

## The essential mosquito-stage P25 and P28 proteins from *Plasmodium* form tile-like triangular prisms

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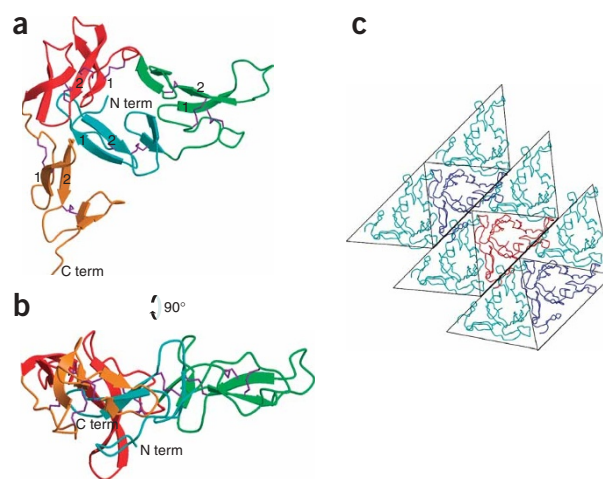
**P25 and P28 proteins are essential for *Plasmodium* parasites to infect mosquitoes and are leading candidates for a transmission-blocking malaria vaccine. The *Plasmodium vivax* P25 is a triangular prism that could tile the parasite surface. The residues forming the triangle are conserved in P25 and P28 from all *Plasmodium* species. A cocrystal structure shows that a transmission-blocking antibody uses only its heavy chain to bind Pvs25 at a vertex of the triangle.**

Most mosquito species are not permissive for infection by parasites of the genus *Plasmodium*. In the few mosquito species that can become infected, most parasites do not survive the mosquito's defenses<sup>1</sup>. As malaria is caused by the few surviving parasites, a better understanding of insect-parasite interactions may lead to strategies that further reduce parasite survival<sup>2</sup>. We set out to study the abundant surface proteins P25 and P28, which are expressed only in the mosquito, are unique to *Plasmodium* and are essential for parasite survival. Gene knockouts of either P25 or P28 alone have little effect on parasite survival, but simultaneous disruption markedly lowers the number of surviving parasites<sup>3</sup>. A P25 transmission-blocking vaccine is in clinical trials<sup>4</sup>. Antibodies elicited by the vaccine are expected to bind their ligand outside the vaccinee. When parasites and antibodies to P25 are taken up in a blood meal by a mosquito, the antibodies inhibit the parasite when it expresses P25 on its surface<sup>5</sup>.

We determined the structure of Pvs25 from an ytterbium-soaked crystal of the yeast-produced Pvs25 used for the vaccine trials (Supplementary Methods and Supplementary Table 1 online). Pvs25 consists of a novel triangular arrangement of four epidermal growth factor (EGF)-like domains tethered on the cell by a glycosylphosphatidylinositol anchor. The shape of Pvs25 is a flat triangular prism with nearly equilateral sides of 50 Å and a thickness of 20 Å (Fig. 1). The P25 structure is the first of a mosquito-stage surface protein from *Plasmodium* and, to our knowledge, of a protein

consisting only of repeated EGF-like domains from any organism. A search with the Dali server<sup>6</sup> indicates that this arrangement of EGF-like domains has no precedent.

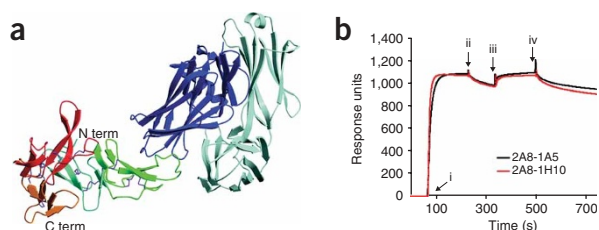
Though common as modules in cell-surface proteins that mediate protein-protein interactions in higher eukaryotes, EGF-like domains occur in only about ten *Plasmodium* surface proteins. The EGF-like domains of P25 and P28 share the EGF family resemblance in having six cysteines linked 1-3, 2-4 and 5-6 along with two central  $\beta$ -strands connected by a B loop (Supplementary Fig. 1 online). Both P25 and P28 lack the 1-3 disulfide in EGF domain 1, and P28 lacks the 5-6 disulfide in EGF domain 4. The overall sequence identity among P25 and P28 proteins varies between 35% and 45%, but the 22 cysteines in P25 and 20 cysteines in P28 and their spacing are conserved in all sequenced *Plasmodium* P25 and P28 proteins (Supplementary Figs. 2 and 3 online).



**Figure 1** Views of Pvs25. (a) The triangular prism formed by domain 1 (light blue), domain 2 (green), domain 3 (red) and domain 4 (gold). The central  $\beta$ -strands in each domain are labeled 1 and 2. The 11 disulfide bonds (violet) are shown. (b) View of the edge of the prism. (c) Pvs25 forms sheets in the crystals. One molecule (red) makes contacts with four  $2_1$  symmetry mates (light blue) and two molecules related by lattice translations (dark blue). The six light blue molecules all have the same triangular face 'up', whereas the other three molecules (red and dark blue) all have the opposite face 'up'. As P25 and P28 molecules are thin prisms, the glycosylphosphatidylinositol anchor could reach the cell membrane whether a molecule was facing 'up' or 'down' on the membrane. N term, N terminus; C term, C terminus.

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**Figure 2** Binding of transmission-blocking antibodies to Pvs25. (a) 2A8 Fab bound via its heavy chain (dark blue) to the B loop of Pvs25 domain 2. The light chain (light blue) does not contact Pvs25. The B loop of Pvs25 domain 3 (red) extends up from the plane of the triangle. (b) Monoclonal antibodies 1H10 and 1A5 do not bind in the presence of prebound 2A8. With Pvs25 immobilized on a Biacore chip, 2A8 was injected at (i) until binding was saturated at (ii), and then the second antibody and 2A8 were injected at (iii) until (iv). Buffer without protein was injected from (ii) to (iii) and from (iv) onward.

To make the triangle, EGF domain 1 forms interfaces with EGF domains 3 and 4 that bury  $610 \text{ \AA}^2$  and  $1,010 \text{ \AA}^2$  of solvent-accessible surface, respectively, and  $1,480 \text{ \AA}^2$  in the combined interface. The residues that interact are conserved in all P25 and P28 sequences. The B loop of domain 1 uses the residues Gln15, Met16, Ser17, His19 and Lys/Glu21 to tuck between the following residues in domains 3 and 4: Glu/Lys99, Ser114, Cys115, Thr135 and Leu139 (Supplementary Fig. 1 and Supplementary Table 2 online). These interactions also help to stabilize the two-disulfide EGF domain 1. The highly conserved interacting residues indicate that the *P. vivax* structure is a good model for all P25 and P28 molecules.

In all the crystals, whether composed of native or reductively methylated Pvs25 with or without a His<sub>6</sub> tag, one Pvs25 molecule makes contacts with six neighbors to form a sheet of molecules (Fig. 1c). Many of these contacts are between highly conserved residues in the P25 and P28 families that are not involved in the protein fold. Fourteen highly conserved residues, nine aspartates or glutamates and five lysines, extend their side chains into the solvent. Four completely conserved glycine residues with normal main chain  $\phi$  and  $\psi$  angles are exposed to solvent in positions in the structure that would accept residues with side chains. Together, these observations suggest that the conserved surface and shape of P25 and P28 may be important for the molecules fitting together, possibly to form structural or protective sheets on the parasite surface. Alternatively, P25 and P28 may bind to one or more receptors.

To understand the binding of an antibody to P25 that inhibits the development of *P. vivax* parasites in mosquitoes, we determined the structure of Fab 2A8 bound to Pvs25. Monoclonal antibodies 2A8, 1H10 and 1A5 were generated using yeast-produced Pvs25 as immunogen, and these bind the parasite surface in immunofluorescence experiments<sup>7</sup>. When mosquitoes are fed *in vitro* on blood mixed with any of the three antibodies and *P. vivax* parasites, the survival of parasites is almost zero (data not shown). In our structure, 2A8 binds a vertex of the Pvs25 triangle, making contacts with the B loop and central  $\beta$ -strands of domain 2 using only its heavy-chain variable domain (V<sub>H</sub>) (Fig. 2a). The 2A8-Pvs25 interaction buries  $1,330 \text{ \AA}^2$ , an

area typical of antibody-protein interactions that use both V<sub>H</sub> and the light-chain variable domain. Residues from all three complementarity-determining loops of V<sub>H</sub> and the hypervariable fourth loop make contacts to 19 Pvs25 residues (Supplementary Fig. 4 and Supplementary Table 3 online). Pvs25 residues Pro63–Asn67 move between  $1.4 \text{ \AA}$  and  $6.3 \text{ \AA}$  upon 2A8 binding, contributing to the shape-complementarity index of 0.79 for the interface, a high value for an antibody-protein antigen complex<sup>8</sup>.

Our measured binding affinities ( $K_d$ ) of 2A8, 1H10 and 1A5 to immobilized Pvs25 are in the range of 1–10 nM. 1H10 and 1A5 are unable to bind Pvs25 that has been prebound with saturating 2A8, indicating that they also bind near or at the B loop of domain 2 (Fig. 2b). The domain 2 B loop of *Plasmodium berghei* P28 has been identified with synthetic peptides as the binding site for the transmission-blocking mAb 13.1 (ref. 9). Proposed mechanisms for the blocking of transmission by antibodies must consider that Fab fragments of mAb 13.1 have potent blocking activity<sup>10</sup>. The short B loop of domain 3 is the other known transmission-blocking epitope, where mAb 4B7 (ref. 11) and mAb 32F81 (ref. 12) bind *Plasmodium falciparum* P25. The B loop of domain 3 extends out of the plane of the P25 or P28 triangle (Figs. 1 and 2). Effective malaria transmission-blocking vaccines should elicit antibodies that bind the B loops of domains 2 and 3 of the P25 or P28 triangular prism.

**Accession codes.** Protein Data Bank: Coordinates have been deposited with the following accession codes: 1Z27 (ytterbium-soaked Pvs25), 1Z1Y (native Pvs25) and 1Z3G (Pvs25–Fab complex).

*Note:* Supplementary information is available on the Nature Structural & Molecular Biology website.

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#### COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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