

An immunoinformatics approach for the design of a multi-epitope subunit vaccine for urogenital schistosomiasis

Olugbenga S. Onile¹, Adeyinka I. Fadahunsi¹, Ameerah A. Adekunle¹, Bolaji F. Oyeyemi² and Chiaka I. Anumudu³

¹ Biotechnology Programme, Department of Biological Sciences, Elizade University, Ilara-Mokin, Ondo State, Nigeria

² Molecular Biology Group, Department Science Technology, The Federal Polytechnic, Ado-Ekiti, Ado-Ekiti, Ekiti State, Nigeria

³ Cellular Parasitology Programme, Department of Zoology, University of Ibadan, Ibadan, Oyo State, Nigeria

ABSTRACT

Discovery of T and B memory cells capable of eliciting long-term immunity against schistosomiasis is important for people in endemic areas. Changes in schistosomes environment due to developmental cycle, induces up-regulation of Heat Shock Proteins (HSPs) which assist the parasite in coping with the hostile conditions associated with its life cycle. This study therefore focused on exploring the role of HSPs in urogenital schistosomiasis to develop new multi-epitope subunit vaccine against the disease using immunoinformatic approaches. The designed subunit vaccine was subjected to in silico antigenicity, immunogenicity, allergenicity and physicochemical parameters analysis. A 3D structure of the vaccine construct was predicted, followed by disulphide engineering for stability, codon adaptation and in silico cloning for proper expression and molecular protein-protein docking of vaccine construct in the vector against toll-like receptor 4 receptor, respectively. Consequently, a 493 amino acid multi-epitope vaccine construct of antigenicity probability of 0.91 was designed. This was predicted to be stable, non-allergenic in nature and safe for human use.

Submitted 6 November 2019

Accepted 25 February 2020

Published 2 October 2020

Corresponding author

Olugbenga S. Onile,
olugbenga.onile@elizadeuniversity.edu.ng

Academic editor

Antonella Prisco

Additional Information and
Declarations can be found on
page 17

DOI 10.7717/peerj.8795

© Copyright

2020 Onile et al.

Distributed under

Creative Commons CC-BY 4.0

OPEN ACCESS

Subjects Bioinformatics, Computational Biology, Parasitology, Immunology, Infectious Diseases

Keywords Immunoinformatics, Urogenital schistosomiasis, Vaccine, Heat shock protein, Multi epitopes

INTRODUCTION

Urinary schistosomiasis has been identified as the main cause of urogenital disease, and the main cause of bladder cancer in developing countries (Onile *et al.*, 2016; Rollinson *et al.*, 2013). In 76 countries, more than 200 million people are estimated to be affected by urogenital schistosomiasis with more than 100 million being urogenital schistosomiasis. In Sub-Saharan Africa, mortality rate of the disease is estimated at 250,000 persons per year (Masamba *et al.*, 2016). In the year 2008, estimated cases of 29 million infected persons were reported in Nigeria which amount to the largest number of infections in Africa (Adenowo *et al.*, 2015) and may have increased (Abdulkadir *et al.*, 2017). Bergquist (2013) reported the spread of the disease to regions that were previously non-endemic.

These include reported cases of urinary schistosomiasis in adolescent refugee from Gambia (*Poddighe et al., 2016*) and 214 male migrant patients *Milesi et al. (2019)* all in Italy. *Uysal et al. (2014)* also reported a rare case of a 22-year-old Nigerian with urinary schistosomiasis in Turkey. Causes of several form of bladder pathologies and tumours in Africa including Nigeria has been linked to urogenital schistosomiasis (*Adebayo et al., 2017; Barsoum, 2013; Onile et al., 2016*). Praziquantel has been the most used drug for treating schistosomiasis since the early 2000s (*Chevalier et al., 2016; Doenhoff, Cioli & Utzinger, 2008*), but the drug has been found to be less effective against the worm in all stages of its life cycle and would not eliminate immature worms within 3–4 weeks of infection (*Alsaqabi & Lotfy, 2014; Aragon et al., 2009; Cioli et al., 2014; Doenhoff, Cioli & Utzinger, 2008*). Also, several cases of drug resistance to praziquantel due to pressure from continuous mass drug administration have now been reported (*Couto et al., 2011; Fallon et al., 1996; Melman et al., 2009*).

By transiting between intra-mammalian, aquatic and snail stages to develop into full maturity, schistosomes face a number of hostile environments throughout their lifecycle (*Mbah et al., 2013*) and heat shock responses have been associated with cellular stress during the movement of parasite from a cooler, low saline and freshwater environment to a warmer, saline environment of a human host (*Devaney, 2006; Mbah et al., 2013*).

Heat shock proteins (HSP) are an important group of molecules involved in a range of biological and developmental processes in schistosomes and other parasites (*Braschi et al., 2006*). Hsp 90 and other HSPs are chaperone proteins that assist proper folding of other proteins. They stabilize proteins against heat stress, and also aid protein degradation. Other functions include stabilizing plethora of proteins required for tumour growth; therefore HSP inhibitors are being investigated as vaccines and anti-cancer drugs (*Das et al., 2019*). Identification of HSPs in cercarial gland secretions and the highest abundance of the protein transcripts in newly transformed schistosomula have been reported (*Ishida & Jolly, 2016*). The role of heat shock response in snail susceptibility to *Schistosoma* infection in the intermediate molluscan host has also been emphasized (*Ishida & Jolly, 2016*). *Onile et al. (2017)*, had reported several HSPs as possible biomarkers for the diagnosis of urinary schistosomiasis, they are also known to play critical roles in innate immunity and adaptive immunity. HSPs can activate specific toll-like receptors (TLRs), provide polypeptides for specific triggering of the acquired immune response and, play a major role in cross-presentation of extracellular antigens. This would result in the induction of CD8+ cytotoxic T-lymphocyte responses (*McNulty et al., 2013*). Antigen presenting cells (APCs) are known for recognizing a wide range of molecular patterns expressed by pathogens called pathogen-associated molecular patterns (PAMPs) during innate immune responses (*El-Din, 2016; Venugopal, Nutman & Semnani, 2009*). TLRs and NOD-like receptors through signaling pathways that induce upregulation of IFNs and other inflammatory cytokines are used by APCs to recognize these PAMPs (*Venugopal, Nutman & Semnani, 2009*). *Zhang et al. (2011)* had reported mice with deficient TLR after infection with *S. japonicum* showed increasing level of parasite egg load.

Heat shock proteins were chosen in this study as target for the development of a new multi-epitope subunit vaccine against urinary schistosomiasis for their role in *Schistosoma* biology.

METHODOLOGY

Retrieval of *Schistosoma haematobium* HSP sequence for vaccine construction

The amino acid sequences of *S. haematobium* HSPs were retrieved from the NCBI protein database (www.ncbi.nlm.nih.gov) and subjected to multiepitope vaccine designing. To test for the proteins ability to induce immune response within a host body, all the retrieved protein sequences were subjected to an antigenicity test using the ANIGENpro database (scratch.proteomics.ics.uci.edu). Antigenic probability of ≥ 0.8 was used to determine which proteins to be chosen for the next step of the multi epitope vaccine construct (Pandey, Bhatt & Prajapati, 2018).

Prediction of cytotoxic T-lymphocytes and helper T-lymphocytes epitope and immunogenicity

To get an immunogenic cytotoxic T-lymphocytes (CTL) epitope with potential of inducing cell mediated immunity and form memory cells, all the three highly antigenic HSP sequences in FASTA format were fed into the NetCTL 1.2 server (<http://www.cbs.dtu.dk/services/NetCTL/>) to predict the CTL epitope at threshold score of 0.75 (using the default setting). The server predicts CTL epitopes from inputted protein sequences based on the training dataset (prediction of MHC-I binding peptides, proteasomal C-terminal cleavage, and Transporter Associated with Antigen Processing (transport efficiency) and only epitopes with combined score greater than 0.75 were selected as CTL epitopes (Khatoon, Pandey & Prajapati, 2017; Pandey, Bhatt & Prajapati, 2018). The selected CTL epitopes (Scores > 0.75) were further analysed in Immune Epitope Design Database (www.iedb.org) for MHC class I immunogenicity prediction. The immunogenicity score determines the probability of eliciting an immune response (higher score means greater probability of immune response and vice versa) (Pandey, Bhatt & Prajapati, 2018).

The helper T-Lymphocytes (HTL) epitopes of 15mer length for mouse allele (H2-IAb, H2-IAd and H2-IEd) were predicted for the HSPs of *S. haematobium* using the Immune Epitope Design Database. The output epitopes were ranked based on lower percentile rank scores that is, the lower the ranked score the higher the binding affinity for HTL receptors (Khatoon, Pandey & Prajapati, 2017). Only epitopes with higher binding affinity (≤ 1.5) with MHC-II were selected for the final multi-epitope vaccine construct. In order to determine the ability of the predicted epitopes in activating Th1 type immune response followed by IFN- γ production, the top 17 HTL epitopes were subjected in FASTA format to the IFN epitope server by using the Motif and SVM hybrid as approach selection and IFN- γ versus other cytokine as model of prediction.

Construction of multi-epitope subunit vaccine

To design a viable vaccine construct capable of inducing innate and adaptive immune response, the high scoring CTL and HTL epitopes from the present immunoinformatics predictions were used. These epitopes were joined together using AAY and GPGPG linkers which were added at the intra-epitope position thereby linking the CTL and HTL epitopes respectively (*Khatoon, Pandey & Prajapati, 2017*). Also, a TLR-4 agonist (RS-09; Sequence: APPHALS) was used adjuvant (*Pandey, Bhatt & Prajapati, 2018; Shanmugam et al., 2012*) and linked with the multi-epitopes (CTL and HTL) using EAAAK linker (*Lee et al., 2014*).

B cell epitopes prediction for *S. haematobium* proteins

The BCPREDS server (ailab.ist.psu.edu/bcpred) was used to predict the linear B-cell epitopes for the final vaccine construct. The 493 amino acid sequence of the final vaccine construct was inputted into the BCPREDS server in plain format followed by the selection of a fixed length epitope prediction method and length of the epitope. The default method of BCPREDS was selected as the prediction method for the amino acid epitopes (*El-Manzalawy, Dobbs & Honavar, 2008; Pandey, Bhatt & Prajapati, 2018*). The specificity threshold was set as default at 75% and conformational epitopes were predicted using the ElliPro server for the tertiary protein structure of the vaccine construct (*Pandey, Bhatt & Prajapati, 2018*).

Prediction of antigenicity, allergenicity and physiochemical parameter of vaccine construct

The potential of the vaccine construct in eliciting immunological response through binding to the B and T cell receptor was determined by first determining the antigenicity of the vaccine construct. The ANTIGENpro server (scratch.proteomics.ics.uci.edu); a sequence-based alignment free and pathogen independent predictor was used to generate the antigenicity index (*Khatoon, Pandey & Prajapati, 2017*).

The non-allergenic potential of the multi-epitope vaccine construct was determined using the AllerTOP v.2.0 server (www.ddg-pharmfac.net/AllerTOP). Several physiochemical parameters of the vaccine construct which include amino acid composition, the theoretical pI, instability index, in-vitro and in-vivo half-life, aliphatic index, molecular weight and grand average of hydropathicity (GRAVY) parameters were all assessed using the ProtParam server (<https://web.expasy.org/protparam/>) (*Onile, 2014*).

Prediction, refinement and validation of the tertiary structure of vaccine construct

The 3D structure of the vaccine construct was predicted using RaptorX structure prediction server (raptorx.uchicago.edu/). This server predicts secondary and tertiary structures as well as contact map, solvent accessibility, disordered regions and binding sites of protein sequences. RaptorX server also assign some confidence scores *P*-value, GDT (global distance test) and uGDT (un-normalized GDT), and modeling error at each

residue to indicate the quality of a predicted 3D mode. In order to improve and refine the predicted 3D model of vaccine construct, the output model of RaptorX server was further modelled using GalaxyRefine server (<http://galaxy.seoklab.org/>) (Heo, Park & Seok, 2013). This server uses CASP10 based refinement approach to reconstruct and repack the protein side chain, followed by dynamics simulation to relax the structure. ProSA-wed (<https://prosa.services.came.sbg.ac.at/prosa.php>) was employed in the tertiary structure validation; also the refinement output was validated using Ramachandran plot analysis (Mordred.bioc.cam.ac.uk/~rapper/rampage.php).

Disulfide engineering for vaccine stability

The Disulfide by Design v2.12 server was used for the in silico disulfide engineering of the refined model 3D structure of the vaccine construct. The refined model was first run to determine residue pairs useful for the purpose of disulphide engineering. Only residue pairs with energy (kcal/mol) less than 2.2 and χ^3 (χ^3) between -87 and $+97$ degree were selected for mutation (Rana & Akhter, 2016). A total of 5 residue pairs were eventually selected for mutation by using the create mutate icon of the Disulfide by Design server.

Codon adaptation and in silico cloning

To determine and optimise the expression rate of the vaccine construct in a proper expression vector, the primary sequence of the vaccine construct was inputted into the Java Codon Adaptation Tool (JCAT) for codon optimization which was performed in the host *Escherichia coli* strain K12. Due to difference in codon usage of *E. coli* and native host *S. haematobium* from where the vaccine construct sequences originated, three different options in the JCAT server were selected which include: avoid rho-independent transcription, prokaryotes ribosome binding site and restriction enzymes cleavage sites. To determine the high-level of protein expression, the output of the JCAT which include codon adaptation Index and percentage GC content were used. *E. coli* pET-28(+) was used as vector to clone the adapted nucleotide sequence (provided by JCAT server) of the final vaccine construct using the restriction cloning module of SnapGene tool (Ali et al., 2017).

Molecular docking of vaccine construct with TLR4

The ClusPro 2.0 server was used for the molecular protein–protein docking to check for the binding affinity between the vaccine construct and TLR-4 receptor (Kozakov et al., 2017; Pandey, Bhatt & Prajapati, 2018). The refined sub unit multi-epitope vaccine protein and TLR-4 PDB file (4G8A) obtained from the RCSB-Protein Data Bank were used as the ligand and receptor respectively.

RESULTS

Retrieved *S. haematobium* HSP sequences for vaccine construct

To design the multi-epitope subunit vaccine for urinary schistosomiasis consisted of seven (7) HSP sequences (HSP90 (KGB37337.1), HSP11 (XP_012799478.1), HSP97

Table 1 Retrieved *S. haematobium* Heat shock protein accession numbers from the NCBI database and their antigenicity scores.

Serial no.	Protein accession no.	Protein name	Antigenicity scores	Selected/non-selected
1	KGB40455.1	60 kDa Heat shock protein	0.669986	Non-selected
2	KGB38609.1	75 kDa Heat shock protein	0.665038	Non-selected
3	KGB41273.1	10 kDa Heat shock protein	0.608315	Non-selected
4	KGB37622.1	Heat shock protein 83	0.665191	Non-selected
5	KGB34481.1	97 kDa Heat shock protein	0.926815	Selected
6	XP_012799478.1	Heat shock protein beta-11	0.895751	Selected
7	KGB37337.1	Activator of 90 kDa Heat shock protein ATPase 1	0.858776	Selected

([KGB34481.1](#)), HSP75 ([KGB38609.1](#)), HSP83 ([KGB37622.1](#)), HSP10 ([KGB41273.1](#)), HSP60 ([KGB40455.1](#)) were retrieved from the NCBI database (<https://www.ncbi.nlm.nih.gov>) in FASTA format. Only three (HSP11, HSP97, HSP90) proteins had an antigenic probability of ≥ 0.8 after retrieved protein sequences were analyzed using ANTIGENpro for antigenicity prediction ([Table 1](#)), consequently they were selected for the vaccine construct.

Predicted CTL, HTL epitope and immunogenicity

By using the NetCTL 1.2 server for prediction of the CTL receptor specific immunogenic epitopes, a total of 23 CTL epitopes (9mer length) were obtained for the inputted three HSP protein sequences and only 12 epitopes with high immunogenicity scores as obtained from IEDB server were chosen as the final CTL epitopes to undergo vaccine designing ([Table 2](#)).

The prediction of the HTL epitope was carried out using the IEDB MHC-II epitope prediction module, all three protein sequences were subjected to the module. The mouse alleles used for prediction were H2-1Ad, H2-1Ed and H2-1Ab. However, only 17 HTL epitopes with lowest percentile with ≤ 1.5 with MHC-II were selected and subjected for the final multi-epitope vaccine construct ([Table 3A](#)). Also, all the 17 HTL epitopes have the capacity of inducing IFN- γ , and this was evident from the positive score obtained from the IFN epitope server output ([Table 3B](#)).

Constructed multi-epitope subunit vaccine

The constructed multi-epitope subunit vaccine consisted of 12 CTL epitopes and 17 HTL epitopes with high binding affinity fused together with the help of AAY, GPGPG and EAAAK linkers. The adjuvant and the CTL epitopes were combined by the EAAAK linkers, the intra-CTL and intra-HTL epitopes were joined by intra-CTL AAY and intra-HTL GPGPG linker respectively to form the final vaccine constructs composed of 493 amino acid residues. For maximum ability of the candidate vaccine to elicit adequate immune response, adjuvant and CTL epitopes were joined together using an EAAAK linker ([Fig. 1A](#)).

Table 2 Predicted cytotoxic T-lymphocyte (CTL) epitopes and their immunogenicity scores for *S. haematobium* as obtained from the immune epitope database.

S/N	Accession ID	Epitopes	Scores	Length	Immunogenicity scores	Selected/non selected
1	KGB34481.1	LDMTEEWLY	0.7760	9	0.35427	Selected
		QTEEIDGTL	0.9769	9	0.33501	Selected
		SIAAGEPTY	0.8120	9	0.19264	Selected
		SQAQLIEEY	1.3793	9	0.15362	Selected
		NSKNAVEEY	1.7785	9	0.14345	Selected
		FTVIEQCLY	2.7477	9	0.0838	Selected
		QLEDMIVQY	1.8465	9	-0.02374	Non-selected
		VTDIVSQQQ	0.8464	9	-0.13985	Non-selected
		FTEPRKIKL	0.7619	9	-0.14494	Non-selected
		FTTKQLNEF	1.0480	9	-0.2746	Non-selected
		SIEVSNMQF	0.8128	9	-0.30903	Non-selected
		2	KGB39720.1	GSDGCFVSF	2.2740	9
3	XP_012797099.1	FSGNITGIF	1.0475	9	0.27699	Selected
		KLDGEANVY	2.2518	9	0.19429	Selected
		GSKIENDLY	2.0995	9	0.16757	Selected
		EVISLIDEY	0.7906	9	0.06843	Selected
		TSSTDGDLV	0.9172	9	0.0511	Selected
		WSDKDATGW	1.0050	9	-0.09881	Non-selected
		YTLLKEDY	1.1013	9	-0.1149	Non-selected
		WTSSTDGDL	0.9051	9	-0.11993	Non-selected
		LAQKNVPAY	0.9310	9	-0.20854	Non-selected
		FLAALKQTY	1.7104	9	-0.23681	Non-selected
AALKQTYGY	0.7829	9	-0.28018	Non-selected		

Note:

Only epitopes with Comb score >0.75 were selected for immunogenicity test and CTL epitopes (12) with high immunogenicity score (+ve) were selected and subjected to the vaccine designing.

Predicted B cell epitopes for *S. haematobium* proteins

A total of 14 epitopes having 0.99 and above probability score with 20mers length were selected as the linear B cell binding epitopes for the final vaccine construct (Table 4). Discontinuous epitopes of 125 amino acids were predicted from the final 3D model of vaccine construct with the score of 0.734 (Fig. 1B).

Predicted antigenicity, allergenicity and physiochemical parameter of the vaccine construct

The antigenicity test of the designed vaccine construct showed the antigenicity probability to be 0.905150, this represents the antigenic nature of the vaccine construct. An allergenicity test carried out for the vaccine construct also showed that the vaccine candidate is non-allergenic in nature.

The prediction of the physiochemical parameters of the vaccine construct was characterized by using the ProtParam server which evaluated for the following parameters;

Table 3 (A) Predicted helper T-lymphocyte (HTL) epitopes and their percentile ranks for *S. haematobium* as obtained from the immune epitope database. (B) Interferon gamma (IFN) Inducing capacity test for the predicted HTL epitopes for urinary schistosomiasis.

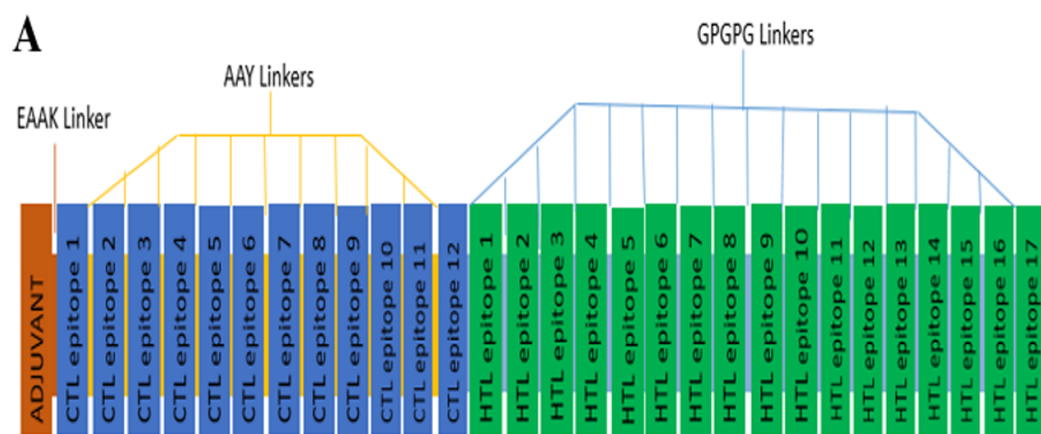
(A) Allele	Seq. no.	Start	End	Epitope	Method	Percentile rank*
H2-IAd	2	54	68	RKVSQIKIVTSRVKA	Consensus (smm/nn)	0.38
H2-IAd	2	53	67	PRKVSQIKIVTSRVK	Consensus (smm/nn)	0.47
H2-IAd	2	52	66	QPRKVSQIKIVTSRV	Consensus (smm/nn)	0.48
H2-IAd	3	435	449	RSLVRIRKVLDSIAA	Consensus (smm/nn)	0.64
H2-IAd	3	500	514	VSQQQAMESVCHPII	Consensus (smm/nn)	0.91
H2-IAd	3	498	512	DIVSQQQAMESVCHP	Consensus (smm/nn)	0.94
H2-IAd	3	499	513	IVSQQQAMESVCHPI	Consensus (smm/nn)	1.03
H2-IAd	3	434	448	NRSLVRIRKVLDSIA	Consensus (smm/nn)	1.04
H2-IAd	3	100	114	CAFQAAICSPAFKVK	Consensus (smm/nn)	1.05
H2-IAd	2	51	65	DQPRKVSQIKIVTSR	Consensus (smm/nn)	1.12
H2-IAd	3	433	447	FNRLVRIRKVLDSI	Consensus (smm/nn)	1.21
H2-IAd	3	99	113	GCAFQAAICSPAFKV	Consensus (smm/nn)	1.23
H2-IAd	3	432	446	NFNRLVRIRKVLDS	Consensus (smm/nn)	1.27
H2-IAd	3	98	112	RGCAFQAAICSPAFK	Consensus (smm/nn)	1.32
H2-IAd	1	214	228	YRVLTTKELVKAFTR	Consensus (smm/nn)	1.35
H2-IAd	1	289	303	TRLFLAQKNVPAYDL	Consensus (smm/nn)	1.37
H2-IAd	3	497	511	TDIVSQQQAMESVCH	Consensus (smm/nn)	1.4

(B) Serial no.	Epitope	Method	Result	Score
Epitope_1	RKVSQIKIVTSRVKA	MERCI	POSITIVE	1
Epitope_2	PRKVSQIKIVTSRVK	MERCI	POSITIVE	1
Epitope_3	QPRKVSQIKIVTSRV	SVM	POSITIVE	0.57919869
Epitope_4	RSLVRIRKVLDSIAA	MERCI	POSITIVE	6
Epitope_5	VSQQQAMESVCHPII	MERCI	POSITIVE	1
Epitope_6	DIVSQQQAMESVCHP	MERCI	POSITIVE	1
Epitope_7	IVSQQQAMESVCHPI	MERCI	POSITIVE	1
Epitope_8	NRSLVRIRKVLDSIA	MERCI	POSITIVE	8
Epitope_9	CAFQAAICSPAFKVK	MERCI	POSITIVE	1
Epitope_10	DQPRKVSQIKIVTSR	SVM	POSITIVE	0.56096058
Epitope_11	FNRLVRIRKVLDSI	MERCI	POSITIVE	9
Epitope_12	GCAFQAAICSPAFKV	MERCI	POSITIVE	1
Epitope_13	NFNRLVRIRKVLDS	MERCI	POSITIVE	8
Epitope_14	RGCAFQAAICSPAFK	MERCI	POSITIVE	2
Epitope_15	YRVLTTKELVKAFTR	MERCI	POSITIVE	11
Epitope_16	TRLFLAQKNVPAYDL	MERCI	POSITIVE	1
Epitope_17	TDIVSQQQAMESVCH	SVM	POSITIVE	0.50508595

Note:

* Only HTL epitopes with higher binding affinity (≤ 1.5) with MHC-II were selected for the final multi-epitope vaccine construct.

The molecular weight was 51409.68 kDa, theoretical pI 9.11, while the total number of negative and positive charge residues were 36 and 49 respectively. The estimated half-life was 4.4 h in mammalian reticulocytes in-vitro, 20 and 10 h in yeast and *E. coli* respectively.



B

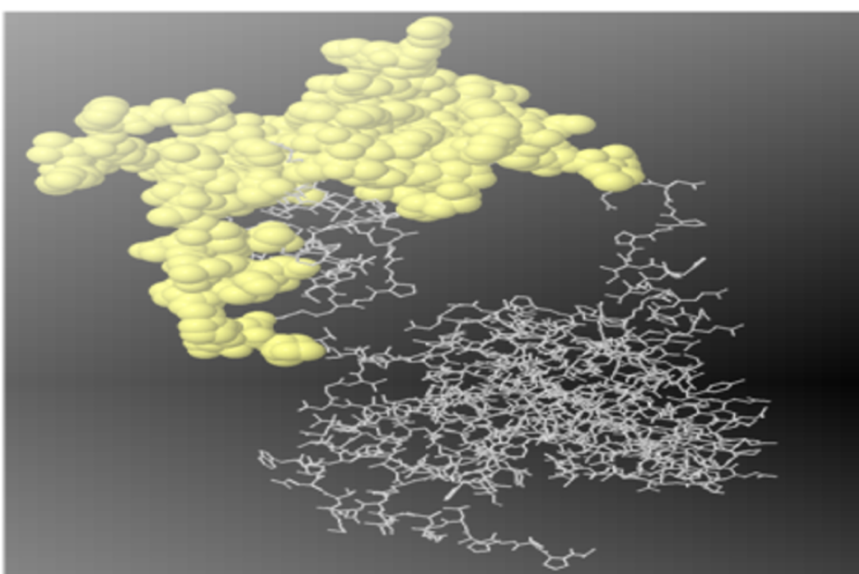


Figure 1 (A) **Diagram of final vaccine construct.** The multi-epitope vaccine sequence consisting of 493 amino acid residues; of which the adjuvant and CTL epitope has been joined by EAAK linker, where AAY and GPGPG linkers were used to join the CTL and HTL epitopes, respectively. (B) Conformation of B-cell epitopes (yellow colour) showing the sequence subunits composed of antigenic epitopes that will come in direct contact with immune receptor. [Full-size !\[\]\(0c0f8cc6eca4f663c17a652926046967_img.jpg\) DOI: 10.7717/peerj.8795/fig-1](https://doi.org/10.7717/peerj.8795/fig-1)

The extinction coefficient was found to be $36790 \text{ M}^{-1} \text{ cm}^{-1}$, at 280 nm measured in water assuming all pairs of cysteine residues are reduced, instability index was given to be 35.01. The value of the aliphatic index was 77.81 while the grand average of hydropathicity (GRAVY) was -0.138 .

Predicted, refined and validated tertiary structure of vaccine construct

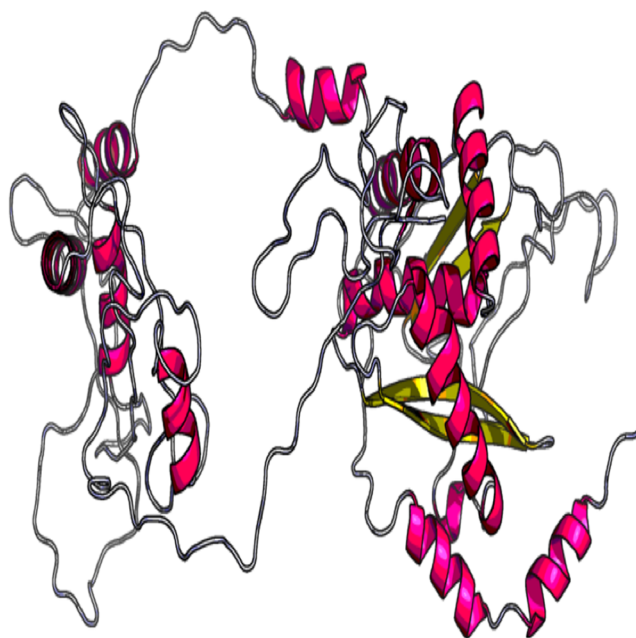
The 3D model and tertiary structure were predicted using RaptorX server (Fig. 2). The best template for the homology modeling was PDB ID: 5ja1A with 493 (100%) amino acid residues modelled as single domain with 4% disorder. The secondary structure information includes helix 31%, Beta sheet 17% and coiled structure 51%. To refine the vaccine construct, GalaxyRefine server was used. Out of all refined models, model 3 was

Table 4 B cell specific epitopes and their score as predicted and obtained from the immune epitope database.

S/N	Position	Epitope	Score
1	170	RVKAGPGGPRKVSQIKIVT	1
2	247	SVCHPIIGPGGDIVSQQQA	1
3	370	LDSIGPGGGCAFQAAICSP	1
4	328	PAFKVKGGPGDQPRKVSQI	1
5	285	AMESVCHPIGPGGNRSLVR	1
6	411	LDSGPGGRGCAFQAAICSP	1
7	191	RVKGGPGGQPRKVSQIKIVT	1
8	145	TSSTDGDLVGGPGRKVSQI	1
9	307	KVLDSIAGPGGCAFQAAIC	1
10	226	KVLDSIAAGPGGVSQQQAM	1
11	442	LTTKELVKAFTRGPGPGTRL	1
12	349	IVTSRGGPGGNRSLVRIRK	1
13	467	NVPAYDLGGPGTDIVSQQQ	1
14	54	IEEYAAYNKNAVEEYAAAYF	0.994
15	14	DMTEEWLYAAYQTEIDGTL	0.753

Note:

Only 20mers epitopes with equal and above 0.99 score were selected.

**Figure 2** Tertiary structure of predicted vaccine construct showing the helix, sheet and coiled region.

Full-size  DOI: 10.7717/peerj.8795/fig-2

selected as the best based on various parameters including GDT-HA (0.9239), RMSD (0.484), MolProbity (2.69), Clash Score (32.7) and Poor rotamers (1.9).

The refinement output was also validated using Ramachandran plot analysis, which revealed that 93.3% of the residue are located in most favoured regions, 5.3% in allowed

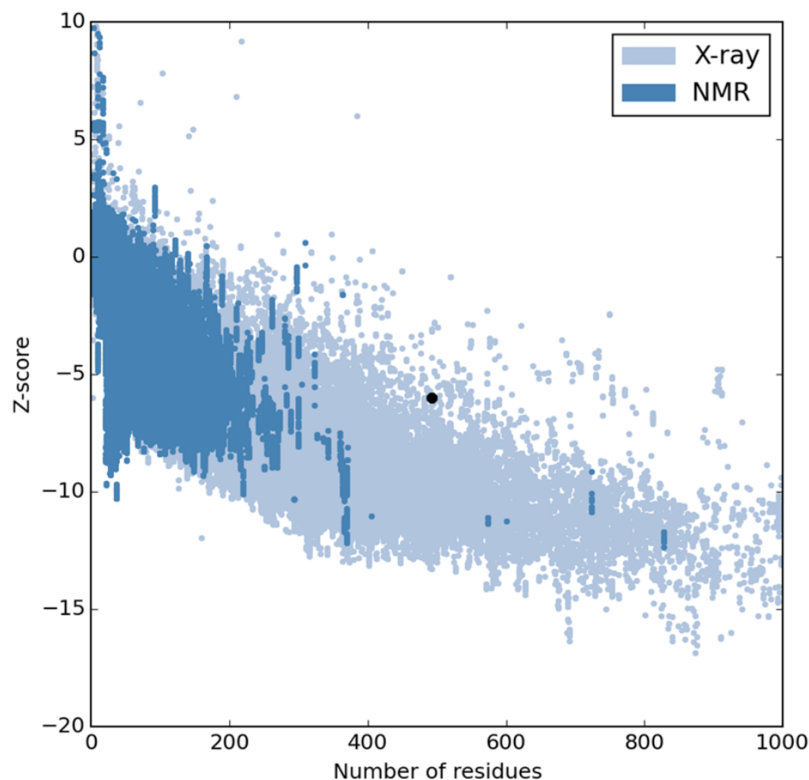


Figure 3 PROSA validation of 3D vaccine structure showing Z-score (-6.01).

Full-size  DOI: [10.7717/peerj.8795/fig-3](https://doi.org/10.7717/peerj.8795/fig-3)

regions and only 1.4% in outlier region and the quality and potential errors in the crude 3D as verified by ProSA-web showed Z-scores of -6.01 for ProSA (Figs. 3 and 4).

Disulfide engineering for vaccine stability

Disulfide engineering was done to stabilize the final model of the vaccine construct using Disulfide by Design v2.12. The result showed a total 58 pairs of residues that are useful for the purpose of disulphide engineering (Fig. 5) but 10 mutations were created from the only five pairs of residues (GLU56-CYS249, TYR60-HIS493, VAL116-GLN242, GLN243-GLU489) that were found with energy value less than 2.2 and Chi3 value range between -87 and +97 degree.

Codon adaptation and in silico cloning

The codon representing the subunit vaccine candidate was adapted to the codons of the *E. coli* K 12 strain using JCAT server and showed that the optimized codon sequence has a length of 1,450 nucleotides with a codon adaptation index (CAI) of 0.95 and average GC content of 54.56%. The adapted codon sequence was later inserted into pET28(+) vector to form the restriction clone (Fig. 6). The adapted codon sequences used in in silico cloning are made up of the following 1,400 nitrogenous bases “GCTCCGCCGCACGC TCTGTCTGAAGCTGCTGCTAAACTGGACATGACCGAAGAATGGCTGTACGCTG CTTACCAGACCGAAGAAATCGACGGTACCCTGGCTGCTTACTCTATCGCTGC

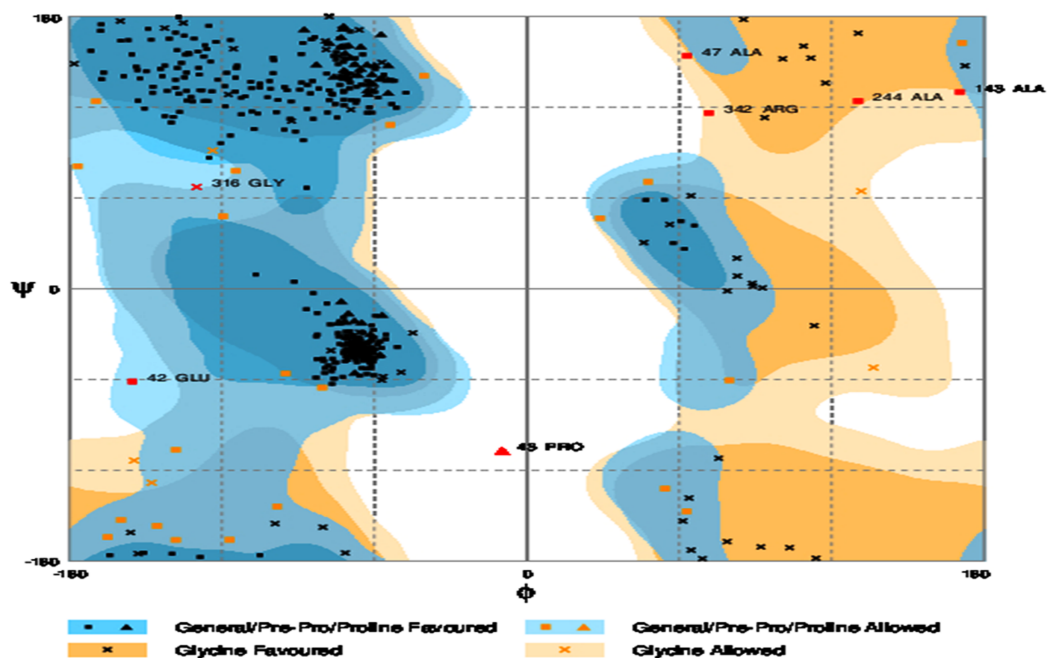


Figure 4 Ramachandran plot formation to the validated 3D modelled structure of vaccine protein. The plot showed 93.3% residues of vaccine protein in favoured region.

Full-size [DOI: 10.7717/peerj.8795/fig-4](https://doi.org/10.7717/peerj.8795/fig-4)

TGGTGAACCGACCTACGCTGCTTACTCTCAGGCTCAGCTGATCGAAGAATACG
 CTGCTTACAACCTCTAAAACGCTGTTGAAGAATACGCTGCTTACTTCACCGTT
 ATCGAACAGTGCCTGTACGCTGCTTACGGTTCTGACGGTTGCTTCGTTTTCTT
 TCGCTGCTTACTTCTCTGGTAACATCACCGGTATCTTCGCTGCTTACAACT
 GGACGGTGAAGCTAACGTTACGCTGCTTACGGTTCTAAAATCGAAAACGA
 CCTGTACGCTGCTTACGAAGTTATCTCTCTGATCGACGAATACGCTGCTTAC
 ACCTCTTCTACCGACGGTGACCTGGTTGGTCCGGGTCCGGGTCTGAAAGTTTC
 TCAGATCAAAATCGTTACCTCTCGTGTTAAAGCTGGTCCGGGTCCGGGTCCG
 CGTAAAGTTTCTCAGATCAAAATCGTTACCTCTCGTGTTAAAGGTCCGGGTCC
 GGGTCAGCCGCGTAAAGTTTCTCAGATCAAAATCGTTACCTCTCGTGTTGG
 TCCGGGTCCGGGTCTTCTCTGGTTTCGTATCCGTAAAGTTCTGGACTCTATC
 GCTGCTGGTCCGGGTCCGGGTGTTTCTCAGCAGCAGGCTATGGAATCTGTT
 TGCCACCCGATCATCGGTCCGGGTCCGGGTGACATCGTTTCTCAGCAGCAG
 GCTATGGAATCTGTTTGCCACCCGGGTCCGGGTCCGGGTATCGTTTCTCAG
 CAGCAGGCTATGGAATCTGTTTGCCACCCGATCGGTCCGGGTCCGGGTAAACG
 TTCTCTGGTTTCGTATCCGTAAAGTTCTGGACTCTATCGCTGGTCCGGGTCCG
 GGTTGCGTTTTCCAGGCTGCTATCTGCTCTCCGGCTTCAAAGTTAAAGGTCC
 GGGTCCGGGTGACCAGCCGCGTAAAGTTTCTCAGATCAAAATCGTTACCTCTC
 GTGGTCCGGGTCCGGGTTCACCCTTCTCTGGTTTCGTATCCGTAAAGTTCTG
 GACTCTATCGGTCCGGGTCCGGGTGTTGCGCTTTCAGGCTGCTATCTGCTC
 TCCGGCTTCAAAGTTGGTCCGGGTCCGGGTAACTTCAACCGTTCTCTGGTT
 CGTATCCGTAAAGTTCTGGACTCTGGTCCGGGTCCGGGTCTGGTTGCGCTT

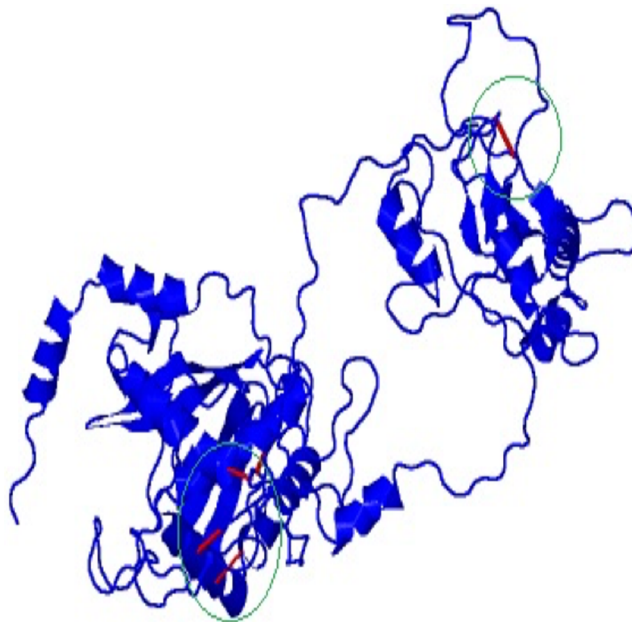


Figure 5 Mutated and stabilized vaccine construct protein by disulphide engineering. The engineered protein showed the disulfide bonds in red colour as presented by the mutated platform of Disulfide by Design server. [Full-size](#) DOI: 10.7717/peerj.8795/fig-5

```
CCAGGCTGCTATCTGCTCTCCGGCTTTCAAAGGTCCGGGTCCGGGTACCGT
GTTCTGACCACCAAAGAAGTGGTTAAAGCTTTCACCCGTGGTCCGGGTCCGGG
TACCCGTCTGTTTCTGGCTCAGAAAAA”.
```

Molecular docking of vaccine construct with TLR4

A total of 29 models were generated from the molecular protein–protein docking between the vaccine construct and the TLR4 receptor using ClusPro 2.0 server. Only one model (000.23) with the lowest energy score (–1,250.0 and highest binding affinity among other predicted docked complex fulfilled the desired criteria for best-docked complex and was eventually selected (Fig. 7).

DISCUSSION

Praziquantel (PZQ) is a pyrazinoisoquinole antihelminthic drug which is one of the few commercially available anti-schistosoma drugs with few known side effects (Aragon *et al.*, 2009). The report of possible drug resistance to praziquantel due to drug pressure has now become of huge public health concern. Several studies have reported remarkable cases of resistance to PZQ which include the development of heritable trait that maintained drug resistance to PZQ in more than six generations (Devaney, 2006; Doenhoff, Cioli & Utzinger, 2008; Fallon *et al.*, 1996; Masamba *et al.*, 2016; William *et al.*, 2001). Only 13% of targeted population received praziquantel treatment, with the drug not capable of preventing reinfection, requires repeated treatment and its characterised by reduction in efficiency among population with heavy infection (Tallima *et al.*, 2017). With the endemicity of schistosomiasis still on the increase across many countries, with increased

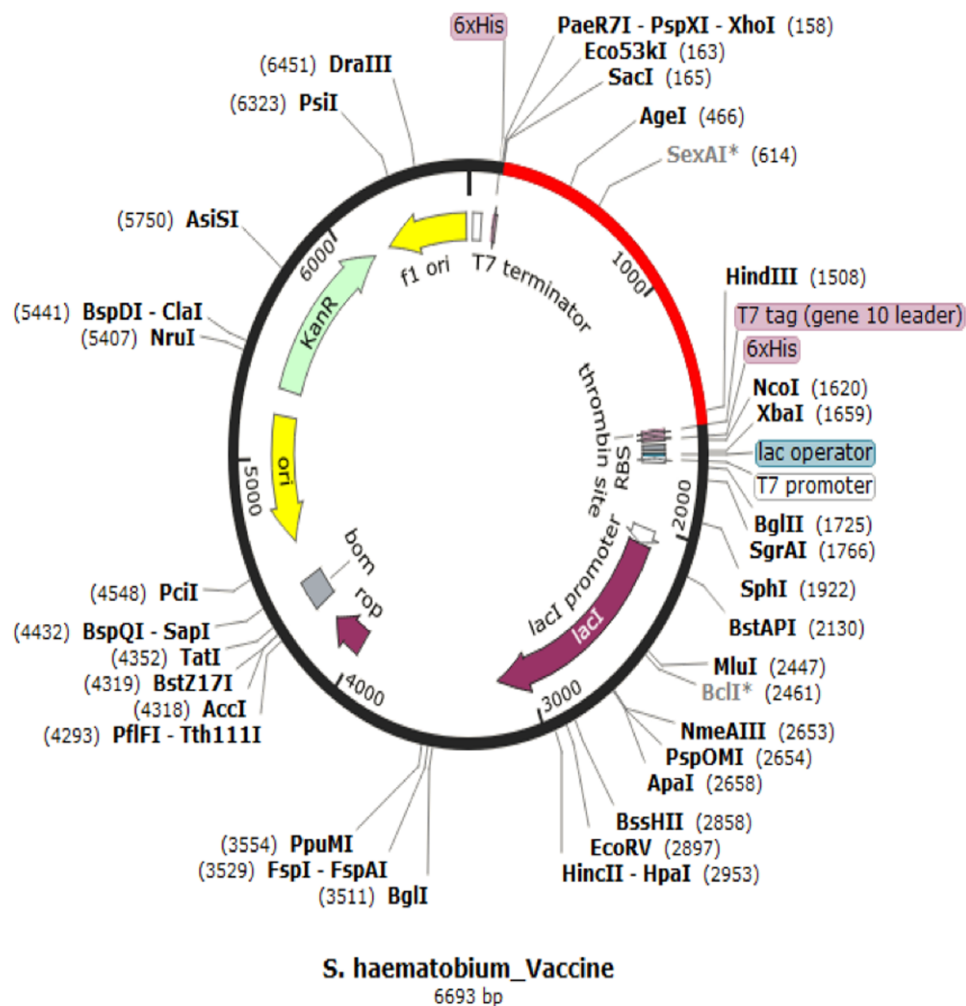


Figure 6 In silico restriction cloning for adapted vaccine construct into pET28a(+) expression vector. The adapted vaccine sequences is shown in red colour as the region of insert while the black circle represent the vector. [Full-size !\[\]\(5f471a71b78d7676bc356df190b88ab4_img.jpg\) DOI: 10.7717/peerj.8795/fig-6](https://doi.org/10.7717/peerj.8795/fig-6)

cases of *Schistosoma* resistance to the most used and reliable drug PZQ. It is now imperative to change the direction of treatment and focus on a lasting line of treatment by targeting the parasitic lifecycle and its different developmental stages, which could well provide a lasting solution to the spread of infection (Masamba *et al.*, 2016). The current study focused on retrieving seven (7) *S. haematobium* HSP sequences for the design of possible multi-epitope subunit vaccine (logistically feasible and safely profiled as described by Shey *et al.* (2019)) construct for the treatment of urinary schistosomiasis using computational approaches. Epitopes-based vaccine will avoid responses against other unsuitable epitopes on antigen, thereby generating more specific immune response against the antigen under consideration (Shey *et al.*, 2019). Only three of these seven HSPs were eventually selected for the design of vaccine due to their predicted high antigenic potential (Khatoon, Pandey & Prajapati, 2017). Masamba *et al.* (2016) has proposed that selective identification of small molecule inhibitors or peptides as an inhibitor of HSPs

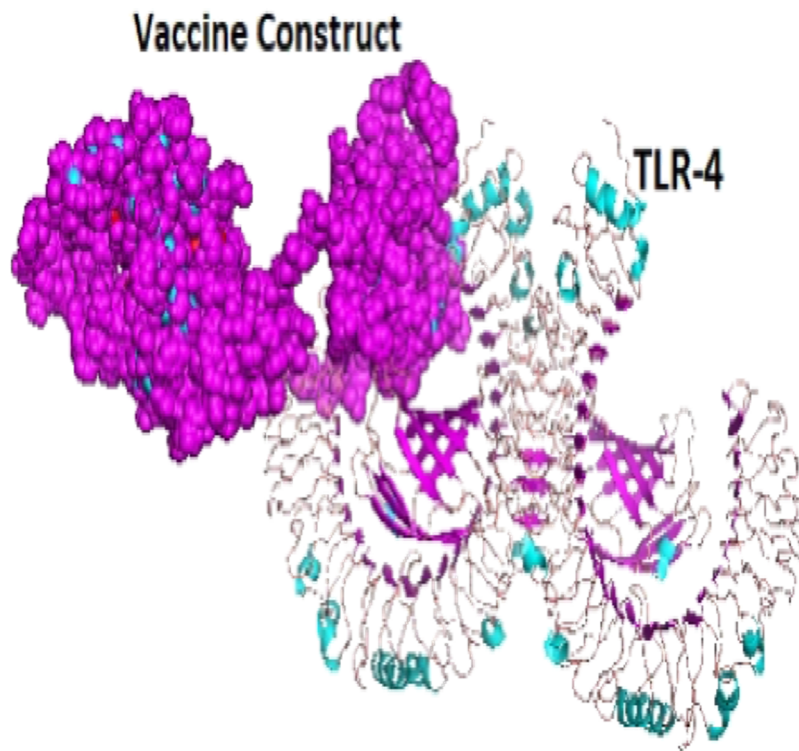



Figure 7 The docked complex showing the vaccine protein (dot) and TLR-4 receptor (cartoon) interaction.

Full-size  DOI: [10.7717/peerj.8795/fig-7](https://doi.org/10.7717/peerj.8795/fig-7)

at the schistosomula stage could be useful in targeting and preventing re-infection of schistosomiasis. Parasites use several mechanisms to upregulate Universal Stress Proteins (including HSPs) which assist the parasite to tolerate different environmental and adverse conditions especially at the schistosomula stage of parasite development (*Mbah et al., 2013*). The HSPs CTL and HTL epitopes with predicted high immunogenicity were selected for inclusion in the vaccine construct. Thus the final vaccine construct had more of high affinities for MHC Class I, II and B-cell epitopes. The CTL-based response targets cells having intracellular viral, bacterial or protozoan infection (*Jordan & Hunter, 2010*) while the HTL-based response is essential for both humoral and cell-mediated immune response (*Pross & Lefkowitz, 2008*). Activated CD8+ cytotoxic T cells are reportedly able to promote death of parasite carrying host MHC class I molecules in its surface (*Zhou et al., 2012*). Also mice immunized with *S. japonicum* 22.6/26GST coupled to Sepharose 4B bead was able to reduce the parasite burden due to an increase in the number of activated CD8+ cells (*Zhou et al., 2012*). Several studies have used mouse model to understand host protective immune response against schistosome infections (*S. mansoni*) and both antibodies and T cells are needed to ensure maximum protection (*Colley & Secor, 2014; Jankovic et al., 1999*). Two previously reported spacer sequences (AAY and GPGPG linkers) were used to improve the designed vaccine (*Shey et al., 2019*) by incorporating between the predicted CTL and HTL epitopes respectively, thereby

producing sequences with minimal junctional immunogenicity leading to the design of a more potent multi-epitope vaccine (Meza et al., 2017; Shey et al., 2019).

The allergenicity and antigenicity test of the designed vaccine predicted its safety for human use and also revealed its potency to inhibit the entrance of Schistosomes into the human host (Ali et al., 2017; Khatoon, Pandey & Prajapati, 2017; Pandey, Bhatt & Prajapati, 2018). Using physiochemical parameters and structural features, the designed vaccine weighed is 51 kDa, which is an average molecular weight for a multi-subunit vaccine (Khatoon, Pandey & Prajapati, 2017). This therefore favours the antigenicity of the vaccine (Khatoon, Pandey & Prajapati, 2017; Pandey, Bhatt & Prajapati, 2018). The theoretical pI of 9.11 showed that the designed vaccine is basic in nature; the obtained higher aliphatic index score of 77.81 classified the vaccine protein as thermostable (Ali et al., 2017; Khatoon, Pandey & Prajapati, 2017), while the negative value of the Grand average of hydropathicity of the vaccine represents its hydrophilic nature. The 3-dimensional structure obtained from the highly accurate homology modeling servers (RaptorX and GalaxyRefine) contain sufficient information about the arrangement of protein amino acid residues, percentage disorder and secondary structures. Also, the Ramachandran plot showed very few outlier residues with most of the residues clustering at very favourable regions thereby ratifying the quality of the overall model as satisfactory. This information was useful in the study of vaccine construct function, dynamics and interaction with ligands and other proteins (Khatoon, Pandey & Prajapati, 2017). To enhance the thermostability of the designed vaccine, novel disulphide bonds were further introduced into the multi-epitope subunit vaccine protein (Rana & Akhter, 2016). In order to express the vaccine construct in the *E. coli* expression system, an in silico cloning was done (Khatoon, Pandey & Prajapati, 2017; Pandey, Bhatt & Prajapati, 2018). Codon adaptation of the designed vaccine was done according to the usage of *E. coli* (strain K12) expression system using JCAT server and found the codon adaptive index to be 0.95 whereas, a score close to 1.0 was described elsewhere as satisfactory (Pandey, Bhatt & Prajapati, 2018). A protein-protein docking analysis was carried out to determine the immune response of TLR-4 agonists against the multi-epitope subunit vaccine designed by minimizing the potential energy of the complete system. The energy minimization ensured conformational stability of designed vaccine-TLR-4 complex by repairing the unnecessary geometry of the structure (Pandey et al., 2017; Pandey et al., 2015). El-Din (2016) has reported decrease in *S. mansoni* worm load, egg load and granuloma size during TLR4 and TLR 9 stimulation with upregulated expression of macrophages. TLR 4 is reportedly known to play significant role in the dendritic cells and macrophages recognition of helminthes products; macrophages production of cytokines and development of Th2 responses (El-Din, 2016; Kane, Jung & Pearce, 2008). In a Th1/Th2 cytokine secretion assay and DNA microarray analysis, activated T cells, up regulation of some cytotoxic genes, followed by increase in parasite egg load have been reported in mice deficient in TLR (Zhang et al., 2011).

CONCLUSION

In this study, we used several immunoinformatics approaches to design vaccine that may be effective against urinary schistosomiasis. Sequences of *S. haematobium* HSPs were

retrieved from the database to design a multi-epitope subunit vaccine containing a CTL, HTL and BCL epitopes of varying length. The designed vaccine was stable with high antigenic properties, high binding affinity for TLR-4 receptor and was found to be non-allergic for human use. However, the designed vaccine requires experimental validation in order to establish its ability in controlling *Schistosoma* infection through the generation of effective immune response and memory.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

The authors received no funding for this work.

Competing Interests

The authors declare that they have no competing interests.

Author Contributions

- Olugbenga S. Onile conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Adeyinka I. Fadahunsi performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Ameerah A. Adekunle conceived and designed the experiments, performed the experiments, analyzed the data, prepared figures and/or tables, authored or reviewed drafts of the paper, and approved the final draft.
- Bolaji F. Oyeyemi conceived and designed the experiments, analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.
- Chiaka I. Anumudu analyzed the data, authored or reviewed drafts of the paper, and approved the final draft.

Data Availability

The following information was supplied regarding data availability:

The HSP sequences available at NCBI: HSP90 ([KGB37337.1](#)), HSP11 ([XP_012799478.1](#)), HSP97 ([KGB34481.1](#)), 212 HSP75 ([KGB38609.1](#)), HSP83 ([KGB37622.1](#)), HSP10 ([KGB41273.1](#)), HSP60 ([KGB40455.1](#)).

Additional data used in this study is available at the RCSB-Protein Data Bank: search term 4G8A.

REFERENCES

- Abdulkadir A, Ahmed M, Abubakar BM, Suleiman IE, Yusuf I, Imam IM, Sule AA, Tela UM, Dogo HM, Yakasai AM, Musa BM. 2017.** Prevalence of urinary schistosomiasis in Nigeria, 1994–2015: systematic review and meta-analysis. *African Journal of Urology* **23(3)**:228–239 DOI [10.1016/j.afju.2016.11.004](#).
- Adebayo AS, Suryavanshi MV, Bhute S, Agunloye AM, Isokpehi RD, Anumudu CI, Shouche YS. 2017.** The microbiome in urogenital schistosomiasis and induced bladder

pathologies. *PLOS Neglected Tropical Diseases* **11**(8):e0005826

DOI [10.1371/journal.pntd.0005826](https://doi.org/10.1371/journal.pntd.0005826).

- Adenowo AF, Oyinloye BE, Ogunyinka BI, Kappo AP. 2015.** Impact of human schistosomiasis in sub-Saharan Africa. *Brazilian Journal of Infectious Diseases: An Official Publication of the Brazilian Society of Infectious Diseases* **19**(2):196–205 DOI [10.1016/j.bjid.2014.11.004](https://doi.org/10.1016/j.bjid.2014.11.004).
- Ali M, Pandey RK, Khatoun N, Narula A, Mishra A, Prajapati VK. 2017.** Exploring dengue genome to construct a multi-epitope based subunit vaccine by utilizing immunoinformatics approach to battle against dengue infection. *Scientific Reports* **7**(1):9232 DOI [10.1038/s41598-017-09199-w](https://doi.org/10.1038/s41598-017-09199-w).
- Alsaqabi SM, Lotfy WM. 2014.** Praziquantel: a review. *Veterinary Science & Technology* **5**:1.
- Aragon AD, Imani RA, Blackburn VR, Cupit PM, Melman SD, Goronga T, Webb T, Loker ES, Cunningham C. 2009.** Towards an understanding of the mechanism of action of praziquantel. *Molecular and Biochemical Parasitology* **164**(1):57–65 DOI [10.1016/j.molbiopara.2008.11.007](https://doi.org/10.1016/j.molbiopara.2008.11.007).
- Barsoum RS. 2013.** Urinary schistosomiasis: review. *Journal of Advanced Research* **4**(5):453–459 DOI [10.1016/j.jare.2012.08.004](https://doi.org/10.1016/j.jare.2012.08.004).
- Bergquist R. 2013.** Closing in on ‘perhaps the most dreadful of the remaining plagues’: an independent view of the multidisciplinary alliance to optimize schistosomiasis control in Africa. *Acta Tropica* **128**(2):179–181 DOI [10.1016/j.actatropica.2013.08.016](https://doi.org/10.1016/j.actatropica.2013.08.016).
- Braschi S, Curwen RS, Ashton PD, Verjovski-Almeida S, Wilson A. 2006.** The tegument surface membranes of the human blood parasite *Schistosoma mansoni*: a proteomic analysis after differential extraction. *Proteomics* **6**(5):1471–1482 DOI [10.1002/pmic.200500368](https://doi.org/10.1002/pmic.200500368).
- Chevalier FD, Le Clec’h W, Eng N, Rugel AR, De Assis RR, Oliveira G, Holloway SP, Cao X, Hart PJ, LoVerde PT, Anderson TJC. 2016.** Independent origins of loss-of-function mutations conferring oxamniquine resistance in a Brazilian schistosome population. *International Journal for Parasitology* **46**(7):417–424 DOI [10.1016/j.ijpara.2016.03.006](https://doi.org/10.1016/j.ijpara.2016.03.006).
- Cioli D, Pica-Mattoccia L, Basso A, Guidi A. 2014.** Schistosomiasis control: praziquantel forever? *Molecular and Biochemical Parasitology* **195**(1):23–29 DOI [10.1016/j.molbiopara.2014.06.002](https://doi.org/10.1016/j.molbiopara.2014.06.002).
- Colley DG, Secor WE. 2014.** Immunology of human schistosomiasis. *Parasite Immunology* **36**(8):347–357 DOI [10.1111/pim.12087](https://doi.org/10.1111/pim.12087).
- Couto FFB, Coelho PMZ, Araújo N, Kusel JR, Katz N, Jannotti-Passos LK, Mattos ACA. 2011.** *Schistosoma mansoni*: a method for inducing resistance to praziquantel using infected *Biomphalaria glabrata* snails. *Memorias do Instituto Oswaldo Cruz* **106**(2):153–157 DOI [10.1590/S0074-02762011000200006](https://doi.org/10.1590/S0074-02762011000200006).
- Das JK, Xiong X, Ren X, Yang J-M, Song J. 2019.** Heat shock proteins in cancer immunotherapy. *Journal of Oncology* **2019**(14):9 DOI [10.1155/2019/3267207](https://doi.org/10.1155/2019/3267207).
- Devaney E. 2006.** Thermoregulation in the life cycle of nematodes. *International Journal for Parasitology* **36**(6):641–649 DOI [10.1016/j.ijpara.2006.02.006](https://doi.org/10.1016/j.ijpara.2006.02.006).
- Doenhoff MJ, Cioli D, Utzinger J. 2008.** Praziquantel: mechanisms of action, resistance and new derivatives for schistosomiasis. *Current Opinion in Infectious Diseases* **21**(6):659–667 DOI [10.1097/QCO.0b013e328318978f](https://doi.org/10.1097/QCO.0b013e328318978f).
- El-Din SAS. 2016.** Role of toll like receptors 4, 5 and 9 ligands in pathogenesis and outcome of intestinal and hepatic Schistosomiasis caused by *Schistosoma mansoni*. *Research Journal of Parasitology* **11**:1–12.
- El-Manzalawy Y, Dobbs D, Honavar V. 2008.** Predicting linear B-cell epitopes using string kernels. *Journal of Molecular Recognition: JMR* **21**(4):243–255 DOI [10.1002/jmr.893](https://doi.org/10.1002/jmr.893).

- Fallon PG, Tao LF, Ismail MM, Bennett JL. 1996. Schistosome resistance to praziquantel: fact or artifact? *Parasitology Today* **12**(8):316–320 DOI [10.1016/0169-4758\(96\)10029-6](https://doi.org/10.1016/0169-4758(96)10029-6).
- Heo L, Park H, Seok C. 2013. GalaxyRefine: protein structure refinement driven by side-chain repacking. *Nucleic Acids Research* **41**(W1):W384–W388 DOI [10.1093/nar/gkt458](https://doi.org/10.1093/nar/gkt458).
- Ishida K, Jolly ER. 2016. Hsp70 may be a molecular regulator of Schistosome host invasion. *PLOS Neglected Tropical Diseases* **10**(9):e0004986 DOI [10.1371/journal.pntd.0004986](https://doi.org/10.1371/journal.pntd.0004986).
- Jankovic D, Wynn TA, Kullberg MC, Hieny S, Caspar P, James S, Cheever AW, Sher A. 1999. Optimal vaccination against *Schistosoma mansoni* requires the induction of both B cell- and IFN-gamma-dependent effector mechanisms. *Journal of Immunology* **162**:345–351.
- Jordan KA, Hunter CA. 2010. Regulation of CD8+ T cell responses to infection with parasitic protozoa. *Experimental Parasitology* **126**(3):318–325 DOI [10.1016/j.exppara.2010.05.008](https://doi.org/10.1016/j.exppara.2010.05.008).
- Kane CM, Jung E, Pearce EJ. 2008. *Schistosoma mansoni* egg antigen-mediated modulation of Toll-like receptor (TLR)-induced activation occurs independently of TLR2, TLR4, and MyD88. *Infection and Immunity* **76**(12):5754–5759 DOI [10.1128/IAI.00497-08](https://doi.org/10.1128/IAI.00497-08).
- Khatoon N, Pandey RK, Prajapati VK. 2017. Exploring *Leishmania* secretory proteins to design B and T cell multi-epitope subunit vaccine using immunoinformatics approach. *Scientific Reports* **7**(1):8285 DOI [10.1038/s41598-017-08842-w](https://doi.org/10.1038/s41598-017-08842-w).
- Kozakov D, Hall DR, Xia B, Porter KA, Padhorney D, Yueh C, Beglov D, Vajda S. 2017. The ClusPro web server for protein–protein docking. *Nature Protocols* **12**(2):255–278 DOI [10.1038/nprot.2016.169](https://doi.org/10.1038/nprot.2016.169).
- Lee SJ, Shin SJ, Lee MH, Lee M-G, Kang TH, Park WS, Soh BY, Park JH, Shin YK, Kim HW, Yun C-H, Jung ID, Park Y-M. 2014. A potential protein adjuvant derived from *Mycobacterium tuberculosis* Rv0652 enhances dendritic cells-based tumor immunotherapy. *PLOS ONE* **9**(8):e104351 DOI [10.1371/journal.pone.0104351](https://doi.org/10.1371/journal.pone.0104351).
- Masamba P, Adenowo AF, Oyinloye BE, Kappo AP. 2016. Universal stress proteins as new targets for environmental and therapeutic interventions of Schistosomiasis. *International Journal of Environmental Research and Public Health* **13**(10):972 DOI [10.3390/ijerph13100972](https://doi.org/10.3390/ijerph13100972).
- Mbah AN, Mahmud O, Awofolu OR, Isokpehi RD. 2013. Inferences on the biochemical and environmental regulation of universal stress proteins from Schistosomiasis parasites. *Advances and Applications in Bioinformatics and Chemistry: AABC* **6**:15–27 DOI [10.2147/AABC.S37191](https://doi.org/10.2147/AABC.S37191).
- McNulty S, Colaco CA, Blandford LE, Bailey CR, Baschieri S, Todryk S. 2013. Heat-shock proteins as dendritic cell-targeting vaccines—getting warmer. *Immunology* **139**(4):407–415 DOI [10.1111/imm.12104](https://doi.org/10.1111/imm.12104).
- Melman SD, Steinauer ML, Cunningham C, Kubatko LS, Mwangi IN, Wynn NB, Mutuku MW, Karanja DMS, Colley DG, Black CL, Secor WE, Mkoji GM, Loker ES. 2009. Reduced susceptibility to praziquantel among naturally occurring Kenyan isolates of *Schistosoma mansoni*. *PLOS Neglected Tropical Diseases* **3**(8):e504 DOI [10.1371/journal.pntd.0000504](https://doi.org/10.1371/journal.pntd.0000504).
- Meza B, Ascencio F, Sierra-Beltrán AP, Torres J, Angulo C. 2017. A novel design of a multi-antigenic, multistage and multi-epitope vaccine against *Helicobacter pylori*: an in silico approach. *Infection, Genetics and Evolution: Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases* **49**:309–317 DOI [10.1016/j.meegid.2017.02.007](https://doi.org/10.1016/j.meegid.2017.02.007).
- Milesi M, Indovina C, Dino O, Di Bella F, Di Lorenzo F, Sanfilippo A, Di Bernardo F, Sodano C, Prestileo T, Immigrant Take Care Advocacy Team Palermo. 2019. Urinary schistosomiasis in migrant population: a case series from a single centre in southern Italy. *Infection* **47**(3):395–398 DOI [10.1007/s15010-018-1244-z](https://doi.org/10.1007/s15010-018-1244-z).

- Onile OS. 2014.** Relationship between physico-chemical parameters and phylogenetics study of human low density lipoprotein receptor-related protein (LRP). *British Biotechnology Journal* 4(3):289–304 DOI 10.9734/BBJ/2014/7176.
- Onile OS, Awobode HO, Oladele VS, Agunloye AM, Anumudu CI. 2016.** Detection of urinary tract pathology in some *Schistosoma haematobium* infected nigerian adults. *Journal of Tropical Medicine* 2016(2):5405207 DOI 10.1155/2016/5405207.
- Onile OS, Calder B, Soares NC, Anumudu CI, Blackburn JM. 2017.** Quantitative label-free proteomic analysis of human urine to identify novel candidate protein biomarkers for schistosomiasis. *PLOS Neglected Tropical Diseases* 11(11):e0006045 DOI 10.1371/journal.pntd.0006045.
- Pandey RK, Bhatt TK, Prajapati VK. 2018.** Novel immunoinformatics approaches to design multi-epitope subunit vaccine for malaria by investigating anopheles salivary protein. *Scientific Reports* 8(1):1125 DOI 10.1038/s41598-018-19456-1.
- Pandey RK, Kumbhar BV, Srivastava S, Malik R, Sundar S, Kunwar A, Prajapati VK. 2017.** Febrifugine analogues as *Leishmania donovani* trypanothione reductase inhibitors: binding energy analysis assisted by molecular docking, ADMET and molecular dynamics simulation. *Journal of Biomolecular Structure and Dynamics* 35(1):141–158 DOI 10.1080/07391102.2015.1135298.
- Pandey RK, Sharma D, Bhatt TK, Sundar S, Prajapati VK. 2015.** Developing imidazole analogues as potential inhibitor for *Leishmania donovani* trypanothione reductase: virtual screening, molecular docking, dynamics and ADMET approach. *Journal of Biomolecular Structure and Dynamics* 33(12):2541–2553 DOI 10.1080/07391102.2015.1085904.
- Poddighe D, Castelli L, Pulcrano G, Grosini A, Balzaretto M, Spadaro S, Bruni P. 2016.** Urinary Schistosomiasis in an adolescent refugee from Africa: an uncommon cause of hematuria and an emerging infectious disease in Europe. *Journal of Immigrant and Minority Health* 18(5):1237–1240 DOI 10.1007/s10903-015-0272-3.
- Pross S, Lefkowitz D. 2008.** *xPharm: the comprehensive pharmacology reference*. Amsterdam: Elsevier, 1–4.
- Rana A, Akhter Y. 2016.** A multi-subunit based, thermodynamically stable model vaccine using combined immunoinformatics and protein structure based approach. *Immunobiology* 221(4):544–557 DOI 10.1016/j.imbio.2015.12.004.
- Rollinson D, Knopp S, Levitz S, Stothard JR, Tchuem Tchuenté L-A, Garba A, Mohammed KA, Schur N, Person B, Colley DG, Utzinger J. 2013.** Time to set the agenda for schistosomiasis elimination. *Acta Tropica* 128(2):423–440 DOI 10.1016/j.actatropica.2012.04.013.
- Shanmugam A, Rajoria S, George AL, Mittelman A, Suriano R, Tiwari RK. 2012.** Synthetic toll like receptor-4 (TLR-4) agonist peptides as a novel class of adjuvants. *PLOS ONE* 7(2):e30839 DOI 10.1371/journal.pone.0030839.
- Shey RA, Ghogomu SM, Esoh KK, Nebangwa ND, Shintouo CM, Nongley NF, Asa BF, Ngale FN, Vanhamme L, Souopgui J. 2019.** In-silico design of a multi-epitope vaccine candidate against onchocerciasis and related filarial diseases. *Scientific Reports* 9(1):4409 DOI 10.1038/s41598-019-40833-x.
- Tallima H, Dvořák J, Kareem S, Abou El Dahab M, Abdel Aziz N, Dalton JP, El Ridi R. 2017.** Protective immune responses against *Schistosoma mansoni* infection by immunization with functionally active gut-derived cysteine peptidases alone and in combination with glyceraldehyde 3-phosphate dehydrogenase. *PLOS Neglected Tropical Diseases* 11(3):e0005443 DOI 10.1371/journal.pntd.0005443.

- Uysal HK, Akgul O, Aliyev E, Tunc MH, Oner YA. 2014.** A rare case of urinary schistosomiasis in Turkey. *Travel Medicine and Infectious Disease* **12**(3):283–285
DOI [10.1016/j.tmaid.2013.11.002](https://doi.org/10.1016/j.tmaid.2013.11.002).
- Venugopal PG, Nutman TB, Semnani RT. 2009.** Activation and regulation of toll-like receptors (TLRs) by helminth parasites. *Immunologic Research* **43**(1–3):252–263
DOI [10.1007/s12026-008-8079-0](https://doi.org/10.1007/s12026-008-8079-0).
- William S, Botros S, Ismail M, Farghally A, Day TA, Bennett JL. 2001.** Praziquantel-induced tegumental damage in vitro is diminished in schistosomes derived from praziquantel-resistant infections. *Parasitology* **122**(1):63–66 DOI [10.1017/S0031182000007137](https://doi.org/10.1017/S0031182000007137).
- Zhang M, Gao Y, Du X, Zhang D, Ji M, Wu G. 2011.** Toll-like receptor (TLR) 2 and TLR4 deficiencies exert differential in vivo effects against *Schistosoma japonicum*. *Parasite Immunology* **33**(4):199–209 DOI [10.1111/j.1365-3024.2010.01265.x](https://doi.org/10.1111/j.1365-3024.2010.01265.x).
- Zhou Y, Zhang H, Sun X-J, Zheng D, Liang Y-J, Luo J, Wang Y, Zhang Z-S. 2012.** Murine CD8(+)T cell cytotoxicity against schistosomula induced by inoculation of schistosomal 22.6/26GST coupled Sepharose 4B beads. *Vaccine* **30**(14):2440–2447
DOI [10.1016/j.vaccine.2012.01.068](https://doi.org/10.1016/j.vaccine.2012.01.068).