

A Zn-Ta Nano with Dual Inhibitory and pseudoperoxidase system Activity toward *Lepidium draba* Peroxidase

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Abstract

Background: Peroxidases play essential roles in plant oxidative metabolism and are increasingly investigated for biotechnological and catalytic applications. Metal-containing nanocomposites can modulate enzymatic activity and may also exhibit enzyme-mimicking catalytic properties.

Methods: The effects of a Zn-Ta nanocomposite on the oxidative activity of *Lepidium draba* peroxidase (LDP) were investigated using enzyme activity assays supported by molecular docking analysis. Enzyme activity was evaluated at different nanocomposite concentrations, and the intrinsic pseudoperoxidase activity of the nanocomposite was assessed under varying temperature and pH conditions.

Results: Zinc was associated with increased LDP activity, likely through interactions with residues near the predicted active site, whereas tantalum-containing treatments showed an inhibitory effect. When combined within the Zn-Ta nanocomposite, an overall reduction in enzyme activity was observed. The highest residual enzymatic activity in the presence of the nanocomposite was recorded at 0.556 mg/mL. The Zn-Ta nanocomposite also exhibited intrinsic pseudoperoxidase activity, with maximum catalytic performance at 55 °C and pH 3.5, and at 70 °C and pH 2.5. Molecular docking confirmed interactions between Zn²⁺, TMB, H₂O₂, and LDP, supporting zinc's role in modulating enzyme activity.

Conclusion: The Zn-Ta nanocomposite demonstrates dual functionality, acting as both an LDP activity modulator/inhibitor and a pseudoperoxidase catalytic system, highlighting its potential for biocatalysis, environmental monitoring, and diagnostic applications.

Keywords: Zn-Ta Nanocomposite, *Lepidium draba*, Nanozyme, Peroxidase Activity, Molecular Docking

1.Introduction

Zinc is an essential element in living organisms, acting as a component of numerous metalloenzymes. It plays critical structural, catalytic, and physiological roles, with approximately 300 enzymes requiring zinc for activity. Zinc also stabilizes the tertiary structure of over 300 proteins, including

zinc finger proteins [1]. This metal is present in all enzyme groups: lyases, hydrolases, ligases/isomerases, transferases, and oxidoreductases [2]. Zinc deficiency can manifest as sexual dysfunction, growth disorders, digestive issues, inflammation, or skin problems [3]. Furthermore, it negatively impacts lymphocyte proliferation, potentially due to zinc's

Conversely, free zinc is toxic to bone tissue, and elevated zinc levels can induce copper deficiency [5, 6]. Studies indicate that high zinc ion concentrations can trigger cell apoptosis and death, possibly by disrupting cellular energy production [7].

Tantalum, discovered in 1802 and first purified in 1903, is a rare, ductile, blue-gray transition metal with an atomic number of 73, an atomic weight of 180.05, and a density of 16.68 g/cm³. Its high melting point (3017°C) makes it suitable for heat-resistant applications in rockets, airplanes, and other high-strength components [8]. While tantalum's bioactivity is still under investigation, its high biocompatibility and low toxicity have led to its exploration as an implant and drug [9]. Tantalum can inhibit enzymes like phosphatases and has been proposed as a peroxidase nanoenzyme, generating hydroxyl radicals from H₂O₂ that cause chromogenic substrate color changes [10].

Peroxidases (EC: 1.11.1.X), iron-containing proteins crucial for electron transfer in oxidative reactions, are essential for all plants and animals [11]. They are widely employed in medical diagnostics, organic synthesis, and biosensors for detecting hydrogen peroxide, alcohols, glucose, choline, and glutamate [12–14]. Peroxidases are generally categorized into three main families: animal, plant, and catalase peroxidases [15–18]. Plant peroxidases, encompassing both prokaryotic and eukaryotic origins, are further subdivided based on structural similarities and evolutionary history into prokaryotic, fungal, and plant-secreted groups [14–18]. Classified into animal, plant, and catalase families, plant peroxidases encompass both prokaryotic and eukaryotic origins, further subdivided into prokaryotic, fungal, and plant-secreted groups [16–18]. Recent research identified and cloned a peroxidase gene from *Lepidium draba* (LDP), a member of the Brassicaceae family. Bioinformatics analysis reveals a high degree of sequence similarity (over 87%) to horseradish peroxidase (HRP), leading to its use as an HRP homolog in medicine and biotechnology due to its catalytic activity and efficiency [19]. Its unique properties, including catalytic activity and folding efficiency, suggest its suitability for medical and biotechnological applications, while studies predict a lower immune response compared to HRP, making it a promising therapeutic candidate [19, 20].

Given the role of peroxidases in cellular metabolism and the importance of understanding metal–enzyme interactions, this study investigates the interaction of LDP with a Zn–Ta nanocomposite. By examining how different nanocomposite concentrations influence enzyme properties—including nanozyme-like activity and molecular interactions—this work provides insight into the mechanisms of enzyme inhibition by metal-containing materials. Such model systems may contribute to a better understanding of enzyme responses to heavy metals and may have potential relevance for environmental monitoring and remediation studies.

2. Materials and Methods

2.1. Materials

All chemicals used in this study were of analytical grade and used without further purification. Potassium dihydrogen phosphate, disodium hydrogen phosphate, sodium dihydrogen phosphate, Tris-base, Tris-HCl, sodium chloride (NaCl), agar, yeast extract, tryptone, ammonium persulfate, N,N,N',N'-tetramethylethylenediamine (TEMED), sodium dodecyl sulfate (SDS), Coomassie Brilliant Blue G-250 and R-250, bis-acrylamide, β -mercaptoethanol, phosphoric acid, sodium acetate, bovine serum albumin (BSA), and isopropyl β -D-1-thiogalactopyranoside (IPTG) were purchased from Merck (Germany). Kanamycin, 3,3',5,5'-tetramethylbenzidine (TMB), 4-aminoantipyrine, hemin, and ammonium hydroxide were obtained from Sigma–Aldrich (USA). Molecular weight protein markers were purchased from Bio-Rad (USA), and Ni-NTA agarose affinity chromatography columns were obtained from Invitrogen (USA). *Escherichia coli* BL21 (DE3) bacterial cells were used as the host strain for recombinant protein expression. The Zn–Ta metal–organic framework (Zn–Ta MOF) nanocomposite was synthesized in-house as described in Section 2.3.

2.2. Expression and purification of recombinant LDP peroxidase

The gene encoding LDP peroxidase was cloned into the pET-28a (+) expression vector [19], providing an N-terminal His-tag, and transformed into *Escherichia coli* BL21 (DE3) bacterial cells. A single colony was used to inoculate Luria–Bertani (LB) medium supplemented with kanamycin (50 μ g/mL) and incubated at 37 °C with shaking at 180 rpm until the optical density at 600 nm (OD₆₀₀) reached approximately 0.6. Protein expression was induced by the addition of isopropyl β -D-1-thiogalactopyranoside (IPTG) to a final concentration of 0.5 mM, followed by incubation at 18 °C for 7 h.

Bacterial cells were harvested by centrifugation at 5,500 rpm for 15 min at 4 °C, and the resulting pellet was stored at –80 °C until further use. For protein extraction, the pellet was resuspended in lysis buffer (50 mM Tris–HCl, pH 8.0, 300 mM NaCl, 10 mM imidazole) and disrupted by sonication on ice using pulse cycles. Cellular debris was removed by centrifugation at 12,000 rpm for 30 min at 4 °C, and the clarified supernatant was subjected to affinity purification.

Recombinant LDP peroxidase was purified using Ni–NTA agarose affinity chromatography under native conditions. The column was equilibrated with lysis buffer, washed with wash buffer containing 20–30 mM imidazole to remove non-specifically bound proteins, and the target protein was eluted using elution buffer containing 250 mM imidazole. Eluted fractions were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE), and fractions containing the

purified enzyme were pooled and used for subsequent experiments.

2.3. Synthesis of Zn–Ta Nanocomposite

The Zn–Ta metal–organic framework (MOF) nanocomposite was synthesized using a co-precipitation method. Briefly, Zn-MOF and Ta-MOF precursors were prepared according to previously published protocols [21, 22]. For the composite synthesis, equimolar amounts of Zn-MOF and Ta-MOF were dissolved in 25 mL of glacial acetic acid under constant stirring. The resulting mixture was subjected to ultrasonication at 200 W for 20 min at room temperature to ensure homogeneous dispersion.

After sonication, the solution was allowed to stand at ambient conditions for 30 min, during which white crystalline Zn–Ta nanocomposite formed. The crystals were collected by centrifugation at 8,000 rpm for 10 min, washed three times with ethanol and deionized water to remove unreacted precursors, and dried under vacuum at 60 °C. The obtained nanocomposite was characterized by X-ray diffraction (XRD), scanning electron microscopy (SEM), and Fourier-transform infrared spectroscopy (FTIR) to confirm crystallinity, morphology, and functional groups.

2.4. Enzyme activity assay

The peroxidase activity of recombinant LDP was measured using 3,3',5,5'-tetramethylbenzidine (TMB) as a substrate in 50 mM phosphate buffer (pH 7.0). The reaction mixture consisted of 70 µL of enzyme solution (0.7 mg/mL), 1 mM H₂O₂, and 0.6 mM TMB, brought to a final volume of 1 mL with phosphate buffer. The change in absorbance at 653 nm was monitored for 180 s using a UV–Vis spectrophotometer. Enzyme activity was calculated using the extinction coefficient of TMB ($\epsilon = 3.9 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$) and expressed as µmol TMB oxidized per minute per milligram of protein (U/mg).

To evaluate the effect of the Zn–Ta nanocomposite on enzyme activity, various concentrations of the nanocomposite (ranging from 0.1 to 1.0 mg/mL) were dispersed in phosphate buffer and sonicated for 20 min to ensure uniform dispersion. The enzyme was added to the nanocomposite suspensions, and the mixture was incubated at room temperature on a shaker. After incubation, samples were centrifuged at 8,000 rpm for 10 min, washed three times with phosphate buffer, and the pellet was resuspended in 500 µL phosphate buffer. Enzyme activity and protein concentration were determined using the Bradford method, with BSA as a standard.

The effect of pH on enzyme activity was assessed by incubating the enzyme with the optimal concentration of the nanocomposite in 50 mM phosphate buffer at pH values ranging from 4.0 to 8.0 at room temperature for 30 min. Temperature effects were studied by incubating the enzyme–nanocomposite mixture in 50 mM phosphate buffer (pH 7.0) at 20–50 °C for 15 min prior to activity assay. All experiments were performed in triplicate, and results are reported as mean $\pm$ SD. Control experiments included enzyme alone, nanocomposite alone, and heat-inactivated enzyme to ensure that observed activity changes were spe-

cifically due to the enzyme–nanocomposite interaction.

2.5. Investigating the peroxidase-like activity of Zn–Ta nanocomposite

The peroxidase-like activity of the Zn–Ta nanocomposite was assessed using TMB and H₂O₂ as substrates. Nanocomposite samples were prepared by dispersing 5 mg of Zn–Ta in 1 mL of Tris–HCl buffer (pH 3.0) and sonicating for 3 min (amplitude 45%, cycle 0.5). Various concentrations of nanocomposite (0.07–1.4 mg/mL) were incubated with 0.6 mM TMB and 1 mM H₂O₂ in Tris–HCl buffer to a total volume of 700 µL. The oxidation of TMB was monitored by measuring the increase in absorbance at 653 nm.

To study the effect of pH, enzyme activity was measured in buffers covering pH 2–7 (glycine, sodium acetate, Tris–HCl, KH₂PO₄), while temperature dependence was evaluated by incubating 0.17 mg/mL nanocomposite with TMB and H₂O₂ at 25–80 °C for 2 min. Kinetic parameters were determined by varying H₂O₂ concentration (0.05–4 mM) and TMB concentration (2–18 µM) at both ambient temperature and the optimal temperature of 55 °C. All experiments were performed in triplicate, and mean $\pm$ SD reported. Controls without nanocomposite were included to verify the catalytic contribution of the MOF.

2.6. Molecular docking

The molecular interactions between LDP peroxidase and its substrates (TMB and H₂O₂) as well as the metal ion Zn²⁺ were analyzed using molecular docking. The 3D structure of LDP peroxidase was modeled using the I-TASSER server due to the absence of a high-resolution crystal structure. Ligand structures were retrieved from PubChem and prepared using PyRx (v0.8). Docking simulations were performed in PyRx using AutoDock Vina with grid boxes defined around the predicted active site. The exhaustiveness parameter was set to 8 to ensure adequate conformational sampling. The top-ranked docking poses were analyzed using Discovery Studio (v2024), LigPlot+, and UCSF Chimera to evaluate hydrogen bonding, hydrophobic interactions, and potential coordination with key catalytic residues. All docking simulations were repeated in triplicate to ensure reproducibility.

2.7. Molecular dynamics simulations

Molecular dynamics-like analyses were performed using the iMODS server (<https://imods.iqf.csic.es/>) to evaluate the flexibility and possible conformational changes of LDP peroxidase upon ligand and nanocomposite binding. Initial docking results were used to generate the starting complexes with TMB, H₂O₂, and Zn–Ta MOF. iMODS applies normal mode analysis (NMA) in internal coordinates to assess protein motion. For each complex, deformability, B-factors, eigenvalues, and the variance of atomic fluctuations were calculated. The generated normal modes allowed identification of flexible regions and potential hydrogen bond interactions between the enzyme and ligands or nanocomposite. Results provided insight into the structural stability and dynamic behavior of LDP peroxidase in response to binding, without performing full-atom MD simulations.

3. Results

3.1. Expression, Purification, and Enzyme Activity

Recombinant *Lepidium draba* peroxidase (LDP) was expressed in *E. coli* using both LB and TB media. The enzyme purified from TB-grown bacterial cells exhibited no detectable activity, likely due to rapid protein overexpression that hindered proper heme incorporation, resulting in a heme-deficient and inactive enzyme. In contrast, expression in LB medium proceeded more gradually, allowing adequate heme incorporation and yielding catalytically active enzyme.

Members of this enzyme family are typically monomeric, with reported molecular weights ranging from approximately 35 to 45 kDa[23]. SDS-PAGE analysis of the purified recombinant LDP revealed a single prominent band at approximately 35 kDa (Fig. 1), which is consistent with the expected molecular weight of the enzyme, including the N-terminal His-tag[24].

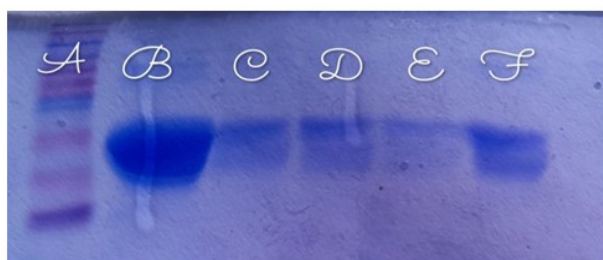


Figure 1. SDS-PAGE analysis of recombinant LDP purification: (A) leader protein; (B) TB medium; (C) LB medium; (D-F) eluted fractions from Ni-NTA agarose using 250 mM imidazole.

3.2. Effect of Zn-Ta nanocomposite on enzyme activity

Control experiments were performed to evaluate the potential background catalytic activity of the Zn-Ta nanocomposite. The nanocomposite alone, in the absence of enzyme, produced negligible TMB oxidation at neutral pH under the assay conditions, indicating that the framework itself did not significantly contribute to the observed enzymatic activity.

The effect of Zn-Ta nanocomposite concentration on the peroxidase activity of LDP was investigated. As shown in Fig. 2, enzyme activity increased with increasing nanocomposite concentration up to 0.556 mg/mL, where the maximum activity was observed. Further increases in nanocomposite concentration did not result in additional enhancement of catalytic activity.

The influence of temperature on enzyme activity in the presence of the nanocomposite was also examined (Fig. 3). Activity measurements conducted at 20, 30, 40, and 50 °C showed that the free enzyme

consistently exhibited higher activity than the enzyme incubated with the Zn-Ta nanocomposite. This observation suggests that interaction with the nanocomposite partially reduces the catalytic efficiency of LDP across the tested temperature range.

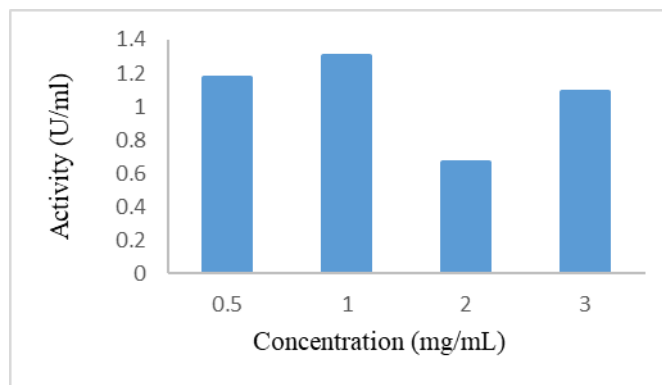


Figure 2: Enzyme activity in the presence of varying Zn-Ta nanocomposite concentrations, showing maximum activity at 0.556 mg/mL.

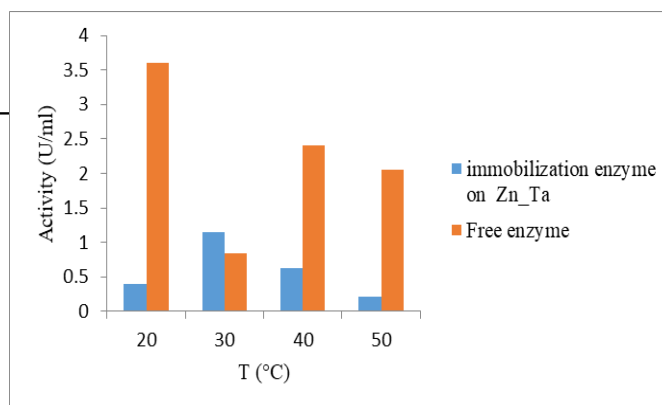


Figure 3: Temperature-dependent activity of free enzyme versus enzyme incubated with nanocomposite (20–50 °C). Nanocomposite reduces enzyme activity at all tested temperatures.

3.3. Peroxidase-like Activity of Zn-Ta Nanocomposite

The intrinsic peroxidase-like activity of the Zn-Ta nanocomposite was evaluated using TMB and H_2O_2 as substrates. Temperature-dependent experiments showed that the nanocomposite exhibited two activity maxima at 55 °C and 70 °C (Fig. 4). Similar optimal temperatures have been reported for related MOF-based catalytic systems, such as Ta-MOF, which showed maximal activity around 55 °C[10]. To maintain consistency and avoid possible thermal instability, subsequent measurements were conducted at 55 °C.

The effect of pH on the catalytic activity of the nanocomposite was further examined using Mix and Tris buffer systems. The highest catalytic activity was observed at 70 °C in Tris buffer (Fig. 5). Un-

der ambient temperature conditions, the optimal pH was 3.5 in Mix buffer, whereas at 70 °C the optimal pH shifted to 2.5 in Tris buffer (Figs. 6–7). These results are consistent with previous reports indicating that many nanozyme systems exhibit higher catalytic efficiency under acidic conditions, such as the Prussian blue/carbon cloth system reported by Niu et al. with an optimal pH near 4.

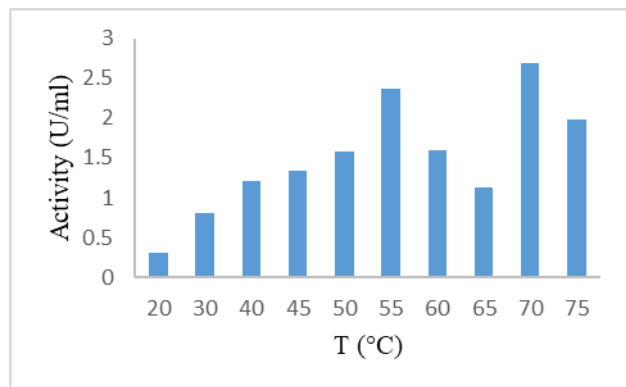


Figure 4: Temperature-dependent peroxidase-like activity of Zn–Ta (20–75 °C), with maxima at 55 °C and 70 °C.

pH dependence was assessed using Mix and Tris buffers at 55 and 70 °C. The highest activity was observed at 70 °C in Tris buffer (Fig. 5). Optimal pH values were 3.5 in Mix buffer at ambient temperature and 2.5 in Tris buffer at 70 °C, comparable to the pH 4 reported by Niu et al. for Prussian blue/carbon cloth [25](Figs. 6–7).

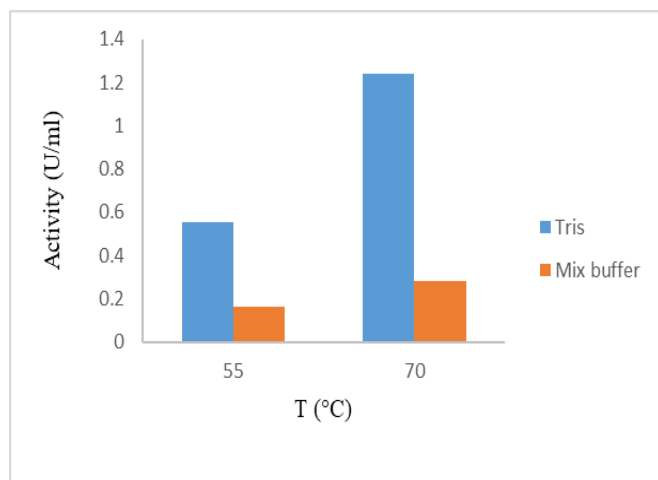


Figure 5: Peroxidase-like activity of Zn–Ta at 55 and 70 °C in Mix and Tris buffers. Tris at 70 °C shows the highest activity.

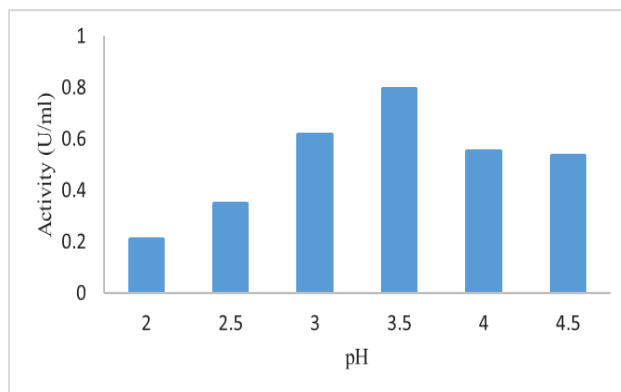


Figure 6: Activity in Mix buffer at ambient temperature.

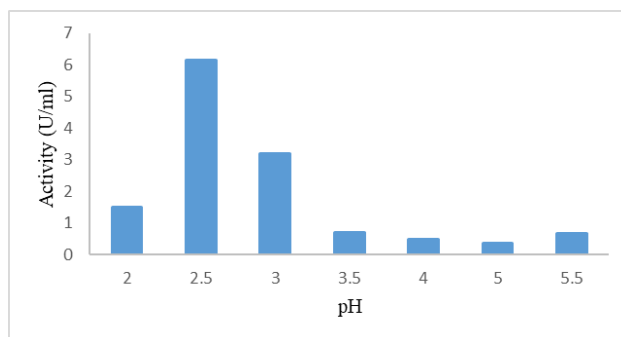


Figure 7: Activity in Tris buffer at 70 °C, highlighting temperature effect on nanocomposite activity.

3.4. Molecular Docking

Docking studies indicated that Zn²⁺ interacts with residues Gly47, Asp42, Ser51, and Val45 with a binding energy of −1.4 kcal/mol. Hydrogen peroxide (H₂O₂) showed interactions with Gly172 and Pro225 with a binding energy of −3.0 kcal/mol, while TMB exhibited the strongest interaction with a binding energy of −9.7 kcal/mol within the predicted active site. Previous studies have reported that several metal ions can inhibit horseradish peroxidase activity; for example, Cu (II) partially inhibits HRP activity[26], and heavy metal ions such as Cd²⁺, Hg²⁺, Pb²⁺ and Zn²⁺ have also been reported to reduce HRP activity [27–29]. Table 1 summarizes docking energies and key interactions for clarity.

3.5. Molecular Dynamics Analysis

The best docking complexes were analyzed using iMODS to predict conformational flexibility and deformation pathways. Eigenvalues for LDP complexes with TMB, H₂O₂, and Zn²⁺ were 1.94622×10^{-4} , 2.998517×10^{-4} , and 2.786694×10^{-4} , respectively, where lower eigenvalues indicate easier deformation. Colored variance bars indicate individual (red) and cumulative (green) motions. Covariance matrices illustrate residue movement correlations: uncorrelated

(white), same direction (red), opposite direction (blue). The elastic network model shows atoms connected by springs, with darker shades indicating stiffer connections. These results demonstrate flexible regions and potential interaction sites for ligands and nanocomposite (Figs.9–11).

Table 1. Docking results for LDP enzyme with Zn²⁺, H₂O₂, and TMB

Ligand	Binding Energy (kcal/mol)	Key Residues
Zn ²⁺	−1.4	Gly47, Asp42, Ser51, Val45
H ₂ O ₂	−3.0	Gly172, Pro225
TMB	−9.7	Active site residues

4. Discussion

Zinc is an essential element that acts as a redox-inert antioxidant and regulates cellular oxidative/antioxidant homeostasis, including glutathione (GSH) metabolism [30]. Micronutrients such as vitamins, trace elements, and antioxidants play crucial roles in managing oxidative stress, tissue regeneration, and immunity [31]. Maintaining zinc homeostasis is important for brain function, as both deficiency and excess can cause damage [30]. High zinc concentrations can be cytotoxic, induce apoptosis, and are linked to prostate cancer [32, 33]. Reactive oxygen species (ROS) like OH, H₂O₂, and O₂ are central to oxidative stress and age-related diseases [30]. Peroxidase enzymes, including *Lepidium draba* peroxidase (LDP), neutralize ROS by oxidizing substrates with hydrogen peroxide. Increasing zinc concentration enhances this activity due to its role in ferric group oxidation [30]. The Zn–Ta nanocomposite contains two metals with distinct functionalities. Zinc enhances LDP activity, while tantalum exhibits inhibitory effects due to its strong affinity for phosphate and hydroxyl groups, which may prevent substrate binding and reduce catalytic turnover [9, 34].

This explains the observed decrease in enzyme activity at higher nanocomposite concentrations, similar to inhibitory effects reported with other metal ions such as mercuric chloride [9]. The concentration of 0.556 mg/mL produced optimal enzyme activity, consistent with these effects. The purified LDP enzyme exhibited a molecular weight of 35 kDa, aligning with previous reports of 40–45 kDa for plant peroxidases [35, 36]. Temperature studies revealed maximal enzyme activity at 20 °C, comparable with literature values around 25 °C [37]. Optimal nanocomposite activity occurred at 55 °C and 70 °C, with pH values of 3.5 at ambient temperature and 2.5 at 70 °C, which aligns with prior findings of 55 °C and pH 4.5 for similar MOFs

[10]. Molecular docking results support these observations. Zinc ions likely bind to active site residues, enhancing substrate interactions, while tantalum ions bind elsewhere, inducing conformational changes that reduce activity [38, 39]. iMODS analyses indicated flexible regions accommodating zinc and TMB, while Ta binding caused localized structural shifts. Covariance and elastic network analyses revealed correlated and anti-correlated motions, highlighting residues sensitive to metal binding. Notably, residues Gly47, Asp42, Ser51, Val45, Gly172, and Pro225 showed interactions consistent with docking predictions, providing a structural explanation for activity modulation. Peroxidase-like nanozymes provide simple and cost-effective methods to assess H₂O₂ and antioxidant capacity [40]. Nanozymes including tantalum-, iron-, carbon-, and precious metal-based compounds, generate hydroxyl radicals that induce color changes in chromogenic substrates, reduced in the presence of antioxidants [10, 41]. Tantalum nanostructures are biocompatible and stable, with applications in sensors, catalysis, nanoelectronics, and biosensing [10]. *Lepidium draba* peroxidase shares significant structural similarity with horseradish peroxidase (HRP), making it suitable for diagnostic and therapeutic applications [20]. Its low immunogenicity further supports potential biomedical use. Given the high catalytic efficiency and unique properties of the Zn–Ta nanocomposite, further optimization of composition and evaluation in vitro and in vivo are warranted [42]. Future studies should employ X-ray crystallography, site-directed mutagenesis, and residue-specific analyses to clarify metal-binding sites and guide the design of more effective enzyme modulators. In conclusion, zinc enhances LDP activity, while tantalum exerts inhibitory effects through structural modulation. Optimal enzyme performance depends on precise control of nanocomposite concentration, pH, and temperature, emphasizing the interplay between structural and environmental factors in enzyme–nanozyme systems.

Comparisons with other nanozymes and detailed molecular analyses suggest that careful tuning of metal composition can maximize activity and stability for biotechnological and therapeutic applications.

5. Conclusion

This study investigated the dual effects of the Zn–Ta nanocomposite on *Lepidium draba* peroxidase using enzyme activity assays, molecular docking, and molecular dynamics simulations. The results demonstrated that zinc enhances enzymatic activity, whereas tantalum exerts inhibitory effects, confirming the modulatory role of the nanocomposite. The enzyme exhibited optimal activity at a nanocomposite concentration of 0.556 mg/mL. Additionally, the Zn–Ta

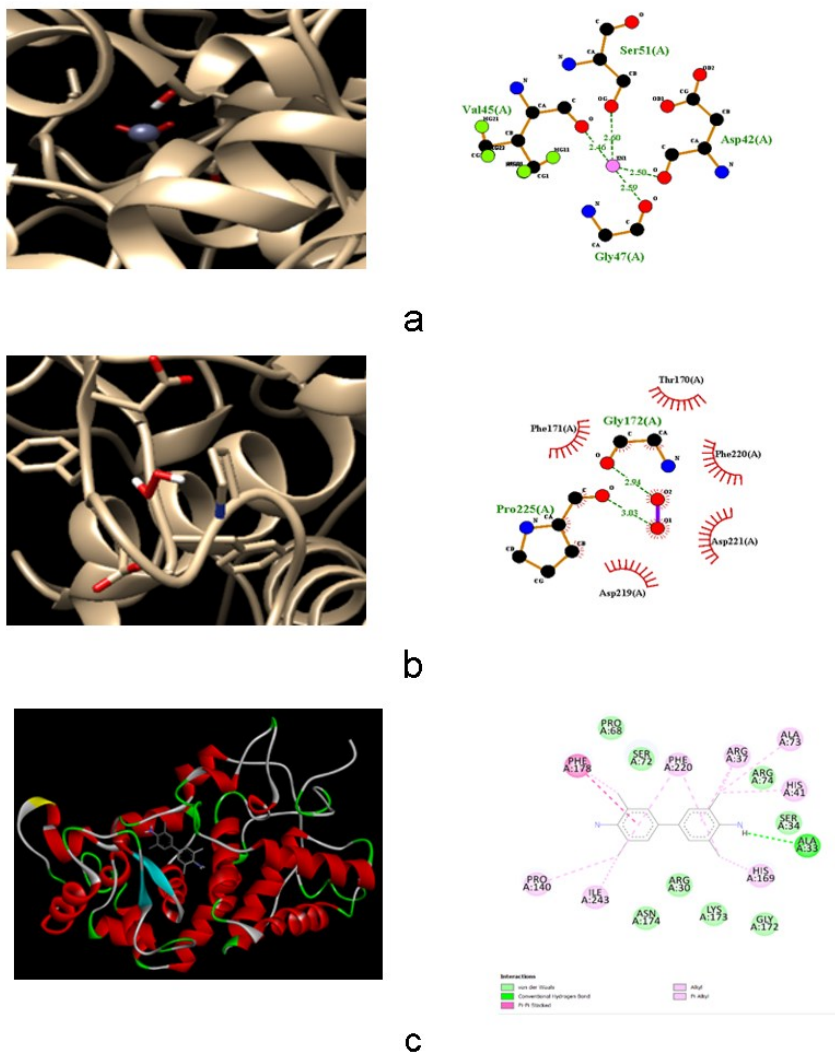


Figure 8: Molecular docking of ligands with LDP peroxidase: (a) Zn²⁺ binding; (b) H₂O₂ binding; (c) TMB

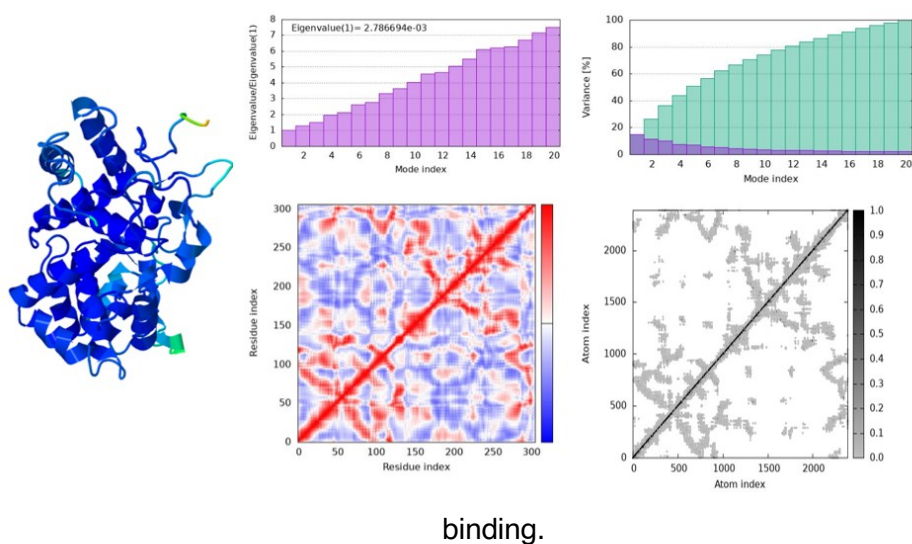


Figure 9: iMODS NMA of LDP with Zn²⁺ (a) Eigenvector, (b) Variance, (c) Covariance matrix, (d) Elastic network.

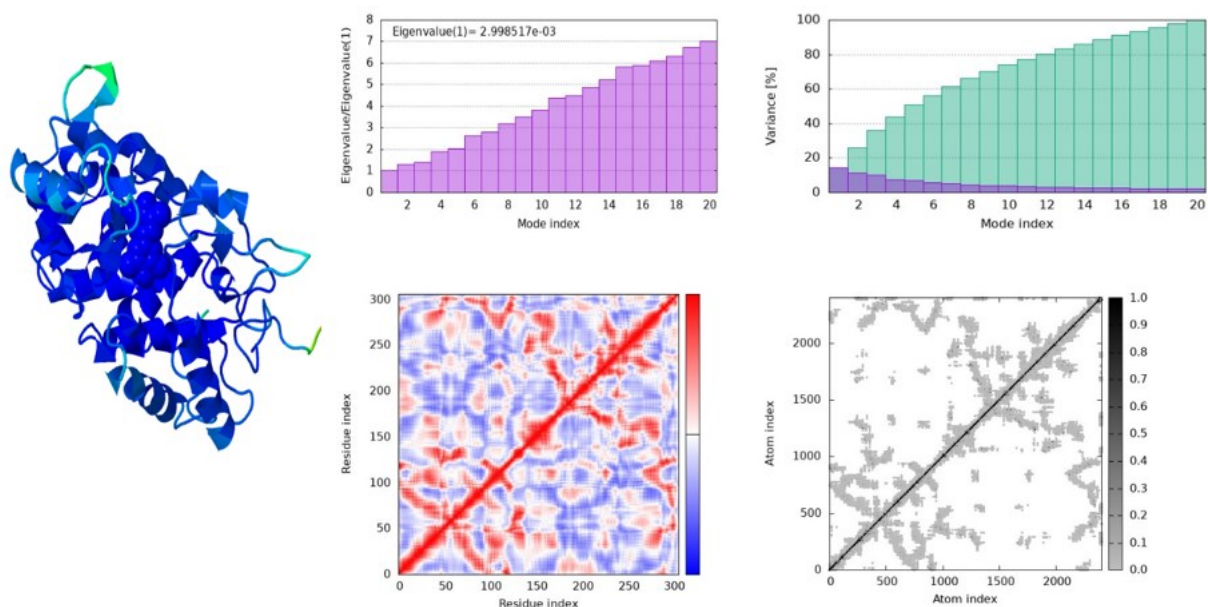


Figure 10: iMODS NMA of LDP with TMB: (a) Eigenvalue, (b) Variance, (c) Covariance matrix, (d) Elastic network.

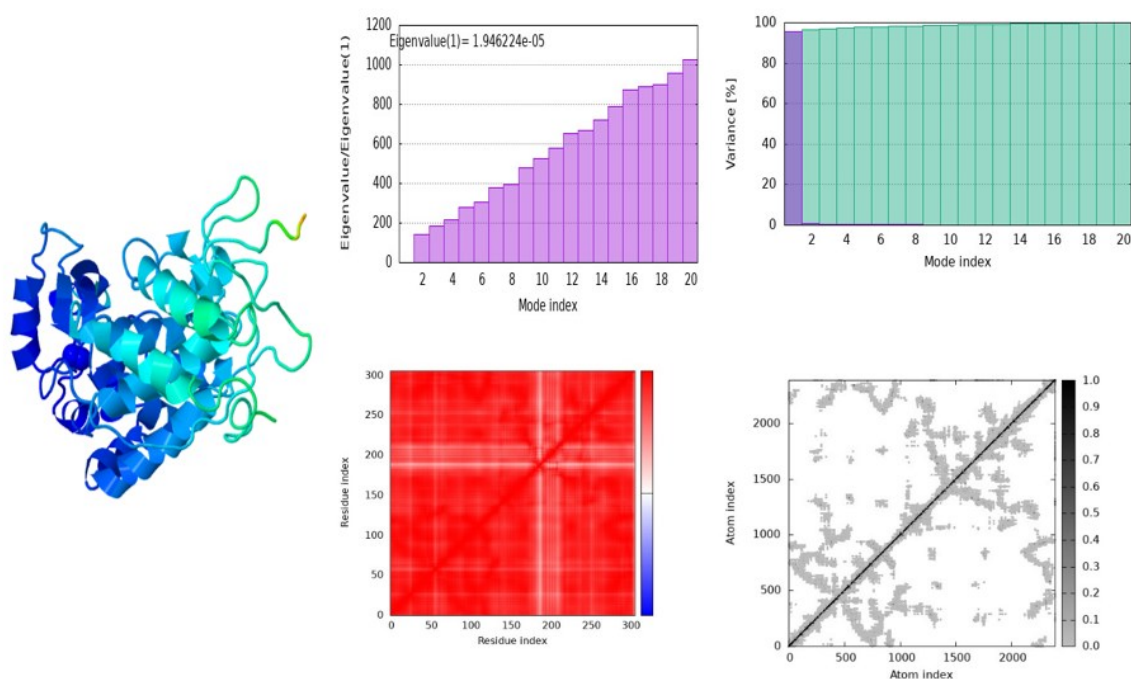


Figure 11: iMODS NMA of LDP with H₂O₂: (a) Eigenvalue, (b) Variance, (c) Covariance matrix, (d) Elastic network.

nanocomposite displayed pseudoperoxidase system activity, with peak performance observed at 55 °C and 70 °C under specific pH conditions (3.5 at ambient temperature and 2.5 at 70 °C).

Molecular docking identified key interactions between Zn, TMB, H₂O₂, and the enzyme, supporting the dual role of the nanocomposite. Molecular dynamics analyses using iMODS further validated these interactions by highlighting conformational flexibility, residue-specific motions, and interaction stability. These results collectively indicate that the Zn-Ta nanocomposite functions both as an enhancer of enzymatic activity (via Zn) and as a modulator/inhibitor (via Ta), offering potential for biotechnological and medical applications.

Declaration of Competing Interest

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

Author contributions

All authors have contributed equally.

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