mode assays, this sensor platform provides both colorimetric and SERS readouts, offering enhanced versatility and sensitivity. The γ -CD host–guest interaction drives nanoparticle aggregation upon fenthion binding, simultaneously suppressing peroxidase-like oxidation of 3,3',5,5'-tetramethylbenzidine and attenuating surface-enhanced Raman scattering (SERS) signals. The dual readouts enable quantitative analysis with detection limits of 7.8 ppb (SERS) and 0.618 ppm (colorimetric). Practical applicability was demonstrated in tomato samples, achieving a SERS detection limit of 0.18 ppm. This cost-effective dual-mode platform demonstrates the potential of γ -CD nanozymes for pesticide residue monitoring by combining simple colorimetric screening with more sensitive SERS-based quantification, offering a complementary approach for food safety and environmental surveillance applications.

Keywords: Colorimetric, SERS, Fenthion, Gamma-cyclodextrin, γ -CD@AgNPs

30 Graphical Abstract



31
 32 Schematic illustration of γ -CD-capped AgNPs forming inclusion complexes with fenthion, inducing γ -
 33 CD@AgNPs aggregation for dual-mode colorimetric-SERS sensing.

Introduction

With global food production expected to decline by 10-25% and the global population projected to grow by around 20%¹, reliance on pesticides is likely to increase substantially to offset reduced crop yields. Organophosphorus pesticides (OPPs) are a type of highly efficient pesticide for the yield of crops and plants². It has been reported that the consumption of OPPs exhibits the highest usage, comprising approximately 33% of the global pesticide consumption, equivalent to 1.65 billion pounds³. This widespread use is alarming due to their acute toxicity, which threatens food safety, human health, and environmental sustainability^{4,5}. Exposure to OPP residues can impair multiple human organs, including the nervous, respiratory, digestive, and hematological systems⁶.

Among OPPs, fenthion is frequently used due to its high insecticidal efficacy^{7,8}. Its persistence and bioaccumulation in soil and water however led to environmental contamination and entry into the food chain⁹. Furthermore, fenthion poses ecological risks by affecting non-target species, including birds and pollinators, thereby disrupting ecosystem stability¹⁰. To mitigate these risks, various countries and regions have implemented strict regulations on fenthion, establishing specific maximum residue limits (MRLs). Around the world, the MRLs for fenthion differ greatly. The European Union sets the limit at 0.01 mg/kg (0.01 ppm)¹¹, while Australia allows up to 5 mg/kg (5 ppm)¹². Consequently, the development of accurate and reliable assays for the detection of fenthion residues at concentrations below the established MRLs is critical for ensuring public health and ecological protection.

Current methods for pesticide residue detection primarily include chromatography, high-performance liquid chromatography (HPLC), enzyme-linked immunosorbent assay (ELISA), and fluorescence spectroscopy¹³. Whilst these techniques are highly sensitive and accurate, many of these methods require expensive instrumentation, labour-intensive protocols, and trained personnel¹⁴, which limit their practicality for on-site rapid detection. Detection methods based on Surface-enhanced Raman Spectroscopy (SERS) have emerged as a highly attractive tool, offering key advantages such as low cost, rapid analysis, and high sensitivity¹⁵. These features make SERS particularly well-suited for on-site or resource-limited applications. However, many conventional SERS systems rely on measuring a single Raman signal without internal referencing, which can limit robustness and make them susceptible to fluctuations from environmental or instrumental factors. To overcome these limitations, multimodal detection strategies are gaining attention. By integrating multiple, complementary signal readouts, these approaches significantly enhance analytical reliability and sensitivity, enabling both qualitative identification and trace level detection of target analytes¹⁶.

SERS provides highly sensitive detection by amplifying the intrinsic 'molecular fingerprint' signals of target molecules adsorbed onto plasmonic nanostructures, offering rapid analysis with minimal sample preparation. Colorimetric assays are simple and intuitive, suitable for on-site screening, while surface-

enhanced Raman spectroscopy (SERS) offers high sensitivity and molecular fingerprinting capabilities, enabling precise quantification of trace analytes. The combination of these methods effectively reduces false positives and improves the analytical accuracy¹⁷. By integrating SERS with colorimetric assays which are known for their intuitive visual output and ease of use, a dual-mode biosensor can be established. This hybrid strategy has been shown to reduce false positives and enhance analytical accuracy through mutually reinforcing detection signals. Beyond improved accuracy, the dual-mode design also strengthens robustness, reliability, and operational efficiency in biomarker detection¹⁸. Owing to the rapid progress in nanotechnology, a variety of nanomaterials with tailored optical and catalytic properties have been developed, enabling their integration into both colorimetric and SERS-based sensing strategies. Noble metal nanoparticles, particularly gold (AuNPs) and silver nanoparticles (AgNPs), exhibit localized surface plasmon resonance (LSPR) under light excitation, resulting in strong light absorption and scattering¹⁹. The size, shape, capping agent, and aggregation state determine the optical colour variations associated with plasmonic light- nanoparticle interactions²⁰. The regulation of AuNP aggregation state has been widely used as a colorimetric sensor for the detection of OPPs and other organic molecules²¹. Upon aggregation, the interparticle distance decreases, leading to plasmon coupling, a redshift in the absorption spectrum, and visible colour changes. Such aggregation-induced optical transitions enable intuitive, visual detection of target analytes. For example, cross-linking and aggregation of metal nanostructures (AuNPs, Au nanorods) have been demonstrated to produce "hot spots" with highly localized electromagnetic fields, significantly amplifying SERS signals and enabling sensitive detection of analytes²². This aggregation-mediated modulation of optical and electromagnetic properties not only facilitates colorimetric and SERS detection, but also influences catalytic activities, offering further opportunities for integrated dual-mode sensing strategies.

In addition to plasmonic enhancement, enzyme-mimicking nanomaterials, so called 'nanozymes', have emerged as powerful tools to augment biosensing capabilities, due to their functional versatility and controllability^{23,24}. Typically, nanozymes catalyse substrate oxidation reactions, generating measurable colorimetric signals that complement optical detection strategies. For instance, Cao et al. employed platinum nanoparticles (PtNPs) as efficient peroxidase mimics to catalyse the oxidation of 3,3',5,5'-tetramethylbenzidine (TMB) by hydrogen peroxide (H₂O₂)²⁵. The catalytic activity of PtNPs was suppressed upon aggregation triggered by the hydrolysis products of acetylcholinesterase (AChE), enabling the development of a highly sensitive PtNP catalysis-based enzyme inhibition assay for the detection of pesticides²¹. Additionally, Yang et al. synthesized Fe single-atom nanozymes (HCNs@Fe SAs) with high peroxidase (POD)-like activity and constructed a portable colorimetric biosensor integrating AChE, thioacetylthiocholine iodide (ATChI), and HCNs@Fe SAs for the detection of AChE activity and OPPs residues²⁶. However, compared to the extensive research on AuNP-based colorimetric sensors and nanozymes, studies focusing on AgNP-based colorimetric systems and Ag nanozymes remain relatively scarce²⁵. This gap points to an opportunity to explore the unique catalytic

and optical properties of silver nanostructures for pesticide detection and the design of dual-mode sensing systems.

In view of this, a dual-mode sensing platform integrating rapid colorimetric screening with sensitive SERS-based detection of fenthion was developed. Gamma-cyclodextrin (γ -CD) was employed in a one-step, green synthesis of Au and Ag nanoparticles (γ -CD@AuNPs and γ -CD@AgNPs), which provided both strong SERS enhancement and intrinsic peroxidase-like catalytic activity. Unlike conventional sensors that rely primarily on signal amplification, the present system integrates molecular recognition (γ -CD host-guest inclusion), catalytic nanozyme activity, and plasmonic SERS enhancement within a single nanoparticle platform. Cyclodextrins are cyclic oligosaccharides that consist of six, seven, or eight glucopyranose units in α , β , and γ forms, respectively. Owing to their unique molecular architecture featuring a hydrophobic inner cavity and a hydrophilic outer surface, cyclodextrins are well known for forming inclusion complexes with guest molecules.²⁷ The catalytic surfaces of γ -CD@AuNPs and γ -CD@AgNPs enabled the oxidation of TMB into oxidised product (oxTMB), accompanied by a visible blue coloration. In the absence of fenthion, the well-dispersed nanoparticles efficiently catalyzed the oxidation of TMB, producing strong colorimetric signals from oxTMB along with enhanced SERS responses. However, in the presence of fenthion, its strong affinity for the γ -CD-coated nanoparticles induces their aggregation, which significantly reduces the available catalytic surface area and consequently suppresses oxTMB production. Consequently, both the colorimetric response and SERS intensity of oxTMB were diminished. This aggregation-induced suppression of catalytic activity established an inverse calibration relationship between fenthion concentration and SERS signal intensity. The developed γ -CD nanozyme dual-mode sensor offers a sensitive and reliable platform for applications in food safety, medical diagnostics, and environmental monitoring, with an example of applicability presented herein.

Results and discussion

Synthesis and characterisation of γ -CD@AuNPs and γ -CD@AgNPs

The γ -CD@AuNPs and γ -CD@AgNPs were synthesized via a one-step green method, as illustrated in Fig. 1a. The reduction of Au^{3+} and Ag^+ ions and the concurrent stabilization of the resulting nanoparticles were facilitated by γ -CD, a natural macrocyclic oligosaccharide, under alkaline conditions (pH 10 - 12) at 60 °C for 20 min. The multifunctional role of cyclodextrins, serving as both reducing and stabilizing agents in noble-metal nanoparticle synthesis, has been well documented in the literature^{28,29}. Owing to its larger cavity size, superior water solubility, and enhanced complexation ability compared to α - and β -cyclodextrins, γ -CD serves as an effective inclusion and encapsulation agent³⁰. Moreover, alkaline conditions facilitate the deprotonation of the -OH groups of CD, which would significantly promote the formation and stabilization of nanoparticles³¹. The formation of γ -CD@AuNPs and γ -CD@AgNPs was evidenced by distinct colour changes, with γ -CD@AuNPs appearing red and γ -CD@AgNPs appearing yellow. These visual observations were further corroborated by absorbance spectroscopy, which revealed characteristic LSPR peaks at 519 nm and 412 nm, respectively (Fig. 1b), confirming successful nanoparticle synthesis.

The morphology of the synthesised γ -CD@AuNPs and γ -CD@AgNPs was characterised by TEM. As shown in Fig. 1c, the TEM image of γ -CD@AuNPs revealed uniformly distributed spherical particles with sizes ranging from 6 to 13 nm, with a mean diameter of 10 ± 2 nm. Similarly, the γ -CD@AgNPs exhibited well-dispersed, spherical nanoparticles with diameters ranging from 20 to 40 nm and an average size of 29 ± 8 nm (Fig. 1d). The surface functionalization of the nanoparticles was further examined by Fourier-transform infrared (FTIR) spectroscopy (Fig. 1e). The spectrum of modified AgNPs and AuNPs was compared with that of original γ -CD to verify the capping of cyclodextrin on the surface of AgNPs and AuNPs. The steric repulsion between CD molecules contributes to the stability of NPs³². Furthermore, the FTIR spectrum of γ -CD-functionalized NPs exhibited a prominent absorption band at 1032 cm^{-1} , assigned to the C-O-C bending vibration of γ -CD³³. The inset in Fig. 1e presents a magnified view of this region, showing that this characteristic peak closely matches that of γ -CD powder, confirming the successful capping of γ -CD on the NPs surface. In addition, across five independently prepared batches, the UV-Vis spectra showed consistent LSPR peak positions (γ -CD@AuNPs: 517–521 nm; γ -CD@AgNPs: 412–418 nm). Following storage at room temperature, the nanoparticles retained their characteristic LSPR features without noticeable peak shifts or spectral broadening (Fig. S1), demonstrating good batch-to-batch reproducibility and optical stability, consistent with previously reported cyclodextrin-stabilized noble metal nanoparticles³⁴. These results confirm the successful synthesis of well-dispersed and uniformly sized γ -CD@AuNPs and γ -CD@AgNPs with stable γ -CD surface modification.

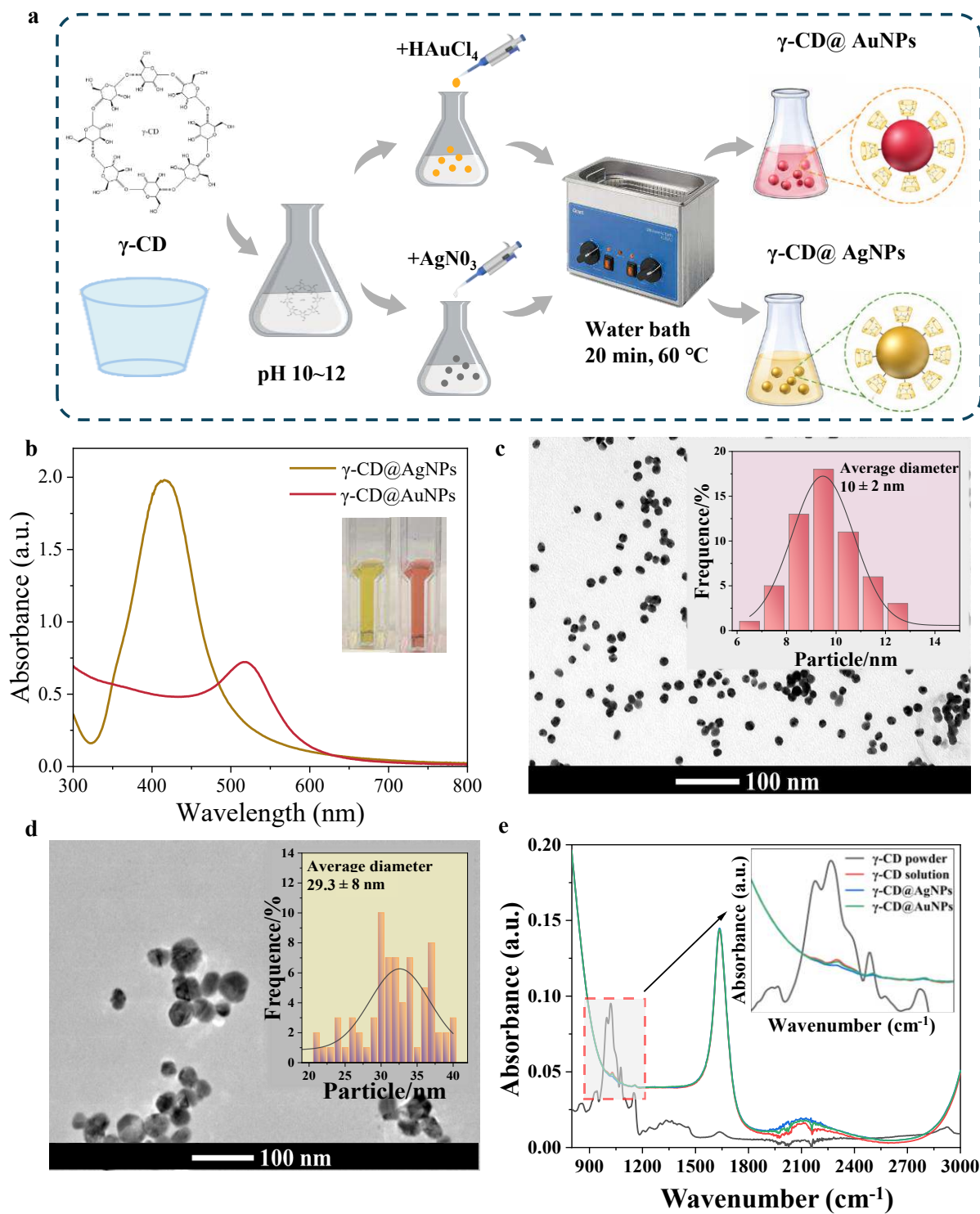


Fig. 1. Characterisation of γ -CD@AuNPs and γ -CD@AgNPs. (a) Schematic representation of the synthesis process for γ -CD@AuNPs and γ -CD@AgNPs. (b) Absorption spectra of γ -CD@AuNPs and γ -CD@AgNPs, showing characteristic LSPR peaks at 519 nm and 412 nm, respectively. The inset displays photographs of the resulting colloidal solutions. (c) TEM image and corresponding particle-size distribution of γ -CD@AuNPs. (d) TEM image and corresponding particle-size distribution of γ -

CD@AgNPs. Particle-size distributions were determined from 100 particles using ImageJ. (e) FTIR spectra of γ -CD powder, γ -CD solution, γ -CD@AuNPs, and γ -CD@AgNPs.

The POD-like activity and SERS signal amplification performance of γ -CD nanozyme

The mechanism by which γ -CD@AuNPs and γ -CD@AgNPs oxidize TMB and enhance the Raman signal is shown in Fig. 2a. The POD-like activity of these γ -CD-capped nanozymes was systematically evaluated using TMB as a chromogenic substrate in the presence of H_2O_2 . Upon oxidation, TMB is converted into its oxidized form (oxTMB), accompanied by a visible colour change from colourless to dark blue. Absorbance spectroscopy (Fig. 2b) revealed two diagnostic absorbance peaks at 370 nm and 652 nm, corresponding to the radical cation (TMB^+) and the charge-transfer complex (CTC), respectively³⁵. Notably, the γ -CD@AuNPs generated a significantly stronger oxTMB signal than that of γ -CD@AgNPs, indicating higher POD-like activity.

To further validate the catalytic performance of the synthesized nanoparticles, we first evaluated the effect of γ -CD nanozyme concentration on POD-like activity. As shown in Fig. S2, both the colour intensity and absorbance at 370 nm increased with higher nanozyme concentrations, confirming the concentration-dependent catalytic behaviour. Following this, steady-state kinetic analyses based on Michaelis–Menten kinetics were conducted over a TMB concentration range of 0–0.45 mM. The kinetic parameters, including the maximum reaction velocity (V_{max}) and the Michaelis constant (K_{m}), were determined (Fig. 2c) and summarized in Table S1. All measurements were performed using identical nanoparticle concentrations, with particle number calculations provided in Supplementary Information Section 1. Although γ -CD@AuNPs exhibited a higher V_{max} ($1.727 \times 10^{-4} \text{ M min}^{-1}$) and a larger K_{m} ($4.2 \times 10^{-2} \text{ mM}$) compared to those of γ -CD@AgNPs ($V_{\text{max}} = 1.994 \times 10^{-5} \text{ M min}^{-1}$; $K_{\text{m}} = 3.6 \times 10^{-2} \text{ mM}$), their substantially higher turnover number (k_{cat}) resulted in a greater overall catalytic efficiency ($k_{\text{cat}}/K_{\text{m}}$) indicating their superior catalytic activity. This observation was further supported by kinetic analyses of POD-like activity over 30 min and catalytic constant (K_{cat}) comparisons (Fig. S3 and Fig. S4), both consistently showing the superior catalytic performance of γ -CD@AuNPs over γ -CD@AgNPs. These results align with previous studies reporting enhanced catalytic efficiency of gold nanoparticles relative to silver counterparts³⁶. This enhancement can be attributed not only to the intrinsic catalytic properties of gold but also to its smaller particle size (10 nm vs. 29 nm, as shown in the TEM images), which results in a higher surface-to-volume ratio and a greater density of catalytically active, low-coordinated surface atoms³⁷. Such small and structurally uniform nanoparticles are widely recognized to provide more accessible active sites for interfacial catalytic reactions.

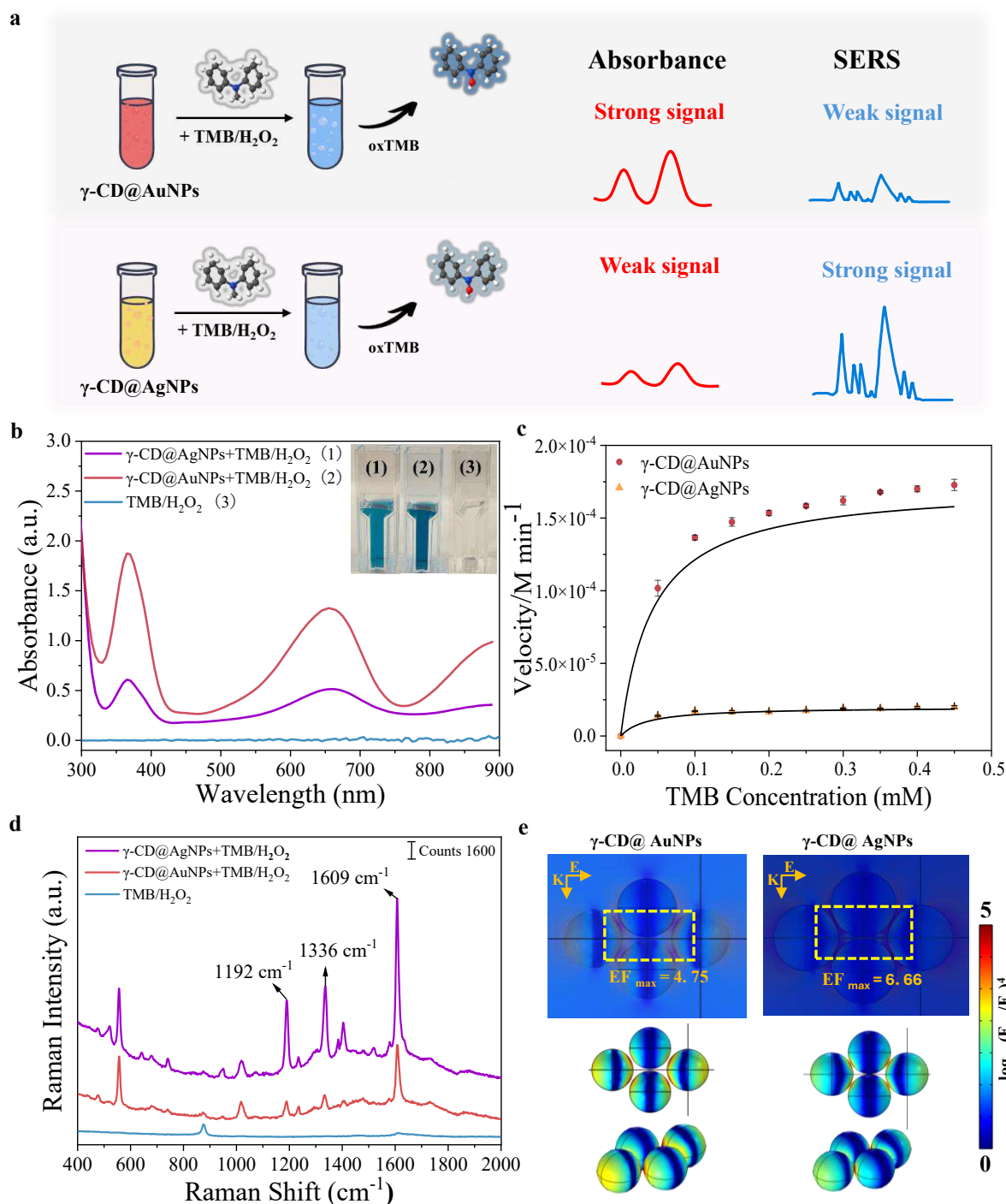
Moreover, a comparison with sodium citrate-reduced gold nanoparticles (Cit-AuNPs), synthesized via the Turkevich method³⁸, demonstrated that γ -CD@AuNPs exhibited superior catalytic activity. The enhanced performance of the γ -CD-synthesized nanoparticles was further confirmed through steady-state kinetics studies, as presented in Fig. S5 and Table S1. In addition to the particle size, CD capping

has been reported to significantly enhance nanozyme catalytic performance by modulating the surface microenvironment³⁹. For example, β -CD-modified Pd@Au nanozymes showed approximately 3.95-fold higher k_{cat} values compared with unmodified Pd@Au, which was attributed to cyclodextrin-assisted enrichment of substrate molecules near the catalytic surface, thereby enhancing TMB binding affinity and facilitating catalytic reaction kinetics. This mechanism is consistent with the enhanced peroxidase-like activity observed for γ -CD-capped nanozymes in this work. Collectively, these findings demonstrate that both γ -CD@AuNPs and γ -CD@AgNPs effectively catalyse the oxidation of TMB in the presence of H_2O_2 , yielding a blue oxidized product and underscoring the potential of γ -CD-mediated synthesis as a green and efficient strategy for developing high-performance nanozymes.

The SERS performance of γ -CD@AuNPs and γ -CD@AgNPs was subsequently evaluated. As shown in Fig. 2d, the Raman spectra of TMB/ H_2O_2 alone exhibited no discernible peaks, indicating the Raman-inactive nature of the unreacted substrate. In contrast, the addition of γ -CD nanozymes resulted in the appearance of three prominent bands at 1609, 1336, and 1190 cm^{-1} , corresponding to aromatic ring stretching and C-H bending, inter-ring C-C stretching, and CH_3 bending, respectively⁴⁰. These results confirm the transformation of Raman-inactive TMB into Raman-active oxTMB species. Interestingly, γ -CD@AgNPs consistently exhibited stronger SERS enhancement than γ -CD@AuNPs, with the 1609 cm^{-1} band showing the most pronounced intensity. A similar trend was also observed using rhodamine 6G (R6G) as a Raman probe (Fig. S6), which further proved that γ -CD@AgNPs exhibited strong SERS signal enhancement performance.

To rationalise this difference, Finite element method (FEM) simulations of electromagnetic field distribution were performed at the 785 nm excitation wavelength, mimicking the experimental scenario. The calculated maximum near-field enhancement (mNE, on $\log_{10}$ scale) was 4.75 for γ -CD@AuNPs arrays and 6.66 for γ -CD@AgNPs arrays (Fig. 2e), indicating stronger LSPR coupling in the silver-based aggregation system. The FEM model building can be seen in Section 2 of the supplementary information and Fig. S7. This enhanced electromagnetic confinement underpins the superior SERS performance of γ -CD@AgNPs. The time-dependent evolution of absorbance (Fig. S8a, b) and SERS (Fig. S8c-d) signals further confirmed the progressive catalytic oxidation of TMB by the nanozymes and revealed distinct differences in the catalytic and SERS responses of the two systems. Collectively, these results demonstrate that γ -CD-capped Au and Ag nanozymes possess dual functionalities, including POD-mimicking catalytic activity and Raman enhancement capability. γ -CD@AuNPs exhibited enhanced catalytic activity compared to both Cit-AuNPs and γ -CD@AgNPs. Specifically, the catalytic efficiency of γ -CD@AuNPs was 1.13-fold higher than that of Cit-AuNPs and 7.4-fold greater than that of γ -CD@AgNPs. However, γ -CD@AgNPs consistently generated stronger SERS enhancement owing to their enhanced electromagnetic field amplification, as supported by the FEM simulations. These results highlight the complementary functional characteristics of the two γ -CD

237 nanozymes, with γ -CD@AuNPs favouring catalytic efficiency and γ -CD@AgNPs providing superior
 238 SERS signal amplification.



239
 240 **Fig. 2.** Evaluation of peroxidase-like activity and SERS signal enhancement by γ -CD@AuNPs and γ -
 241 CD@AgNPs. (a) Mechanism diagram of γ -CD@AuNPs and γ -CD@AgNPs showing TMB oxidation
 242 for both colorimetric and SERS signal amplification. (b) Absorbance spectra demonstrating peroxidase-
 243 like catalytic activity of γ -CD@AuNPs and γ -CD@AgNPs. (c) Steady-state kinetic analysis of γ -
 244 CD@AuNPs and γ -CD@AgNPs. (d) SERS spectra showing signal enhancement of oxidized TMB

generated by the catalytic activity of γ -CD@AuNPs and γ -CD@AgNPs. (e) FEM simulations of SERS electromagnetic nearfield enhancement distribution around γ -CD@AuNPs and γ -CD@AgNPs, illustrating the mechanism of SERS enhancement due to LSPR effects.

Catalytic and SERS responses of γ -CD nanozymes in the presence of fenthion

Moving forward, the catalytic and SERS responses of γ -CD@AuNPs and γ -CD@AgNPs in the presence of fenthion were comparatively investigated to understand the different behaviours of the two nanozymes (Fig. 3). Fig. 3a illustrates the proposed responses of γ -CD@AuNPs and γ -CD@AgNPs in the presence of fenthion. In both systems, γ -CD serves as both a stabilizing ligand and a molecular recognition element. The catalytic responses of both nanozymes decreased progressively with increasing fenthion concentration. For γ -CD@AuNPs, the intensity of absorbance at 370 and 652 nm gradually decreased (Fig. 3b), whereas γ -CD@AgNPs exhibited a substantially larger reduction in intensity under identical conditions (Fig. 3c). A similar concentration-dependent trend was observed in the SERS measurements (Fig. 3d and e). The Raman intensity decreased progressively with increasing fenthion concentration for both nanozymes, with γ -CD@AgNPs displaying a markedly greater signal change than γ -CD@AuNPs.

TEM images provided direct structural evidence for the concentration-dependent evolution of the nanoparticle assemblies (Fig. 3f–k). In the absence of fenthion, both γ -CD@AuNPs (Fig. 3f) and γ -CD@AgNPs (Fig. 3i) were well dispersed. Small aggregates were observed at 1 ppm (Fig. 3g and j), followed by extensive clustering at 10 ppm (Fig. 3h and k). Notably, γ -CD@AgNPs formed substantially larger and denser aggregates than γ -CD@AuNPs at the same fenthion concentration (Fig. 3h and k), consistent with the more pronounced catalytic and SERS responses observed above. This aggregation reduces the accessible catalytic surface area and disrupts the uniform dispersion of nanozymes, thereby impairing their catalytic and optical performance.

Overall, the catalytic, SERS and TEM results consistently indicate that γ -CD@AgNPs undergo more extensive nanoparticle aggregation than γ -CD@AuNPs in the presence of fenthion, which is accompanied by larger changes in both catalytic activity and Raman intensity. The different behaviours of the two nanozymes can be rationalised by several complementary factors. First, γ -CD acts as both a stabilising ligand and a molecular recognition element. Its toroidal structure, consisting of a hydrophobic cavity and a hydrophilic exterior, enables host–guest inclusion with fenthion while maintaining colloidal stability⁴¹, thereby increasing the probability of analyte-mediated interparticle association. Second, the larger size of γ -CD@AgNPs (29 ± 8 nm) compared with γ -CD@AuNPs (10 ± 2 nm) may make their colloidal state more sensitive to analyte-induced interparticle interactions. The Ag surface also provides favourable coordination sites for sulphur-containing functional groups present in fenthion, including phosphorothioate (P=S) and thioether groups^{42,43}, which may further facilitate aggregation-mediated signal modulation. Similar Ag–S interactions have been reported for sulphur-

containing organophosphate pesticides and proposed to contribute to nanoparticle aggregation and optical signal modulation^{44,45}. In addition, despite the aggregation-induced attenuation of the Raman signal, γ -CD@AgNPs retained substantially stronger SERS enhancement than γ -CD@AuNPs owing to the intrinsically stronger electromagnetic field enhancement of Ag nanostructures, as supported by FEM simulation (Fig. 2e). Therefore, γ -CD@AgNPs offer a more responsive platform where γ -CD host-guest recognition, Ag-S interactions, and plasmonic coupling collectively contribute to fenthion detection. Based on these advantages, γ -CD@AgNPs were selected for the subsequent development of the colorimetric-SERS dual-mode sensing platform for fenthion detection.

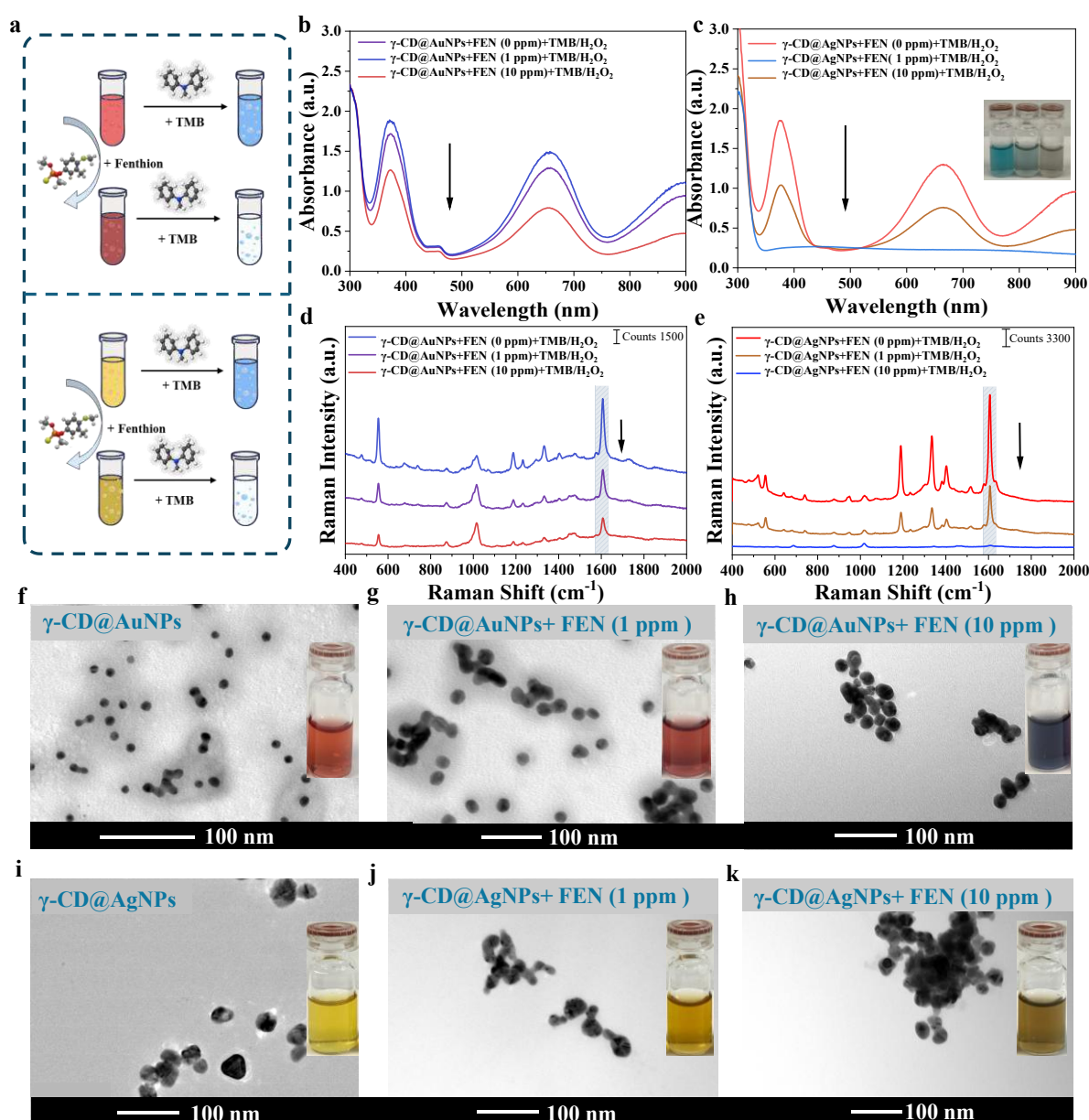


Fig. 3. Comparative colorimetric and SERS responses of γ -CD@AuNPs and γ -CD@AgNPs toward fenthion and their aggregation behaviours. (a) Schematic illustration of γ -CD@AuNPs and γ -CD@AgNPs acting as nanozymes for the colorimetric detection of fenthion. UV-Vis absorption spectra

of the TMB oxidation system catalysed by γ -CD@AuNPs (b) and γ -CD@AgNPs (c) in the absence and presence of fenthion (1 and 10 ppm), showing the inhibition of oxTMB formation upon fenthion addition. Insets show the corresponding colour changes. SERS spectra of the corresponding systems, demonstrating the differential responses of γ -CD@AuNPs (d) and γ -CD@AgNPs (e) following fenthion addition. (f–h) TEM images of γ -CD@AuNPs and (i–k) γ -CD@AgNPs before and after exposure to fenthion (1 and 10 ppm), demonstrating concentration-dependent nanoparticle aggregation.

Colorimetric and SERS-based detections of fenthion

As illustrated in Fig. 4, γ -CD@AgNPs exhibit a concentration-dependent dual optical response to fenthion, enabling both colorimetric and SERS detection. The proposed sensing mechanism is illustrated in Fig. 4a. At low fenthion concentrations, nanoparticles remain well-dispersed, maintaining strong LSPR signals and robust POD-like catalytic activity. In contrast, higher fenthion concentrations induce aggregation via interactions between the aromatic ring and phosphorothioate group (P=S) of fenthion and the nanoparticle surface. This aggregation leads to redshifted and broadened LSPR bands, a distinct shoulder in the 450–700 nm region, and a visible colour change from yellow to greenish brown (Fig. 4b). Simultaneously, the catalytic oxidation of TMB to blue oxTMB is progressively suppressed as fenthion concentration increases, resulting in colour fading from deep blue to light blue. The absorbance spectra (Fig. 4c) confirmed this trend, with a marked reduction in the 370 nm peak. The corresponding calibration curve (Fig. 4d) exhibited a clear linear relationship ($y = -0.1892x + 2.30$, $R^2 = 0.97$) over a detection range of 125 ppb to 12.5 ppm. The LOD was determined to be 618 ppb, calculated using the standard $3\sigma/m$ criterion, where σ represents the SD of blank measurements, consistent with the previous studies^{46,47}. This enables a colorimetric detection strategy that is both visually discernible and quantitatively robust. To further enhance the sensitivity of this detection platform, SERS was employed in subsequent analyses.

Quantitative analyses of fenthion were conducted across various concentrations using the SERS platform. Analysis of the full SERS spectra (Fig. 4e) and the oxTMB band at 1609 cm^{-1} (Fig. 4f) provides clear evidence that increasing the concentration of fenthion led to a decreased SERS signal coming from oxTMB; therefore, inverse sensitivity could be observed. Prior to analysis, the detection time was optimised (Fig. S9), with 5 min yielding the highest signal intensity. A linear inverse relationship was observed between SERS intensity at 1609 cm^{-1} and fenthion concentration over the range of 1 ppb to 1 ppm ($y = -344x + 979.65$, $R^2 = 0.96$). This method enabled quantitative determination of trace fenthion, achieving a LOD as low as 7.76 ppb. The SERS sensor demonstrated high sensitivity for fenthion confirming its potential for monitoring trace-level pesticide residues.

It should be noted that the two detection modes provide complementary analytical functions rather than identical sensitivity. The colorimetric response enables simple and low-cost screening without complex instrumentation, whereas the SERS mode provides enhanced sensitivity and molecular fingerprint

information for quantitative fenthion analysis. Therefore, the dual-mode strategy is intended to provide a tiered detection approach, where colorimetric analysis supports preliminary screening and SERS enables lower-concentration confirmation.

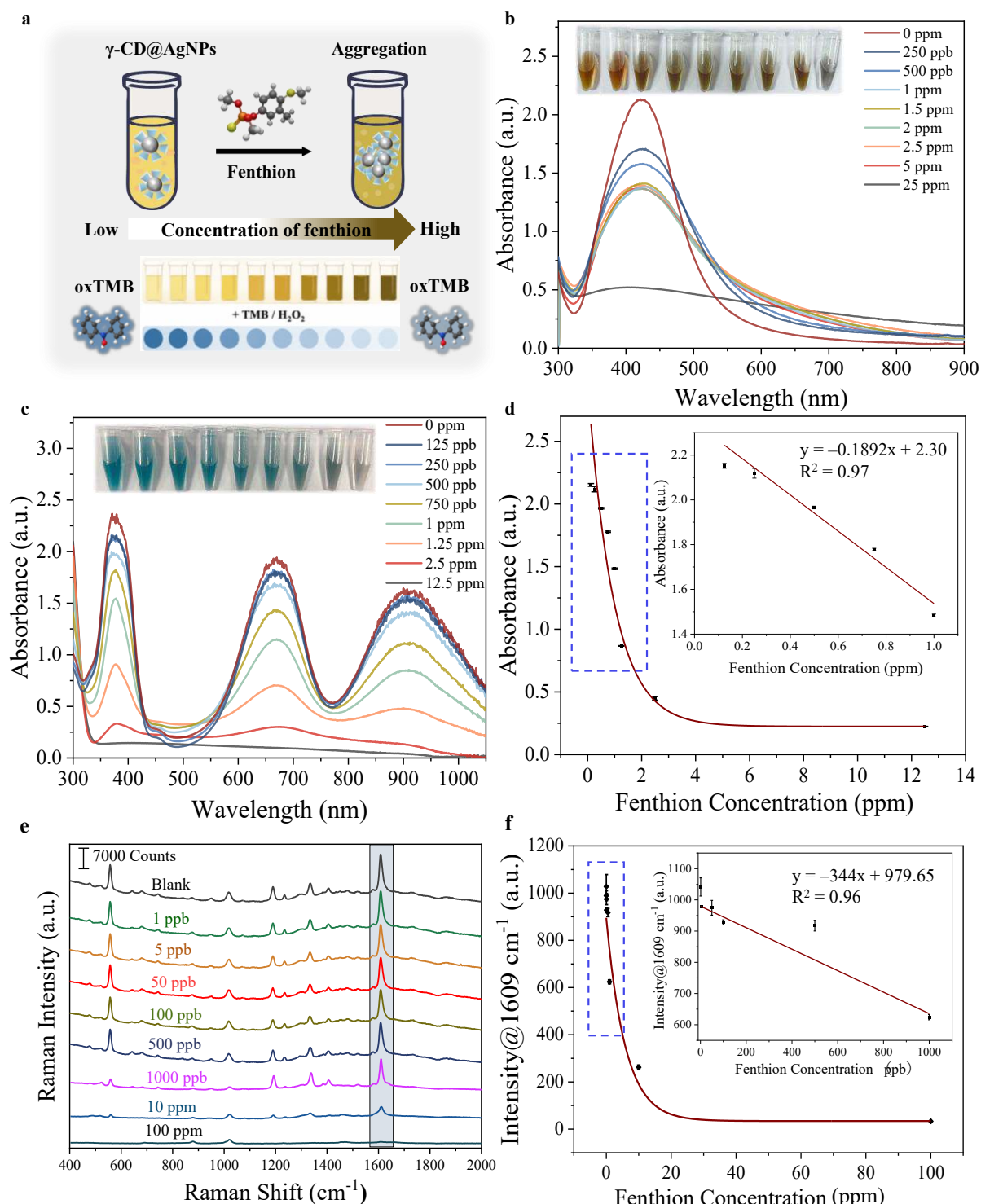


Fig. 4. (a) Schematic representation of the colorimetric sensing mechanism for fenthion detection using γ -CD@AgNPs. (b) Absorption spectra of γ -CD@AgNPs incubated with fenthion. (c) Absorbance spectra of oxTMB in the presence of fenthion. (d) Calibration curve of absorbance value and fenthion concentration. (e) Raman spectra of oxTMB in the presence of fenthion. (f) Calibration curve of SERS intensity and fenthion concentration.

concentration of fenthion. Error bars represent standard error of the mean ($n = 3$). (e) Full Raman spectra showing characteristic oxTMB peaks, with Raman intensity inversely correlated with fenthion concentration. (f) SERS measurements at 1609 cm^{-1} at various concentrations (1 ppb - 100 ppm), with an inset highlighting the linear range between 1 and 1000 ppb. Error bars represent standard error of the mean ($n = 3$).

Selectivity of the SERS-sensor for fenthion detection

The selectivity of the developed SERS was systematically evaluated using OPPs, including chlorpyrifos, diazinon, fenthion, and profenofos, as well as representative pesticides from other major classes, namely methomyl (carbamate), deltamethrin (pyrethroid), and imidacloprid (neonicotinoid). The molecular structures of all tested representative pesticides are illustrated in Fig. 5a. All four OPPs compounds possess a thionophosphoryl ($\text{P}=\text{S}$) group, and their sulphur atoms are capable of forming covalent or semi-covalent $\text{Ag}-\text{S}$ bonds with the $\gamma\text{-CD@AgNPs}$ surface. Since the OPPs share the most similar core structures and functional groups with fenthion, they represent the most challenging interferents for selectivity evaluation. SERS spectra collected after exposure to each pesticide (1 ppm) and the blank control are shown in Fig. 5b, with characteristic signals observed around 1609 cm^{-1} . Profenofos, chlorpyrifos and diazinon produced relatively strong and comparable intensities at this band, whereas fenthion generated significantly weaker signal. This indicates that fenthion induces the strongest interaction with the $\gamma\text{-CD@AgNP}$ system under the tested conditions, resulting in the most pronounced nanoparticle aggregation and suppression of the oxTMB SERS signal.

Among CD-based inclusion studies, chlorpyrifos is the most frequently reported guest molecule⁴⁸⁻⁵⁰. For example, Lucas-Abellán et al. showed that chlorpyrifos forms higher-order complexes with $\beta\text{-CD}$, substantially, enhancing chlorpyrifos's aqueous solubility and formulation stability⁴⁹. In addition, $\alpha\text{-CD}$ -capped AgNPs have been reported as colorimetric probes for chlorpyrifos detection based on LSPR band shifts induced by noncovalent interactions between chlorpyrifos molecules and nanoparticle surfaces⁵¹. Furthermore, the study by Chadha et al. demonstrated that $\gamma\text{-CD}$ capped noble-metal nanoparticles can be used as selective nanoprobe for chlorpyrifos, indicating their appreciable affinity for $\gamma\text{-CD}$ coatings⁵⁰. In our experiments, $\gamma\text{-CD}$ also interacted with chlorpyrifos, aligning with these studies. Importantly, however, a key finding of our work is that fenthion exhibited a substantially stronger interaction with $\gamma\text{-CD@AgNPs}$ (Fig. 5b). This enhanced selectivity toward fenthion can be rationalized by the combined contribution of $\gamma\text{-CD}$ -mediated host-guest interaction and fenthion- $\gamma\text{-CD@AgNPs}$ surface coordination. From a molecular recognition perspective, the molecular size and conformational flexibility of fenthion are more compatible with the $\gamma\text{-CD}$ cavity ($7.5\text{-}8.3\text{ \AA}$), facilitating favorable hydrophobic inclusion^{52,53}, whereas chlorpyrifos suffers steric hindrance from its trichloropyridyl ring, resulting in weaker inclusion. In addition, the stronger aggregation observed for fenthion might be attributed to its two sulphur donor sites ($\text{P}=\text{S}$ and an aryl thioether), which facilitate

stronger Ag-S coordination^{54,55}, whereas chlorpyrifos mainly relies on P=S binding and CD inclusion due to the lack of the thioether group, resulting in weaker interaction under the same conditions^{56,57}. This combined molecular recognition and surface coordination mechanism may explain the enhanced selectivity toward fenthion and the aggregation-induced dual-mode signal observed in this work.

Statistical analysis using one-way ANOVA followed by Dunnett's test demonstrated that the SERS intensities for fenthion were significantly different from the blank control (**** $P < 0.0001$), while profenofos and deltamethrin exhibited a weaker but still significant difference (** $P < 0.01$) (Fig. 5c). In contrast, pesticides from other chemical classes, including methomyl and imidacloprid, showed no statistically significant difference in SERS intensity compared with the blank control ($P > 0.05$). To further validate the selectivity observed among structurally similar OPPs, visual inspection of the mixtures (Fig. S10) revealed a distinct colour change in the γ -CD@AgNPs upon exposure to fenthion, which was not observed with the other OPPs at equivalent concentrations. Notably, in the 100 ppm fenthion samples, aggregated clusters of γ -CD@AgNPs settled at the bottom of the tubes, and the solution colour changed to dark grey. The distinct colour change and settling (aggregation) observed only with fenthion, even at lower concentrations indicate a preferential analytical response and stronger apparent interaction with fenthion under the detection conditions. This aggregation behaviour can be further supported by the above TEM analysis, which revealed small aggregates at 1 ppm (Fig. 3j) and extensive clustering at 10 ppm (Fig. 3k). In contrast, no such aggregation was observed for the other OPPs. Although other OPPs pesticides may also interact with the nanocomposite surface, the combination of optimal γ -CD cavity fit and strong multidentate Ag-S coordination leads to the most pronounced aggregation and consequent signal suppression for fenthion. Collectively, these results underscore the high selectivity of the γ -CD@AgNPs-TMB/ H_2O_2 system for fenthion under identical experimental conditions, highlighting its potential as a sensitive and practical SERS-based probe for fenthion detection.

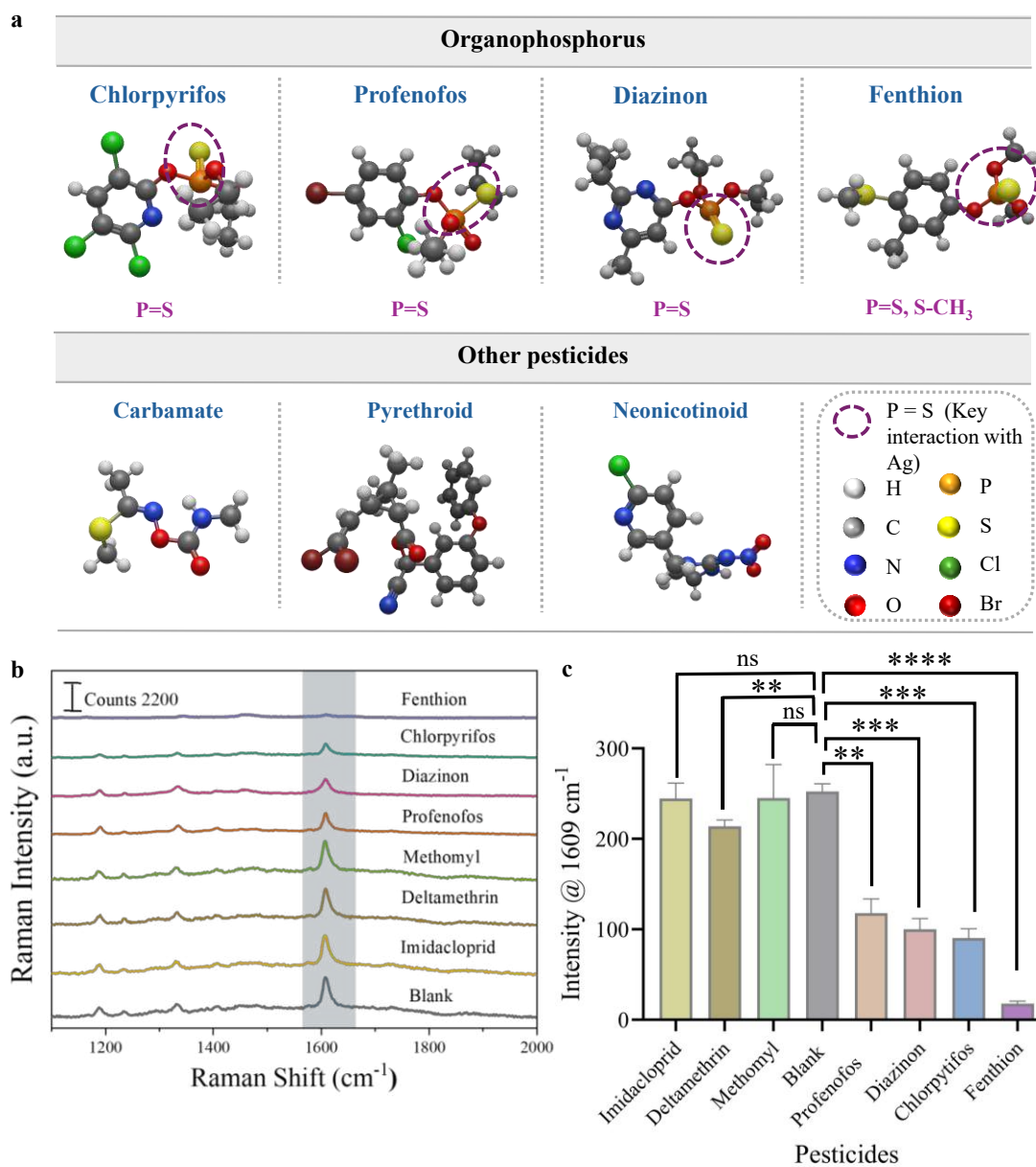


Fig. 5. Selectivity of γ -CD@AgNPs- H_2O_2 /TMB system for fenthion detection. (a) Molecular structures of representative pesticides: OPPs (chlorpyrifos, profenofos, diazinon, fenthion), carbamate (methomyl), pyrethroid (deltamethrin), and neonicotinoid (imidacloprid). (b) SERS spectra of the γ -CD@AgNPs- H_2O_2 /TMB system after exposure to each pesticide (1 ppm) and to a blank solution. The shaded region highlights the oxTMB marker band at 1609 cm^{-1} employed for quantitation. (c) Raman intensity at 1609 cm^{-1} ; error bars represent standard deviation ($n = 3$). Statistical significance was determined by one-way ANOVA followed by Dunnett's multiple-comparisons test, with each pesticide treatment compared with the blank control. ns, $P \geq 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$ versus the blank control.

Detection of fenthion in spiked tomato samples

The practical applicability of the γ -CD@AgNPs/ H₂O₂-TMB-based SERS sensor was demonstrated through the detection of fenthion residues on the surface of tomato peels using a non-destructive swab sampling method. As illustrated schematically in Fig. 6a, this approach enabled direct assessment of surface-bound pesticide residues without the need for complex sample preparation. Prior to analysis, the swab extraction solvents were compared (Fig. S11), with methanol providing a stronger oxTMB signal intensity than acetonitrile and thus was selected for subsequent extractions. Tomato samples were spiked with known concentrations of fenthion, and SERS spectra were obtained after swabbing the spiked tomato surfaces (Fig. 6b). A linear relationship was obtained ($y = -293.15x + 992$, $R^2 = 0.95$) over the range of 5 ppb to 2 ppm (inset, Fig. 6c). The LOD for fenthion was 0.18 ppm (0.18 mg/kg) following surface swabbing and SERS analysis. For reference, the MRL for fenthion in tomatoes established by the Australian government is 5 mg/kg¹², highlighting the potential of the method for sensitive monitoring of surface fenthion residues.

In the spiked recovery experiments, the recoveries ranged from 88.5 % -118 %, with RSD < 6.4 % (Table S2), which corroborates the accuracy and reliability of the swab-based SERS analysis method. The influence of the tomato matrix on SERS quantification was evaluated by comparing the analytical sensitivities (slopes) of the calibration curve obtained in tomato extract with that obtained in buffer. The matrix effect was calculated as 85.21% (See Supplementary Information Section 3 for the calculation details), indicating that the SERS response in tomato was $\approx$ 15% lower than in the clean solvent system, which is considered a mild suppression, as values within the range of 80–120% are generally regarded as reflecting acceptable matrix influence in analytical chemistry^{58,59}. Compared to conventional extraction-based techniques, the swab-assisted γ -CD@AgNPs-H₂O₂/TMB system offers advantages in terms of simple sampling and minimal sample preparation while enabling surface residue collection without cutting or homogenising the food sample, making it a viable tool for food safety monitoring. Overall, this work developed a dual-mode sensing platform for sensitive and selective detection of fenthion, integrating simple colourimetric screening with highly sensitive SERS quantification using γ -CD-capped AgNPs. This platform provides a cost-effective and environmentally friendly strategy for pesticide residue monitoring, with considerable potential for future adaptation to diverse food matrices and broader food safety and environmental surveillance applications.

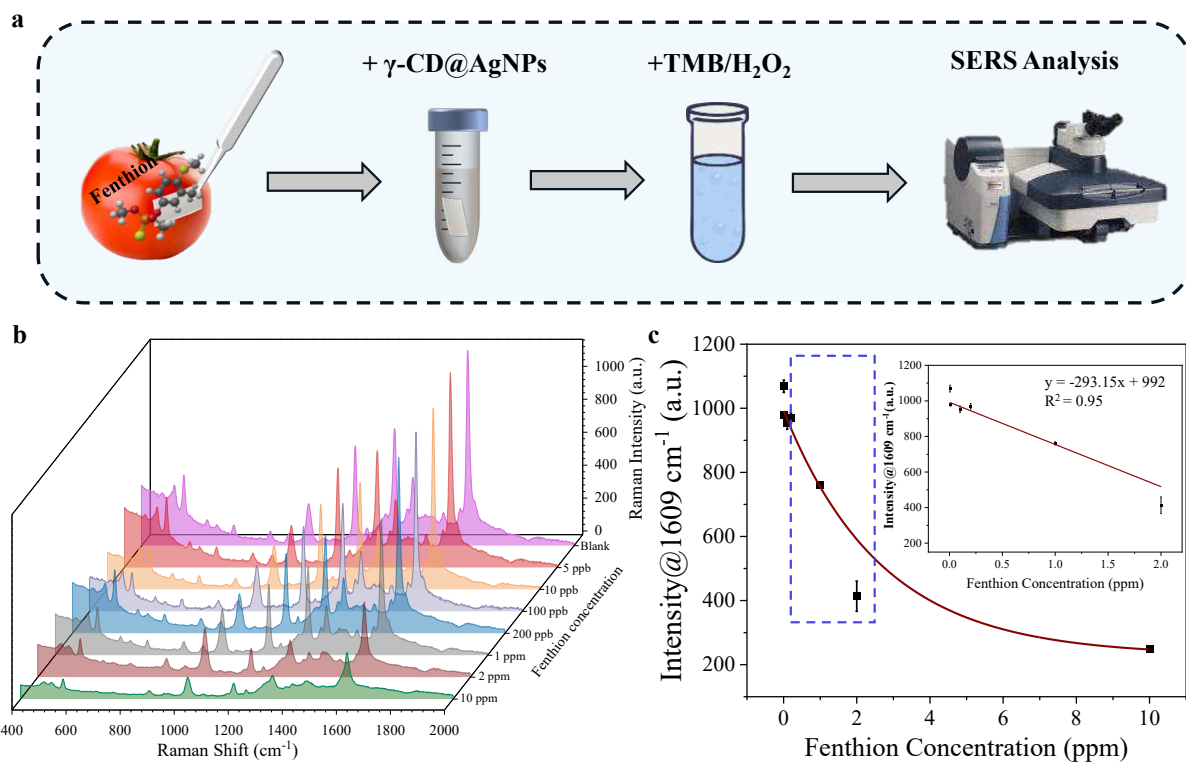


Fig. 6. Detection of fenthion residues at varying concentrations on tomato surfaces by SERS. (a) Schematic illustration of the swab-based sampling method and corresponding SERS detection of pesticides released from the swab. (b) SERS spectra obtained from tomato surfaces swabbed with different concentrations of fenthion, together with an unspiked tomato sample as the blank control. (c) Correlation between fenthion concentration and SERS intensity following swabbing.

Methods

Chemicals and reagents

Sodium citrate tribasic dihydrate ($C_6H_5Na_3O_7 \cdot 2H_2O$), gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$, 99.9%), silver nitrate ($AgNO_3$), sodium hydroxide ($NaOH$), hydrochloric acid (HCl), sodium acetate (CH_3COONa), rhodamine 6G, 3,3',5,5'-tetramethylbenzidine (TMB), hydrogen peroxide (H_2O_2), γ -cyclodextrins (γ -CD), acetic acid (CH_3COOH), fenthion ($C_{10}H_{15}O_3PS_2$), chlorpyrifos ($C_9H_{11}Cl_3NO_3PS$), profenofos ($C_{11}H_{15}BrClO_3PS$), diazinon ($C_{12}H_{21}N_2O_3PS$), methomyl ($C_5H_{10}N_2O_2S$), deltamethrin ($C_{22}H_{19}Br_2NO_3$), imidacloprid ($C_9H_{10}ClN_5O_2$) and 96-well flat-bottom microtitre plates were all purchased from Sigma Aldrich (UK). Organic tomatoes were purchased from Sainsbury's (UK).

Instrumentation analysis

Absorbance spectroscopy measurements were performed using a Cary 60 spectrophotometer (Agilent Technologies, USA). Microtiter plate absorbance measurements were performed using a Safire2 TECAN microplate reader. SERS measurements were carried out using a DXR2 Raman microscope operated with an excitation laser light at 785 nm (Thermo Fisher Scientific, UK). Fourier-transform infrared (FTIR) spectroscopy measurements were conducted on a Nicolet iS50 FTIR spectrometer (Thermo Fisher Scientific, UK). Transmission electron microscopy (TEM) measurements were performed using a Joel JEM 1400 Plus model, operated at 200 kV (Thermo Fisher, Scientific, UK).

Synthesis of γ -CD@AuNPs and γ -CD@AgNPs

To synthesise γ -CD-functionalised nanoparticles, 10 mL of 7 mM γ -CD was first prepared by dissolving γ -CD in deionised water (dH_2O). Subsequently, 0.5 mL of 2.0 M $NaOH$ was added to the solution to adjust the pH to the range of 10-12. After stirring for 2 min, 7 mL of the corresponding metal salt solution, i.e. either 3 mM $HAuCl_4$ or 3 mM $AgNO_3$, was added to the mixture. The reaction mixtures were then thoroughly mixed and heated in a water bath. After 20 min, the solutions exhibited a red-wine colour for γ -CD@AuNPs and dark yellow colour for γ -CD@AgNPs, indicating the successful formation of the corresponding metal nanoparticles.

Kinetic analysis of γ -CD@AuNPs and γ -CD@AgNPs peroxidase-like activity

The POD-like catalytic activity of the nanozymes was assessed via a TMB-based colorimetric assay. Kinetic analysis was performed by varying TMB concentrations from 0 to 0.45 mM (final concentration) in the presence of 6% H_2O_2 . Each reaction was conducted in a 96-well flat-bottom microtiter plate with a total volume of 300 μ L, consisting of 75 μ L of H_2O_2 , 75 μ L of TMB, and 150 μ L of γ -CD-coated nanoparticles (either AuNPs or AgNPs). The absorbance was measured at 370 nm. Kinetic parameters for TMB oxidation catalyzed by γ -CD@AuNPs or γ -CD@AgNPs were calculated using the Michaelis–Menten model:

$$V = \frac{V_{max} \times [S]}{K_m + [S]}$$

Where V and V_{max} stand for the initial and the maximum initial reaction velocity, respectively. K_m corresponds to the Michaelis-Menten constant, and [S] represents the concentration of TMB.

Colorimetric and SERS-based detections of fenthion

A stock solution of fenthion (1000 ppm) was prepared in methanol and stored at 4 °C. Working solutions, ranging from 1 ppb to 100 ppm, were freshly prepared by diluting the stock in 60% methanol (v/v, MeOH:H₂O = 60:40). For the colorimetric assay, 250 µL of fenthion solution at the desired concentration was mixed with 250 µL of γ-CD@AgNPs in a microcentrifuge tube and incubated overnight at room temperature to ensure sufficient host–guest inclusion and Ag–S interaction. Subsequently, 250 µL of 1.25 mM TMB and 250 µL of 6% H₂O₂ were added, mixed thoroughly, and incubated for 5 min at room temperature. Colour changes were recorded visually and by absorption spectrophotometry.

For SERS measurements, 150 µL of the γ-CD@AgNP–fenthion mixture was transferred to a 96-well microtiter plate, followed by the sequential addition of 150 µL of 1.25 mM TMB and 6% H₂O₂. The reaction was incubated for 5 min at room temperature. A blank control (γ-CD@AgNPs with TMB and H₂O₂, without fenthion) was used for the Raman measurement to optimize signal intensity. Each sample was measured in triplicate. Raman spectra were acquired using a 785 nm laser source with a 10× objective lens over the range of 400–2000 cm^{−1} and processed using OMNIC™ software (Thermo Fisher Scientific, UK). The SERS intensity at 1609 cm^{−1} was recorded and used to construct the standard calibration curve for fenthion quantification. The limit of detection (LOD) was calculated using the standard equation:

$$LOD = \frac{3\sigma}{m}$$

where σ represents the standard deviation (SD) of the blank signal and m is the slope of the calibration curve. The SD value was obtained from six independent blank measurements (n = 6) performed under identical experimental conditions.

Selectivity studies and statistical analysis

Selectivity was evaluated with four commonly used OPPs, including chlorpyrifos, profenofos, diazinon, and fenthion, as well as three representative pesticides from other major chemical classes, including methomyl (carbamate), deltamethrin (pyrethroid), and imidacloprid (neonicotinoid). The three-dimensional molecular structures of the pesticides were generated using Avogadro 2 and visualized using the standard CPK colour scheme. All analytes were tested at a concentration of 1 ppm under identical experimental conditions. The SERS intensity at the 1609 cm^{−1} band was recorded for each

replicate (n = 3). Statistical analysis was performed using Graphpad Prism 10.0 software (GraphPad, California, USA). Differences among groups were analysed using one-way ANOVA followed by Dunnett's multiple-comparisons test, with each pesticide treatment compared with the blank control. Statistical significance was defined as ns, $P \geq 0.05$, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$, $****P < 0.0001$.

Detection of fenthion in spiked tomato samples

Prior to spiking, the tomatoes were thoroughly washed with dH₂O and subsequently swab-cleaned using ethanol, then air dried at room temperature. Fenthion standard solutions were prepared at concentrations ranging from 5 ppb to 100 ppm in 60% methanol. A volume of 5 μ L of each solution was uniformly applied to approximately 1 cm² on the surface of the tomatoes using a micropipette. The samples were subsequently air-dried to facilitate solvent evaporation and ensure pesticide adherence. For residue collection, sterile cotton swabs were pre-soaked in methanol and gently swabbed across the tomato surface evenly for ~40 times in a consistent sweeping motion to collect surface-bound pesticide. Each swab was immersed in 1.0 mL of 60 % methanol and vortexed for 2 min to release the pesticide into the solvent phase. A 75 μ L aliquot of the supernatant was then mixed with 75 μ L of γ -CD@AgNPs and incubated overnight. Subsequently, the mixture was reacted with 150 μ L of 1.25 mM TMB and 6% H₂O₂, and SERS was used to detect and quantify fenthion residue. Herein, the final fenthion concentration was calculated by adjusting the subsequent dilution occurring at each step. For the surface swab method, recovery (%) is calculated as:

$$\text{Recovery (\%)} = \frac{C_{\text{measured}}}{C_{\text{spiked}}} \times 100$$

Where C_{measured} is the fenthion concentration determined from the calibration curve, and C_{spiked} represents the known fenthion level applied to tomato surface. The repeatability of the SERS measurements was evaluated using the relative standard deviation (RSD). RSD (%) was calculated as $(\text{SD}/\text{mean}) \times 100$, based on the concentrations obtained from three replicate measurements (n = 3).

528 **Data availability**

529 **The data supporting the findings of this study are available from the corresponding author**
530 **upon reasonable request.**

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717 **Ethics declarations**

718 Competing interests

719 The authors declare that they have no known competing financial interests or personal relationships that
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