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Immunoinformatics studies and design of a novel multi-epitope peptide vaccine against *Toxoplasma gondii* based on calcium-dependent protein kinases antigens through an *in-silico* analysis

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Purpose: Infection by the intracellular apicomplexan parasite *Toxoplasma gondii* has serious clinical consequences in humans and veterinarians around the world. Although about a third of the world's population is infected with *T. gondii*, there is still no effective vaccine against this disease. The aim of this study was to develop and evaluate a multimeric vaccine against *T. gondii* using the proteins calcium-dependent protein kinase (CDPK)1, CDPK2, CDPK3, and CDPK5.
Materials and Methods: Top-ranked major histocompatibility complex (MHC)-I and MHC-II binding as well as shared, immunodominant linear B-cell epitopes were predicted and linked using appropriate linkers. Moreover, the 50S ribosomal protein L7/L12 (adjuvant) was mixed with the construct's N-terminal to increase the immunogenicity. Then, the vaccine's physicochemical characteristics, antigenicity, allergenicity, secondary and tertiary structure were predicted.
Results: The finally-engineered chimeric vaccine had a length of 680 amino acids with a molecular weight of 74.66 kDa. Analyses of immunogenicity, allergenicity, and multiple physiochemical parameters indicated that the constructed vaccine candidate was soluble, non-allergenic, and immunogenic, making it compatible with humans and hence, a potentially viable and safe vaccine candidate against *T. gondii* parasite.
Conclusion: *In silico*, the vaccine construct was able to trigger primary immune responses. However, further laboratory studies are needed to confirm its effectiveness and safety.

Keywords: Immunoinformatics, *Toxoplasma gondii*, CDPK1, CDPK2, CDPK3, CDPK5, *In silico*

Introduction

Toxoplasma gondii is a compulsory intracellular protozoan having a place in the Apicomplexa phylum and infects a vast spectrum of people and warm-blooded animals [1]. Serological research's reported that one-third of the universe people are infected with *T. gondii* [2]. It can infect many warm-blooded creatures, such as humans, wild animals, livestock, and birds [3]. The only definitive hosts are felids; humans and a wide variety of other warm-blooded species are intermediate hosts [4]. *Toxoplasma* disease is obtained by accidental utilization of oocysts shed from cats in sullied water or vegetables, ingestion of tissue cysts presents in undercooked contaminated meat, intrinsically from pregnant moms to the embryo, and seldom by organ transplantation and blood transfusion [5]. Toxoplasmosis in immunocompetent patients, appears no indications, though in immunocom-

promised patients, it has serious side effects, like nerve and eye problems and even death [6]. There are usually no obvious clinical symptoms in healthy people, but it can cause serious harm to the fetus or to immunocompromised patients, such as those with acquired immunodeficiency syndrome (AIDS) [7]. In specific, it can cause retinochoroiditis and toxoplasmic encephalitis in people and fetus removal in animals [7]. Toxoplasmosis is one of the most causes of death in immunocompromised and dangerous tumor patients but too it can lead to a destroying impact on the livestock's generation and health, especially little household ruminants [8]. The available drugs to treat toxoplasmosis are combination of pyrimethamine and sulfadiazine which are not successful and they have severe side effects [9]. Moreover, in spite of the significance of toxoplasmosis, only an approved chemical vaccine (Toxovax) is available to treat and prevent toxoplasmosis in animals. Using this vaccine, because of its low efficacy and extreme side effects has been constrained [10]. Therefore, discovering and developing a successful vaccine are very important and fundamentally needed to limit *T. gondii* infection. To date, different techniques, counting inactivated vaccines, live attenuated vaccines, and subunit vaccines are used to avoid toxoplasmosis [11]. They can lead to some success, but toxoplasmosis prevention needs a better strategy. The epitope-oriented vaccines using various epitopes instead of whole antigens can prevent bacterial and viral infections [12]. Nowadays, the vaccines with base of epitope, which use variant epitopes instead of complete antigens, are proposed to prevent bacterial and viral infections [12]. In fact, these vaccines, which stimulate both humoral and cellular immunity, able to create preservative immunity against infections [13]. Thus, the recognition and isolation of efficient epitopes that motivate the immune system is considered an important step in the development of epitope-based vaccines. The most important household proteins, known as calcium-dependent protein kinases (CDPKs) are a kind of threonine/serine kinases that are present in protists and plants, such as apicomplexan parasites, and ciliates [14]. There are different CDPKs in apicomplexan protists, particularly in *T. gondii*. The CDPKs as important $Ca2^{+}$ signaling mediators take part in outer membrane motility, parasite host cell egress, and cell invasion and division [15]. The CDPK family is a promising option for medications to treat *Toxoplasma* and a suitable option for designing vaccines [16]. In several studies, vaccination with CDPK1 [17], CDPK3 [18], CDPK2 [19], CDPK6 [20], and CDPK5 [21], and caused strong cellular and humoral responses and increased the survival time in mice. *T. gondii* CDPK1 protein is moderated among apicomplexans, included in critical biological function, counting the control of the parasite's life cycle at stages subordinate on microneme secretion [22]. CDPK2 plays a basic part in amylopectin digestion system. CDPK5 is significant in the calcium-dependent egress of parasites from host cells and vital for formation of latent steps in the mice brains [16]. CDPK3 is fundamental for the quick acceptance of parasite departure and the foundation of inveterate contamination in mice, and it is the key to the harmfulness of the parasite *in vivo* [23]. Prediction of epitopes is exceedingly vital to determine the immunogenicity of an antigen in vaccine design. Thus, bioinformatics instruments and online assets offer assistance to investigators in recognizing and predicting the possible epitopes of B- and T-cells [24]. Bioinformatics as an accurate, fast, and cost-effective science can merge biology and computers to analyze biological information. In recent times, numerous online devices and servers have been produced utilizing the bioinformatics science capable of isolating the best major histocompatibility complex (MHC)-II, MHC-I, and B-cell epitopes of antigenic cases [25]. Thus, we analyzed some important features of CDPK1, CDPK2, CDPK3, and CDPK5 proteins using different bioinformatics servers to design of a multiepitope peptide vaccine against *T. gondii*.

Materials and Methods

Ethics approval

This study was approved by the Ethical Committee of Shahed University (IR.SHAHED.REC.1402.139).

Collecting parasite protein sequences

The amino acid sequences related to the considered *T. gondii* proteins, such as CDPK1 (accession no., EPT31305.1), CDPK2 (accession no., EPT27057.1), CDPK3 (accession no., EPT27420.1), and CDPK5 (accession no., EPT26997.1) were collected from the National Center for Biotechnology Information (NCBI) database (https://www.ncbi.nlm.nih.gov/) in FASTA format. Subsequently, the protein antigenicity was then predicted via the ANTIGENpro server (http://scratch.proteomics.ics.uci.edu/).

Predicting linear B lymphocyte epitope

To predict the B-cell epitopes ABCpred server was applied. The sequences were input in the ABCpred server (https://webs.iiitd.edu.in/raghava/abcpred/ABC_submission.html) to recognize antigens that can actuate antibodies and B-cell response. To predict B-cell epitopes, this database uses an artifi-

cial neural network. The length of the window used for prediction was between 12 and 16, plus a threshold of 0.51. The epitope parts that were the same as the parts recognized by the final $CD8^+$ T-cells and were not causing allergies were chosen to make the final vaccine.

Predicting MHC-II and MHC-I epitopes

To determine these epitopes, the IEDB (http://tools.iedb.org/main/tcell/) server was applied to analyze amino acid sequences of CDPK1, CDPK2, CDPK3, and CDPK5. The primary MHC-I and MHC-II epitopes were considered the common outputs of these tools. The servers applied to predict MHC-II and MHC-I epitopes were adjusted according to their default settings. The prediction was done using Select full HLA reference set. Three peptides with top affinity to MHC molecules which had the minimum percentile ranks were chosen for each allele.

Vaccine designing

Using particular peptide linkers, the ultimate vaccine construct was planned via the eligible linear B lymphocyte (LBL), helper T-lymphocyte (HTL), and cytotoxic T-lymphocytes (CTL) epitopes. The epitopes were connected via the KK linker for both LBL and HTL and the AAY linker for CTL epitopes. An EAAK linker connected the 50S ribosomal protein L7/L12 (NCBI ID: P9WHE3) to the developed vaccine N-terminus as an adjuvant [26], which improves the strength of the immune reactions to a specific antigen.

Physiochemical effects, solubility, allergenicity, and antigenicity prediction of the developed vaccine

The physicochemical factors, like molecular weight, the number of amino acids, atomic composition of amino acid, theoretical isoelectric point (pI), extinction coefficients, grand average of hydropathicity (GRAVY), estimated half-life, instability, and aliphatic index, and of developed vaccine construct were assessed using the ProtParam web-server (http://web.expasy.org/protparam/). We can determine the protein stability using the protein instability index. If the stability index is 40 or higher, it means the protein is not stable. The ANTIGENpro server was employed to predict the vaccine's efficacy based on its antigenicity identification. ANTIGENpro assesses antigenicity of proteins by a sequence-based and alignment-free approach. This can be regarded as the primary predictor of the protein antigenicity by reactivity data resulting from protein microarray analysis (http://scratch.proteomics.ics.uci.edu/explanation.html#ANTIGENpro). Assessing whether a protein possesses the potential to elicit an allergic reaction by stimulating a detrimental immune response was accomplished through the utilization of the AllergenFP server. Moreover, SOLpro (http://scratch.proteomics.ics.uci.edu/) anticipated solubility.

Predicting secondary and tertiary structure and refinement

The developed vaccine construct secondary structure was determined by PSIPRED (http://bioinf.cs.ucl.ac.uk/psipred/) online tool. PSIPRED is a common server to predict protein secondary structure, using two feedforward neural networks for the analysis of PSIBLAST-produced protein output with high accuracy. Throughout the analysis, we maintained the server settings in their default values.

Using SWISS-MODEL (https://swissmodel.expasy.org/), the tertiary structure of the vaccine was designed, and then the anticipated model underwent refinement by the GalaxyRefined server (https://galaxy.seoklab.org/cgi-bin/submit.cgi?type=REFINE). The Ramachandran plot and the SWISSMODEL program (https://swissmodel.expasy.org/assess) validated the three-dimensional (3D) proposed model. A Ramachandran plot shows high-energy areas for backbone dihedral angles against amino acid residues in a protein structure (https://swissmodel.expasy.org/assess/help). The model overall quality illustrated as an X-ray, NMR plot, Z-score, and local quality plot was evaluated using the ProSA-web server (https://prosa.services.came.sbg.ac.at/prosa.php).

Reverse translation and codon optimization

The amino acid sequence was back translated into nucleotide sequence using reverse translate tool of Sequence Manipulation Suite server (https://www.bioinformatics.org/sms2/rev_trans.html). Then, the Java Codon Adaptation Tool (JCat) server performed codon optimization to increase the generation of the developed vaccine construct in a proper expression system [27]. This phase is done because the genetic code possesses several options to encode each amino acid. The vaccine construct coding sequences were optimized for codon for protein expression in the *Escherichia coli* (strain K12) host. The percentage of GC content codon and the adaptation index (CAI) are obtained from the JCat server. The appropriate CAI score is 1.0; nonetheless, a score of ≥0.8 is regarded as good, while the GC content is from 30% to 70%.

Results

Antigenic prediction and protein retrieval

For *T. gondii* antigens, CDPK1, CDPK2, CDPK3, and CDPK5,

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with lengths of respectively 582, 711, 537, and 682 amino acids, were prepared. The antigenicity assessment using the ANTIGENpro server approved their antigenic nature to be >0.5% threshold with corresponding values of respectively 0.7660, 0.6068, 0.7600, and 0.6067 for CDPK1, CDPK2, CDPK3, and CDPK5.

B-cell prediction

The ABCpred server predicted B-cell epitopes. We used several conserved genomic sequences as input templates approved by the transmembrane property test. From the produced epitopes, merely 3 B-cell epitopes from each antigen were selected for further studies (Table 1).

MHC-II and MHC-I epitope prediction

The prediction results of MHC-I and MHC-II epitopes are shown in Tables 2 and 3, from which the three best epitopes from each antigen were considered for vaccine design.

Developing multi-epitope vaccine

The ultimate vaccine construct contained 36 immunogenic epitopes (12 HTL, 12 CTL, and 12 LBL) joined via suitable linkers. Moreover, the 50S ribosomal protein L7/L12 (adjuvant) was mixed with the construct's N-terminal to increase the immunogenicity. The EAAAK linker linked the adjuvant to the epitope sequence. The diagram in Fig. 1 illustrates the design of the final vaccine.

Table 1. ABCpred server identified a B-cell epitope from the CDPKs protein

	Rank	Sequence	Start position	Score
CDPK1	1	HEWIQTYTKEQISVDV	380	0.94
	2	STLGGAAGEPRSRGHA	6	0.89
	3	SSTELATIFGVSDVDS	534	0.89
CDPK2	1	SGEIYWEPIPTNRVVT	75	0.94
	2	DADGDGHITFDEFCGL	658	0.94
	3	TGSIDYTEFIAACLHQ	592	0.94
CDPK3	1	MGSKLTTLEETKELTQ	373	0.93
	2	TAYYIAPEVLRKKYDE	239	0.92
	3	TKRDSMPMTPGMYITQ	52	0.91
CDPK5	1	ATRCSTPCNMFSGRNT	52	0.94
	2	GFCVSTPRTEKASATR	39	0.94
	3	AAAVGEGNTKRGLDDF	76	0.91

CDPK, calcium-dependent protein kinase.

Table 2. Prediction of MHC-I binding epitopes

	Rank	Start	End	Length	Peptide	Score
CDPK1	1	478	486	9	VEHEVDQVL	0.98
	2	156	164	9	RQVLSGITY	0.98
	3	207	216	10	KMKDKIGTAY	0.97
CDPK2	1	485	493	9	AEQALRHAW	0.99
	2	95	103	9	TSSVIENVW	0.98
	3	284	292	9	QEIEIMKSL	0.98
CDPK3	1	181	189	9	KQVLSGTTY	0.98
	2	429	437	9	IEAEVDHIL	0.98
	3	324	332	9	AEEALNHPW	0.97
CDPK5	1	667	675	9	SPLARARAL	0.99
	2	477	486	10	RPERPIPARL	0.98
	3	183	191	9	SPQPEAPVF	0.98

MHC, major histocompatibility complex; CDPK, calcium-dependent protein kinase.

Table 3. Prediction of MHC-II binding epitopes

	Rank	Start	End	Length	Peptide	Score	Percentile rank
CDPK1	1	455	469	15	LIEGYKELMRMKGQD	0.98	0.01
	2	456	470	15	IEGYKELMRMKGQDA	0.98	0.01
	3	494	508	15	NGYIEYSEFVTVAMD	0.49	0.02
CDPK2	1	225	239	15	RSAFILANTGPITNY	0.91	0.01
	2	224	238	15	KRSAFILANTGPITN	0.92	0.01
	3	202	216	15	RRHSISTQAADEAAG	0.94	0.01
CDPK3	1	72	86	15	YQRVKKLGS	0.95	0.01
	2	385	399	15	FRQLDNNGD	0.98	0.01
	3	237	251	15	YYIAPEVLR	0.87	0.05
CDPK5	1	522	536	15	MLHDAFAALDTNADG	0.98	0.01
	2	524	538	15	HDAFAALDTNADGVL	0.98	0.01
	3	523	537	15	LHDAFAALDTNADGV	0.99	0.01

MHC, major histocompatibility complex; CDPK, calcium-dependent protein kinase.

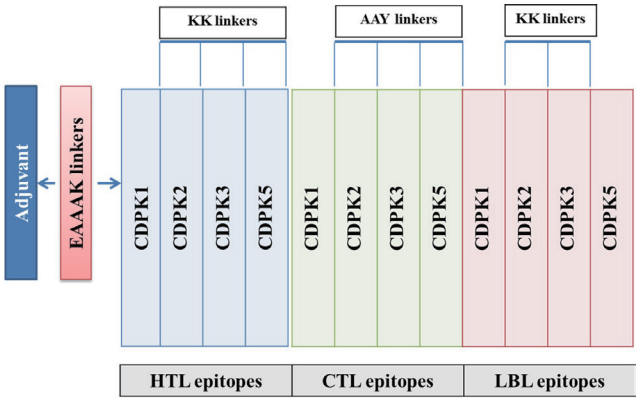

Fig. 1. A diagram showing the *Toxoplasma gondii* vaccine's multi-epitope design. From left to right, the candidate vaccine construct contains the adjuvant, helper T-lymphocyte (HTL), cytotoxic T-lymphocytes (CTL), and linear B lymphocyte (LBL).

Physiochemical effects, allergenicity, antigenicity, and solubility prediction of the developed vaccine

The vaccine's physicochemical characteristics can be seen in Table 4. The vaccine molecular weight was estimated as 74.66 kDa (theoretical pI=9.21). The vaccine instability index was predicted to be 30.37, indicating its stability. Also, the GRAVY score and aliphatic index were -0.467 and 75.51, respectively, indicating hydrophilic vaccine nature. Also, the vaccine's immunological potency was measured by the antigenicity score (0.9099). The construct was soluble and non-allergenic with a solubility score of 0.829940 (Table 4).

Table 4. Prediction of the physiochemical properties, antigenicity, allergenicity, and solubility

Characteristic	Finding	Remarks
No. of amino acids	680	Suitable
Molecular weight	74,668.52	High
Theoretical isoelectric point	9.21	
Chemical formula	$C_{3324}H_{5325}N_{907}O_{1002}S_{20}$	-
Extinction coefficient (at 280 nm in H20)	70,960	-
Estimated half-life (mammalian reticulocytes, *in vitro*)	30 hr	-
Estimated half-life (yeast, *in vivo*)	>20 hr	-
Estimated half-life (*Escherichia coli, in vivo*)	>10 hr	-
Instability index of vaccine	30.37	Stable
Aliphatic index of vaccine	75.51	Thermostable
Grand average of hydropathicity (GRAVY)	-0.467	Hydrophilic
Antigenicity	0.909992	Antigenic
Allergenicity	No	Non-allergen
Solubility	Soluble	0.611924

GRAVY, grand average of hydropathicity.

Secondary structure prediction

Alpha helices, random coils, and Beta strands were measured using PSIPRED server. The PSIPRED server could predict 60.29 alpha helices, 2.64% beta strands, and 37.05% random coils (Table 5).

Tertiary structure prediction, refinement, and validation

The SWISS-MODEL's online server determined and con-

Table 5. The secondary structure of the vaccine construct

Features	PSIPRED server	
	Amino acids	Percentage
Alpha helix	410	60.29
Beta strand	18	2.64
Random coil	252	37.05

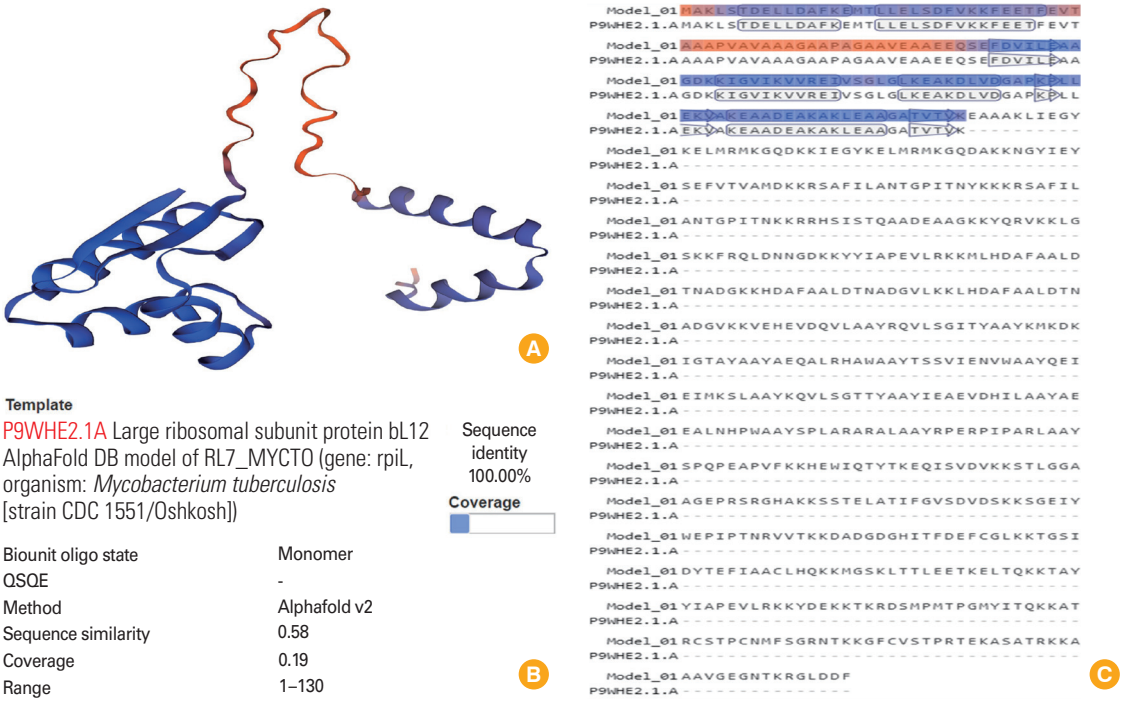

Fig. 2. Analysis of the three-dimensional (3D) structure created with SWISS-MODEL for the design of a vaccine for multiple epitopes. **(A)** Prediction of the 3D structure of multi-epitope vaccine construct. **(B)** Coverage and identity of the sequence. **(C)** Model-template alignment.

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structed the designed protein 3D structure. The SWISS-MODEL results for the designed protein found seven templates that matched the target sequence. So, we chose the model that had the highest similarity and coverage (Fig. 2). After the SWISS-MODEL made a model prediction, it was further refined via the Galaxy Refine server. Of the five generated refined models, model 2 was selected for further analysis since it has the lowest RSMD score (0.377), GDT-HA score (0.9654), MolProbity score (1.484), and a Rama favored region of 96.1, indicating a better quality than the raw model (Fig. 2B). The z-score indicating overall model quality was -4.11 and many residues were in the favored region. The 3D structure quality showed an improvement (z-score of -4.27) following refinement (Fig. 3A, B). According to protein validation prior to refinement, in the primary model, 84.38% of residues were in favored, 2.13% in allowed and 1.56% in outlier areas. Following the 3D model refinement, 96.09% of residues were in the favored, 0.78% in allowed, and 1.06% in outlier areas of the Ramachandran plot (Fig. 3C, D).

Codon optimization and *in silico cloning*

After reverse translation of the protein sequence, the codon adaptation was performed as per the *E. coli* K12 strain. The CAI-value and GC% of the initial sequence were estimated as 0.58 and 53.52%, respectively, whereas they were improved in the codon adapted sequence as per 1.0 and 40.08%, respectively.

Discussion

Toxoplasmosis is a big problem for both human society and the livestock industry [28]. So, developing an effective vaccine

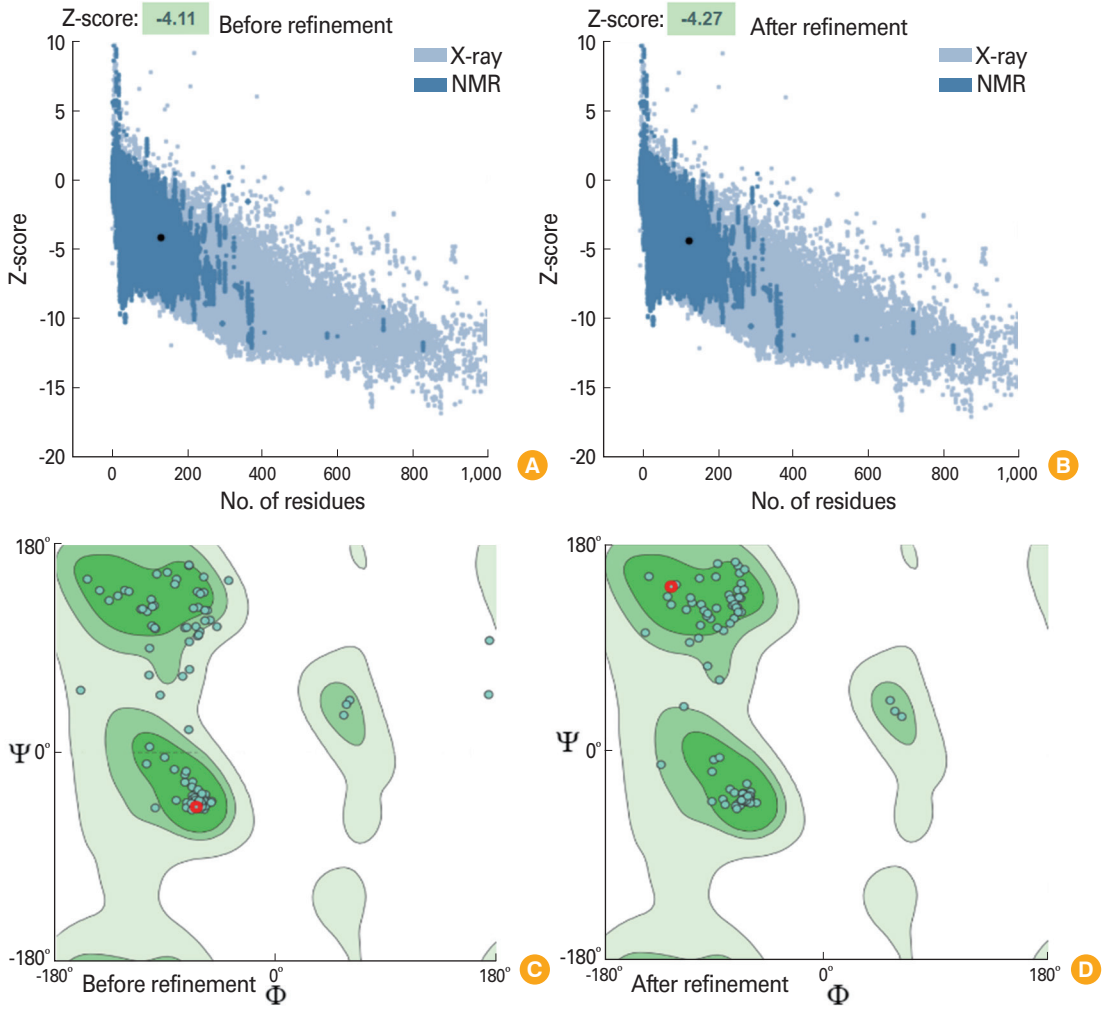

Fig. 3. Ramachandran plot validation of the multi-epitope vaccine design in a three-dimensional (3D) model. **(A, B)** The 3D structure of the predicted vaccine's z-score plot, both before and after ProSA-web server refinement. The z-score of the original model was -4.11; after refining, the result was -4.27. **(C, D)** Ramachandran plot-based multi-epitope vaccine design analysis.

to combat toxoplasmosis in both animals and humans remains a significant challenge for scientists [28]. Bioinformatics tools are now being used more for rational vaccine design. This is beneficial in various ways such as: (1) Properly targeting, long-lasting immunity with desirable polarity in cellular components; (2) time- and cost-effectiveness; and (3) eliminating unfavorable responses through epitope-based specific construct design. However, the *in-silico* results are only theoretical and need to be approved through experiments conducted in a laboratory [29]. Multi-epitope vaccines are gaining considerable attention in the field of vaccine development owing to their multiple benefits. These benefits include being very specific, safe, stable, and easy to produce and store [26]. We used a robust immunoinformatics method to develop a new multi-epitope vaccine for *T. gondii*. The amino acid sequences related to the targeted proteins (CDPK1, CDPK2, CDPK3, and CDPK5) were obtained from the NCBI database. In order to determine their antigenic potential, the gathered sequences were submitted to the ANTIGENpro server. The proteins had scores indicating they could cause antigenicity, so they could trigger a strong reaction from the immune system in the body.

When *Toxoplasma* infection occurs, dendritic cells, neutrophils, and macrophages migrate to the infection site. They sense the parasite primarily through toll-like receptors and release interleukin (IL)-12, a Th1-type cytokine, which further activates T $CD4^+$ and $CD8^+$ cells. Additionally, they induce the secretion of interferon-γ, another Th1-type cytokine, by natural killer cells. The latter stops the parasite from multiplying inside the host cells and from spreading the infection further. Furthermore, in order to mount a particular humoral response, IL-4, a Th2-type cytokine, would be an essential marker of B-cell promotion and differentiation [30]. The possible linear T- and B-lymphocyte epitopes were anticipated and HTL ($CD4^+$) activated the plasma B-lymphocytes creating memory and antibody B-lymphocytes. It moreover enacts the CTL ($CD8^+$) and macrophages that destroy the infectious agents or targeted antigens [31]. MHC I and MHC II should access epitopes to create the required immune reaction [32]. The B-lymphocyte epitopes are critical to activate the cells of the immune system and actuating humoral and antibody-mediated immunity. LBL (n=12), CTL (n=12), and HTL (n=12) epitopes that could pass the toxicity, allergenicity, and antigenicity tests were selected in the ultimate vaccine development. The multi-epitope vaccine was created by connecting 12 CTL, 12 HTL, and 12 LBL epitopes using the AAY, KK, and KK linkers, respectively. These linkers are vital components in vaccine development as they aid in the expression, folding, and stability of the vaccine [33]. The AAY linker conjugated CTL epitopes to affect protein stability by giving the proteasomal cleavage location; decreasing immunogenicity and improving epitope presentation [34]. LBC and HTC epitopes were conjugated by KK (bi-lysine) to protect the independent immunogenic activities of epitopes [35]. The multi-epitope vaccine developed an adjuvant because it can improve vaccine stability, antigen immune reaction, and longevity than T- and B-cell epitopes with no adjuvant [36]. The adjuvant was 50S ribosomal protein L7/L12 as it can enhance the vaccine immunogenicity and is connected to the protein sequence by the EAAAK linker, which is inflexible and negligibly meddling with the vaccine [37]. The final vaccine had 680 amino acids that could be easily mass-produced and synthesized.

According to the allergenicity and antigenicity results, the vaccine construct was nonallergenic and antigenic and could be used. The vaccine construct molecular weight was 74 kDa showing the vaccine's optimal molecular weight (approximately >40–50 kDa). In fact, little molecules are expelled from tissues by the blood, although proteins increase the efficiency of lymphatic absorption by increasing molecular weight. In this way, the contact with B and T lymphocytes within the lymph nodes increases [37]. Thus, the vaccine construct solubility was predicted by a solubility evaluation tool for determining the construct solubility in the *E. coli* host, which was confirmed (0.611). An aliphatic index of 75.51 (>70) recommends thermostability over a wide temperature range [38]. The protein structure conformation is maintained by the protein α-helices and β-turns with high hydrogen bond energy enabling favorable interactions with antibodies [38]. The SOPMA server showed 11.76% β-strands, 53.09% α-helices, and 25.74% random coils for vaccine secondary structure. It had 37.05% random coils, 2.64% β-strands, and 60.29% α-helices predicted by the PSIPRED server. The high ratio of β-strands and α-helices indicates high-energy hydrogen bonding, maintaining the protein structure and strong interactions between antibodies. Nonetheless, a linear sequence is not sufficient to predict the complexes and immunogenicity of MHC and T-cell receptors, associated with the protein spatial structure; hence, the SWISS-MODEL server predicted the 3D structure. Structure prediction is useful to determine the structure with a close association with the vaccine protein structure, and refinement of the 3D structure can help obtain parity with experimental accuracy [39]. Galaxy refinement can repackage and reconstruct side chains within the 3D model, improving the quality of molecu-

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lar dynamics simulations [40]. The 3D structure validation using Ramachandran plot analysis on the SWISS-MODEL indicated locating 96.09% of the amino acid residues in the most desirable area.

The strengths of our study are using robust immunoinformatics methods and updated immunological information critical to designing a vaccine. The LBL, CTL, and HTL epitopes were used to develop the vaccine with an N-terminal adjuvant, and based on immunoinformatics assessment, the vaccine construct was satisfactory regarding antigenicity, toxicity, allergenicity, and physicochemical effects. Immune simulation measurements have demonstrated the capacity to trigger and cause a broad and strong immune response. Although our research was according to an integrated computational pipeline, no *in vitro* and *in vivo* assessment is an important limitation. Thus, the vaccine constructs a protective effect for *T. gondii* infection and its safety need to be verified by more experimental studies.

In conclusion, we designed a potential multiepitope vaccine for *T. gondii* with different immunoinformatics tools. It showed satisfactory physiochemical properties, antigenicity evolution, toxicity, allergenicity, immunogenicity, and solubility. Future studies are needed to indicate the vaccine constructs protective efficacy against *T. gondii* infection and their safety. Further assessment using antigens based on biological criteria is needed.

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