Structure of malaria invasion protein RH5 with erythrocyte basigin and blocking antibodies

Katherine E. Wright1, Kathryn A. Hjerrild2, Jonathan Bartlett1, Alexander D. Douglas2, Jing Jin2, Rebecca E. Brown2, Joseph J. Illingworth3, Rebecca Ashfield2, Stine B. Clemmensen3, Willem A. de Jongh3, Simon J. Draper2 & Matthew K. Higgins1

Invasion of host erythrocytes is essential to the life cycle of Plasmodium parasites and development of the pathology of malaria. The stages of erythrocyte invasion, including initial contact, apical reorientation, junction formation, and active invagination, are directed by coordinated release of specialized apical organelles and their parasite protein contents. Among these proteins, and central to invasion by all species, are two parasite protein families, the reticulocyte-binding protein homologue (RH) and erythrocyte-binding like proteins, which mediate host–parasite interactions. RH5 from Plasmodium falciparum (PfRH5) is the only member of either family demonstrated to be necessary for erythrocyte invasion in all tested strains, through its interaction with the erythrocyte surface protein basigin (also known as CD147 and EMMPRIN)1–14. Antibodies targeting PfRH5 or basigin efficiently block parasite invasion in vitro5–9, making PfRH5 an excellent vaccine candidate. Here we present crystal structures of PfRH5 in complex with basigin and two distinct inhibitory antibodies. PfRH5 adopts a novel fold in which two three-helical bundles come together in a kite-like architecture, presenting binding sites for basigin and inhibitory antibodies at one tip. This provides the first structural insight into erythrocyte binding by the Plasmodium RH protein family and identifies novel inhibitory epitopes to guide design of a new generation of vaccines against the blood-stage parasite.

Each Plasmodium species contains at least one RH protein. These are often large, of low sequence complexity and with no homology to proteins of known structure. PfRH5 is unusual in being significantly shorter than its homologues (~60 kDa for PfRH5 vs 200–375 kDa for other RH proteins). It lacks their carboxy-terminal transmembrane segment, but associates peripherally with the membrane and with PfRH5 interacting proteins (PfrIpr)10. Although it shares only ~20% pairwise identity with other PfRH proteins3,11, PfRH5 is remarkably conserved, with five common non-synonymous single nucleotide polymorphisms (SNPs)7,8,12. Crucially, antibodies raised against one PfRH5 variant neutralise parasites of all tested heterologous strains, containing these and other less common SNPs8,9, and anti–PfRH5 monoclonal antibodies that neutralise parasites of all tested heterologous strains, containing these and other less common SNPs8,9, and anti–PfRH5 monoclonal antibodies that prevent parasite growth in vitro can directly block the PfRH5–basigin interaction13. Moreover, acquisition of anti–PfRH5 antibodies during natural infection correlates with clinical outcome and these antibodies can also inhibit parasite growth in vitro13. These findings have generated intense excitement about PfRH5 as a next-generation blood-stage malaria vaccine target and emphasized the need for structural information to guide rational immunogen design.

Theoretically, PfRH5 required a protein construct lacking flexible regions but still capable of binding basigin. Long disordered regions were predicted within residues 1–140 and 248–296 (Extended Data Fig. 1a), and in cultured parasite lines, PfRH5 is processed by removal of the amino terminus to generate a ~45 kDa fragment13,14. We therefore designed PfRH5ANL, encompassing residues 140–526 but lacking 248–296, and showed that it binds basigin by surface plasmon resonance with an affinity of 1.3 μM (Fig. 1c), comparable to the affinity of full-length PfRH5 for basigin (1.1 μM)15.

To ensure that PfRH5ANL contains epitopes required to elicit an inhibitory immune response, we raised rabbit polyclonal IgG and tested their ability to neutralise parasites by a growth-inhibitory activity (GIA) assay (Fig. 1d). IgG raised against PfRH5ANL protein showed a potent inhibitory effect, similar to that of IgG raised by immunisation of rabbits with viral vectors expressing full-length PfRH5, or full-length PfRH5 recombinant protein16,17. We also tested binding of PfRH5ANL to a panel of mouse monoclonal antibodies previously characterized for PfRH5 binding and growth-inhibitory activity15. PfRH5ANL bound to growth-inhibitory antibodies including QA1, QA5 and 9AD4, but not to non-inhibitory 4BA7 and RB3 (Extended Data Fig. 2). Thus, PfRH5ANL induces a growth-inhibitory immune response, and contains the epitopes targeted by inhibitory antibodies.

For structural studies, PfRH5ANL was mixed with basigin or fragments of growth-inhibitory monoclonal antibodies, 9AD4 or QA1. The

![Figure 1](image-url)
complexes were trimmed using endoproteinase GluC and lysines chemically methylated before crystallization. Crystals formed and data were collected to 3.1 Å (PfRH5–basigin), 2.3 Å (PfRH5–9AD4) and 3.1 Å resolution (PfRH5–QA1). Structures were determined using molecular replacement (Extended Data Table 1).

PfRH5 adopts a rigid, flat, ‘kite-shaped’ architecture with a pseudo-twofold rotation symmetry and no similarity to known structures (Fig. 1a). Each half is predominantly built from a three-helical bundle, with the outermost helices containing significant kinks or breaks. The N-terminal half begins with a short, two-stranded β-sheet that crosses the long axis of the kite at its centre. This is followed by a single, short helix and two long, kinked helices connected by the truncated loop (containing 58 residues in full-length PfRH5). The C-terminal half is simpler, consisting of three long helices that span the entire length of the domain and finishing with a flexible C terminus. One disulphide bond (C345–C351) stabilizes the loop that links the two halves of the structure, while another links the second and third helices (C224–C317), leaving one unpaired cysteine (C329).

PfRH5 is predominantly rigid, with five copies in the three different crystal forms aligning with an r.m.s.d. of 0.9 Å over 95% of residues (Extended Data Fig. 1b). Only the C terminus (residues 496–end) and the loop linking helices 4 and 5 (residues 396–406) adopt different positions in different crystal forms. A molecular envelope derived from small angle X-ray scattering (SAXS) analysis of full-length PfRH5 in solution exhibits a similar flat structure (Fig. 1b, Extended Data Fig. 3). This envelope is elongated relative to PfRH5ANL, most probably owing to residues missing in this construct or not ordered in the crystal structure (22 residues at the C terminus, the flexible loop, and perhaps part of the extended N terminus).

As members of the Plasmodium RH family share little sequence identity, sequence alignments and structure-based threading were used to predict whether other members contain the PfRH5 fold. In each protein analysed (P. falciparum RH1, RH2a, RH2b, RH3 and RH4; Plasmodium vivax RBP-1 and RBP-2; Plasmodium reichenowi Rh5; and Plasmodium yoelii Py01365), N-terminal PfRH5-like domains were identified with high confidence, despite sequence identities of 14–22% and a lack of totally conserved residues or disulphide bonds (Extended Data Fig. 4a). Similar residues are located primarily in the interior of the domain, where they may stabilize the fold (Extended Data Fig. 4b). In PfRH4, the only other RH protein with a known erythrocyte receptor, the complement receptor 1 (CR1) binding fragment contains the putative PfRH5 fold46. These PfRH5-like domains are therefore excellent candidates for ligand-binding modules in other RH proteins.

Basigin binds at the tip of PfRH5, distant from the flexible loop and C terminus, with both domains and the intervening linker directly contacting PfRH5 (Fig. 2a, d). Most of the contact area (~1,350 Å²) occurs through hydrogen bonds between the backbone of strands A and G of the basigin N-terminal domain and loops at the tip of PfRH5 (Extended Data Table 2a). PfRH5 residues F350 and W447 stabilize this interaction through hydrogen bonds between the backbone of strands A and G of PfRH5 and the basigin side chains. The limited involvement of basigin side chains will reduce the potential for basigin escape mutants that prevent PfRH5 binding and impair parasite invasion.

The basigin C-terminal domain and H102 in the linker also directly contact PfRH5 (Extended Data Table 2a). The three loops at the tip of the basigin C-terminal domain (linking strands B and C, strands D and E and strands F and G) interact with the second and fourth helices of PfRH5 through hydrogen bonds and a hydrophobic patch contributed by residues VPP from the BC loop. However, flexibility of the basigin linker allows different orientations of the C-terminal domains in the two copies in the asymmetric unit of the crystal. Chain B interacts through the BC and DE loops (~650 Å² interface) while chain D interacts through the BC and FG loops (~480 Å²) (Fig. 2b).
greater 7, 8, 12. These SNPs are distributed across the structure, but do not
found in 227 field isolates, and only five at frequencies of 10% or
partially fits, consistent with a flexible interaction with PfRH5.
N-terminal domain fit the SAXS envelope, the C-terminal domain only
plexes. Flexibility is also predicted from SAXS analysis of the com-
binders currently available for inhibiting parasite growth5. Crystal struc-
matic binding in vitro block PfRH5–basigin binding and parasite growth. 9AD4 does not block
fragments from three inhibitory monoclonal antibodies were studied
trations during invasion remains to be elucidated.
To identify inhibitory epitopes, complexes of PRfH5ANL with Fab
fragments from three inhibitory monoclonal antibodies were studied by
crystallography and SAXS. QA1 and QA5 were previously shown to
block PRfH5–basigin binding and parasite growth. 9AD4 does not block
PRfH5–basigin binding in vitro, but is one of the most effective anti-
bodies currently available for inhibiting parasite growth1. Crystal struc-
tures of PRfH5 bound to QA1 and QA4 were confirmed by SAXS, while
a model for PRfH5–QA5 was derived from SAXS analysis, guided by a
previously identified linear epitope (residues 201–213 from helix 2)3.
The antibodies bind to three distinct sites, close to the vertex of PRfH5
(Fig. 3, Extended Data Fig. 8, Extended Data Table 2b, c). QA1 binds
to loops at the PRfH5 tip, overlapping the basigin N-terminal domain
binding site. QA5 predominantly interacts with PRfH5 helix 2, over-
lapping the basigin C-terminal domain binding site. In contrast, 9AD4
binds helices 2 and 3, close to, but not overlapping, either basigin bind-
ing site. This is likely to allow intact 9AD4 IgG to impede erythrocyte
binding when PRfH5 and basigin are both membrane-tethered. This
reveals inhibitory epitopes in or close to the basigin binding sites that
can be targeted to block parasite invasion.
In summary, PRfH5 adopts a novel architecture formed, as in many
families of parasite surface proteins14, from a robust α-helical scaffold.
This maintains the overall fold by retaining residues required for helical
packing, while allowing significant surface sequence variation. Sequence
homology identifies this fold at the N terminus of other RH proteins,
where it is likely to act as a ligand-binding module. Characterization of
the PRfH5–basigin complex prompts a range of future experiments
to investigate the role of PRfH5 in erythrocyte invasion. Furthermore,
 monoclonal antibodies that block parasite growth bind at or close to
the basigin-binding site. Immunogens containing these regions of PRfH5
will be important components of a vaccine to prevent P. falciparum
erythrocyte invasion, thereby crippling the parasite responsible for the
deadliest form of human malaria.

Online Content Methods, along with any additional Extended Data display items and Source Data, are available in the online version of the paper; references unique to these sections appear only in the online paper.

Received 15 June; accepted 28 July 2014.
Published online 17 August 2014.

1. Cowman, A. F. & Crabb, B. S. Invasion of red blood cells by malaria parasites. Cell 124, 755–766 (2006).

2. Tham, W. H., Healer, J. & Cowman, A. F. Erythrocyte and reticulocyte binding-like proteins of Plasmodium falciparum. Trends Parasitol. 28, 23–30 (2012).

3. Baum, J. et al. Reticulocyte-binding protein homologue 5 - an essential adhesin involved in invasion of human erythrocytes by Plasmodium falciparum. Int. J. Parasitol. 39, 371–380 (2009).

4. Crosnier, C. et al. Basigin is a receptor essential for erythrocyte invasion by Plasmodium falciparum. Nature 480, 534–537 (2011).

5. Douglas, A. D. et al. Neutralization of Plasmodium falciparum merozoites by antibodies against PRfH5. J. Immunol. 192, 245–258 (2014).

6. Douglas, A. D. et al. The blood-stage malaria antigen PRfH5 is susceptible to vaccine-inducible cross-strain neutralizing antibody. Nature Commun. 2, 601 (2011).

7. Williams, A. R. et al. Enhancing blockade of Plasmodium falciparum erythrocyte invasion: assessing combinations of antibodies against PRfH5 and other merozoite antigens. PLoS Pathog. 8, e1002991 (2012).
A full-length recombinant *Plasmodium falciparum* PfRH5 protein induces inhibitory antibodies that are effective across common PfRH5 genetic variants. *Vaccine* **31**, 373–379 (2013).

Bacterially expressed full-length recombinant *Plasmodium falciparum* RH5 protein binds erythrocytes and elicits potent strain-transcending parasite-neutralizing antibodies. * Infect. Immun.**82**, 152–164 (2014).

An EGF-like protein forms a complex with PfRh5 and is required for invasion of human erythrocytes by *Plasmodium falciparum*. *PLoS Pathog.**7**, e1002199 (2011).

A novel reticulocyte-binding family homolog of *Plasmodium falciparum* that binds to the erythrocyte, and an investigation of its receptor. *PLoS ONE**3**, e3300 (2008).

Analysis of *Plasmodium falciparum* diversity in natural infections by deep sequencing. *Nature**487**, 375–379 (2012).

Naturally acquired antibodies specific for *Plasmodium falciparum* reticulocyte-binding protein homologue 5 inhibit parasite growth and predict protection from malaria. *J. Infect. Dis.**209**, 789–798 (2014).

Complement receptor 1 is the host erythrocyte receptor for *Plasmodium falciparum* PfRh4 invasion ligand. *Proc. Natl Acad. Sci. USA**107**, 17327–17332 (2010).

Erythrocyte binding protein PfRH5 polymorphisms determine species-specific pathways of *Plasmodium falciparum* invasion. *Cell Host Microbe**4**, 40–51 (2008).

Various PfRH5 polymorphisms can support *Plasmodium falciparum* invasion into the erythrocytes of owl monkeys and rats. *Mol. Biochem. Parasitol.**187**, 103–110 (2013).

RH5-Basigin interaction plays a major role in the host tropism of *Plasmodium falciparum*. *Proc. Natl Acad. Sci. USA**110**, 20735–20740 (2013).

Sequence variation and structural conservation allows development of novel function and immune evasion in parasite surface protein families. *Protein Sci.**23**, 354–365 (2014).

**Acknowledgements**

M.K.H. is a Wellcome Trust Investigator (101020/Z/13/Z). K.E.W. is funded by a Wellcome Trust PhD studentship. S.J.D. holds a UK Medical Research Council (MRC) Career Development Fellowship (G1000527), and is a Jenner Investigator and Lister Institute Research Prize Fellow. The project was also funded by the European Vaccine Initiative (EVI) (InnoMalVac); the UK MRC (MR/K025954/1); the European Community’s Seventh Framework Programme (FP7/2007-2013, grant agreement number 242095 – EVIMalaR); and a Wellcome Trust Training Fellowship (089455/2/09/z to ADD). We thank J. Furze and D. Alanine; D. Staunton and E. Lowe; A. Round (ESRF); and R. Flaig and J. Brandao-Neto (Diamond Light Source).

**Author Contributions**

K.E.W. purified and crystallized the proteins, collected and analysed SAXS data, and performed surface plasmon resonance and analytical ultracentrifugation analysis. M.K.H. and K.E.W. prepared crystals for data collection and solved the structures. W.A.J. and S.B.C. made S2 cell lines, and K.A.H. and J.J.I. purified proteins. A.D.D. and J.B. provided hybridomas. J.J., R.E.B. and R.A. designed and performed parasite assays and ELISAs. K.E.W., M.K.H. and S.J.D. designed the project, analysed the data, and wrote the paper.

**Author Information**

Atomic coordinates and structure factors are deposited at the Protein Data Bank with accession codes 4U0Q, 4U0R and 4U1G. Reprints and permissions information is available at www.nature.com/reprints. The authors declare competing financial interests: details are available in the online version of the paper. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to M.K.H. (matthew.higgins@bioch.ox.ac.uk) and S.J.D. (simon.draper@ndm.ox.ac.uk).
METHODS
Basigin cloning, expression and purification. A section of the basigin gene encoding immunoglobulin domains 1 and 2 of the short isoform (residues 22–205) was amplified from complementary DNA using primers 5′-CCGGATCCTGGCGGAAACCGTGTCG-3′ and 5′-CCCATATGGATGGTGGACGTCCCTTGAGG-3′. PCR products were cloned into a modified pET15b vector (Novagen), which encodes an N-terminal hexa-histidine tag followed by a tobacco etch virus (TEV) protease cleavage site. TEV cleavage leaves an additional glycan at the N terminus from the cleavage site.

Basigin was expressed in bacterial strain Origami B (DE3) (Novagen) by incubation overnight at 25 °C after induction with 1 mM IPTG. The protein was purified by nickel-nitrilotriacetic acid (Ni2+)–NTA; Quagen affinity chromatography, followed by buffer exchange into PBS using a PD-10 desalting column (GE Healthcare), and overexpression of His-tagged TEV protease at 4 °C, before a second Ni2+-NTA column. The flow-through was concentrated using an Amicon Ultra centrifugal filter device (molecular mass cutoff, 3,000 Da). Finally, gel filtration was performed with a Superdex 200 16/60 column (GE Healthcare) in 20 mM HEPES (pH 7.5) and 150 mM NaCl.

Expression and purification of 9AD4, QA1 and QA5. Anti-PfRH5 monoclonal antibodies were described previously.2,3 Hybridomas expressing 9AD4, QA1 and QA5 were grown in Dulbecco’s modified Eagle’s medium (DMEM; Sigma) supplemented with 4 mM 1-glutamine (Sigma), 0.01 M HEPES (Life Technologies), 100 U penicillin and 0.1 mg ml−1 streptomycin (Sigma), and 20% fetal calf serum (Gibco). They were then transferred into CD Hybridoma medium (Life Technologies) and supplemented with 10% fetal calf serum (Gibco) and 1.5 mg ml−1 lactate dehydrogenase. Percentage growth inhibition is expressed relative to wells containing IgG from control immunised rabbits. The mean of the three replicate wells was taken to obtain the final data for each individual rabbit at each tested IgG concentration. Experiments were performed twice against each strain of parasite with very similar results. A representative experiment is shown in Fig. 1d. The mean ± standard error of mean are plotted for the results obtained with the purified IgG from the four immunised rabbits.

ELISA. Maxisorp plates were coated with anti-PfRH5 mouse monoclonal antibody (50 μl per well at 5 μg ml−1) and left at 4 °C overnight. Following six washes in PBS with 0.05% Tween-20 (PBS-T), plates were blocked with 150 μl per well Casein block solution (Pierce) for 1 h, followed by another six PBS-T washes. PfRH5NL protein was added to wells in triplicate (50 μl per well) at four dilutions in blocking buffer (900, 200, 50 and 12.5 ng ml−1) and incubated at room temperature for 2 h. Following another six PBS-T washes, plates were incubated with polyclonal anti-full-length PfRH5 rabbit serum3 diluted 1:1,000 in blocking solution, 50 μl per well for 1 h. Following another PBS-T wash, plates were incubated with goat anti-rabbit IgG alkaline phosphatase diluted 1:5,000 in blocking solution, 50 μl per well for 1 h. Following a final six PBS-T washes, and two PBS washes, plates were developed and optical density read at 405 nm as previously described.

Crystallization, data collection and data processing of PfRH5 complexes. Crystallization was achieved using vapour diffusion in sitting drops. A TTP Labtech Mosquito crystal robot was employed to mix 100 nl of each protein complex at 8 mg ml−1 with 100 nl of well solutions from commercially available crystal screens. Crystals were first obtained in conditions from the Proplex (Rhino–basigin), JCSG+ (RHSAN–9AD4) and Morpheus (RHSAN–QA1) crystal screens (Molecular Dimensions). The well solution for PfRH5–basigin was 0.2 M lithium sulphate, 0.1 M MES (pH 6.0), and 20% (w/v) PEG 4000. For PfRH5–9AD4, the well solution was 24% (w/v) PEG 1500 and 20% (w/v) glycerol. PfRH5–QA1 was crystallized in a 0.12 M mixture of 1:6-hexanediol, 1-butanol, 1,2-propanediol, 2-propanol, 1,4-butanediol, 1,3-propanediol; 0.1 M MES-imidazole (pH 6.5); 20% (w/v) PEG 550 MME; 10% (v/v) 1,4-butanediol, 1,3-propanediol; 0.1 M MES-imidazole (pH 6.5); and 20% (w/v) PEG 550 MME; 10% (v/v) 1,4-butanediol, 1,3-propanediol; 0.1 M MES-imidazole (pH 6.5); and 20% (w/v) PEG 550 MME; and at 18 °C for PfRH5–QA1. Crystal seeds were generated by adding crystals in well solution to a Seed Bead (Hampton Research) and vortexing for 15 s. New crystal drops were then set up by mixing 100 nl of each protein complex at 8 mg ml−1 with 100 nl of well solutions from commercially available crystal screens. Crystals were first obtained in conditions from the Proplex (Rhino–basigin), JCSG+ (RHSAN–9AD4) and Morpheus (RHSAN–QA1) crystal screens (Molecular Dimensions). The well solution for PfRH5–basigin was 0.2 M lithium sulphate, 0.1 M MES (pH 6.0), and 20% (w/v) PEG 4000. For PfRH5–9AD4, the well solution was 24% (w/v) PEG 1500 and 20% (w/v) glycerol. PfRH5–QA1 was crystallized in a 0.12 M mixture of 1:6-hexanediol, 1-butanol, 1,2-propanediol, 2-propanol, 1,4-butanediol, 1,3-propanediol; 0.1 M MES-imidazole (pH 6.5); 20% (w/v) PEG 550 MME; 10% (v/v) 1,4-butanediol, 1,3-propanediol; 0.1 M MES-imidazole (pH 6.5); and 20% (w/v) PEG 550 MME; 10% (v/v) 1,4-butanediol, 1,3-propanediol; 0.1 M MES-imidazole (pH 6.5); and 20% (w/v) PEG 550 MME; and at 18 °C for PfRH5–QA1. Crystal seeds were generated by adding crystals in well solution to a Seed Bead (Hampton Research) and vortexing for 15 s. New crystal drops were then set up by mixing 100 nl of 8 mg ml−1 protein complex, 50 nl of well solution, 50 nl of seeds, and 50 nl of Silver Bullets or Silver Bullets Bio additives (Hampton Research).

RHSAN–basigin crystals were cryoprotected by transfer into well solution supplemented with 25% ethylene glycol, then cryoooled by plunging into nitrogen. PfRH5–9AD4 crystals were directly cryoocooled. PfRH5–QA1 crystals were cryoocooled in well solution with a total of 32% (w/v) PEG 550 MME and 16% (w/v) PEG 20,000 before cryocooling.

Data were collected at the Diamond Light Source, on beamline I04-1 for the PfRH5–basigin complex (wavelength 0.92 Å), and on beamline I04 for both the PfRH5–Fab complexes (wavelength 0.97949 Å). Data reduction was performed using iMosflm22,23 and scala24 from the CCP4 processing suite25. A composite of the light chain from PDB entry 3Q9Q and the heavy chain from 3H5W was used as
a molecular replacement model for 9AD4 in Phaser\textsuperscript{34}. Density modification was performed with PARROT\textsuperscript{35}. Buccaneer\textsuperscript{36} was employed to build helices into initial PRHR5AN density, initiating a cycle of model building and refinement using REFMAC\textsuperscript{37}, BUSTER\textsuperscript{38}, and Coot\textsuperscript{39}. PRHR5AN was used as a molecular replacement model in Phaser\textsuperscript{40}, together with basigin (PDB code 3B5H) for the PRHR5AN–basigin structure, or with a composite of the heavy chain from 2ZN9 and the light chain from 1172 for the PRHR5AN–QA1 structure. Refinement was accomplished with BUSTER\textsuperscript{38}. Ramachandran statistics, determined using PROCHECK\textsuperscript{41}, were as follows: for PRHR5AN–basigin, 93% of residues fell in the allowed regions and 7% in the additional allowed region; for PRHR5AN–9AD4, 93.9% were in the allowed region, 5.8% were in the additional allowed region, and 0.3% were allowed region; and for PRHR5AN–QA1, 86.0% were in the allowed region, 13.3% were in the additional allowed region, and 0.7% were in the generously allowed region. There were no residues in the disallowed region of the Ramachandran plot in any of the structures.

Alignment of RH protein sequences. Sequences of \textit{P. falciparum} RH1, RH4, RH2a, RH2b, and RH3; \textit{P. vivax} RBP-1 and RBP-2; \textit{P. reichenowi} RH5; and \textit{P. yoelii} Py0135 were aligned against PRHR5 using the Clustal Omega server\textsuperscript{42}, and an alignment figure generated with ESPript\textsuperscript{34}. The homologous portion of each sequence was then threaded onto the PRHR5 structure using the Phyre2 server\textsuperscript{35}.

Small-angle X-ray scattering data collection and processing. Small-angle X-ray scattering (SAXS) data were collected at beamline BM29 at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). Scattering was detected using a scattering (SAXS) data were collected at beamline BM29 at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). Scattering was detected using a 2D Pilatus image reader at 20 °C.

Full-length PRHR5–basigin, PRHR5–QA1 and PRHR5–QA5 complexes were prepared by mixing PRHR5 and 9AD4 in equimolar amounts for 90 min at 25 °C. The samples for sedimentation velocity experiments were full-length PRHR5–basigin, PRHR5–QA1 and PRHR5–QA5 complexes. Refinement was accomplished with BUSTER\textsuperscript{38}.

Analitical ultracentrifugation. The analytical ultracentrifugation experiments were carried out using a Beckman An-60 Ti rotor in a Beckman Optima XL-1 analytical ultracentrifuge. During the time course of a sedimentation equilibrium experiment, degradation of full-length PRHR5 was observed. Therefore experiments were performed using PRHR5AN. Basigin, PRHR5AN and a gel-filtered PRHR5AN–basigin complex were analysed at 22 μM, 9 μM, and 9 μM, respectively. The run proceeded for 20 h at different speeds and scans recorded the ultraviolet absorbance at 280 nm. The buffer density and viscosity, together with sample partial specific volumes, were calculated as above\textsuperscript{43}. The data were fitted to an ideal monodisperse model using SEDPHAT\textsuperscript{44}, with the assumption that at concentrations of ~10 times the Kd, PRHR5AN is fully complexed with basigin.
**Extended Data Figure 1 | PfRH5 disorder predictions and structural alignment.**

**a.** Long-range disorder was predicted by POODLE-L\(^{19}\) and was used to determine domain boundaries for the PfRH5\_NL crystallization construct. The disorder predictions are shown above the sequence of PfRH5, with values of >0.5 indicative of disorder. The residues visible in the PfRH5\_NL crystal structure are shown below the PfRH5 sequence as secondary structure elements (sheets as blue arrows, and helices as tubes in rainbow colouring) linked by blue lines. The missing loop (248–296) is shown as a break in the blue line. The secretion signal sequence is indicated (black underline).

**b.** Two copies of PfRH5 from the PfRH5–basigin structure (red and orange), two copies from the PfRH5–QA1 structure (blue and cyan), and one copy from PfRH5–9AD4 (green) structure were aligned using Coot\(^{31}\), giving an r.m.s.d. of 1.7 Å. The C terminus and the loop between helices 4 and 5 were the only regions showing significant differences. For the remaining 95% of PfRH5, the r.m.s.d. is 0.9 Å.
Extended Data Figure 2 | Investigation of the interaction of PfRH5NL with a panel of mouse monoclonal antibodies using ELISA. Five monoclonal antibodies that bind to PfRH5 were coated on an ELISA plate and probed using PfRH5NL at concentrations of 12.5, 50, 200 or 800 ng ml⁻¹. Antibodies 9AD4, QA1 and QA5 interacted with PfRH5NL while RB3 and 4BA7 did not. Indeed, RB3 and 4BA7 bind to the flexible N terminus and the truncated loop, respectively, both features lacking in PfRH5NL. The error bars are standard error of mean (n = 3).
Extended Data Figure 3 | SAXS analysis of the PfRH5–basigin complex.

a, The theoretical scattering calculated from the average of 20 ab initio reconstructions (continuous lines, with PfRH5 in orange and PfRH5–basigin in blue) plotted with the experimental scattering intensity curves (diamonds). The data are presented as the natural logarithm of the intensity. Guinier plots are displayed in the inset.

b, The distance distribution function, $P(r)$, of PfRH5 (orange) and PfRH5–basigin (blue).

c, To the left, the crystal structure of PfRH5$_{DNL}$ (yellow) was docked into the average ab initio SAXS envelope of full-length PfRH5 (grey). Extra density corresponding to some or all of the truncated regions is visible at the bottom of the kite-like structure, near the C terminus. To the right, the crystal structure of PfRH5$_{DNL}$–basigin is docked into the average ab initio SAXS envelope of full-length PfRH5–basigin (grey).

d, Summary of SAXS parameters. The radius of gyration ($R_g$) was determined from the Guinier plot using AutoRg$^{37}$, and the maximum particle dimension (Dmax) and the Porod volume$^{39}$ were calculated using GNOM$^{37}$. An estimate of the molecular weight was obtained by dividing the Porod volume by 1.7. Ab initio modelling was used to generate 20 shape reconstructions from the data. The normalized spatial discrepancy parameter (NSD) diagnoses the similarity of these models$^{42}$. The models were averaged and the fit of the average model to the experimental data are indicated by the $\chi$ value.

|       | $R_g$ (nm) | Dmax (nm) | Porod volume (nm$^3$) | MW$_{app}$ (kDa) | NSD       | $\chi$ |
|-------|------------|-----------|------------------------|-----------------|-----------|--------|
| PfRH5 | 3.7        | 11.9      | 104.5                  | 61.4            | 0.692±0.036 | 1.01   |
| PfRH5: basigin | 4.1 | 13.8 | 138.3 | 81.3 | 0.715±0.034 | 0.74   |

PfRH5$_{ANL}$ is yellow. In dark blue and cyan are basigin molecules from the two PfRH5$_{ANL}$–basigin complexes in the asymmetric unit, superimposed based on the structure of PfRH5$_{ANL}$.
Extended Data Figure 4 | A conserved PfRH5-like fold in other Plasmodium RH proteins. a, P. falciparum RH1, RH4, RH2b, RH2a and RH3 (a pseudogene); P. vivax RBP-1 and RBP-2; P. reichenowi RH5; and P. yoelii Py01365 were aligned using Clustal Omega33 and were threaded using the Phyre2 server35, giving more than 98% confidence of fold conservation over 260 residues in each case. The secondary structure of PfRH5 is shown below the sequence in a rainbow colour scheme as in Fig. 1a. Residues from PfRH5 that interact with basigin, QA1 and 9AD4 are indicated above the sequence by blue, red or green stars, respectively. Cysteine residues that make disulphide bonds are indicated by pink numbers, with residues sharing the same number forming a disulphide bond. b, PfRH5 is shown in yellow, with residues similar among RH proteins (from the alignment in a) highlighted as pink sticks. The majority of the similar residues appear to play a structural role stabilizing the architecture of the domain.
Extended Data Figure 5 | Location of PfRH5 polymorphisms, and residues of PfRH5 and basigin implicated in host tropism.  

a, b, Indicated are the locations of PfRH5 SNPs that are common (10% frequency or greater; red sticks) or uncommon (blue sticks) among 227 field isolates, as well as additional SNPs observed in lab strains (green sticks). Basigin (blue) is shown in addition to PfRH5 (yellow). SNPs Y203, I204, N347, Y358 and E362 are localized in or near the PfRH5–basigin interface. Not visible in this orientation is lab strain polymorphism K429. c, Highlighted are basigin residues F27, Q100 and H102 (orange sticks). Mutation of F27 or Q100, or an insertion adjacent to H102, all change the affinity for PfRH5. Also shown are two SNPs of PfRH5, namely N347 and I204 (pink sticks), found in the PfRH5–basigin binding interface and linked to the strain’s ability to invade Aotus monkey erythrocytes.
Extended Data Figure 6 | Arrangement of two PfRH5–basigin complexes in the asymmetric unit of the crystal. One complex, shown in yellow (PfRH5) and blue (basigin), interacts with the second, shown in silver (PfRH5) and cyan (basigin), primarily through packing between the two C-terminal domains of basigin. The two C termini of basigin are in close proximity (top view).
Extended Data Figure 7 | Analysis of the PfRH5–basigin complex using analytical ultracentrifugation.  

**a, b.** Sedimentation velocity analysis. The continuous sedimentation coefficient distributions that best fit the data are shown for basigin (top), full-length PfRH5 (middle), and a gel-filtered PfRH5–basigin complex (bottom). The inset shows the fitting residuals.

**c, d.** Sedimentation equilibrium analysis. PfRH5ΔN (residues 140–526), basigin, and a gel-filtered PfRH5ΔN–basigin complex were analysed. The runs lasted 20 h at different speeds, as indicated in the inset legends. Ultraviolet absorbance was monitored at 280 nm. The residuals are shown below fitted data. The calculated molecular weights are consistent with the formation of a 1:1 complex between PfRH5ΔN and basigin.
Extended Data Figure 8 | SAXS of PfRH5 in complex with growth-inhibitory Fab fragments. **a**, The theoretical scattering calculated from the average of 20 ab initio reconstructions (continuous lines, with PfRH5 in orange, PfRH5–9AD4 in green, PfRH5–QA1 in red, and PfRH5–QA5 in blue) plotted with the experimental scattering intensity curves (black diamonds). The data are presented as the natural logarithm of the intensity. The Guinier plots are displayed in the inset. **b**, The distance distribution function, \( P(r) \), with colours as in **a**. The crystal structures of PfRH5–QA1 (left) and PfRH5–9AD4 (middle) were docked into the corresponding full-length PfRH5–Fab envelopes (grey). PfRH5ANL is shown in yellow, QA1 in red, and 9AD4 in green. PfRH5ANL and a Fab fragment (cyan) were docked into the PfRH5–QA5 SAXS envelope to generate a model of the PfRH5–QA5 structure (right). **d**, A summary of SAXS parameters. The radius of gyration (\( R_g \)) was determined from the Guinier plot using AutoRg\(^{37} \), and the maximum particle dimension (\( D_{\text{max}} \)) and the Porod volume\(^{39} \) were calculated using GNOM\(^{37} \). An estimate of the molecular weight was obtained by dividing the Porod volume by 1.7. Ab initio modelling was used to generate 20 shape reconstructions from the data. The normalized spatial discrepancy parameter (NSD) diagnoses the similarity of these models\(^{42} \). The models were averaged and the fit of the average model to the experimental data are indicated by the \( \chi \) value.
Extended Data Table 1 | Crystallographic data collection and refinement statistics

|                                | PIRH5ANL-basigin | PIRH5ANL-9AD4  | PIRH5ANL-QA1 |
|--------------------------------|------------------|----------------|--------------|
| **Data collection**            |                  |                |              |
| Space group                    | P2₁2₁2₁          | P2₁2₁2₁        | P2₂₁2₁       |
| Cell dimensions                |                  |                |              |
| \( a, b, c \) (Å)              | 75.28, 109.36,   | 39.79, 86.66,  | 65.12, 137.30,|
|                                | 151.95           | 324.04         | 228.58       |
| \( \alpha, \beta, \gamma \) (°) | 90.00, 90.00,    | 90.00, 90.00,  | 90.00, 90.00,|
|                                | 90.00            | 90.00          | 90.00        |
| Resolution (Å)                 | 67.46 – 3.10 (3.27-3.10) | 41.86-2.30 (2.42-2.30) | 47.24-3.10 (3.27-3.10) |
| \( R_{ppm} \)                  | 0.080 (0.297)    | 0.047 (0.375)  | 0.049 (0.380) |
| \( I/\sigma I \)               | 9.4 (2.7)        | 9.8 (2.0)      | 9.2 (2.3)    |
| Completeness (%)               | 99.8 (99.8)      | 96.2 (83.3)    | 95.5 (96.3)  |
| Redundancy                     | 6.1 (6.2)        | 5.0 (4.4)      | 4.5 (4.6)    |
| **Refinement**                 |                  |                |              |
| Resolution (Å)                 | 67.46-3.10       | 41.86-2.30     | 47.24-3.10   |
| No. reflections                | 23357            | 49336          | 36259        |
| \( R_{work}/R_{free} \) (%)   | 21.71 / 24.45    | 22.10 / 25.83  | 23.6 / 28.1  |
| No. atoms                      | 7629             | 6005           | 11478        |
| Wilson B-factors               | 48.0             | 41.1           | 90.1         |
| R.m.s deviations               |                  |                |              |
| Bond lengths (Å)               | 0.010            | 0.014          | 0.013        |
| Bond angles (°)                | 1.5              | 1.7            | 1.9          |
### Extended Data Table 2 | Description of interactions between PfRh5 with basigin or monoclonal antibodies QA1 and 9AD4

#### a

| Chain | PRH5 Residue | Group | Chain | Basigin Residue | Group | Interaction |
|-------|--------------|-------|-------|----------------|-------|-------------|
| A / C | S197         | side chain | B / D | Q100           | side chain NH2 | hydrogen bond |
| A / C | S197         | side chain | B / D | E84            | side chain   | hydrogen bond |
| A / C | F350         | side chain | B / D | N98            | backbone NH   | hydrophobic  |
| A / C | N352         | side chain CO | B / D | N98          | backbone CO   | hydrophobic  |
| A / C | R357         | side chain | B / D | V26            | backbone CO   | hydrophobic  |
| A / C | W447         | side chain | B / D | T28            | backbone NH   | hydrophobic  |
| A / C | R448         | side chain | B / D | T25            | side chain   | hydrophobic  |
| A / C | T449         | side chain | B / D | V26            | backbone NH   | hydrophobic  |
| A / C | T449         | backbone NH | B / D | V26          | backbone CO   | hydrophobic  |

**Linker:**

| Chain | PRH5 Residue | Group | Chain | Basigin Residue | Group | Interaction |
|-------|--------------|-------|-------|----------------|-------|-------------|
| A / C | Y200         | side chain | B / D | H102          | side chain | hydrogen bond |

**C-terminal domain:**

| Chain | PRH5 Residue | Group | Chain | Basigin Residue | Group | Interaction |
|-------|--------------|-------|-------|----------------|-------|-------------|
| A / C | hydrophobic  |       | B / D | V131          | side chain | hydrophobic  |
| A     | hydrophobic  |       | B     | P133          | side chain | hydrophobic  |
| A     | D207         | side chain | B     | Q164          | side chain NH2 | hydrophobic  |
| C     | E362         | side chain | D     | K191          | side chain | hydrophobic  |
| C     | E362         | side chain | D     | S190          | side chain | hydrophobic  |

#### b

| Chain | PRH5 Residue | Group | Chain | QA1 Residue | Group | Interaction |
|-------|--------------|-------|-------|-------------|-------|-------------|
| A / D | K196         | side chain | B / E | N101        | side chain CO | hydrogen bond |
| A / D | K196         | side chain | B / E | D31         | backbone CO   | hydrogen bond |
| A / D | S197         | backbone NH | B / E | D53         | side chain   | hydrophobic  |
| A / D | Y346         | side chain | B / E | Y33         | side chain   | pi stacking  |
| A / D | N348         | side chain CO | B / E | G105        | backbone NH   | hydrophobic  |
| A / D | N352         | side chain | B / E | Y33         | side chain   | hydrophobic  |
| A / D | N352         | side chain | B / E | Y59         | side chain   | hydrophobic  |
| A / D | N354         | backbone NH | B / E | Y57         | side chain   | hydrophobic  |
| A / D | K452         | side chain | B / E | D104        | side chain   | hydrophobic  |

**Light chain:**

| Chain | PRH5 Residue | Group | Chain | QA1 Residue | Group | Interaction |
|-------|--------------|-------|-------|-------------|-------|-------------|
| A / D | N347         | side chain NH2 | C / F | S95         | backbone CO | hydrogen bond |
| A / D | N348         | backbone NH | C / F | Y36        | side chain | hydrophobic  |
| A / D | N349         | backbone NH | C / F | Y34        | side chain | hydrophobic  |
| A / D | N349         | side chain CO | C / F | W96        | side chain | hydrophobic  |
| A / D | F350         | side chain | C / F | Y34        | side chain | hydrophobic  |
| A / D | Q451         | backbone CO | C / F | Y34        | side chain | hydrophobic  |

#### c

| Chain | PRH5 Residue | Group | Chain | 9AD4 Residue | Group | Interaction |
|-------|--------------|-------|-------|--------------|-------|-------------|
| A     | A205         | backbone CO | B     | Y103        | backbone CO | hydrogen bond (H2O) |
| A     | F209         | side chain | B     |             | hydrophobic | hydrophobic |
| A     | Y335         | side chain | B     | Y103        | backbone CO | hydrogen bond |
| A     | N338         | side chain CO | B     | W107        | side chain | hydrophobic  |
| A     | L339         | side chain | B     |             | hydrophobic | hydrophobic |
| A     | E341         | side chain | B     | S52         | side chain | hydrogen bond |
| A     | E341         | side chain | B     | M54         | backbone CO | hydrophobic  |
| A     | E341         | side chain | B     | A55         | backbone CO | hydrophobic  |
| A     | E341         | side chain | B     | Y56         | backbone CO | hydrophobic  |
| A     | Q342         | side chain CO | B     | N53         | side chain NH2 | hydrophobic  |
| A     | hydrophobic  |         | B     | F101        | side chain | hydrophobic  |

**Light chain:**

| Chain | PRH5 Residue | Group | Chain | 9AD4 Residue | Group | Interaction |
|-------|--------------|-------|-------|--------------|-------|-------------|
| A     | K212         | side chain | C     | Y32        | backbone CO | hydrogen bond |
| A     | D331         | side chain | C     | Y32        | side chain | hydrophobic  |
| A     | N334         | side chain NH2 | C     | Y31        | side chain | hydrophobic (H2O) |