EDUCAÇÃO CIÊNCIA E SAÚDE (ISSN 2358-7504)
Artigo Original
http://doi.org/10.20438/ecs.v13i1.771

IN SILICO STRUCTURAL CHARACTERIZATION AND MOLECULAR DYNAMICS OF *TRYPANOSOMA RANGELI* GP63: INSIGHTS FROM ALPHAFOLD MODELING

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Abstract

The *Trypanosoma rangeli* surface metalloprotease GP63 (TrGP63) holds diagnostic relevance due to its cross-reactivity with *Trypanosoma cruzi* homologs. However, its structural characterization remains limited. This study aimed at the in silico structural characterization of TrGP63, employing computational modeling and molecular dynamics (MD) simulations to validate the protein's dynamic behavior. Three-dimensional prediction was performed using AlphaFold, and the model's quality was assessed by confidence metrics (pLDDT > 90; pTM = 0.95) and stereochemical analysis (PROCHECK). Conformational stability was investigated through 25 ns MD simulations (GROMACS, GROMOS57a4 force field). The generated model exhibited high accuracy and identified the conserved catalytic motif HEXXH. The MD simulations demonstrated the structural stability of TrGP63. Radius of Gyration (Rg) and Root-Mean-Square Deviation (RMSD) analyses indicated rapid system accommodation and maintenance of an equilibrated conformational state. Fluctuation analysis (RMSF) revealed rigidity in the central domains, including the catalytic site, and greater flexibility in the C-terminal region. These results provide the first molecular dynamics-validated structural model of TrGP63. Furthermore, structural and electrostatic divergences, specifically its predominantly neutral catalytic cleft and less conserved C-terminal region, suggest distinct substrate specificities. Consequently, these regions are prime targets for designing species-specific epitopes, facilitating the accurate differential diagnosis of Chagas disease.

Keywords: *Trypanosoma rangeli*, GP63, AlphaFold modeling, molecular dynamics, structural characterization.

Resumo

A metaloprotease de superfície GP63 de *Trypanosoma rangeli* (TrGP63) possui relevância diagnóstica devido à sua reatividade cruzada com homólogos de *Trypanosoma cruzi*. Contudo, sua caracterização estrutural permanece limitada. O presente estudo objetivou a caracterização estrutural *in silico* da TrGP63, empregando modelagem computacional e simulações de dinâmica molecular (DM) para validar o comportamento dinâmico da proteína. A predição tridimensional foi realizada utilizando o AlphaFold, e a qualidade do modelo foi aferida por métricas de confiança (pLDDT > 90; pTM = 0,95) e análise estereoquímica (PROCHECK). A estabilidade conformacional foi investigada por simulações de DM de 25 ns (GROMACS, campo de força GROMOS57a4. O modelo gerado apresentou alta acurácia e identificou o motivo catalítico conservado HEXXH. As simulações de DM demonstraram a estabilidade estrutural da TrGP63. As análises de Raio de Giração (Rg) e Desvio Quadrático Médio (RMSD) indicaram a rápida acomodação do sistema e a manutenção de um estado conformacional equilibrado. A análise de flutuação (RMSF) revelou rigidez nos domínios centrais, incluindo o sítio catalítico, e maior flexibilidade na região C-terminal. Esses resultados fornecem o primeiro modelo estrutural da TrGP63

validado por dinâmica molecular. Além disso, divergências estruturais e eletrostáticas, especificamente a fenda catalítica predominantemente neutra e a região C-terminal menos conservada, sugerem especificidades de substrato distintas. Consequentemente, essas regiões são alvos principais para o desenho de epítopos espécie-específicos, facilitando o diagnóstico diferencial preciso da doença de Chagas.

Palavras-chaves: *Trypanosoma rangeli*, GP63, AlphaFold modelagem, dinâmica molecular, caracterização estrutural.

1 Introduction

The hemoflagellate protozoan *Trypanosoma rangeli* is widely distributed in Central and South America, infecting diverse mammal species, including humans, and is transmitted by triatomines of the genus *Rhodnius* (Grisard et al., 1999; Santos et al., 2024). Although classically considered non-pathogenic to vertebrate hosts, *T. rangeli* shares the same geographical distribution area, vectors, and reservoirs with *Trypanosoma cruzi*, the etiological agent of Chagas Disease (Guhl; Vallejo, 2003; Maia, 2023). This coexistence represents a significant diagnostic challenge, as *T. rangeli* presents high antigenic similarity with *T. cruzi*, resulting in cross-reactions in serological assays and, consequently, false-positive diagnoses for Chagas Disease (Guhl; Vallejo, 2003; Pereira, 2021).

Among the proteins involved in this cross-reactivity, the GP63 metalloprotease family, also known as leishmanolysin in *Leishmania*, stands out. Structurally, GP63 is a zinc-dependent metzincin characterized by a highly conserved HEXXH catalytic motif, which is strictly required for its proteolytic activity. The protein is predominantly attached to the parasite's cell membrane via a glycosylphosphatidylinositol (GPI) anchor, although secreted isoforms are also common. GP63 is recognized as a major virulence factor in this genus, acting in multiple biological processes. In *Leishmania*, it plays a pivotal role in modulating parasite-host interactions by cleaving a broad spectrum of host substrates. This enzymatic activity facilitates immune response evasion, confers resistance to complement-mediated lysis through the degradation of complement cascade proteins, and promotes macrophage phagocytosis (Silvestrini et al., 2024; ONWAH et al., 2026).

In *T. cruzi*, GP63 homologs exhibit a complex gene repertoire and are differentially expressed at various stages of the parasite life cycle. They are intimately associated with cell invasion mechanisms, contributing to the degradation of the host extracellular matrix and enabling tissue traversal. This versatile proteolytic capability highlights the evolutionary relevance of the GP63 family in the physiology, adaptation, and pathogenesis of trypanosomatids (Kulkarni; Olson; Engman, 2009; Cuevas et al., 2003; Berná et al., 2025).

Despite the functional importance attributed to GP63 in related parasites, knowledge regarding this protein in *T. rangeli* remains quite limited. Genomic data indicate that the parasite possesses an extensive GP63 gene repertoire, with evidence of differential expression throughout its evolutionary stages (Pereira, 2021). However, the structural and functional particularities of these metalloproteases remain poorly understood. Elucidating the three-dimensional structure of *T. rangeli* GP63 is essential for understanding its biological function and for identifying specific epitopes that may

support the development of more precise differential diagnostic methods (Calixto et al., 2013; Maia, 2023).

The experimental determination of protein structures by techniques such as X-ray crystallography or nuclear magnetic resonance (NMR) is a time-consuming and technically challenging process. In this context, the emergence of artificial intelligence-based tools, such as AlphaFold, has transformed structural molecular biology by enabling the prediction of structures with a high degree of accuracy (Jumper et al., 2021). Protein prediction accuracy can be assessed by molecular dynamics (MD) simulations. MD thus constitutes a powerful computational resource for the refinement and validation of predicted models, allowing the investigation of structural stability, conformational flexibility, and dynamic behavior of proteins in a simulated aqueous environment (Araya et al., 2023; Varadi et al., 2024).

Therefore, the present study aims to perform the first high-quality *in silico* structural characterization of the *T. rangeli* GP63 metalloprotease. Through the three-dimensional prediction of the protein using AlphaFold, followed by detailed structural analyses and MD simulations, we sought to evaluate its stability and dynamic behavior (Garrido-Rodríguez et al., 2024). The results presented herein provide a solid structural basis for future functional investigations and for the identification of specific antigenic regions of *T. rangeli*, contributing to the advancement of knowledge regarding this protozoan.

2 Material and Methods

2.1 Acquisition of the TrGP63 sequence and primary analyses

The sequence of the TrGP63 surface glycoprotein was obtained from the UniProt database (accession number: A0A422MZ47). The region corresponding to the mature form of the protein was submitted to analysis by the ProtParam program (ExPASy), with the objective of determining physicochemical parameters, including theoretical molecular mass, isoelectric point, and amino acid composition. The prediction of the presence of a signal peptide was performed using SignalP 6.0 (Teufel et al., 2022), while the potential GPI-type anchoring site was identified using PredGPI (Pierleoni et al., 2008).

2.2 Comparative sequence analysis

The alignment between the TrGP63 sequence and its ortholog *Leishmania major* GP63 (LmGP63) was performed using the Clustal Omega software, available on the EMBL-EBI platform. Global identity and similarity indices were examined, as well as the conservation of catalytic and structural residues, with emphasis on the HEXXH motif and disulfide bridge-forming cysteine residues. The interpretation of structural conservation was based on previous studies on trypanosomatid metalloproteases (Chaudhuri; Chang, 1989; Yao et al., 2003; Steinkraus et al., 1993; Calixto et al., 2013).

2.3 Three-dimensional structural modeling

Considering the low sequence identity between TrGP63 and the crystallographic structures available in the Protein Data Bank (PDB), homology modeling was not considered adequate. Thus, the three-dimensional structure of TrGP63 was predicted using AlphaFold (Jumper et al., 2021), a tool based on deep neural networks capable of generating models with near-experimental precision. The obtained model was evaluated using the pTM (Predicted Template Modeling score) and pLDDT (Predicted Local Distance Difference Test) metrics, parameters employed to estimate the global and local reliability of structural prediction (Wróblewski; Kmiecik, 2024). The stereochemistry and geometric quality of the model were examined with the PROCHECK program (Laskowski et al., 1993), utilizing the distribution of φ and ψ torsion angles represented in the Ramachandran plot, in order to verify the conformational adequacy of the model.

2.4 Structural analysis and identification of the catalytic site

The three-dimensional architecture of TrGP63 was inspected using UCSF ChimeraX software, allowing the characterization of the N-terminal, central, and C-terminal subdomains. The catalytic site was identified based on structural comparison with LmGP63 (PDB ID: 1LML), evidencing the conserved HEXXH motif and the third histidine involved in Zn^{2+} ion coordination. Zinc coordination distances were determined from direct geometric measurements, while disulfide bridges were identified and constructed using the covalent bond analysis module of UCSF ChimeraX.

2.5 Molecular Dynamics Simulation

The conformational stability and structural flexibility of TrGP63 in aqueous solution were evaluated by MD simulations performed in the GROMACS 2025.3 software, using the GROMOS54a7 force field (Schmid et al., 2011). The system was solvated in a cubic box containing SPC model water molecules, maintaining a minimum distance of 10 Å between the protein and the box edges. Na^+ and Cl^- ions were added to neutralize the total charge of the system. The energy minimization process was conducted with 5,000 steps using the Steepest Descent method, followed by an equilibration step in two stages: (i) NVT ensemble for 100 ps, and (ii) NPT ensemble for 100 ps, both maintained at a temperature of 310 K. The production simulation was executed for 25 ns, with an integration step of 2 fs. The structural stability of the system was evaluated using RMSD (root-mean-square deviation), RMSF (root-mean-square fluctuation per residue), and Rg (radius of gyration) parameters, calculated with GROMACS internal tools.

2.6 Electrostatic analysis and structural comparison

The electrostatic potential of the TrGP63 molecular surface was calculated employing the APBS (Adaptive Poisson–Boltzmann Solver) program. The structural comparison between the three-dimensional models of TrGP63 and LmGP63 was performed in UCSF ChimeraX, and the superposition between the structures was evaluated by the RMSD, in order to estimate the degree of conformational similarity between both proteins.

3 Results and Discussion

3.1 Obtaining of the TrGP63 sequence and preliminary analyses

The initial characterization of TrGP63 was performed based on its primary sequence, whose analysis by the ProtParam program estimated an approximate molecular mass of 66 kDa. The prediction of signaling regions obtained with SignalP software (Teufel et al., 2022) indicated the segment between Met1 and Ala22 as the probable signal peptide, responsible for directing the protein to the secretory pathway (Fig. 1). Complementarily, PredGPI software (Pierleoni et al., 2008) predicted the residues downstream of Ser494 as removed for the anchoring of a glycosylphosphatidylinositol (GPI) group (Fig. 1). The concordance between these analyses indicates that TrGP63 exhibits the structural determinants typical of *T. rangeli* surface proteins.

MRHTPYVLLLLLLLCCFTGCFAATEHI**C**IFDRISRKAGPPLRAIVRELPDRERGGTQVLT
ASVSDWAPIRFKVFSEDMNNPSKYCTAAGDFRSDLLARTLFCRQQDFFTAEKKSIILNRM
LPEAMQLHIDRLHVKPEMRPVVVPFFSSGTPCGKFEIPSSHHTAGVYGADMVLYAAAAPI
EGTTLAWAVRCFELPDGRPVVGVINIGPHSVTDSEFSVRVAAHEIAHAIGFGTNVFEEKE
MIKTIPEVRGKKNVVVVSSPKTLEKTRAHFKCTSAPGMELEDEGGGGMVLSHWKRRNAKD
ELMAAINGAGYYTALTMAAFEDMGFFRAQWSMAEQMSWGSNSGCELLTEKCLTNGVTRYP
EMFCSSPRNLMKCASDRLALGRCKTTTFPNPLPATFQYFTNPRRGGLLGDLMDYCPYIRP
FRNTHCVYGDADVMRGSRIGPRSRCFKVDGLRDSFGFTGDVCAEVSCDNDTVSVRYLGDD
VWHACPEGKRITPSGRFRGGKIVCPRRIEVCPQLQKNGGDREDANKGTDGGAKTDPNLDV
THLHSVLFPLVSTAFFFVVAVGAVW

Figure 1. Primary sequence of TrGP63. Residues indicated in green correspond to the signal peptide. Amino acids in blue represent the propeptide, while the cysteine residue in bold marks the cysteine residue involved in the cysteine switch mechanism. Regions in red indicate segments cleaved during GPI anchor addition, and the catalytic site is composed of the amino acids highlighted in violet. Finally, residues in black represent the mature form of the protein, resulting from post-translational modifications.

Source: the authors.

The alignment of the TrGP63 amino acid sequence with its ortholog in *L. major* (LmGP63) revealed, in the N-terminal region, a conserved domain corresponding to the propeptide (Ala23–Ser64). This domain contains the cysteine residue Cys28 (Fig. 1), fundamental for the cysteine switch mechanism, responsible for modulating catalytic activity. This process prevents the newly synthesized enzyme from exhibiting proteolytic activity on intracellular components of the parasite, ensuring that its activation occurs only after appropriate proteolytic processing (Xu et al., 2017).

3.2 Conservation of GP63 sequences

Comparative studies described in the literature demonstrate that GP63s from different *Leishmania* species maintain a high degree of structural and functional conservation (Berná et al., 2025). A notable exception is observed in *Leishmania guyanensis*, whose predicted sequence presents an absence of the five cysteine residues located in the C-terminal portion. To date, there is no experimental evidence elucidating the effects of this

variation on structural stability or on the enzyme's catalytic activity (Steinkraus et al., 1993). Considering this information, a global alignment involving TrGP63 and LmGP63 sequences was performed (Fig. 2). The alignment revealed broad conservation along the entire sequence, including residues composing the catalytic HEXXH motif and the third histidine involved in active site coordination. Generally, about 40% of residues remain identical among the analyzed proteins, highlighting the conservation of 18 cysteines, 19 glycines, and 12 prolines, amino acids that play an essential role in maintaining conformational integrity and enzyme stability.

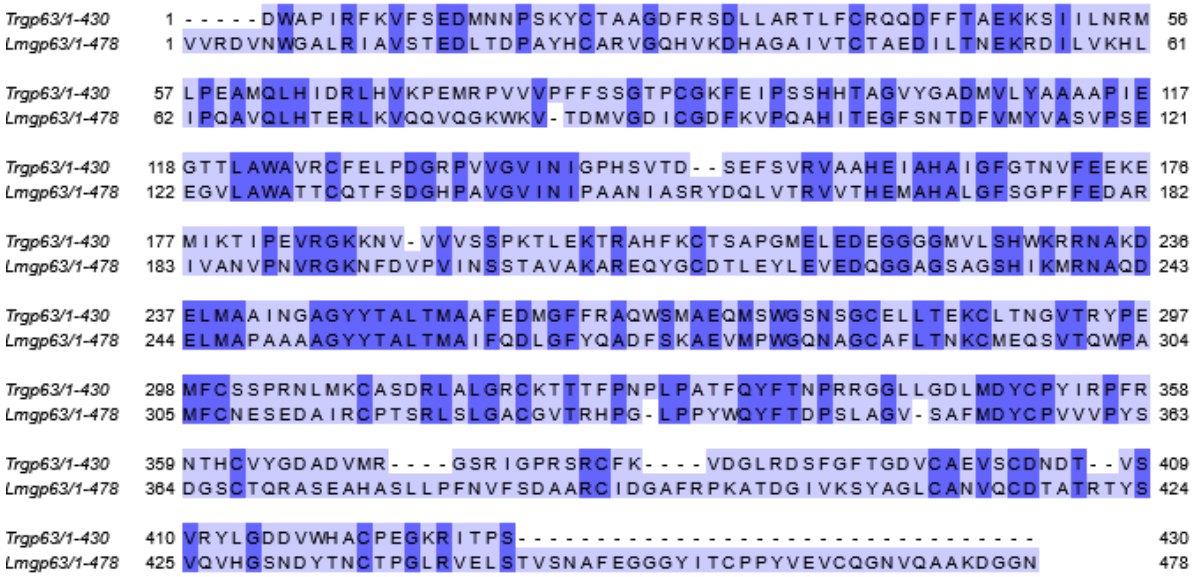

Figure 2. Global alignment of TrGP63 and LmGP63. The displayed sequences correspond to the mature form of the proteins. Residues were colored according to identity (identical residues in blue), allowing better visualization of the degree of conservation between sequences.

Source: the authors.

3.3 Optimization and validation of the model

The search for structures homologous to TrGP63 was conducted using BLAST (Basic Local Alignment Search Tool) and the Protein Data Bank (PDB). Only one homologous structure was identified, LmGP63 (PDB ID: 1LML), presenting identity and similarity of 38% and 53%, respectively. The low level of identity between sequences rendered comparative modeling unsuitable for constructing the three-dimensional model. Thus, the 3D model of TrGP63 was generated using AlphaFold, which employs deep neural networks for structural prediction. The obtained structure presented a Predicted Template-Modeling score (pTM) value of 0.95. The pTM is a global confidence metric used by AlphaFold to estimate model accuracy, ranging from 0 (low reliability) to 1 (high reliability) (Meier; Söding, 2015). The pLDDT (Predicted Local Distance Difference Test) value was greater than 90, reinforcing the high quality and reliability of the predicted structure (Mariani et al., 2013). Stereochemical evaluation was conducted using the Procheck software (Laskowski et al., 1993), which presented satisfactory results, with no residues located in unfavorable regions (Fig. 3).

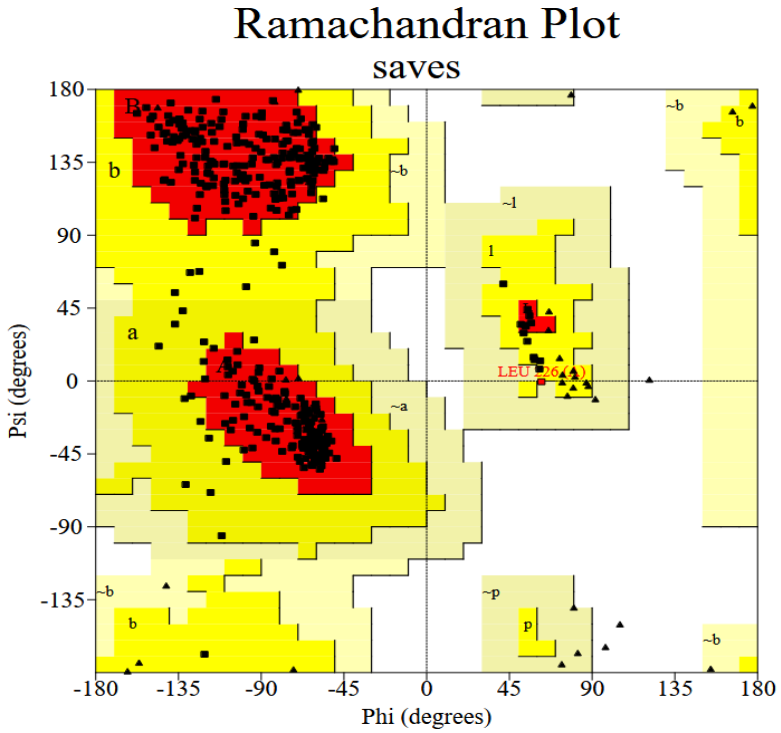

Figure 3. Ramachandran Plot. Residues in most favorable regions (A,B,L): 335 (91.3%); residues in additionally allowed regions (a,b,l,p): 31 (8.4%); residues in generously allowed regions (~a,~b,~l,~p): 1 (0.3%); residues in disallowed regions: 0 (0%).

Source: the authors.

3.4 General structure of TrGP63

The TrGP63 protein presents a compact three-dimensional conformation, organized into three main subdomains: N-terminal, central, and C-terminal (Fig. 4). The N-terminal subdomain, comprised between residues Asp1 and Phe167, exhibits an architecture compatible with the typical catalytic module of zinc-dependent proteases. Located in this region are the residues comprising the catalytic HEXXH motif, His159, Glu160, and His163, corresponding to the conserved motif characteristic of metalloproteinases (Di Leo et al., 2023).

The central subdomain, comprised between residues Gly168 and Glu285, exhibits a compact core formed by α-helices and β-strands organized in an antiparallel manner. This portion of the protein connects to the C-terminal subdomain via a single disulfide bridge. In metzincins, the extended catalytic motif HEXXHXXGXXH contains a glycine residue inserted into a structural loop that functions to position the third histidine residue in the active site, enabling its coordination to the zinc atom. In TrGP63, however, there is an insertion of 61 residues between Gly166 and His228, the latter also being involved in metal coordination, a characteristic conserved among all known leishmanolysins (Schlagenhauf et al., 1998; Razzazan et al., 2008; Calixto et al., 2013). The organization of residues interacting with the zinc ion follows the same pattern observed in other proteases of the same family. The metal ion is coordinated to the side chains of histidines 159, 163, and 228, with distances of 2.4 Å, 2.4 Å, and 1.52 Å, respectively (Fig. 5).

Furthermore, a water molecule also integrates the catalytic site, acting as a nucleophilic agent in the attack on the substrate during catalysis.

Figure 4. Three-dimensional structure of TrGP63 obtained using AlphaFold. Representation of the tertiary conformation of the protein, highlighting the N-terminal, Central, and C-terminal subdomains, indicated by red, blue, and green colors, respectively.

Source: the authors.

The C-terminal subdomain, comprised between residues Lys286 and Asn430 (Fig. 4), gathers six of the nine disulfide bridges identified in the molecule (Fig. 6), which confers high structural stability. The residues located at the extreme of this region form a loop corresponding to the segment destined for glycosylphosphatidylinositol (GPI) anchoring.

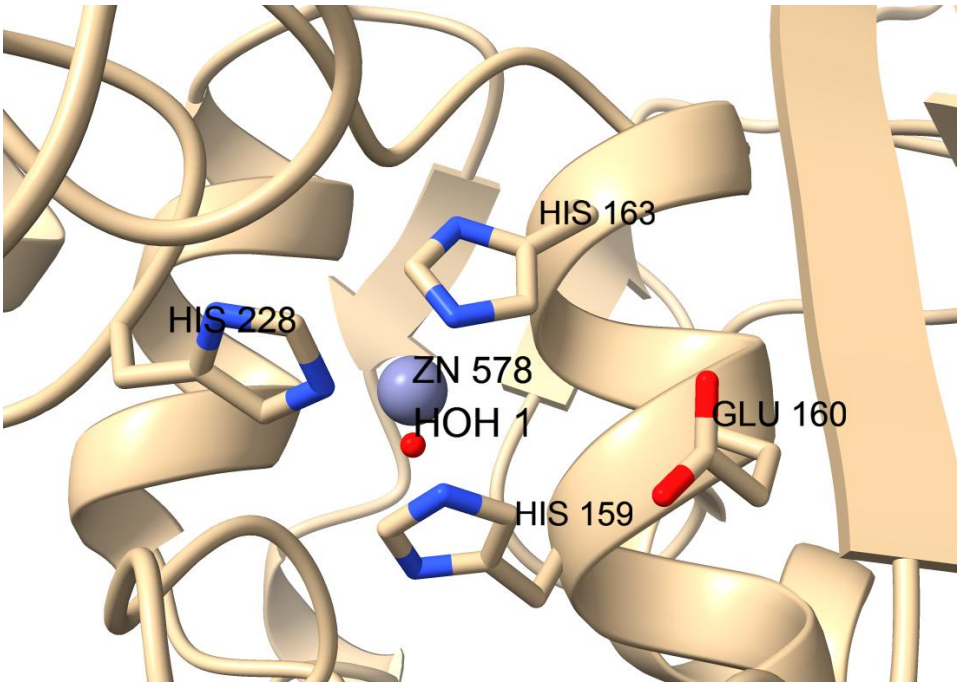

Figure 5. Catalytic center. Illustration of the TrGP63 active site, showing the three histidine residues and the catalytic water molecule coordinated to the zinc ion. The glutamate residue plays a fundamental role in polarizing the water molecule, favoring the nucleophilic attack that occurs during catalysis. This structural arrangement is typical of zinc-dependent metalloproteases belonging to the metzincin family and is indispensable for peptide bond cleavage and maintenance of the enzyme's hydrolytic activity.

Source: the authors.

3.5 Structural conservation

With the aim of evaluating the degree of structural conservation between TrGP63 and LmGP63, a direct comparison between the three-dimensional structures was conducted (Fig. 7). The resulting alignment revealed high similarity between the proteins, especially in the N-terminal and central subdomains, which exhibited robust structural correspondence. In contrast, the C-terminal subdomain presented lower conservation, an expected result, given that differences in this region have been widely reported among diverse GP63s, including between isoforms belonging to the same species (Hernández-Ramírez et al., 2024).

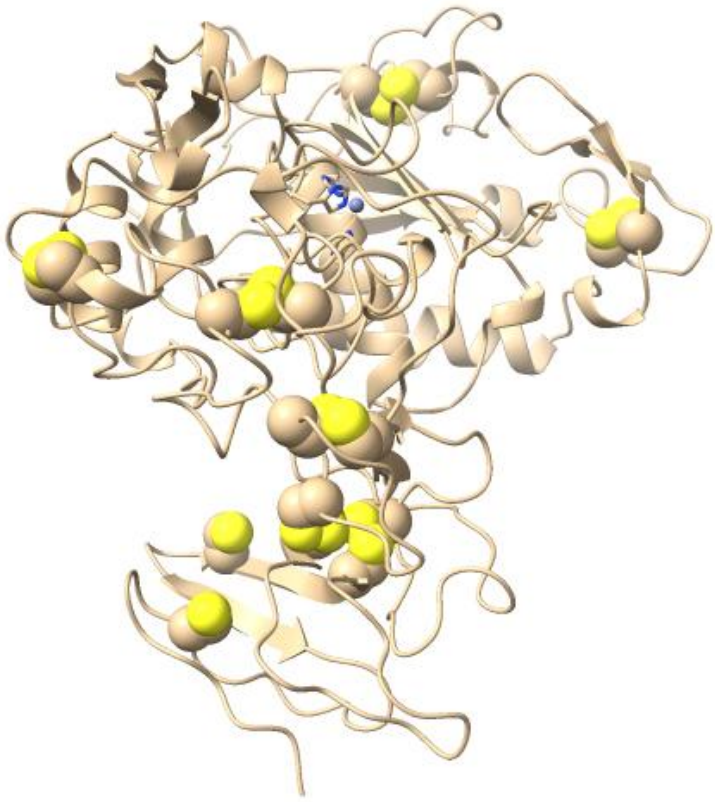

Figure 6. Three-dimensional structure of TrGP63. Yellow spheres indicate cysteine residues responsible for disulfide bridge formation. Among the nine identified bridges, six are concentrated in the C-terminal domain, suggesting greater stability and structural rigidity in this portion of the protein. Source: the authors.

The RMSD value obtained between TrGP63 and LmGP63 structures was 0.942 Å, evidencing strong conformational similarity between them. Considering that enzyme-substrate affinity is determined not only by active site conformation but also by electrostatic complementarity at the interaction interface (Grassmann et al., 2023), analysis of surface charge distribution was performed (Fig. 8).

The analysis of electrostatic potential revealed a predominance of neutral charges around the area corresponding to the catalytic cleft of TrGP63, attributed to the presence of uncharged residues. This charge distribution pattern differs from that observed in LmGP63 (Schlagenhauf; Etges; Metcalf, 1998), indicating that both enzymes likely do not share affinity for similar substrates in vertebrate and invertebrate hosts.

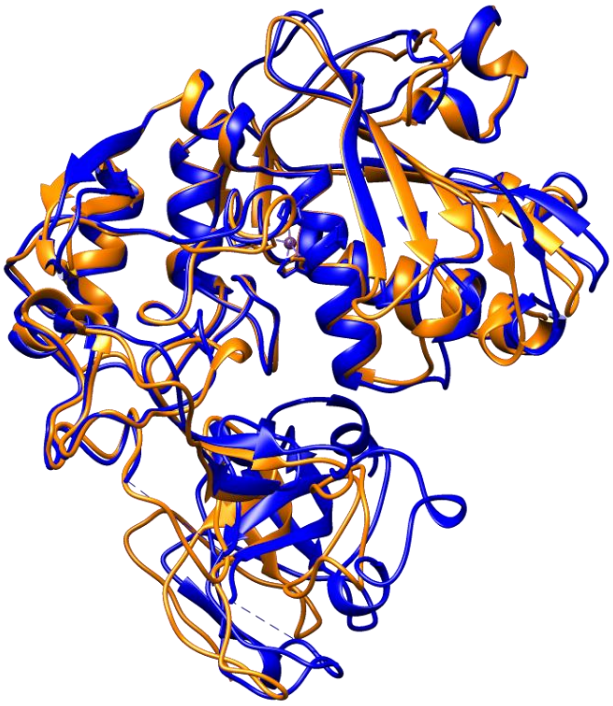

Figure 7. Structural alignment. Structures represented in blue and orange correspond to LmGP63 and TrGP63 proteins, respectively. The alignment revealed an RMSD value of 0.942 Å, indicating a high degree of structural conservation.

Source: the authors.

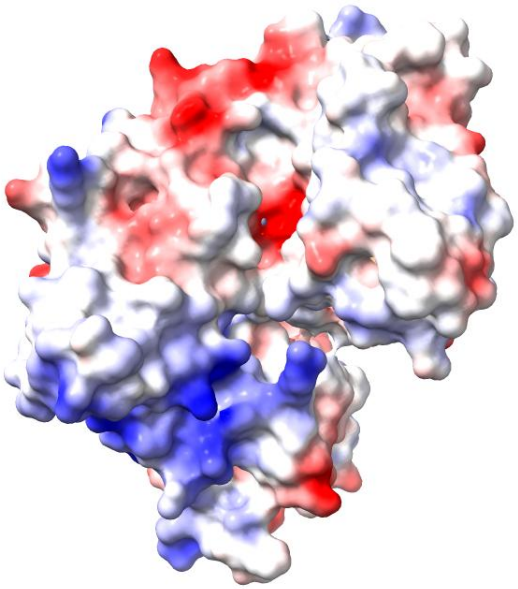

Figure 8. Surface charge distribution of TrGP63. Regions with positive, negative, and neutral charges are represented in blue, red, and white, respectively. Source: the authors**.**

3.6 Analysis of TrGP63 structural stability by molecular dynamics

The structural stability of TrGP63 over the 25 ns simulation was evaluated through analysis of Rg, RMSD, and RMSF. Together, these parameters provided an integrated view of the metalloprotease's conformational behavior during the dynamic regime. The Rg profile remained mostly stable throughout the simulation, varying between approximately 2.35 and 2.60 nm (Fig. 9). An initial increase up to ~5 ns was observed, followed by stabilization between 2.45–2.50 nm, suggesting that the protein reaches a relatively consistent compacted state after initial structural relaxation events. A discrete increase between 20–23 ns indicates a possible late conformational rearrangement, which does not compromise overall stability but may reflect adjustments in flexible or solvent-exposed regions.

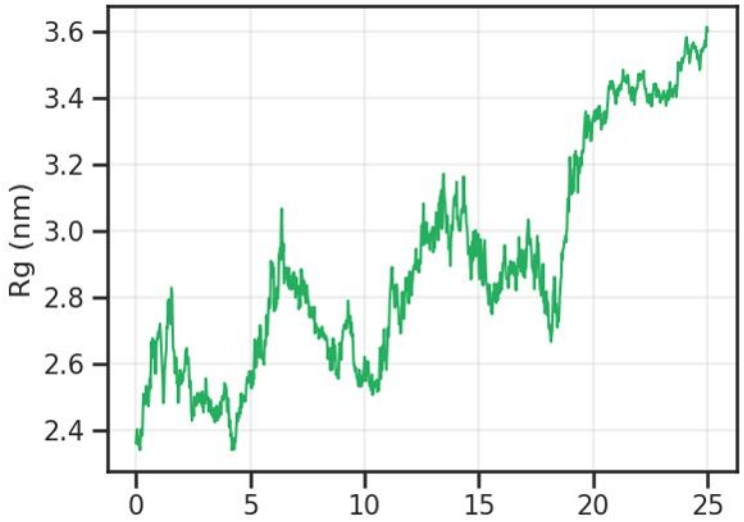

Figure 9. Temporal variation of the radius of gyration of TrGP63 during the 25 ns simulation, indicating the global compaction behavior of the structure. Stabilization is observed after the first nanoseconds, suggesting maintenance of conformational integrity throughout the dynamic regime.

Source: the authors.

Rg values in this range are compatible with surface metalloproteases containing globular catalytic domains and more flexible C-terminal regions, as previously observed for GP63 homologous isoforms in *Leishmania* and *Trypanosoma* (Camargo et al., 2025). The RMSD presented a progressive increase up to about 6 ns, subsequently stabilizing between 0.55–0.75 nm (Fig. 10). This behavior is typical of proteins undergoing an initial accommodation phase after immersion in a solvated environment, subsequently reaching a metastable state. The absence of abrupt variations after ~10 ns reinforces that the protein did not undergo significant global structural deviations, maintaining the three-dimensional framework compatible with that predicted for M8 family metalloproteases. RMSD values within this range are expected for multi-domain metalloprotease simulations and have been reported in comparative studies involving *Leishmania* spp. GP63 (Mercado-Camargo et al., 2020).

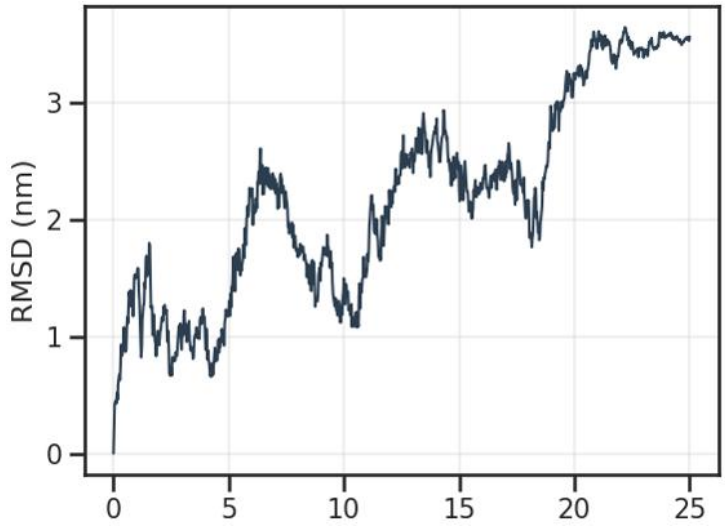

Figure 10. Backbone RMSD of TrGP63. The initial increase followed by stabilization indicates the process of structural accommodation and subsequent conformational equilibrium during molecular dynamics.

Source: the authors.

The RMSF analysis revealed low fluctuation for most of the polypeptide chain (0.15–0.30 nm), indicating a high degree of rigidity in the central structural domains, where the Zn^{2+}-dependent catalytic site is located (Fig. 11). As expected, the greatest fluctuations were observed in the C-terminal region (residues >350), reaching up to 0.6 nm. This region is described, in homologous metalloproteases, as more flexible and frequently associated with anchoring functions and/or membrane interaction, and may present partial structural disorder (Bianchini et al., 2006).

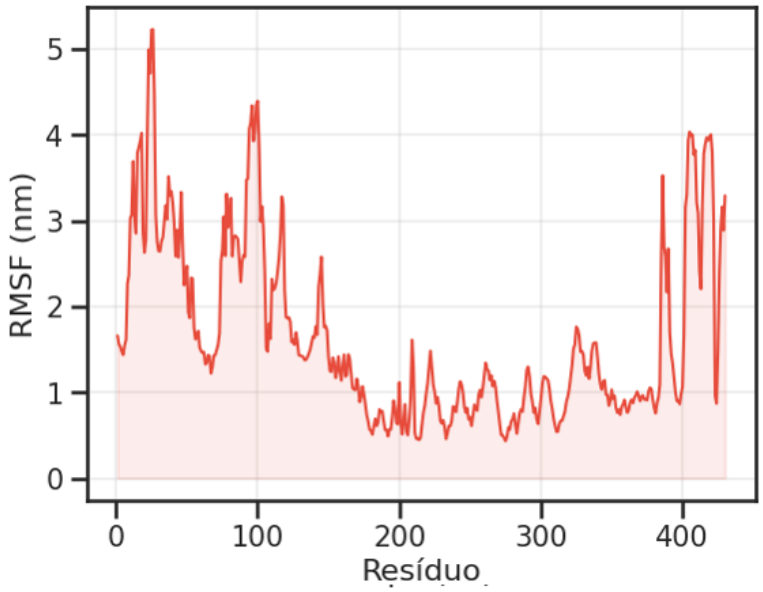

Figure 11. RMSF of TrGP63 residues, evidencing rigid regions in the central domain and greater flexibility in the C-terminal. Fluctuation peaks correspond to exposed loops and intrinsically dynamic structural segments.

Source: the authors.

Furthermore, small fluctuation peaks distributed along the sequence may reflect surface loops, essential for substrate recognition dynamics and conformational plasticity, characteristics already attributed to the GP63 mechanism of action in trypanosomatids (Mousavi et al., 2025).

4 Conclusion

This study established the first in silico structural model of the TrGP63 metalloprotease, filling a critical gap in the characterization of *T. rangeli* antigens. The structure, generated by AlphaFold, demonstrated high accuracy and stereochemical quality. Subsequent validation by MD confirmed the conformational stability of the model, with the system reaching rapid conformational equilibrium (RMSD ~0.55–0.75 nm) and maintaining its compactness (Rg ~2.45–2.50 nm). The RMSF analysis corroborated the rigidity of the central catalytic domains and the expected high flexibility of the C-terminal region, associated with the GPI anchor.

However, the central reflection of this work emerges from the identified divergences. The analysis of electrostatic potential exposed a predominantly neutral catalytic cleft in TrGP63, distinct from its homolog, suggesting a probable divergence in substrate specificity and host interaction. This distinction, combined with the lower conservation of the C-terminal subdomain, represents the fundamental advance of the study. These regions of structural and electrostatic divergence are rational and primary targets for in silico prospecting and the design of species-specific epitopes, paving the way for the development of recombinant antigens capable of, finally, providing an accurate differential diagnosis for Chagas Disease or providing a deeper understanding of disease pathogenesis.

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