

A Step towards Malaria Vaccine DBP Engagement with DARC of Erythrocyte

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Abstract

Plasmodium vivax is causative agent of malaria. In *Plasmodium vivax* Duffy binding protein (DBP) is most ligand for merozoite invasion of human erythrocyte surface by DARC. Thus, it is most suitable protein for development of vaccine. Initial infection can stop threw malaria vaccine DBP has great role of a family of related proteins found in different malaria species that are found in host cell invasion. There is only reason of malaria diseases in human being is ligand between DBP and DARC. We studied using bioinformatics tool structure which joins DBP to DARC. After many decades of research, there is still zero result ineffective malaria vaccine. We tried to find out a way that target conserved region of DBP to block the PvDBP-DARC attachment. Inhibiting of attachment DBP with DARC by monoclonal antibodies or artificial peptide chain same as DBP may lead us a step towards malaria vaccine.

Keywords: *Plasmodium vivax*, erythrocyte, vaccine

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INTRODUCTION

Plasmodium vivax is found more commonly worldwide than other malaria species. Thus, it has great role in morbidity and mortality at least 2.85 billion people infected by *Plasmodium vivax* every year [1]. Malaria is most important tropical disease in Asia and South Africa. At least 80 million individuals worldwide suffer from malaria vivax. *Plasmodium vivax* is endemic outside Africa, and it causes substantial morbidity worldwide [2]. *Plasmodium vivax* Duffy binding protein (DBP) is a 140 kDa protein secreted by micromeres of a parasite organelles at the apical end of the merozoite as it invades erythrocytes [3] invasion of RBC by *P. vivax* is a particularly attractive vaccine target because it depends on a single host cell receptor, the Duffy antigen receptor for chemokines (DARC) [4].

DARC negative people are resistance for *Plasmodium vivax* infection. *Plasmodium vivax* invasion in reticulocyte depended upon interaction between Duffy binding proteins with DARC. *Plasmodium vivax* DBP during invasion on the surface of red blood cell binds the Duffy antigen receptor for chemokines DARC [5, 6]. DBP is a great choice against *Plasmodium vivax* vaccine [7]. Interaction

between DBP with DARC made by tight junction can be seen in DBP structure [8]. Grimberg and his team have shown that antibodies to recombinant PvDBP generated by rabbits and human serum can inhibit *Plasmodium vivax* invasion in red blood cell [9].

PvDBP STRUCTURE ANALYSIS

While studying *Plasmodium vivax* Duffy binding protein region II (PvDBP II) to the surface of Duffy antigen receptor for chemokines (DARC), we found that DBP interact with DARC involves amino acid molecules as initial attachment of merozoite on the red blood cell surface [10].

Plasmodium vivax DBP is necessary for erythrocytes invasion. Many amino acid and molecules are required to attach the Duffy binding protein with DARC.

Aim and Object

The main aim of this research was to detailed bioinformatics study of Duffy binding protein which helps us to search specific molecule or any synthetic peptide chain with DARC before *Plasmodium vivax* merozoite invade to erythrocyte. It might be used as prophylactic treatment and may a step towards vaccine development.

STUDY OF ERYTHROCYTE BINDING PROTEIN STRUCTURE

While studying of 3D structure of Duffy binding protein on erythrocyte surface. We got pdb id 4nuv, which had two parts:

1. Erythrocyte binding protein or Duffy receptor (A, B)
2. Atypical chemokine receptor or Duffy antigen receptor for chemokine (C, D).

These structures of *Plasmodium vivax* Duffy receptor which attached to Duffy antigen receptor for chemokine (DARC). Biochemical function of erythrocyte binding protein is only receptor activity. It causes pathogenesis in biological process.

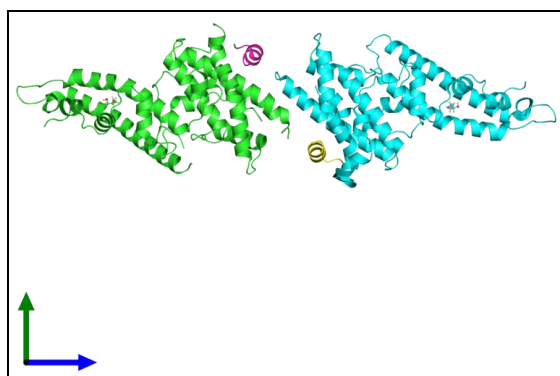


Fig. 1: The structure Pdb id 4nuv demonstrates two chain of Duffy receptor, two chain of atypical chemokine receptor and two molecules of glycerol.

Structure analysis of atypical chemokine receptor showed two chains C and D. Length of atypical chemokine receptor was 34 amino acid, theoretical weight was 3.63 kDa and uniprot id was Q-16570.

This 3D view of pdb id 4nuv show interaction between Duffy receptor or erythrocyte or Duffy binding protein (DBP) chain A, B, and

atypical chemokine receptor or Duffy antigen receptor for chemokine (DARC) chain C, D with amino acid and polypeptide. Chain A and chain B was connected by ligand glycerol in figure 1.

Interfaces Summary for 4nuv

There are four types of colored chains which show the attachment site of interactions. Each circle diameter is in relation of surface of align chain. Figure 2.

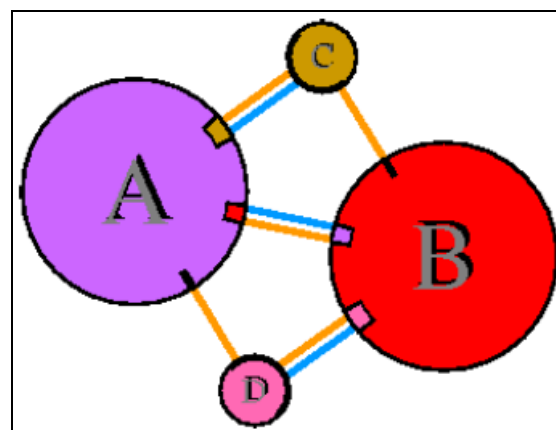


Fig. 2: Schematic Diagram of Interactions between Protein Chains. Interacting Chains was joined by Colored Lines, Each Representing a Different Type of Interaction, as per the Key below. The Area of Each Circle was Proportional to the Surface Area of the Corresponding Protein Chain. The Extent of the Interface Region on Each Chain was Represented by a Colored Wedge whose Color Corresponds to the Color of the Other Chain and whose Size Signified the Interface Surface Area.

Statistics for all the interfaces are given in Table 1 in detail.

Table 1: Interface Statistics.

Chain	No. of interface chain residues	No. of interface area (Å)	No. of salt bridge	No. of disulphide bonds	No. of hydrogen bonds	Non-bonded contacts
A-B	7:5	407:420	-	-	3	19
A-C	10:9	586:653	-	-	2	42
B-D	11:10	577:655	-	-	4	55
A-D	3:2	109:111	-	-	-	5
B-C	1:1	76:72	-	-	-	2

Here we describe interaction of each chain of 4NUV whereas chain A and B are Erythrocyte binding protein which found on plasmodium vivax surface and take part to attach to chain C, D which are Duffy Antigen Receptor for Chemokine (DARC) which found on human erythrocyte. Step wise description of each chain is listed below.

Protein-Protein Interface: Chain A and Chain B

The colored chains show the attachment site of interactions. Each circle diameter is in relation of surface of align chain. Figure 3.

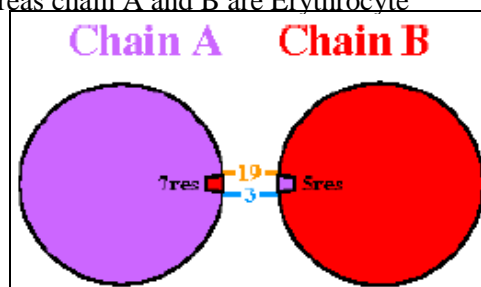


Fig. 3: Analysis of Instruction between Protein Chain A and B. Interacting Chains was joined by Colored Line, each Represent Different type of Interaction as per the Key Below. The Area of Each Circle was Proportional to the Surface of the Corresponding Chain.

Statistics are given Table 2.

Table 2: Analysis of Interface Statistics.

Chain	No. of interface chain residues	No. of interface area (Å)	No. of salt bridge	No. of disulphide bonds	No. of hydrogen bonds	Non-bonded contacts
A	7	407				
B	5	420			2	19

Protein-Protein Interfaces of 4NUV Chain A Chain B

There are showed list of amino acid of chain A and chain B which interacted with each other. Figure 4

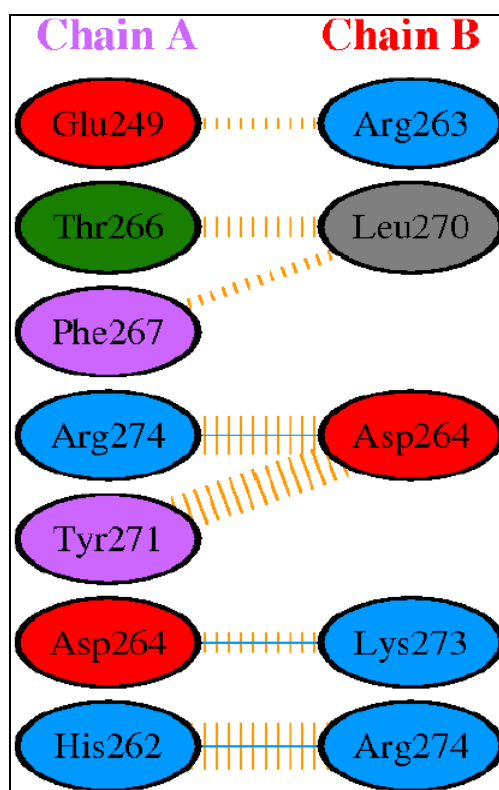


Fig. 4: The Number of H-bond Lines between Any Two Residues Indicates the Number of Potential Hydrogen Bonds between Them. For Non-bonded Contacts, Which can be Plentiful, the Width of the Striped Line is Proportional to the Number of Atomic Contacts.

Residue Colors: Positive (H, K, R); negative (D, E); S, T, N, Q = neutral; A, V, L, I, M = aliphatic; F, Y, W = aromatic; P, G = Pro & Gly; C = cysteine.

Protein-Protein Interface: Chain A and Chain C

The colored chains show the attachment site of interactions. Each circle diameter is in relation of surface of align chain. Figure 5.

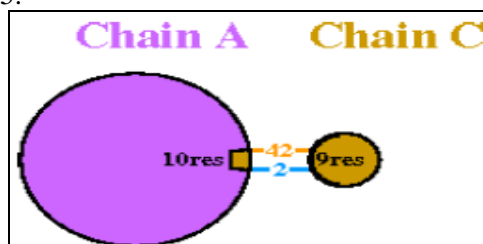


Fig. 5: Schematic Diagram of Interactions between Protein Chains. Interacting Chains are joined by Colored Lines, Each Representing a Different Type of Interaction, as per the Key Below. The Area of Each Circle is Proportional to the Surface Area of the Corresponding Protein Chain. The Extent of the Interface Region on Each Chain is represented by the Black Wedge whose Size Signifies the Interface Surface Area.

Statistics for this interface are given in Table 3.

Table 3: Analysis of this interface is given below

Chain	No. of interface chain residues	No. of interface area(A)	No. of salt bridge	No. of disulphide bonds	No. of hydrogen bonds	Non-bonded contacts
A	10	586				
			-	-	2	42
C	9	635				

Interface between protein chains A and protein chain C

The Duffy binding protein chain A always interacts with chain C of duffy antigen receptor of chemokine. Here we showed attachment between chain A and chain C in Figure 6.

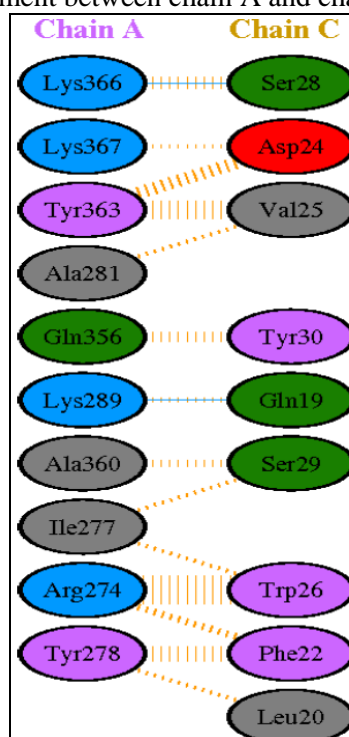


Fig. 6: The Number of H-bond Lines between any Two Residues Indicates the Number of Potential Hydrogen Bonds between Them. For Non-bonded Contacts, Which can be Plentiful, the Width of the Striped Line is Proportional to the Number of Atomic Contacts.

Residue Colors: Positive (H, K, R); negative (D, E); S, T, N, Q = neutral; A, V, L, I, M = aliphatic; F, Y, W = aromatic; P, G = Pro & Gly; C = cysteine.

Interface between protein chain A and protein chain D

There are no hydrogen bond between chain A and chain D. Only 5 nonbonded contacts present between them. The colored protein molecular chains demonstrate the attachment site of interactions. Each circle diameter is in relation of surface of align chain in Figure 7.

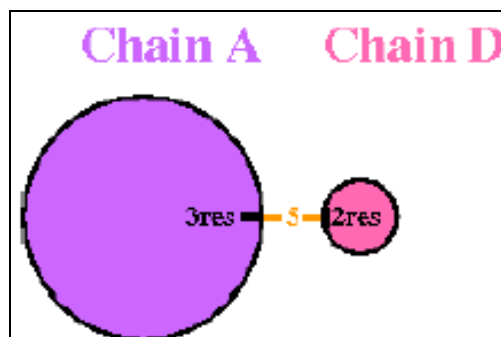


Fig. 7: Schematic Diagram of Interactions between Protein Chains Interacting Chains are Joined by Colored Lines, Each Representing a Different Type of Interaction, as per the Key Below. The Area of Each Circle is Proportional to the Surface Area of the Corresponding Protein Chain. The Extent of the Interface Region on Each Chain is represented by the Black Wedge whose size signifies the Interface Surface Area.

Statistics for this interface are given in Table 4.

Table 4: Analysis of this interface is given below.

chain	No. of interface chain residues	No. of interface area(A)	No. of salt bridge	No. of disulphide bonds	No. of hydrogen bonds	Non-bonded contacts
A	3	109				
			-	-	-	5
D	2	111				

Interaction between protein chains A with protein chain D

There are no hydrogen bond between chain A and chain D. Only 5 nonbonded contacts present between them in Figure 8.

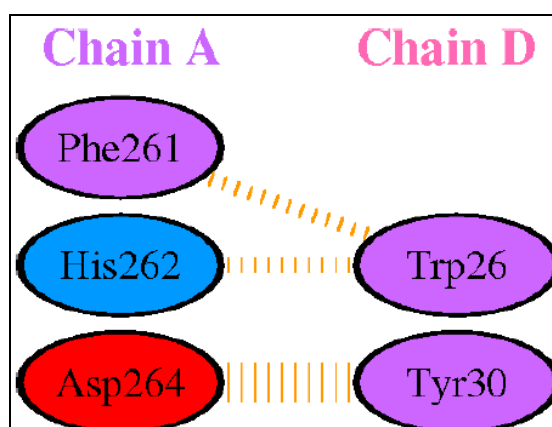


Fig. 8: The Number of Non-bonded Contacts, Which can be Plentiful, the Width of the Striped Line is Proportional to the Number of Atomic Contacts.

Interaction protein chain B with protein chain C

There are no hydrogen bond between chain B and chain C. Only 2 nonbonded contacts present between them. The colored protein molecular chains demonstrate the attachment site of interactions. Each circle diameter is in relation of surface of align chain in figure 9.

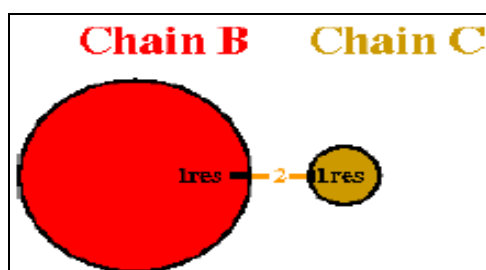


Fig. 9: Schematic Diagram of Interactions between Protein Chains Interacting Chains are Joined by Colored Lines, Each Representing a Different type of Interaction, as per the Key Below. The Area of Each Circle is Proportional to the Surface Area of the Corresponding Protein Chain. The Extent of the Interface Region on Each Chain is represented by the Black Wedge Whose size signifies the Interface Surface Area.

Statistics for this interface are given Table 5.

Table 5: Analysis of this interface is given below.

Chain	No. of interface chain residues	No. of interface area(A)	No. of salt bridge	No. of disulphide bonds	No. of hydrogen bonds	Non-bonded contacts
B	1	76				
			-	-	-	2
C	1	72				

Interaction protein chains B with protein chain C

There are no hydrogen bond between chain B and chain C. Only 5 non bonded contacts present between them. Figure 10

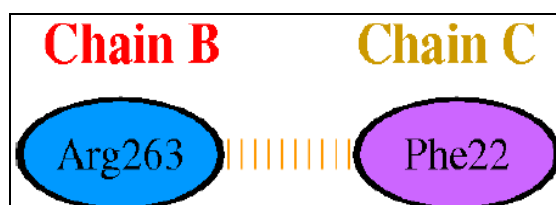


Fig. 10: The Number non-bonded Contacts, which can be Plentiful, the Width of the Striped Line is Proportional to the Number of Atomic Contacts.

Intraction between protein chain B with protein chain D

There are four hydrogen bond between chain B and chain D and 55 non bonded contacts present between them. The colored protein molecular chains demonstrate the attachment site of interactions. Each circle diameter is in relation of surface of align chain in figure 11.

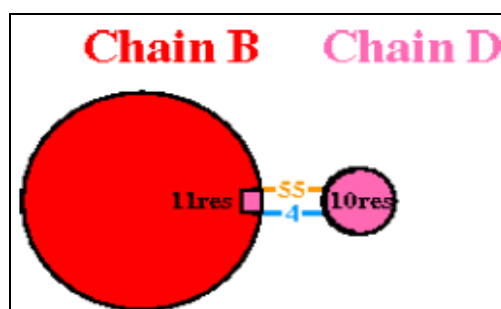


Fig. 11: Schematic Diagram of Interactions between Protein Chains. Interacting Chains are joined by Colored Lines, Each Representing a Different Type of Interaction, as per the Key Below. The Area of Each Circle is Proportional to the Surface Area of the Corresponding Protein Chain. The Extent of

the Interface Region on Each Chain is represented by the Black Wedge whose Size Signifies the Interface Surface Area.

Statistics for this interface are given in Table 6.

Table 6: Analysis of this interface is given below

Chain	No. of interface chain residues	No. of interface area(A)	No. of salt bridge	No. of disulphide bonds	No. of hydrogen bonds	Non-bonded contacts
B	11	577	-	-	4	55
D	10	655				

Interaction protein chain B with protein chain D

There are four hydrogen bond between chain B and chain D and 55 non bonded contacts present between them. The colored protein molecular chains demonstrate the attachment site of interactions. Each circle diameter is in relation of surface of align chain. Figure 12

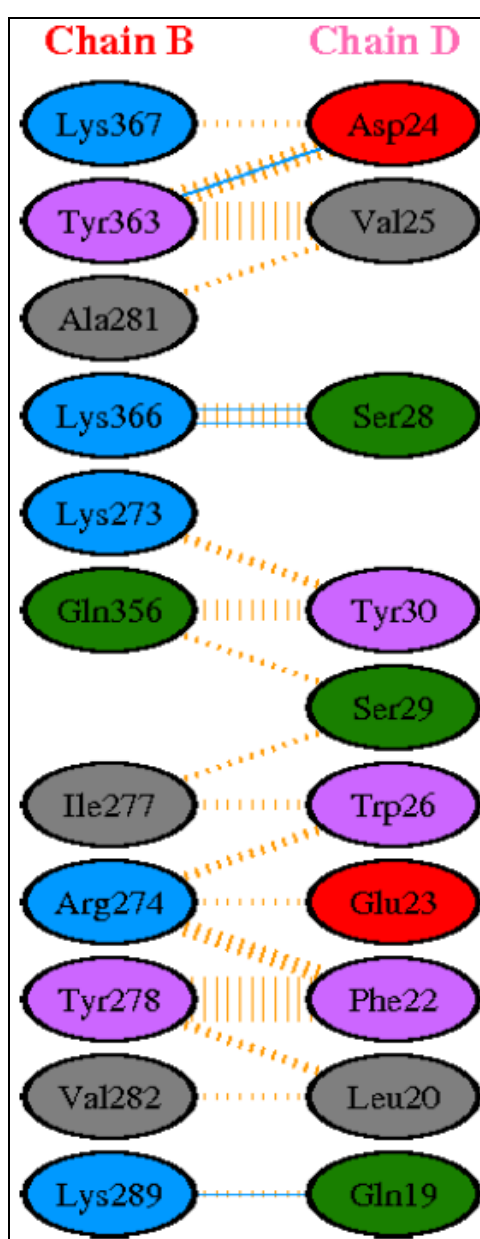


Fig. 12: The Number of H-bond Lines between any Two Residues Indicates the Number of Potential Hydrogen Bonds between Them. For Non-bonded Contacts, Which can be Plentiful, the Width of the Striped Line is Proportional to the Number of Atomic Contacts.

Here are the twenty amino acid with their and one-letter abbreviations. Mnemonic names may help you to remember the one-letter codes, but are not the correct names.

Ala A Alanine	Met M Methionine	Ile I Isoleucine
Arg R Arginine	Phe F	Ser S Serine
Asn N	Phenylalanine	Thr T Threonine
Asparagine	Pro P Proline	Trp W
Asp D Aspartic acid	Gln G Glutamine	Tyr Y Tyrosine
Cys C Cysteine	Glu E Glutamic acid	Val V Valine
Leu L Leucine	Gly G Glycine	
Lys K Lysine	His H Histidine	

CONCLUSION

In this study, we can analysis of erythrocyte binding protein with erythrocyte Duffy antigen receptor for chemokines (DARC). We analyzed that these attachment sites can be used for development vaccine or a prophylactic treatment of malaria. We focused here to develop antibodies against plasmodium vivax merozoite antigen for origination of plasmodium vivax. The challenges is that find out suitable antibody to inhibit or block erythrocyte binding protein to erythrocytes. I am very hopeful to break this bond in my further practical research. For inhabitation of PvDBP-DARC junction antibody testing may be suitable alternative for stopping malaria in human being.

My aim is to make such antigen which survive after death of erythrocyte and engage with new erythrocyte and the cycle is going on. To get the conclusion of binding channel in between malaria parasite and erythrocyte I thought the study of this mechanism using bioinformatics tool. Malaria vivax erythrocyte binding protein uses the Duffy blood group antigen on erythrocyte surface as a receptor. Erythrocyte binding protein (EBP) is most suitable ligand for invading erythrocytes.

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