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Research paper

Analysis of Apical Membrane Antigen (AMA)-1 characteristics using bioinformatics tools in order to vaccine design against *Plasmodium vivax*

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A R T I C L E I N F O

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A B S T R A C T

Plasmodium vivax, an intracellular protozoan, causes malaria which is characterized by fever, anemia, respiratory distress, liver and spleen enlargement. In spite of attempts to design an efficient vaccine, there is not a vaccine against *P. vivax*. Notable advances have recently achieved in the development of malaria vaccines targeting the surface antigens such as Apical Membrane Antigens (AMA)-1. AMA-1 is a micronemal protein synthesized during the erythrocyte-stage of *Plasmodium* species and plays a significant role in the invasion process of the parasite into host cells. *P. vivax* AMA-1 (PvAMA-1) can induce strong cellular and humoral responses, indicating that it can be an ideal candidate of vaccine against malaria. Identification and prediction of proteins characteristics increase our knowledge about them and leads to develop vaccine and diagnostic studies. In the present study several valid bioinformatics tools were applied to analyze the various characteristics of AMA-1 such as physical and chemical properties, secondary and tertiary structures, B- cell and T-cell prediction and other important features in order to introduce potential epitopes for designing a high-efficient vaccine. The results demonstrated that this protein had 57 potential PTM sites and only one transmembrane domain on its sequence. Also, multiple hydrophilic regions and classical high hydrophilic domains were predicted. Secondary structure prediction revealed that the proportions of random coil, alpha-helix and extended strand in the AMA-1 sequence were 53.74%, 27.22%, and 19.4%, respectively. Moreover, 5 disulfide bonds were predicted at positions 14–21aa, 162–192aa, 208–220aa, 247–265aa and 354–363aa. The data obtained from B-cell and T-cell epitopes prediction showed that there were several potential epitopes on AMA-1 that can be proper targets for diagnostic and vaccine studies. The current study presented interesting basic and theoretical information regarding PvAMA-1, being important for further studies in order to design a high-efficiency vaccine against malaria.

1. Introduction

Malaria is a life-threatening disease, caused by heamoparasites belonging to *Plasmodium* genus, affecting a large number of people worldwide. The disease is, in fact, the most significant and common of the tropical deathlike diseases where it causes heavy losses and death in children and pregnant women. It has been reported that there are over 100 species of *Plasmodium* infecting a variety of animal species including mammals, birds, and reptiles (WHO, 2015). Human malaria is caused by *Plasmodium* species which two of them; *P. vivax*, and *P. falciparum*, lead to the greatest concern (McFadden, 2019). Although, *P. vivax* is included few of the estimated cases worldwide; it is responsible for about a half of all malaria cases outside sub-Saharan Africa, being the most widespread *Plasmodium* species. *P. vivax* has a unique life cycle as sporozoites injected into the blood flow may stay in hepatocytes for a long time (hypnozoites) (WHO, 2015). It invades reticulocytes and uses about 75%–80% of the hemoglobin as a nutrient source. In spite of fewer deaths compared to *P. falciparum*, *P. vivax* has remained as a significant cause of morbidity and mortality, particularly in Asia and America (Price et al., 2007). Malaria is characterized by fever, anemia, headache, myalgia, nausea, vomiting, respiratory distress, liver and spleen enlargement (Oh et al., 2001). Treatment and control of *P. vivax* have become a serious challenge due to drug and vector resistance. Furthermore, wide distribution, antigen variation, relapsing (occurs months to years after infection), and co-infection with *P. falciparum* has led to a renewed interest in development of a vaccine against *P. vivax* (Herrera et al., 2007). Despite many attempts to present a vaccine against malaria, there is not an efficient vaccine yet. It may be because of challenges facing vaccine developers including identifying protective antigens (Hill et al., 2010). Notable advances have recently

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achieved in the development of malaria vaccines targeting the surface antigens such as Apical Membrane Antigens (AMA)-1, Circumsporozoite proteins (CSP), merozoite surface proteins (MSP) and Duffy-binding protein (DBP) (Dobaño et al., 2009).

AMA-1, a 66 kDa polypeptide (83 kDa in *P. falciparum*), is a well-known micronemal protein synthesized during the last 4 h of the erythrocytic phase in *Plasmodium* species and other apicomplexan parasites which can play a significant role in the invasion process of the parasite into host cells (Remarque et al., 2008). AMA-1 is an integral membrane protein with 556–563 amino acids and 16 cysteine residues, comprising of an ectoplasmic domain, a transmembrane domain, and a C-terminal cytoplasmic domain based on the disulfide-bonding pattern (Nair et al., 2002). *P. vivax* AMA-1 (PvAMA-1) is considered an important target for the immune system which is able to induce strong cellular and humoral responses, thereby making it an ideal candidate of the vaccine against malaria (Narum et al., 2000; Obaldia 3rd. et al., 2017). Identification and prediction of protein epitopes characteristics can increase our knowledge about them and leads to develop in vaccine and diagnostic studies. Bioinformatics, a computer science-related field, is applied to analyze and predict gene and protein sequencing data. It helps compare genetic and genomic data and analyze the biological pathways and networks (Iurescia et al., 2012). Previously, bioinformatics-based studies have been performed to identified genetically attenuated vaccine targets (Kumar et al., 2015) and subunit vaccine candidates (Pichugin et al., 2018; Pritam et al., 2019).

In spite of the importance of PvAMA-1 and its characteristics in vaccine development, there are no comprehensive studies to reveal general properties, secondary and tertiary structures, prediction of B-cell and T-cell epitopes using valid bioinformatics servers. Therefore, the present study was targeted toward the analyzing of the characteristics of AMA-1 utilizing bioinformatics tools to introduce potential epitopes for designing a high-efficient vaccine against *P. vivax*.

2. Materials and methods

2.1. Physical and chemical properties

The gene and amino acids sequences of PvAMA-1 were derived from the GeneBank, National Centre for Biotechnology Information (NCBI), available at (https://www.ncbi.nlm.nih.gov/nuccore/262479636). Basic physico-chemical properties of PvAMA-1 protein consisting of amino acids number, molecular weight (MW), theoretical isoelectric point (pI), Amino acid composition, Total number of negatively and positively charged residues, Atomic composition, extinction coefficients, estimated half-life in mammal's reticulocytes, yeast and *Escherichia coli*, instability index, aliphatic index and grand average of hydropathicity (GRAVY) were analyzed.

2.2. Prediction of Post-Translational Modification (PTM) and transmembrane domains

NetPhos 3.1 Server (http://www.cbs.dtu.dk/services/NetPhos/) and CSS-Palm Online Service (http://csspalm.biocuckoo.org/online.php) were applied to predict the phosphorylation and the acylation sites, respectively. Also, transmembrane domains of PvAMA-1 were predicted using TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM-2.0/).

2.3. Prediction of subcellular localization and hydrophilicity/hydrophobicity

To predict subcellular localization and hydrophilicity/hydrophobicity of the PvAMA-1 protein, we used PSORT II prediction (http://psort.hgc.jp/form2.html) and ProtScale (https://web.expasy.org/protscale/) servers, respectively.

2.4. Secondary structures analysis and 3D model constructed

Garnier-Osguthorpe-Robson (GOR) secondary structure prediction online service (Garnier et al., 1996) (https://npsa-prabi.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_gor4.html) was utilized to predict the secondary structure of PvAMA-1. It predicts three main classes of motifs building the secondary structure of a protein: alpha-helix, extended strand, and random coil. Also, we used SWISS-MODEL (Waterhouse et al., 2018) (https://swissmodel.expasy.org/) to construct three-dimensional models of the AMA-1 sequence. Furthermore, disulfide bond prediction of the protein was done using the DiNNNA online service (http://clavius.bc.edu/~clotelab/DiANNA/).

2.5. Prediction of B cell epitopes

Reliable bioinformatics tools including BCPREDS (B-cell epitope prediction server) (http://ailab.ist.psu.edu/bcpred/predict.html), ABCpred (Artificial neural network based B-cell epitope prediction) (http://crdd.osdd.net/raghava/abcpred/ABC_submission.html), Bcepred (B- cell epitope prediction) and IEDB (Immune Epitope Database) servers were employed to analyze and predict B cell epitopes on the AMA-1 protein.

BCPRED was applied to predict the continuous B-cell epitopes by recruiting a combination of a subsequence kernel (SSK) and a support vector machine (SVM) approach (El-Manzalawy et al., 2008). It takes a single amino acid sequence in a plain format as input, and each epitope receives a score. Epitopes with a high score are presented in a table. By default, the program runs with the following features: Epitope length: 20 amino acids, Classifier specificity: 75% and the use of overlap filter.

Bcepred, an online server for Prediction of linear B-cell epitopes, was used to predict B cell epitopes. This server predicts epitopes using physico-chemical properties of a protein sequence such as hydrophilicity, flexibility/mobility, accessibility, polarity, exposed surface and turns with 58.7% accuracy at a threshold of 2.38 (Saha and Raghava, 2004). The amino acid residue segment with a score above the threshold is considered as predicted B-cell epitope. ABCpred is used to predict B cell epitope(s) in an antigen sequence, using Artificial Neural Network (ANN) with 65.93% accuracy (Saha and Raghava, 2006). This software orders predicted B cell epitopes according to their score obtained by a trained recurrent neural network. The outputs of ABCpred are presented in graphical and tabular forms. In graphical form, the epitopes are colored in blue, making it easy for the users to see B-cell epitope on protein quickly. Also, the peptides with a score above the threshold are ranked in a table. Based on the server, peptides with a higher score may have a higher possibility to be considered as an epitope (Saha and Raghava, 2006). Default parameters of the program were applied for the present study.

Moreover, IEDB (http://tools.iedb.org/bcell/) was applied for predicting continuous antibody epitope from the AMA-1 sequences. It uses the following methods to predict B cell epitopes: hydrophilicity (Parker et al., 1986), beta-turn (Chou and Fasman, 1978), surface accessibility (Emini et al., 1985), flexibility (Karplus and Schulz, 1985), Bepipred linear epitope prediction (Larsen et al., 2006) and antigenicity (Kolaskar and Tongaonkar, 1990). The output of IEDB presents as graphs and tables. On the graphs, the Y-axes depict for each residue the correspondent score while the X-axes depict the residue positions in the sequence. The tables provide values of calculated scores for each residue.

2.6. Prediction of MHC-I and MHC-II binding epitopes

Two online servers NetMHCcons 1.1 (Karosiene et al., 2012) (http://www.cbs.dtu.dk/services/NetMHCcons/) and NetMHCIIpan 3.2 (Jensen et al., 2018) (http://www.cbs.dtu.dk/services/NetMHCIIpan/) were applied to predict peptides binding to MHC (major histocompatibility complex) class I and class II molecules, respectively. NetMHCcons provides a possibility for the user to choose MHC molecule from a long list of alleles or alternatively upload the MHC protein sequence of interest. NetMHCIIpan provides a prediction for the three human MHC class II isotypes HLA-DR, HLA-DP, and HLA-DQ, as well as mouse molecules (H-2) (Andreatta and Nielsen, 2018).

The prediction values are given in the half maximal inhibitory concentration (IC50), in nano Molars, and as %Rank. In NetMHCcons a peptide is considered as a strong binder if the %Rank is below 0.5% or the binding affinity (IC50) is below 50 nM. Also, the peptide is considered as a weak binder if the %Rank is below 2% or the binding affinity (IC50) is below 500 nM. In NetMHCIIpan, peptides with %Rank below 2% and 10% are considered strong and weak binders, respectively.

Ten frequently occurring alleles including HLA A01:01, HLA-A02:01, HLA-A03:01, HLA-A24:02, HLA-A26:01, HLA-B07:02, HLA-B08:01, HLA-B27:05, HLA-B39:01 and HLA-B40:01 as human MHC class I molecules and 10-allele HLA reference set consisting of DRB1_0301, DRB1_0701, DRB3_0101, DRB5_0101, DPA10201-DPB10101, DPA10301-DPB10402, DPA10201-DPB11401, DQA10301-DQB10302, DQA10101-DQB10501, DQA10501-DQB10301 as human MHC class II molecules were selected. By default, the length of predicted peptides in NetMHCcons and NetMHCIIpan methods were 9 and 15 amino acids, respectively.

2.7. Prediction of Cytotoxic T Lymphocyte (CTL) epitopes

CTL epitopes were predicted using CTLpred online server (Bhasin and Raghava, 2004b) (http://www.imtech.res.in/raghava/ctlpred/index.html). It is a direct method for prediction of CTL epitopes which uses information of T cell epitopes instead of MHC binders. The method is based on elegant machine learning techniques including Artificial Neural network (ANN) and support vector machine (SVM). The prediction was employed based on the combined method with 76% accuracy (ANN + SVM). By default, ANN and SVM cutoff scores were set at 0.51 and 0.36, respectively. The cutoff value is used to differentiate the epitopes and non-epitopes.

2.8. Validation of the bioinformatics tools used to predict B and T cell epitopes

To check the validation of the bioinformatics tools, they were applied on the *P. vivax* rhoptry neck protein 2 (PvRON2). The prediction of B and T cell epitopes of this antigen were previously performed by López et al. (2018). The validation of the used bioinformatics tools was approved.

3. Results

3.1. Secondary structures analysis and 3D model constructed

Prediction of secondary and 3D structures of a protein has a significant effect on Biological function of it. The data extracted from GOR secondary structure prediction online server demonstrated that the proportions of random coil, alpha-helix and extended strand in the AMA-1 sequence were 53.74% (302/562), 27.22% (153/562) and 19.4% (107/562), respectively (Fig. 1). Results from SWISS-MODEL to predict and construct 3D models for PvAMA-1 showed that 115 templates were found to match the target sequence. A model with the highest sequence identity (97.3%) and the highest coverage among all templates was selected using SWISS-MODEL. It included 61% protein from 43 to 474 amino acids. More information about the result extracted from SWISS-MODEL such as the 3D model predicted for PvAMA-1, protein global quality estimate, sequence identity and coverage, model-template alignment, and local quality estimate was pictured in Fig. 2.

3.2. Prediction of B cell epitopes

Epitope prediction can give researchers significant information to identify immunogenic peptides and design of new potential vaccines. Linear B cell epitopes of PvAMA-1 were predicted using BCPREDS server in which segment with a score above the threshold was considered as predicted B-cell epitope. The higher score indicates the higher binding affinity. Ten epitopes with a high score were presented in Table 1. Moreover, the epitopes predicted by Bcepred server based on one of the chemo-physical parameters including Flexibility, Hydrophilicity, Accessibility, Turns, Exposed Surface, Polarity, and Antigenic Propensity were shown in Table 2. These parameters are commonly used to choose potential epitopes of an antigen.

Additionally, ABCpred server was used to predict B cell epitopes of PvAMA-1 which ranked the predicted epitopes (above the threshold value) according to their scores. The higher score of the peptide represents the higher probability to be an epitope. This server predicted 31 epitopes on our sequence. The highest score (0.96) was for linear epitope KESIKCPCEPEHISNS. Ten epitopes with higher scores were sorted in Table 3. Furthermore, the results of IEDB prediction were depicted in Fig. 3. The threshold, minimum and maximum scores for used parameters were as follows: beta-turn (1.001, 0.596, 1.366), surface accessibility (1.000, 0.030, 5.213), flexibility (1.002, 0.873, 1.114), antigenicity (1.013, 0.856, 1.277), and hydrophilicity (2.039, −6.600, 6.900). Given the results mentioned above, there are some potential B cell epitopes on the AMA-1 sequence that can be proper targets for further diagnostic and vaccine studies.

3.3. Prediction of MHC-I and MHC-II binding epitopes

The data obtained from NetMHCcons (for MHC-I binding epitopes) and NetMHCIIpan (for MHC-II binding epitopes) were presented in Table 4 and 5, respectively. Ten frequently alleles were selected for both MHC. The servers present data in a table containing used alleles, predicted peptide, Affinity/ IC50 (nM), % Rank and binding level (strong or weak).

For each allele, three peptides with high affinity to MHC molecules were chosen. Overall, results showed that there are several epitopes on the AMA-1 protein which can strongly bind to MHC class I and class II molecules. Most strong binders belonged to HLA-B58–01 (MCH I) and HLA-DPA10201-DPB11401 (MHC II).

3.4. Prediction of CTL epitopes

CTLpred predicted and ranked epitopes based on their scores. The information of 10 high-score CTL epitopes predicted by CTLpred was summarized in Table 6.

4. Discussion

AMA-1 is one of the most promising candidates for vivax malaria vaccine based on its effect on animal models and inducing strong immune responses that restrain the growth of the parasite in vitro (Kocken et al., 1999). One of the most fundamental steps to design and develop an effective protein-based vaccine is to analyze the characteristics of the antigen using bioinformatics tools. In the current study, different bioinformatics tools were applied to check various aspects of PvAMA-1 protein in order to gain more information about this protein which can be significantly useful in vaccine design investigations. To the best of our knowledge, this is the first study to identify and predict B-cell and T-cell epitopes and some other characteristics of PvAMA-1 using various bioinformatics tools, hence; there were few studies to compare the results with them. The molecular weight (MW) of the AMA-1 was predicted to be 64.5 Kd. Also, we calculated the aliphatic index of PvAMA-1 (66.3). This index indicates the relative volume occupied by aliphatic side chains (alanine, valine, isoleucine, and leucine). This

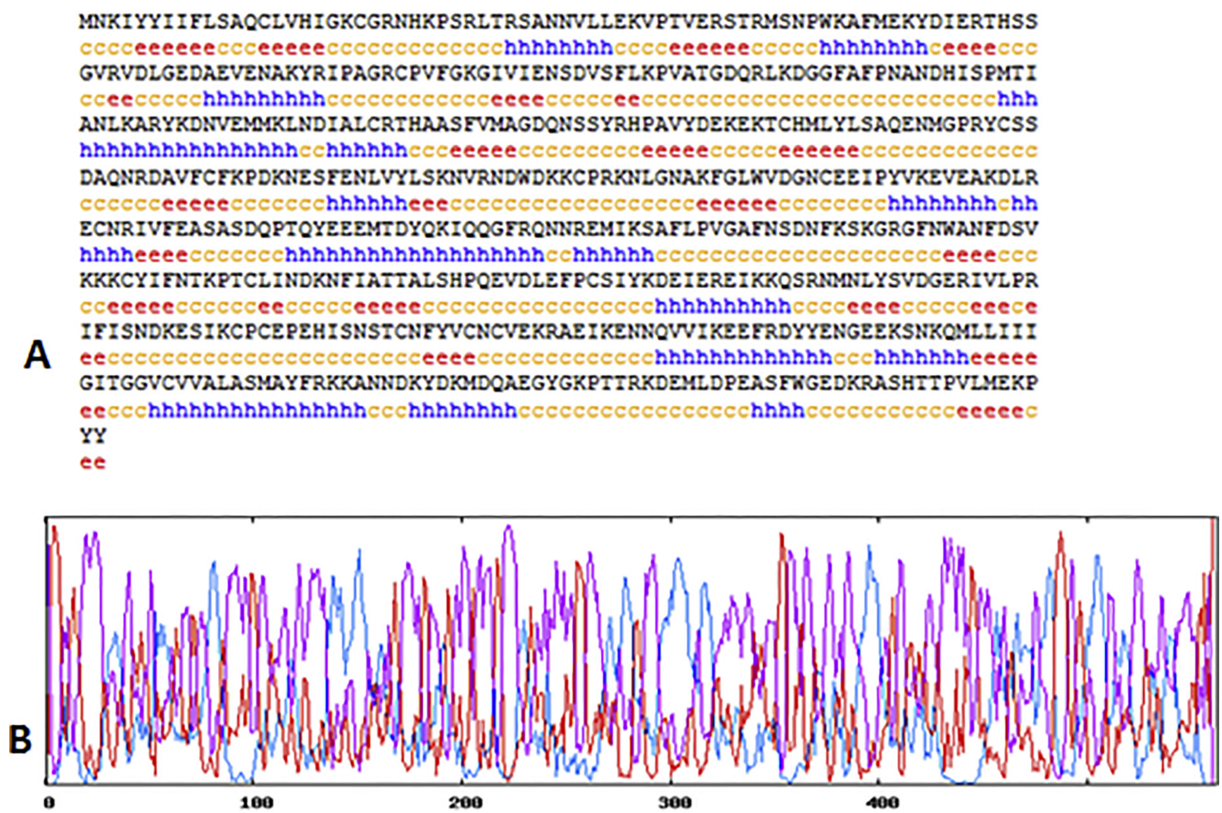

Fig. 1. Analysis of Secondary structure of AMA-1 using GOR IV. A: Predicted secondary structure (h: helix, e: extended strand, c: coil). B: Graphical frame of secondary structure prediction.

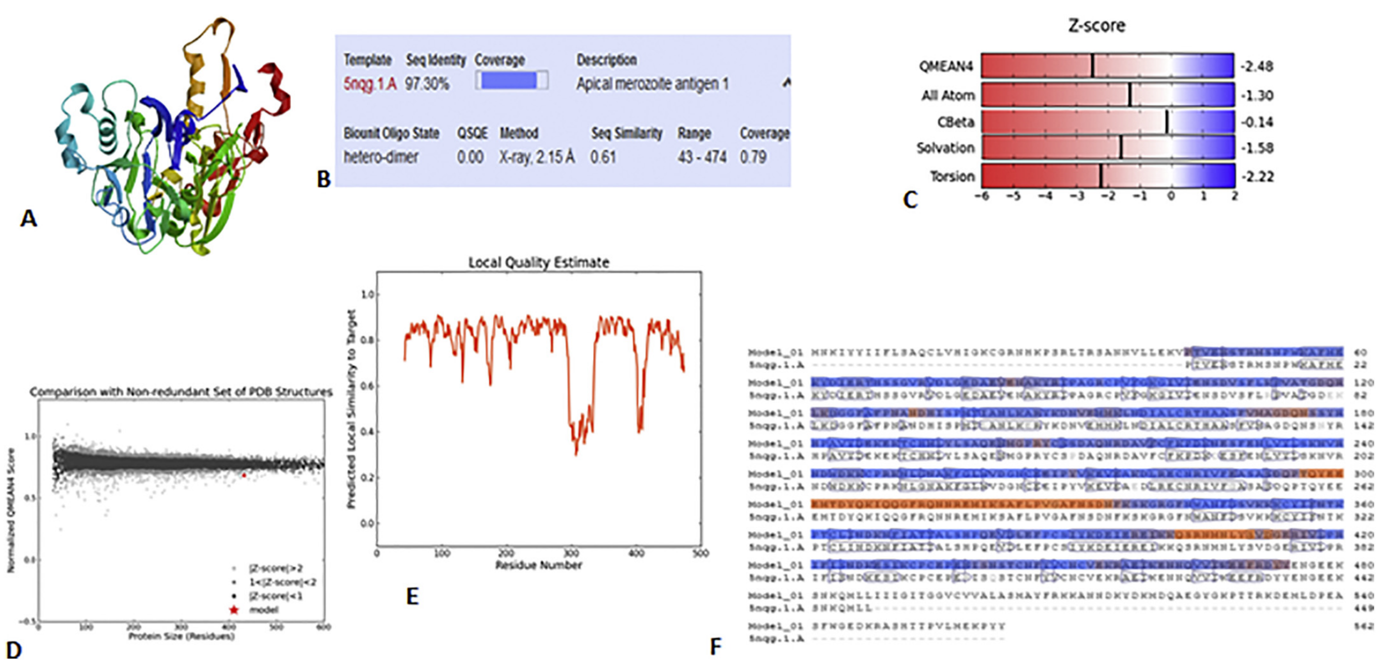

Fig. 2. Analysis of 3D structure constructed for AMA-1 using SWISS_MODEL. A: Tertiary structure prediction of AMA-1 protein. B: Sequence identity and coverage. C: Global Quality Estimate; QMEAN, a composite estimator, includes four individual terms Cβ atoms, all atoms, solvation, and torsion. Positive values indicate that the model scores higher than experimental structures on average. Negative numbers indicate that the model scores lower than experimental structures on average. The QMEAN *Z*-score itself is shown on top. D: Comparison with non-redundant set of PDB structures: The x-axis shows protein length (number of residues). The y-axis is the normalized QMEAN score. Every dot represents one experimental protein structure. Black dots are experimental structures with a normalized QMEAN score within 1 standard deviation of the mean (|Z-score| between 0 and 1), experimental structures with a |Z-score| between 1 and 2 are grey. Experimental structures that are even further from the mean are light grey. The actual model is represented as a red star. E: Local quality: for each residue of the model (reported on the x-axis), the expected similarity to the native structure (y-axis). Typically, residues showing a score below 0.6 are expected to be of low quality. F: Model-Template Alignment. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Linear B-Cell epitopes predicted on PvAMA-1 using *BCPREDS.*

Position	Epitope	Score
543	WGEDKRASHTTPVLMEKPYY	0.999
288	EASASDQPTQYEEEMTDYQK	0.984
460	ENNQVVIKEEFRDYYENGEE	0.979
113	PVATGDQRLKDGGFAFPNAN	0.975
173	GDQNSSYRHPAVYDEKEKTC	0.950
257	FGLWVDGNCEEIPYVKEVEA	0.944
78	EDAEVENAKYRIPAGRCPVF	0.933
41	KVPTVERSTRMSNPWKAFME	0.920
424	SNDKESIKCPCEPEHISNST	0.893
520	DQAEGYGKPTTRKDEMLDPE	0.858
325	LPVGAFNSDNFKSKGRGFNW	0.840
379	PQEVDLEFPCSIYKDEIERE	0.836

Table 3
B-Cell epitopes predicted on PvAMA-1 using *ABCpred* (artificial neural network).

Rank	Sequence	Start position	Score
1	KESIKCPCEPEHISNS	427	0.96
2	EGYGKPTTRKDEMLDP	523	0.94
3	NSSYRHPAVYDEKEKT	176	0.93
4	AEVENAKYRIPAGRCP	80	0.91
5	AQENMGPRYCSSDAQN	199	0.90
6	KARYKDNVEMMKLNDI	144	0.90
7	PEASFWGEDKRASHTT	538	0.89
8	PEHISNSTCNFYVCNC	436	0.89
9	LPRIFISNDKESIKCP	418	0.87
10	MLLIIIGITGGVCVVA	485	0.86

result showed that the AMA-1 protein may be more stable at different temperatures. Additionally, in the present study, GRAVY (−0.67) and Instability indexes (30.5) of AMA-1 were counted. The negative value of GRAVY indicates the protein hydrophilicity. The instability index provides an estimate of the stability of a protein in a test tube. The AMA-1 sequence was classified as a stable protein because the value smaller than 40 is predicted as stable.

PTMs play a vital role in the mechanisms of cellular control (Lee et al., 2009). Also, identification of phosphorylation sites on proteins is a great tool to analyze signaling networks and functional relationships between signaling proteins (Dephoure et al., 2013). Therefore, in the present study phosphorylation and acylation sites on the AMA-1 were predicted. The results demonstrated that there were 57 potential PTM sites including 54 phosphorylation sites and 3 acylation sites on our sequence which may affect protein function and activity. Moreover, prediction of subcellular location showed that cytoplasm was the major place for PvAMA-1 in the cell. Subcellular localization of proteins into different organelles has a great effect on the survival and growth of living cells. Thus, identification of the location of proteins in eukaryotic cells is a significant step to find out their role in cell life (Singh and Mittal, 2016). In the present study, the prediction of hydrophilicity/hydrophobicity showed more than 10 hydrophilic domains on the AMA-1 sequence. Hydrophilic side chains move toward the outside of the protein because they have an affinity with water. Prediction of hydrophilicity/hydrophobicity of a protein can be a useful tool to understand the folding and stability of protein structures (Durell and Ben-Naim, 2017). It is also a great step to know about secondary structure, protein interaction sites and prediction of antigenic epitopes. In addition, we predicted five disulfide bonds in the AMA-1 sequence using DiNNNA server. Disulfide bonds, also called disulfide bridges, play a key role in protein structure and function (Winther and Thorpe, 2014) and protein-protein interactions (Meitzler et al., 2013). Sequences containing disulfide bonds equals/less than five are easier to predict accurately than those with more than five bonds. Accurate prediction of disulfide bonds can reduce the conformational space to improve the 3D structure modeling of proteins and protein folding (Yang et al., 2015). Prediction of protein structures is a key to increase our understanding of a protein function, which provides information regarding how to control, affect or modify the protein. The secondary structure of a protein has a crucial effect on the epitopes (Shaddel et al., 2018). The results indicated that random coil was included about half of the protein structure following by alpha-helix and extended strand. It is believed that the random coil is looser, trend to twist, presented on the protein surface, and maybe a potential epitope (Zhang et al., 2014). Alpha-helix and beta-turn, usually located in the internal of the protein, contain high chemical-bond energy which maintains the protein structure. It seems that they do not act as epitopes. Also, given the importance of tertiary structure in the biological function of proteins, we constructed the 3D structure of PvAMA-1 using SWISS-MODEL server. It is worth noting that prediction of tertiary structure can significantly help understand the proteins structures and connection between structural and functional aspects of proteins.

Epitope, a part of an antigen, is identified by B cell, T-cells and molecules of the host immune system. Only a few amino acid residues comprising an epitope (instead of the whole protein) are enough to induce protective responses, thus; prediction or identification of this

Table 2
B-Cell epitopes predicted on PvAMA-1 using *Bcepred* based on different parameters.

Parameter	Epitope sequence
Flexibility	VHIGKCGRNHKPSRLTRSA, VPTVERSTRM, YDIERTHS, IVIENSD, VMAGDQN, AVYDEKEK, RYCSSDAQ, FCFKPDKN, VYLSKNVRNDWDKKCPRKNL, QGFRQNN, AFNSDNFKSKGRG, ANFDSVKK, DEIEREIKKQSR, NLYSVDG, IFISNDKES, EHISNST, DYYENGEEKSNK, AYFRKKANN, EGYGKPTTRK, SFWGEDKR
Hydrophilicity	GKCGRNHKPSR, ERTHSSG, DLGEDAEVENA, GDQRLKDG, KARYKDN, VYDEKEKTCH, MAGDQNSSYR, CSSDAQNRDA, KPDKNESFEN, SKNVRNDWDKKCPRKN, VDGNCEEI, KEVEAKD, EASASDQPTQYEEEMTDY, RQNNREM, NSDNFKSKG, DSVKKKC, KDEIERE, KQSRNMN, ISNDKESIK, EIKENNQ, DYYENGEEKSNKQM, RKKANNDKYDKMDQAEGYGKPTTRKDEM, GEDKRASHT
Accessibility	KCGRNHKPSRLTRSANN, EKVPTVERSTRMSNPWKAFMEKYDIERTHSSG, EDAEVENAKYRIP, TGDQRLKDGG, ANLKARYKDNVEM, GDQNSSYRHPAVYDEKEKTCHM, YLSAQENMGPRYCSSDAQNRDA, CFKPDKNESFEN, YLSKNVRNDWDKKCPRKNLGNAK, EEIPYVKEVEAKDLRECNR, SASDQPTQYEEEMTDYQKIQQGFRQNNREMIKS, FNSDNFKSKGRGF, NFDSVKKKCY, SHPQEVDLE, IYKDEIEREIKKQSRNMNLYSVDGER, FISNDKESIKCP, EPEHISN, VEKRAEIKENNQVVIKEEFRDYYENGEEKSNKQML, MAYFRKKANNDKYDKMDQAEGYGKPTTRKDEMLDPE, FWGEDKRASHTTP, LMEKPYY
Turns	FPNANDHIS, GDQNSSYR, GAFNSDNFKS, HISNSTCNF, KANNDKY
Exposed Surface	RNHKPSRLTR, EKYDIERT, NLKARYKDNVE, VYDEKEKTCH, FKPDKNESFE, SKNVRNDWDKKCPRKNL, KEVEAKDLRE, QPTQYEEEMTDYQKIQQ, FRQNNREMIK, SDNFKSKGR, FDSVKKKCY, YKDEIEREIKKQSRNMN, NDKESIK, EKRAEIKENNQ, KEEFRDYYENGEEKSNKQML, YFRKKANNDKYDKMDQ, GKPTTRKDEMLD, EDKRASH, MEKPYY
Polarity	HIGKCGRNHKPSRLTR, EKVPTVERSTRMS, WKAFMEKYDIERTHSSG, RVDLGEDAEVENAKYRI, GDQRLKDG, NLKARYKDNVE, AVYDEKEKTCHML, FKPDKNESFEN, VRNDWDKKCPRKNL, EEIPYVKEVEAKDLRECNRIV, PTQYEEEMTDY, FRQNNREMIKSA, NFKSKGRGF, FDSVKKKCY, IYKDEIEREIKKQSRNM, ERIVLPR, NDKESIKCPCEPEHISN, CNCVEKRAEIKENNQVVIKEEFRDYYENGEEKSNKQM, MAYFRKKANNDKYDKMDQAE, GKPTTRKDEMLDPE,FWGEDKRASHTT
Antigenic Propensity	KIYYIIFLS, QCLVHIGKCG, NVLLEKVPTV, HSSGVRVDL, RCPVFGKGIV, VSFLKPV, TCHMLYLS, VFCFKPD, FENLVYLSKNV, CEEIPYVKEVE, SVKKKCYIF, TKPTCLI, LSHPQEVDLEFPCSIYK, RIVLPRIFI, ESIKCPCEPEH, STCNFYVCNCVEKR, VVIKEEF, QMLLIIIGITGGVCVVAL, SHTTPVL

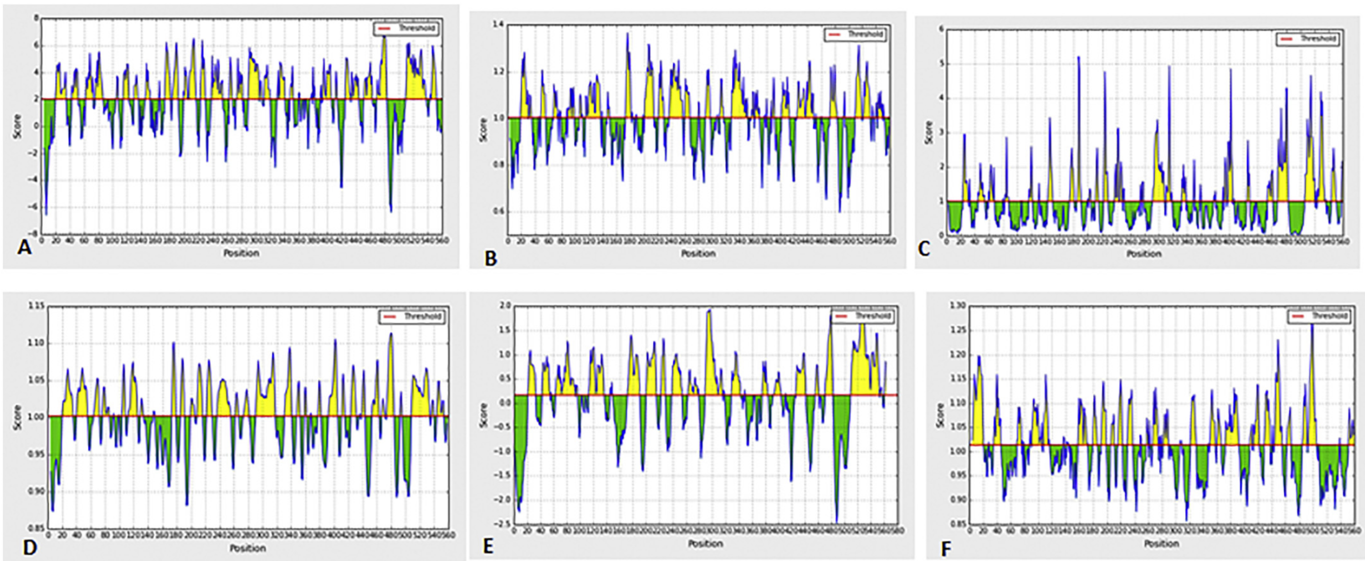

Fig. 3. The output of IEDB server based on parameters including. A: hydrophilicity, B: beta-turn, C: surface accessibility, D: flexibility, E: Bepipred linear epitope prediction, and F: antigenicity. The residue with a higher score representing that the residue might have a higher probability to be a part of the epitope (those residues are colored in yellow on the graphs). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 4
Prediction of MHC-I binding epitopes on PvAMA-1 using *NetMHCcons*.

Allele	Peptide	IC50 (nM)	%Rank	Binding level
HLA-A01:01	ISNSTCNFY	93.08	0.15	SB
	VVALASMAY	759.40	0.50	SB
	SSYRHPAVY	767.66	0.50	SB
HLA-A02:01	VLLEKVPTV	3.62	0.05	SB
	QMLLIIIGI	112.49	3.00	WB
	MIKSAFLPV	128.08	3.00	WB
HLA-A03:01	ASMAYFRKK	57.20	0.40	SB
	RQNNREMIK	116.20	0.80	WB
	AVFCFKPDK	120.68	0.80	WB
HLA-A26:01	NANDHISPM	110.68	0.25	SB
	TIANLKARY	801.61	0.80	WB
	ESFENLVYL	1000.67	1.00	WB
HLA-B07:02	KPSRLTRSA	23.56	0.25	SB
	IPAGRCPVF	38.96	0.40	SB
	GPRYCSSDA	52.18	0.50	SB
HLA-B08:01	MIKSAFLPV	39.38	0.15	SB
	MMKLNDIAL	41.12	0.17	SB
	IALCRTHAA	63.05	0.25	SB
HLA-B39:01	TRSANNVLL	31.04	0.25	SB
	NKIYYIIFL	283.70	1.50	WB
	KRASHTTPV	307.68	1.50	WB
HLA-B40:01	IENSDVSFL	46.07	0.50	SB
	REMIKSAFL	18.47	2.00	SB
	AEIKENNQV	344.70	1.00	WB
HLA-B58:01	RSTRMSNPW	5.59	0.05	SB
	KSKGRGFNW	21.03	0.30	SB
	VALASMAYF	22.80	0.30	SB
HLA-B15:01	RLKDGGFAF	23.56	0.15	SB
	RMSNPWKAF	42.94	0.40	SB
	SSYRHPAVY	48.63	0.50	SB

Table 5
Prediction of MHC-II binding epitopes on PvAMA-1 using *NetMHCIIpan*.

Allele	Peptide	IC50 (nM)	%Rank	Binding level
DRB1_0301	VEMMKLNDIALCRTH	128.30	2.50	WB
	EMMKLNDIALCRTHA	133.65	2.50	WB
	MMKLNDIALCRTHAA	135.93	3.00	WB
DRB3_0101	VFCFKPDKNESFENL	71.51	1.40	SB
	RNMNLYSVDGERIVL	73.71	1.50	SB
	MNLYSVDGERIVLPR	75.01	1.50	SB
DRB5_0101	PMTIANLKARYKDNV	21.06	0.70	SB
	MTIANLKARYKDNVE	22.41	0.80	SB
	SPMTIANLKARYKDN	23.17	0.90	SB
HLA-DPA10201-DPB10101	NREMIKSAFLPVGAF	117.57	2.00	SB
	REMIKSAFLPVGAFN	129.02	2.50	WB
	NNREMIKSAFLPVGA	138.23	3.00	WB
HLA-DPA10301-DPB10402	NREMIKSAFLPVGAF	148.59	1.60	SB
	REMIKSAFLPVGAFN	162.47	1.90	SB
	NNREMIKSAFLPVG	182.23	2.50	WB
HLA-DPA10201-DPB11401	KNFIATTALSHPQEV	232.09	0.25	SB
	NDKNFIATTALSHPQ	242.99	0.25	SB
	DKNFIATTALSHPQE	250.44	0.25	SB
HLA-DQA10301-DQB10302	RTHAASFVMAGDQNS	863.02	2.50	WB
	CRTHAASFVMAGDQN	901.84	3.00	WB
	GVRVDLGEDAEVENA	943.81	3.00	WB
HLA-DQA10101-DQB10501	EKTCHMLYLSAQENM	627.69	7.50	WB
	KTCHMLYLSAQENMG	762.34	9.50	WB
	RNMNLYSVDGERIVL	755.64	9.50	WB
HLA-DQA10501-DQB10301	IIGITGGVCVVALAS	55.59	1.40	SB
	IGITGGVCVVALASM	58.24	1.50	SB
	IIIGITGGVCVVALA	59.05	1.50	SB
HLA-DRB1*07:01	NDKNFIATTALSHPQ	23.85	0.70	SB
	INDKNFIATTALSHP	23.88	0.70	SB
	DKNFIATTALSHPQE	26.21	0.90	SB

substantial segment of amino acid residues can be a key to understand the immune and pathogenesis mechanisms of a pathogen and importantly in designing of epitope-based vaccines and immunodiagnostic test (Desai and Kulkarni-Kale, 2014). Antibodies play an undeniable role in protection against erythrocyte-form of *Plasmodium* genus and cause reduction of parasitemia in the later stages of the infection (Mueller et al., 2013). In the current study, we chose reliable bioinformatics tools for predicting linear B-cell epitopes in PvAMA-1 in order to select potential targets for further malarial vaccines studies.

The use of various indexes and predicting tools results in a more accurate prediction of structures and epitopes of a protein (Kringelum et al., 2012); therefore, in the present study, we used several valid bioinformatics tools such as BCPREDS, ABCpred, Bcepred, and IEDB to predict linear B-cell epitopes on PvAMA-1. The results demonstrated that there were potential epitopes in this protein indicating that it can be a promising candidate in vaccine design after being tested in animal models. Our results indicated that more than 10 potential antigen epitopes were predicted in PvAMA-1 by BCPREDS. Based on the results

Table 6
Predicted CTL epitopes of PvAMA-1 using *CTLpred* (combined method).

Peptide rank	Start position	Sequence	Score (ANN/SVM)
1	120	RLKDGGFAF	0.52/1.65
2	478	EEKSNKQML	1.00/1.03
3	413	GERIVLPRI	0.81/1.10
4	155	KLNDIALCR	0.94/0.90
5	145	ARYKDNVEM	0.92/0.91
6	423	ISNDKESIK	0.94/0.87
7	334	NFKSKGRGF	0.89/0.90
8	336	KSKGRGFNW	0.66/1.08
9	227	ESFENLVYL	0.79/0.93
10	480	KSNKQMLLI	0.82/0.87

extracted from ABCpred, of 31 epitopes predicted on AMA-1, 10 epitopes can be used as a vaccine target.

Furthermore, we used Bcepred to predict linear B-cell epitopes. It predicts B cell epitopes using physical and chemical properties including hydrophilicity, flexibility/mobility, accessibility, polarity, exposed surface, and turns. In this study, several B-cell epitopes were predicted on PvAMA-1 using any of the properties. Based on the properties, the accuracy of Bcepred can be varied from 52.92% and 57.53%. It has been proved that the highest accuracy (58.70%) was achieved when combining four amino acid properties (hydrophilicity, flexibility, polarity, and exposed surface) (Saha and Raghava, 2004).

Some parameters of proteins including hydrophilicity, flexibility, accessibility, turns, exposed surface, polarity, and antigenic propensity have been correlated with the location of continuous epitopes. In our study, the results obtained from IEDB showed some potential epitopes on PvAMA-1. Bueno et al. (2011), identified a highly antigenic B cell epitope within PvAMA-1 vaccine candidate using BepiPred server and reported its serological reactivity during natural infection. They reported that there was no homology between this specific immunodominant region and genome sequence of mice and humans which makes this epitope a proper target in pre-clinical and clinical trials. Any of the bioinformatics tools used in the current study indicated that there were several potential B-cell epitopes in PvAMA-1 protein which might represent promising polypeptidic vaccine candidates against vivax malaria. Choosing an epitope eliciting protective antibody responses after immunization, could be a promising step to design more effective vaccines (Remarque et al., 2008).

MHC molecules present T-cell epitopes to T-cells. Binding of peptides to the MHC is a key stage in the process of T-cell antigen presentation and, thus; a significant factor in the selection of potential epitopes. The results of prediction of MHC binding epitopes showed that some epitopes predicted on PvAMA-1 can strongly bind to MHC I and II. These findings are consistent with the data from other studies (Arévalo-Herrera and Herrera, 2001; Bhasin and Raghava, 2004a; Parra-López et al., 2006). The prediction and characterization of both CD8 and CD4 T cell epitopes on a protein present significant data to understand the infection pathogenesis (Tchernev and Orfanos, 2006). In addition, it has an important effect on the development of epitope-based vaccines against infectious agents (Reche et al., 2006).

In this study, 10 potential CTL epitopes were predicted using CTLpred. The identification of peptides stimulating Cytotoxic T Lymphocytes (CTLs) is a major problem in process of vaccines design. Since, all MHC binders may not act as T cell epitopes, hence; a highly accurate prediction method for CTL epitopes is needed. The use of artificial neural network and support vector machine is explored to solve the problem. Here, machine learning techniques SVM and ANN have been used to develop a CTL epitope prediction method. The consensus and combined prediction resulted in improvement of specificity and sensitivity respectively along with accuracy. The best accuracy and sensitivity are for consensus and combined prediction approaches respectively, which are significantly higher compared to the individual methods (ANN and SVM). *P. vivax* is an intracellular parasite infecting erythrocytes and hepatocytes, therefore; T-cell mediated cellular immunity plays a critical role against the infection (Mueller et al., 2013).

Designing a protective vaccine against *P. vivax* is still a significant need. Identification of specific antigens and epitopes inducing protective responses is a critical challenge in vaccine development (Guy et al., 2018). AMA-1 has been considered as a promising target of vaccine stimulating protective cellular and humoral responses against malaria in animal models (Narum et al., 2000). Prediction and characterization of AMA-1 biological characteristics in particular, antigenic and immunogenic epitopes, would be an important step to overcome the barriers observed in vaccine development against malaria (Bueno et al., 2011).

5. Conclusion

One of the first measures to design an ideal vaccine is to identify potential antigen inducing robust protective responses. To get this purpose, an accurate and comprehensive analysis of the antigen using bioinformatics tools is essential. The present study tried to reveal important aspects of PvAMA-1 protein in terms of physical and chemical features, structures, hydrophilicity, antigenicity, B cell, and T cell epitopes and etc. using various and reliable bioinformatics tools. To our knowledge, it is the first study conducted for a comprehensive prediction and analysis of physical, chemical and biological characteristics of PvAMA-1. All the previous data demonstrates that this protein contains potential epitopes and can be used as a promising candidate for a vaccine against vivax malaria. The current study presented interesting basic and theoretical information regarding PvAMA-1, being important for further in vivo studies in order to design a high-efficiency vaccine against malaria.

Ethics approval and consent to participate

This article does not contain any studies with human participants or animals performed by any of the authors.

Conflict of interests

The authors declare that they have no conflict of interest.

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References

Andreatta, M., Nielsen, M., 2018. Bioinformatics tools for the prediction of T-cell epitopes. Methods Mol. Biol. 1785, 269–281. https://doi.org/10.1007/978-1-4939-7841-0_18.
Arévalo-Herrera, M., Herrera, S., 2001. Plasmodium vivax malaria vaccine development. Mol. Immunol. 38, 443–455.
Bhasin, M., Raghava, G.P., 2004a. SVM based method for predicting HLADRB1* 0401 binding peptides in an antigen sequence. Bioinformatics 20, 421–423.
Bhasin, M., Raghava, G.P.S., 2004b. Prediction of CTL epitopes using QM, SVM and ANN techniques. Vaccine 22, 3195–3201.
Bueno, L.L., Lobo, F.P., Morais, C.G., Mourão, L.C., de Ávila, R.A., Soares, I.S., Fontes, C.J., Lacerda, M.V., Chavez-Olórtegui, C., Bartholomeu, D.C., Fujiwara, R.T., Braga, E.M., 2011. Identification of a highly antigenic linear B cell epitope within *Plasmodium vivax* apical membrane antigen 1 (AMA-1). PLoS One 6, e21289. https://doi.org/10.1371/journal.pone.0021289.
Chou, P.Y., Fasman, G.D., 1978. Prediction of the secondary structure of proteins from their amino acid sequence. Adv. Enzymol. Relat. Areas Mol. Biol. 47, 45–148.
Dephoure, N., Gould, K.L., Gygi, S.P., Kellogg, D.R., 2013. Mapping and analysis of phosphorylation sites: a quick guide for cell biologists. Mol. Biol. Cell 24, 535–542. https://doi.org/10.1091/mbc.E12-09-0677.
Desai, D.V., Kulkarni-Kale, U., 2014. T-cell epitope prediction methods: an overview.

Methods Mol. Biol. 1184, 333–364. https://doi.org/10.1007/978-1-4939-1115-8_19.
Dobaño, C., Sedegah, M., Rogers, W.O., Kumar, S., Zheng, H., Hoffman, S.L., Doolan, D., 2009. *Plasmodium*: mammalian codon optimization of malaria plasmid DNA vaccines enhances antibody responses but not T cell responses nor protective immunity. Exp. Parasitol. 122, 112–123.
Durell, S.R., Ben-Naim, A., 2017. Hydrophobic-hydrophilic forces in protein folding. Biopolymers 107 (8). https://doi.org/10.1002/bip.23020.
El-Manzalawy, Y., Dobbs, D., Honavar, V., 2008. Predicting linear B-cell epitopes using string kernels. J. Mol. Recognit. 21, 243–255. https://doi.org/10.1002/jmr.893.
Emini, E.A., Hughes, J.V., Perlow, D.S., Boger, J., 1985. Induction of hepatitis a virus-neutralizing antibody by a virus-specific synthetic peptide. J. Virol. 55, 836–839.
Foroutan, M., Ghaffarifar, F., Sharifi, Z., Dalimi, A., Pirestani, M., 2018. Bioinformatics analysis of ROP8 protein to improve vaccine design against *Toxoplasma gondii*. Infect. Genet. Evol. 62, 193–204.
Garnier, J., Gibrat, J.F., Robson, B., 1996. GOR secondary structure prediction method version IV. Methods Enzymol. 266, 540–553.
Guy, Andrew J., Irani, Vashti, Richardsand, Jack S., Ramsland, Paul A., 2018. Structural patterns of selection and diversity for Plasmodium vivax antigens DBP and AMA1. Malar. J. 17 (183). https://doi.org/10.1186/s12936-018-2324-3.
Herrera, S., Corradin, G., Arevalo-Herrera, M., 2007. An update on the search for a *Plasmodium vivax* vaccine. Trends Parasitol. 23, 122–128.
Hill, A.V., Reyes-Sandoval, A., O'Hara, G., Ewer, K., Lawrie, A., Goodman, A., Nicosia, A., Folgori, A., Colloca, S., Cortese, R., Gilbert, S.C., Draper, S.J., 2010. Prime-boost vectored malaria vaccines: progress and prospects. Hum. Vaccin. 6, 78–83.
Iurescia, S., Fioretti, D., Fazio, V.M., Rinaldi, M., 2012. Epitope-driven DNA vaccine design employing immunoinformatics against B-cell lymphoma: a biotech's challenge. Biotechnol. Adv. 30, 372–383. https://doi.org/10.1016/j.biotechadv.2011.06.020.
Jensen, K.K., Andreatta, M., Marcatili, P., Buus, S., Greenbaum, J.A., Yan, Z., Sette, A., Peters, B., Nielsen, M., 2018. Improved methods for predicting peptide binding affinity to MHC class II molecules. Immunology 154, 394–406. https://doi.org/10.1111/imm.12889.
Karosiene, E., Lundegaard, C., Lund, O., Nielsen, M., 2012. NetMHCcons: a consensus method for the major histocompatibility complex class I predictions. Immunogenetics 64, 177. https://doi.org/10.1007/s00251-011-0579-8.
Karplus, P.A., Schulz, G.E., 1985. Prediction of chain flexibility in proteins – a tool for the selection of peptide antigens. Naturwissenschafren 72, 212–213.
Kocken, C.H.M., Dubbeld, M.A., Van Der Wel, A., Pronk, J.T., Waters, A.P., Langermans, J.A.M., Thomas, A.W., 1999. High-level expression of plasmodium vivax apical membrane antigen 1 (AMA-1) in Pichia pastoris: strong immunogenicity in Macaca mulattaImmunized with P. vivax AMA-1 and Adjuvant SBAS2. Infect. Immun. 67, 43–49.
Kolaskar, A.S., Tongaonkar, P.C., 1990. A semi-empirical method for prediction of antigenic determinants on protein antigens. FEBS Lett. 10, 172–174.
Kringelum, J.V., Lundegaard, C., Lund, O., Nielsen, M., 2012. Reliable B cell epitope predictions: impacts of method development and improved benchmarking. PLoS Comput. Biol. 8 (12).
Kumar, H., Frisschknecht, F., Mair, G.R., Gomes, J., 2015. In silico identification of genetically attenuated vaccine candidate genes for Plasmodium liver stage. Infect. Genet. Evol. 2015 (36), 72–81.
Larsen, J.E., Lund, O., Nielsen, M., 2006. Improved method for predicting linear B-cell epitopes. Immunome Res. 2 (2).
Lee, T.Y., Hsu, J.B., Chang, W.C., Wang, T.Y., Hsu, P.C., Huang, H.D., 2009. A comprehensive resource for integrating and displaying protein post-translational modifications. BMC Res. Notes 2, 111.
López, C., Yepes-Pérez, Y., Díaz-Arévalo, D., Patarroyo, M.E., Patarroyo, M.A., 2018. The in vitro antigenicity of *Plasmodium vivax* Rhoptry neck protein 2 (PvRON2) B- and T epitopes selected by HLA-DRB1 binding profile. Front. Cell. Infect. Microbiol. 8, 156.
McFadden, G.I., 2019. Plasmodium: more don'ts. Trends Parasitol. 35, 4–6.
Meitzler, J.L., Hinde, S., Bánfi, B., Nauseef, W.M., de Montellano, P.R., 2013. Conserved cysteine residues provide a protein-protein interaction surface in dual oxidase (DUOX) proteins. J. Biol. Chem. 8, 7147–7157. https://doi.org/10.1074/jbc.M112.414797.
Mueller, I., Galinski, M.R., Tsuboi, T., Arevalo-Herrera, M., Collins, W.E., King, C.L., 2013. Natural acquisition of immunity to Plasmodium vivax: epidemiological observations and potential targets. Adv. Parasitol. 81, 77–131. https://doi.org/10.1016/B978-0-12-407826-0.00003-5.
Nair, M., Hinds, M.G., Coley, A.M., Hodder, A.N., Foley, M., Anders, R.F., Norton, R.S., 2002. Structure of domain III of the blood-stage malaria vaccine candidate, Plasmodium falciparum apical membrane antigen 1 (AMA1). J. Mol. Biol. 322, 741–753.
Narum, D.L., Ogun, S.A., Thomas, A.W., Holder, A.A., 2000. Immunization with parasite-derived apical membrane antigen 1 or passive immunization with a specific monoclonal antibody protects BALB/c mice against lethal *Plasmodium yoelii yoelii* YM blood-stage infection. Infect. Immun. 68, 2899–2906.
Obaldia 3rd., N., Stockelman, M.G., Otero, W., Cockrill, J.A., Ganeshan, H., Abot, E.N., Zhang, J., Limbach, K., Charoenvit, Y., Doolan, D.L., Tang, D.C., Richie, T.L., 2017. A *Plasmodium vivax* plasmid DNA- and adenovirus-vectored malaria vaccine encoding blood-stage antigens AMA1 and $MSP1_{42}$ in a prime/boost heterologous immunization regimen partially protects Aotus monkeys against blood-stage challenge. Clin. Vaccin. Immunol. 5, 24. https://doi.org/10.1128/CVI.00539-16.
Oh, M., Shin, H., Shin, D., Kim, U., Lee, S., Kim, N., Choi, M.H., Chai, J.Y., Choe, K., 2001. Clinical features of vivax malaria. Am. J. Trop. Med. Hyg. 65, 143–146.
Parker, J.M., Guo, D., Hodges, R.S., 1986. New hydrophilicity scale derived from high-performance liquid chromatography peptide retention data: correlation of predicted surface residues with antigenicity and X-ray-derived accessible sites. Biochemistry 25, 5425–5432.
Parra-López, C., Calvo-Calle, J.M., Cameron, T.O., Vargas, L.E., Salazar, L.M., Patarroyo, M.E., Nardin, E., Stern, L.J., 2006. Major histocompatibility complex and T cell interactions of a universal T cell epitope from *Plasmodium falciparum* Circumsporozoite protein. J. Biol. Chem. 281, 14907–14917.
Pichugin, A., Zarling, S., Perazzo, L., Duffy, P.E., Ploegh, H.L., Krzych, U., 2018. Identification of a novel CD8 T cell epitope derived from Plasmodium berghei protective liver-stage antigen. Front. Immunol. 29, 91.
Price, R.N., Tjitra, E., Guerra, C.A., Yeung, S., White, N.J., Anstey, N.M., 2007. Vivax malaria: neglected and not benign. Am. J. Trop. Med. Hyg. 77, 79–87.
Pritam, M., Singh, G., Swaroop, S., Singh, A.K., Singh, S.P., 2019. Exploitation of reverse vaccinology and immunoinformatics as promising platform for genome-wide screening of new effective vaccine candidates against Plasmodium falciparum. BMC Bioinformatics 4, 468.
Reche, P.A., Keskin, D.B., Hussey, R.E., Ancuta, P., Gabuzda, D., Reinherz, E.L., 2006. Elicitation from virus-naive individuals of cytotoxic T lymphocytes directed against conserved HIV-1 epitopes. Med. Immunol. 5, 1.
Remarque, E.J., Faber, B.W., Kocken, C.H., Thomas, A.W., 2008. Apical membrane antigen 1: a malaria vaccine candidate in review. Trends Parasitol. 24, 74–84.
Saha, S., Raghava, G.P., 2004. BcePred:prediction of continuous B-cell epitopes in antigenic sequences using Physico-chemical properties. Artif. Immune Syst. 3239, 197–204.
Saha, S., Raghava, G.P., 2006. Prediction of continuous B-cell epitopes in an antigen using recurrent neural network. Proteins 65, 40–44.
Shaddel, M., Ebrahimi, M., Tabandeh, M.R., 2018. Bioinformatics analysis of single and multi-hybrid epitopes of GRA-1, GRA-4, GRA-6 and GRA-7 proteins to improve DNA vaccine design against *Toxoplasma gondii*. J. Parasit. Dis. 42, 269–276. https://doi.org/10.1007/s12639-018-0996-9.
Singh, S., Mittal, A., 2016. Transmembrane domain lengths serve as signatures of organismal complexity and viral transport mechanisms. Sci. Rep. 6, 22352. https://doi.org/10.1038/srep22352.
Tchernev, G., Orfanos, C.E., 2006. Antigen mimicry, epitope spreading and the pathogenesis of pemphigus. Tissue Antigens 68, 280–286.
Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F.T., de Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T., 2018. SWISS-MODEL: homology modelling of protein structures and complexes. Nucleic Acids Res. 46, 296–303. https://doi.org/10.1093/nar/gky427.
Winther, J.R., Thorpe, C., 2014. Quantification of thiols and disulfides. Biochim. Biophys. Acta 1840, 838–846. https://doi.org/10.1016/j.bbagen.2013.03.031.
World Health Organization, 2015. World Malaria Report 2014. World Health Organization.
Yang, J., He, B.J., Jang, R., Zhang, Y., Shen, H.B., 2015. Accurate disulfide-bonding network predictions improve ab initio structure prediction of cysteine-rich proteins. Bioinformatics 31, 3773–3781. https://doi.org/10.1093/bioinformatics/btv459.
Zhang, N.Z., Huang, S.Y., Zhou, D.H., Xu, Y., He, J.J., Zhu, X.Q., 2014. Identification and bioinformatic analysis of a putative calcium-dependent protein kinase (CDPK6) from *Toxoplasma gondii*. Genet. Mol. Res. 18, 10669–10677. https://doi.org/10.4238/2014.