# Literature review on DBLMSP and DBLMSP2 in *Plasmodium*

November 10, 2022

### Conventions

Throughout the report I refer to DBLMSP and DBLMSP2 as **DBs**.

All gene names use the stable IDs from PlasmoDB release 53.

Important references are highlighted in the bibliography.

## 1 Current state of knowledge relevant to DBs

### 1.1 Genomic location and domain structure of DBs

In *P. falciparum*, DBLMSP and DBLMSP2 are two genes located 16kbp apart on chromosome 10 (DBLMSP: Pf3D7_10_v3:1,412,641..1,416,363(+); DBLMSP2: Pf3D7_10_v3:1,432,498..1,434,786(+)). They are part of a set of 8 tandemly arrayed genes spanning 32kbp on chromosome 10 that all share an N-terminal 'NLRNA/G' sequence [55]. [55] call them 'MSP3-like', and proposed renaming them MSP3.1-MSP3.8. In this system MSP3.4 and MSP3.8 are the names for what is now stably referred to as DBLMSP and DBLMSP2, respectively. Six of the eight genes, including DBLMSP and DBLMSP2, possess a polymorphic C-terminal domain called SPAM ([36]; Pfam: PF07133). In MSP3 (MSP3.1 in the MSP3-like classification), SPAM was found to mediate protein oligomerisation [18]. The genes GLURP and LSA-1 are located 5' and 3' of the MSP3-like gene cluster, respectively.

In contrast to the other MSP3-like genes, DBs also possess a Duffy-Binding Like (DBL) domain (Pfam: PF05424), located between the N-terminal NLR sequence and C-terminal SPAM. DBL is named after the

initial discovery that *Plasmodium vivax* requires the so-called Duffy antigen/chemokine receptor (DARC) on human red-blood cells for cell invasion [37]. In *P. vivax* and *P. knowlesi*, a gene called Duffy Binding Protein (DBP) is responsible for binding DARC.

## 1.2 DBL domains in *P. falciparum*

In addition to the DBs, the DBL domain is present in a number of other *P. falciparum* (*Pf*) proteins. EBA175 was first identified as a protein mediating RBC invasion via binding to the RBC receptor glycophorin A (GYPA) [42], a RBC surface glycoprotein ('glyco' means sugars attached). EBA175 binding requires a sialic acid (e.g. Neu5Ac) attached to GYPA [42], and antibodies raised against EBA175 inhibit merozoite invasion of RBCs [54]. EBA175 contains two tandemly arrayed DBL domains [1] called F1 and F2 that mediate GYPA binding [61]. A number of genes paralogous to EBA175 exist that also have tandem F1/F2 DBL domains: EBA140, EBA165, EBA181, EBL1(fig. 1; [2]). EBA140 also mediates merozoite invasion of RBCs [35] by binding to the lycophorin C receptor linked to sialic acid [31]. EBA165 is functional in all *Laverania* species but is inactivated in *Pf* and is thus a pseudogene [50]. EBL1 and EBA181 are less well understood. EBL1 may bind to glycophorin B [26], and EBA181 binds RBCs but the receptor is unknown [16].

The presence of multiple EBA paralogs is thought to mediate functional redundancy, allowing merozoites from different strains to invade RBCs with different surface receptors (e.g. lacking glycophorin A and B [22]) or differently modified surface receptors (e.g. with sialic acid removed [39, 57]). Alternative use of different EBAs for invasion was also shown to be epigenetically mediated for EBA-140, with expression switched on or off across isogenic subclones [8]. Another paralogous family or proteins, the reticulocyte-binding homologs (RH), is located in the same apical organelles as EBAs (rhoptries and micronemes). RHs are also involved in RBC invasion and displays functional redundancy [19]. RHs and EBAs are thought to function cooperatively in RBC invasion [32]. Because both protein families are recognised by the immune system, alternative invasion pathways likely provide adaptability against immune system targeting of specific proteins [66].

DBL domains are also found in the *var* genes, a family of paralogs specific to the subgenus *Laverania* [45, 44] (of which *Pf* is a member, but not *P. vivax*). *var* genes are mostly located in chromosome subtelomeres, with 60

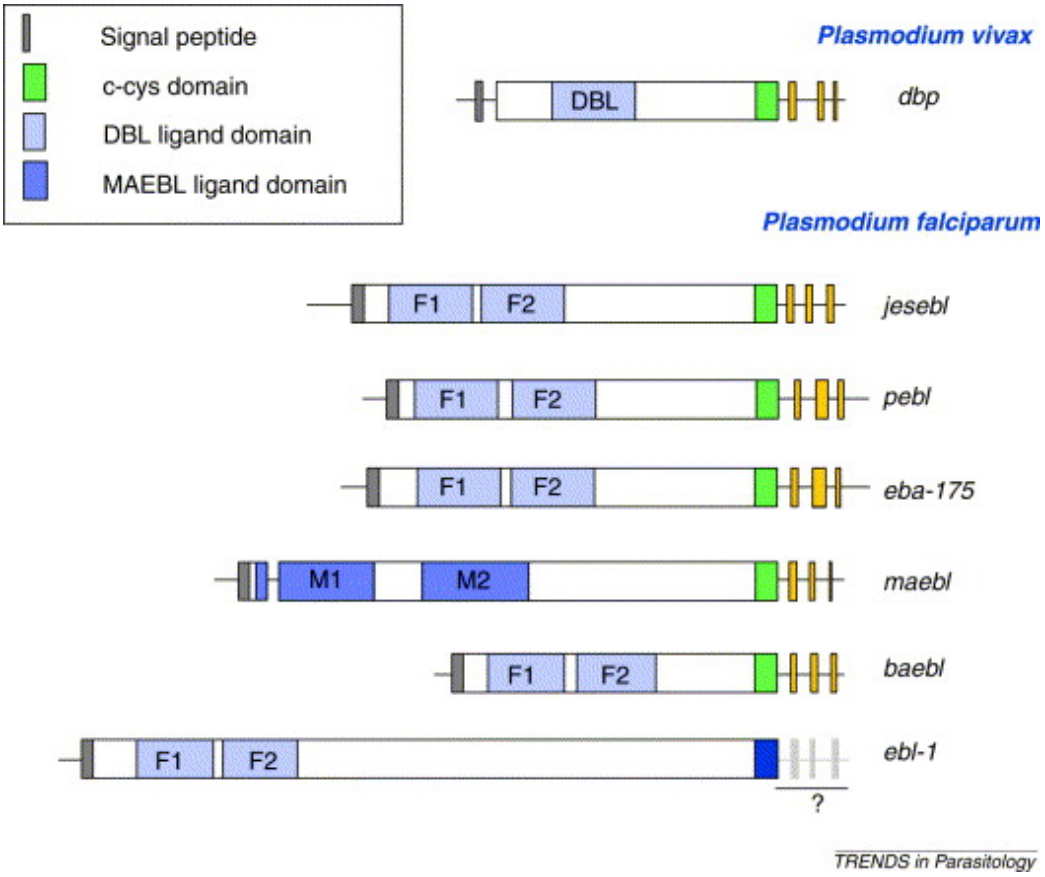


Figure 1: DBL domains in the erythrocyte-binding like gene family. F1 and F2 are tandem DBL domains homologous to *P. vivax* DBL. All genes except EBL1 possess a conserved C-terminal cysteine rich domain. ID mapping: EBA181(*jesebl*), EBA165(pebl), EBA140(baebl). Source: [2].

copies in the *Pf* genome [59, 15]. During the ring stage of the intraerythrocytic cycle (IEC), *Pf* expresses only one or a few *var* genes at a time, and during the schizont and trophozoite stages of the IEC, a single *var* protein product (called *PfEMP1*) is found exported at the surface of the iRBC [7]. The 5' end of *var* genes contains multiple DBL and CIDR domains mediating adherence to other cells (fig. 2). PfEMP1 allows iRBS to bind to uninfected red blood cells (rosetting), endothelial cell receptors in tissues, and to platelets enabling iRBC clumping [38]. The specific combination of DBL and CIDR domains determines the set of bound human receptors and is associated with malaria disease severity [TODO citations]. Different domain combinations are produced via meiotic recombination during sexual reproduction [58], but also by non-allelic recombination [14] during mitosis [10] (and thus during a single infection). Recombination occurs in a structured way between subgroups of *var* genes based on genomic context (subtelomeric, central), gene orientation and flanking non-coding sequence [29]. Of all the DBL/CIDR domains, only DBL-$\alpha$ is found in every *var* gene [56].

PfEMP1 is recognised by the human immune system [TODO: cite]. *Pf* evades immune system recognition and clearance by switching which *var* gene is expressed during the course of infection [52]. [TODO epigenetically mediated].

## 1.3 Biological function of DBs

### 1.3.1 DB localisation and RBC binding

DBLMSP is expressed at the late schizont and merozoite stages and localises to the merozoite surface [65]. [65] showed that COS7 (monkey-derived) cells made to express the DBL and SPAM domains of DBLMSP bound RBCs. Binding was abolished by treating RBCs with trypsin or neuraminidase or by adding immune sera from DBLMSP-immunised mice. Using ELISA, [65] also showed recombinant DBL and SPAM domains were both bound by human immune sera from malaria-exposed, but not malaria-naive, individuals. This suggests DBLMSP is involved in RBC binding by merozoites. However, *in vitro* RBC invasion is only reduced by 25% in the presence of high levels of anti-DBLMSP antibodies([53] Fig. 4B), and RBC invasion efficiency is not reduced in DBLMSP knock-out parasite lines [53, 9], showing DBLMSP is not essential for RBC invasion.

[24] find DBLMSP2 is expressed on the surface of schizonts and mero-

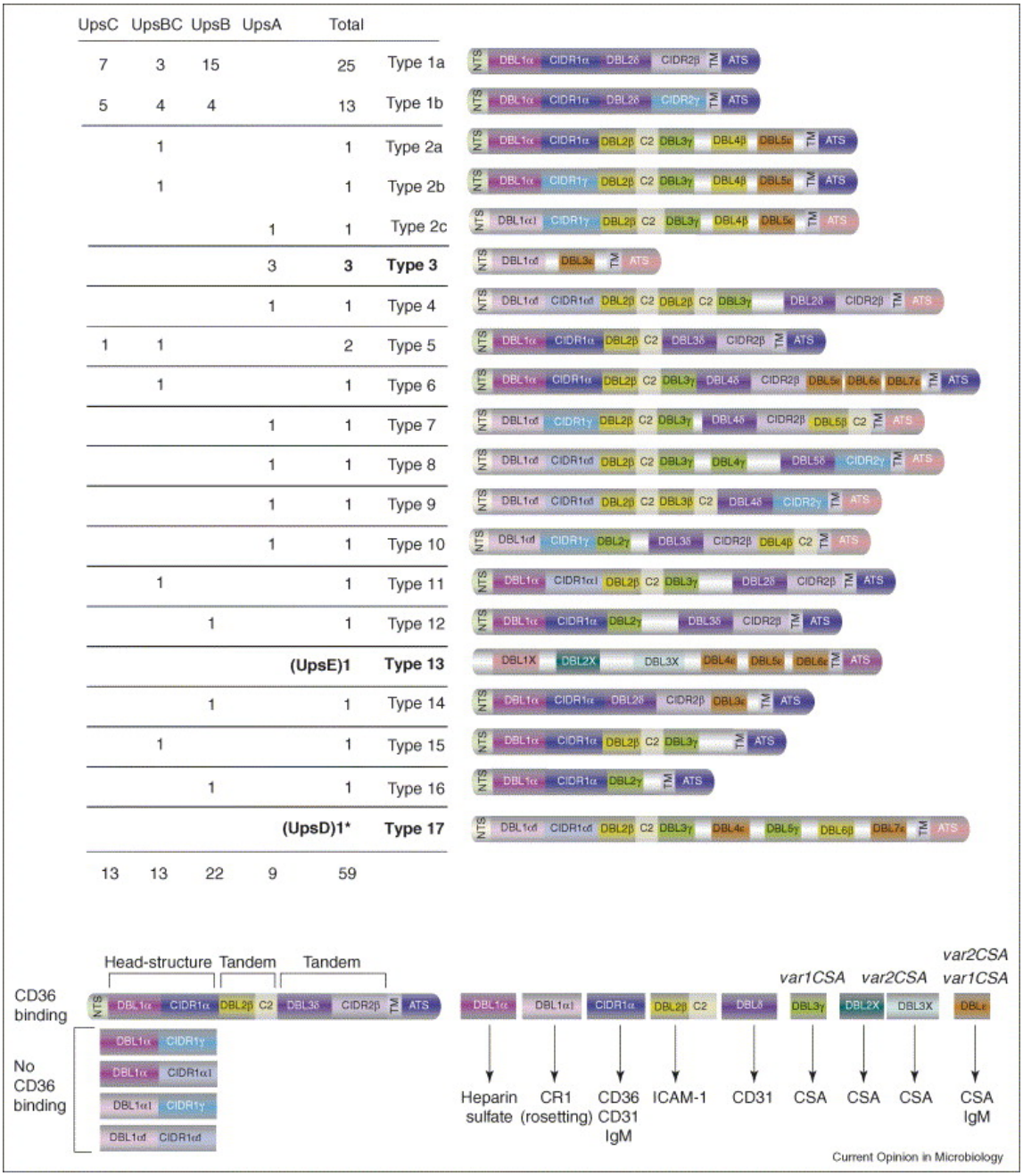


Figure 2: DBL domains in the *var* gene family. *var* genes have a N-terminal segment (NTS), transmembrane (TM) domain and acidic terminal segment (ATS), and *var* types are assigned by combination of DBL and CIDR domains. The left-hand panel gives counts of *var* types in the *Pf* 3D7 genome by promoter type (*UpsA*-*UpsE*). The bottom panel lists bound human proteins. Source: [28].

zoites using anti-DBLMSP2 antibodies, and that full-length DBLMSP2 and the DBL domain only bind to RBCs. There are potential caveats to these results. In contrast to [65], who expressed DBLMSP on COS7 cells and assayed RBC binding, [24] added purified DBLMSP2 to RBCs together with *Pf* post-invasion culture supernatant, so the DBLMSP2 in pulldowns could have been in complex with other *Pf* proteins binding to RBCs. [24] also do not find that DBLMSP binding to RBCs is trypsin or neuraminidase sensitive, whereas [65] do. Finally, Gavin Wright and Cecile Crosnier at the Sanger Institute could not replicate DBLMSP2 binding to RBCs (unpublished, personal communication).

### 1.3.2 DBLMSP2 and gametocytogenesis

DBLMSP2 transcripts are present at very low levels in bulk RNA-seq of clinical and laboratory isolates ([46] Sup. Table S3, [3]). Using immunofluorescent labeling of DBLMSP2, [3] showed that DBLMSP2 is only present on the surface of a minority of schizonts (fig. 3). This is consistent with repressive chromatin factors being found at the DBLMSP2 locus: histone mark H3K9me3 [33] and heterochromatin protein 1 (HP1; [13]). These marks are not widespread in the genome and are typically associated with clonally variant expression of virulence gene families (e.g. *var*,*rif*,*stevor*,*clag* [33]). In *Pf*, HP1 epigenetically regulates both the single, mutually exclusive expression of *var* genes and sexual commitment via the induction of the *ap2-g* transcription factor [5], which is essential for sexual differentiation of *Pf* into gametocytes [27]. HP1 forms a complex with the *PfGDV1* protein [12], another early marker of gametocytogenesis [11]. Consistent with this, [12] found that conditional activation of *PfGDV1* induces *ap2-g* expression and gametocytogenesis, likely by antagonising HP1-based repression. Remarkably, DBLMSP2 is one of the 8 genes induced by *PfGDV1* ( [12] Fig. 2). However, in two other studies DBLMSP2 did not appear as part of the transcriptional signature of sexual committment defined using single-cell RNA-seq ([49] Supplementary Table 3; [6] Supplementary File 2). It is thus unclear still whether DBLMSP2 plays a role in gametocytogenesis.

### 1.3.3 DBLMSP2 and drug resistance

DBLMSP2 has also been implicated in drug resistance. A GWAS study using SNP array genotyping found variants in DBLMSP2 associated with resistance

to the antimalarial halofantrine [63]. [63] functionally validated the association by showing that episomal (plasmi-mediated) DBLMSP2 overexpression increases resistance to halofantrine and structurally related compounds (mefloquine and lumefantrine), and that *Pf* strains with higher DBLMSP2 copy-number are also more resistant. Given that DBLMSP and DBLMSP2 occur in tandem and can share sequence [34], I think WGS-based CNV quantification is required to validate cross-strain CNV differences in DBLMSP2. Subsequently, [64] found that knocking-out DBLMSP2 reduced $IC_{50}$ (drug concentration giving a 50% parasite growth reduction) to the these three antimalarials by about 50%, and that a specific SNP (C591S) in the SPAM domain approximately doubles $IC_{50}$ for all three antimalarials.

### 1.3.4 DB complex formation

All six 'MSP3-like' proteins (which includes the DBs) localise to the merozoite surface [55]. Despite this, all 'MSP3-like' proteins lack transmembrane domains and GPI anchors characteristic of membrane-anchored proteins. One 'MSP3-like' protein, MSP6, was found to occur bound to GPI-mediated membrane-anchored MSP1 [62]. This was also found for another merozoite surface protein, MSP7 [47]. Subsequently, [30] found that DBs are also found in complex with MSP1. MSP1 is the most abundant merozoite surface protein [17] and is proteolytically processed into four, non-covalently linked subunits during merozoite maturation. Upon merozoite entry into RBCs, the MSP1 complex is cleaved by the PfSUB2 protease [23], leaving the GPI-anchor MSP1 fragment bound to the merozoite surface while the rest is shed [4, 23]. Antibodies against MSP1 block invasion [4] and are associated with protection from malaria [43]. AMA1, a microneme protein involved in merozoite reorientation post RBC attachment and a major vaccine target, is also shed following PfSUB2 cleavage [23]. Interestingly, EBA175 is also shed post-merozoite invasion via proteolytic cleavage by the PfROM4 protein [40]. Subsequent work showed shed EBA175 mediates uninfected RBC clustering via its two tandem DBL domains, and that RBC clustering enhances merozoite reinfection and immune system evasion [48] (see fig. 4 for an illustrative model).

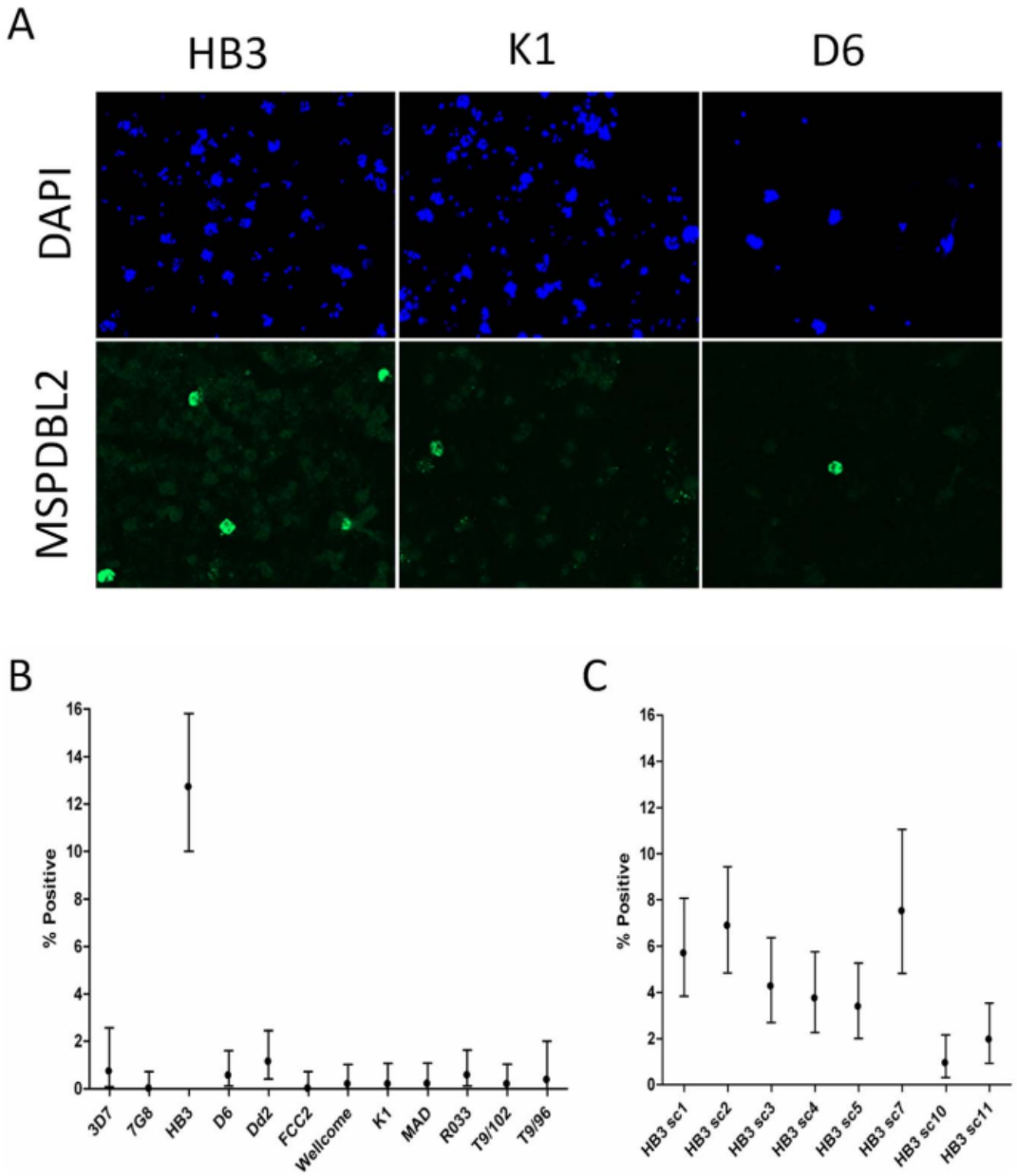


Figure 3: DBLMSP2 is expressed in a subset of schizonts. (A) DAPI (parasite nuclear DNA) and DBLMSP2 immunofluorescent staining with anti-DBLMSP2 antibodies shows DBLMSP2 is present at the surface of a small number of schizonts in three independent cultured isolates (HB3, K1, D6). (B) The HB3 parasite line has the highest fraction of DBLMSP2-positive schizonts. Source: [3]

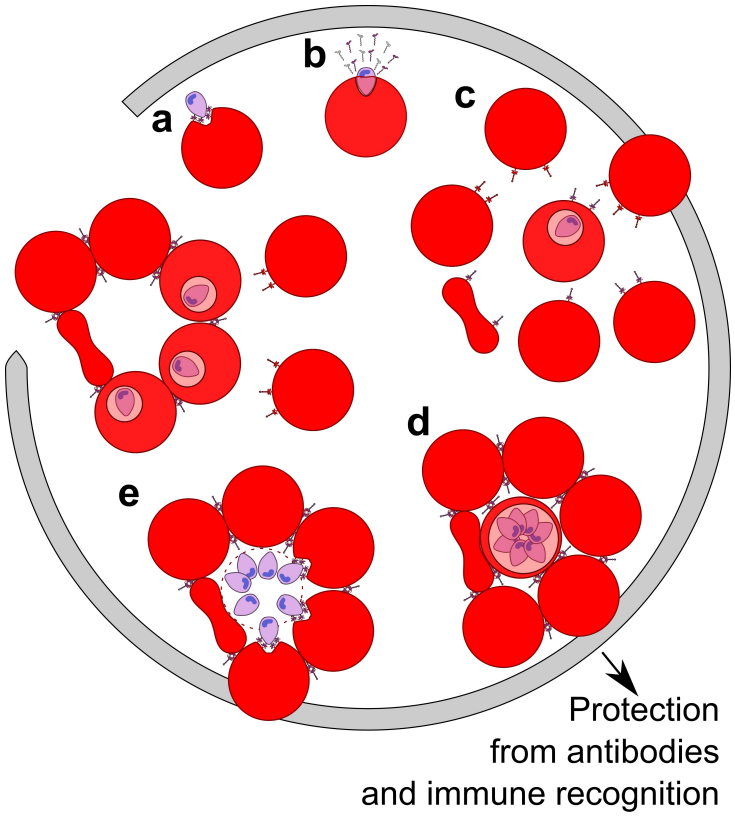


Figure 4: EBA175 shed from merozoites post RBC invasion mediates RBC clustering. Model from [48]. The two DBL domains from EBA175 mediate RBC clustering by binding to GYPA on the RBC surface. RBC clustering reduced antibody-based (anti-AMA1 and anti-RH5) growth inhibition (illustrated in d) and enhanced parasite growth (illustrated in e).

### 1.3.5 Immune system interactions

[55] find IgGs in immune serum from people in malaria-endemic Senegal react against a conserved C-terminal region in all six 'MSP3-like' proteins, suggesting cross-reactive immunity to all can arise.

No RBC binding partners for DBs have been identified to date, and conflicting evidence exists regarding what kind of RBCs DBs bind ([65] vs [24]). Further, from personal communication, Cecile Crosnier and Gavin Wright did not observe DB binding to RBCs.

## 1.4 Genetic diversity of DBs

# 2 Questions of interest around DBs

$\delta$: doable $\epsilon$: uncertain

## 2.1 Biological function

1. [$\delta$] What is the sequence and structure relationship between DBL domain in DBs and other Pf DBL domains? (see [51] for an analysis of DBL homology blocks, with MEME motifs, showing parallel between EBA-175 and VAR2CSA DBL structures (Fig.5))

2. [$\epsilon$] Can we identify putative human protein interactants, can we dock DBs to them, and can we evaluate goodness of fit compared to known DBL-human protein interactions?

3. [$\epsilon$] Can we find putative structural interactants expressed in human cell types likely to be in DB environment when DBs are expressed? (e.g. bone marrow for DBLMSP2 in early gametocytes

4. [$\delta$] How often are DBs not functional (nonsense mutations) in natural pops, and is there a diff. between DBLMSP and DBLMSP2? (Note premature stop codons are known in DBLMSP: [60]; [41] Table 1)

5. [$\delta$] What is the phylogenetic distribution of DBs?

## 2.2 Location of polymorphisms

1. [$\delta$] Do we see conserved/hypervariable sites at the DNA level?

2. [$\delta$] Can we map polymorphisms onto DB structures and identify structurally conserved or variable regions? (BioStruct can do this [20], see [21] for examples in *Pf*

## 2.3 Gene conversion and recombination

1. [$\epsilon$] Can we identify and quantify gene conversion events between DBs? (Note, could maybe also do this in tandemly arrayed genes clag 3.1 and clag 3.2, this has been observed in [25])

## 2.4 Drug resistance

1. [$\delta$] Is the drug resistance SNP in DBLMSP2 SPAM domain found in DBLMSP sequences (or other 'MSP3-like' proteins with SPAM domains)?

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