Structure of *Plasmodium falciparum* Rh5/CyRPA/Ripr invasion complex

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Plasmodium falciparum causes the severe form of malaria with significant mortality in humans. Blood stage merozoites of _P. falciparum_ invade erythrocytes and this requires multiple parasite ligand–host receptor interactions. This includes binding of the PfRh5/CyRPA/PfRipr complex with erythrocyte receptor basigin [1,2], an essential step for entry into human erythrocytes. We show the PfRh5/CyRPA/PfRipr complex binds erythrocyte cell line JK-1 significantly better than PfRh5 alone through insertion of PfRh5 and PfRipr into host membranes as a high molecular weight complex. Here we report a subnanometer resolution cryo-electron microscopy structure of PfRh5/CyRPA/PfRipr complex revealing the organisation of this essential invasion complex, the mode of interactions between members of the complex and show CyRPA is a critical mediator for complex assembly. The structure identified blades 4-6 of the CyRPA β-propeller as contact sites for PfRh5 and PfRipr. The limited contacts between PfRh5/CyRPA and CyRPA/PfRipr was consistent with dissociation of PfRh5 and PfRipr from CyRPA for membrane insertion. Comparision of the PfRh5-basigin crystal structure to the PfRh5/CyRPA/PfRipr cryo-EM structure suggests PfRh5 and PfRipr are positioned parallel to the erythrocyte membrane prior to membrane insertion. This provides new information on the function of this complex revealing insights into invasion of _P. falciparum_.

Invasion of *P. falciparum* merozoites into erythrocytes requires the ligand PfRh5 that binds to host receptor basigin [1]. PfRh5 forms a ternary complex with PfRipr and CyRPA at the merozoite/erythrocyte interface [2,3]. This complex is linked to formation of a pore between the merozoite and erythrocyte membrane through which Ca^{2+} can pass [2,4].

To understand the function of the PfRh5/CyRPA/PfRipr complex we expressed the PfRipr protein and it forms a complex with recombinant PfRh5 and CyRPA (Fig. 1a). This complex migrates at 480 kDa in blue native electrophoresis compared to PfRipr with an apparent molecular weight of 242 kDa (Fig. 1a). We used Anion exchange chromatography (AEC) to separate uncomplexed PfRipr from ternary complexes and the peak contained the three proteins PfRh5/CyRPA/PfRipr confirming formation of a stable complex (Fig. 1b). Recombinant PfRh5/CyRPA/PfRipr complex was equivalent to endogenous complex purified from *P. falciparum* as both migrated at 480 kDa (Fig. 1c). Chemical crosslinked PfRh5/CyRPA/PfRipr complex migrated at 212 kDa (Extended Data Fig. 1a) indicating a 1:1:1 stoichiometric ratio suggesting migration on native-PAGE was due to an elongated shape as confirmed by negative stain electron microscopy (Extended Data Fig. 1b).

Basigin ectodomain lacking the transmembrane region did not bind the PfRh5/CyRPA/PfRipr complex in solution but bound PfRh5 (Fig. 1d, Extended Data Fig. 1c and d) [5]. However, full-length basigin including the lipid embedded transmembrane region bound the ternary complex (Fig. 1d, Extended Data Fig. 1e and f). Affinity of interaction showed PfRh5, PfRh5/CyRPA and PfRh5/CyRPA/PfRipr complex bound to full-length basigin with a similar affinity of 200 nM (Fig. 1e, Extended Data Table S1). However, PfRh5-basigin and PfRh5/CyRPA/PfRipr-basigin interaction had an additional higher affinity state (50 nM) with a slower off rate (Fig. 1e, Extended Data Table S1), which was more prevalent in PfRh5/CyRPA/PfRipr-basigin interactions reflected by the ratio of low affinity K_{D1} to high affinity K_{D2} suggesting significant conformational changes (Extended Data Table S2). These conformational changes in PfRh5 were

confirmed using hydrogen–deuterium exchange MS (HXMS) which showed the disulfide loop (Cys345-Cys351), forming part of the basigin binding site [6], had a bimodal distribution of deuterium exchange consistent with two states, with the detected protein sequence also undergoing significant conformational changes (Fig. 2a). Therefore PfRh5 undergoes conformational changes during binding to basigin that are stabilised in the ternary complex requiring interaction with lipid micelles surrounding the receptor transmembrane helix for efficient binding (Extended data Fig. 1c-d and extended data Fig. 1e-f).

We next showed the PfRh5/CyRPA/PfRipr complex bound to basigin on erythroid line JK-1 [7] (Fig. 2b, Extended data Fig. 1h-I and 2). PfRh5 bound to JK-1 cells, in a basigin-dependent manner, with an approximately two-fold higher binding compared to JK-1ΔBSG cells (Fig. 2b). Neither PfRipr nor CyRPA bound to JK-1 or JK-1ΔBSG cells (Fig. 2b). PfRh5/CyRPA showed no significant JK-1 binding suggesting this assay detects high affinity binding events and that CyRPA interferes with interaction of PfRh5 with basigin for the binary complex (Fig. 2b). PfRh5/CyRPA/PfRipr was detected on the surface of JK-1 cells at higher levels than PfRh5 alone indicating the ternary complex bound JK-1 cells at significantly higher efficiency, consistent with the relative contribution of high affinity binding sites (Fig. 1e, Table S2). Additionally, the number of JK-1 cells detected containing bound PfRipr increased dramatically for the ternary complex indicating its association was dependent on its presence in the complex. Whilst an increased level of CyRPA could be detected on JK-1 cells when bound in the ternary complex it was not as significant as PfRh5 and PfRipr suggesting dissociation from the complex during binding to basigin. Therefore binding of PfRh5/CyRPA/PfRipr to basigin

initiates molecular events mediating increased association of PfRh5/PfRipr with erythrocyte membranes.

Due to the requirement of lipid micelles for PfRh5/CyRPA/PfRipr-basigin interactions (Fig 1d, Extended data Fig. 1e and f), we hypothesized the ternary complex inserts into erythrocyte membranes upon binding to basigin. To test this we measured the ability of proteins to lyse erythrocytes as an indication of membrane insertion activity [8]. Lysis activity was observed when erythrocytes were incubated with PfRh5/CyRPA/PfRipr complex (Fig. 2c), whilst no significant activity was detected with single or binary components. Therefore PfRh5/CyRPA/PfRipr disrupts the erythrocyte membrane using excess, non-physiological concentration of proteins, however, the concentration of the ternary complex during merozoite invasion would be precisely controlled and not result in erythrocyte lysis.

Differential solubility in detergent was used to confirm insertion of proteins into erythrocyte membranes (Fig. 2d). For PfRh5, PfRh5/CyRPA and ternary complexes, a proportion of the PfRh5 pool was in the triton-X100 detergent resistant membrane (DRM) fraction (Fig. 2d, Extended data Fig. 1j), indicating high-molecular weight species embedded within highly-ordered and condensed membrane. When added as a ternary complex PfRipr was also found in DRM fractions indicating it was also inserted into the membrane. However, CyRPA, was in the soluble fraction suggesting, after binding to basigin, the PfRh5/CyRPA/PfRipr complex disassembles with CyRPA excluded from the membrane whilst PfRh5 and PfRipr insert (Fig. 2d). Erythrocyte membrane-associated PfRh5/PfRipr migrated as a single band of high-molecular weight (~700 kDa) indicating they were in the same complex as oligomers (Fig. 2e). However,

121 insertion of PfRh5/PfRipr into the membrane did not alter permeability to Ca^{2+} (Extended
122 data Fig. 3) suggesting additional proteins are required for pore formation between
123 erythrocyte membranes and the apical end of invading merozoites [2,4].

124 We used cryo-EM to obtain structural insights into the PfRh5/CyRPA/PfRipr
125 complex (Extended data Fig. 4). Three dimensional classification resulted in separation
126 of two populations corresponding to CyRPA/PfRipr and PfRh5/CyRPA/PfRipr
127 complexes (Extended data Fig. 4c). Fourier Shell Correlation (FSC) reported global
128 resolution of binary and ternary complexes at 5.07 Å and 7.17 Å, respectively (Extended
129 data Fig. 4 d-e). Local resolution suggested PfRh5 was flexible with the basigin binding
130 site being most flexible, while CyRPA and PfRipr region were more stable (Extended
131 data Fig. 4f-g, Extended Data Table S3). Densities corresponding to the six blade β-
132 sheets of the CyRPA β-propeller in the binary map were resolved including several β-
133 strands within blade 1, 3 and 6 of the β-propeller (Extended data Fig. 5a-b). The ternary
134 map densities corresponding to the six blade β-sheets of the CyRPA β-propeller [9,10] were
135 resolved as were the six α-helices of PfRh5. (Fig. 3a-b and Extended data Fig. 5c) [5,6].

136 The PfRh5/CyRPA/PfRipr complex was composed of a stoichiometric ratio of
137 1:1:1 with an elongated shape whereby CyRPA constitutes the core stabilising PfRh5 and
138 PfRipr on the opposite sides of the ternary complex (Fig. 3c-d, Extended Data video 1,
139 Extended Data Fig. 1a). The basigin binding site of PfRh5 consisting of the α2 and α4
140 helices, the disulphide loop (Cys345-Cys351) [6] was located at the tip of the ternary
141 complex opposite to PfRipr, which is solvent exposed and thus CyRPA and PfRipr did
142 not contact basigin (Fig. 3c-d). This was consistent with the K_D of PfRh5 and

PfRh5/CyRPA/PfRipr complex for basigin being similar demonstrating the ternary complex interacts with basigin via PfRh5 (Fig. 1e).

The CyRPA binding site of PfRh5 was at the tip of the α-helical scaffold opposite the basigin binding site (Fig. 4a). Density for the C-terminal tail of PfRh5 and part of the α7 helix was inserted into the central cavity of the CyRPA β-propeller (Fig. 4a and Extended data Fig 5d). At this contact site, the α5 and α7 helices of PfRh5 presents a hydrophobic groove enriched in hydrophobic residues to CyRPA (Fig 4a and Extended data Fig 5e-f). Two loops (B4 loop and B4B5 connecting loop) presented by blades 4 and 5 of the CyRPA β-propeller enriched with several aromatic residues (Y185, F187 and F226) are inserted in this groove of PfRh5 (Fig 4a and Extended data Fig 5e-f). Upon binding of PfRh5 to CyRPA, it is likely the B5 loop located on blade 5 of CyRPA became disordered to accomodate occupancy by the α5 helix of PfRh5 (Extended data Fig. 5g). Crosslinking studies detected an interaction between the C-terminus of PfRh5 immediately proceeding helix α7 with blade-1 of CyRPA consistent with the cryo-EM structure (Fig 4, Extended data Fig 5h).

Whilst the PfRipr model could not be built *de-novo* due to resolution of the map and presence of primarily β-sheet structures (Extended data Fig. 6a), several secondary structural elements were clearly visible at the CyRPA/PfRipr contact interface within the ternary complex. Secondary structure predictions indicated two putative α-helices (residues 196-211 and residues 364-373) residing in the N-terminus of PfRipr (Extended data Fig. 6b), while the rest of the amino acid sequence at the C-terminus (residues 373-1086) was predicted to contain loops and β-strands including eight epidermal growth factor-like repeats (EGFs 3-10). At the interface of the CyRPA/PfRipr, density for a 4-

turn α-helix could be observed that contacts blade 6 of the CyRPA β-propeller (Fig. 4b). The length of this α-helix density suggests it corresponds to residues 196-211 of PfRipr. In addition, density for a β-strand of PfRipr forms an inter-molecular β-sheet interaction with blade 6 of the CyRPA β-propeller (Fig. 4b). Therefore, blades 4-5 and 6 of the CyRPA β-propeller provide contact sites for PfRh5 and PfRipr, respectively (Fig. 4c).

A crystal structure of PfRh5-basigin complex is available [6], enabling alignment of the PfRh5/CyRPA/PfRipr cryo-EM structure to the PfRh5-basigin crystal structure (Extended Fig. 6c). The superimposed structures suggest the PfRh5/CyRPA/PfRipr complex was positioned parallel to the erythrocyte membrane (Fig. 4d). This orientation in relation to the erythrocyte membrane along with the conformationl changes detected when bound to basigin (Fig 1e), could facilitate membrane insertion of PfRh5 and PfRipr. Interestingly, the C-terminal helical bundle of PfRh5 is structurally similar to the N-terminal coiled-coil domain of SipB (Root mean square deviation of 3.4 Å over 144 residues) of the bacterial type III secretion system and possesses amphipathic property [11] (Extended data Fig. 6d-e). The membrane inserted PfRh5/PfRipr complex, along with other unidentified parasite proteins, may be involved in the formation of a pore enabling invading merozoites to inject components into the erythrocyte cytoplasm.

It is likely the conformational changes observed for PfRh5 in the ternary complex can be blocked during merozoite invasion by inhibitory antibodies. A monoclonal antibody to PfRh5 (9AD4), that inhibits invasion, has been identified that does not block the basigin-PfRh5 interaction, these monoclonal antibodies inhibit invasion and may act by interference with conformational changes blocking the function of the complex [12] (Extended data Fig. 6c.). Additionally, other monoclonal antibodies that bind PfRh5 and

CyRPA could sterically interfere with docking of the PfRh5/CyRA/PfRipr complex to the erythrocyte membrane preventing the membrane insertion of PfRh5 and PfRipr (Extended data Fig. 6c) [5,10]. This raises a route to identify epitopes for antibodies blocking conformational changes and membrane insertion trapping the complex in an inactive state and inhibiting invasion (Supplementary discussion). Collectively, this data lays the foundation to understand molecular details for invasion of *P. falciparum* into erythrocytes and will be important for design of vaccines against this disease.

Figure Legends

Fig. 1. PfRh5, CyRPA and PfRipr form a ternary complex. a, Size Exclusion Chromotography (SEC) peaks of PfRh5/CyRPA/PfRipr (red) or PfRipr (blue). Red asterisk, PfRh5/CyRPA/PfRipr; green asterisk, CyRPA/PfRipr; blue asterisk, PfRipr. **b,** AEC elution of PfRh5/CyRPA/PfRipr and separation of PfRipr. **c,** Purification of PfRh5/CyRPA/PfRipr from parasites. Experiments **a-c** repeated at least 3 times with biological independent samples and were reproducible. **d,** Ectodomain (left) and full-length basigin (right) with transmembrane and DDM micelle. **e,** SPR measuring interaction of PfRh5, PfRh5/CyRPA and PfRh5/CyRPA/PfRipr complexes with full-length basigin.

Fig. 2. PfRh5/CyRPA/PfRipr complex inserts into membranes. a, HXMS analysis of PfRh5 showing deuterium incorporation across peptide spanning disulfide loop Cys345-Cys351 (left) and detected peptides (right). **b,** FACS analyses of PfRh5, PfRipr, CyRPA, PfRh5/CyRPA and PfRh5/CyRPA/PfRipr binding to JK-1 and JK-1ΔBSG cells. Shown

is plot of % positive cells detected after incubation with protein(s). **c,** Haemolytic activity of PfRh5, CyRPA, PfRipr, PfRh5/CyRPA and PfRh5/CyRPA/PfRipr complexes. For **b – c,** n=3 with experiments performed at least 3 times with biological independent samples and were reproducible. Bar graphs show mean values with standard deviation. Student t-test used to calculate statistical significance with two tailed *p* value. **d,** Differential solubilization of PfRh5, PfRh5/CyRPA and PfRh5/CyRPA/PfRipr with erythrocytes. Samples were separated on non-reducing SDS-PAGE analysed by western-blot. * high-molecular weight species. **e,** Pelleted erythrocyte membranes after and insertion of PfRh5 and PfRipr detected by native-PAGE immuno-blotting analyses. For **d-e,** experiments repeated 3 times with biological independent samples and reproducible.

Fig. 3. Organization of PfRh5/CyRPA/PfRipr ternary complex. a, EM density of CyRPA region of PfRh5/CyRPA/PfRipr complex (left) and a cross section showing resolution of 6 bladed β-sheets (right). **b,** EM density of PfRh5 region of PfRh5/CyRPA/PfRipr (top) and cross section showing resolution of 5 α-helices (bottom). **c-d,** 3D reconstruction of PfRh5/CyRPA/PfRipr at global resolution of 7.17 Å with map shown in (**c)** and refined model in (**d)**.

Fig. 4. Interactions between PfRh5/CyRPA and CyRPA/PfRipr a, EM density of CyRPA (red) bound to PfRh5 (blue). B4 and B4B5 loops of CyRPA were inserted into hydrophobic groove of PfRh5 formed by α5 and α7 helices. **b,** EM density of CyRPA (red) bound to PfRipr (yellow) (left). Contact between CyRPA and PfRipr magnified (right) showing inter-molecular β-sheet interaction and binding of PfRipr N-terminal α-

helix to blade 6 of CyRPA β-propeller. **c,** Model of PfRh5/CyRPA/PfRipr showing contacts between PfRh5/CyRPA and CyRPA/PfRipr. **d.** Model of molecular events for binding and insertion of PfRh5/CyRPA/PfRipr complex. Ternary complex binds basigin via a lower affinity binding site, an interaction requiring membrane lipid. Upon initial interaction with basigin, a conformational change in PfRh5 led to a high-affinity interaction and exposure of amphipathic helical domain in PfRh5, leading to oligomerization and insertion of PfRh5 and PfRipr into erythrocyte membrane, whilst CyRPA is excluded.

Extended Data

Extended Data Figure 1: Stoichiometry of the PfRh5/CyRPA/PfRipr complex, interaction with soluble or full length basigin and JK-1 cells. a, Chemical crosslinking of PfRh5/CyRPA/PfRipr complex by DSS analyzed on an SDS-PAGE. **b,** Negative stain EM of purified PfRh5/CyRPA/PfRipr ternary complex showing the elongated shape of the complex. **c,** SEC analysis of mixture containing recombinant PfRh5/CyRPA/PfRipr complex and soluble basigin. Soluble basigin was eluted separately from the ternary complex indicating no binding to basigin. **d,** Immuno-precipitation of PfRh5/CyRPA-HA/PfRipr from parasite schizont stage extract using anti-HA resins could not pull down soluble basigin. **e,** Native PAGE analysis of the SEC (left) and IEX (right) eluted

fractions showing the migration of the quaternary complex comprised of PfRh5/CyRPA/PfRipr/full length basigin close to ~ 600 kDa. **f,** Western blot analysis of the IEX elutions indicated the presence of full length basigin in the complex. **g**, SEC analysis showed PfRh5 and PfRipr are eluted separately from each other indicating no complex formation in the absence of CyRPA. **h,** Labelling of JK1 and JK-1ΔBSG cells with anti-CD147 (basigin) and analysis using flow cytometry. **i,** Analysis of PfRh5 (blue line), PfRh5/CyRPA (red line) and PfRh5/CyRPA/PfRipr (green line) binding to JK-1 and JK-1ΔBSG (ΔBSG) cells. **j,** Differential solubilization showing peripheral membrane protein (spectrin), integral membrane protein (glycophorin) and detergent resistant membrane protein flotillin were localised in the sodium bicarbonate soluble, TX100 soluble and TX100 insoluble fractions respectively. For experiments in a-j they were repeated 3 times with biological independent samples and were reproducible.

Extended Data Figure 2: Gating strategy for FACS analysis. a, Representative flow cytometry plots of unstained JK1 cells and cells stained with anti-CD147-APC. Population of cells were gated on using forward and side scatter (top panels). Doublet exclusion was performed using FSC-A and FSC-H (middle panels). Voltage used for the APC channel (anti-CD147) was set using unstained cells where the negative population was positioned $<10^3$ (bottom panels). Experiments were repeated 3 times independently and reproducible.

b, Representative flow cytometry plots of JK1 and JK1ΔBSG cells in the presence or absence of recombinant proteins. Cells were incubated with either no protein, single recombinant protein, the binary Rh5/CyRPA complex or with the ternary

Rh5/CyRPA/Ripr, followed by the subsequent incubation of the respective primary and secondary antibodies as indicated. Experiments were repeated 3 times with biological independent samples and were reproducible.

Extended Data Figure 3: The PfRh5/CyRPA/PfRipr complex does not stimulate Ca^{2+} flux across the erythrocyte membrane. a, FACS kinetic plot of online stimulation of Fluo-4-loaded **erythrocytes** stimulated with a dilution series of the Ca^{2+} ionophore A23187 (1 μM – 0.031 μM). Equivolume of a two-fold concentration of A23187 was added to erythrocytes loaded with Fluo-4 at 10 sec and fluorescence monitored continuously for 1 min 30 sec. Equivolume of a 2x concentration of PfRh5 only or preassembled PfRh5/CyRPA/PfRipr complex also added at 10 sec after the start of acquisition. PfRh5 and PfRh5/CyRPA/PfRipr complex –bound erythrocytes were then re-measured at 4 min and 6 min. At 7 min post addition of PfRh5/CyRPA/PfRipr to Fluo-4-loaded erythrocytes were re-challenged with 1 μM of A23187. **b,** Kinetic plot of samples where PfRh5 alone and PfRh5/CyRPA/PfRipr complex was added, plotted only with stimulation with 0.031 μM A23187. **c,** Graphical representation of mean fluorescence intensity values at 80 sec, as above. n=3, experiments were repeated 3 times with biological independent samples and were reproducible.

Extended Data Figure 4: Cryo-EM single particle analysis of PfRh5/CyRPA/PfRipr complex a, A micrograph post drift correction and dose-weighting. **b,** Reference-free 2D class averages. **c,** 3D classification resulted in separation of the binary CyRPA/PfRipr complex (left) and the ternary PfRh5/CyRPA/PfRipr complex (right). **d,** Fourier-shell-

correlation (FSC) curves indicating the overall resolutions of the ternary PfRh5/CyRPA/PfRipr (blue) and binary CyRPA/PfRipr (red) reconstructions. **e,** FSC curves between final refined PfRh5/CyRPA/PfRipr ternary model and full map excluding unbuilt region of PfRipr density (black); between model refined in half map 1 and the reconstruction from that same half ($FSC_{work,}$ blue); and between model refined in half map 1 and reconstruction from half map 2 ($FSC_{test,}$ red) for the PfRh5/CyRPA/PfRipr ternary complex **f-g,** Local resolution estimation color specdtrum of the ternary PfRh5/CyRPA/PfRipr map **(f)** and the binary CyRPA/PfRipr map **(g).**

Extended Data Figure 5: Cryo-EM densities of CyRPA in the binary complex and PfRh5 in the ternary complex a, EM density showing the top view of the CyRPA β-propeller (left) and a cross section of the same region showing the resolution of the 6-bladed β-sheets of CyRPA (right). **b,** Density of β-strands resolved in blade-1, 3 and 6 of the CyRPA β-propeller. **c,** EM densities showing the individual α-helices (α2-7) of PfRh5.

d, Model showing α7 helix of PfRh5 inserted into central cavity of CyRPA. **e,** Hydrophobic residues (L393, L397, F494, I498) form groove of PfRh5 in contact with aromatic residues (Y185, F187, F226) presented by B4 and B4B5 loops of CyRPA. **f,** Models showing hydrophobic groove of PfRh5 and binding aromatic residues of CyRPA (Y185, F187, F226) shown in orange. **g,** Density maps and the refined atomic models showing the disordered B5 loop in CyRPA upon binding of PfRh5 (left), the corresponding region showing the ordered B5 loop in CyRPA in the absence of PfRh5 (middle) and the two superimposed blade-5 β-sheets of CyRPA in the absence (blue) and

presence (red) of PfRh5 (right). **h,** Tandem mass spectra of DSS cross-linked peptides identified from tryptic digestion of gel purified PfRh5/CyRPA/PfRipr complex. High-resolution spectra from Q-Exactive MS for two cross-linked peptides between PfRh5$^{520-526}$ and CyRPA$^{37-50}$. Experiments were repeated 3 times with biological independent samples and were reproducible.

Extended Data Figure 6: EM density map of PfRipr in the ternary complex and orientation of the PfRh5/CyRPA/PfRipr complex on the erythrocyte membrane . a, Density map corresponding to PfRipr (yellow) and CyRPA (red) showing several β-sheets in PfRipr. **b,** Seondary structure prediction using the Phyre2 server indicated two putative, high-confidence α-helices (in dash-rectangles) reside in the N-terminus of PfRipr [13]. **c,** The cryo-EM structure of PfRh5/CyRPA/PfRipr overlaid with the crystal structure of PfRh5-basigin (BSG) complex. The overlaid structures suggest the PfRh5/CyRPA/PfRipr complex is positioned parallel with the erythrocyte membrane prior to insertion. The crystal structures of CyRPA-C12, CyRPA-8A7 and PfRh5-9AD4 antigen-antibodies complexes were also overlaid with the PfRh5/CyRPA/PfRipr cryo-EM structure. The overlaid structures suggest these monoclonal antibodies function to inhibit the docking of the invasion complex to the erythrocyte membrane. **d,** The crystal structure of the N-terminal domain of SipB is superimposed with the C-terminal helical bundles of PfRh5. Over 144 residues of SipB were aligned with PfRh5-C-term with a RMSD of 3.4 Å. **e,** The PfRh5 C-terminal helical bundle containing the α4 - α7 helices are shown in cartoon, and surface representations. Hydrophobic residues lining one side of the helical bundle are colored in red whereas hydrophilic residues lining the opposite side of the helical bundle are coloured in blue.

Extended Data Table S1. Kinetic constants derived from fitting SPR sensorgrams.

Extended Data Table S2. Kinetic constants derived from fitting SPR sensograms.

Extended Data Table S3. Cryo-EM data collection, refinement and validation statistics.

Extended Data video 1. 3-D reconstruction of the PfRh5/CyRPA/PfRipr complex.

Methods

Protein expression and purification.

The expression and purification of recombinant PfRh5 and PfCyRPA have been described previously [5,9]. Full-length PfRipr (amino acids 20–1086) was expressed in *Drosophila* S2 cells (ExpreS^{2}ion Biotechnologies) and purified using the Strep-Tactin®XT purification system [14]. Full length basigin was expressed in SF21 cells as per supplier's manual as a C-terminally FLAG-tagged fusion protein. Full length basigin was extracted from the membrane of SF21 cells in lysis buffer (40 mM Tris, 150 mM NaCl, 1% DDM, pH 8.5) and clarified supernatant containing DDM solublised basigin was incubated with FLAG resins at 4^{o}C for 2 hr to enable binding. Basigin bound resins were washed and eluted in elution buffer (20 mM Tris, 150 mM NaCl, 0.4 mM DDM and 100 µg/ml FLAG peptides, pH 8.5). Eluted fractions contatining full length basigin was further purified by size exclusion chromatography using a Superose 6 10/300 size exclusion column in elution buffer (20 mM Tris, 150 mM NaCl, 0.4 mM DDM, pH 8.5).

For preparation of PfRh5/CyRPA/PfRipr complex, individual components were mixed at 1:1:1 molar ratio at room temperature for 1 hr. Sample was injected onto a Superose 6 10/300 size exclusion column in elution buffer (20 mM Tris, 250 mM NaCl, pH 8.5) and

the PfRh5/CyRPA/PfRipr complex separated from uncomplexed components. Fractions containing eluted PfRh5/CyRPA/PfRipr complex were pooled and diluted in ion exchange buffer A (20 mM Tris, 25 mM NaCl, pH 8.5) followed by loading of the sample onto a HiTrap Q HP column. After extensive wash in buffer A, PfRh5/CyRPA/PfRipr complex was eluted in a linear gradient of buffer A and buffer B (20 mM Tris, 1 M NaCl, pH 8.5). This resulted in the separation of free Ripr from the ternary complex.

Surface plasmon resonance

Surface Plasmon resonance binding assays were performed using BIAcore 4000 instruments in an SPR buffer (10 mM HEPES, 150 mM NaCl, 3.4 mM EDTA, 0.005% Tween-20, pH 7.4). Basigin, including the transmembrane helix, was immobilised as the ligand on a CM5 sensor chip surface by amine coupling. Hydrodynamic addressing was used to immobilise basigin on spots 1 and 5 of a single flowcell at densities of 7725 RU and 3834 RU respectively. Sensograms were double referenced by subtracting spot 2 or spot 4 that had been blocked with ethanolamine (spot 2 from 1 or 4 from 5), and a blank SPR buffer only sample. Analyte protein samples (PfRh5 alone, PfRh5/CyRPA binary and PfRh5/CyRPA/PfRipr ternary complexes) were reconstituted in SPR buffer at various concentrations (2 μM to 2 nM) to derive binding affinities and injected for 150 sec with 500 sec dissociation time. The sensor surface was regenerated with glycine buffer (10 mM glycine pH 2.1) between each cycle before repeating analyte injections. Sensorgrams were initially fitted to a Langmuir specific 1-site binding model and then a

heterogeneous ligand binding model, if appropriate, to derive on and off rates and the dissociation constants (K_D).

To determine the affinity of basigin binding to PfRh5-only, PfRh5/CyRPA binary and the PfRh5/CyRPA/PfRipr ternary complex SPR experiments were performed immobilising basigin on the sensor surface, flowing the various PfRh5 complexes as the analyte. Since PfRh5 and PfRipr do not interact in the absence of CyRPA (Extended data Fig. 1g), the binding affinity of PfRh5/PfRipr binary complex for basigin could not be measured. Initially, the SPR curves were analysed by a 1:1 binding model and then a heterogeneous ligand model for the interaction. The 1:1 binding model gave K_D values 240 nM, 180 nM and 130 nM for PfRh5-only, PfRh5/CyRPA binary and the PfRh5/CyRPA/PfRiprrespectively (Extended Data Table S1). Visual inspection of the 1:1 binding model fit to the raw data indicated that PfRh5-only and PfRh5/CyRPA binary were in reasonable agreement with the data, however the PfRh5/CyRPA/PfRipr ternary complex showed a poor fit. HXMS (Fig. 2a) experiments provided evidence for two distinct populations of apo-PfRh5 conformers and electron microscopy local resolution analysis also indicated the basigin binding site is the most flexibile part of the ternary complex (Extended data Fig. 4f). This provided justification for analysis by a heterogeneous ligand model, fitting the data to two independent sites with two distinct on and off rates (k_{a1}, k_{a2}, k_{d1} *and* k_{d2}) and providing two K_D values (K_{D1} and K_{D2}), one for each site. The PfRh5/CyRPA-basigin binding showed minor differences between the two sites with similar K_D values 200 and 240 nM (Fig. 1e, Extended Data Table S2), which were comparable to the values from the 1:1 binding model. However, both the PfRh5-basigin and PfRh5/CyRPA/PfRipr-basigin interactions showed clear differences between

the two sites with a low affinity site that was comparable to the 1:1 binding model affinity (around 200 nM), and an additional higher affinity binding site with slower on and off rates and a 4-fold lower K_D around 50 nM (Fig. 1e, Extended Data Table S2). This suggested two discrete conformations for PfRh5 and the PfRh5/CyRPA/PfRipr ternary complex, in agreement with the HDMS and elctron microscopy data, that have differing affinities for basigin. Comparing the contribution of R_{max} to each fit showed that with PfRh5-only the low affinity site dominated with the high affinity site contributing 10% (9:1 ratio) of the fit (Extended Data Table S2). This ratio was decreased with the PfRh5/CyRPA/PfRipr ternary complex with the high affinity site contributing 30% (7:3 ratio) of the fit (Extended Data Table S2), providing an explanation for the poor fit to the 1:1 binding model. Additionally this indicates that the high affinity conformation is stabilised in the ternary state.

Cell lines

Plasmodium falciparum strain 3D7 was obtained from Prof David Walliker at Edinburgh University and was validated using whole genome sequencing. The erythroleukemia cell line JK-1 was obtained from the Leibniz Institute Deutsche Sammlung von Mikroorganismen und Zellkulturen collection of microorganisms and cell cultures (catalog no. ACC347). Its identity was confirmed by detection of specific proteins on the surface such as basigin. All cell lines were tested for negative for Mycoplasma contamination.

Antibodies

Antibodes raised against PfRh5, CyRPA and PfRipr were generated in the Cowman laboratory and were previously published[3,5,9].

Flow cytometry-based cell binding assay

All binding and antibody incubations were performed at room temperature for 1 hr in 50 µl. Washes were performed in PBS supplemented with 1% (w/v) bovine serum albumin (BSA) and spun at 1,000 x g for 1 min. In all conditions, an equivalent molarity of PfRh5 at 4 µM was used. The binary PfRh5-PfCyRPA and ternary PfRh5/CyRPA/PfRipr protein complexes were created by combining equimolar amounts of proteins in PBS/1% BSA for one hour at room temperature. JK1 of JK1-ΔBSG cells were washed twice and $1x10^6$ cells per binding condition were used. PfRh5, the binary PfRh5-PfCyRPA and ternary PfRh5/CyRPA/PfRipr protein complexes were added separately to cells for 1 hr at room temperature. After binding, the cells were washed and incubated with specific primary antibodies (0.2 mg/ml of monoclonal anti-PfRh5, 0.05 mg/ml of polyclonal anti-PfRipr or 0.05 mg/ml of polyclonal anti-CyRPA). After two washes, Alexa Fluor 488-conjugated goat anti-mouse or goat anti-rabbit secondary antibodies (1:100; Life Technologies) were added. The cells were washed three times and resuspended in 150 µl PBS before analysis with the LSRII flow cytometer (BD Biosciences). 30,000 events were recorded and results were analysed using the FlowJo software. The background signal induced by the primary and secondary antibodies in the absence of protein was subtracted from the corresponding positive fluorescent signals.

Haemolytic assay

Erythrocytes were washed in Phosphate-buffer saline (PBS) 4 times before incubation with buffer control or 1.6 μM of purified recombinant proteins (PfRh5, PfRipr, CyRPA, PfRh5/CyRPA binary and PfRh5/CyRPA/PfRipr ternary complexes at 37^{o}C for 24 hr with shaking. Unlysed cells were pelleted by centrifugation at 6000 rpm for 1 min. The absorbance on the supernatant containing the released hemoglobin was measured at 405 nm.

Differential solubilization of proteins in erythrocyte membrane

Remaining cell pellets from the haemolytic assay were washed in PBS 4 times to release soluble proteins and subsequently pelleted by centrifugation at 6000 rpm at 4^{o}C for 1 min. The PBS washed cell pellets were treated with Na_sCO_3 pH 11.5 to release peripheral membrane associated proteins or Triton X100 to release integral membrane proteins. Centrifugation at 40,000 rpm at 4^{o}C for 20 min was performed to isolate the Na_sCO_3 and Triton X100 soluble and insoluble fractions for western-blot analysis.

Ca^{2+} flux measurements using FACS

Erythrocytes were resuspended in Ringers buffer at 1% hematocrit and labelled with x concentration of Fluo-4 for 30 min at room temperature. Erythrocytes were then further diluted in Ringers buffer to 0.1% hematocrit and aliquoted into 200 μl in FACS tubes. Ca^{2+} ionophore A23187 was diluted in Ringers buffer to 2x final concentration. PfRh5 alone (conc) or PfRh5/CyRPA/PfRipr (conc) were mixed and diluted in buffer x. Flourescence of Fluo-4-loaded erythrocytes were then acquired on a LSRII FACS analyser (BD Biosciences). Samples were analysed in FlowJo v8.

Hydrogen-Deuterium Exchange MS (HXMS)

Sample stock solutions were diluted to 40 pmol/µL protein concentration with 100 mM NaCl, 20 mM HEPES, pH 7.5. 2 µL protein solution was transferred into a 10 mm autosampler vial (Thermo Scientific), 38 µL of deuterium buffer and 2 µL of of quench buffer (1.5% v/v formic acid) where used. 12 µL acidified protein was injected into the sample loop and subsequently digested, desalted and separated online using the Agilent Technologies 1200 series Capillary LC System. The injected sample was delivered to an immobilized pepsin column (Poroszyme Immobilized Pepsin Cartridge, 2.1 mm x 30 mm, cat. number 2-3131-00, Applied Biosystems) at a flow rate of 50 µL/min buffer A1 (5% v/v methanol, 0.2 % v/v formic acid in MiliQ water, pH 2.5) using an Agilent Technologies 1200 series pump, which equated to a digestion time of two minutes. The online digestion and subsequent separation steps were performed at 1°C by storing lines, pepsin column, C18-trap and valve (Agilent Technologies 1200 series) in a 120 litre fridge (Westinghouse). The flow was diverted by a two-position ten-port valve and a binary pump (Agilent Technology 1200 series). The resulting peptic peptides were trapped on a C18 trap column (0.5 mm x 5 mm, ReproSil-Pur C18-AQ 5 µm, Dr. Maisch) and desalted with 95% buffer A2 (0.2% v/v formic acid in MiliQ water) and 5% buffer B2 (95% v/v acetonitrile, 0.2% v/v formic acid) at a flow rate of 5 µL/min. A 10 minute linear gradient (5-55% buffer B2) starting after 3.2 min was applied to elute the peptides. The eluate was directed into a Thermo LTQ XL Hybrid Ion Trap-Orbitrap mass spectrometer with an ESI source operated at a capillary temperature of 180 °C, and a spray voltage of 1.8 kV using a 3 µm ID conductive coated pulled ESI emitter tip (New

Objective, Woburn USA). Mass spectra were acquired over the m/z range 350-2000 using the orbitrap analyser. For peptide identification the five most abundant ions per scan were fragmented and analysed in the ion trap. For each sample run, spectra were acquired for 20 minutes and the system was flushed and re-equilibrated after every sample measurement by injecting MiliQ water and performing a blank run. Each sample was analysed in triplicate. In order to identify peptides and determine sequence coverage, the acquired MS/MS data was subjected to a protein database search, including a customised database featuring the sequence of PfRh5 recombinant protein.

The HXMS analysis revealed PfRh5 exists in multiple different conformations. Peptides spanning the disulfide loop (Cys345-Cys351), that form part of the basigin binding site [6], showed a bimodal distribution of deuterium exchange suggesting this region of the protein exists in two distinct conformational states (Fig. 2a). Additionally, most of the detected protein sequence appeared to undergo significant exchange of deuterium over the time course suggesting dynamic changes in most regions of the protein and an absence of disordered regions (Fig. 2a).

Cross-linking Mass Spectrometry (XLMS)

Protein samples were manually excised from preparative SDS-PAGE gels and subjected to manual in-gel reduction, alkylation, and tryptic digestion. All gel samples were reduced with 10 mM DTT (Sigma) for 30 min, alkylated for 30 min with 50 mM iodoacetamide (Sigma) and digested with 375 ng trypsin gold (Promega) for 16 hr at 37°C. The extracted peptide solutions were then acidified (0.1% formic acid) and concentrated to 10 µl by centrifugal lyophilisation using a SpeedVac AES 1010 (Savant).

Extracted peptides were injected and fractionated by reversed-phase liquid chromatography on a nanoACQUITY UHPLC system (Waters, USA) using a nanoACQUITY C18 250 mm × 0.075 mm I.D. column (Waters, USA) with a linear 90-min gradient at a flow rate of 300 nl/min from 98% solvent A (0.1% Formic acid in Milli-Q water) to 35 % solvent B (0.1% Formic acid, 99.9% acetonitrile). The nano-UHPLC was coupled on-line to a Q-Exactive Orbitrap mass spectrometer equipped with a nano-electron spray ionization source (Thermo Fisher Scientific, Bremen, Germany). High mass-accuracy MS data was obtained in a data-dependent acquisition mode with the Orbitrap resolution set at 70,000 and the top-ten multiply charged species selected for fragmentation by HCD. The stepped (N)CE voltage was set to 19.5, 26, 32.

Raw files were analysed using MaxQuant1,2 (version 1.5.3.30) [15,16]. The database search was performed using the Uniprot *P. falciparum* (isolate 3D7) database plus common contaminants with strict trypsin specificity allowing up to 2 missed cleavages. The minimum peptide length was 7 amino acids. Carbamidomethylation of cysteine was a fixed modification while N-acetylation of proteins N-termini and oxidation of methionine were set as variable modifications. During the MaxQuant main search, precursor ion mass error tolerance was set to 4.5 ppm and fragment ions were allowed a mass deviation of 20 ppm [15,16]. PSM and protein identifications were filtered using a target-decoy approach at a false discovery rate (FDR) of 1%. MaxQuant APL files were converted to MGF files using the APL to MGF convertor software (https://www.wehi.edu.au/people/andrew-webb/1298/apl-mgf-converter) [15]. Cross-linked peptides were identified from the MGF files using StavroX software (version 3.6.0.1) [16].

Lysines, protein N-termini, serines, threonines, and tyrosines were set as reaction sites of the cross-linker NHS-esters. Trypsin was set as the enzyme allowing for 3 missed cleavages at lysines and 2 at arginines. Precursor precision was set at 10 ppm with fragment ion precision set at 20 ppm.

Electron microscopy

Negative stain EM was performed at the Bio21 Advanced Microscopy Facility, the University of Melbourne and Ramaciotti Centre for Cryo-EM, Monash Univesrity. 3 µl of purified PfRh5/CyRPA/PfRipr complex was incubated on glow-discharged holey carbon grids (Quantifoil 1.2/1.3) with a 5 nm continuous carbon support layer for 30 sec. Excess sample was removed by blotting on a filter paper, and grids were wash in water before staining in 1 % uranyl acetate solution for 30 sec. Grids were air-dried and transferred to a FEI TF30 electron microscope operated at 200 kV with images recorded at a calibrated magnification of 20,500 at defocus values ranged from 1-2 µm.

For cryo-EM, frozen samples were transported on dry ice to Janelia CryoEM facility, Ashburn, VA. Prior to grid preparation, an aliquot of protein was thawed on ice immediately followed by glycerol removal using a 0.5 ml 100k mwco Amicon filtration unit (Millipore, Darmstadt Germany) in a 4°C table-top centrifuge at 2000 rcf for minimum of 5 cycles. 3.2µl of sample diluted in glycerol-free buffer was applied to a glow-discharged 200-mech quantifoil 1.2/1.3 Au grid (Quantifoil, Großlöbichau Germany) then rapidly plunge-frozen into a liquid ethane bath on a Vitrobot (FEI company, part of Thermo Fisher Scientific, Hillsboro, OR).

Grids were imaged on a 300 kV FEI Titan Krios cryo electron microscope (FEI Company) equipped with a spherical aberration corrector, an energy filter (Gatan GIF Quantum) and a post-GIF Gatan K2 Summit direct electron detector. Images were taken on the K2 camera in dose-fractionation mode at a calibrated magnification of 48077, corresponding to 1.04 Å per physical pixel (0.52 Å per super-resolution pixel). The dose rate on the specimen was set to be 9.25 electron per $Å^2$ per second and total exposure time was 10 s, resulting in a total dose of 92.5 electrons per $Å^2$. With dose fractionation set at 0.2 s per frame, each movie series contained 50 frames and each frame received a dose of 1.85 electrons per $Å^2$. An energy slit with a width of 20 eV was used during data collection. Fully automated data collection was carried out using SerialEM with a nominal defocus range set from −1.5 to −3 μm.

A first round of data collection and processing indicates that there are a limited number of projection views of the sample. To get more projection views of the sample, four different data sets were collected with the compu-stage tilted at different angles. To be specific, 6104 movie series were collected at 0 degree tilt; 2485 movie series were collected at 30 degree tilt; 1709 movies were collected at 40 degree tilt and 2676 movies were collected at 45 degree tilt.

Image Processing

Beam-induced motion were measured, corrected, and dose-weighted at 1.85 electron/$Å^2$ per frame with data binned by 2 using cisTEM [17]. CTF determination for each movie series was calculated by amplitude averaging of every 3 frames using cisTEM. Automated particle picking using ab inito mode was carried out in cisTEM on all the

602 micrographs and particle stacks were extracted for each data set. For data collected at 30,
603 40 and 45 degree tilt, local CTF correction was performed for each particle using GCTF
604 [18]. Multiple rounds of reference-free 2D classification with CTF correction was
605 performed for each data set in cisTEM to throw away bad particles. Particles from good
606 representative 2D classes from all four datasets were combined to form a new stack of
607 752,018 particles. This new particle stack was loaded into cryosparc to generate ab initio
608 3D models [19]. The resultant initial models and heterogeneous refinement in cryosparc
609 indicated that the particles belong to two different populations: 70.9% of particles are
610 CyRPA/PfRipr binary complex and 29.1% are PfRh5/CyRPA/PfRipr ternary complex.
611 The two populations were separately imported into cisTEM for further 3D refinement.
612 Fourier Shell Correlation at a criteria of 0.143 reported resolution 5.07 Å for the binary
613 complex and 7.17 Å for the ternary complex.
614
615 **Model building and refinement**
616 The crystal structures of PfRh5 (PDB ID: 4WAT) [5] and CyRPA (PDB ID: 5TIK) [9] were
617 individually docked into the PfRh5/CyRPA/PfRipr ternary map using UCSF Chimera [20]
618 The fitted PfRh5 and CyRPA models were manually refined in Coot [21]. Since densities
619 corresponding to the N-terminal β-hairpin and part of the C-terminal tail of PfRh5 were
620 disordered, these domains were therefore removed from the model. The N-terminal α-
621 helix and β-strand of PfRipr that contact blade-6 of CyRPA were manually built as a
622 poly-alanine model guilded by the density map in Coot. After manual building in Coot,
623 the model of PfRh5/CyRPA/PfRipr was globally real space refined and minimized in
624 Phenix [22] using the PfRh5/CyRPA/PfRipr density map. During the course of manual

model building and global refinement in phenix, torsion, rotamer, Ramachandran, C-β deviation restraints and secondary structure restraints were applied throughout. After model refinement, Bsoft package was used to calculate FSC curves between refined atomic models and density maps [23]. All structural figures were generated in UCSF Chimera [20] and pymol www.pymol.org.

Data availability

All relevant data are available from the authors and/or are included with the manuscript. Atomic coordinates and the cryo-EM density maps have been deposited in the Protein Data Bank under accession number 6MPV and the Electron Microscopy Data Bank under accession number EMD-9192 and EMD 9193.

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Author Contribution

WW performed biochemistry and cryo-EM and wrote the manuscript. RH and ZY performed cryo-EM and analysis. JH, TH, VS, TMMS, TJ and WAdJ performed protein purification. RWB and PEC performed plasmon surface resonance. SM and WHT performed FACS analysis. CJT performed Ca^{2+} uptake experiments. DH, JJS and AIW

649 performed Mass Spectrometry. UK and MJT made JK-1ΔBSG and JK-1 cells. All
650 authors and assisted with manuscript preparation. AFC was responsible for project
651 strategy, management, data interpretation and writing the manuscript.
652

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656 **References**

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659 1 Crosnier, C. *et al.* Basigin is a receptor essential for erythrocyte invasion by
660 *Plasmodium falciparum*. *Nature* **480**, 534-537, doi:nature10606 [pii]
661 10.1038/nature10606 (2011).
662 2 Volz, J. C. *et al.* Essential Role of the PfRh5/PfRipr/CyRPA Complex during
663 *Plasmodium falciparum* Invasion of Erythrocytes. *Cell Host Microbe* **20**, 60-71,
664 doi:10.1016/j.chom.2016.06.004 (2016).
665 3 Chen, L. *et al.* An EGF-like protein forms a complex with PfRh5 and is required for
666 invasion of human erythrocytes by *Plasmodium falciparum*. *PLoS Pathog.* **7**,
667 e1002199, doi:10.1371/journal.ppat.1002199 (2011).
668 4 Weiss, G. E. *et al.* Revealing the Sequence and Resulting Cellular Morphology of
669 Receptor-Ligand Interactions during *Plasmodium falciparum* Invasion of Erythrocytes.
670 *PLoS Pathog.* **11**, e1004670, doi:10.1371/journal.ppat.1004670 (2015).
671 5 Chen, L. *et al.* Crystal structure of PfRh5, an essential *P. falciparum* ligand for
672 invasion of human erythrocytes. *Elife* **3**, doi:10.7554/eLife.04187 (2014).
673 6 Wright, K. E. *et al.* Structure of malaria invasion protein RH5 with erythrocyte basigin
674 and blocking antibodies. *Nature*, doi:10.1038/nature13715 (2014).
675 7 Kanjee, U. *et al.* CRISPR/Cas9 knockouts reveal genetic interaction between strain-
676 transcendent erythrocyte determinants of *Plasmodium falciparum* invasion. *Proc Natl*
677 *Acad Sci U S A* **114**, E9356-E9365, doi:10.1073/pnas.1711310114 (2017).
678 8 Blocker, A. *et al.* The tripartite type III secreton of Shigella flexneri inserts IpaB and
679 IpaC into host membranes. *J. Cell Biol.* **147**, 683-693 (1999).
680 9 Chen, L. *et al.* Structural basis for inhibition of erythrocyte invasion by antibodies to
681 *Plasmodium falciparum* protein CyRPA. *Elife* **6**, doi:10.7554/eLife.21347 (2017).

10 Favuzza, P. *et al.* Structure of the malaria vaccine candidate antigen CyRPA and its complex with a parasite invasion inhibitory antibody. *Elife* **6**, doi:10.7554/eLife.20383 (2017).

11 Barta, M. L. *et al.* The structures of coiled-coil domains from type III secretion system translocators reveal homology to pore-forming toxins. *J. Mol. Biol.* **417**, 395-405, doi:10.1016/j.jmb.2012.01.026 (2012).

12 Douglas, A. D. *et al.* Neutralization of *Plasmodium falciparum* merozoites by antibodies against PfRH5. *J. Immunol.* **192**, 245-258, doi:10.4049/jimmunol.1302045 (2014).

13 Kelley, L. A., Mezulis, S., Yates, C. M., Wass, M. N. & Sternberg, M. J. The Phyre2 web portal for protein modeling, prediction and analysis. *Nat. Protoc.* **10**, 845-858, doi:10.1038/nprot.2015.053 (2015).

14 Douglas, A. D. *et al.* A PfRH5-based vaccine is efficacious against Heterologous strain blood-stage *Plasmodium falciparum* infection in aotus monkeys. *Cell Host Microbe* **17**, 130-139, doi:10.1016/j.chom.2014.11.017 (2015).

15 Cox, J. & Mann, M. MaxQuant enables high peptide identification rates, individualized p.p.b.-range mass accuracies and proteome-wide protein quantification. *Nat. Biotechnol.* **26**, 1367-1372, doi:10.1038/nbt.1511 (2008).

16 Cox, J. *et al.* Andromeda: a peptide search engine integrated into the MaxQuant environment. *J. Proteome Res.* **10**, 1794-8105, doi:10.1021/pr101065j (2011).

17 Grant, T., Rohou, A. & Grigorieff, N. cisTEM, user-friendly software for single-particle image processing. *Elife* **7**, doi:10.7554/eLife.35383 (2018).

18 Zhang, K. Gctf: Real-time CTF determination and correction. *J Struct Biol* **193**, 1-12, doi:10.1016/j.jsb.2015.11.003 (2016).

19 Punjani, A., Rubinstein, J. L., Fleet, D. J. & Brubaker, M. A. cryoSPARC: algorithms for rapid unsupervised cryo-EM structure determination. *Nat. Methods* **14**, 290-296, doi:10.1038/nmeth.4169 (2017).

20 Pettersen, E. F. *et al.* UCSF Chimera--a visualization system for exploratory research and analysis. *J. Comput. Chem.* **25**, 1605-1612, doi:10.1002/jcc.20084 (2004).

21 Emsley, P., Lohkamp, B., Scott, W. G. & Cowtan, K. Features and development of Coot. *Acta Crystallogr. D. Biol. Crystallogr.* **66**, 486-501, doi:10.1107/S0907444910007493 (2010).

22 Adams, P. D. *et al.* Ensemble refinement, CABLAM. *Computational Crystallogr. Newsletter* **4**, 28-32 (2013).

23 Heymann, J. B. & Belnap, D. M. Bsoft: image processing and molecular modeling for electron microscopy. *J. Struct. Biol.* **157**, 3-18, doi:10.1016/j.jsb.2006.06.006 (2007).

Extended data figures and tables
https://www.nature.com/articles/s41586-018-0779-6#Sec18