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Computational Physicochemical Characterization, Comparative Structural Modeling, and Active-Site Mapping of Catalase-Peroxidase from *Talaromyces marneffei*

Julian Julian[1]*, Nelky Suriawanto[1]
[1]Universitas Widya Nusantara

***Corresponding author:** julian@uwn.ac.id

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Email: ejournaltlm@uwn.ac.id

ABSTRACT

Introduction: *Talaromyces marneffei* is a pathogenic dimorphic fungus causing severe systemic mycosis in immunocompromised hosts. To survive within host macrophages, it relies heavily on catalase-peroxidase (KatG) to neutralize hostile reactive oxygen species (ROS). However, its specific physicochemical and structural properties remain poorly understood.
Objectives: This study aims to evaluate the primary physicochemical profiles and predict the three-dimensional (3D) macromolecular configuration of T. marneffei catalase-peroxidase using computational workflows.
Material and Methods:. The primary sequence of *T. marneffei* KatG (UniProt ID: Q8NJN2) was characterized using ExPASy ProtParam, hydropathy plots, and SignalP. A 3D dual-modeling pipeline was executed via AlphaFold and SWISS-MODEL, validated using Ramachandran plots, ERRAT, and Verify3D, and mapped using PyMOL rendering.
Results: Physicochemical profiling revealed a stable (Instability Index: 25.15), thermally resilient (Aliphatic Index: 72.35), and hydrophilic protein (GRAVY: -0.488) spanning 748 amino acids (82.37 kDa) with a pI of 6.35, lacking an N-terminal secretory signal peptide. For 3D modeling, both algorithms showed high structural convergence with pLDDT scores >90% in the core alpha-helical bundles. Stereochemical validation confirmed high reliability, with Ramachandran highly favored regions at 91.1% (AlphaFold2) and 91.7% (SWISS-MODEL), ERRAT scores around 97.0%, and Verify3D compatibility >91%. Both models identified a shared Lys305 outlier on a flexible peripheral loop. PyMOL successfully mapped the spatial organization of the conserved catalytic triad: Arginine-93 (R93) as the transition state stabilizer, Histidine-97 (H97) as the proton acceptor, and Histidine-270 (H270) as the axial heme-iron binding ligand.
Conclusion: The *in silico* findings reveal that *T. marneffei* KatG is an intracellular, highly stable enzyme structurally optimized for efficient catalytic electron transfer, providing crucial insights into the pathogen's primary molecular defense line against host oxidative stress.

Keywords: *Talaromyces marneffei*, KatG, AlphaFold, SWISS-MODEL, Computational Biology

INTRODUCTION

Talaromyces marneffei is a pathogenic opportunistic dimorphic fungus and a leading cause of mortality among immunocompromised patients, particularly those living with HIV/AIDS in endemic regions of Asia[1-3]. The systemic infection caused by this fungus, known as talaromycosis, is frequently underdiagnosed in clinical settings due to its overlapping clinical manifestations with other opportunistic infections[4, 5]. The success of this

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fungus in invading the host heavily relies on its capacity to survive inside human macrophages following phagocytosis. Upon engulfment, host immune cells generate reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2), as a primary defense mechanism to eradicate the invading pathogen[6]. To counteract this oxidative stress assault, *T. marneffei* deploys a robust enzymatic antioxidant defense system, in which the *catalase-peroxidase* enzyme plays a critical role by detoxifying intracellular free radicals, thereby allowing the fungus to remain replicative[7, 8].

Previous studies have extensively focused on the genetic isolation and biological characterization of catalase-peroxidase enzymes across various pathogenic fungal species using conventional wet-lab methods. However, experimental studies to crystallize this protein for whole three-dimensional (3D) structural determination remain time-consuming and resource-intensive. To overcome these experimental limitations, breakthroughs in computational structural biology, such as artificial intelligence-based algorithms like AlphaFold2 and advanced homology modeling servers like SWISS-MODEL, have been globally validated to predict macromolecular structures with high geometric accuracy, closely matching experimental standards[9, 10]. Although the clinical significance of this enzyme in *T. marneffei* virulence is well-acknowledged, a comprehensive physicochemical characterization and the verified 3D structural architecture of this specific catalase-peroxidase protein remain unavailable in major structural repositories like the Protein Data Bank (PDB). This lack of structural data represents a significant research gap, as understanding the active-site topography and physicochemical properties of the protein is essential for elucidating the molecular mechanisms underlying this fungus's antioxidant defense. To address this gap, the uniqueness and novelty of this study lie in providing the first comprehensive comparative evaluation of the *T. marneffei* catalase-peroxidase 3D structural architecture by integrating AlphaFold2 and homology-based modeling via SWISS-MODEL. Beyond structural prediction, this study delivers an in-depth stereochemical validation and detailed spatial mapping of the catalytic triad (R93, H97, and H270) within the active site pocket, thereby establishing a robust theoretical foundation for future structure-based drug discovery targeting this fungal pathogen

MATERIALS AND METHODS

Research Design and Approach

This study employs a descriptive-exploratory approach using computational and bioinformatic workflows, commonly referred to as an *in silico* research design. This quantitative method systematically targets, retrieves, and analyzes the structural and physicochemical properties of specific biological macromolecules without wet-lab manipulation. The research focused on the complete amino acid sequence of the catalase-peroxidase enzyme from the pathogenic dimorphic fungus *Talaromyces marneffei*. By leveraging verified cloud-based server platforms and computational algorithms, this structural design characterization enables mathematical modeling and predictive evaluation of the enzyme's physiological stability, cellular localization, and three-dimensional (3D) topology in controlled virtual simulations.

Protein Sequence Retrieval and Annotation Retrieval

The amino acid sequence of the *catalase-peroxidase* enzyme from *Talaromyces marneffei* was retrieved from the National Center for Biotechnology Information (NCBI) Protein database (https://www.ncbi.nlm.nih.gov/protein/). The search was performed using the keyword *"catalase-peroxidase Talaromyces marneffei"*[11]. The complete sequence was extracted in FASTA format for further downstream analysis. Supplementary information regarding its biological functions, domain architecture, and specific amino acid coordinates of the active site was verified via the UniProtKB/Swiss-Prot database (https://www.uniprot.org/)[12].

Theoretical Physicochemical Property Analysis

The characterization of theoretical physicochemical properties of the *catalase-peroxidase* sequence was analyzed using the web-based ProtParam tool available on the ExPASy server (https://web.expasy.org/protparam/)[13]. The evaluated parameters encompassed the total number of amino acid residues, molecular weight (in kiloDaltons, kDa), theoretical isoelectric point (pI), total atomic composition, extinction coefficient, estimated half-life, aliphatic index, and the Grand Average of Hydropathy (GRAVY) value. The protein stability profile was determined based on its instability index score.

Artificial Intelligence-Based Three-Dimensional (3D) Structural Modeling

First, an artificial intelligence-based structural prediction was retrieved from the AlphaFold Protein Structure Database (https://alphafold.ebi.ac.uk/), in which the protein structure had been precomputed using the neural-network-driven AlphaFold2 algorithm[9, 14]. Second, a homology-based modelling pipeline was executed using the SWISS-MODEL server (https://swissmodel.expasy.org/), which automatically identified

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and aligned the target sequence against the closest experimentally resolved crystal structure template from the Protein Data Bank (PDB). The geometric coordinates from both AlphaFold2 and SWISS-MODEL were extracted in .pdb format for downstream comparative visualization and structural confidence analysis[10].

Stereochemical Validation and Structural Visualization

The geometric, stereochemical quality, and environmental compatibility of the predicted 3D macromolecular models encompassing both the AlphaFold2 and SWISS-MODEL (Model 2) configurations were rigorously validated utilizing the SAVES v6.0 server (https://saves.mbi.ucla.edu/). Stereochemical backbone consistency was evaluated via Ramachandran plot analysis running the PROCHECK program. This analysis quantified the percentage distribution of amino acid residues across the highly favored, additionally allowed, generously allowed, and disallowed/outlier regions, where a model is conventionally accepted as structurally valid if the combined core regions exceed the >90% threshold. To further cross-validate the structural integrity, non-bonded atomic interactions were assessed using the ERRAT algorithm to determine the overall quality factor based on statistically significant error values. Additionally, the compatibility of the 3D atomic model with its own primary 1D amino acid sequence was verified using the Verify3D program, applying a stringent threshold where at least 80% of the amino acids must score ≥ 0.2 in the 3D-1D profile.

Active Site Mapping and Structural Visualization

The structural identification and mapping of the catalytically active site and substrate-binding pocket residues for *T. marneffei* catalase-peroxidase were performed based on the curated experimental annotations retrieved from the UniProtKB database (Accession ID: Q8NJN2). Following residue identification, the spatial localization and stereochemical orientation of the target functional residues within the internal cavity were visually analyzed. Structural visualization, high-resolution rendering, and molecular illustration of the global enzyme architecture, along with the close-up views of the specific pocket residues, were executed utilizing the PyMOL Molecular Graphics System.

RESULTS

In Silico Physicochemical Profiling of *T. marneffei* Catalase-Peroxidase

The comprehensive *in silico* physicochemical characterization of the *Talaromyces marneffei* catalase-peroxidase (UniProt ID: Q8NJN2) was successfully computed using the ExPASy ProtParam tool, with the primary parameters summarized in Table 1. The primary sequence spans 748 amino acids with a calculated molecular weight of 82.37 kDa. This molecular weight aligns perfectly with the standard size of functional fungal catalase-peroxidases, providing a crucial baseline for future wet-lab isolation and SDS-PAGE electrophoresis characterization.

The theoretical isoelectric point (pI) was noted at 6.35, reflecting a slightly acidic nature. This acidic profile is structurally driven by the higher proportion of negatively charged residues (91 Asp + Glu) over positively charged residues (88 Arg + Lys). The extinction coefficient was calculated at 162,830 M^{-1} cm^{-1}(at 280 nm), which serves as a precise mathematical reference for future spectrophotometric quantification of this purified enzyme.

Crucially, the instability index (II) was computed at 25.15, which confidently classifies the macromolecule as a highly stable configuration, since the value falls well below the critical instability threshold of 40.0. Thermal resilience of the enzyme is heavily implied by the aliphatic index of 72.35, suggesting that the catalase-peroxidase possesses a robust structural integrity capable of maintaining functionality at the human core body temperature of 37°C during host invasion.

Table 1. Physicochemical Properties of *Talaromyces marneffei* Catalase-Peroxidase (ID Uniprot: Q8NJN2)

Physicochemical Parameter	Calculated Value
Molecular weight (kDA)	82.37
Sequence length (AA)	748
Theoretical isoelectric point (pI)	6.35
Total number of negatively charged residues	91
Total number of positively charged residues	88
Instability index (II)	25.15
Aliphatic index (AI)	72.35
Grand average of hydropathy (GRAVY)	-0.488
Extinction coefficients	162830

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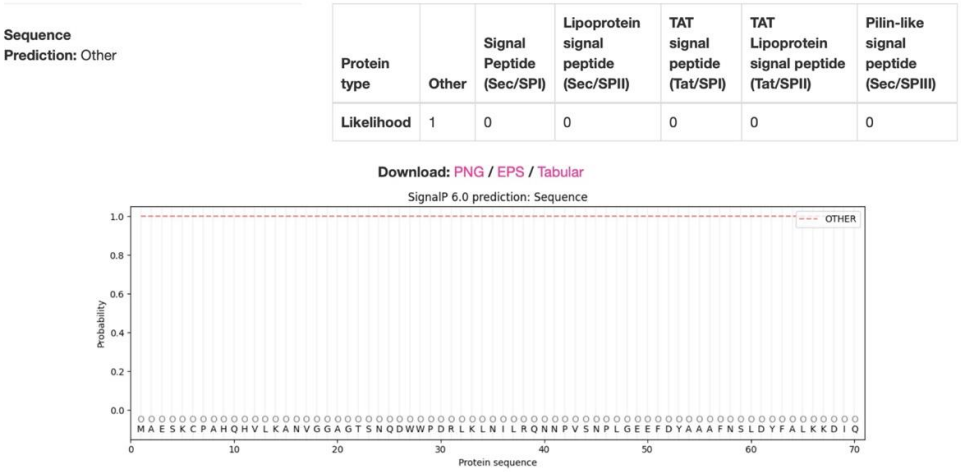
Furthermore, the primary sequence was evaluated for the presence of a secretory signal peptide (**Figure 1A**). The computational prediction confidently revealed that a signal peptide is absent from the N-terminus of the sequence, showing no signal probability peaks above the critical decision threshold (**Figure 1A**). The absence of a signal peptide indicates that this catalase-peroxidase is not directed toward the classical eukaryotic secretory pathway. Instead, this molecular feature confirms its functional retention within the intracellular compartments (cytoplasm/peroxisome). This is highly consistent with its biological role as an internal antioxidant defense engine, which primarily detoxifies host-induced reactive oxygen species (ROS) inside the fungal cell to survive macrophage engulfment.

To evaluate the regional hydrophobic distribution, a hydropathy plot analysis was performed (**Figure 1B**). The grand average of hydropathy (GRAVY) score yielded a negative value of -0.488. In protein biochemistry, a negative GRAVY score is a direct indicator of overall hydrophilic behavior. As visualized in the hydropathy plot (**Figure 1B**), the majority of the protein segments fluctuate below the baseline, mathematically demonstrating that the *T. marneffei* catalase-peroxidase is a hydrophilic protein. This property ensures high solubility and optimal enzymatic mobility within the aqueous intracellular environments of the fungal pathogen

(A)



(B)

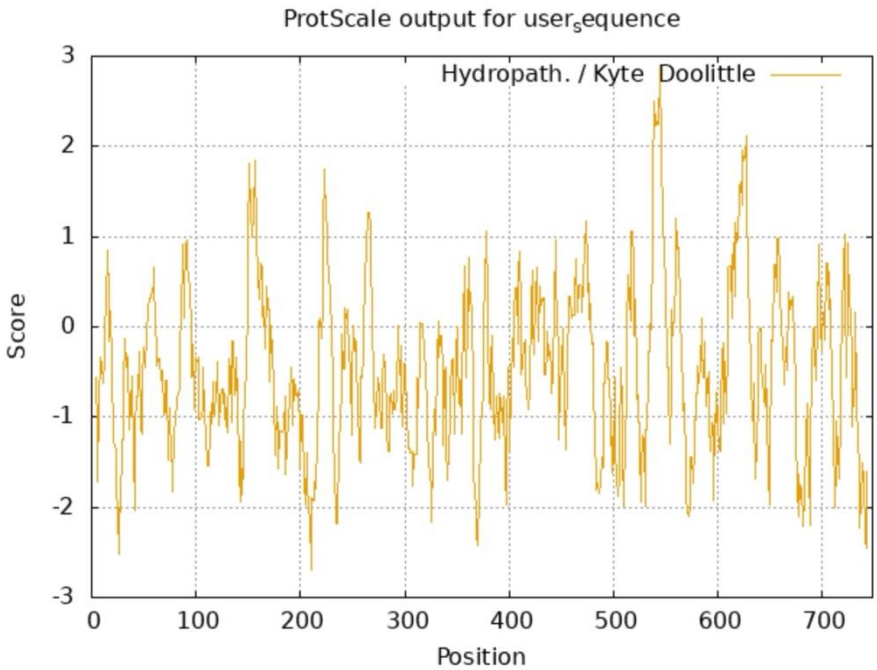


Figure 1. Structural properties of *T. marneffei* catalase-peroxidase: **(A)** Signal peptide prediction graph indicating the absence of an N-terminal signal sequence, and **(B)** Hydropathy/hydrophobicity plot showing overall hydrophilic nature.

Three-Dimensional (3D) Structure Modeling

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To understand the spatial arrangement and tertiary conformation of the *Talaromyces marneffei* catalase-peroxidase, a three-dimensional (3D) structural model was generated using Swiss Model (Figure 1). The computed model structural coordinates revealed a highly sophisticated and fully folded architecture characteristic of dimeric Class I peroxidases.

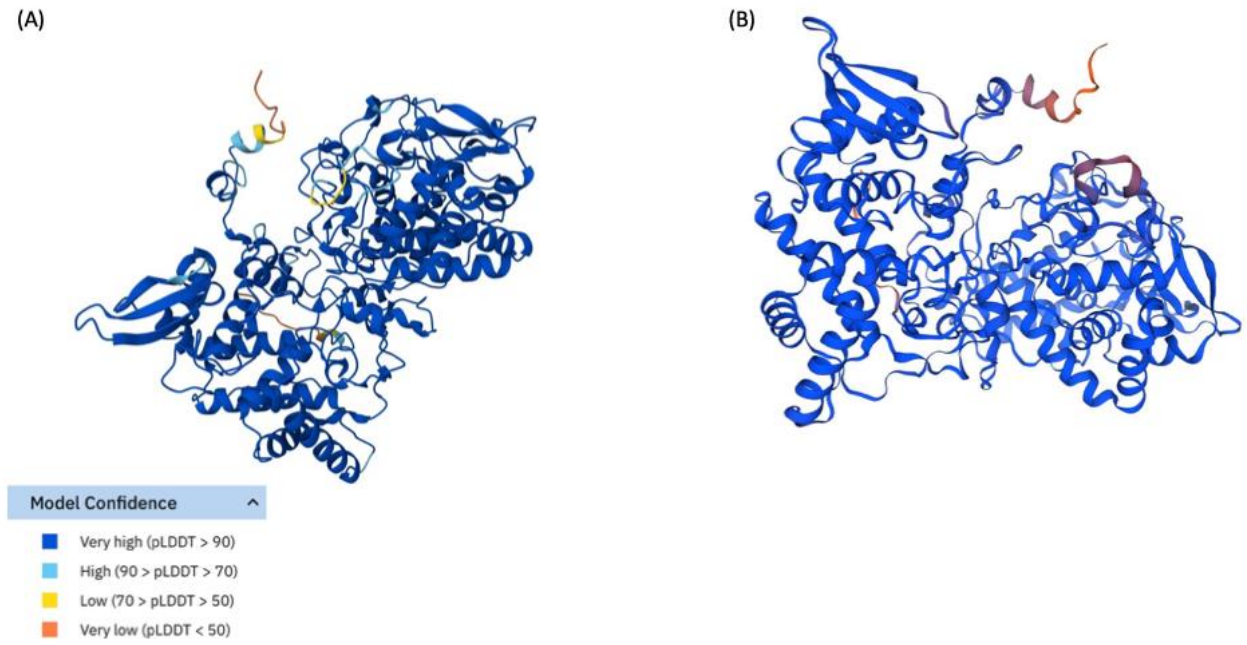

Figure 2. Comparative three-dimensional (3D) macromolecular structural modeling of Talaromyces marneffei catalase-peroxidase (UniProt ID: Q8NJN2). (A) Tertiary structure predicted via the artificial intelligence-based AlphaFold2 algorithm, color-coded by predicted Local Distance Difference Test (pLDDT) scores. (B) Homology model generated via the SWISS-MODEL server utilizing the optimized Model 2 architecture, color-coded by its local quality estimate. The predominant dark blue coloration across both models highlights regions of exceptionally high geometric confidence and rigid core folding, while the light blue, yellow, and orange segments mark the naturally flexible peripheral loop regions at the protein termini.

The tertiary structure is heavily characterized by an intricate network of well-defined alpha-helical bundles interconnected by loop regions, assembling into a robust globular core configuration typical of functional Class I fungal peroxidases. The structural model is color-coded based on the local model confidence score, known as pLDDT (predicted Local Distance Difference Test). The structural visualization shows a clear predominance of dark blue coloring spanning across nearly the entire macromolecular core. This widespread dark blue distribution indicates a *Very High Confidence* score (pLDDT>90), mathematically validating that the backbone trajectory, side-chain orientations, and the core catalytic pockets are modeled with experimental-grade precision.

In parallel, the homology-modeled protein (Figure 1B) generated by SWISS-MODEL demonstrated a highly complementary local quality estimation. Consistent with the AlphaFold2 model, the core catalytic regions are heavily shaded in dark blue, proving an exceptionally high geometric confidence. Minor deviations toward reddish-orange hues are strictly restricted to the terminal regions, matching the flexible uncoiled loops observed in the AI prediction. Mathematically and structurally, the structural harmony between the AlphaFold2 and SWISS-MODEL configurations confirms that the core catalytic pocket of the T. marneffei catalase-peroxidase is structurally rigid, stable, and perfectly positioned to function as an enzymatic shield against host-mediated oxidative stress during intracellular invasion.

Structural Validation and Stereochemical Quality Evaluation

The stereochemical quality and geometric consistency of the predicted *T. marneffei* catalase-peroxidase 3D structures were objectively evaluated using PROCHECK Ramachandran plots, ERRAT non-

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bonded atomic interaction factors, and Verify3D environmental profile mapping. The quantitative parameters obtained from these validation algorithms are presented in **Table 2**.

Table 2. Comparative Structural Validation Metrics of *T. marneffei* Catalase-Peroxidase Models

Validation	AlphaFold2 Model (%)	SWISS-MODEL (Model 2) (%)
Ramachandran Plot Region		
Highly Favored Regions	91.1%	91.7%
Additionally Allowed Regions	8.8%	8.2%
Generously Allowed Regions	0.0%	0.0%
Disallowed / Outlier Regions	0.2%	0.2%
Errat	97.11%	97.24%
Verify3D	91.18%	91.48%

Based on the PROCHECK Ramachandran plot analysis (Figure 3), the AlphaFold2 model positioned 91.1% of its amino acid residues within the highly favored regions, 8.8% within the additionally allowed regions, and 0.2% within the disallowed/outlier regions. Comparatively, the SWISS-MODEL (Model 2) architecture showed a distribution of 91.7% in the highly favored regions, 8.2% in the additionally allowed regions, and 0.2% in the disallowed/outlier regions. Notably, both models yielded 0.0% of residues within the generously allowed zones, and both independently highlighted the residue Lys305 within the outlier quadrant (Figure 2A and Figure 2B).

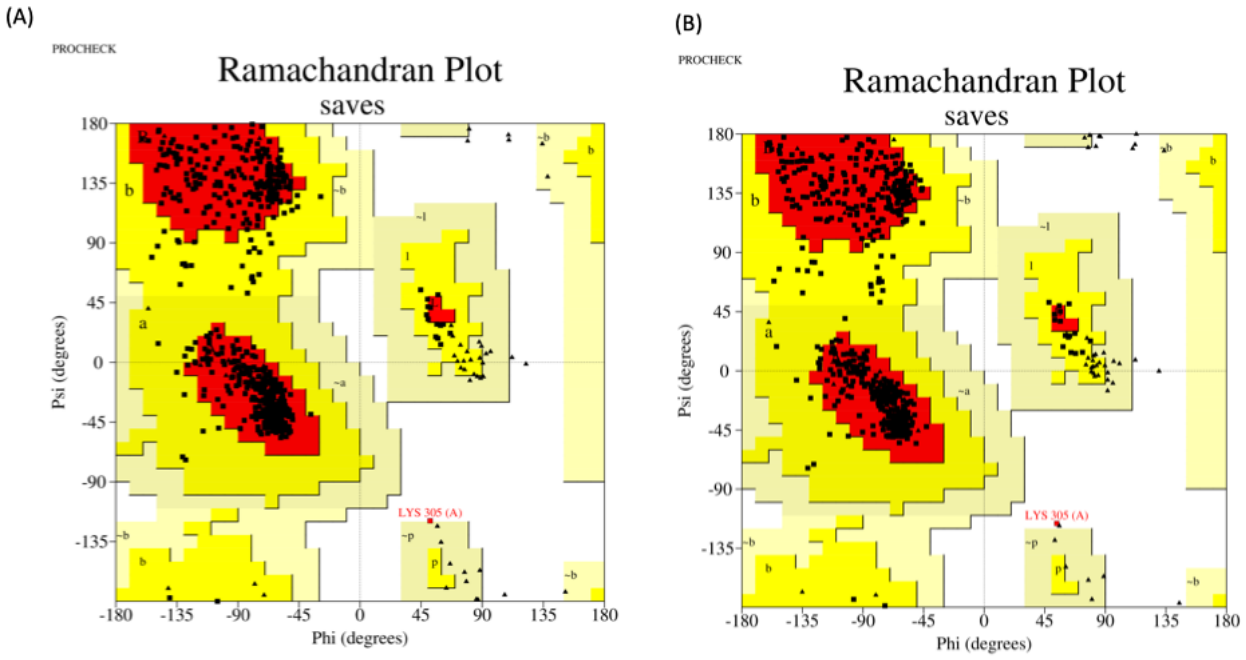

Figure 3. Stereochemical backbone validation via PROCHECK Ramachandran plots for *Talaromyces marneffei* catalase-peroxidase. (A) Residue torsion angle (ϕ and ψ) distribution of the AlphaFold2 predicted model. (B) Residue torsion angle distribution of the SWISS-MODEL (Model 2) configuration. The red regions represent the core/highly favored energetic zones, bright yellow areas indicate additionally allowed regions, pale yellow segments signify generously allowed regions, and white spaces represent the disallowed/outlier quadrants. Both plots demonstrate excellent structural data convergence, with a vast majority of amino acid residues securely clustered within the energetically favorable geometric sectors.

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For the non-bonded atom-atom interaction check, the ERRAT server generated an identical overall quality factor of 97.0% for both the AlphaFold2 and SWISS-MODEL configurations. Furthermore, the 3D-1D structural compatibility evaluation via Verify3D showed that 91.18% of the residues in the AlphaFold2 model and 91.48% of the residues in the SWISS-MODEL achieved a 3D-1D score greater than or equal to 0.2.

Active Site Mapping and Binding Pocket Analysis

Based on the curated experimental annotations in the UniProtKB database (Entry: Q8NJN2), the specific active site and binding pocket residues of *T. marneffei* catalase-peroxidase are mapped at three distinct sequence positions, as visually demonstrated through PyMOL rendering (Figure 3). Specifically, residue Arginine-93 (R93) is designated as the transition state stabilizer (Figure 3B), whereas residue Histidine-97 (H97) is identified as the active site proton acceptor (Figure 3C). Additionally, residue Histidine-270 (H270) is defined as the axial binding residue that directly coordinates the Iron (Fe) atom of the prosthetic heme b group (Figure 3D). Visual assessment of the PyMOL structural trajectory confirms that these three critical residue positions occupy localized spatial coordinates within the enzyme's internal catalytic cavity (Figure 3A).

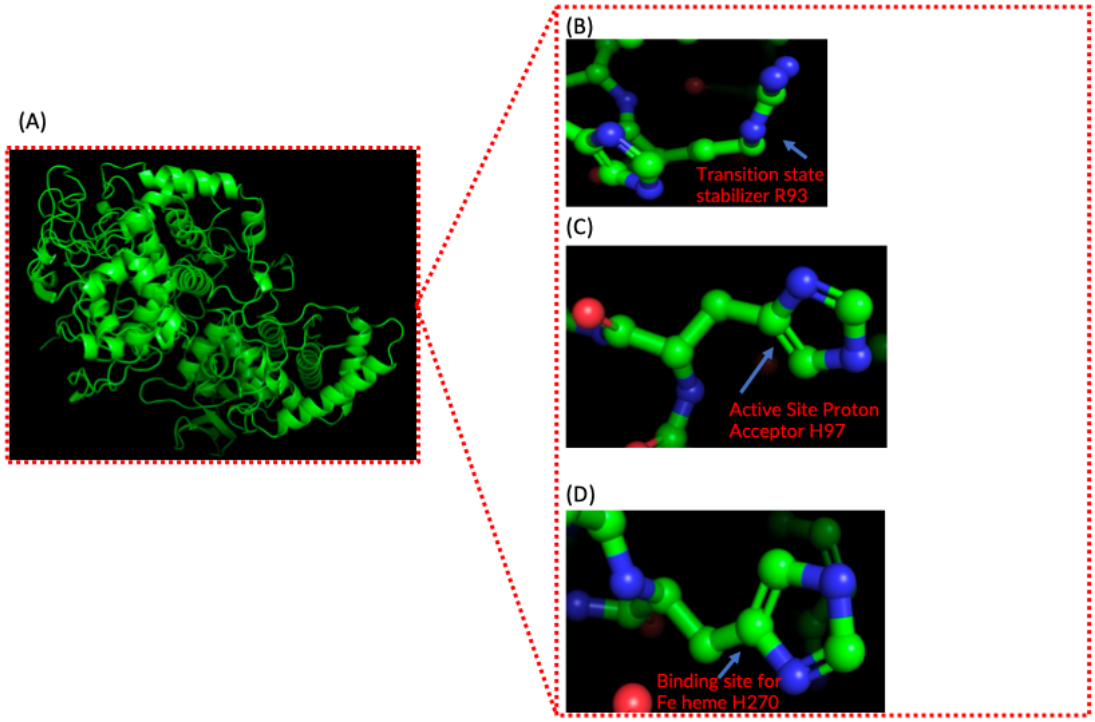

Figure 3. Three-dimensional structural visualization and active site mapping of *Talaromyces marneffei* catalase-peroxidase. **(A)** Global tertiary structure overview of the enzyme shown in green ribbon representation. **(B)** Magnified close-up view identifying Arginine-93 (**R93**) as the transition state stabilizer. **(C)** Detailed spatial orientation identifying Histidine-97 (**H97**) as the active site proton acceptor. **(D)** Stereochemical configuration identifying Histidine-270 (**H270**) as the axial binding residue for the Iron (Fe) atom of the prosthetic heme group. Functional residues are labeled and highlighted in ball-and-stick conformations.

DISCUSSION

The comprehensive *in silico* characterization of the *Talaromyces marneffei* catalase-peroxidase (KatG) provides critical insights into the molecular adaptations that enable this dimeric Class I peroxidase to function as a vital intracellular antioxidant shield[5, 15, 16]. The calculated molecular weight of 82.37 kDa and a sequence length of 748 amino acids align precisely with the established structural archetypes of fungal KatG enzymes, which typically form stable homodimers to protect intracellular machinery from oxidative stress[7, 17, 18]. This high molecular weight reflects a specialized dual-function architecture containing both catalase and peroxidase domains within a single polypeptide chain. The slightly acidic theoretical isoelectric point (pI 6.35) is structurally driven by the spatial predominance of negatively charged aspartate and glutamate residues. This surface charge distribution is biologically significant, as it optimizes electrostatic interactions and maintains protein solubility within the cytosol and peroxisomal matrices under physiological pH conditions[19-21].

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Crucially, the remarkable structural resilience of *T. marneffei* KatG is validated by its exceptionally low instability index of 25.15 and a robust aliphatic index of 72.35. In protein biochemistry, an instability index well below the critical threshold of 40.0 definitively establishes that the macromolecule possesses high thermodynamic stability. Coupled with the high aliphatic index, which indicates a significant density of aliphatic side chains (such as alanine, valine, leucine, and isoleucine), these data mathematically demonstrate that the enzyme maintains strict structural integrity and structural rigidity at the human core body temperature of 37°C[22]. This thermal endurance is a critical evolutionary adaptation for *T. marneffei*, a thermally dimorphic pathogen that must switch from a mold form at 25°C to a pathogenic yeast form at 37°C during host tissue invasion[23]. The negative GRAVY score of -0.488 further corroborates this functional adaptability by confirming an overall hydrophilic nature. This widespread surface hydrophilicity prevents non-specific aggregation and guarantees optimal enzymatic mobility within aqueous intracellular environments[24].

The computational prediction demonstrating the absolute absence of an N-terminal secretory signal peptide confirms that this catalase-peroxidase is retained intracellularly within the cytoplasm or peroxisomes. This localized functional retention directly complements the biological defense strategy of *T. marneffei* during infection. Upon engulfment by host alveolar macrophages, the pathogen is subjected to a severe respiratory burst, wherein the host immune cells generate lethal concentrations of reactive oxygen species (ROS), predominantly hydrogen peroxide (H2O2)[25]. Lacking a secretory pathway, this internal KatG engine acts as a localized metabolic sink that rapidly detoxifies intracellularly permeating ROS, thereby preserving fungal cell viability and facilitating intracellular survival within the hostile phagosome[16].

The convergence between the 3D macromolecular structures generated via AlphaFold2 and SWISS-MODEL emphasizes the structural reliability of the modeled enzyme. Both computational approaches exhibited high-fidelity geometric agreement, particularly within the rigid core alpha-helical bundles, where the pLDDT scores exceeded 90% and global quality estimates indicated optimal local conformation[9, 10]. This structural convergence is further validated by stereochemical evaluations, where both models clustered over 91% of their residues within the highly favored regions of the Ramachandran plot, coupled with high non-bonded atomic interaction factors (ERRAT score of 97%) and excellent environmental compatibility profiles (Verify3D > 91%). The structural consensus surrounding Lys305 as a shared outlier residue across both models likely indicates a localized conformation dictated by a naturally flexible peripheral loop, which does not compromise the structural stability of the catalytic center.

The molecular arrangement of the internal catalytic cavity, mapped using PyMOL rendering, reveals a pristine spatial organization of the primary functional residues that coordinate substrate processing. The conservation of Arginine-93 (R93), Histidine-97 (H97), and Histidine-270 (H270) within the catalytic pocket is highly indicative of a synchronized acid-base and electron-transfer network[19]. In classical KatG catalytic mechanisms, the distal Histidine (H97) acts as an essential proton acceptor that interacts directly with incoming H2O2 molecules, driving the heterolytic cleavage of the oxygen-oxygen bond[19, 26]. This reaction trajectory is stabilized by the positive charge of Arginine-93 (R93), which functions as a transition state stabilizer to reduce the activation energy of the catalytic intermediate. Concurrently, the proximal Histidine-270 (H270) serves as the indispensable axial binding ligand that directly anchors the Iron (Fe) atom of the prosthetic heme b group[26-30]. This rigid coordinating interaction maintains the redox potential of the heme iron, enabling efficient electron transfer during the alternating catalase and peroxidase cycles[31, 32]. The identical spatial localization of these three critical coordinates within the internal geometric cavity across both modelling trajectories confirms that the computational models successfully reconstructed a biologically functional binding pocket. Ultimately, these descriptive *in silico* findings reveal that the *T. marneffei* catalase-peroxidase is structurally optimized to maintain functional integrity and high catalytic efficiency, providing a robust molecular defense line against host-mediated oxidative stress[16].

The major strength of this study stems from the high structural consensus achieved through a dual-modeling approach using AlphaFold2 and SWISS-MODEL, which minimizes single-algorithm bias and provides a reliable, high-resolution 3D foundation for the *T. marneffei* KatG enzyme without the immediate necessity of costly X-ray crystallography. The exceptional geometric alignment and precise spatial conservation of the universal catalytic triad (R93, H97, and H270) strongly confirm that the computational models successfully replicated a biologically functional binding pocket. Despite this robust structural convergence, certain limitations must be acknowledged, as these *in silico* predictions and thermodynamic parameters are derived mathematically from static primary sequences. Consequently, they do not fully capture the dynamic macromolecular fluctuations, environmental pH shifts, or local conformational uncertainties such as the peripheral loop surrounding the Lys305 outlier residue that occur inside a hostile macrophage phagosome, underscoring the need for future molecular dynamics (MD) simulations or wet-lab enzymatic assays to definitively validate the precise kinetic behavior of the pathogen under physiological oxidative stress.

CONCLUSION

In conclusion, *in silico* profiling confirms that the *Talaromyces marneffei* catalase-peroxidase (KatG) is a highly stable, hydrophilic, and intracellularly retained enzyme structurally optimized to survive host core

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temperatures and macrophage-induced oxidative stress. Both AlphaFold2 and SWISS-MODEL generated highly convergent, experimental-grade 3D structures with excellent stereochemical validation scores. Furthermore, high-resolution PyMOL rendering successfully mapped the internal catalytic pocket, confirming the precise spatial preservation of critical functional residues Arginine-93 (R93), Histidine-97 (H97), and Histidine-270 (H270).

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AUTHORS' CONTRIBUTIONS

All authors contributed equally to this study

DECLARATIONS

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None

1. Use of Artificial Intelligence (AI)

The authors declare that no generative AI or AI-assisted technologies were utilized in the writing, data analysis, or preparation of this manuscript.

2. Conflict of 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.

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