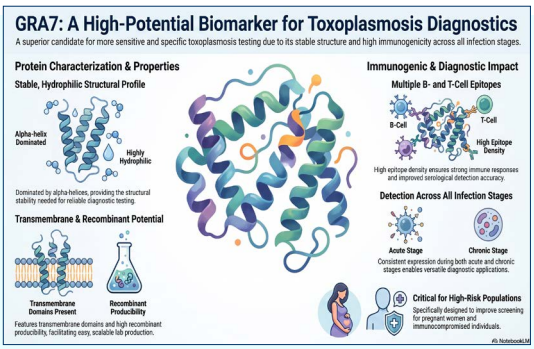
http://ijmpes.com
doi:10.34172/ijmpes.6282
Open Access
Original Article
APMI Press
Aras Part Medical International Press
A scientific publishing imprint of PMC Pharmaceutique France
Int J Med Parasitol Epidemiol Sci 2026;7(3): 171-182
eISSN 2766-6492
International Journal of Medical Parasitology & Epidemiology Sciences

In Silico Analysis and Immunogenicity Assessment of the *Toxoplasma gondii* Dense Granule Antigen 7 (GRA7)

Siti Fadilla[1,2], Muhammad Luqman Habibi[1], Nuning Winaris[3,4], and Loeki Enggar Fitri[3,4*]

[1]Master Program in Biomedical Science, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia
[2]Medical Doctor Profession Education, Faculty of Medical and Health Science, Maulana Malik Ibrahim State Islamic University, Malang, Indonesia
[3]Department of Clinical Parasitology, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia
[4]AIDS, Toxoplasmosis, Opportunistic Disease, and Malaria Research Group, Faculty of Medicine, Universitas Brawijaya, Malang, East Java, Indonesia"

Abstract



Introduction: *Toxoplasma gondii* is a parasite of the protozoan type and is intracellular. These parasites can cause the occurrence of toxoplasmosis in infected individuals. These protozoa are zoonotic and have a global prevalence. The method of enforcing diagnosis in this parasite serologically, molecularly, and microscopically has limitations, namely in the values of sensitivity, specificity, and feasibility. Dense Granule Antigen 7 (GRA 7), which is a protein secreted by *T. gondii* during the infection stage in the acute and chronic phases, can elicit a strong immune response, so GRA 7 can be used as a biomarker and is very promising.

Methods: This study used a method in the form of in silico. This method was performed to characterize the physiochemical properties, secondary and tertiary structures, post-translational modification, and immunogenic epitopes of GRA7.

Results: In silico analysis found that GRA7 is hydrophilic. In terms of structural stability, GRA 7 is dominated by alpha-helix. Furthermore, GRA7 contains a transmembrane domain and several predicted epitope regions that may be recognized by B cells and T cells. All of these findings support that GRA7 has the potential to be a potent antigen. In addition, the recombinant productivity of GRA7 increases its application as a diagnostic and on a wide scale.

Conclusions: Overall, GRA7 shows strong potential as a reliable antigen for serological diagnosis of toxoplasmosis due to its high sensitivity and specificity, especially among vulnerable groups such as pregnant women and individuals with weakened immune systems.

Keywords: *Toxoplasma gondii*, GRA7, In Silico Analysis, Serological Diagnosis.

Received: June 17, 2026, **Revised:** July 23, 2026, **Accepted:** July 30, 2026, **ePublished:** September 10, 2026

Introduction

Toxoplasma gondii (*T. gondii*) is an obligate intracellular protozoan parasite and the main cause of toxoplasmosis, a zoonotic disease that is estimated to infect nearly one-third of people worldwide, with prevalence rates differing among geographic regions (1). Humans can be infected by this parasite through several main pathways, transmission can occur through several routes, including consuming raw or undercooked meat containing tissue cysts, ingestion of eggs or vegetables contaminated with oocysts, or vertical transfer from an infected mother to the fetus through the placenta (2). In its life cycle, *T. gondii* manifests in three different biological forms, namely takizoites that are invasive and trigger acute infections, bradyzoites that settle inside tissue cysts, and sporozoites in oocysts that are released with cat feces (2).

To detect *T. gondii* infection, the medical world applies serological, molecular, and microscopic methods, each of which has its own advantages and limitations. The most commonly used method in the laboratory is serological testing, specifically the ELISA technique, to detect the presence of IgM and IgG antibodies. Although this method is highly sensitive and effective for initial screening, the interpretation of the results is often challenging because IgM antibodies may remain detectable in the body for several months, making it difficult for doctors to distinguish between a recent infection and a past infection (3,4). Meanwhile, the presence of IgG antibodies only indicates past exposure without being able to confirm an active infection. To overcome this constraint, IgG avidity testing is sometimes performed to help estimate the time of infection, although its reliability will decrease if the

*Corresponding Author: Loeki Enggar Fitri, Email: lukief@ub.ac.id

test is done too early (5). As a more specific alternative, molecular approaches, particularly Polymerase Chain Reaction (PCR), are used to identify *T. gondii* DNA in various biological samples, including blood, cerebrospinal fluid, and amniotic fluid. This PCR technique is very valuable for early diagnosis because it has high specificity, especially for patients with immune system disorders (immunocompromise) and in cases of suspected congenital toxoplasmosis (6,7). However, the PCR method still has its own drawbacks, such as the possibility of producing false-negative results if the number or load of parasites in the sample is too low, and requires the support of special and sophisticated laboratory facilities (8).

Microscopic examination of tissue samples was actually able to detect the presence of *T. gondii* directly. However, this method is rarely applied in routine clinical procedures due to its invasive nature, low level of sensitivity, and lack of direct therapeutic benefits to patients. In addition to these technical constraints, the application of this method is also limited by strict ethical regulations, such as the need to obtain approval from the Institutional Review Board (IRB), the existence of informed consent, and the obligation to reduce risks that can be accepted by patients, except in certain high-risk medical situations (9).

Given the high rate of spread of toxoplasmosis globally and the adverse impact it can cause, innovation in creating more practical and accurate diagnostic instruments is needed. One potential breakthrough is to utilize specific antigens as *T. gondii* biomarkers. Dense Granule Antigen 7 (GRA7), a protein excreted by the parasite, shows much more promising advantages over other antigens for immunological and diagnostic needs as biomarkers. GRA7 has very strong immunogenic properties because it can stimulate both humoral and cellular immune responses. This makes it an ideal modality for serology-based detection. Unlike other antigens that only appear in certain life cycles, GRA7 is produced consistently in both the tachyzoite (acute) and bradyzoite (chronic) phases, making it reliable at various stages of infection. In addition, GRA7 has the potential to be a vaccine candidate because it has been shown to trigger a protective immune response in experimental animals, as well as being active in host-pathogen interactions, including modulating immune signaling pathways such as NF-κB and activating inflammasomes. This combination of unique characteristics is what positions GRA7 as the flagship protein for the development of diagnostic tools as well as future toxoplasmosis therapy (10–12).

Dense Granule proteins, particularly GRA7, are not yet commercially available as diagnostic kits for toxoplasmosis, although GRA7 accumulates in the parasitophorous vacuole during tachyzoite infection of host cells. In contrast, when the intracellular parasite is in the bradyzoite form, GRA7 can be found in the cytoplasm of infected cells. This characteristic is highly valuable for the recognition of Tc/CD8+ cells (cytotoxic T cells), as GRA7 is processed and displayed through the MHC class I antigen presentation pathways (13). GRA proteins are abundantly secreted in infected patients, making them detectable in blood samples during the early stages of infection. Therefore, GRA7 holds great potential as a target protein for detecting toxoplasmosis infection (14).

The development of bioinformatics technology today has opened up a new paradigm in infectious disease research, including in the treatment of toxoplasmosis. The use of in silico analysis tools, computer-based modeling, and biological prediction simulations now allow researchers to identify and map the characteristics of potential antigens targeted as diagnostic targets. Considering the widespread occurrence of *T. gondii* infection worldwide and the weaknesses of current conventional diagnostic methods, an in-depth exploration of GRA7 as a serological biomarker is very urgent in order to produce a more sensitive, precise, and valid detection tool. Through this bioinformatics approach, antigen validation processes such as GRA7 can be carried out more innovatively and efficiently before being implemented in the clinical realm. Therefore, this study focuses on the in silico method to thoroughly explore the biophysical features as well as the configuration of local and three-dimensional protein structures, post-translational modification, and mapping of immunogenic epitopes possessed by the GRA7 protein.

Materials and Methods

The methodology applied in this study refers to Karimipour-Saryazdi et al (2024). In order to ensure the relevance and accuracy of the analysis, several forms of modification and specific adjustments were deliberately applied so that all stages of work continued to run in accordance with the main objectives of this study (15).

Amino Acid Sequence

The TgGRA7 protein sequence was obtained by accessing amino acid data available in the National Center for Biotechnology Information (NCBI) database. The data is downloaded in the form of a FASTA format and refers to the specific accession number ABE69202. The sequence was accessed through the official website of the NCBI (https://www.ncbi.nlm.nih.gov) (16).

Physicochemical Characteristics

The molecular and chemical characteristics of the TgGRA7 protein were assessed with the aid of ExPASy ProtParam (https://web.expasy.org/protparam/) (17). Through this platform, a series of structural and biochemical parameters are evaluated. The evaluation was carried out including the calculation of the total the total number of amino acids, estimated molecular mass, and predicted isoelectric point (pI). Additionally, several calculations were performed to determine the predicted protein half-life, the overall count of residues carrying positive and negative charges, the predicted protein stability score, light absorption coefficient, aliphatic residue index, and grand average hydropathicity value (GRAVY) score in order to understand the fundamental properties of the protein.

Post-Translational Modification Sites

Possible post-translational modification regions in the TgGRA7 protein were identified using two platforms, namely the NetAcet 1.0 and NetPhos 3.1 servers. The NetAcet 1.0 (https://services.healthtech.dtu.dk/services/NetAcet-1.0/) device was used to map the possible presence of N-acetylation sites in protein structures (18). The process of tracking potential areas for phosphorylation processes involving serine, threonine, and tyrosine amino acid residues was assessed through the NetPhos 3.1 prediction platform (https://services.healthtech.dtu.dk/services/NetPhos-3.1/) (19).

Prediction Methods for Transmembrane Domain, Signal Peptide, and Subcellular Localization

The membrane-spanning segments of TgGRA7 were identified through the TMHMM v.2.0 prediction platform (https://services.healthtech.dtu.dk/services/TMHMM-2.0/) (20). To examine whether TgGRA7 contains an N-terminal signal peptide and to determine the possible cleavage position, the sequence was further analyzed using SignalP 6.0. The probable intracellular location of the protein was subsequently assessed with PSORT II (https://psort.hgc.jp/form2.html) (21).

Secondary and Tertiary Structure Prediction

The local structural elements of TgGRA7 were examined through the GOR4 web-based prediction tool (https://npsa.lyon.inserm.fr/cgi-bin/npsa_automat.pl?page = /NPSA/npsa_gor4.html) (22). For three-dimensional modeling, the protein sequence was submitted to SWISS-MODEL (https://swissmodel.expasy.org/) (23), where its structure was generated using a homology-based approach according to sequence similarity with available templates. The reliability of the obtained 3D model was then checked using a Ramachandran plot generated by PROCHECK through the SAVES v6.0 server (https://saves.mbi.ucla.edu/) (24). Moreover, the three-dimensional structure prediction and the structural flexibility prediction of TgGRA7 were carried out based on the AI platform AlphaFold2 (https://alphafold.ebi.ac.uk/) by calculating its local accuracy and model confidence score using per-residue pLDDT score (25).

B-cell Epitope and Antigenicity Prediction

Potential linear B-cell epitope regions in TgGRA7 were identified by submitting its amino acid sequence to the BcePred web server (http://crdd.osdd.net/raghava/bcepred/) (26). The analysis used multiple protein characteristics as prediction parameters, including residue flexibility, water affinity, surface accessibility, turn-forming tendency, exposed regions, polarity, and antigenicity potential. Furthermore, antigenicity was predicted using IEDB (https://tools.iedb.org/main/) (27) and ANTIGENPro (https://scratch.proteomics.igb.uci.edu/) (28) to evaluate potential antigenic regions and identify peptide segments that may contribute to the immunogenic properties of the TgGRA7 protein.

Protein–Protein Interaction Network Analysis

The network of predicted protein interactions involving TgGRA7 was evaluated to determine its possible functional relationships with other Toxoplasma gondii proteins. The network was constructed using the STRING database, where *Toxoplasma gondii* was designated as the target organism (29). During the process, the TgGRA7 protein is positioned as the main input to predict various potential interactions based on the functional association data that has been recorded. Once the interaction data was obtained, visual mapping as well as network topology analysis were executed using the Cytoscape 3.0 software (30). This analysis process aims to map the interaction profile of TgGRA7, including in calculating the degree value and identifying the centrality position of the protein in the predicted network architecture.Through the integration of the two platforms, the connectivity patterns between TgGRA7 and other functional proteins can be observed and understood. This makes it easier to identify the strategic role of TgGRA7 as a hub/key node in the parasite's biological network. The entire series of modeling processes, starting from the formation of an initial STRING-based network for *T. gondii* organisms to the final visualization through Cytoscape 3.0, can be observed.

Results

GRA7 Protein Basic Analysis

The FASTA-formatted amino acid sequence of GRA7 was obtained from the NCBI database with the accession ID ABE69202.1. Bioinformatic analysis showed that this protein consists of 236 amino acid residues, has an estimated molecular mass of 25,944.89 Da, and possesses a theoretical isoelectric point of 5.33. The sequence includes 30 basic residues, namely arginine and lysine, as well as 39 acidic residues, represented by aspartic acid and glutamic acid. GRA7 is predicted to contain 3,614 atoms, with an extinction coefficient of 4.47 $M^{-1}cm^{-1}$ in water at 280 nm. Its estimated half-life varies across biological systems, reaching approximately 30 hours in mammalian reticulocytes under in vitro conditions, exceeding 20 hours in yeast cells, and lasting more than 10 hours in E. coli. Based on ExPASy ProtParam evaluation, GRA7 falls into the unstable protein category due to its instability index value of 49.97. In addition, the aliphatic index of 71.19 reflects moderate heat stability, whereas the GRAVY score of −0.594 indicates that GRA7 tends to be hydrophilic.

Prediction of Post-Translational Modification Sites in GRA7

Post-translational modification sites in the GRA7 protein were predicted using the web tools NetAcet-1.0 (DTU Health Tech, 2005) and NetPhos 3.1 (DTU Health Tech, 1999), respectively. Analysis using NetAcet 1.0 identified a single possible acetylation region in GRA7. In contrast, NetPhos 3.1 revealed 34 predicted phosphorylation positions, including 3 tyrosine, 14 threonine, and 17 serine residues (**Figure 1**).

```
      MARHAIFFALCVLGLVAAALPQFATAATASDDELMSRIRNSDFFDGQAPV   #     50
      DSLRPTNAGVDSKGTDDHLTTSMDKASVESQLPRREPLETEPDEQEEVHF   #    100
      RKRGVGSDAEVTDDHIYEEHTDRKVVPRKSEGKRSFKDLLKKLALPAVGM   #    150
      GASYFAADRILPELTEQQQTGDEPLSTGQNVSTVIGFAALAAAVAFLGLG   #    200
      IKRTYRHFSPRKNRSRQPAPEHEVPESGEDREDARQ                 #    250
%1    ............................S.....S....S.........   #     50
%1    .....T.....S........TS....S..S.........T..........   #    100
%1    ......S....T....Y...T........S....S................   #    150
%1    ...................T.....S......T..................   #    200
%1    ...T....S.....S...........S.........
```

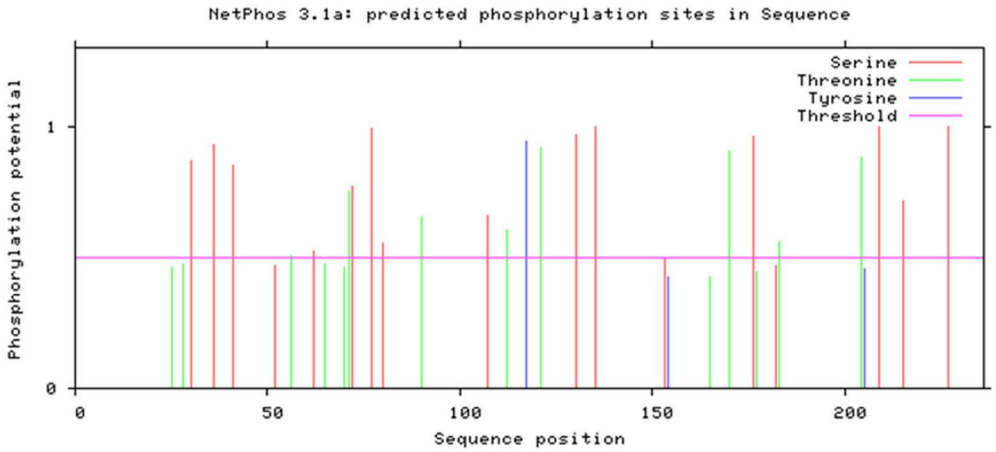


Figure 1. Computational assessment of predicted phosphorylation regions in GRA7 (a) The protein amino acid sequence is 250 residues long, displayed in blocks of 50 residues. Below the sequence, there are five lines labeled %1, which show the predicted potential phosphorylation sites. In the %1 lines, the symbols have the following meanings: (S) Serine, (T) Threonine, (Y) Tyrosine are predicted to be phosphorylated. (.) Indicates no phosphorylation is predicted at that position. (b) The horizontal axis represents the location of amino acid residues across the protein sequence. The Y-axis shows the phosphorylation potential, ranging from 0 to 1. The horizontal purple line represents the phosphorylation prediction threshold. Only predictions with potentials above this line are considered positive (valid).

Transmembrane Domain, Signal Peptide, and Subcellular Localization

The TMHMM v.2.0 analysis provided by the Technical University of Denmark showed that showed that the GRA7 sequence contains two transmembrane domains (Figure 2). In addition, PSORT II predicted the subcellular localization of GRA7, with 13% assigned to the Golgi apparatus, 17.4% endoplasmic reticulum, 34.8% extracellular, including cell wall, and 34,8% plasma membrane.

Based on the TMHMM analysis, the GRA7 protein is predicted to contain two transmembrane helices, indicating its potential association with cellular membranes. In addition, the prediction of a possible N-terminal signal sequence suggests that GRA7 may undergo targeting through the secretory pathway. Subsequent evaluation using SignalP was performed to confirm whether the protein contains a signal peptide and to determine the possible cleavage position within its N-terminal region **(Figure 3).**

Prediction of the Secondary and Tertiary Structures

The GOR4 web-based tool was used to evaluate the secondary structure profile of GRA7. This analysis identified the dominant structural components of the protein, such as alpha-helical regions, random coil segments, and extended strand formations. The GOR4 results showed that the GRA7 sequence consisted of 40.25% alpha-helix (95/236), 51.69% random coil (122/236), and 8.05% extended strand (19/236), respectively (**Figure 4**).

```
# WEBSEQUENCE Length: 236
# WEBSEQUENCE Number of predicted TMHs:  2
# WEBSEQUENCE Exp number of AAs in TMHs: 44.63309
# WEBSEQUENCE Exp number, first 60 AAs:  22.08916
# WEBSEQUENCE Total prob of N-in:        0.89520
# WEBSEQUENCE POSSIBLE N-term signal sequence
WEBSEQUENCE     TMHMM2.0        inside       1     4
WEBSEQUENCE     TMHMM2.0        TMhelix      5    27
WEBSEQUENCE     TMHMM2.0        outside     28   178
WEBSEQUENCE     TMHMM2.0        TMhelix    179   201
WEBSEQUENCE     TMHMM2.0        inside     202   236
```

Figure 2. Analysis of the transmembrane domain of GRA7. The protein sequence is 236 amino acids long. The predicted number of transmembrane helices is 2. The average number of amino acids expected to be located within transmembrane helices is 44.63. The average number of amino acids in transmembrane helices located within the first 60 amino acids is 22.09. The total probability that the N-terminal end is located inside the cell is 89.5%.

3D Model Forecast and Assessment

The prediction of the 3D structure of GRA7

The three-dimensional conformation of GRA7 was constructed through the SWISS-MODEL platform. The modeling process produced two structural candidates, and the model showing the greatest sequence similarity was chosen for further interpretation. The selected model demonstrated 92.8% sequence identity and complete coverage of the GRA7 sequence, spanning residues 1 to 236. The SWISS-MODEL result, including the alignment between the target sequence and template as well as the generated 3D protein structure, is shown in Figure 5a. A separate structural prediction was also performed using

AlphaFold, as illustrated in Figure 5b. The reliability of the AlphaFold-derived model was then examined using the predicted Local Distance Difference Test (pLDDT) score, with this metric having a range from 0 to 100. It is evident that high pLDDT scores can be observed for the core domains of the structure (pLDDT > 70, colored blue), which is consistent with the stable alpha helices detected by the GOR4 and SWISS-MODEL algorithms. On the other hand, low to very low pLDDT scores are seen within the N-terminal and C-terminal parts, as well as in the loop regions (pLDDT < 70; pLDDT < 50, respectively, colored yellow, orange, and red). According to the AlphaFold protocol, such low pLDDT scores are strongly correlated with intrinsically disordered regions or segments that exhibit high structural flexibility. From the standpoint of the immunobiology of the target protein, such flexible regions are extremely beneficial since they significantly increase the presentation of dynamic epitopes, rendering the protein highly accessible to the host's immune response.

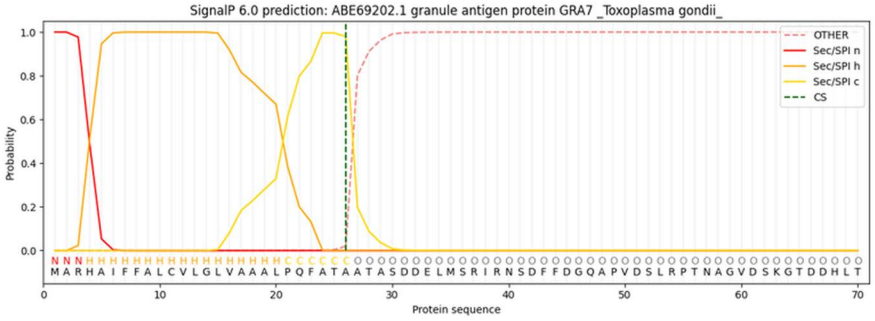
Ramachandran Plot Analysis

The Ramachandran plot analysis for the GRA7 structure model shows how the phi (φ) and psi (ψ) torsion angles are distributed across amino acid residues in the generated 2D model (**Figure 6**).

The results indicate that most of the residues fall within the allowed conformational regions, with 80% of them in the favored region, suggesting that the predicted structure is stable and aligns with the conformational predictions typically found in natural proteins. Although there are a few points that fall into the disallowed region, their number is relatively small and does not affect the overall stability and validity of the structure. This confirms that the GRA7 model generated from bioinformatics prediction has acceptable quality and can be used for further analysis.

Prediction of Linear B-cell Epitopes and Antigenicity of the GRA7 Protein

Continuous B-cell epitope candidates in the GRA7 protein were identified through analysis with the BcePred platform. This prediction was performed by evaluating several sequence-based features, such as structural flexibility, hydrophilic character, residue accessibility, turn tendency, surface exposure, polarity, and antigenic potential. The prediction results generated by BcePred are presented in Table 1. These predicted epitopes may contribute to the



Protein type	Other	Signal Peptide (Sec/SPI)
Likelihood	0.0002	0.9997

Figure 3. Prediction of Sec/SPI Signal Peptide in the GRA7 Protein. SignalP 6.0 prediction results revealed that the GRA7 protein contains a Sec/SPI-type signal peptide with a very high confidence level (99.97%) with a predicted cleavage site between amino acid residues 26 and 27. The presence of this signal peptide indicates that GRA7 is targeted to the classical secretory pathway, which is dependent on the Sec system and processed by Signal Peptidase I (SPI).

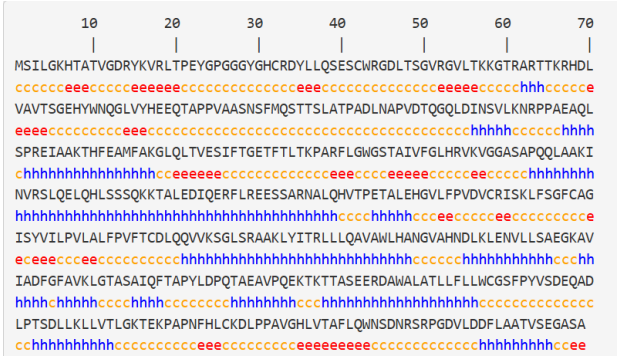


Figure 4. Predicted secondary structure by the GOR4 online service. The top row represents the amino acid sequence (protein residues). The bottom row shows the predicted secondary structure elements for each residue, color-coded as follows: blue indicates h (alpha-helix), red indicates e (beta-strand or extended strand), and orange indicates c (coil, unstructured region)

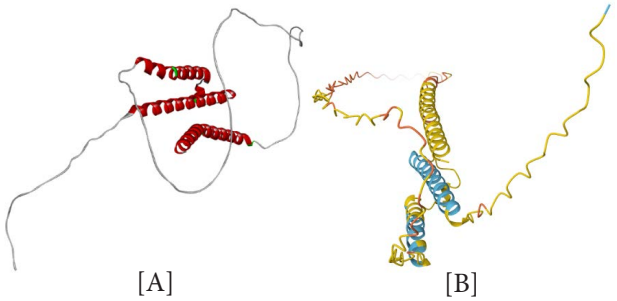


Figure 5. A. Result from the web server SWISS-MODEL. The GRA7 protein's 3D model. **B**. AlphaFold server-generated structural model of 3D TgGRA7 structure. This structure is highlighted according to its per-residue confidence level as determined using the pLDDT value: dark blue represents a very high confidence level (pLDDT > 90), light blue represents a confident prediction (90 > pLDDT > 70), yellow represents a low-confidence prediction (70 > pLDDT > 50), and orange/red represents a very low confidence level (pLDDT < 50), which directly relates to high flexibility and Intrinsically Disordered Regions (IDRs).

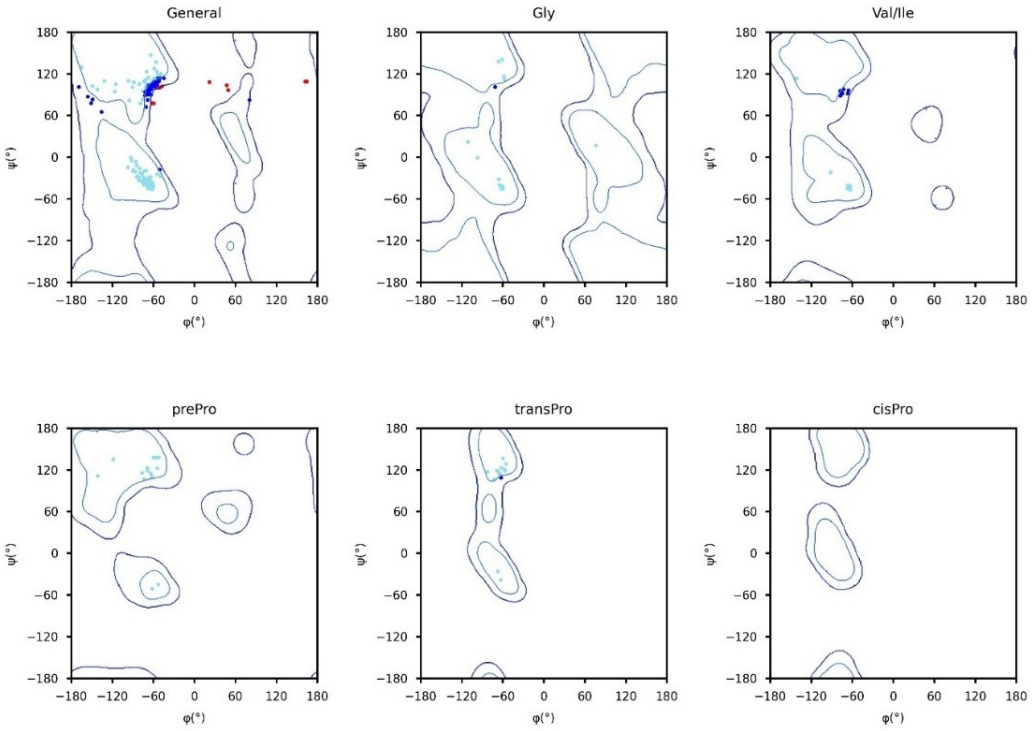


Figure 6. 2D Ramachandran plot for GRA7 structure analysis

Table 1. Epitope prediction of the GRA7 protein using multiple criteria from the BcePred web server.

Prediction Parameter	Epitope Sequence
Flexibility	AGVDSKGT; HFRKRGV; KVVPRKSEGKRSF; RHFSPRKNRSR; EVPESGEDR
Hydrophilicity	ATASDDE; VDSKGTDDH; ETEPDEQEE; DAEVTDD; EEHTDRK; RKSEGKRS; TEQQQTGDEP; PESGEDREDARQ
Accessibility	ESQLPRREPLETEPDEQEE; YEEHTDRKVVPRKSEGKRS; ELTEQQQTGDE; IKRTYRHFSPRKNRSRQPAP; PESGEDREDARQ
Turns	NOT AVAILABLE
Exposed surface	QLPRREPLETEPDEQEE; EEHTDRKVVPRKSEGKRSFKDLLKKL; FSPRKNRSRQP; EDREDARQ
Polarity	PRREPLETEPDEQEEVHFRKRGV; DDHIYEEHTDRKVVPRKSEGKRSFK; IKRTYRHFSPRKNRSRQ; PEHEVPESGEDREDARQ
Antigenic propensity	LCVLGLV

antigenic properties of GRA7.

The antigenicity analysis of the GRA7 protein shows that this protein has a good potential to function as an antigen recognized by the immune system, which is highly relevant for diagnostic or vaccine development. According to the graph generated from IEDB and ANTIGENPro (Figure 7), most segments of the GRA7 protein have antigenicity scores above the threshold value, with an average score of 1.016 and a maximum value of 1.257, indicating that this protein is quite effective in triggering an immune response. The green and yellow colors on the graph represent segments with good antigenicity potential, where yellow indicates areas with higher scores.

Further predictions identify several antigenic peptides in the GRA7 protein that may trigger an immune response (Table 2), such as HAIFFALCVLGVAAALPQPATA at positions 4-26 (length 23), QAPVDSL at positions 47-53 (length 7), and VSTVIGFAALAAAVAFLGLG at positions 181-200 (length 20). These peptides have potential as epitopes that may be applied in the design of serological assays or vaccines to detect and combat *Toxoplasma gondii* infection.

The Interaction of GRA7 Protein Network and Its Central Role in the Immune System

Degree is a parameter used in network analysis to measure how many proteins interact with other proteins. The higher the degree value, the more interactions the protein has. In this graph, GRA7 is marked in red, indicating the highest degree compared to other proteins like RON4, ROP18, BN1205_093730, MIC10, and MIC8, which have lower degree values, shown by lighter colors (**Figure 8**).

Discussion

In this study, several characteristics of the GRA7 protein were analyzed. The MW of GRA7 is 25.944.89 Da and its amino acid sequence consists of 236 residues. Regarding its immunogenic potential, the GRA7 is large enough and structurally complex enough to form stable antigenic determinants and elicit a strong immune response, as proteins with MW between 5-10 kDa are generally weak immunogens (31). GRA7 is considered a relatively large protein, with an estimated molecular mass of 25.9 kDa and a size exceeding 20 kDa. These two characteristics are commonly linked to stronger antigenic potential, allowing the protein to be more easily recognized by the immune system and to induce an antibody response. The instability index was applied to assess protein stability, and the value obtained for GRA7 was 49.97, which classifies it theoretically

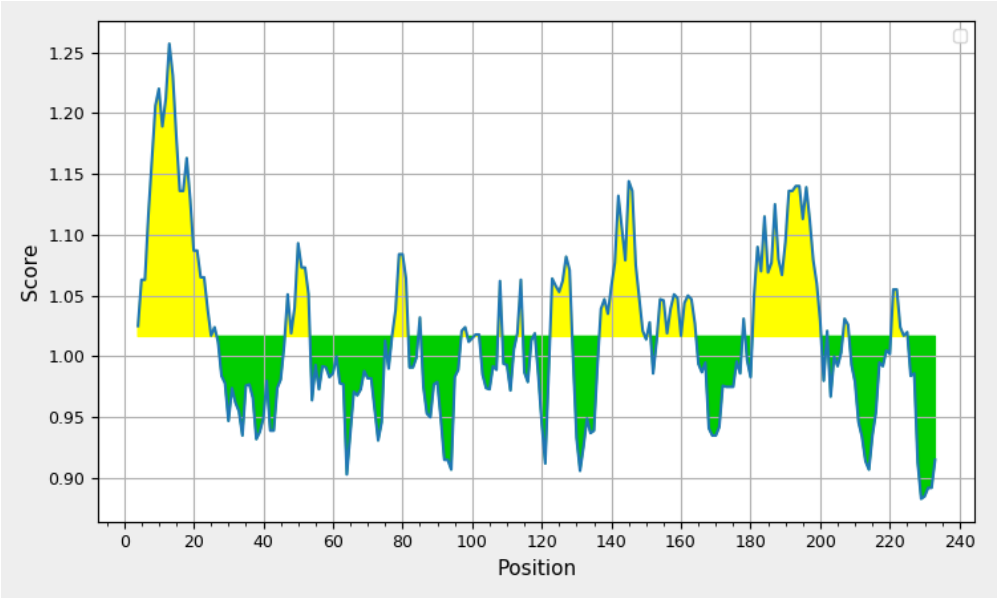


Figure 7. The prediction of the antigenicity of the GRA7 protein

Table 2. Predicted Antigenic Peptides in the GRA7 Protein

No.	Start	End	Peptide	Length
1	4	26	HAIFFALCVLGLVAAALPQFATA	23
2	47	53	QAPVDSL	7
3	123	128	RKVVPR	6
4	137	149	KDLLKKLALPAVG	13
5	154	164	YFAADRILPEL	11
6	181	200	VSTVIGFAALAAAVAFLGLG	20

as an unstable protein. However, in an immunological environment this trait may represent structural flexibility which is beneficial for epitope exposure, consistent with the prevalence of random coils and loop areas identified in the secondary and tertiary structure investigations (32).

Furthermore, the hydropathicity and aliphatic index assessments produced values of −0.594 and 71.19, respectively. The negative GRAVY score further supports that GRA7 is hydrophilic, interacting well with surrounding water molecules, and highlights hydrophobic interaction tendencies that reinforce the identification of transmembrane domains and the proposed involvement of GRA7 in protein–membrane interactions and modulation of host immune signalling, including NF-κB activation and inflammasome pathways. The aliphatic index of 71.19 suggests a moderate thermal stability, which supports the structural integrity of the α-helix-dominant configuration predicted by the SWISS-MODEL 3D model in this work. The theoretical isoelectric point (pI) of 5.33, however, suggests a slightly acidic character that physiologically may favour GRA7 stability and solubility in the host intracellular milieu, consistent with its function as a dense granule secretory protein active in the parasitophorous vacuole (33). Therefore, GRA7 can be seen as a secretory protein that is transported across cell membranes or targeted to certain organelles by the classical secretion pathway. This secretory property implies that the protein is not restricted

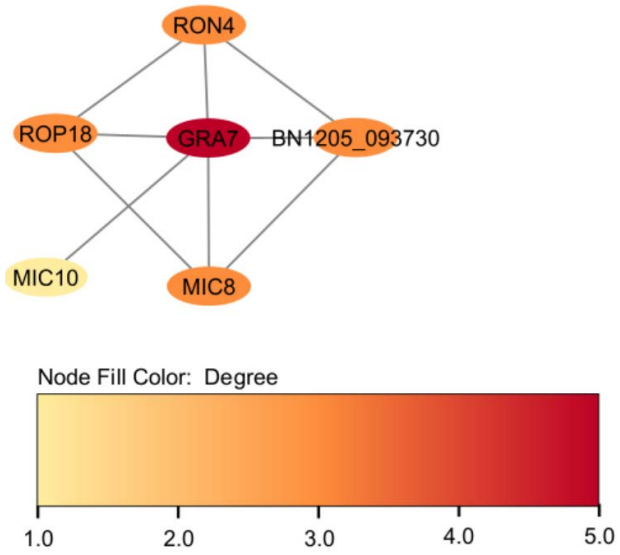


Figure 8. The protein interaction network places GRA7 at the center, as it shows the highest degree value.

to hidden intracellular places, such as only within the parasite nucleus or cytoplasm, but can instead be exposed to settings accessible to the host immune system. Therefore, this characteristic favours the potential of GRA7 as a good vaccine antigen candidate, as exposed proteins usually have more power to trigger immune responses involving both humoral and cellular mechanisms (34). The estimated half-life of about 30 hours in eukaryotic cells suggests that GRA7 is available for a sufficient time period to have effective interactions with the host immune system. This is consistent with the discussion that this antigen is present during both the tachyzoite and bradyzoite developmental stages, thus allowing the immune system to recognise it during the progression of infection. This distinguishes GRA7 from other antigens that are generally stage-specific and provides GRA7 with a diagnostic benefit by allowing

the detection of infection in multiple phases. The *T. gondii* GRA7 antigen has demonstrated strong sensitivity and specificity as a diagnostic marker candidate because this protein was constantly expressed throughout the active stage of infection and it had significant immunogenic characteristics. Foroutan et al. (2019) revealed that GRA7 is a better diagnostic tool compared to other antigens notably for the diagnosis of acute infections. Therefore, the application of GRA7 antigen in serological or molecular-based approaches is crucial to get prompt and accurate diagnosis in high-risk individuals (35). GRA7 consistently showed better diagnostic performance than the other 2 recombinant dense granule antigens, GRA6 and GRA14, in detecting IgG antibodies against *T. gondii* in human sera from the Philippines, according to another study by Ybañez and Nishikawa (2022) (36). The *T. gondii* GRA7 antigen has shown various benefits as an approach for diagnosing toxoplasmosis, especially in distinguishing between acute and chronic phases of infection. Identification of IgM antibodies to GRA7 is indicative of an acute infection and assessment of anti-GRA7 IgG avidity can be useful to differentiate recent infections from previous infections. The key advantage of GRA7 is the high expression in the initial stage of infection and its ability to induce a robust humoral immune response, which makes it very appropriate for identifying IgG and IgM antibodies (35–37). The study by Teimouri et al. (2019) further supports the efficiency of GRA7 in separating acute from chronic toxoplasmosis which is important in clinical decision making, especially in pregnant women and immunocompromised patients. Thus, GRA7 is a promising option for the development of sensitive, specific and widely applicable diagnostic methods for toxoplasmosis (38).

Post-translational modifications (PTMs) contribute significantly to the regulation of biological activity. To evaluate the potential acetylation and phosphorylation sites in the GRA7 protein, two web-based prediction platforms, NetAcet-1.0 and NetPhos 3.1, were used. The analysis identified 35 possible PTM sites in GRA7, consisting of a single predicted acetylation position and 34 potential phosphorylation regions. These molecular changes may contribute to the regulation of specific protein functions and overall protein activity (39).

GRA7 was predicted to contain two transmembrane domains and was recognized by antigen-presenting cells, which may contribute in the stimulation of T- and B-lymphocyte responses and trigger a rapid immune response. SignalP 6.0 prediction results showed that the GRA7 protein possesses a Sec/SPI-type signal peptide with a very high confidence score (0.9997) with the expected cleavage location between amino acid residues 26 and 27. This signal peptide clearly suggests that GRA7 follows the traditional secretory pathway via the Sec system, sending the protein to the endoplasmic reticulum before secretion or targeting to particular compartments (40). During the invasion of host cells, T. gondii releases dense granule proteins such as GRA7 into the parasitophorous vacuole. These proteins contribute to the re gulation of immune responses in the host. From an immunological perspective, proteins that are secreted or displayed on the vacuolar surface are more likely to be processed by antigen-presenting cells (APCs), displayed through MHC class I and II pathways, and subsequently identified by T and B lymphocytes (41). The detection of a signal peptide and the secretory characteristics of GRA7 provide important advantages for vaccine development. Naturally secreted antigens tend to induce stronger protective immune responses because they can be recognized and processed more rapidly by the adaptive immune system. Experimental studies have also shown that immunization with recombinant GRA7 (rGRA7), particularly when encapsulated in nanoparticles or combined with suitable adjuvants, can stimulate protective antibody-mediated and cell-mediated immune responses during acute *T. gondii* infection. Thus, the SignalP prediction provides not only a bioinformatic level of support for the secretory nature of GRA7 but also a compelling molecular basis for its application as a prospective vaccine candidate and diagnostic biomarker (36). Additionally, PSORT II prediction of subcellular localisation indicated that GRA7 was largely localised in the extracellular space and the plasma membrane. This extracellular localisation is fully consistent with its role as a secreted protein released into the host milieu, increasing its accessibility to host immune cells for the rapid activation of immunity (42).

GRA7 is a secreted protein and also has a high degree, closeness centrality and betweenness centrality in the *T. gondii* protein-protein interaction network. This suggests that GRA7 is a highly central and important protein, which can link proteins that are not directly connected, indicating its potential function in controlling crucial biological processes and altering host–pathogen interactions (34). Protein-protein interaction network research identified GRA7 as a hub protein with high degree value and high centrality scores (closeness and betweenness centrality). These topological network metrics show that GRA7 is a major connection in the *T. gondii* interactome, mediating complicated interactions with other virulence factors and host defence mechanisms. Recent findings indicate that GRA7 contributes to the control of innate immunity in the host by influencing NF-κB pathway activation and inflammasome regulation. In addition, phosphorylation mediated by protein kinase C-α (PKCα) enhances the ability of GRA7 to interact with adaptor molecules, such as ASC, also known as apoptosis-associated speck-like protein containing a CARD and phospholipase D1 (PLD1). This interaction enhances inflammasome formation, increases IL-1β production, and strengthens the host antimicrobial response. In addition, TRAF6 has been shown to associate with GRA7 through the adaptor protein MyD88, thereby stimulating NF-κB activation and promoting the transcription of genes encoding pro-inflammatory cytokines (43).

Moreover, to characterize secondary structural features, particularly alpha-helical regions and beta-turn formations, and integrating them into structural modeling is essential

for accurately predicting the protein's three-dimensional conformation. The GOR4 prediction shows that GRA7 protein contains 8.05% extended strands, 51.69% random coils and 40.25% alpha-helices. The existence of random coils indicates that GRA7 contains flexible areas that could promote interaction with other molecules, particularly in the context of immunological recognition. The large relative percentage of alpha-helices indicates structural stability and implies that these areas may play essential functions in maintaining the protein shape and antigenicity. (35,38). Overall, this structural composition supports the potential of GRA7 as an effective antigenic candidate for diagnostic applications in toxoplasmosis.

This study used the SWISS-MODEL platform to generate a predicted three-dimensional structure of the GRA7 protein. SWISS-MODEL is a web-accessible interactive platform used to generate 3D protein structure models. The predicted GRA7 structure was mainly composed of alpha-helices, indicated in red. These helical regions contribute to maintaining the protein's structural stability and are often involved in immune-related interactions, including antibody epitope binding. The grey areas around and connecting the helices are coils or unstructured regions (random coils). These regions are usually flexible and allow dynamic movement that can help recognition of antigens by immune cells (e.g., contact with T-cell receptors or MHC molecules). The structural elements are lengthy loops, which are not helices and are not beta-sheets. These long loops could harbour potential linear epitopes that can be recognised by antibodies. The high content of α-helix and the overall structural order suggest that GRA7 is highly conformationally stable, which is significant for its antigen function and its potential use in diagnosis or vaccine production. The combination of stable helices and flexible loops makes GRA7 a good antigen candidate, as it has a surface that is stable but flexible enough to bind to the immune system. The structural reliability of the generated 3D model was further examined through Ramachandran plot analysis, which indicated that 80% of the residues occupied the favored conformational regions. This high proportion suggests that the predicted structural conformation is stereochemically stable and reliable, which lays a solid foundation for subsequent functional and epitope mapping assessments.

Furthermore, the architecture and physicochemical dynamics of TgGRA7, analysed using SWISS-MODEL, have been significantly corroborated by the AlphaFold prediction (44). Homology modelling provides a good spatial view of the protein structure, but the residue-wise confidence score (pLDDT) from AlphaFold gives us an insight into the dynamical properties of the protein. The very confident scores (blue regions, pLDDT > 70) in the core alpha-helices show how structurally robust these regions are. On the other hand, the long stretches of residues depicted in yellow, orange and red colour (pLDDT < 50) within the N- and C-terminal regions as well as in the extended loop sections gives unequivocal evidence of the intrinsically disordered regions (IDRs) (45). In the context of structural vaccinology, the lack of structural conformational stability is a functional feature of these disordered areas that preserves their flexibility and accessibility in cellular environments. Therefore, the fundamentally disordered areas are highly exposed as antibody targets. This dynamical feature is thus bridging the correlation between high coil content anticipated by GOR4 and high antigenicity of the epitopes (46).

BcePred was applied to identify potential linear B-cell epitope regions, while ANTIGENPro was utilized to assess the antigenic potential of the protein, confirming GRA7's substantial immunogenic potential. The overall ANTIGENPro average score of 1.016 is unusually high and suggests very high chance of antigenicity. Linear epitopes represented by high scoring particular peptide segments are very promising. It is expected that these targeted peptide sequences will be well recognised by host antibodies, giving a reasonable basis for the production of highly specific diagnostic tools or subunit vaccinations.

The study indicates that the GRA7 serves as a key element of a protein complex with the virulence kinase ROP18 and pseudokinases ROP8/2 on the cytosolic side of the parasitophorous vacuole membrane. GRA7 directly binds GTP-bound forms of the immunity-related GTPase Irga6 and induces rapid polymerisation and subsequent accelerated disassembly, leading to an increased turnover of IRG. GRA7 deletion alone produces little modification in virulence, but the combined deletion of GRA7 and ROP18 results in significantly increased IRG recruitment, higher parasite clearance by IFN-γ-activated macrophages and total attenuation of acute virulence in mice. These results demonstrate that GRA7 works together with ROP18 to subvert IRG-dependent host immunity by modifying the polymerisation kinetics of IRGs and so improving parasite survival (47). The study demonstrates that GRA7 serves as a key element of the *T. gondii* ROP5/ROP18 kinase complex and contributes specifically to suppressing the immunity-related GTPase Irga6. GRA7 directly binds to ROP5 and improves ROP18-mediated phosphorylation of Irga6, while also helping recruit ROP5 to the parasitophorous vacuole membrane. Although, the formation of the ROP5-ROP18 complex does not depend on the presence of GRA7, its deletion markedly decreases Irga6 phosphorylation, increases IRG accumulation on the vacuole, reduces parasite survival in cells activated by IFN-γ and decreases its virulence in mouse models. Together, these findings identify GRA7 as an Irga6-specific effector that modulates ROP18 activity and a crucial component of *T. gondii*'s approach to disrupt the IRG-mediated host defence system (48).

TgGRA7 appears as the essential component in the protein-protein interaction network, whose importance is far more than just being a vascular structural protein. Protein-protein interaction network analysis revealed that TgGRA7 has a notably high degree value. The high degree value is a key signal that the protein in question is a protein hub or the spot where multiple routes of parasites meet. This finding is of particular interest since it implies

a connection of GRA7 with other organellar proteins of different origin. In particular, this is the case with RON4, from the rhoptries, and MIC8 and MIC10, from the microneme. (43).

The presence of BN1205_093730 gene in this network further supports the suggested role of a hypothetical protein not yet explored in helping to increase the functional capacity of GRA7. As an internal signal regulator, TgGRA7 presents a unique aspect of being able to recruit certain molecules from within the host, including forming associations with rhoptry proteins and making the host's immune response paralysed (49). The fact that GRA7 regulates the activity of kinases such as ROP18 and this illustrates the evolutionary importance of the centrality of protein interactions is not coincidence (47). Hence, the prominence of TgGRA7 in this interaction network confirms its importance as a possible therapeutic target and vaccination development (11).

From a diagnostic perspective, recombinant rGRA7 shows strong diagnostic sensitivity and specificity for identifying *T. gondii* infection in humans and animals during both acute and chronic stages. Several comparative investigations indicated that the diagnostic performance of serological tests using rGRA7 (ELISA or ICT) was superior or equal to that of other GRA antigens including GRA6 and GRA14 (3,4). These results suggest GRA7 as a promising diagnostic antigen for quick test development or for use in multi-epitope antigen panels. GRA7 has also been tested as a subunit vaccination candidate. The level of protection differs across studies, but immunization with rGRA7, either alone or combined with other antigens such as GRA12, has been reported to stimulate antibody-mediated and cell-mediated immunity responses which may offer limited protection during acute *T. gondii* infection (5). However, the protective efficacy is heavily dependent on the choice of adjuvant, delivery mechanism and antigen composition, indicating the need for further optimisation. Together, the evidence that is piling up supports the dual role of GRA7 as an immunomodulatory effector and diagnostic biomarker, making it an interesting target for improving *T. gondii* diagnostic tools and subunit vaccine development. The merging of genetic studies with applied immunodiagnostics could boost our understanding of the processes of GRA7 and speed up the translational applications in toxoplasmosis research (6,50).

Toxoplasma gondii can infect many types of hosts, including humans. In individuals with normal immune function, *T. gondii* infection usually does not cause noticeable symptoms. However, severe outcomes may occur in vulnerable groups, including pregnant women, developing fetuses, individuals with HIV/AIDS, and organ transplant recipients. For this reason, early identification of *T. gondii* infection is essential to prevent severe outcomes, including miscarriage, congenital defects, and neurological or visual impairment (48). Thus, there is a need for sensitive, specific and easily accessible diagnostic procedures. GRA7 is a secretory antigen secreted by *T. gondii* during the active phase of infection (31). GRA7 is a useful serological biomarker for numerous reasons. It can stimulate strong humoral and cellular immune responses (51-55). This protein is antigenic and is recognised by patients' antibody in acute as well as chronic phase of infection. Therefore, it is a suitable candidate for creation of diagnostic kits. Antibodies against GRA7 can be found in the early phases of infection and survive for a long time, so that acute infections and reactivation of latent infection can be recognised. GRA7 has been demonstrated to have great sensitivity and specificity in serological tests such as ELISA compared to other antigens, decreasing the possibility of false positives or cross reactivity with other parasite illnesses. GRA7 can be manufactured recombinantly allowing the creation of recombinant antigen based diagnostics which are more cost effective, stable and suited for mass production (35).

Conclusions

Bioinformatics investigation shows that GRA7 comprises a mostly alpha-helical structure with transmembrane domains and several locations for post-translational modifications that could modulate its biological activity. The existence of flexible random coils and prolonged loops further boosts its potential to efficiently engage with immunological components. Its stable tertiary structure makes it an ideal candidate for immunological recognition. In addition, GRA7 allows the creation of scalable, reliable diagnostic tools. Thus, GRA7 appears to be a promising candidate for developing sensitive and specific serological methods, particularly for the early detection of toxoplasmosis among vulnerable populations, particularly pregnant women and immunocompromised patients.

Acknowledgements

This paper forms part of the first author's thesis in the Master's Program in Biomedical Sciences, Faculty of Medicine, Universitas Brawijaya. The authors sincerely thank Anggia Noor Ramadhani for her valuable assistance in data analysis.

Authors' Contribution

Conceptualization: Loeki Enggar Fitri, Nuning Winaris
Data curation: Siti Fadilla, Muhammad Luqman Habibi
Formal analysis: Siti Fadilla
Funding acquisition: Loeki Enggar Fitri
Investigation: Siti Fadilla, Muhammad Luqman Habibi
Methodology: Siti Fadilla, Loeki Enggar Fitri, Nuning Winaris
Project administration: Nuning Winaris
Resources: Loeki Enggar Fitri
Software: Siti Fadilla
Supervision: Loeki Enggar Fitri, Nuning Winaris
Validation: Loeki Enggar Fitri, Nuning Winaris
Visualization: Siti Fadilla
Writing–original draft: Siti Fadilla, Muhammad Luqman Habibi, Nuning Winaris, Loeki Enggar Fitri
Writing–review & editing: Siti Fadilla, Muhammad Luqman Habibi, Nuning Winaris, Loeki Enggar Fitri

Data Availability Statement

The data supporting this study can be obtained from the corresponding authors upon reasonable request.

Competing Interests

The authors confirm that they have no competing interests related

to this work.

Ethical Approval

Ethical clearance for the study was reviewed and obtained from 274/EC/KEPK/07/2024 at the Universitas Brawijaya.

Funding

This research received funding support from the Competitive Funding for the Research Ecosystem of Professors from the Institute for Research and Community Service, Universitas Brawijaya, Indonesia, under grant number 00929.8/DST/UN10.A0501/B/PT.01.03.2/2026.

References

1. Mushlih M, Nurfitriana A, Ningsih KW, Azizah N, Arian NL. Comparison of *Toxoplasma gondii* identification using PCR method and ELFA method. Meditory 2020;8(2):101-8. doi:10.33992/m.v8i2.1266
2. Ariyeni D, Helvinda W. Ocular toxoplasmosis. J Kesehat Andalas 2020;9(1):133-40.
3. Kim MJ, Park SJ, Park H. Trend in serological and molecular diagnostic methods for *Toxoplasma gondii* infection. Eur J Med Res 2024;29(1):520. doi:10.1186/s40001-024-02055-4
4. Vargas-Villavicencio JA, Cañedo-Solares I, Correa D. Anti-*Toxoplasma gondii* IgM long persistence: what are the underlying mechanisms? Microorganisms 2022;10(8):1659. doi:10.3390/microorganisms10081659
5. Garnaud C, Fricker-Hidalgo H, Evengård B, Álvarez-Martínez MJ, Petersen E, Kortbeek LM, et al. *Toxoplasma gondii*-specific IgG avidity testing in pregnant women. Clin Microbiol Infect 2020;26(9):1155-60. doi:10.1016/j.cmi.2020.04.014
6. Gazzonis AL, Marino AM, Garippa G, Rossi L, Mignone W, Dini V, et al. *Toxoplasma gondii* seroprevalence in beef cattle raised in Italy: a multicenter study. Parasitol Res 2020;119(11):3893-8. doi:10.1007/s00436-020-06878-y
7. Brenier-Pinchart MP, Robert-Gangneux F, Accoceberry I, Pichard S, Garnaud C, Fricker-Hidalgo H, et al. Multicenter comparative assessment of the TIB MolBiol *Toxoplasma gondii* detection kit and four laboratory-developed PCR assays for molecular diagnosis of toxoplasmosis. J Mol Diagn 2021;23(8):1000-6. doi:10.1016/j.jmoldx.2021.05.010
8. de Oliveira Azevedo CT, do Brasil PE, Guida L, Lopes Moreira ME. Performance of polymerase chain reaction analysis of the amniotic fluid of pregnant women for diagnosis of congenital toxoplasmosis: a systematic review and meta-analysis. PLoS One 2016;11(4): e0149938. doi:10.1371/journal.pone.0149938
9. Olson EM, Lin NU, Krop IE, Winer EP. The ethical use of mandatory research biopsies. Nat Rev Clin Oncol 2011;8(10):620-5. doi:10.1038/nrclinonc.2011.114
10. Ihara F, Fereig RM, Himori Y, Kameyama K, Umeda K, Tanaka S, et al. *Toxoplasma gondii* dense granule proteins 7, 14, and 15 are involved in modification and control of the immune response mediated via NF-κB pathway. Front Immunol 2020;11:1709. doi:10.3389/fimmu.2020.01709
11. Koh HJ, Kim YR, Kim JS, Yun JS, Jang K, Yang CS. *Toxoplasma gondii* GRA7-targeted ASC and PLD1 promote antibacterial host defense via PKCα. PLoS Pathog 2017;13(1): e1006126. doi:10.1371/journal.ppat.1006126
12. Sun HC, Deng PM, Fu Y, Deng JH, Xie RH, Huang J, et al. Protective efficacy of *Toxoplasma gondii* GRA12 or GRA7 recombinant proteins encapsulated in PLGA nanoparticles against acute *Toxoplasma gondii* infection in mice. Front Cell Infect Microbiol 2023;13:1209755. doi:10.3389/fcimb.2023.1209755
13. Kusumaningsih P. Pengembangan antibodi poliklonal dari stadium takizoit *Toxoplasma gondii* isolat WTA. Jurnal Kesehatan Terpadu 2018;2(1):31-6. doi:10.36002/jkt.v2i1.444
14. Arab-Mazar Z, Javadi Mamaghani A, Fallahi S, Rajaeian S, Koochaki A, Seyyed Tabaei SJ, et al. Immunodiagnosis and molecular validation of *Toxoplasma gondii*-recombinant dense granular (GRA) 5 protein for the detection of toxoplasmosis in hemodialysis patients. Semin Dial 2021;34(5):332-7. doi:10.1111/sdi.12981
15. Karimipour-Saryazdi A, Ghaffarifar F, Dalimi A, Foroutan M, Horton J, Sadraei J. In silico analysis of the ROP29 protein as a vaccine candidate against *Toxoplasma gondii*. J Parasitol Res 2024;2024:1918202. doi:10.1155/2024/1918202
16. National Institutes of Health (NIH). National Center for Biotechnology Information (NCBI). Available from: https://www.ncbi.nlm.nih.gov/. Accessed June 13, 2025.
17. Gasteiger E, Hoogland C, Gattiker A, Duvaud S, Wilkins MR, Appel RD, et al. Protein identification and analysis tools on the ExPASy server. In: Walker JM, ed. The Proteomics Protocols Handbook. Totowa, NJ: Humana Press; 2005. p. 571-607. doi:10.1385/1-59259-890-0:571
18. Kiemer L, Bendtsen JD, Blom N. NetAcet: prediction of N-terminal acetylation sites. Bioinformatics 2005;21(7):1269-70. doi:10.1093/bioinformatics/bti130
19. Blom N, Gammeltoft S, Brunak S. Sequence and structure-based prediction of eukaryotic protein phosphorylation sites. J Mol Biol 1999;294(5):1351-62. doi:10.1006/jmbi.1999.3310
20. Krogh A, Larsson B, von Heijne G, Sonnhammer EL. Predicting transmembrane protein topology with a hidden Markov model: application to complete genomes. J Mol Biol 2001;305(3):567-80. doi:10.1006/jmbi.2000.4315
21. Nakai K, Horton P. PSORT: a program for detecting sorting signals in proteins and predicting their subcellular localization. Trends Biochem Sci 1999;24(1):34-6. doi:10.1016/s0968-0004(98)01336-x
22. Combet C, Blanchet C, Geourjon C, Deléage G. NPS@: network protein sequence analysis. Trends Biochem Sci 2000;25(3):147-50. doi:10.1016/s0968-0004(99)01540-6
23. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, Gumienny R, et al. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res 2018;46(W1): W296-303. doi:10.1093/nar/gky427
24. Colovos C, Yeates TO. Verification of protein structures: patterns of nonbonded atomic interactions. Protein Sci 1993;2(9):1511-9. doi:10.1002/pro.5560020916
25. Varadi M, Anyango S, Deshpande M, Nair S, Natassia C, Yordanova G, et al. AlphaFold Protein Structure Database: massively expanding the structural coverage of protein-sequence space with high-accuracy models. Nucleic Acids Res 2022;50(D1): D439-44. doi:10.1093/nar/gkab1061
26. Saha S, Raghava GPS. BcePred: prediction of continuous B-cell epitopes in antigenic sequences using physico-chemical properties. In: Nicosia G, Cutello V, Bentley PJ, Timmis J, eds. Artificial Immune Systems. Berlin: Springer; 2004. p. 197-204. doi:10.1007/978-3-540-30220-9_16
27. Vita R, Mahajan S, Overton JA, Dhanda SK, Martini S, Cantrell JR, et al. The immune epitope database (IEDB): 2018 update. Nucleic Acids Res 2019;47(D1): D339-43. doi:10.1093/nar/gky1006
28. Cheng J, Randall AZ, Sweredoski MJ, Baldi P. SCRATCH: a protein structure and structural feature prediction server. Nucleic Acids Res 2005;33(Suppl 2): W72-6. doi:10.1093/nar/gki396
29. Szklarczyk D, Kirsch R, Koutrouli M, Nastou K, Mehryary F, Hachilif R, et al. The STRING database in 2023: protein-protein association networks and functional enrichment analyses for any sequenced genome of interest. Nucleic Acids Res 2023;51(D1): D638-46. doi:10.1093/nar/gkac1000
30. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res 2003;13(11):2498-504. doi:10.1101/gr.1239303
31. Pedroza-Escobar D, Castillo-Maldonado I, González-Cortés T, Delgadillo-Guzmán D, Ruíz-Flores P, Cruz JHS, et al. Molecular bases of protein antigenicity and determinants of

immunogenicity, anergy, and mitogenicity. Protein Pept Lett 2023;30(9):719-33. doi:10.2174/0929866530666230907093339
32. Sganzerla Martinez G, Dutt M, Kumar A, Kelvin DJ. Multiple protein profiler 1.0 (MPP): a webserver for predicting and visualizing physiochemical properties of proteins at the proteome level. Protein J 2024;43(4):711-7. doi:10.1007/s10930-024-10214-z
33. Asghari A, Majidiani H, Fatollahzadeh M, Nemati T, Shams M, Azizi E, et al. Insights into the biochemical features and immunogenic epitopes of common bradyzoite markers of the ubiquitous *Toxoplasma gondii*. Infect Genet Evol 2021;95:105037. doi:10.1016/j.meegid.2021.105037
34. Sun HC, Huang J, Fu Y, Hao LL, Liu X, Shi TY. Enhancing immune responses to a DNA vaccine encoding *Toxoplasma gondii* GRA7 using calcium phosphate nanoparticles as an adjuvant. Front Cell Infect Microbiol 2021;11:787635. doi:10.3389/fcimb.2021.787635
35. Foroutan M, Zaki L, Tavakoli S, Soltani S, Taghipour A, Ghaffarifar F. Rhomboid antigens are promising targets in the vaccine development against *Toxoplasma gondii*. EXCLI J 2019;18:259-72. doi:10.17179/excli2018-1993
36. Ybañez RH, Nishikawa Y. Comparative performance of recombinant GRA6, GRA7, and GRA14 for the serodetection of *T. gondii* infection and analysis of IgG subclasses in human sera from the Philippines. Pathogens 2022;11(2):277. doi:10.3390/pathogens11020277
37. Saha S, Raghava GP. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins 2006;65(1):40-8. doi:10.1002/prot.21078
38. Teimouri A, Modarressi MH, Shojaee S, Mohebali M, Zouei N, Rezaian M, et al. Detection of *Toxoplasma*-specific immunoglobulin G in human sera: performance comparison of in-house Dot-ELISA with ECLIA and ELISA. Eur J Clin Microbiol Infect Dis 2018;37(8):1421-9. doi:10.1007/s10096-018-3266-y
39. Teimouri A, Abbaszadeh Afshar MJ, Mohtasebi S, Jafarpour Azami S, Alimi R, Keshavarz H. Assessment of an in-house enzyme-linked immunosorbent assay and IgG avidity test based on SAG1 and GRA7 proteins for discriminating between acute and chronic toxoplasmosis in humans. J Clin Microbiol 2021;59(8): e0041621. doi:10.1128/jcm.00416-21
40. Ferra B, Holec-Gąsior L, Grążlewska W. *Toxoplasma gondii* recombinant antigens in the serodiagnosis of toxoplasmosis in domestic and farm animals. Animals (Basel) 2020;10(8):1245. doi:10.3390/ani10081245
41. Teimouri A, Goudarzi F, Goudarzi K, Alimi R, Sahebi K, Foroozand H, et al. *Toxoplasma gondii* infection in immunocompromised patients in Iran (2013-2022): a systematic review and meta-analysis. Iran J Parasitol 2022;17(4):443-57. doi:10.18502/ijpa.v17i4.11271
42. Mohammadhasani F, Dalir Ghaffari A, Asadi M. Comprehensive bioinformatics assessments of the ROP34 of *Toxoplasma gondii* to approach vaccine candidates. Discov Appl Sci 2024;6(10):501. doi:10.1007/s42452-024-06189-2
43. Swapna LS, Stevens GC, Sardinha-Silva A, Hu LZ, Brand V, Fusca DD, et al. ToxoNet: a high confidence map of protein-protein interactions in *Toxoplasma gondii*. PLoS Comput Biol 2024;20(6): e1012208. doi:10.1371/journal.pcbi.1012208
44. Borkakoti N, Thornton JM. AlphaFold2 protein structure prediction: implications for drug discovery. Curr Opin Struct Biol 2023;78:102526. doi:10.1016/j.sbi.2022.102526
45. Akdel M, Pires DEV, Pardo EP, Jänes J, Zalevsky AO, Mészáros B, et al. A structural biology community assessment of AlphaFold2 applications. Nat Struct Mol Biol 2022;29(11):1056-67. doi:10.1038/s41594-022-00849-w
46. da Silva PC, Martínez L. Intrinsically disordered malaria antigens: an overview of structures, dynamics and molecular simulation opportunities. J Braz Chem Soc 2024;35(10): e-20240102. doi:10.21577/0103-5053.20240102
47. Hermanns T, Müller UB, Könen-Waisman S, Howard JC, Steinfeldt T. The *Toxoplasma gondii* rhoptry protein ROP18 is an Irga6-specific kinase and regulated by the dense granule protein GRA7. Cell Microbiol 2016;18(2):244-59. doi:10.1111/cmi.12499
48. Mao F, Yang Y, Chen Y, Zhang Q, Ding X, Ni B, et al. Seroprevalence and risk factors of *Toxoplasma gondii* infection among high-risk populations in Jiangsu province, Eastern China. Front Cell Infect Microbiol 2021;11:783654. doi:10.3389/fcimb.2021.783654
49. Dunn JD, Ravindran S, Kim SK, Boothroyd JC. The *Toxoplasma gondii* dense granule protein GRA7 is phosphorylated upon invasion and forms an unexpected association with the rhoptry proteins ROP2 and ROP4. Infect Immun 2008;76(12):5853-61. doi:10.1128/iai.01667-07
50. Brenier-Pinchart MP, Filisetti D, Cassaing S, Varlet-Marie E, Robert-Gangneux F, Delhaes L, et al. Molecular diagnosis of toxoplasmosis: multicenter evaluation of the *Toxoplasma* RealCycler universal PCR assay on 168 characterized human samples. J Mol Diagn 2022;24(6):687-96. doi:10.1016/j.jmoldx.2022.03.009
51. Villard O, Cimon B, L'Ollivier C, Fricker-Hidalgo H, Godineau N, Houze S, et al. Serological diagnosis of *Toxoplasma gondii* infection: recommendations from the French National Reference Center for Toxoplasmosis. Diagn Microbiol Infect Dis 2016;84(1):22-33. doi:10.1016/j.diagmicrobio.2015.09.009
52. Garedaghi Y, Shojaee S, Khaki A, Hatef A, Ahmadi Ashtiani HR, Rastegar H, et al. Modulating effect of *Allium cepa* on kidney apoptosis caused by *Toxoplasma gondii*. Adv Pharm Bull 2012;2(1):1-6. doi:10.5681/apb.2012.001
53. Garedaghi Y, Bahavarnia SR. Repairing effect of *Allium cepa* on testis degeneration caused by *Toxoplasma gondii* in the rat. Int J Womens Health Reprod Sci 2014;2(2):80-9. doi:10.15296/ijwhr.2014.12
54. Garedaghi Y, Firozivand Y. Assessment of pregnant women toxoplasmosis by ELISA method in Miandoab city, Iran. Int J Womens Health Reprod Sci 2017;5(1):72-5. doi:10.15296/ijwhr.2017.13
55. Garedaghi Y, Safarmashaei S. Survey of *Toxoplasma* contamination in kidney recipient patients by ELISA method and comparison it with control group in Tabriz (East-Azerbaijan), Iran. Adv Environ Biol 2011;5(4):769-72.