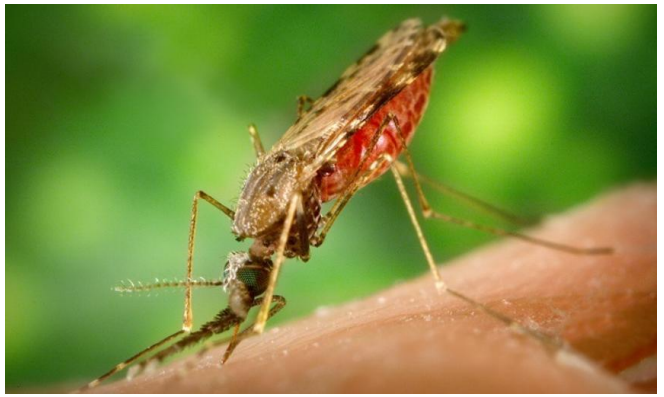
High-throughput isolation and characterisation of monoclonal antibodies against PfRH5

Dr Kirsty McHugh
Draper group
Blood-stage Malaria

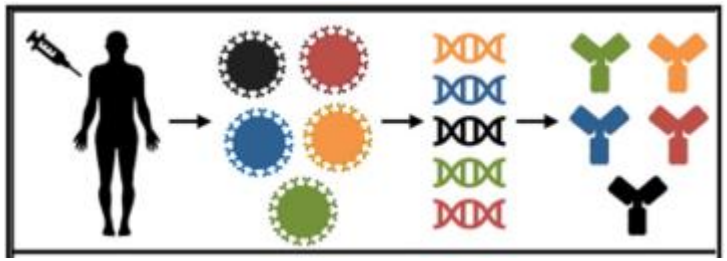
Presentation Outline

- **The Draper Blood-Stage Malaria Group –** our research

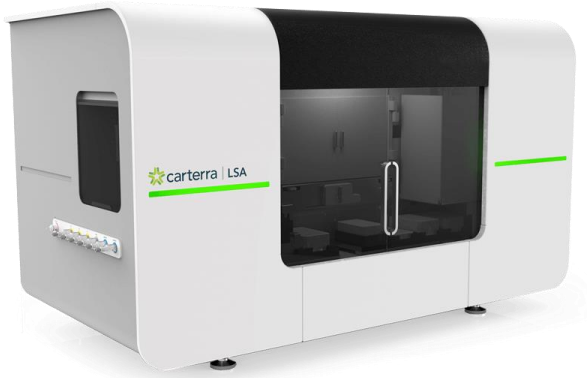


- **Blood-stage malaria –** RH5

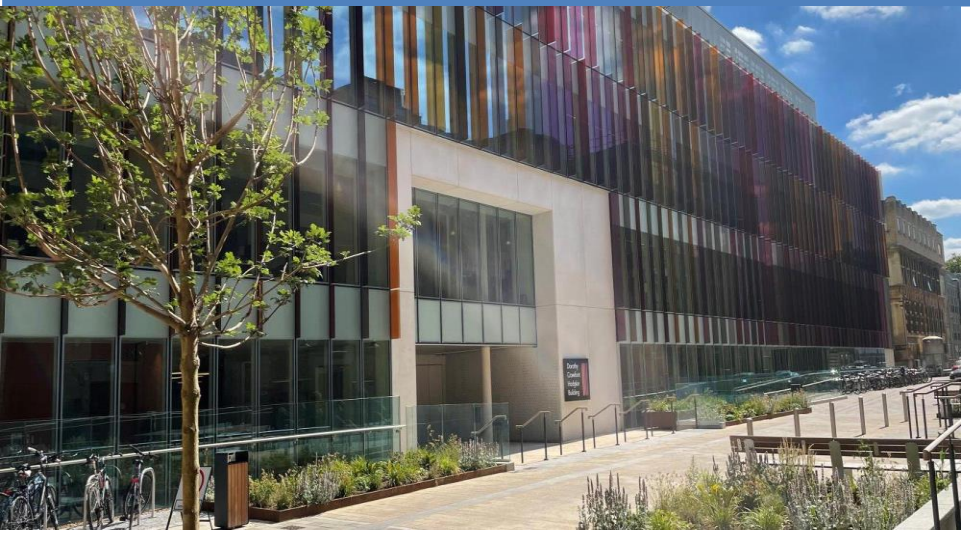
- **HT pipeline for isolation and characterisation of mAbs**



- **Carterra LSA HT-SPR platform**
  - Epitope binning
  - HT-SPR kinetics
  - Peptide epitope mapping
  - Antibody quantitation



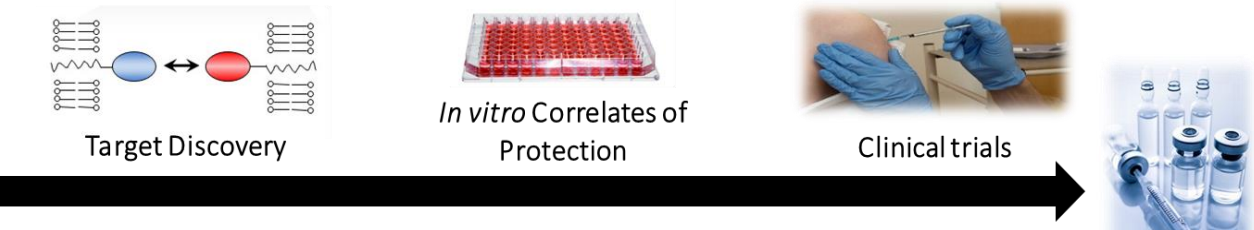
Draper Lab – Blood-stage Malaria



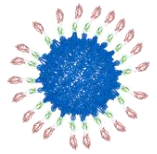
Dorothy Crowfoot Hodgkin Building

Vaccine Development and Antibody Immunology – Malaria

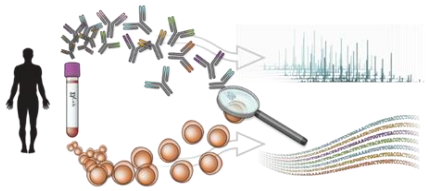
- >20 years' experience in malaria vaccine design and testing
  - Preclinical vaccine development
  - Early-phase clinical trials
- Very **active clinical team**:
  - 16 early phase trials to date, 4 in progress
  - developed CHMI models for *P.falciparum* and *P.vivax* to test vaccine efficacy
- **Research interests cover:**
  - **Rational vaccine design**
  - **Quantitative antibody Immunology**
  - **mAb isolation and characterisation**
    - Inform next generation vaccine design
    - Develop blood-stage mAb therapeutics



Structure-Guided and Innovative Design

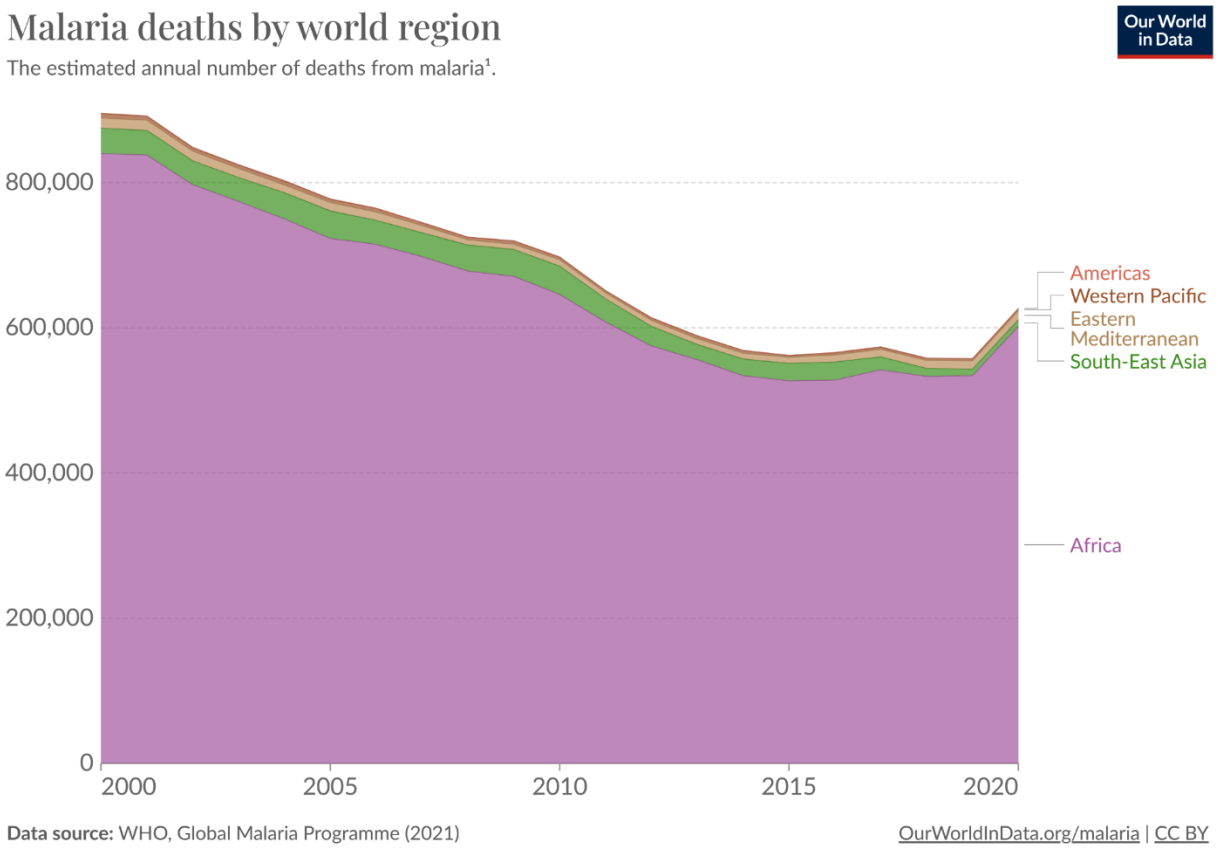


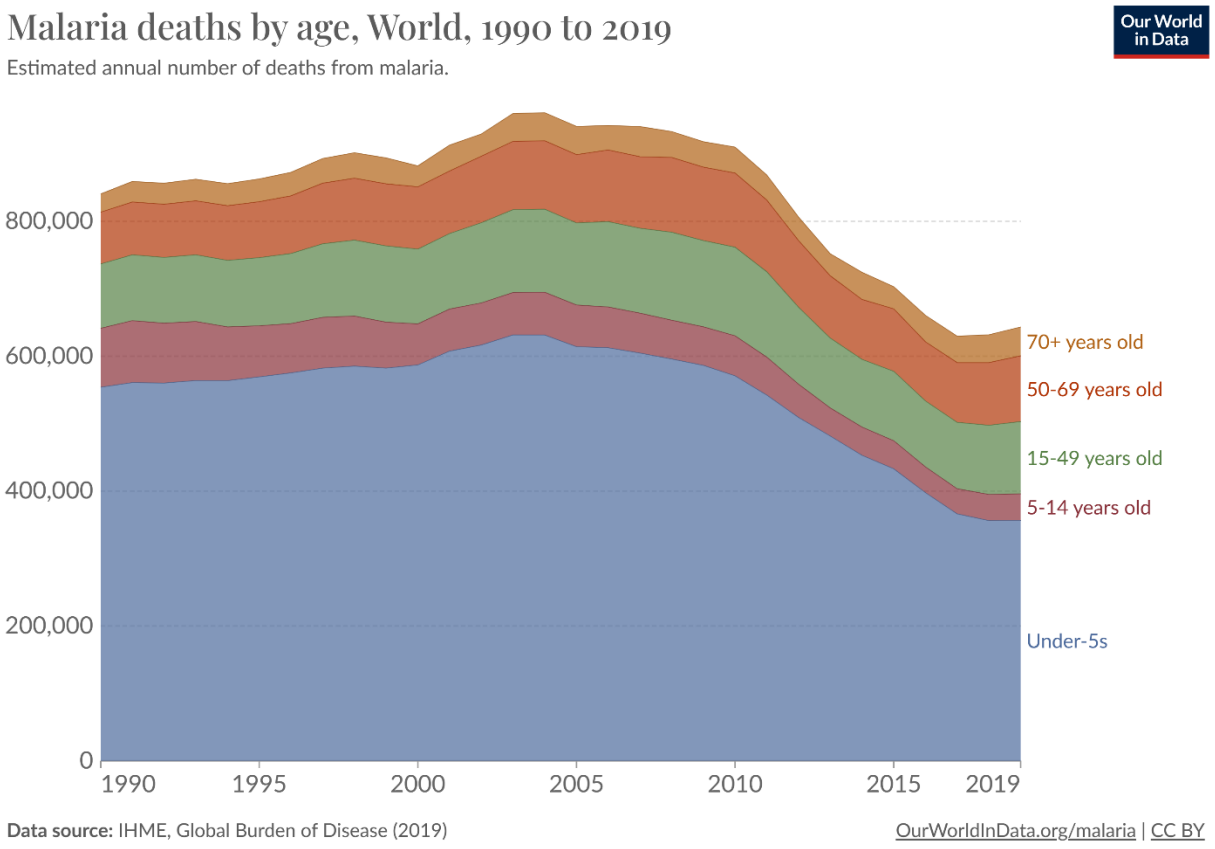
Human Antibody Immunology



Malaria – A global health problem

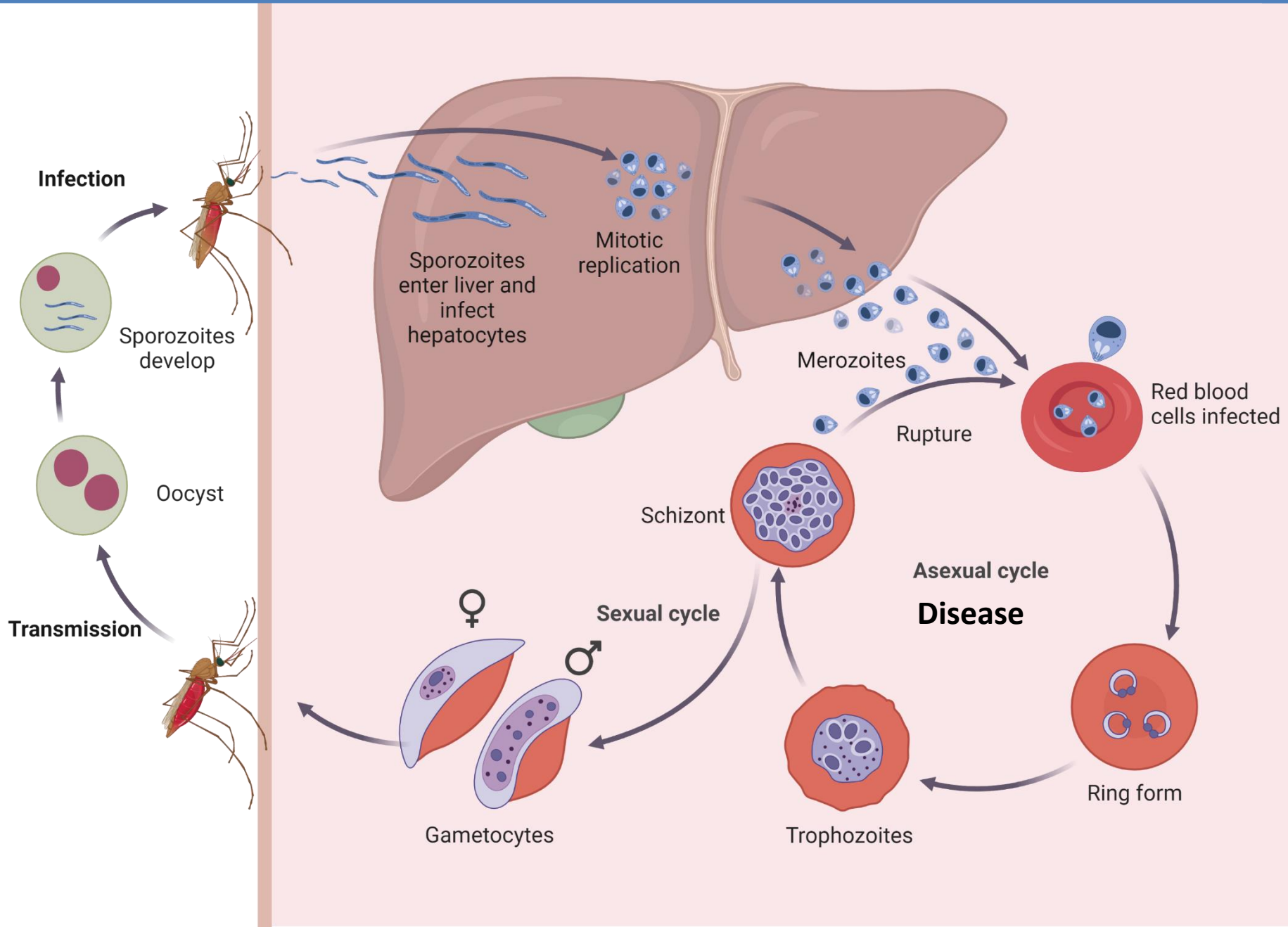
In 2022, there were an estimated **249M malaria cases**, and over **600,000 deaths**



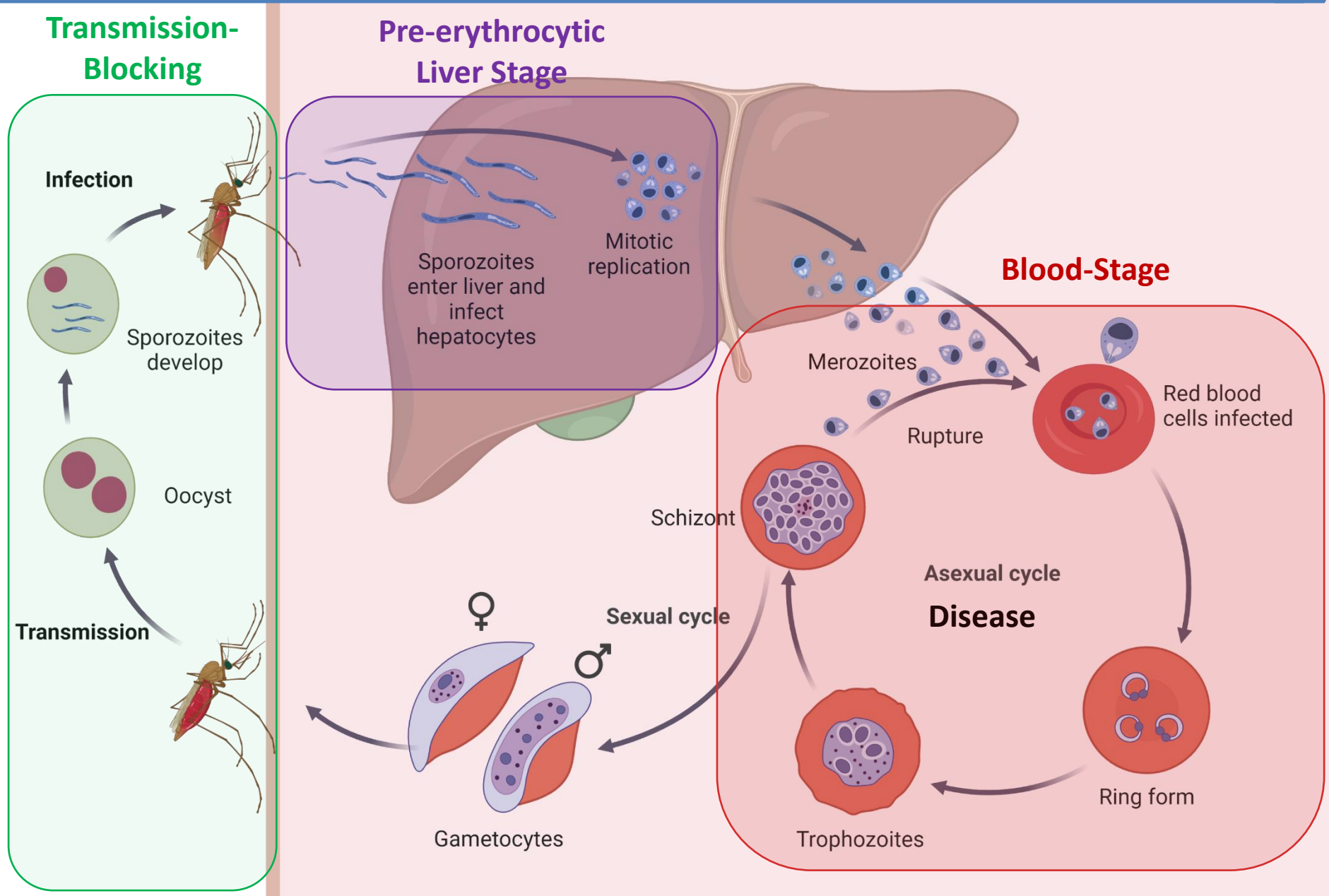


1. Malaria: Malaria is a life-threatening disease caused by parasites that are transmitted by female Anopheles mosquitoes. There are five parasite species that cause malaria in humans. Two of these species – P. falciparum and P. vivax – pose the greatest threat. The first symptoms – fever, headache and chills – usually appear 10 to 15 days after the infective mosquito bite and may be mild and difficult to recognize as malaria. Left untreated, P. falciparum malaria can progress to severe illness and death within 24 hours.

The Malaria Life Cycle



The Malaria Life Cycle



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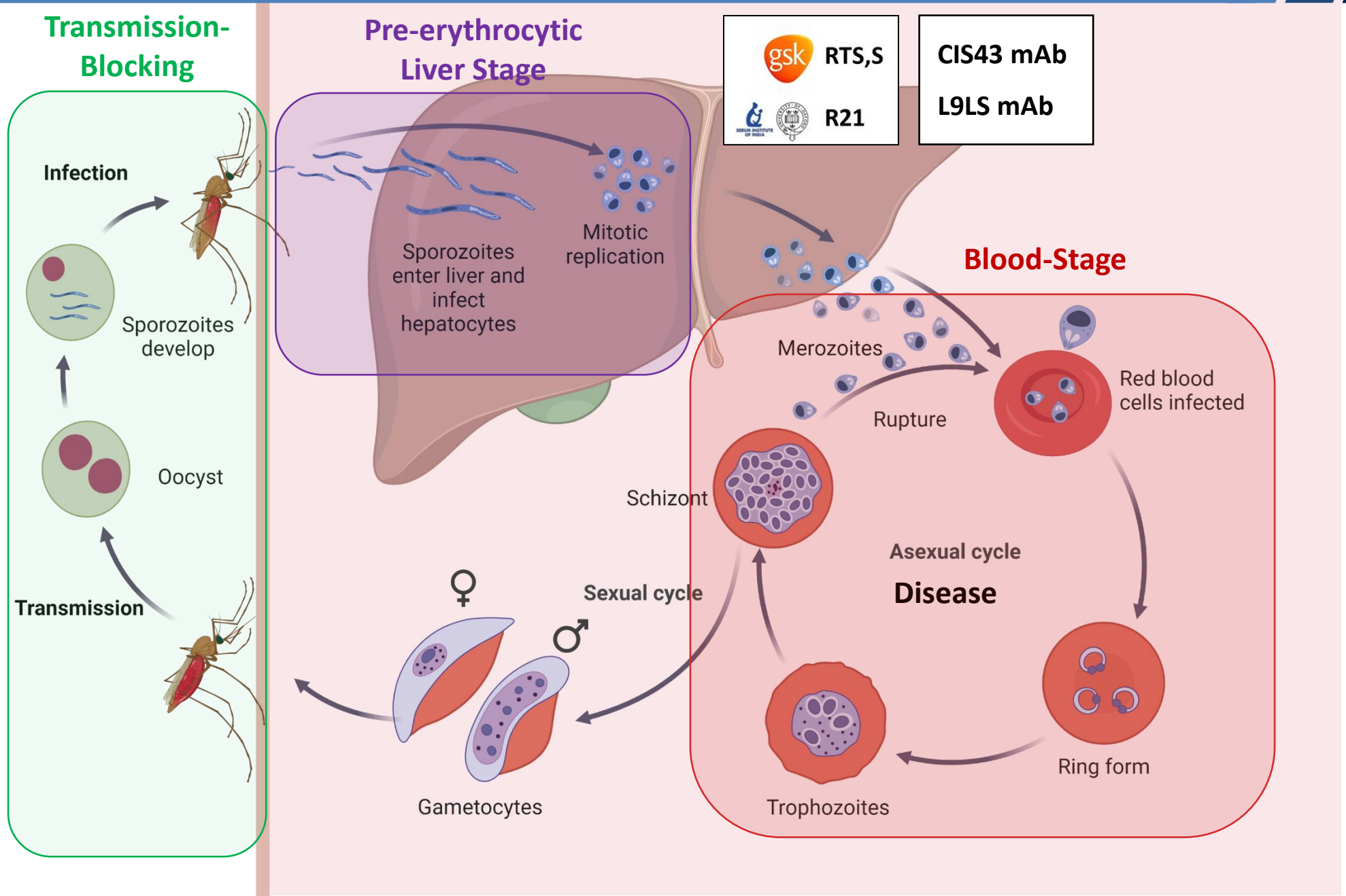

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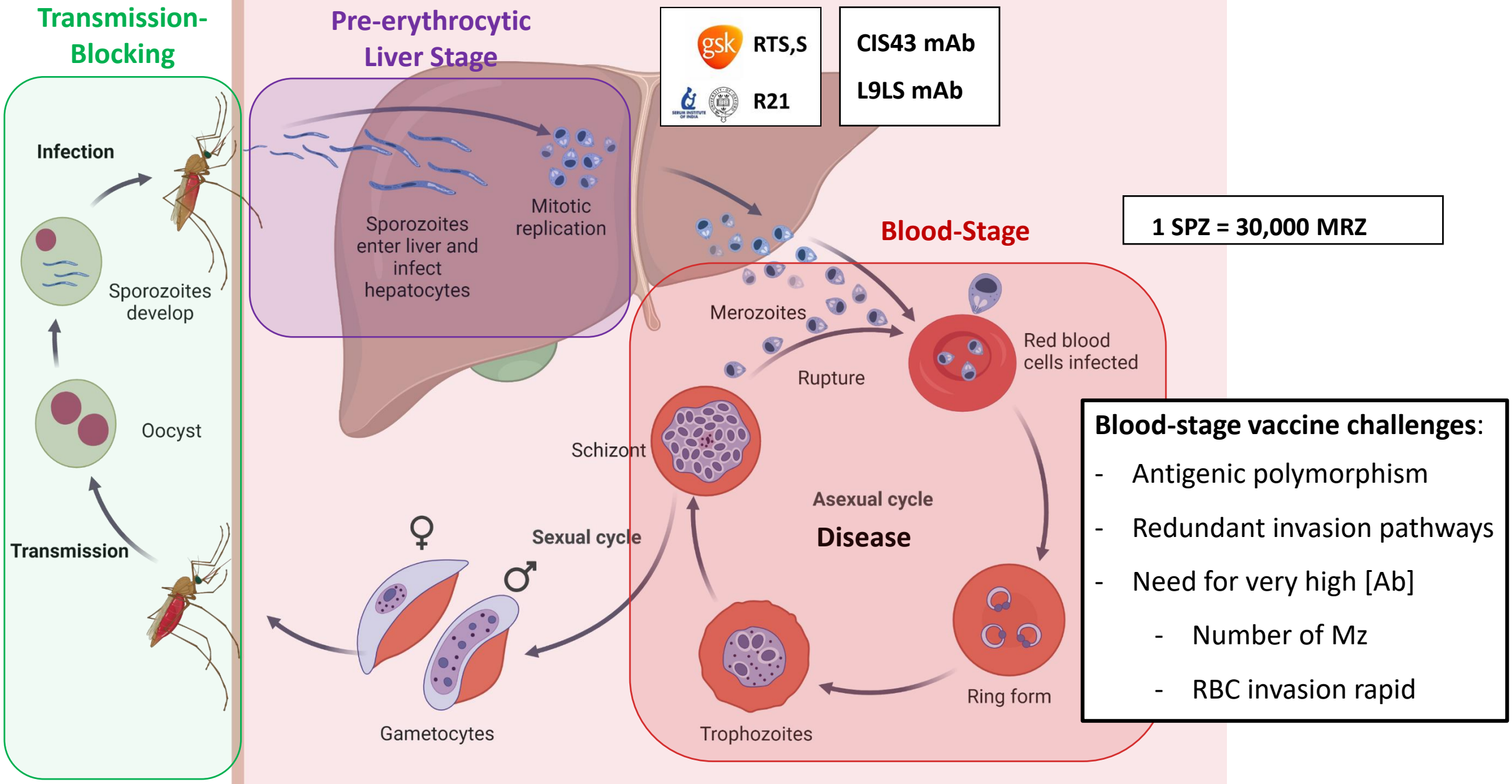
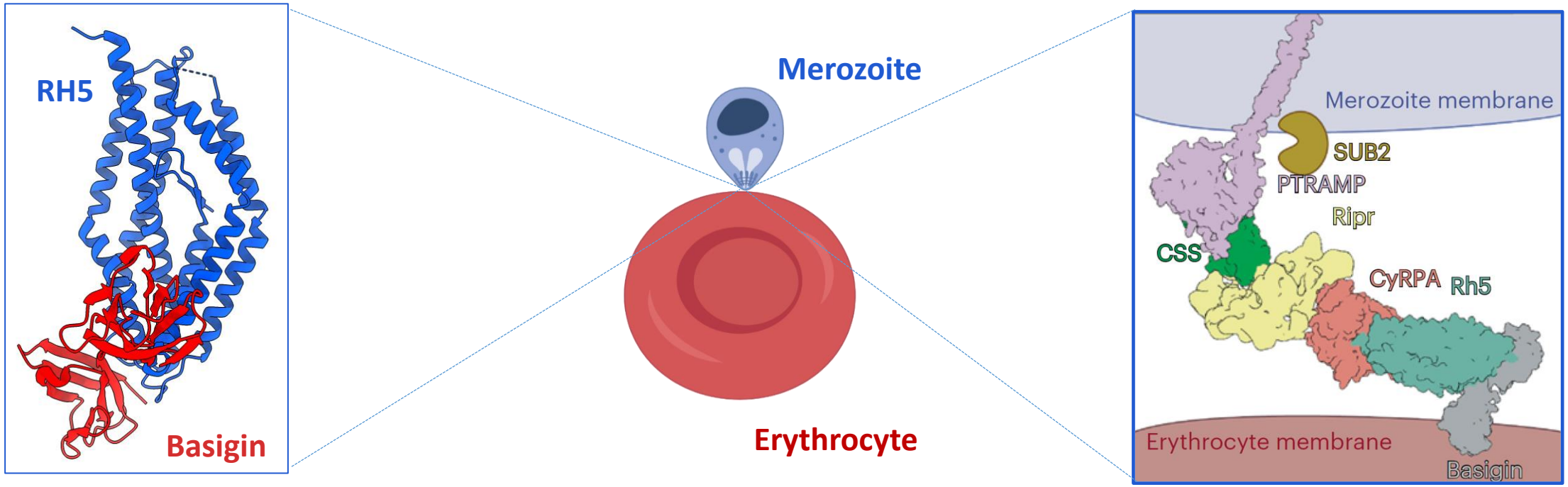


PfRH5 – a Conserved Blood-Stage Target

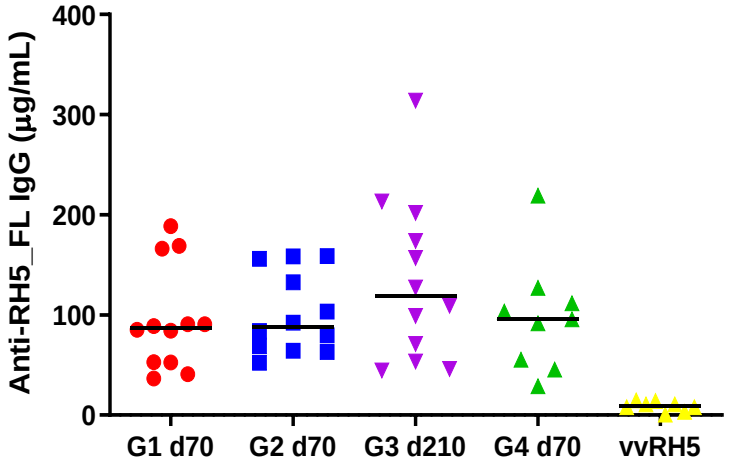


- **RH5 - the first highly conserved merozoite target identified susceptible to vaccine-induced antibodies** (Douglas *et al.* 2011, Nat Commun)
- **Forms an essential interaction with basigin (CD147) on the red blood cell surface** (Crosnier *et al.* 2011, Nature)
- **Structure reported of RH5 bound to basigin and neutralising mAbs** (Wright *et al.* 2014, Nature)
- **"PCRCR" invasion complex – 5 essential conserved proteins (PTRAMP, CSS, RIPR, CyRPA, RH5)** (Wong *et al.* 2019, Nature; and Scally *et al.* 2022 Nat Microbiol)

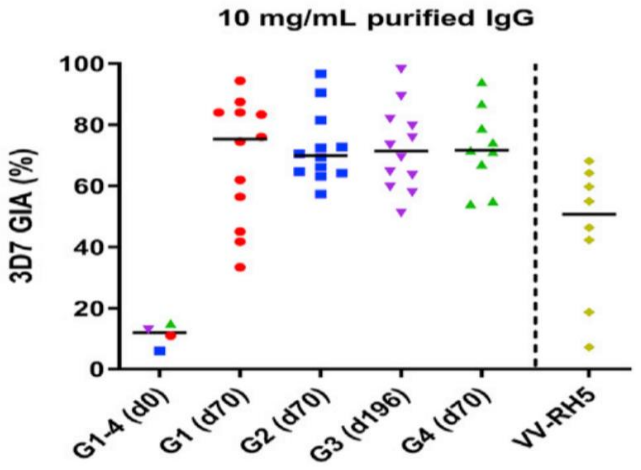
RH5 in the Clinic

- 4 early phase clinical trials of RH5-based vaccines have been completed.
- RH5 has been delivered using different platforms/formulations in the UK (**VAC057, VAC063**) and in Tanzania (**VAC070, VAC080**)

Peak (d70) RH5 IgG ELISA



Peak (d70) IgG GIA

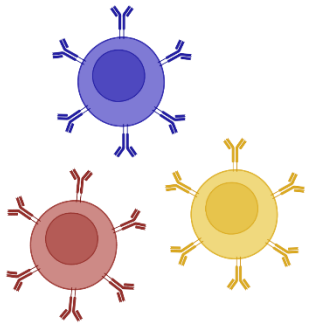


- In UK trials, the VAC063 RH5 protein-in-adjuvant (AS01, GSK) vaccine gave improved antibody quantity (ELISA) and functional activity (GIA) compared with the VAC057 viral vectors

VAC063 volunteers were selected for mAb isolation to map the RH5 epitope landscape

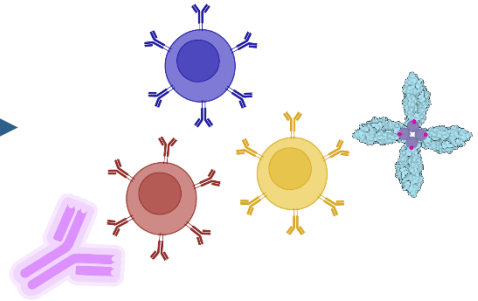
Antibody discovery - mAb isolation pipeline

B-Cell Enrichment

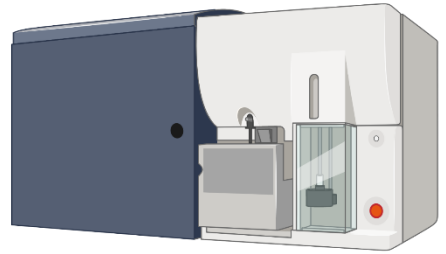


Antigen Probe Staining

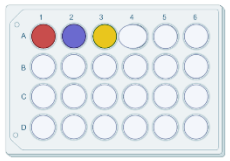
Phenotypic Marker Staining



FACS

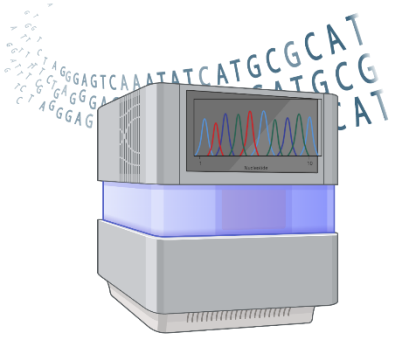


Single B Cell Sorting



RT-PCR

Sequencing (Human Ig Primers)

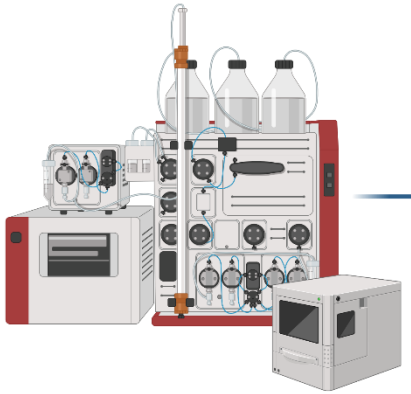


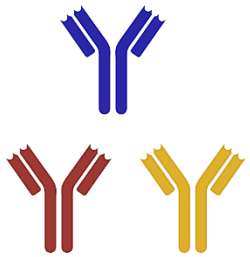
Cloning (Twist Bio/ In-house)

IgG1 Vector

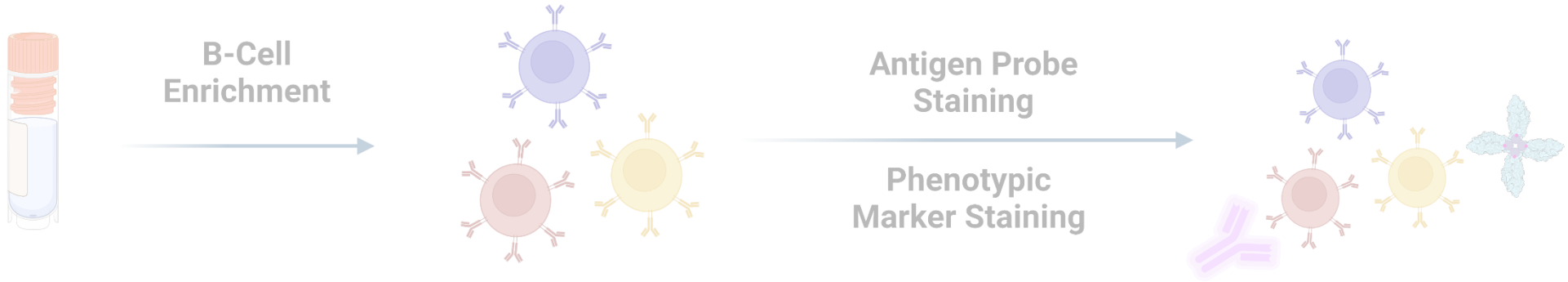
Expression HEK293

Purification

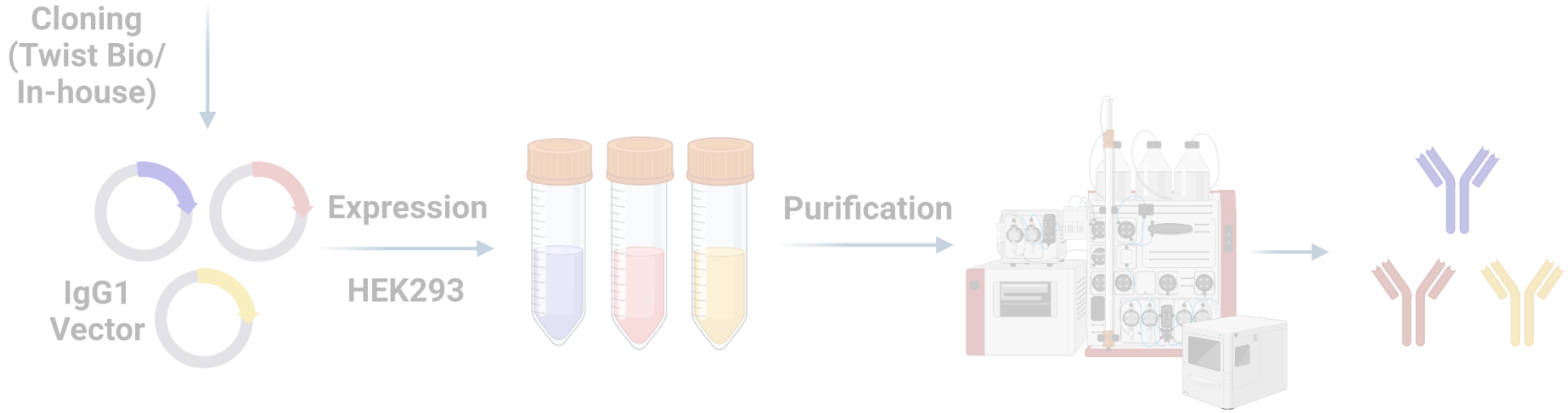




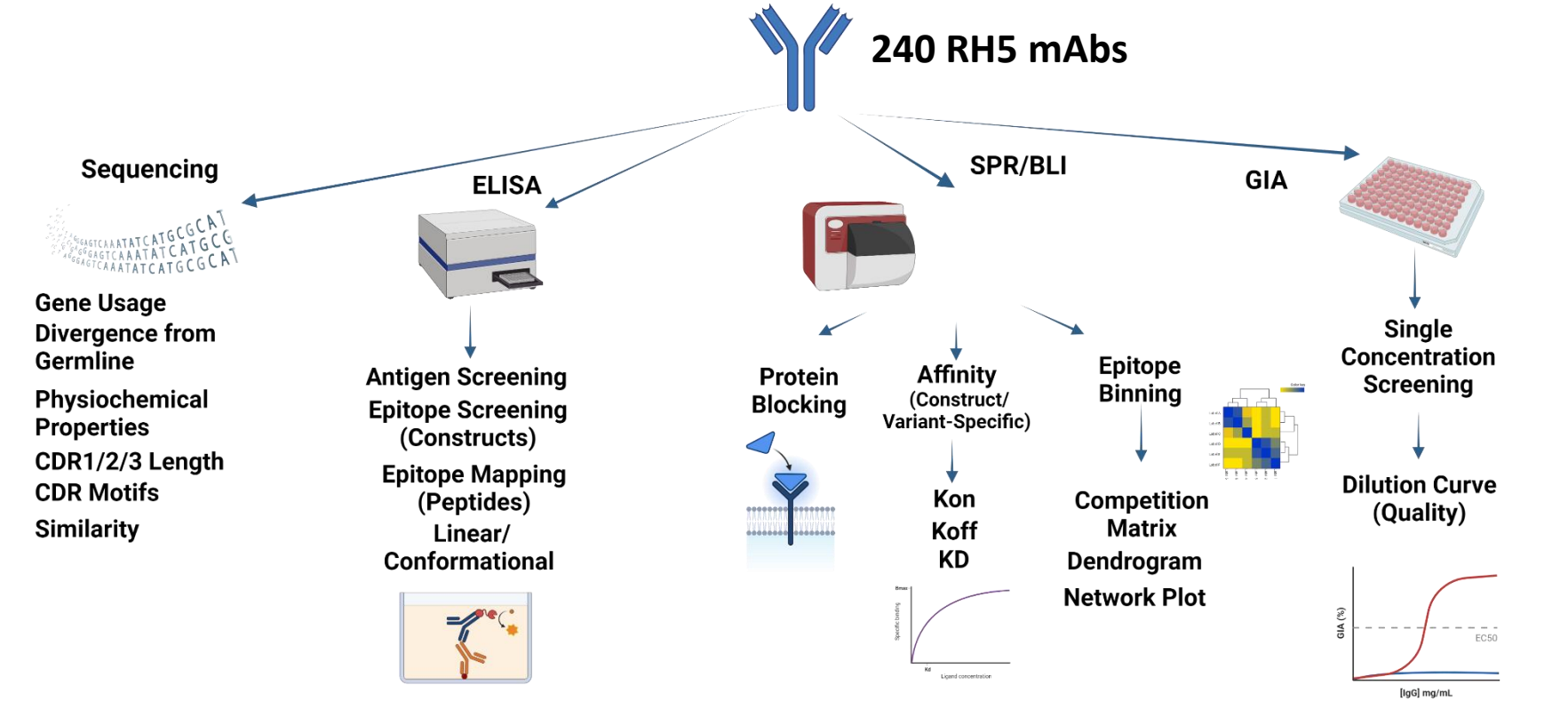
Antibody discovery - mAb isolation pipeline



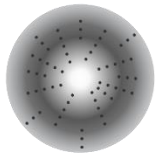
21 VAC063 volunteers → 240 mAbs



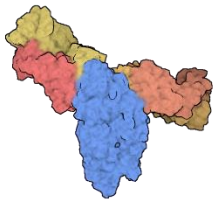
mAb characterisation pipeline



Structural Determination

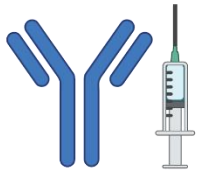


Antibody Combinations (Synergy/Antagonism)

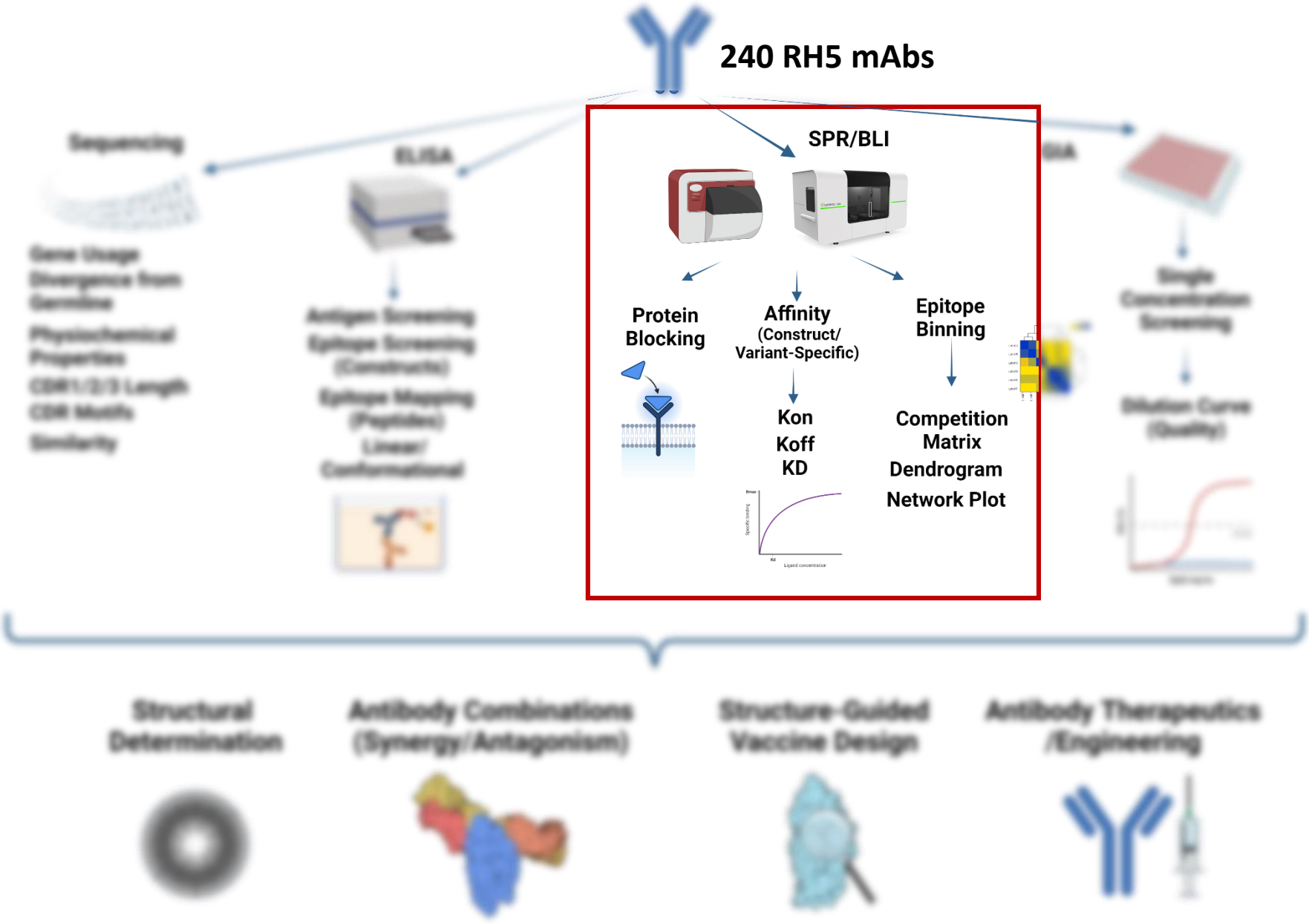


Structure-Guided Vaccine Design

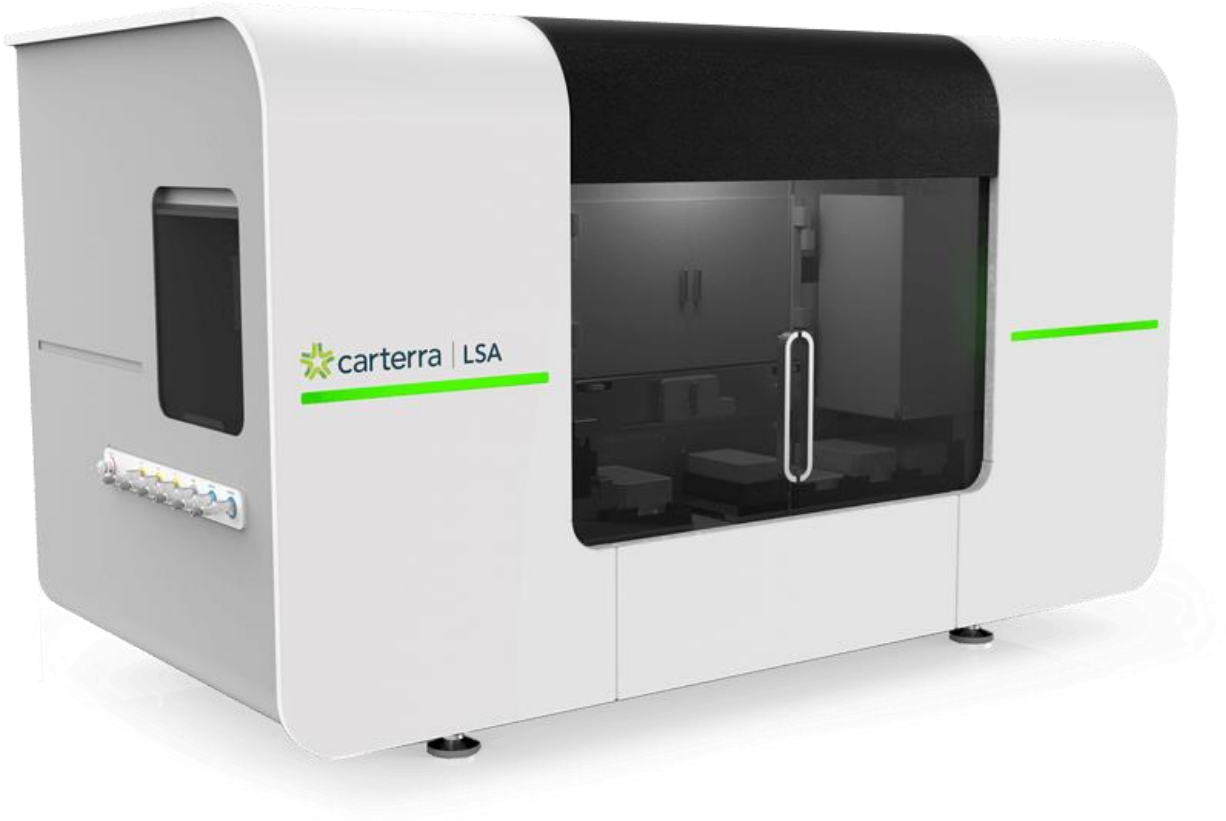
Antibody Therapeutics /Engineering



mAb characterisation pipeline



The Carterra LSA: for HT-SPR kinetics and epitope binning



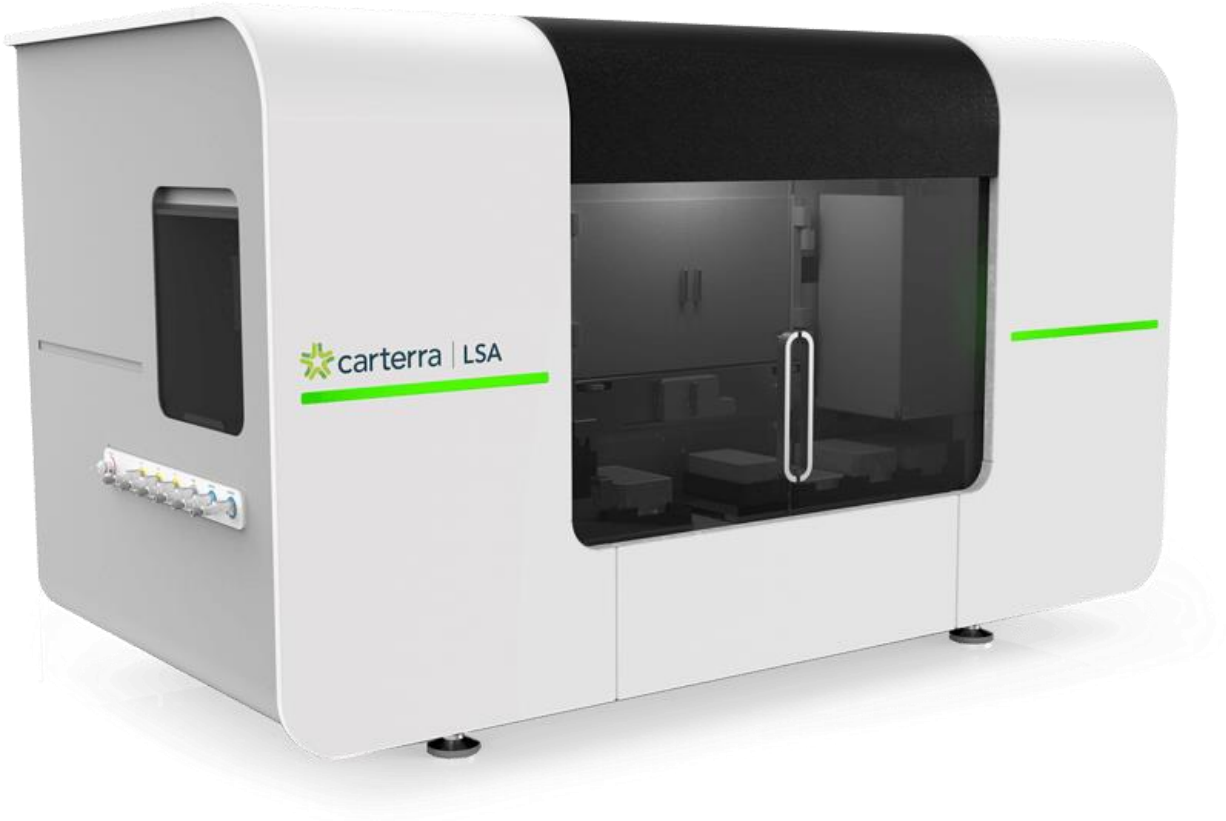
- Screen up to **384 clones** simultaneously in a single experiment
- Relatively quick – kinetics from 384 ligand array in 8h (per antigen)
- Use only a small amount of sample:
  - <1ug of each mAb for kinetics coupled ligand array
  - <30ug of mAb for epitope binning

UKRI Medical Research Council

MRC equipment grant

carterra®

The Carterra LSA: for HT-SPR kinetics and epitope binning



1. Epitope binning
2. Kinetics
3. Peptide epitope mapping
4. Quantitative serum antibody analysis

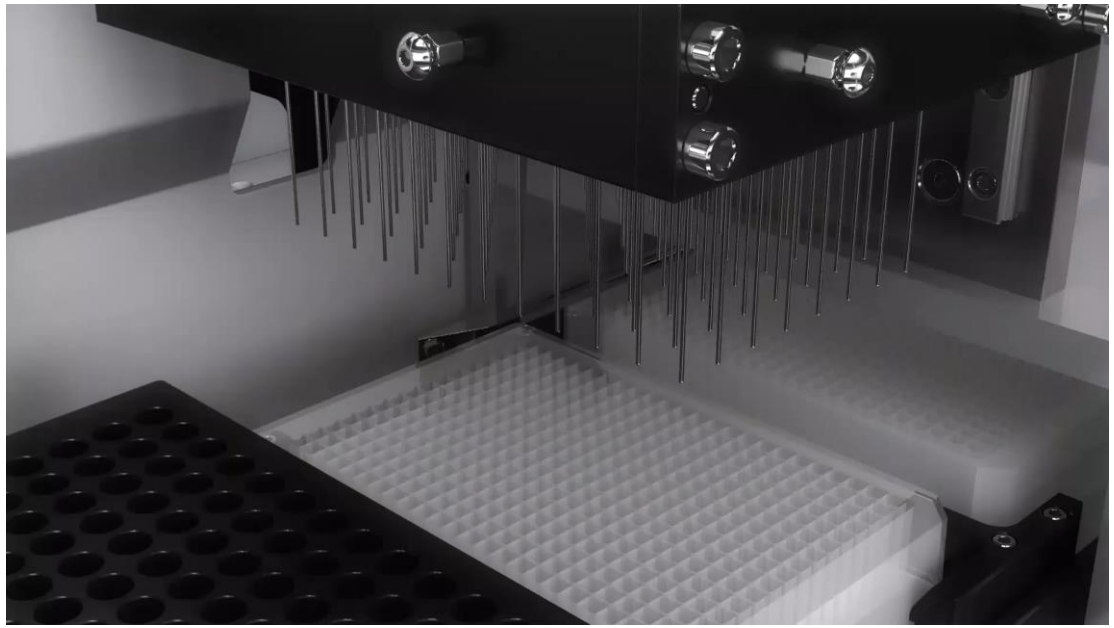
UKRI Medical Research Council

MRC equipment grant

carterra®

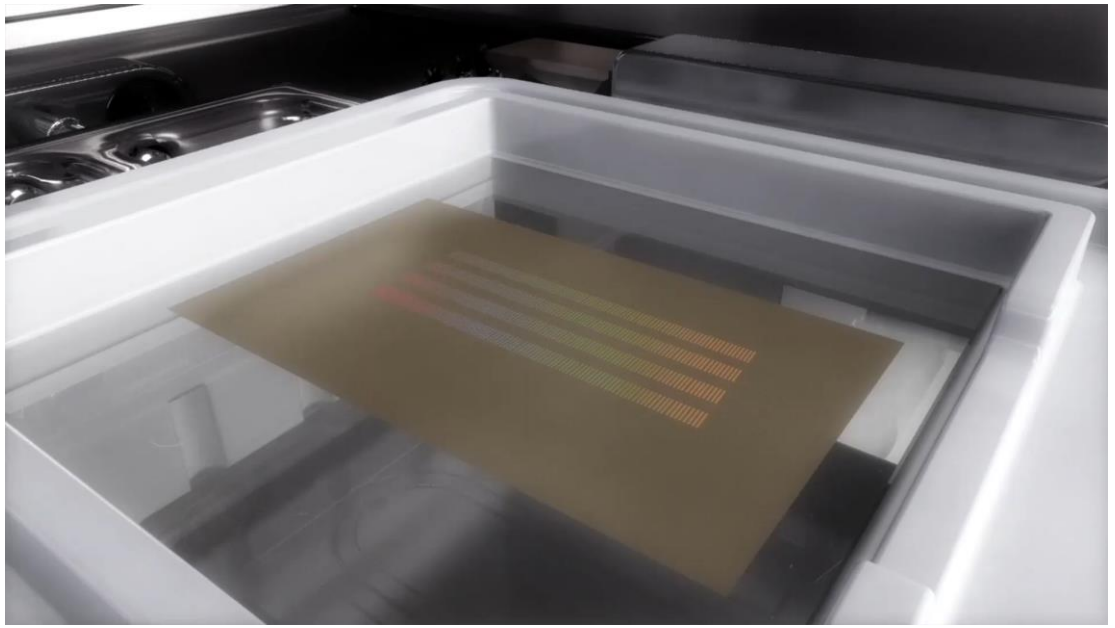
Traditional SPR with a gold surface integrated with advanced fluidics

Print head/Multi-channel mode



- 96 needles lowered into a ligand plate to takes up samples
- Print head then docks onto gold surface to capture/couple the array using **bidirectional flow**
- This can be repeated up to 4 times to create a 384 array of:
  - unique ligands, replicates, a concentration series

Single-channel mode



- Single flow cell docks to deliver buffer blanks, antigens, and regeneration buffer over the entire ligand array in single injections

carterra

1. EPITOPE BINNING – experiment overview

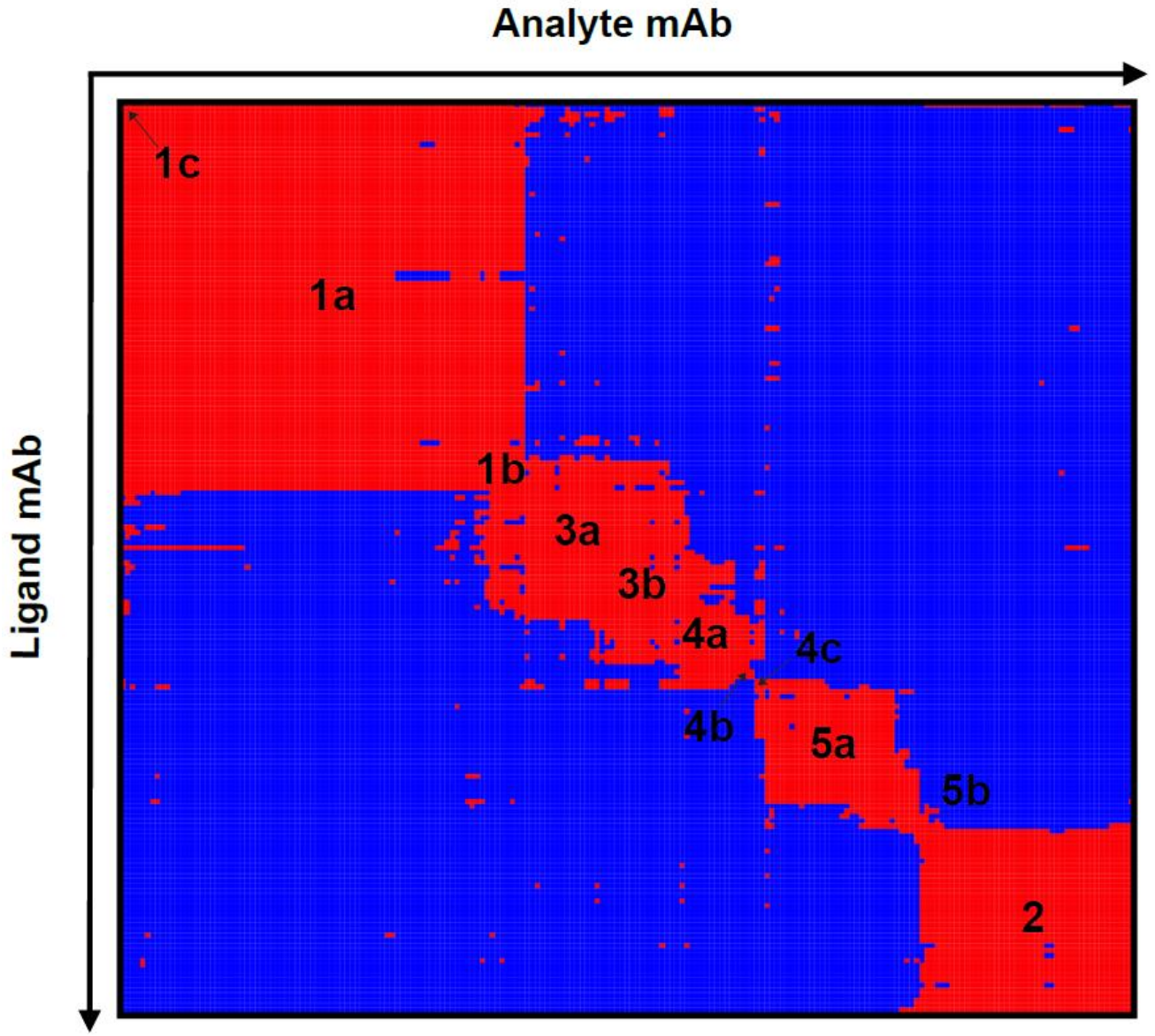
247 mAbs were coupled to a chip to create the ligand array

RH5 analyte was injected over the whole array with the single flow cell (SFC)

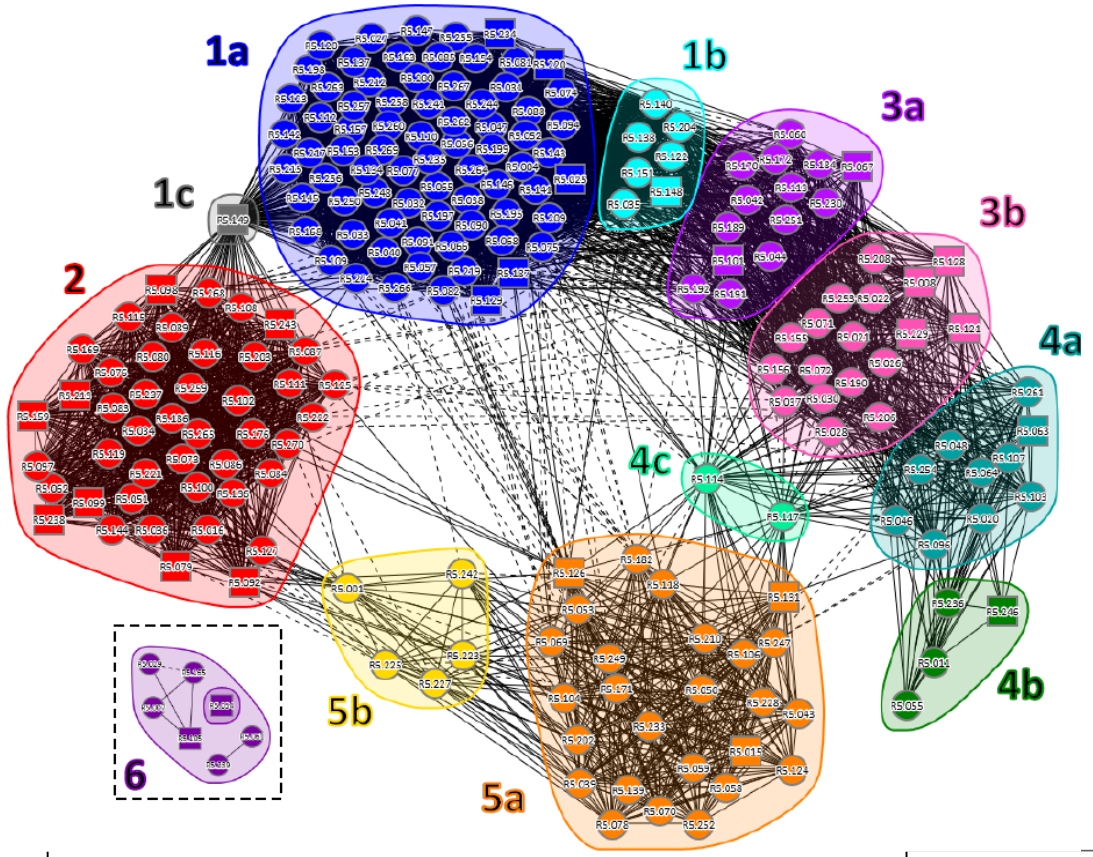
The 1st of the coupled antibodies was injected as an analyte

Following binding, the ligand array was regenerated for subsequent antigen then antibody cycles with the remaining panel

Data is processed using the Epitope software to generate a heatmap

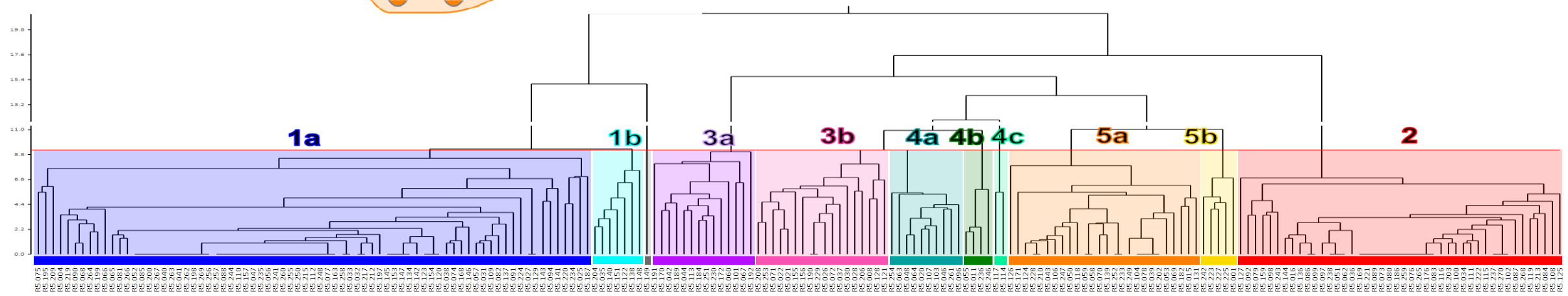


The Epitope Landscape of RH5



- mAbs have been grouped into **12 epitope communities**, and **6 super communities** according to competition relationships

- Nuanced blocking behaviours can be picked up: sub-communities and cross-talking antibodies



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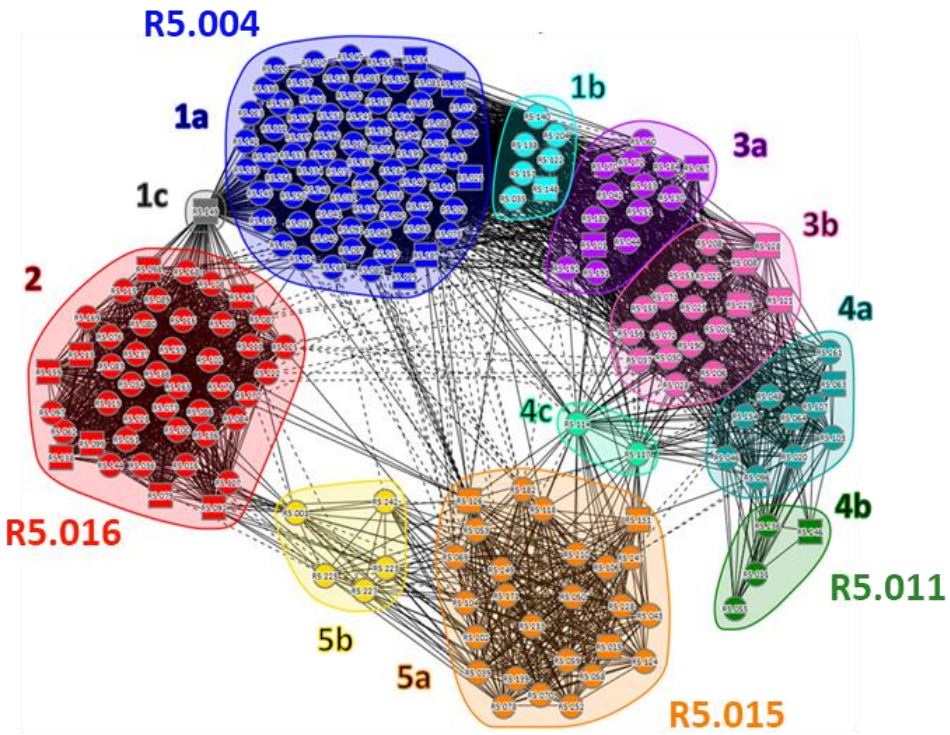
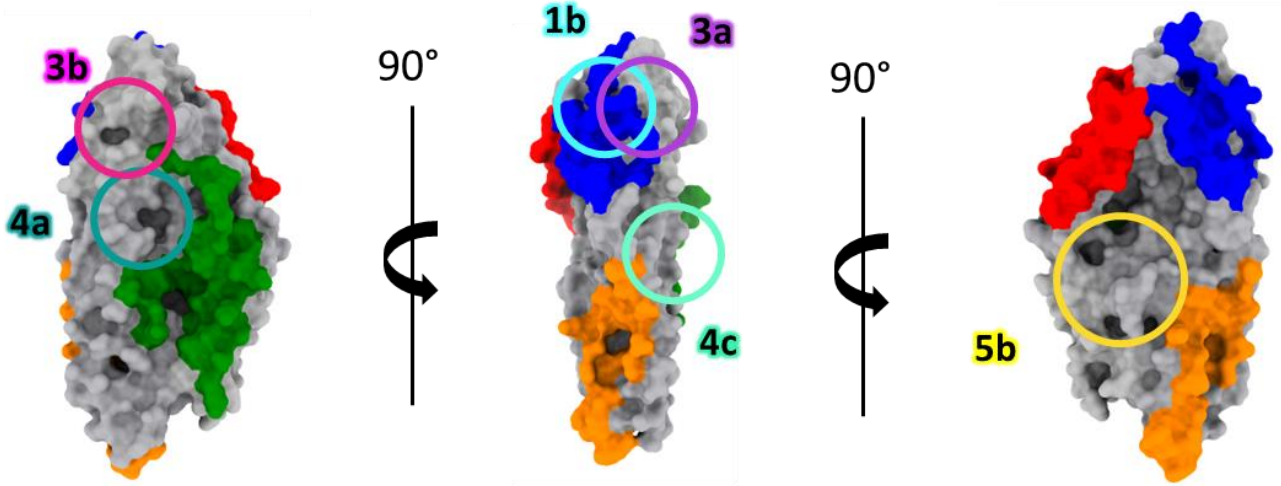
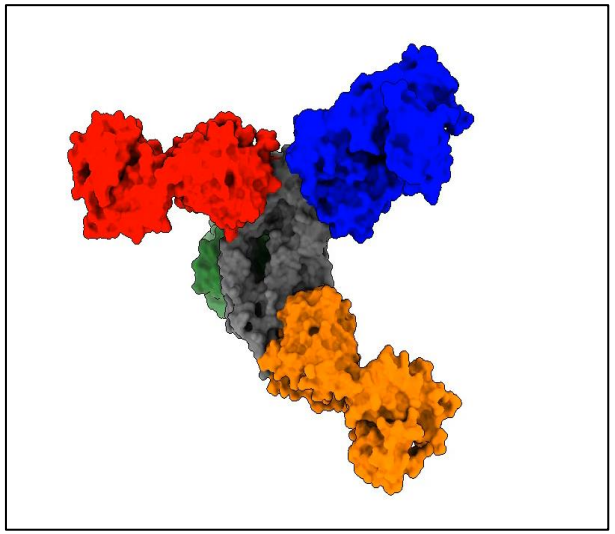
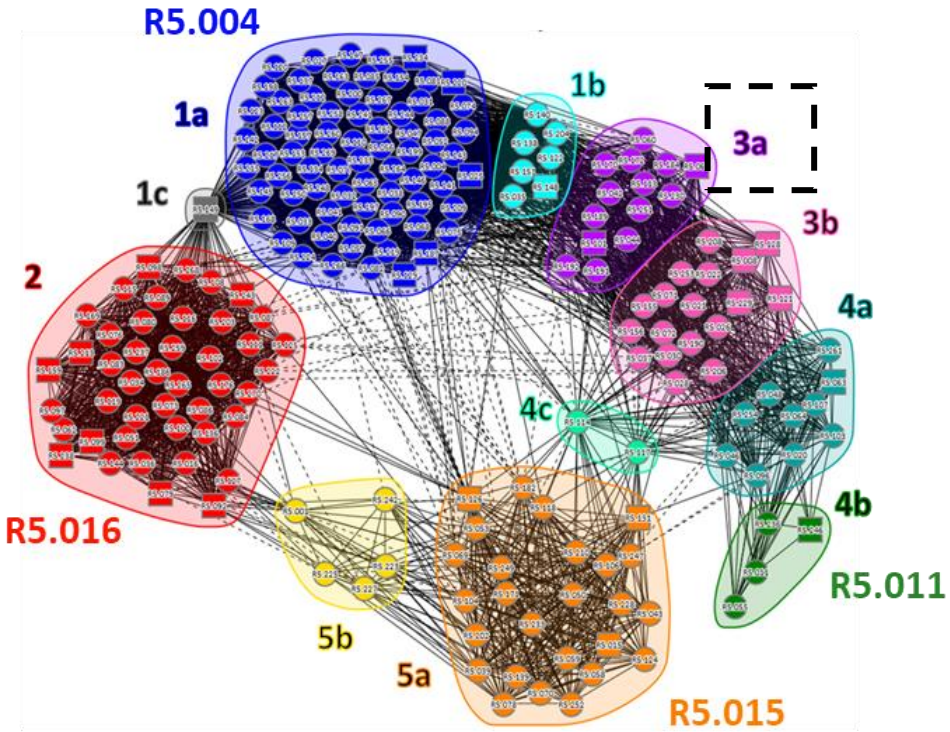


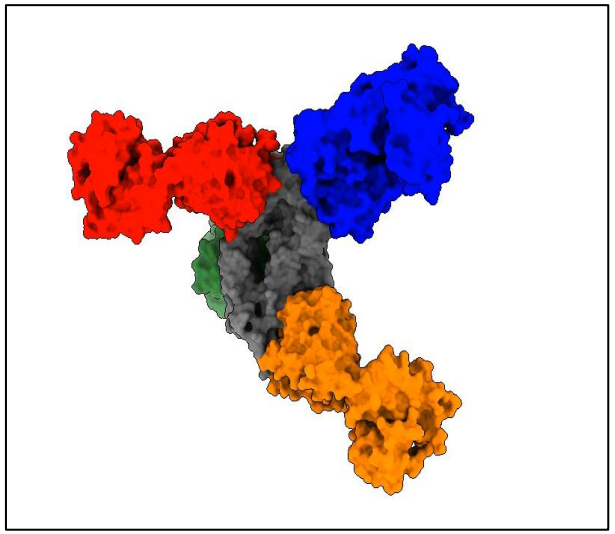


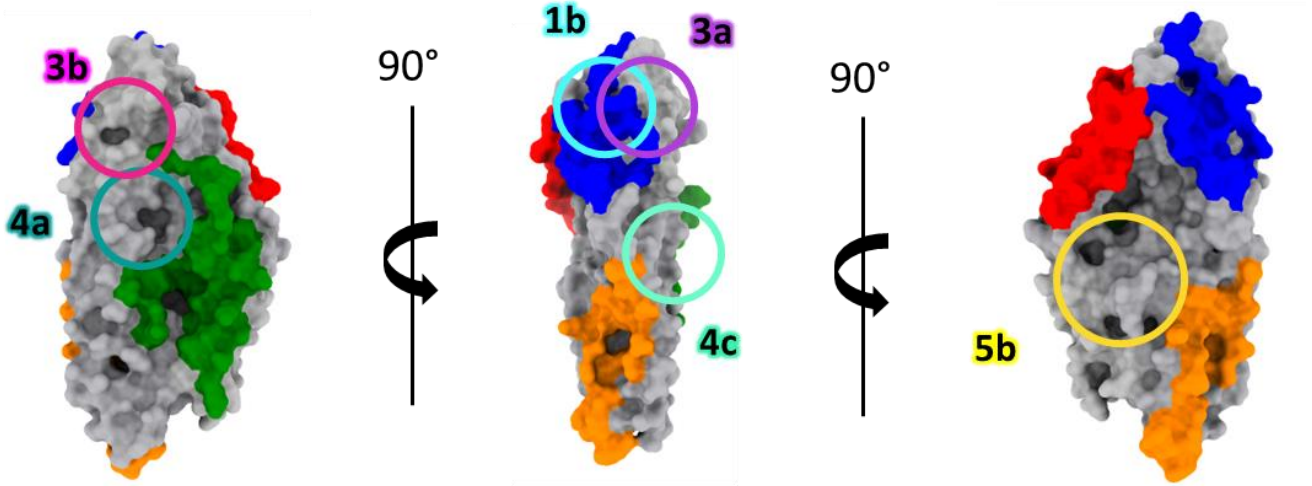


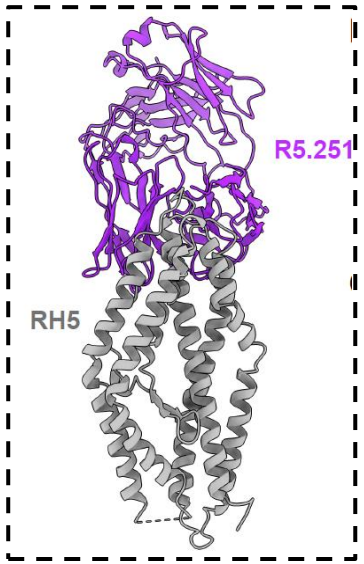
- 4 mAbs from 4 communities have previously defined structural epitopes (Alanine et al 2019)
- Potential binding site regions for remaining communities can be predicted based on the overlapping competition profiles
- **Binning informs the down-selection of antibodies** for structural studies to precisely define the missing epitopes

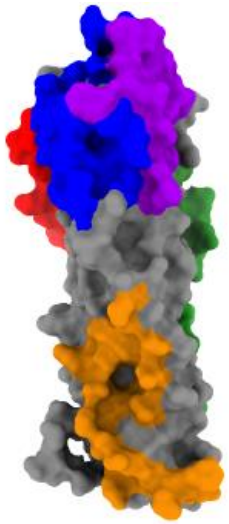
The Epitope Landscape of RH5





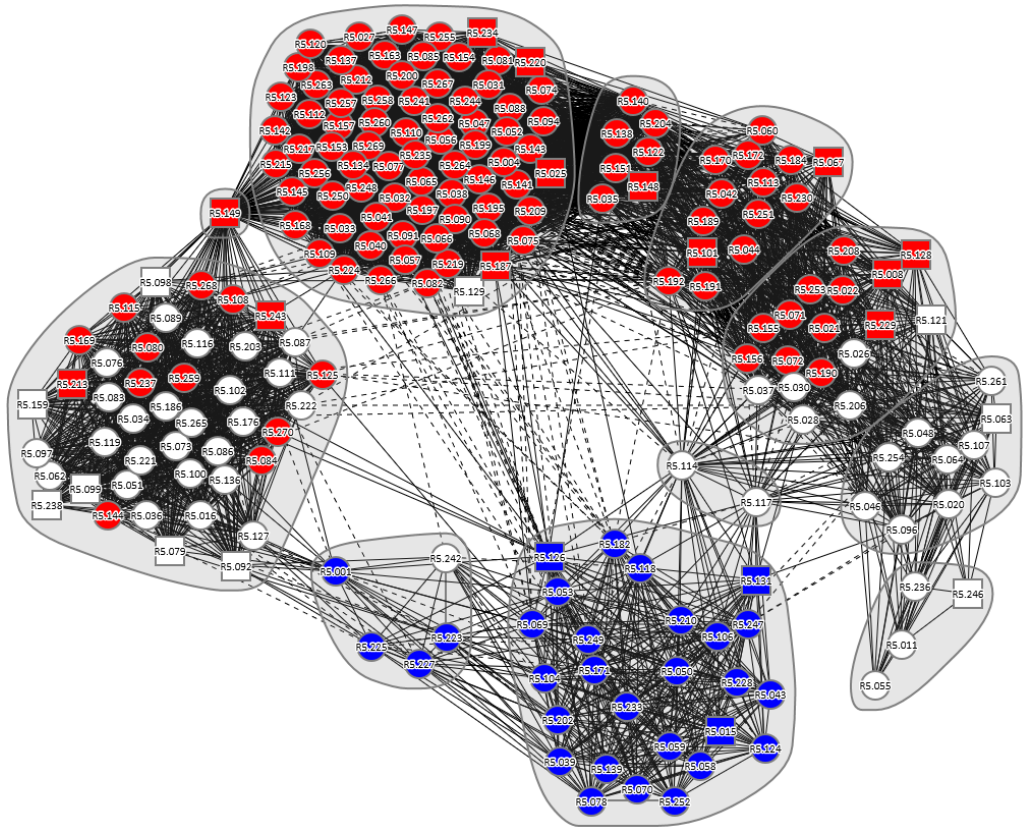




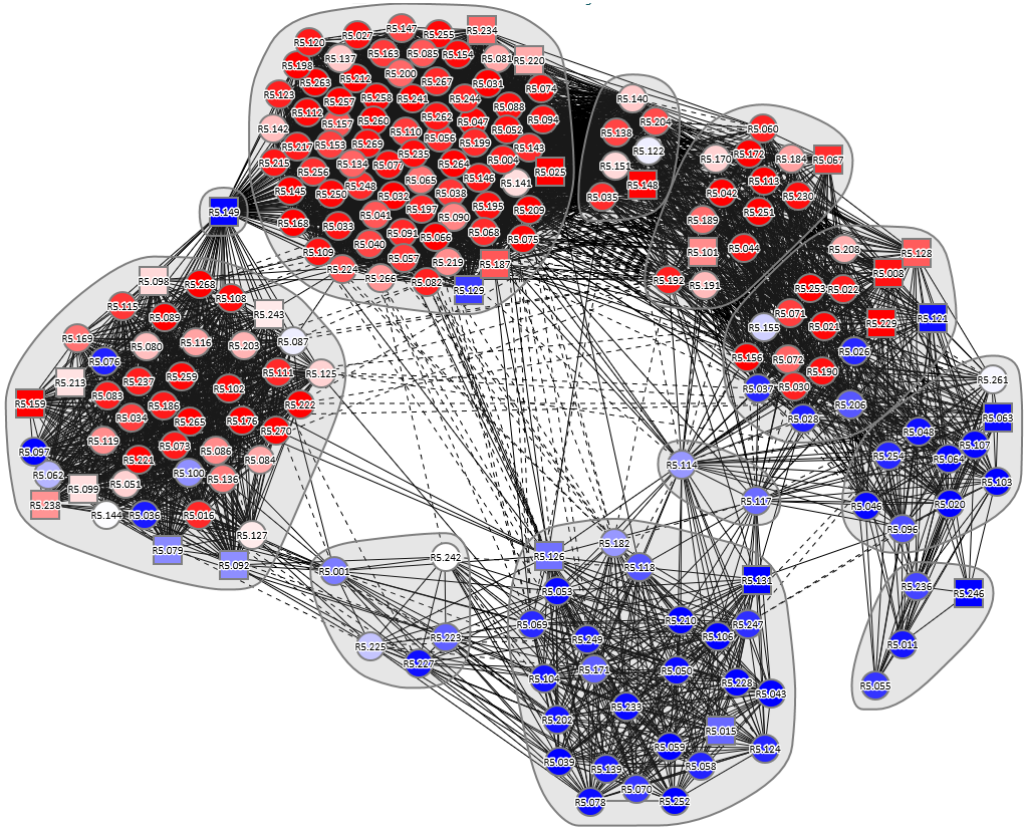


Combining epitope with function

Protein blocking activity
as measured by BLI



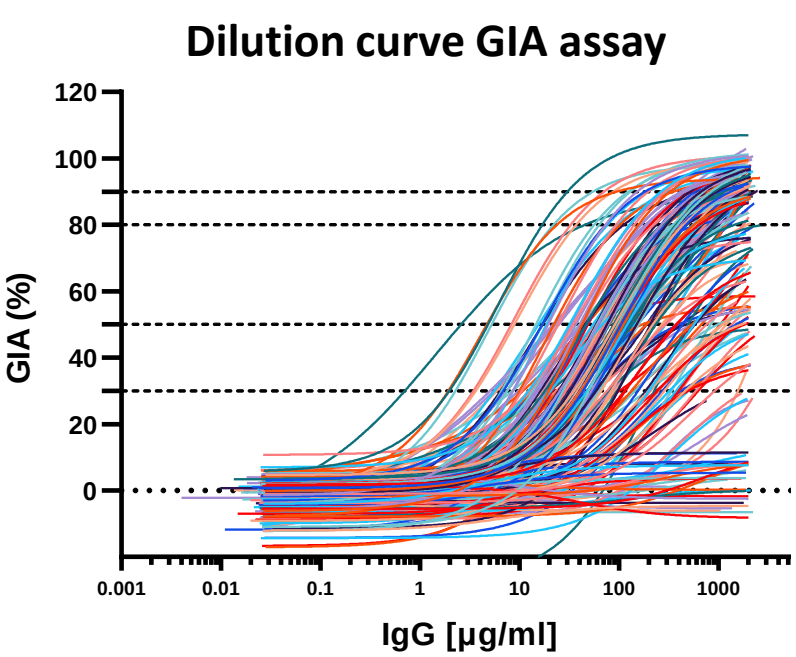
Parasite inhibition activity
as measured by GIA assay

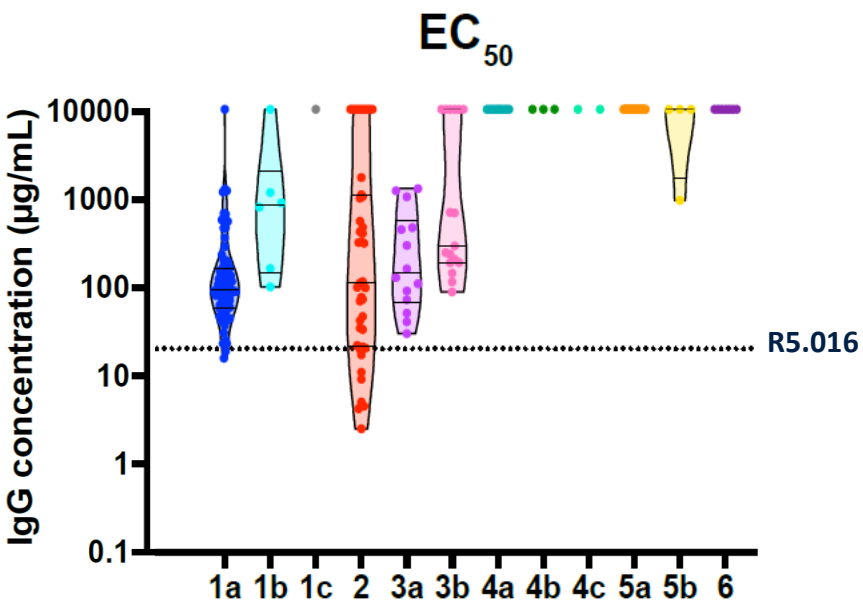


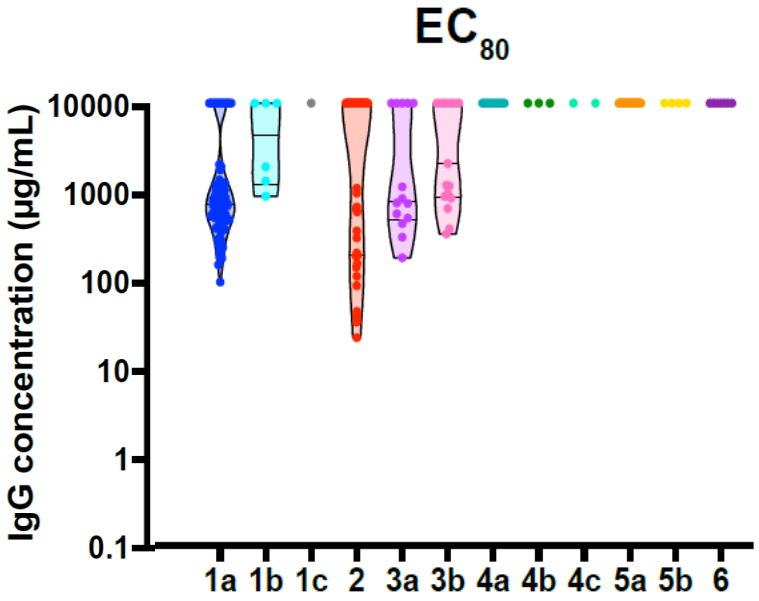
Basigin Blocking CyRPA Blocking No Blocking

0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100%

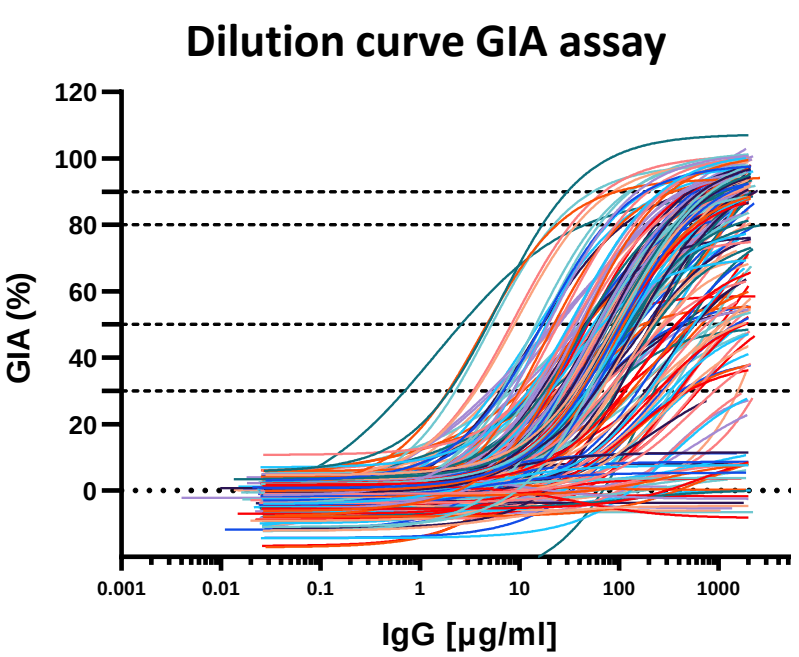
Combining epitope with function: GIA +ve mAbs

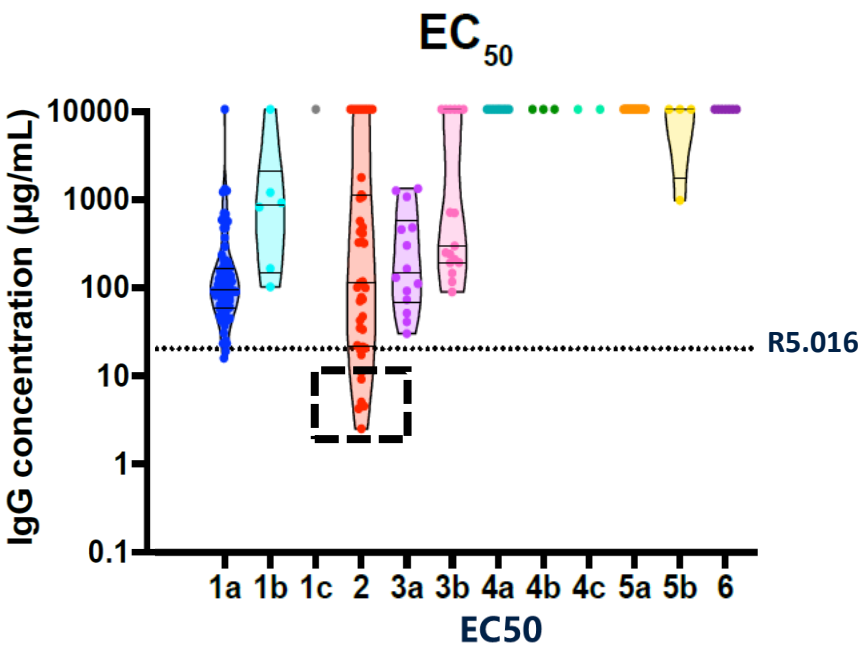


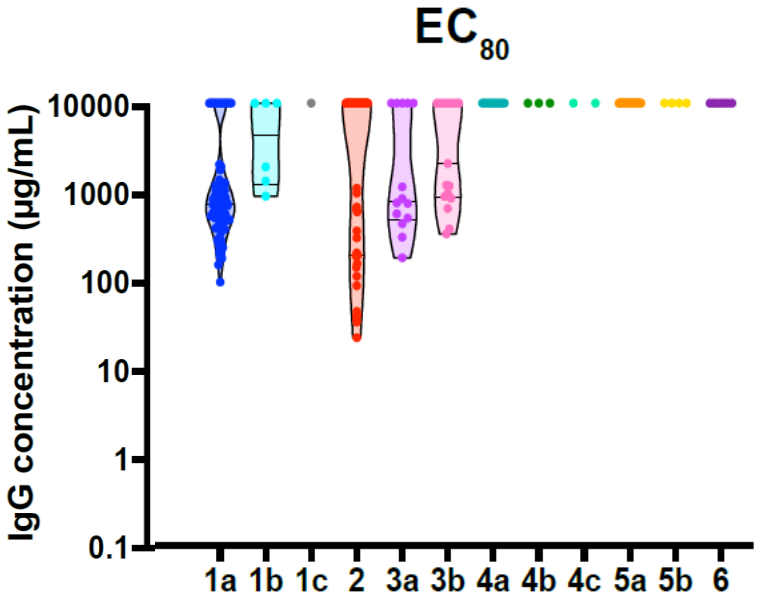


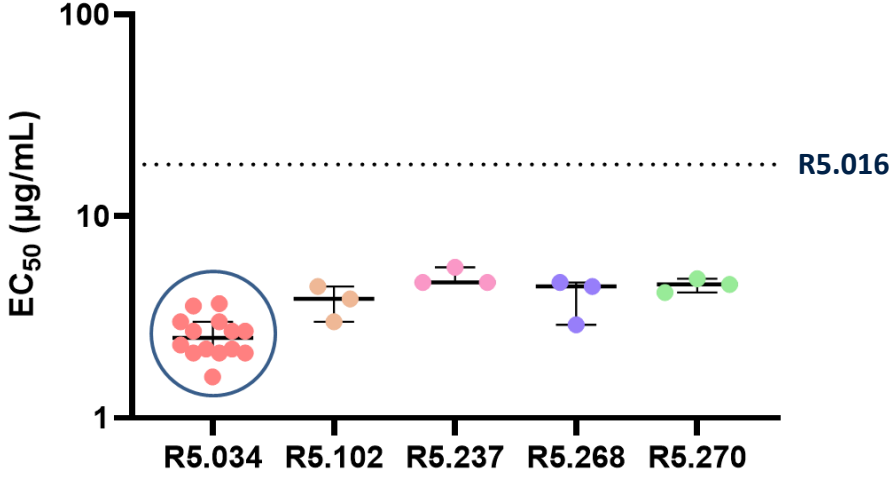


Combining epitope with function: GIA +ve mAbs



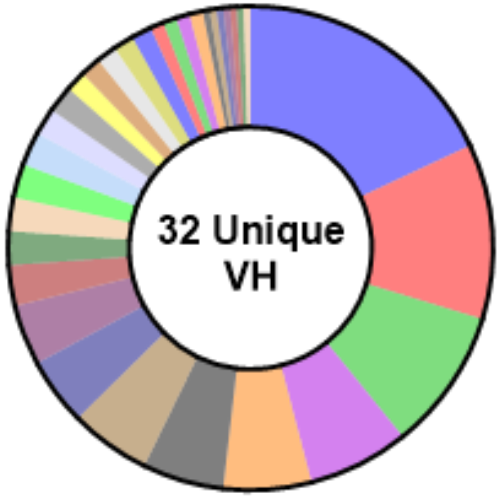


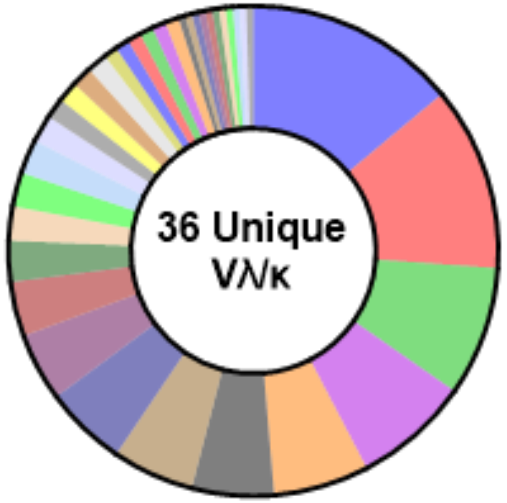


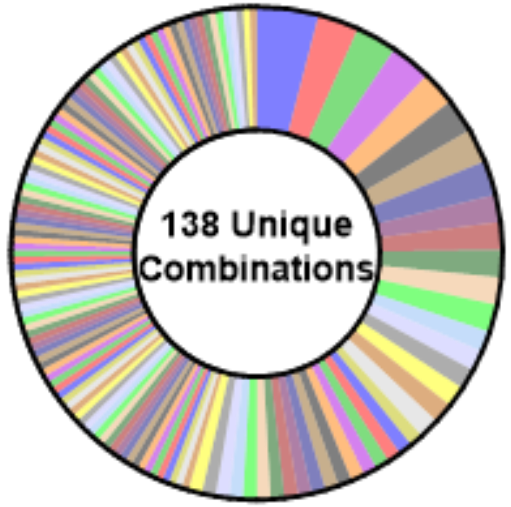


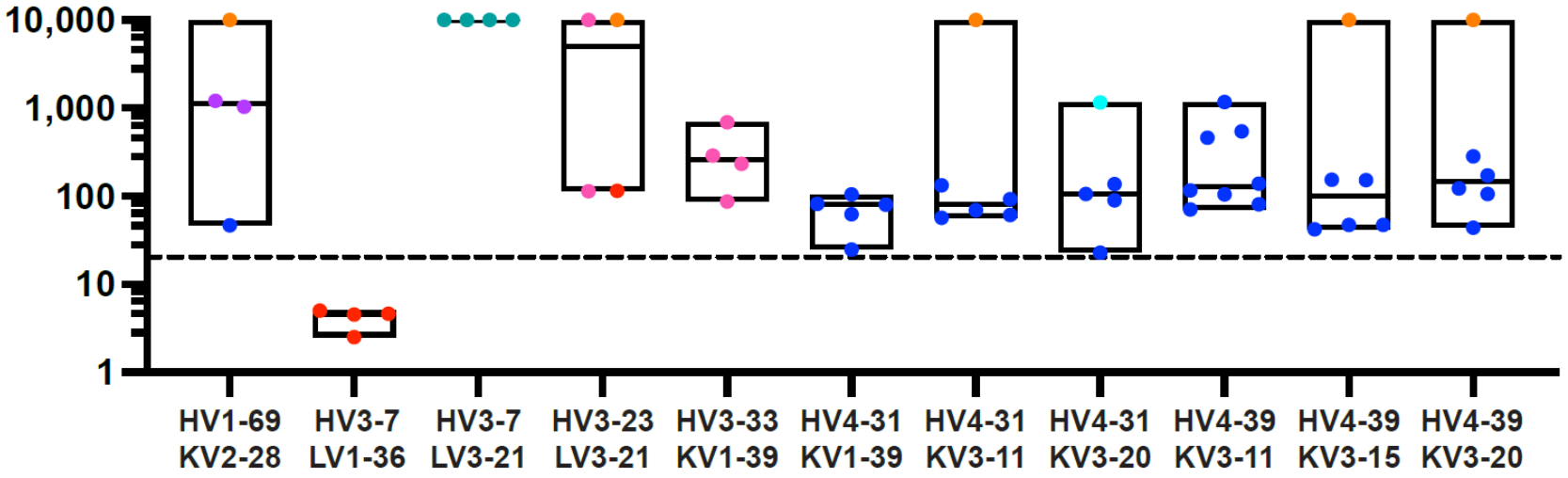
~7X improvement over R5.016 benchmark

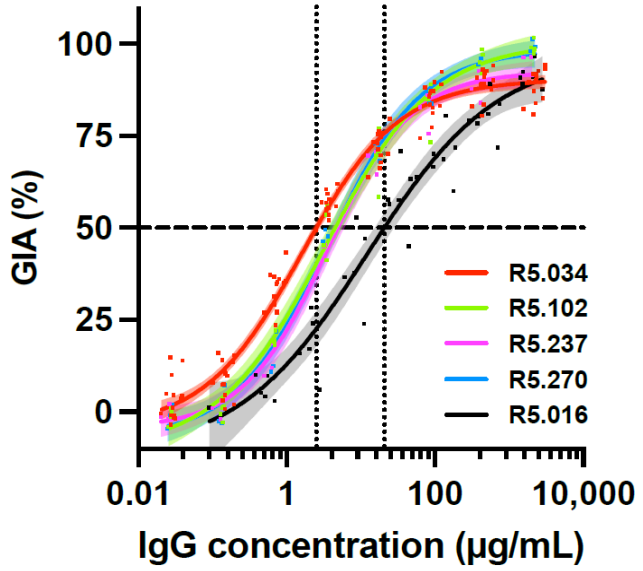
Sequence Predicts a Potent Public Clonotype











2. KINETICS – experiment overview

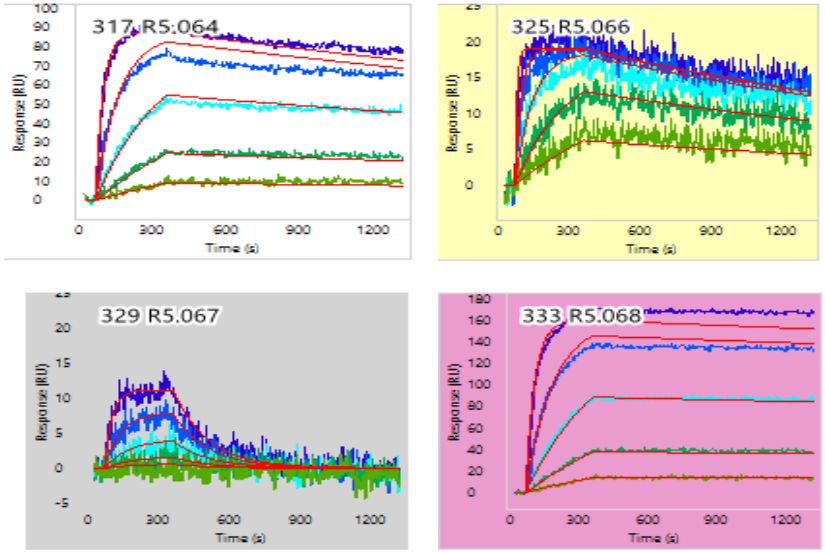
mAbs were captured to a CMDP chip via an Fc-lawn in multichannel mode to create a 384 ligand array

RH5 analyte was injected as a 3-fold dilution series starting at 100nM over the whole array with the single flow cell (SFC)

Following each set of analyte injections (lowest to highest conc), the ligand array was recaptured

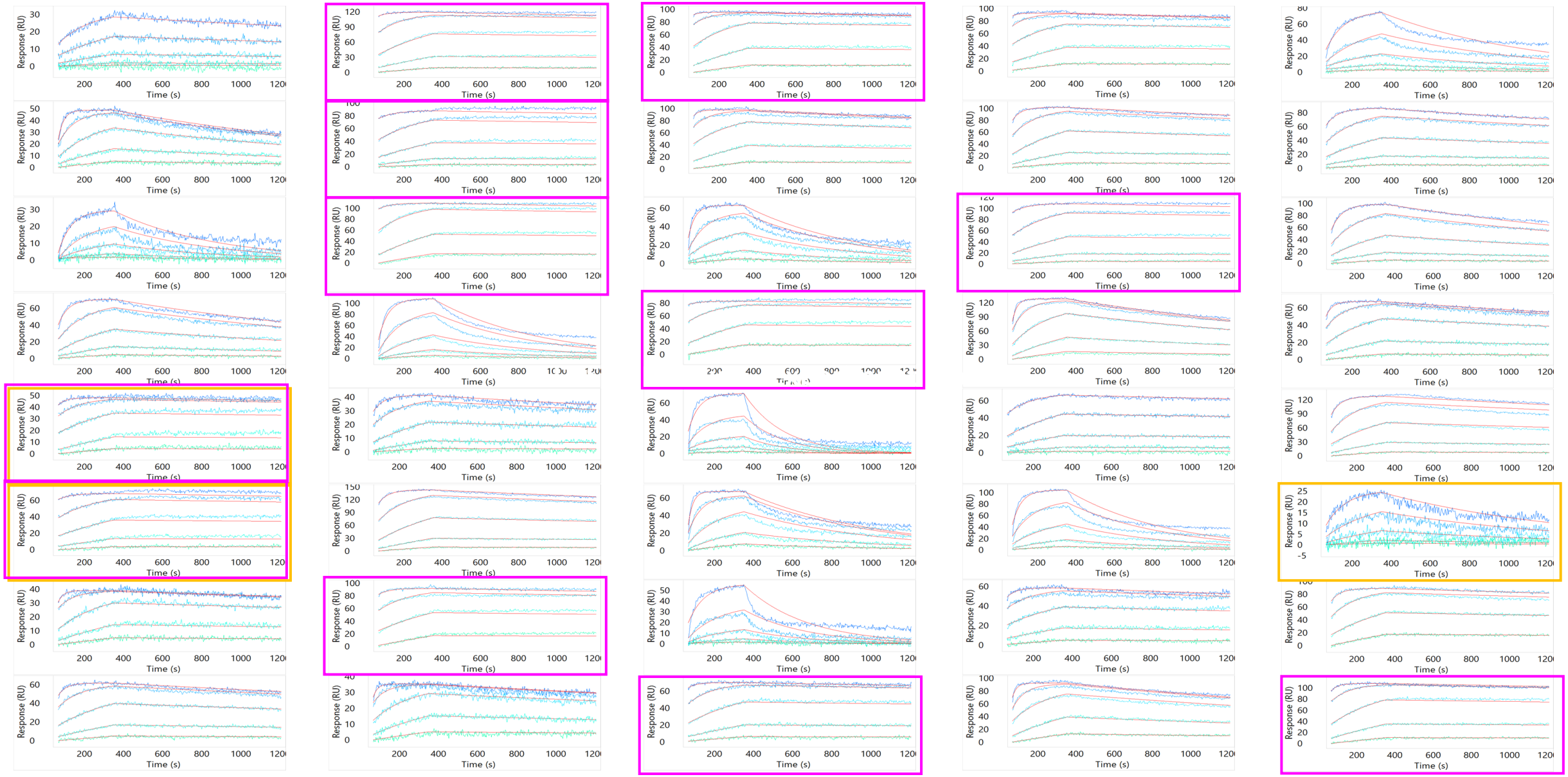
Kinetic parameters using the Carterra Kinetics Software package

Data was referenced, double-referenced and then fitted to the Langmuir model



Software flags ligands for further investigation: **yellow residuals >7% Rmax ; pink dissociation <5%, grey <20RUs**

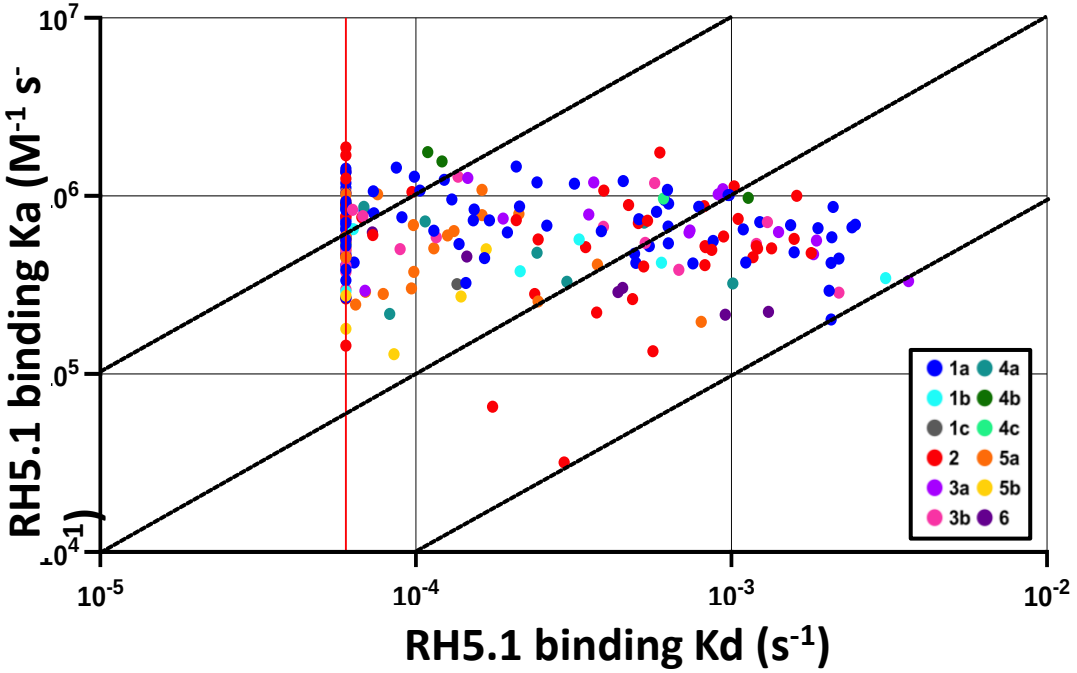
RH5 mAb Kinetics: Antibodies R5.001 to R5.056

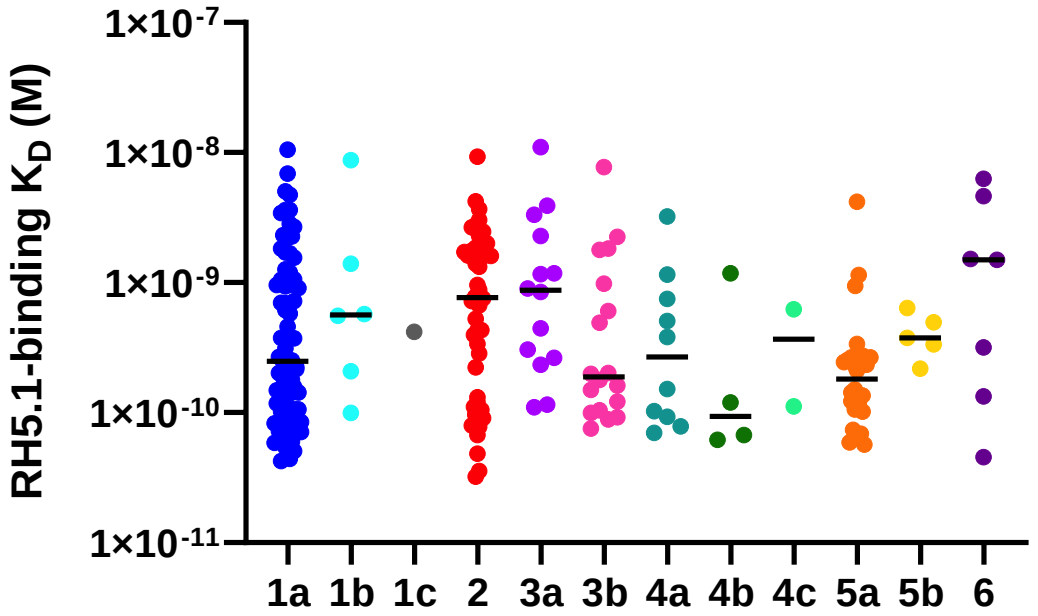


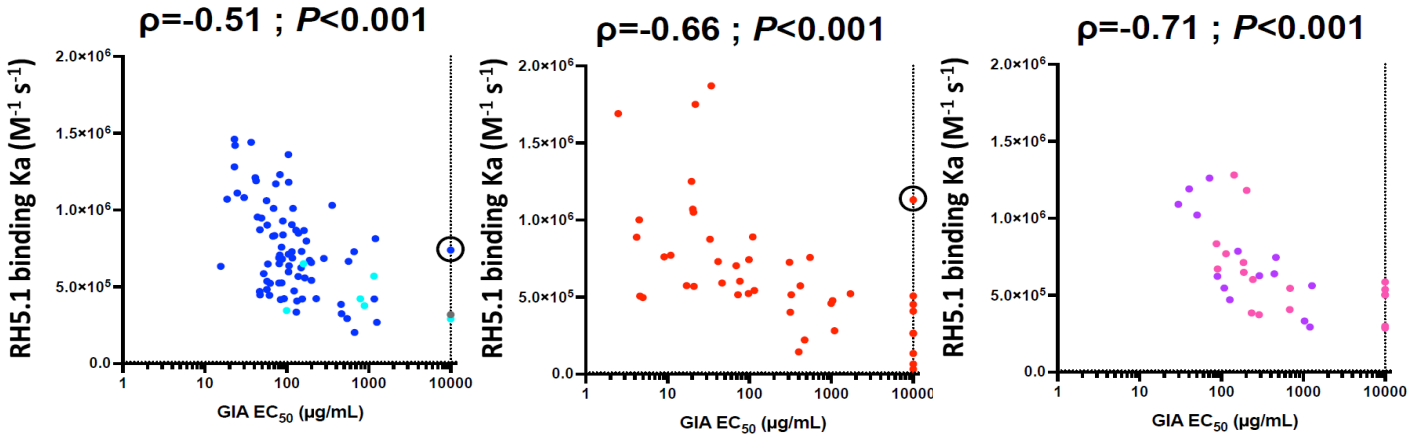
Flags: yellow residuals >7% Rmax ; pink dissociation <5%

carterra

RH5 mAb Kinetics: Antibody Ka rates correlate with GIA activity

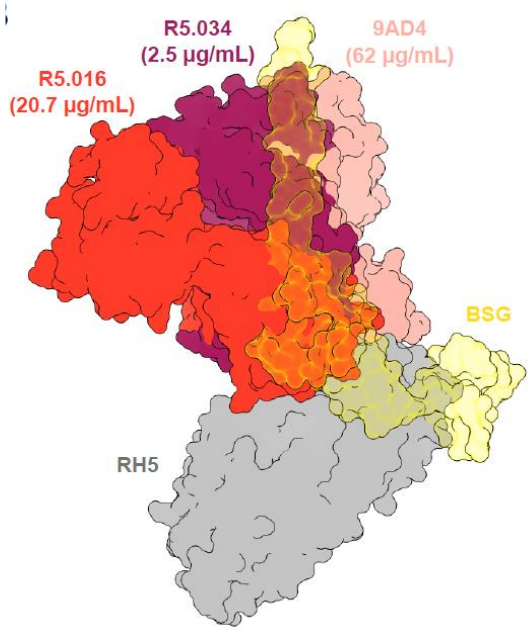


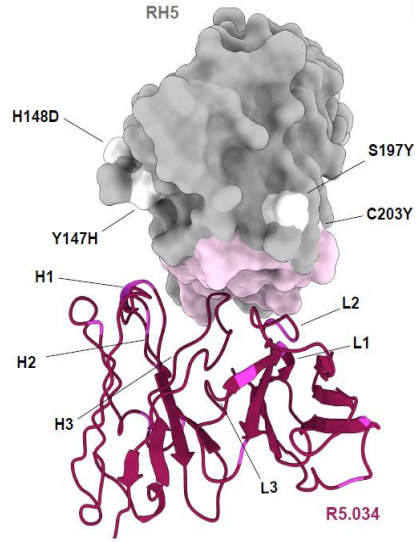


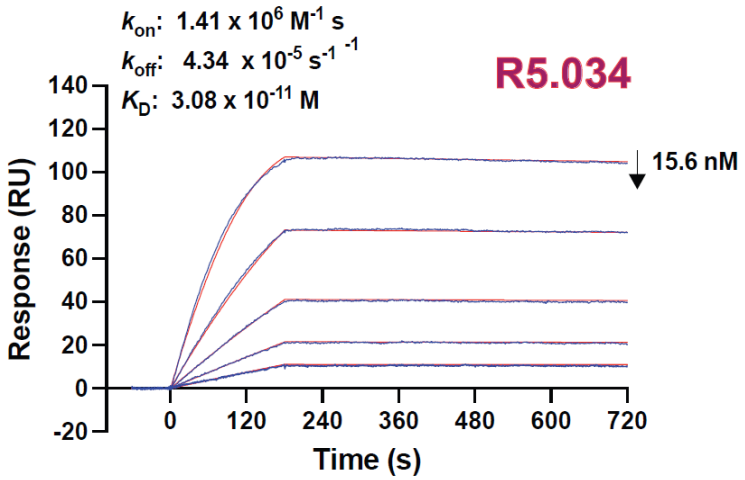


Speed of RH5 binding is a major determinant of mAb potency

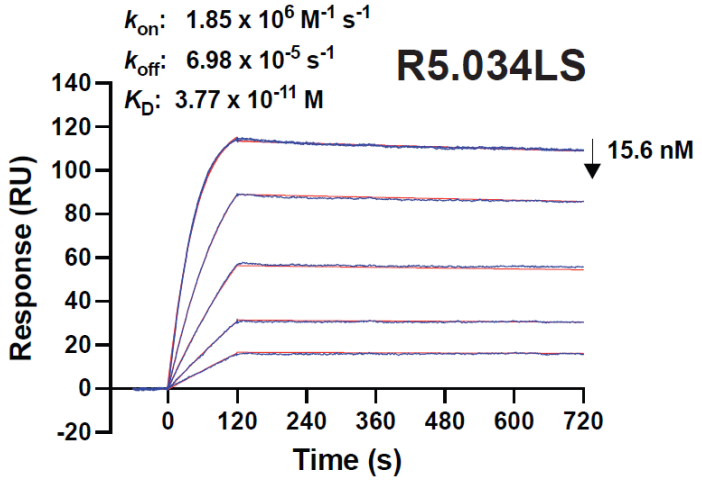
R5.034 – leading blood-stage mAb candidate for clinical development

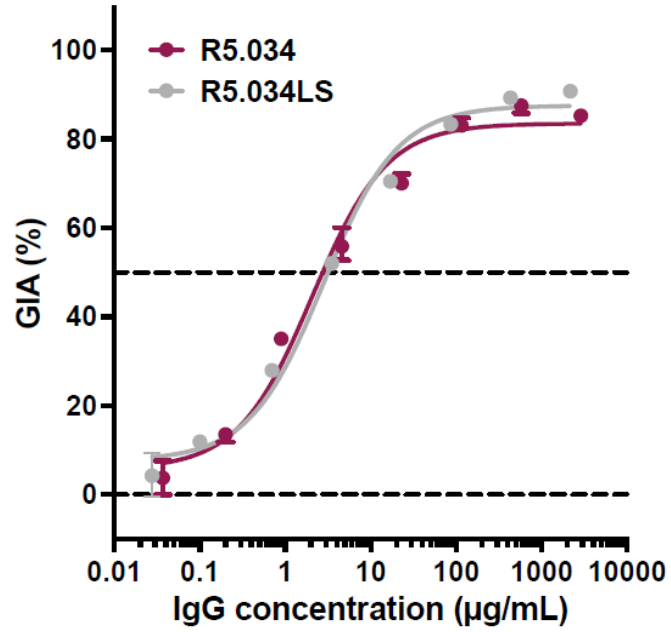


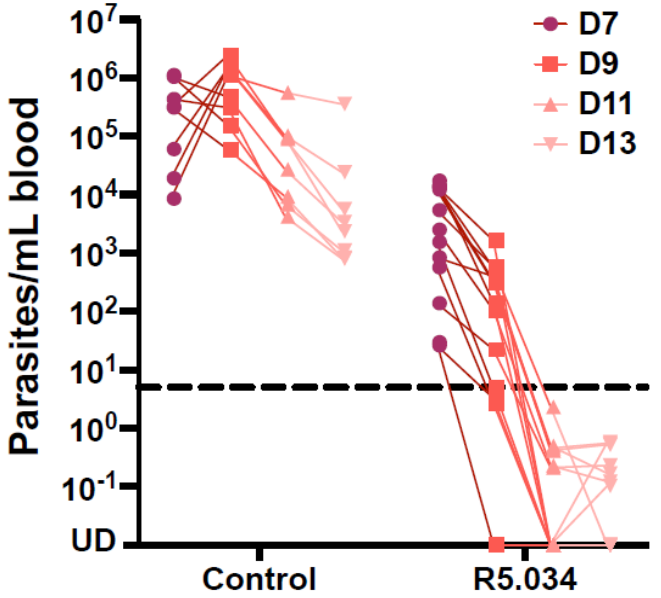




R5.034 kinetic parameters by HT-SPR		
ka (M-1 s-1)	kd (s-1)	KD (M)
1.75E+06	5.37E-05	3.10E-11







Summary

- We have established a pipeline for mAb isolation and HT characterisation that sets a paradigm for our future mAb campaigns

- We have characterised the epitopes on RH5 to a detailed resolution

- Combining epitope information with functionality, kinetics and sequencing information reveals determinants of potency which will aid next generation vaccine design

- We have identified several mAb candidates, including R5.034, for potential therapeutic development

Analysis of the Diverse Antigenic Landscape of the Malaria Invasion Protein RH5 Identifies a Potent Vaccine-Induced Human Public Antibody Clonotype

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Ababacar Diouf

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