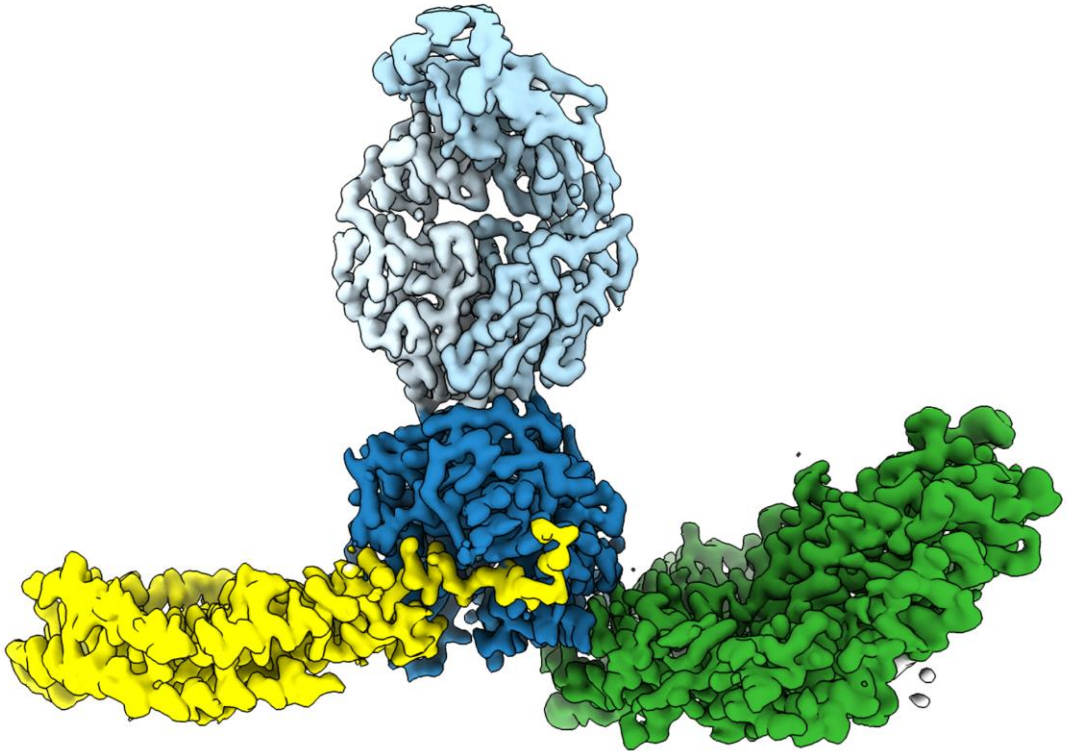
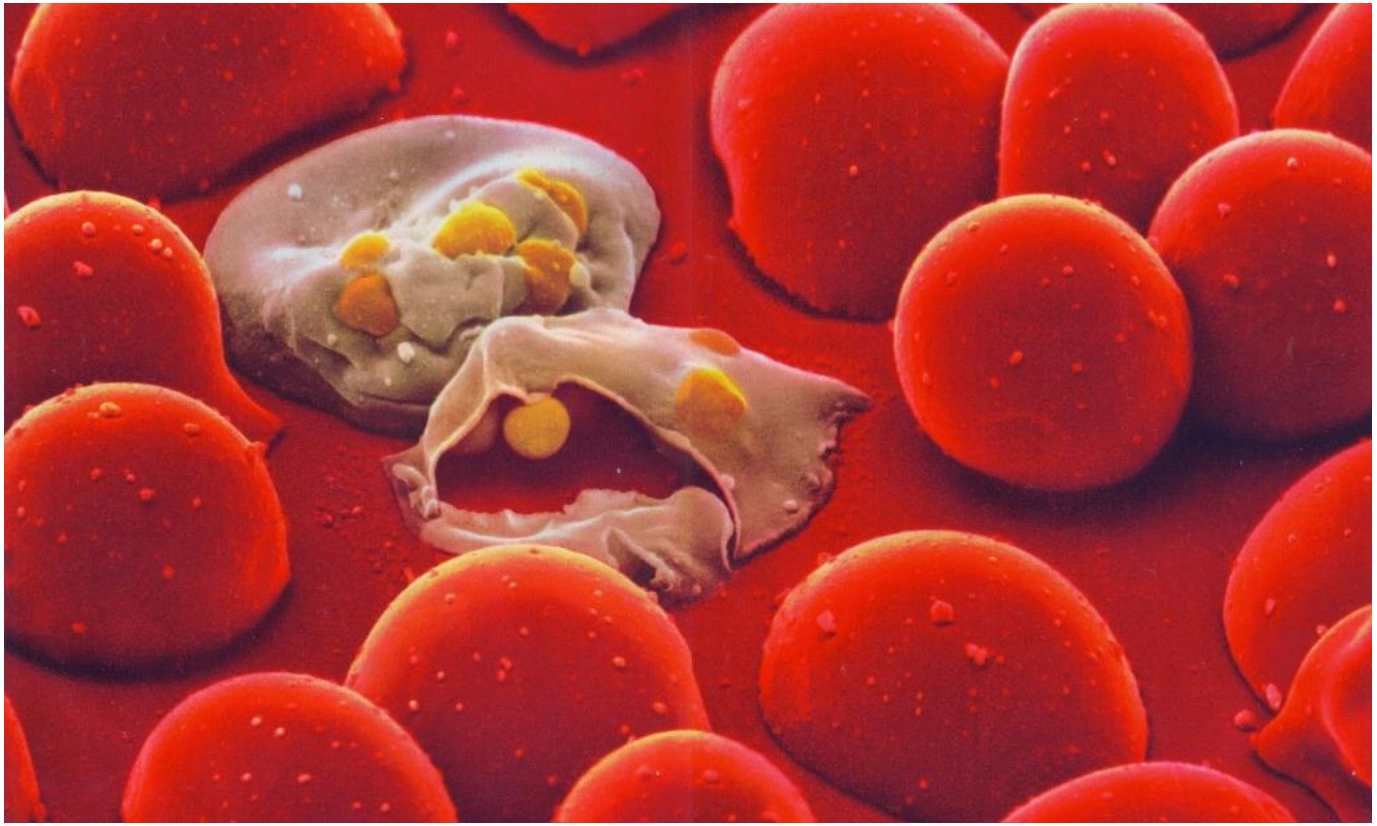
Structure-guided approaches to malaria vaccine design

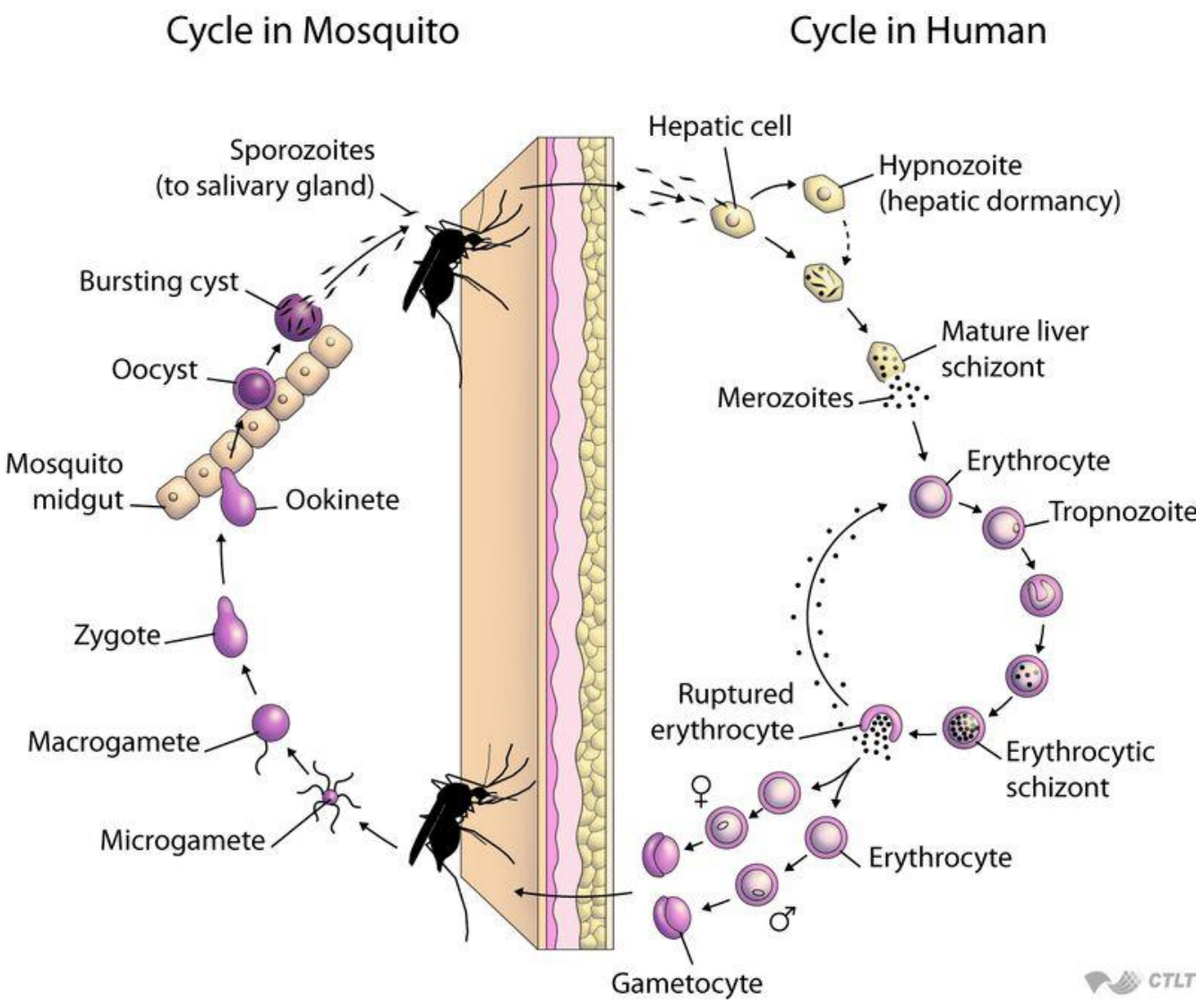


Malaria parasites



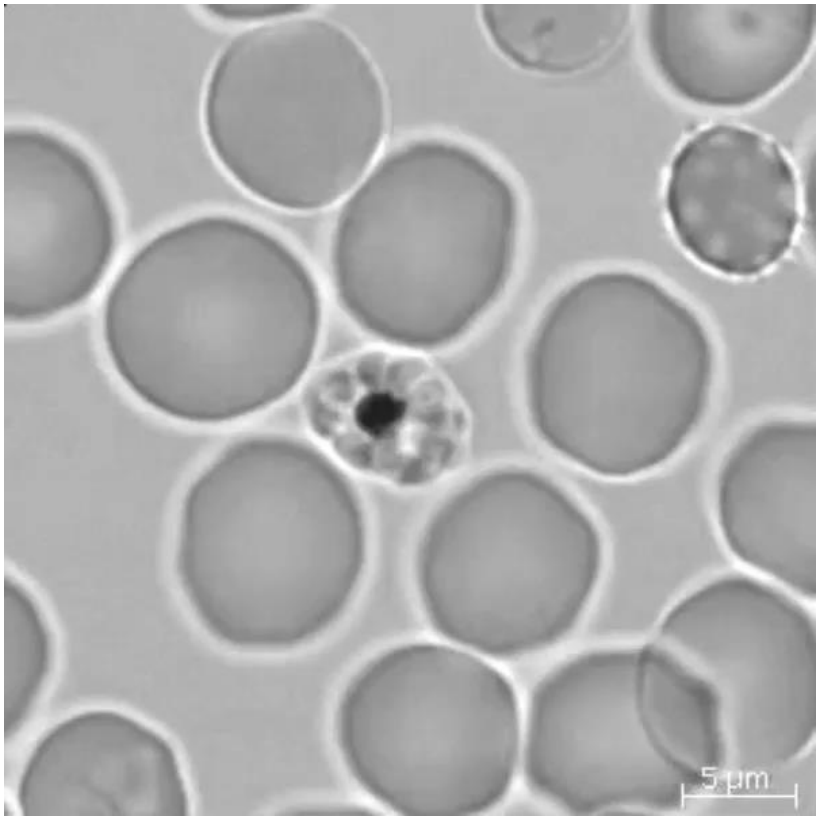
In 2021, malaria led to ~620,000 deaths and ~250 million cases. 77% of deaths were children <5 years old.

Sites of vulnerability in the malaria life-cycle



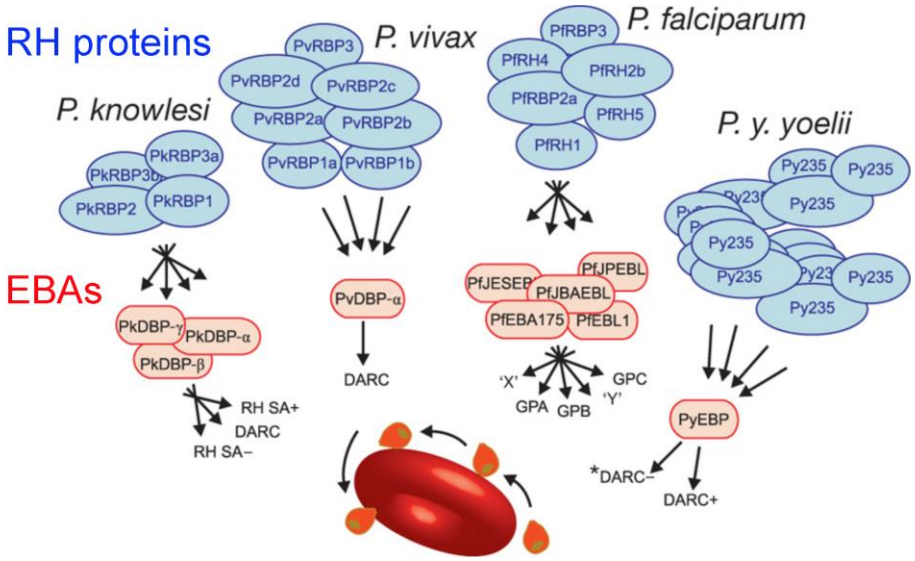
Red blood cell invasion

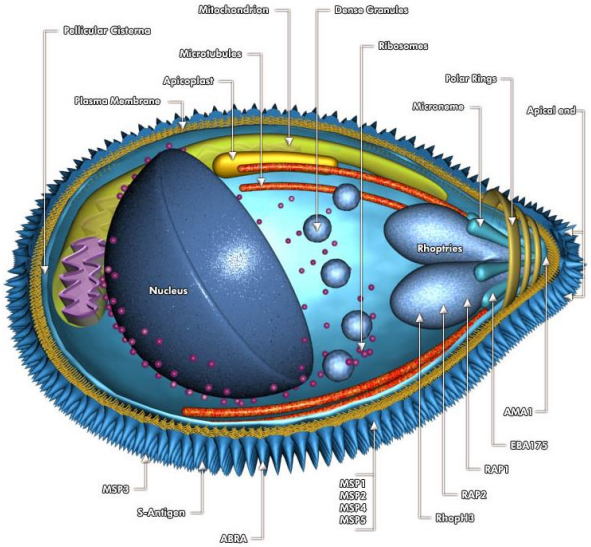
A rapid invasion process



Parasites take only ~20 seconds to invade.

A redundant invasion machinery deployed only when required



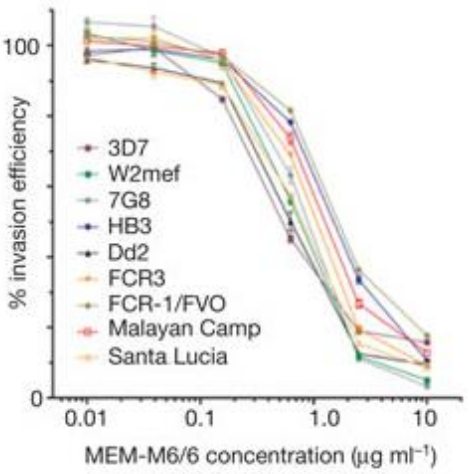


Invasion is mediated by a redundant set of host-parasite interactions and the machinery is held within the apical organelles of the parasite, deployed when needed.

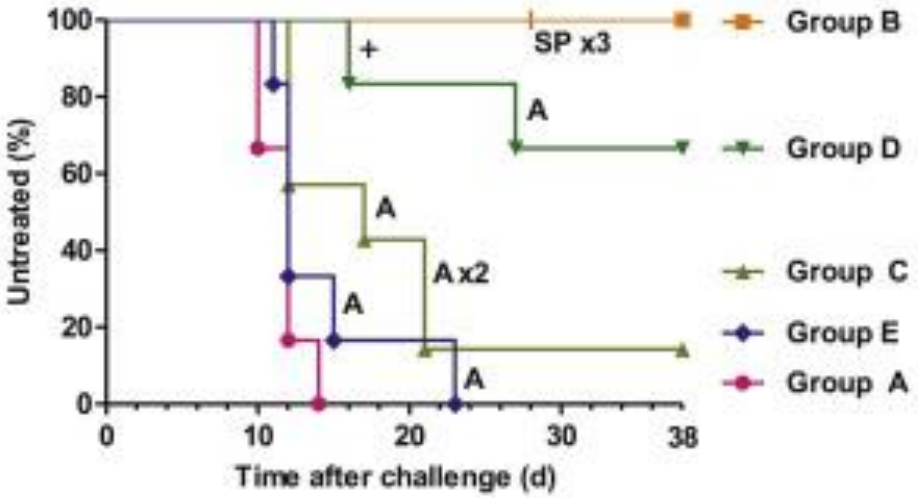
Why is it hard to target blood stage malaria with a vaccine?

- A redundant system of polymorphic invasion proteins allows switching to a different pathway when one pathway is blocked by antibodies.
- The process of invasion is fast. Parasites enter a new erythrocyte within ~20 seconds of emerging from the previous cell.
- Invasion proteins are held within organelles, away from immune detection, until they are required. Their exposure is transient.
- High concentrations of inhibitory antibodies are required for protection….aim for antibody quality as well as quantity.

PfRH5 brings hope



Crosnier et al, 2011

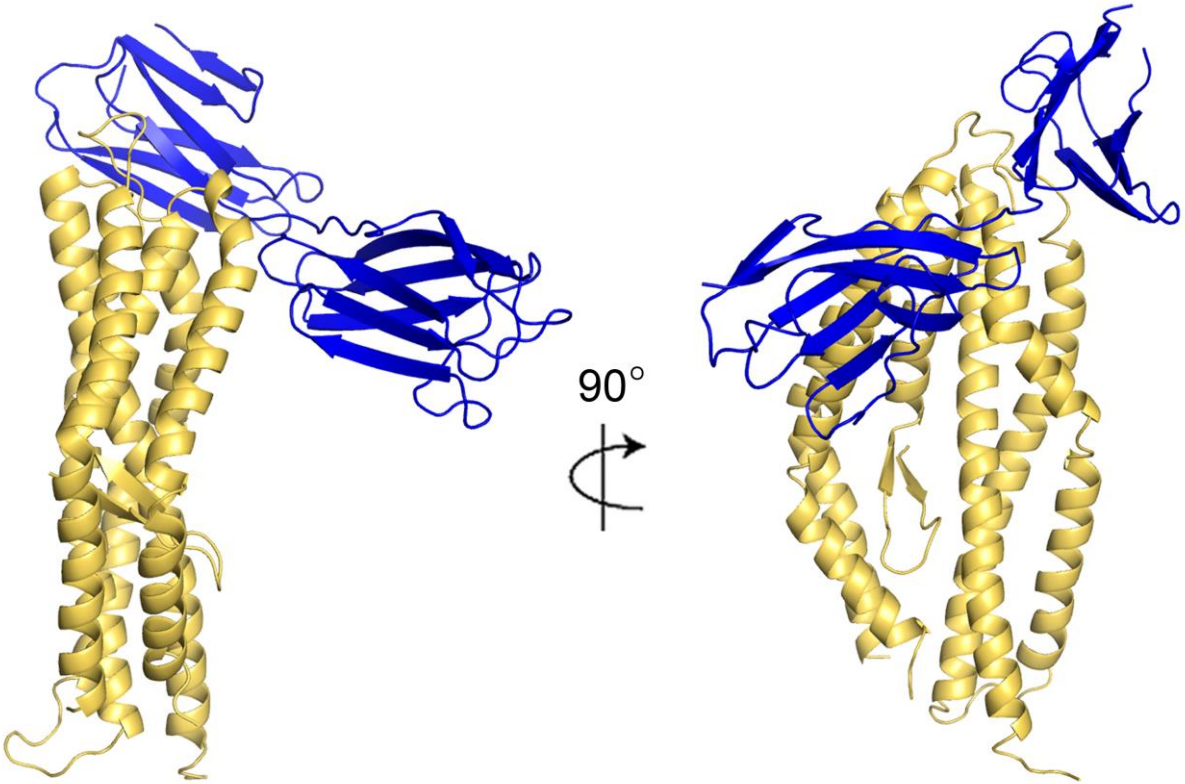


Douglas et al, 2015

- PfRH5 is essential for invasion by all tested strains.
- It binds to basigin on the erythrocyte surface.
- Immunization of aotus monkeys is protective.

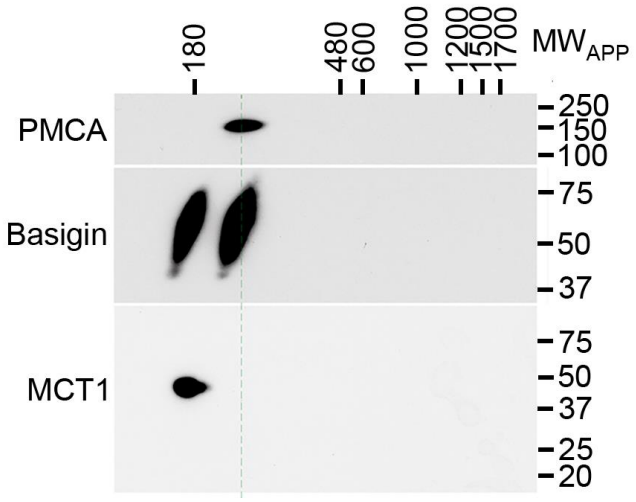
What do we know about the structure and function of PfRH5 and its binding partners?

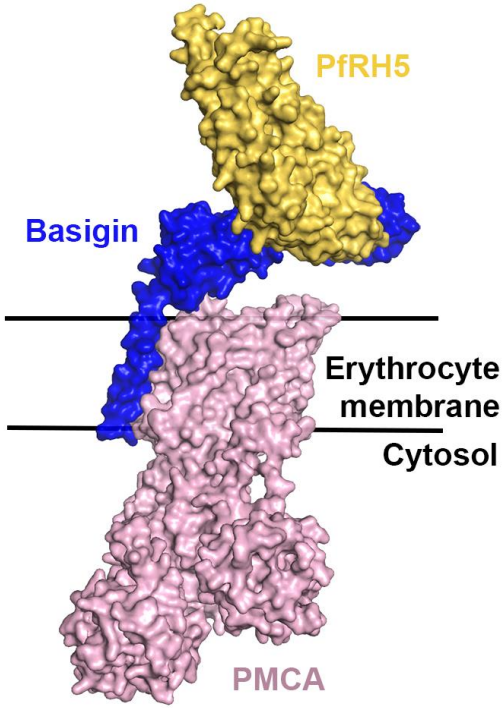
How does PfRH5 bind to basigin?

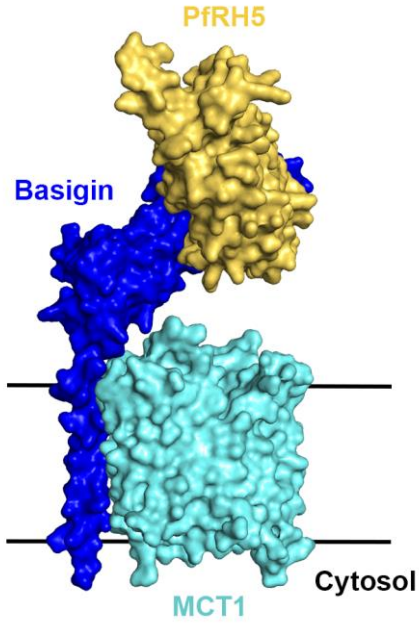


Wright et al (2014) Nature

Basigin forms complexes with membrane transporters PMCAs and MCT1







Jamwal et al (2022) bioRXIV

PfRH5 functions as part of a five-component complex, PfPCRCR

nature microbiology

Article

https://doi.org/10.1038/s41564-022-01261-2

PCRCR complex is essential for invasion of human erythrocytes by *Plasmodium falciparum*

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Check for updates

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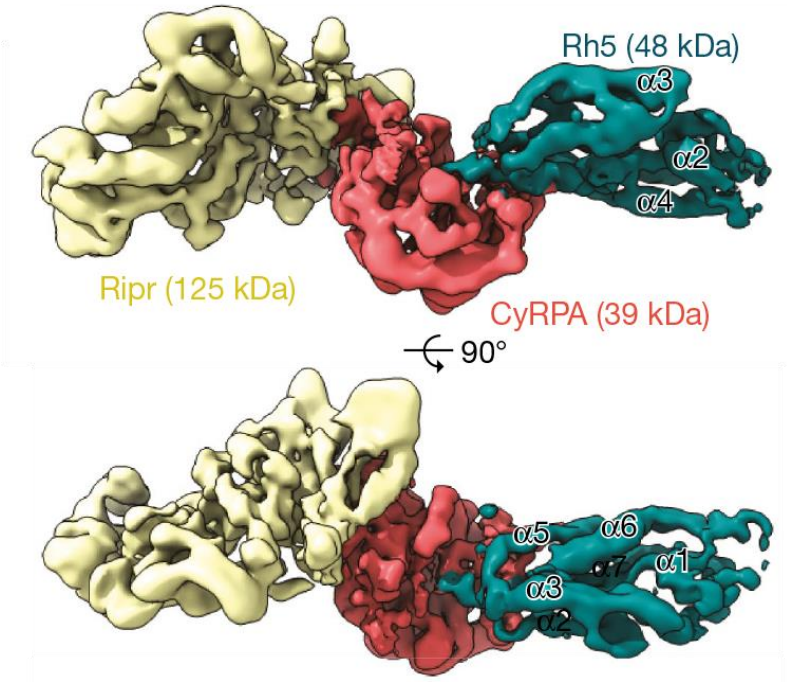
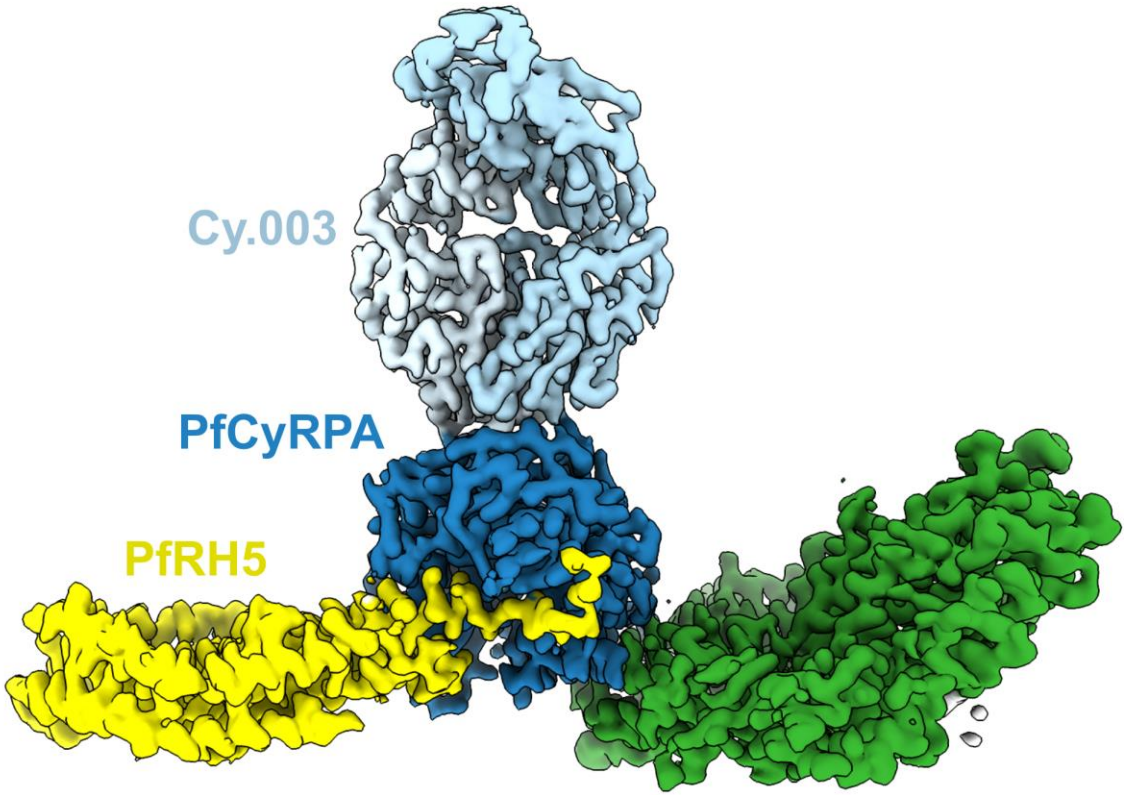
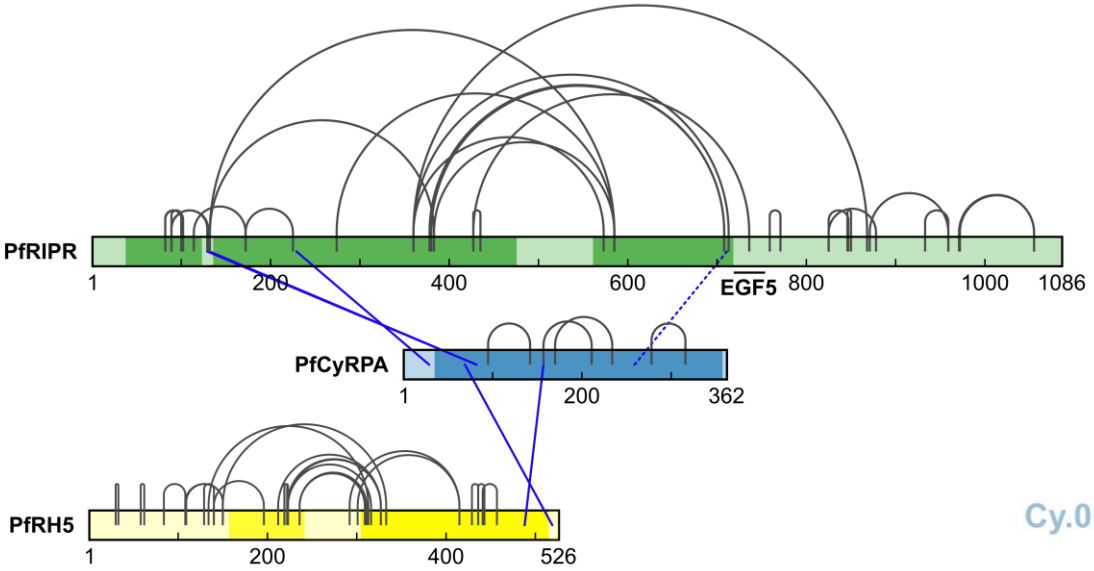


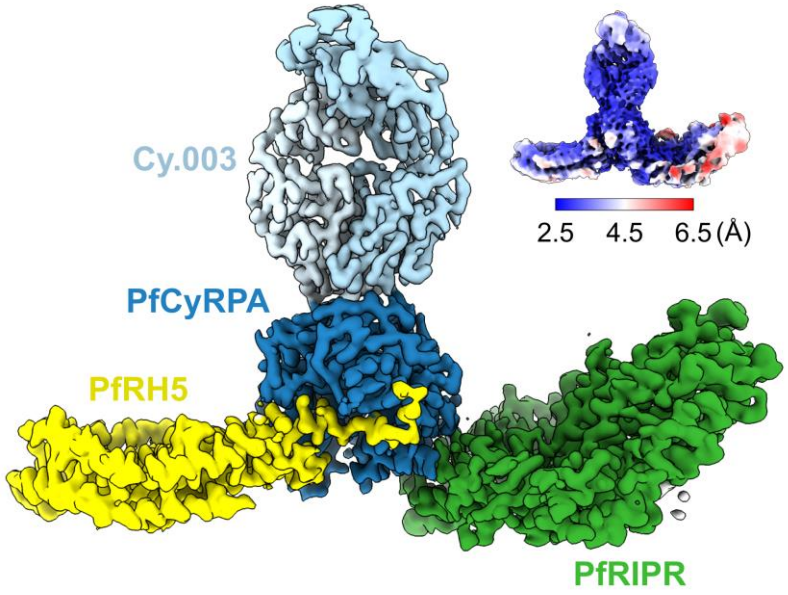
The structure of the PfRCR complex



Farrell et al (2023) BioRXIV

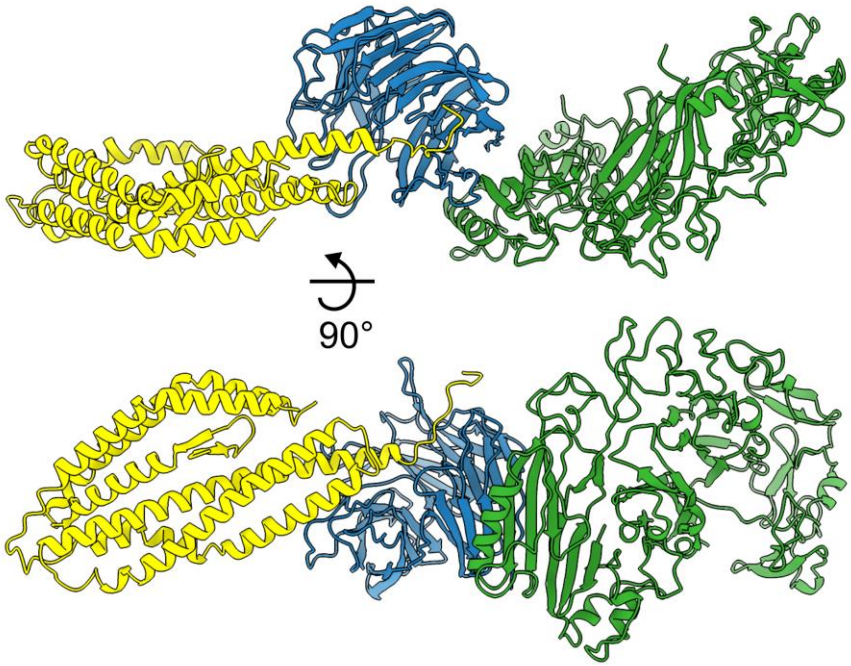
Structure-guided building by AlphaFold and cross-linking mass spectrometry

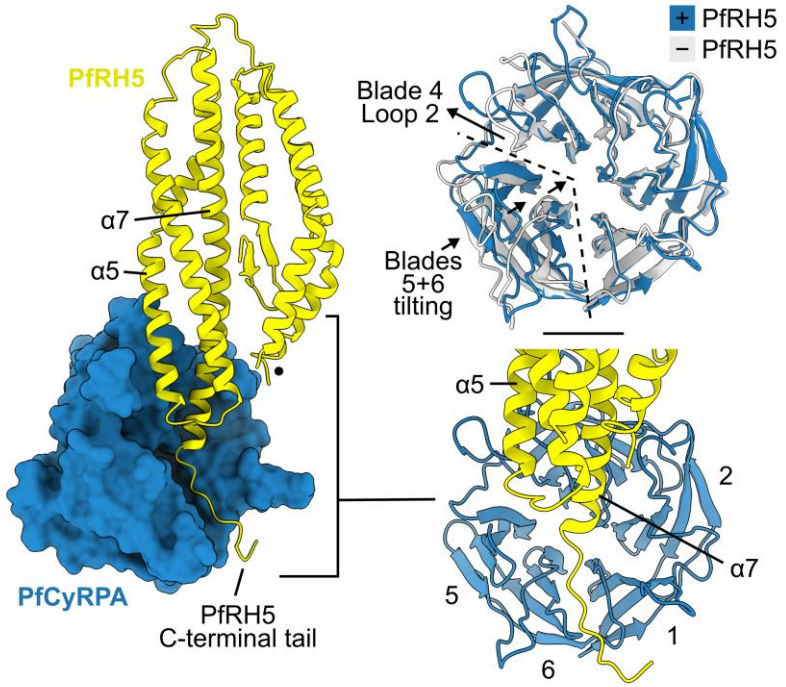




Farrell et al (2023) BioRXIV

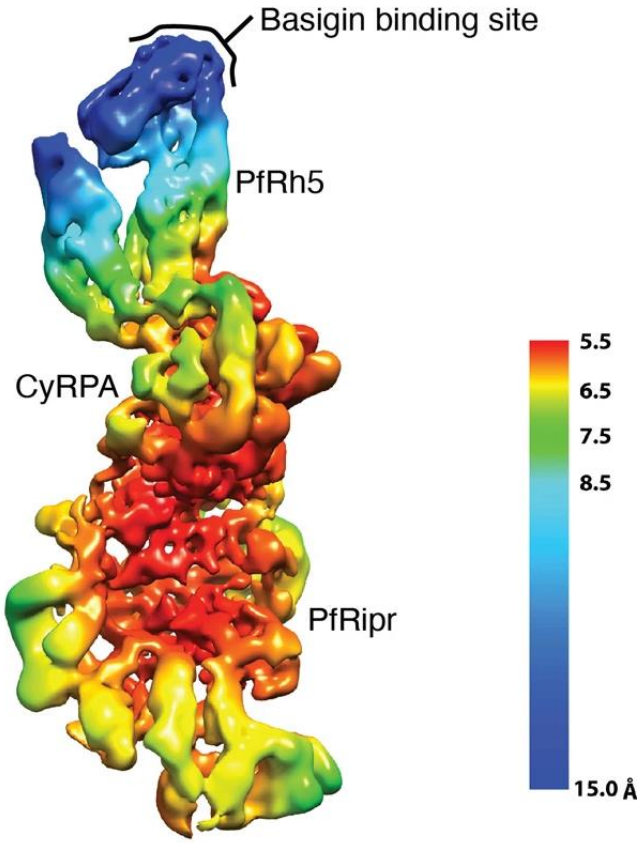
The structure of the PfRCR complex

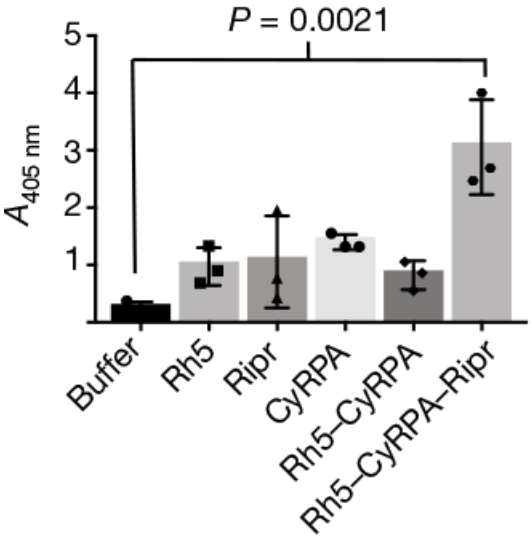


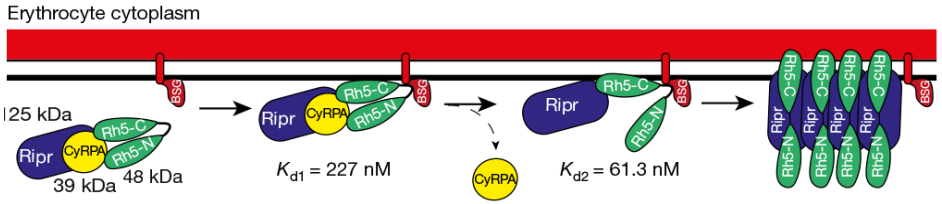


Farrell et al (2023) BioRXIV

Does PfRH5 form a pore?

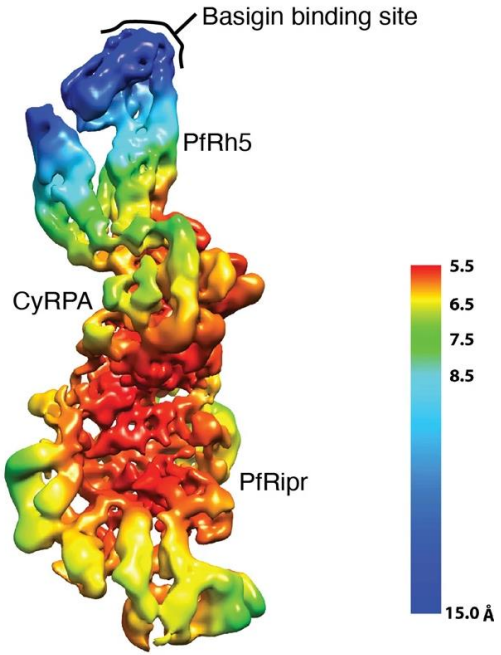


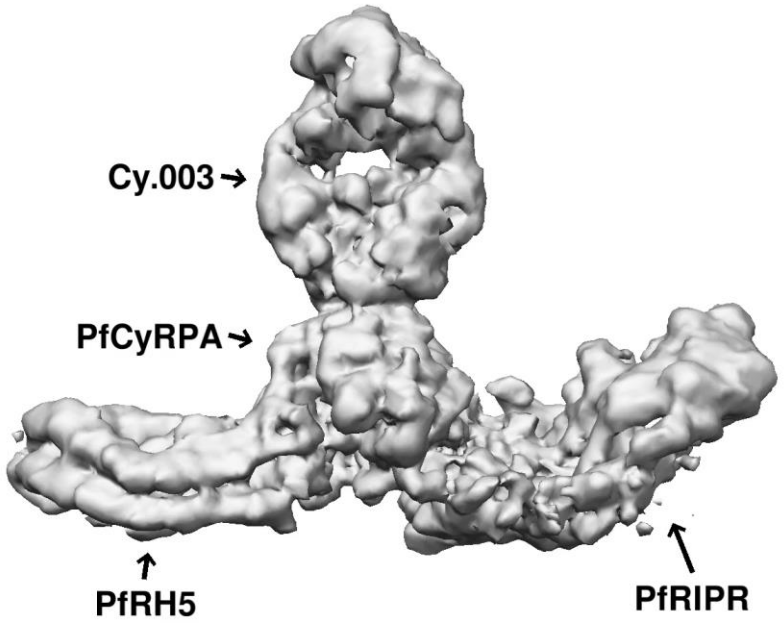




Wong et al (2019) Nature

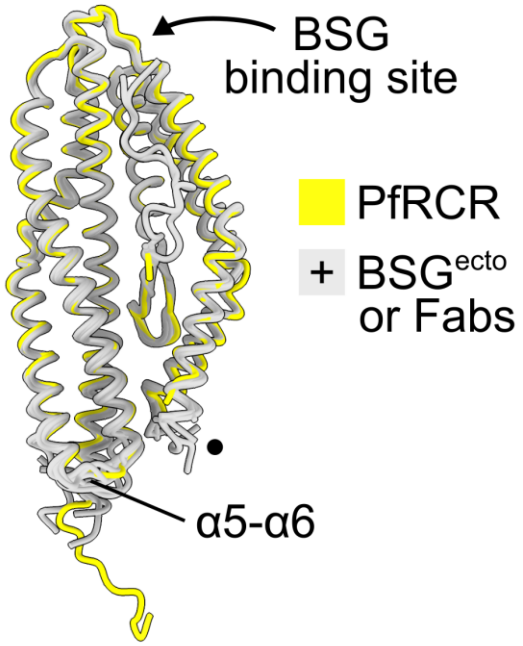
A dynamic protein complex will appear low resolution towards the ends if not corrected

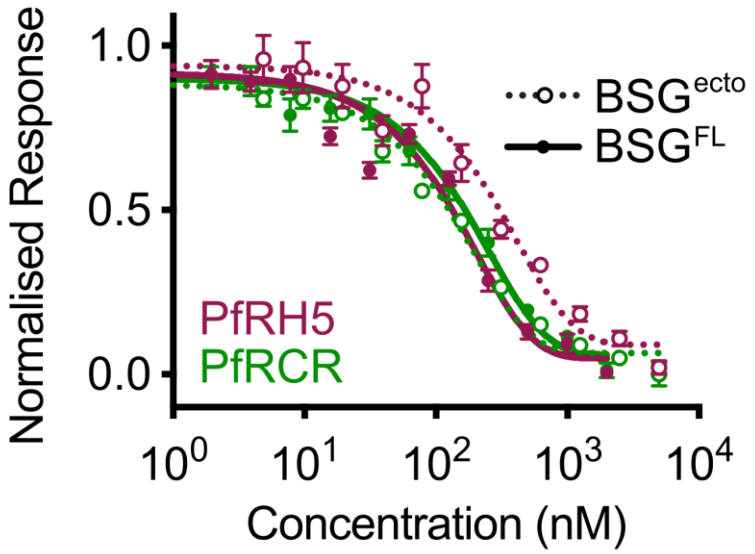




Farrell et al (2023) BioRXIV

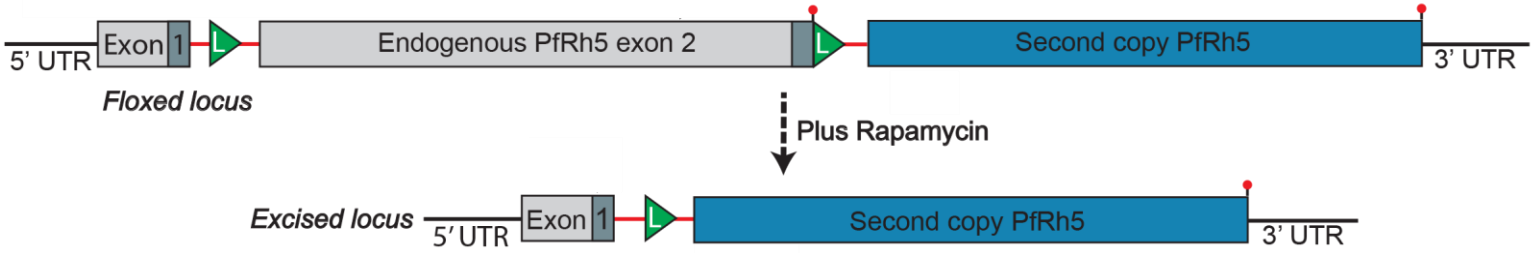
PfRH5 in PfRCR is unchanged in conformation and still binds basigin

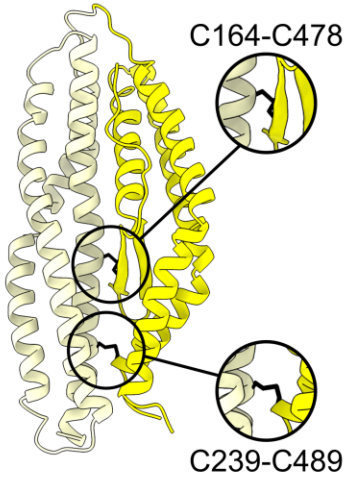


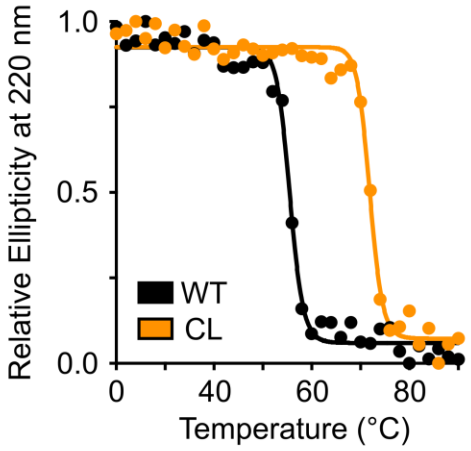


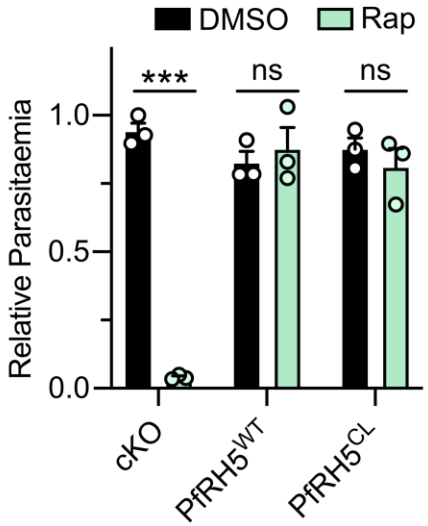
Farrell et al (2023) BioRXIV

Cys-locked PfRH5 can mediate normal invasion efficiency



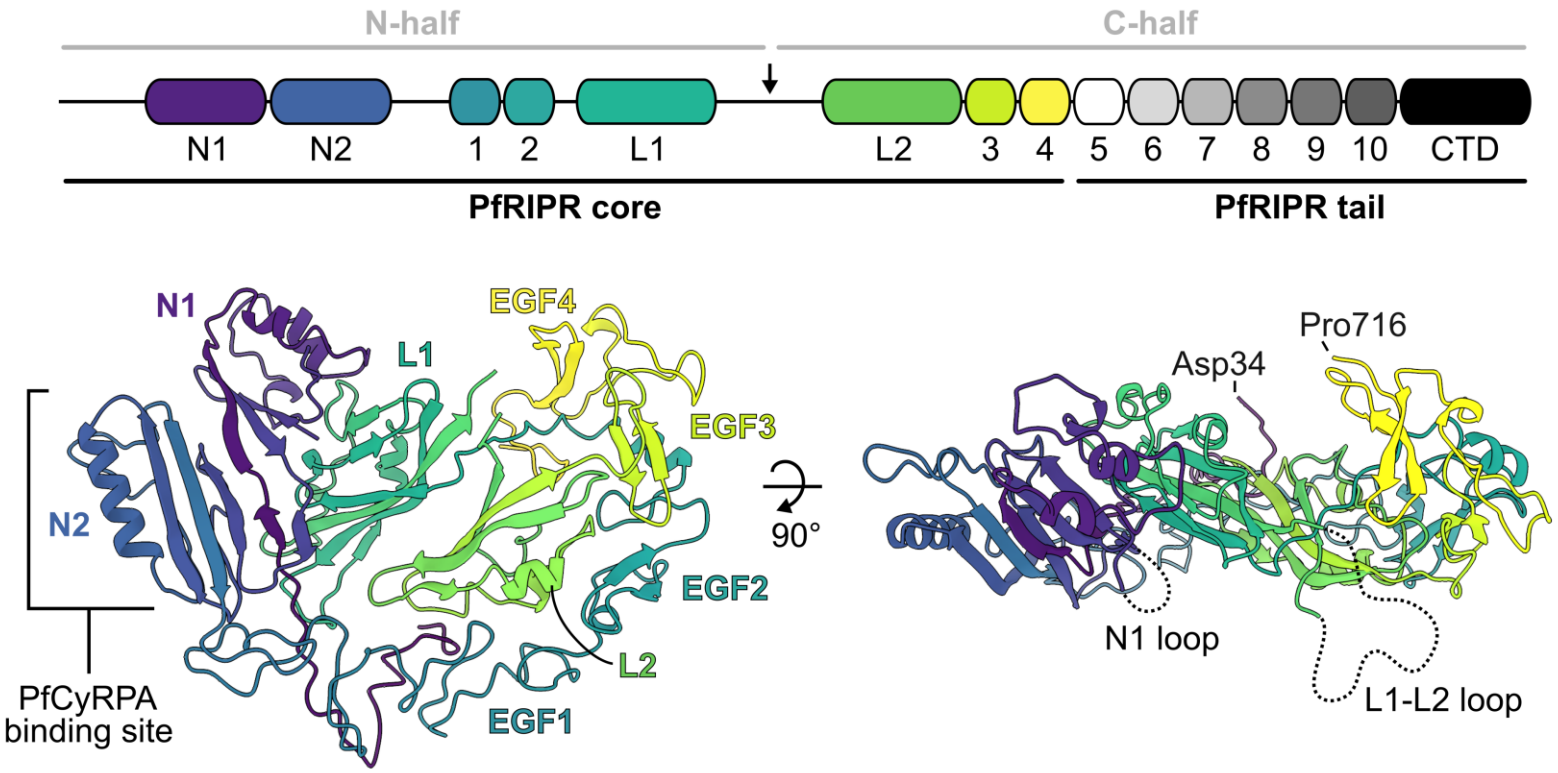






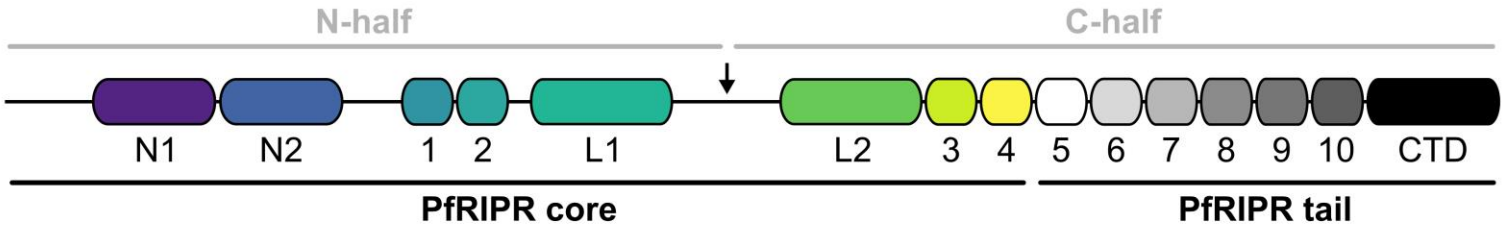
Farrell et al (2023) BioRXIV

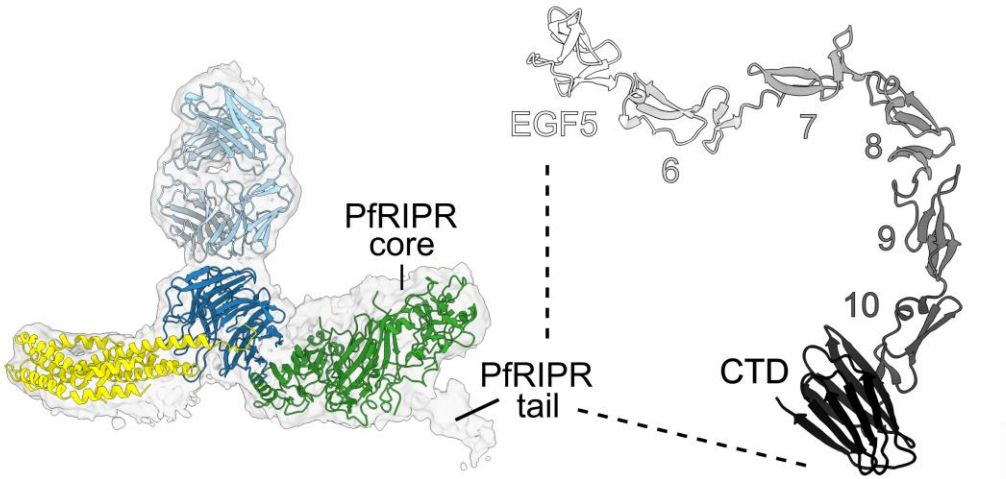
Revealing the structure of PfRIPR



Farrell et al (2023) BioRXIV

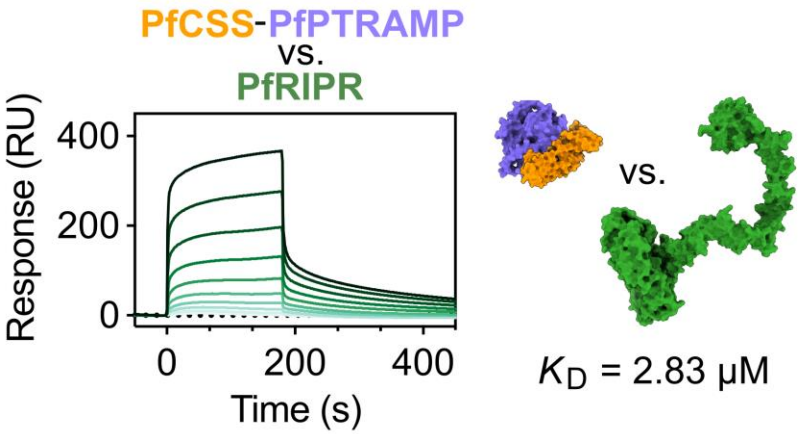
Revealing the structure of PfRIPR

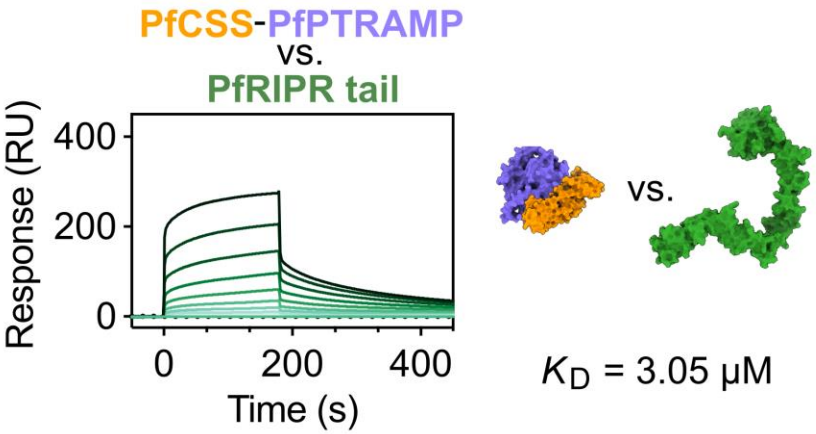




Farrell et al (2023) BioRXIV

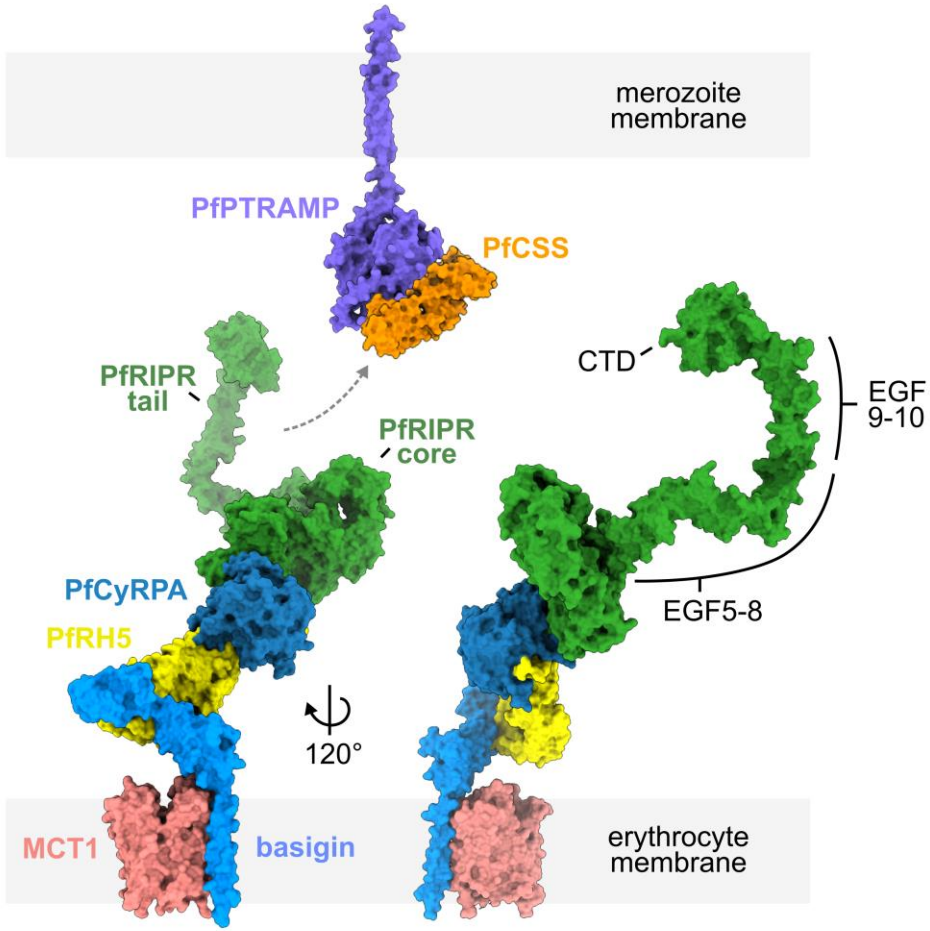
The PfRIPR tail binds to PfCSS and PfPTRAMP





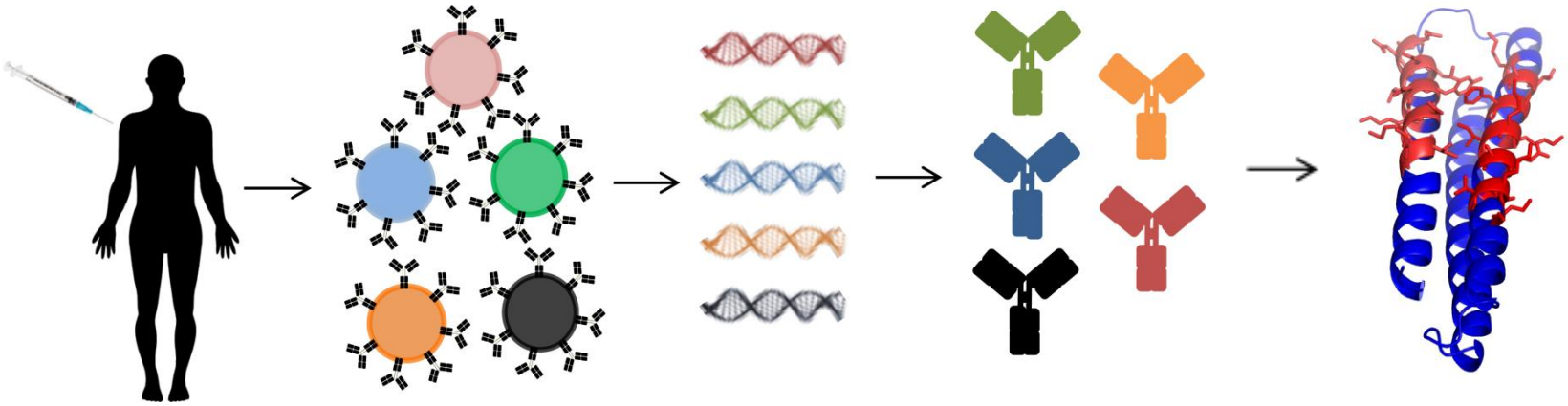
Farrell et al (2023) BioRXIV

PfPCRCR bridges parasite and erythrocytes membranes



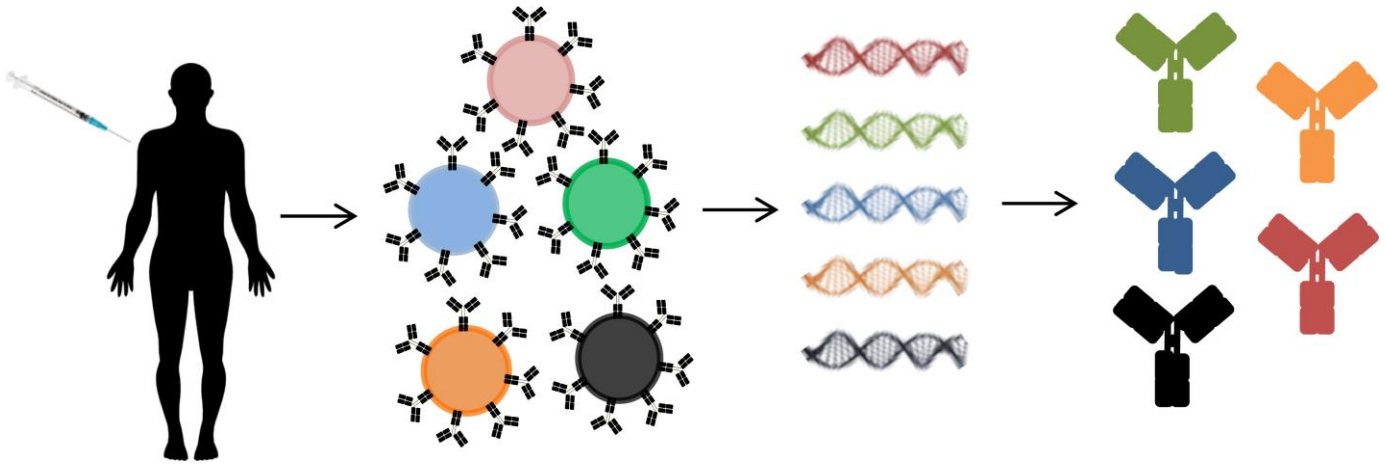
Farrell et al (2023) BioRXIV

Applying structural vaccinology to malaria



How do the most effective PfRCR-targeting antibodies work?

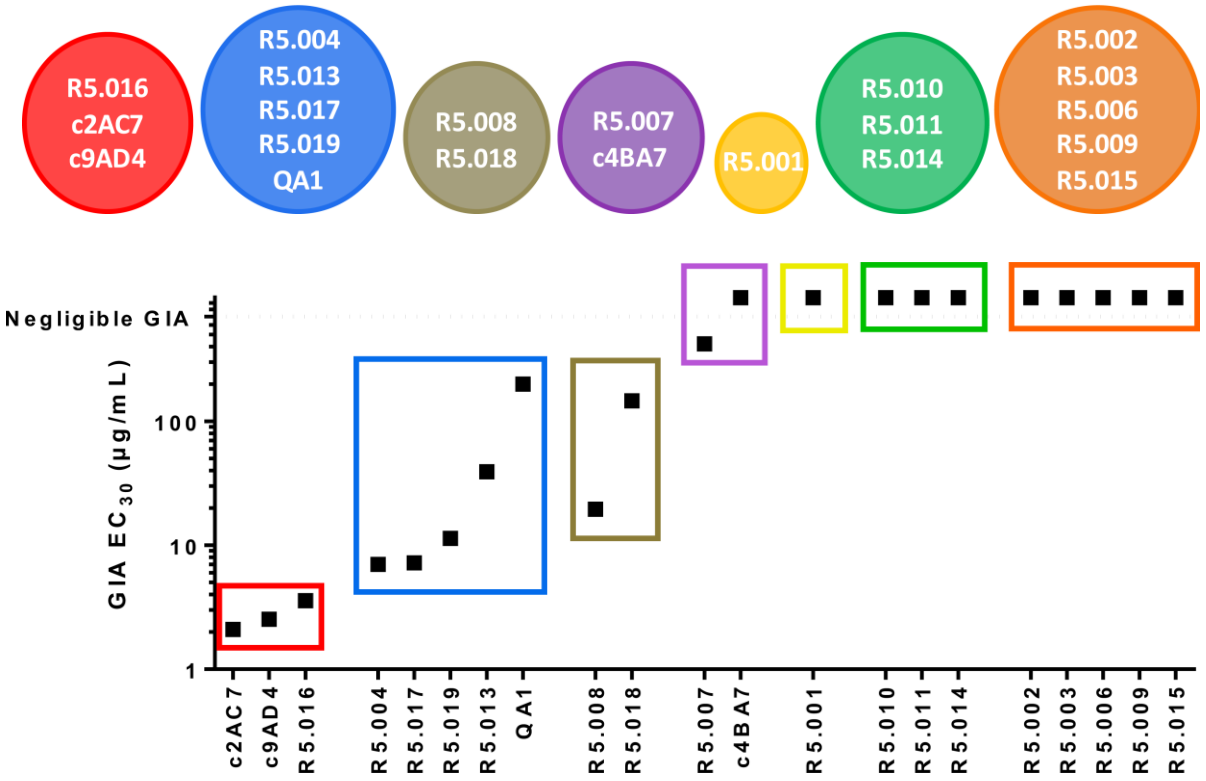
Understanding the human antibody response to PfRH5 vaccination



Human volunteers were immunized with PfRH5 as part of a vaccination trial in Oxford. After single B cell sorting, human mAbs were cloned and tested.

Alanine et al (2019) Cell

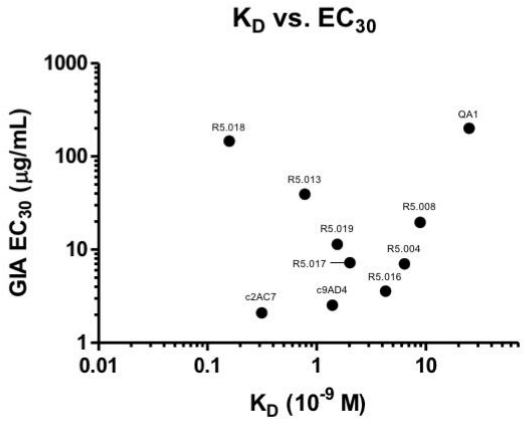
Three epitope bins for neutralizing antibodies



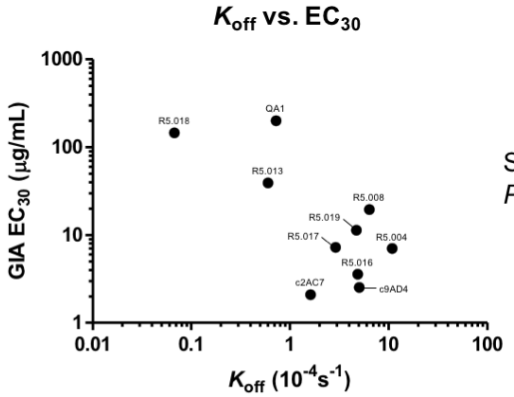
A panel of mAbs were classified into groups and tested for their ability to prevent erythrocyte invasion.

Alanine et al (2019) Cell

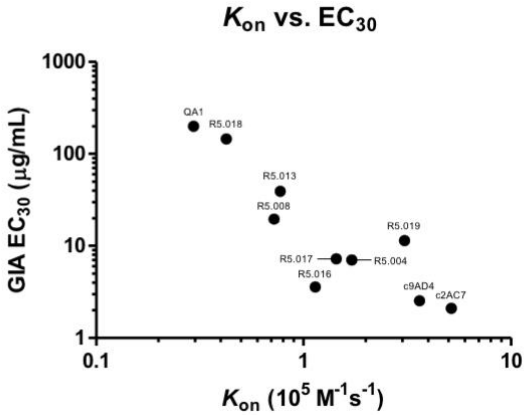
The importance of on-rate



Spearman ρ = 0.20
P-value: 0.583



Spearman ρ = -0.515
P-value: 0.133

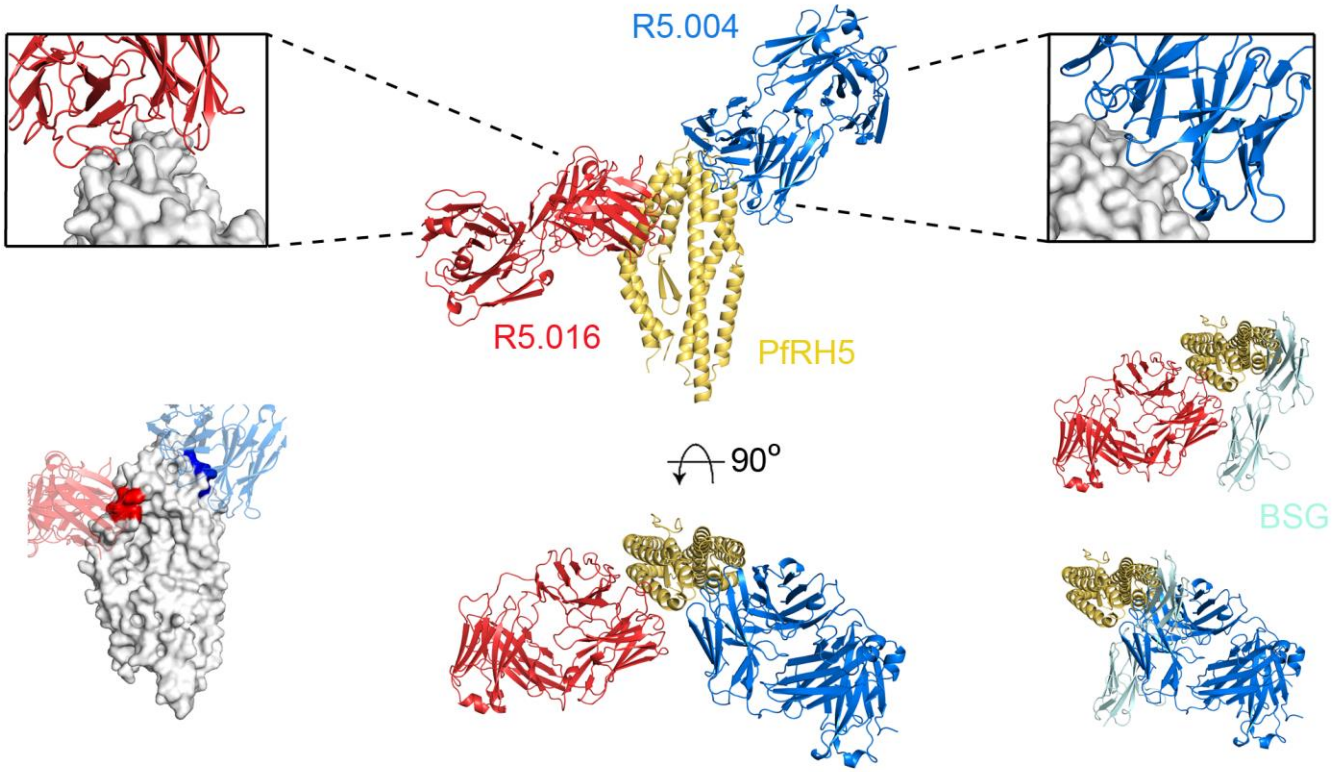


Spearman ρ = -0.88
P-value: 0.001

On-rate is the best predictor of efficacy for neutralising antibodies.

Alanine et al (2019) Cell

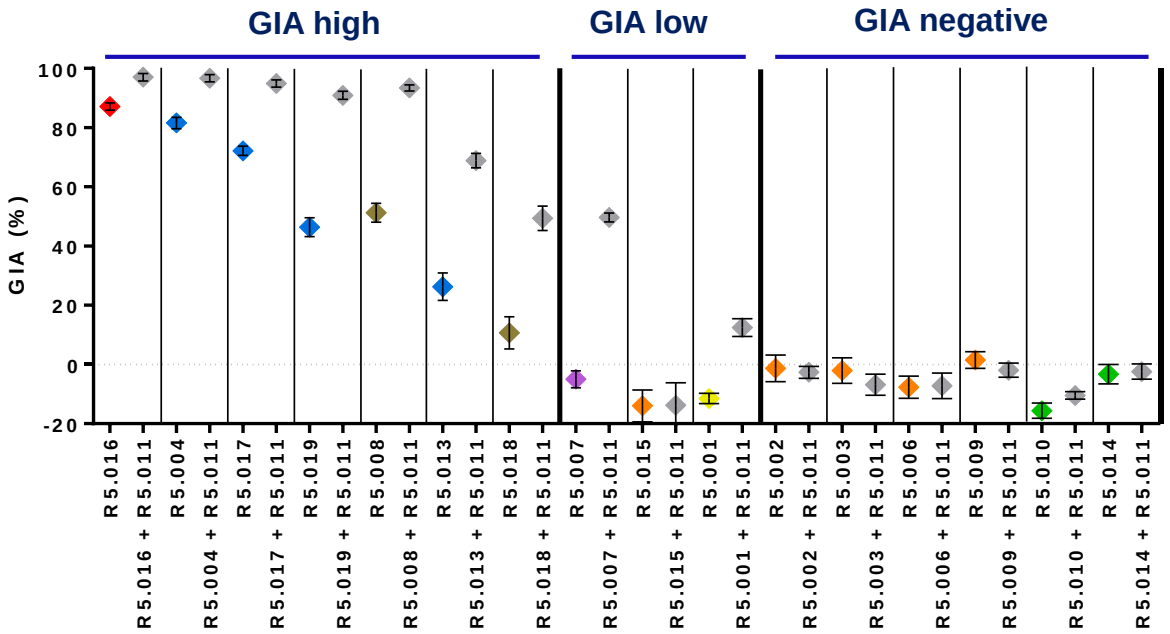
Epitopes for inhibitory mAbs



The two major groups of inhibitory mAbs have epitopes within the top half of PfRH5.

Alanine et al (2019) Cell

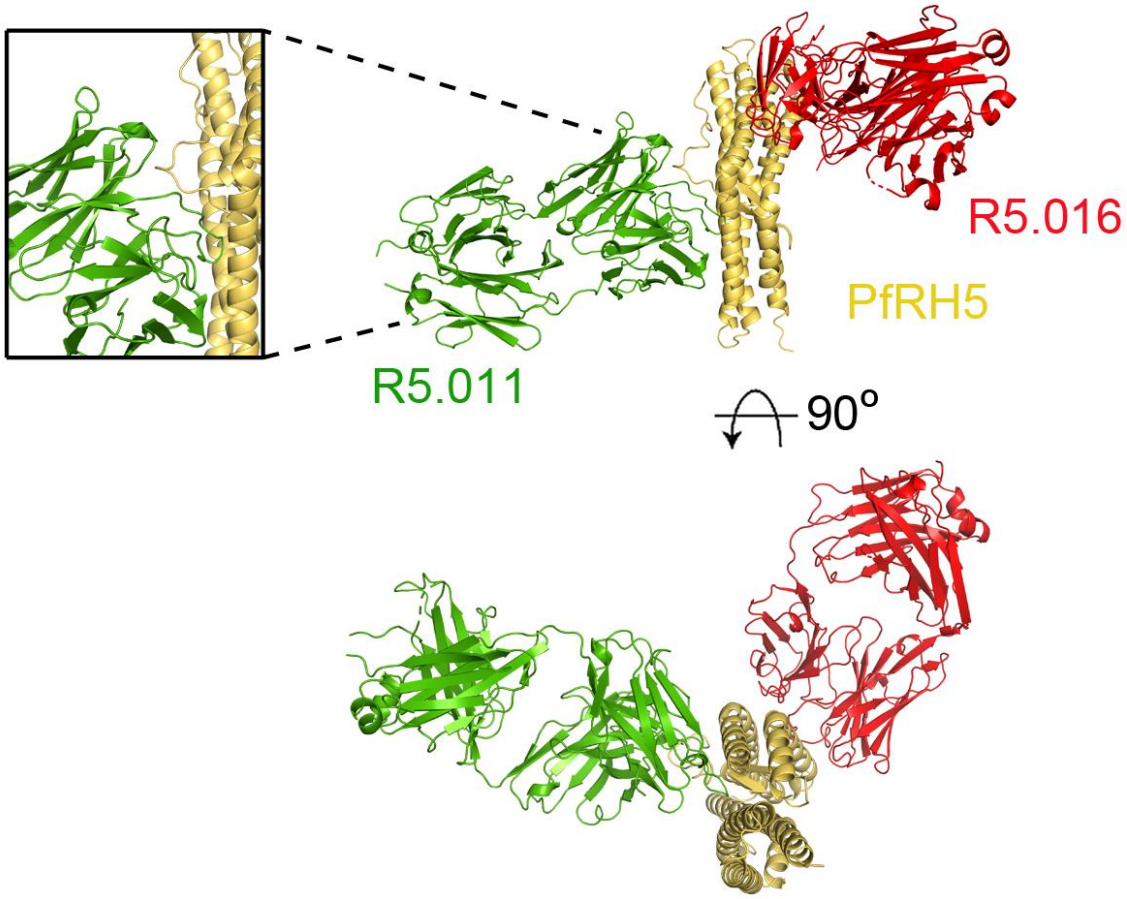
Non-neutralising mAbs can synergise with neutralizing antibodies



R5.011 has no detectable inhibitory activity but potentiates the activity of all neutralising mAbs.

Alanine et al (2019) Cell

How do potentiating mAbs work?

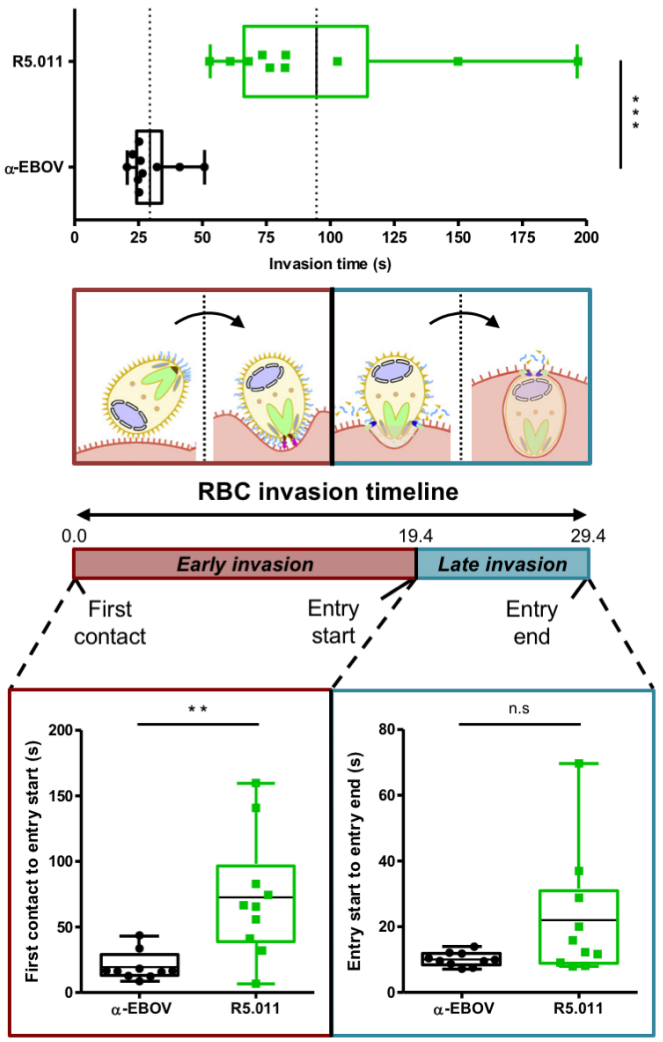


Alanine et al (2019) Cell

Potentiating mAbs slow erythrocyte invasion

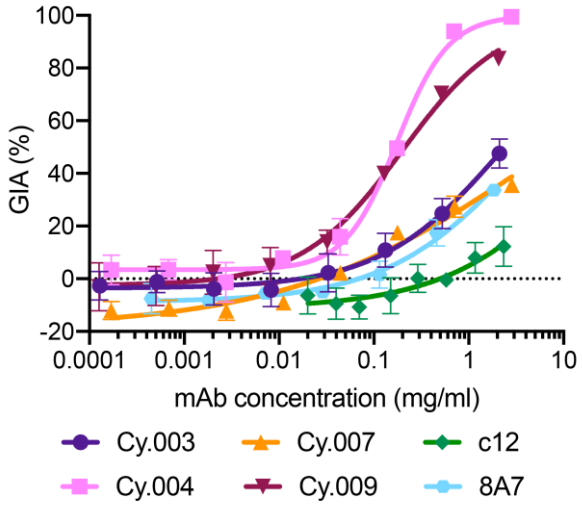
Video microscopy shows that R5.011 increases the time taken for invasion by 3-4 fold.

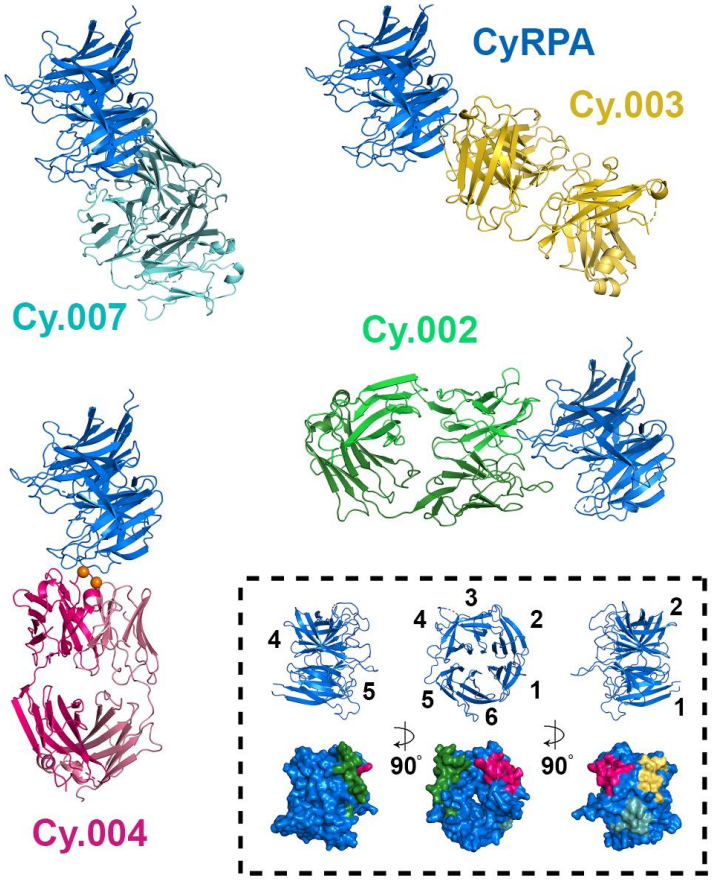
This allows inhibitory mAbs longer to bind.



Alanine et al (2019) Cell

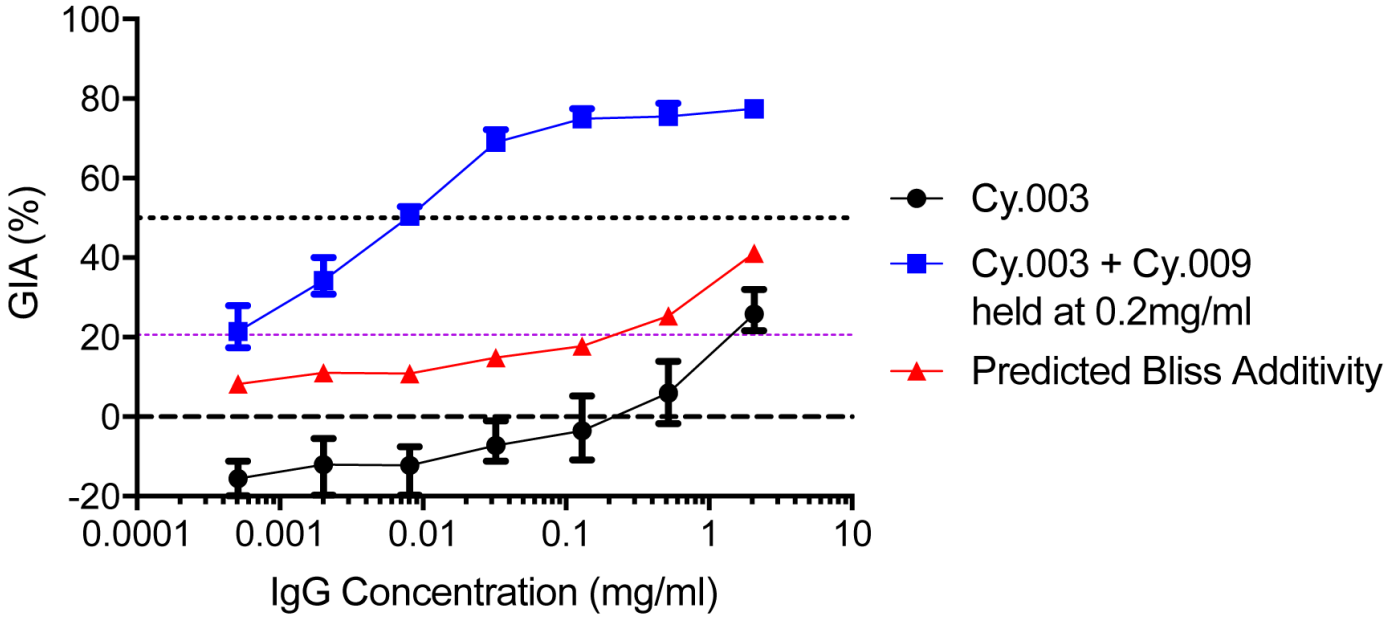
Neutralising antibodies which target PfCyRPA bind to blades 1 and 2 of the β-propeller





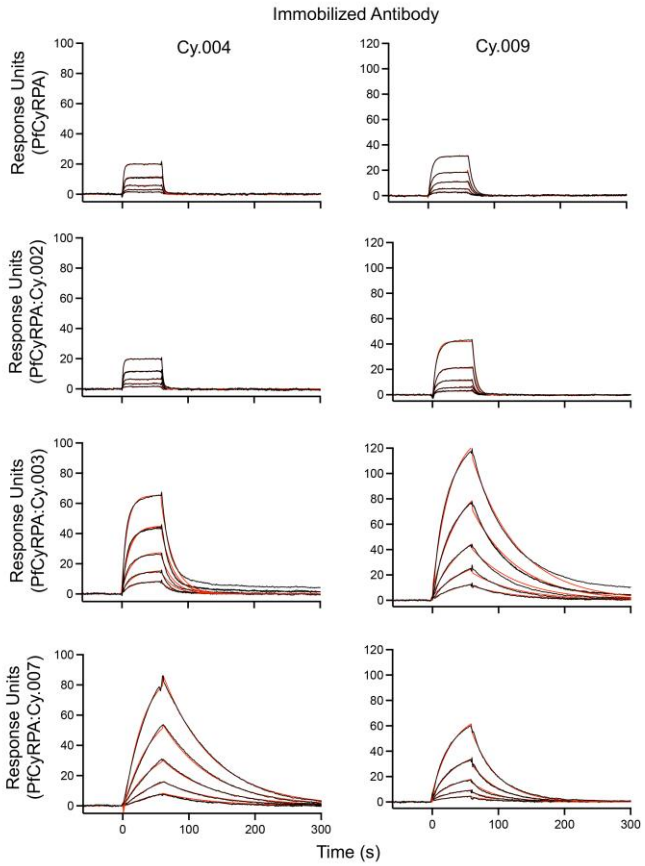
Ragotte et al (2022) Nature Comms

CyRPA-targeting antibodies can also show synergy



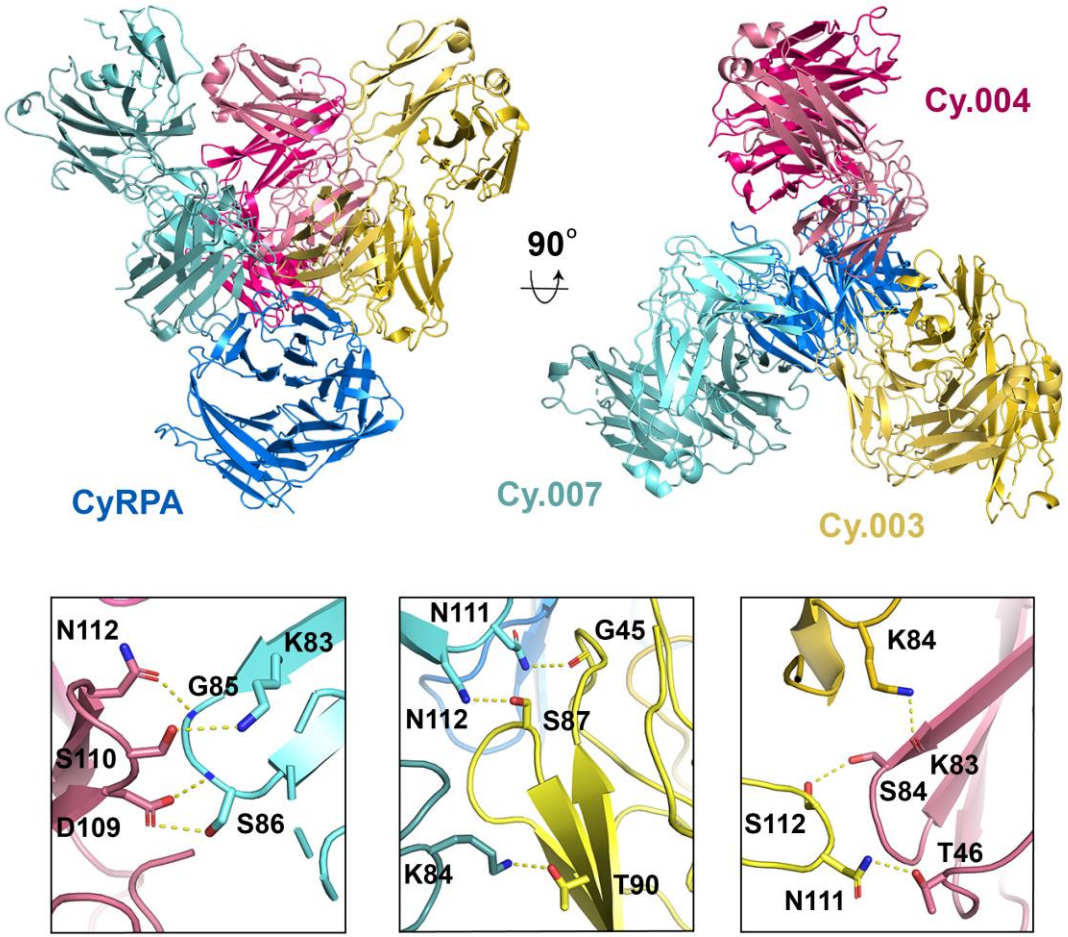
Ragotte et al (2022) Nature Comms

Synergy is associated with improved binding kinetics and affinity



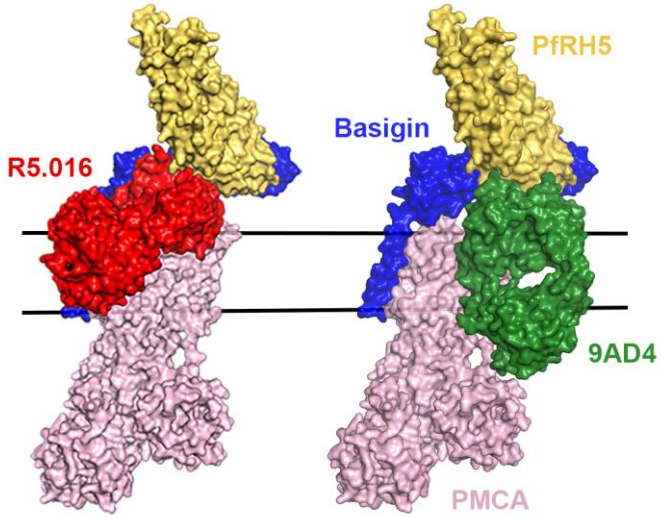
Ragotte et al (2022) Nature Comms

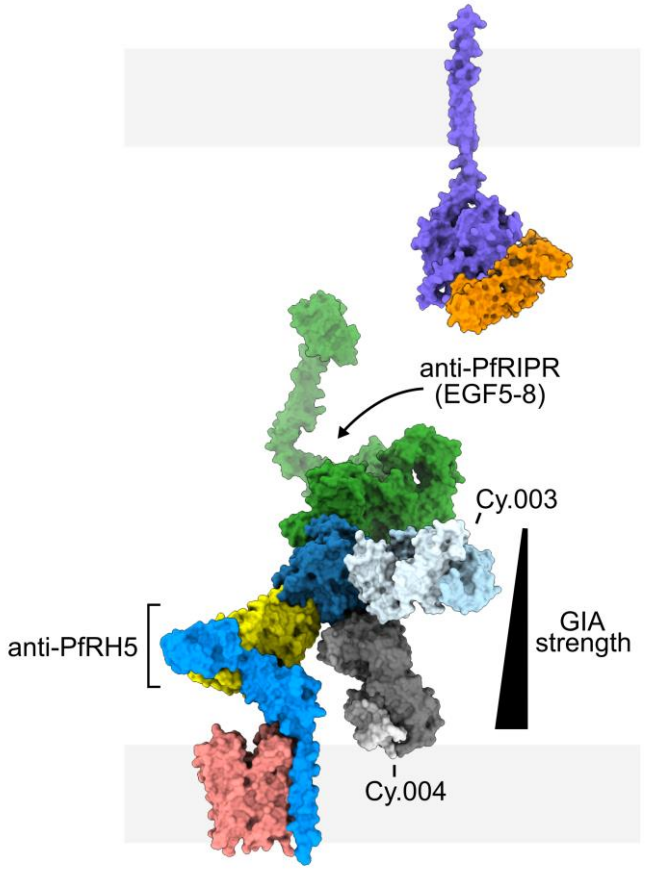
Synergy results from lateral interactions between antibodies



Ragotte et al (2022) Nature Comms

Neutralising antibodies against PfRH5 and PfCyRPA appear to block erythrocyte binding

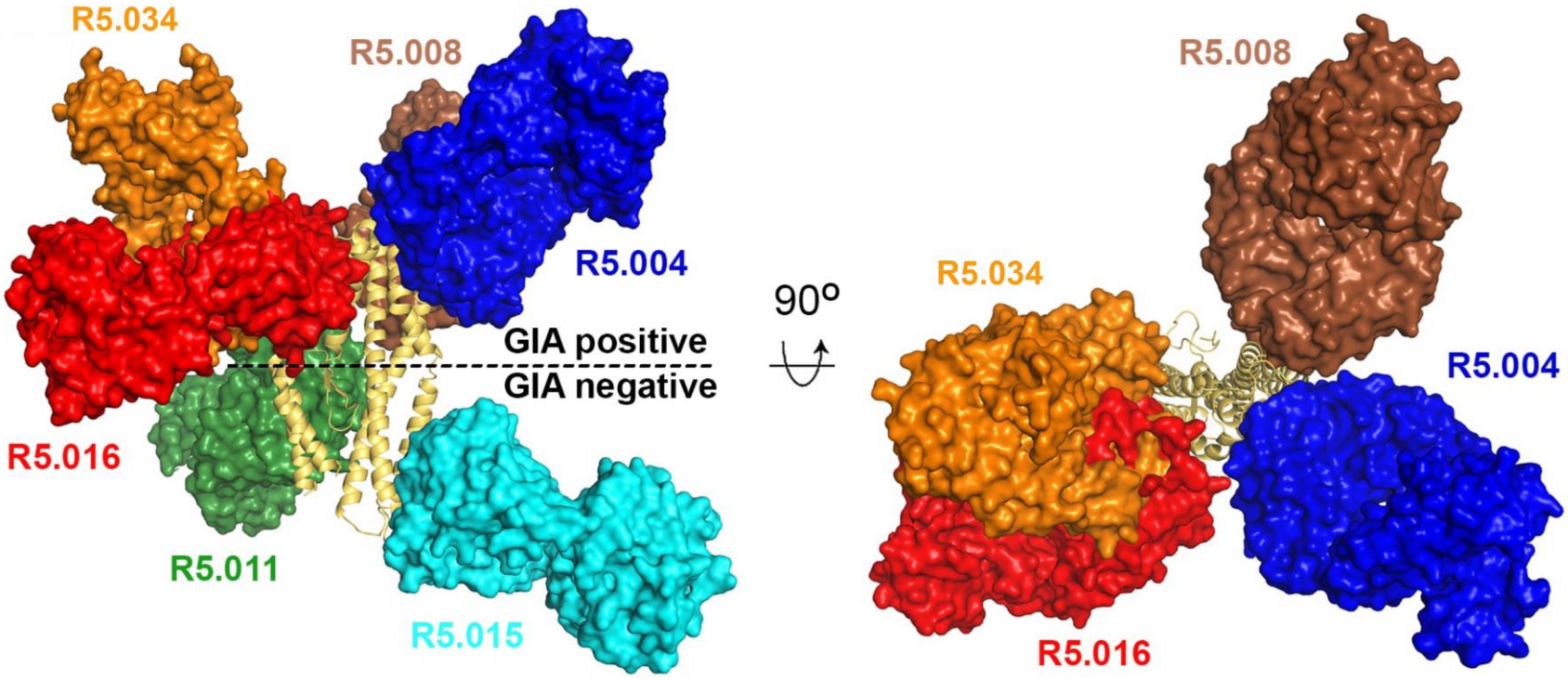




Jamwal et al (2022) BioRXIV, Farrell et al (2023) BioRXIV

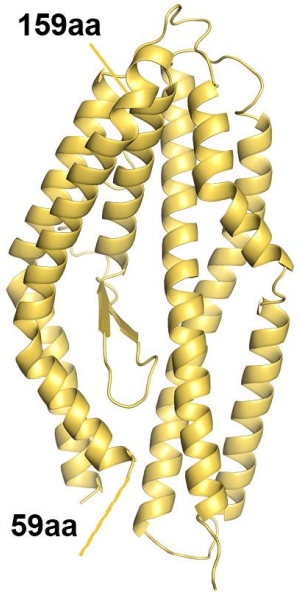
Can we use this information to design improved vaccine candidates?

A reminder of the neutralizing epitopes of PfRH5



Alanine et al (2019) Cell; Ragotte et al (2022) Nature Comms and Farrell et al (2023) BioRXIV

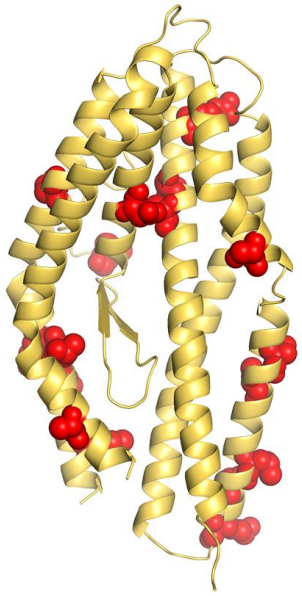
A series of PfRH5-based immunogens



RH5.1

Full-length

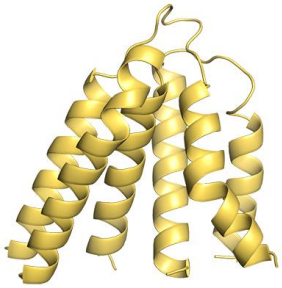
Used in vaccine trials



RH5.2

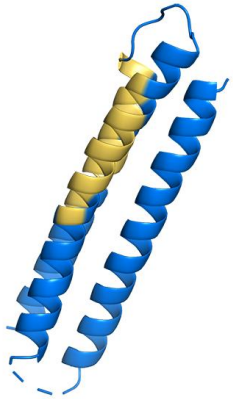
Truncated and thermally stabilised

Campeotto et al (2017)



RH5top

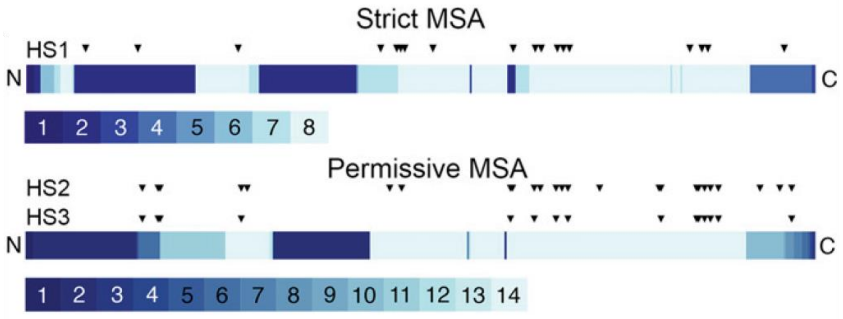
In design and production

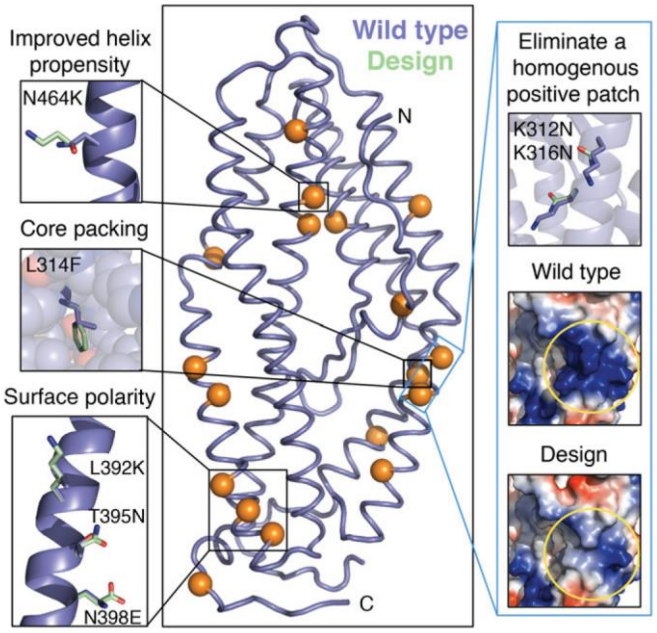


RH5 bundle (B4)

Focused immunogen designed to present single epitope

Structure-guided thermal stabilisation of RH5 to generate RH5.2



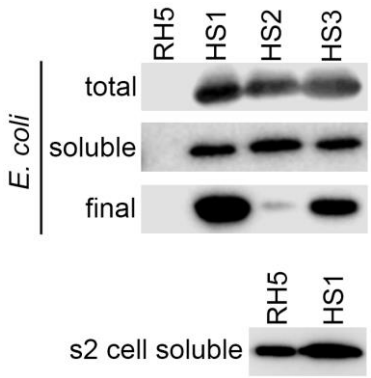


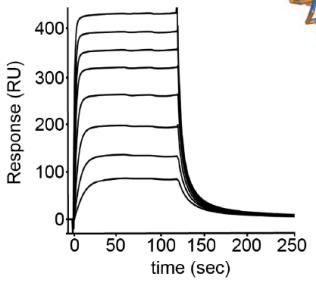
All variable residues in a multiple sequence alignment, minus those which form key epitopes, are allowed to vary.

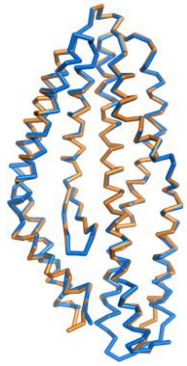
Rosetta energy function is used to score the effect on protein stability, iteratively building more stable sequences.

Campeotto, Goldenzweig et al (2017) PNAS

Structure-guided thermal stabilisation of RH5 to generate RH5.2

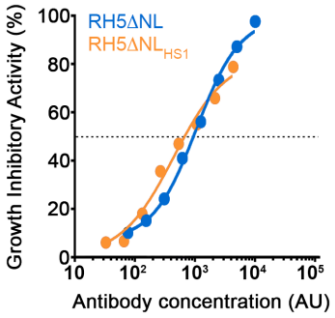


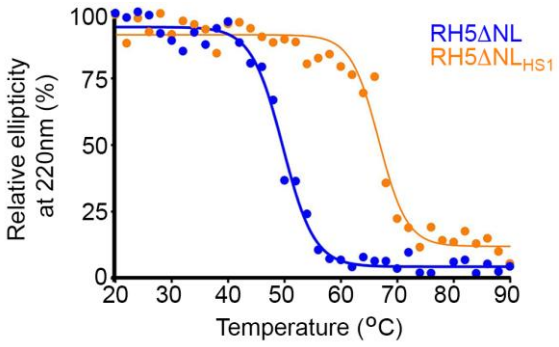




The final design retains functional properties and structure.

It expresses functionally in *E. coli* and has ~15°C improved thermal stability.





Campeotto, Goldenzweig et al (2017) PNAS

Conclusions

- The structure of PfRCR reveals formation of a bridge which links parasite and erythrocyte.

- Structural studies of the most effective PfRH5 and PfCyRPA-targeting neutralizing antibodies reveal the epitopes which should be presented by a vaccine and hint at the complexity.

- Structure-guided design allows us to generate improved vaccine immunogens which elicit much higher quality responses.

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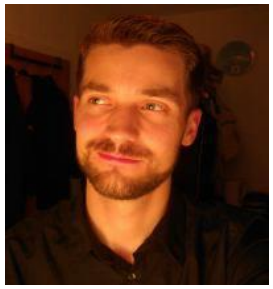
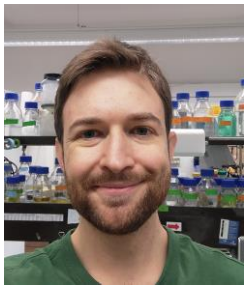


Frank Lennartz

Richard Zhou

Tom Harrison



Stephan Hirshi

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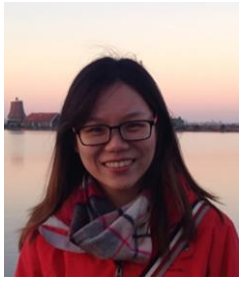

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