High-throughput characterisation of the cattle antibody response to foot-and-mouth disease virus using virus-like particles

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Biotechnology and Biological Sciences Research Council

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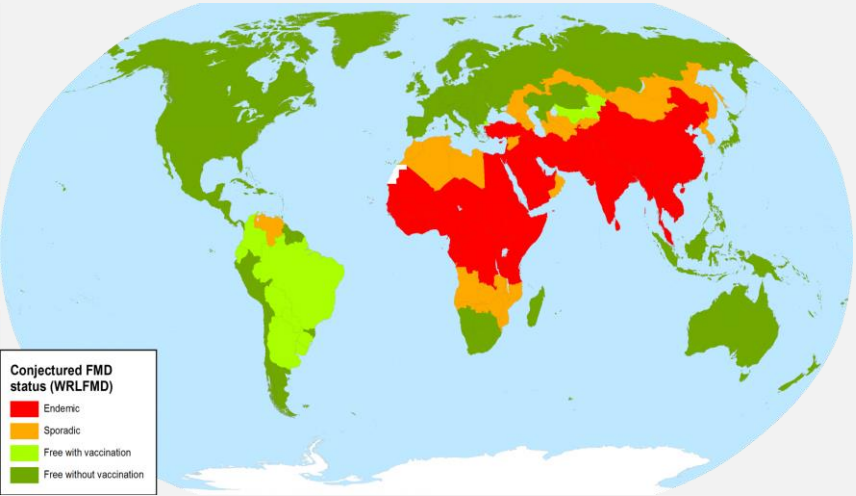
The Pirbright Institute : Who are we?

- World leading research institute and reference laboratory
- Viruses and immune systems of livestock
  - Host/virus interaction
  - Disease control and prevention
  - Vaccinology
  - Epidemiology
- Livestock antibody hub & Immunological Toolbox

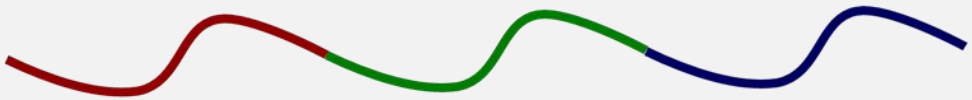
Foot-and-mouth disease virus (FMDV)

- Cloven hoofed animals
  - Vesicles in mouth/on feet
  - Fever
  - Lethargy
- Highly contagious
- Global food security threat
- Economically damaging
  - Germany 2025 estimate €1 billion



- Endemic in parts of Africa, Asia & Middle East (sporadic Europe)
- 7 serotypes
- Vaccines available
  - limited cross protection
  - duration ~6 months
  - difficult to manufacture
  - unstable (cold chain)

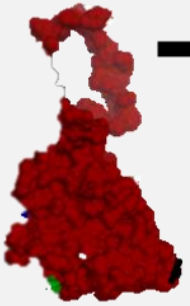
Virus-like-particles: Enabling research at low containment

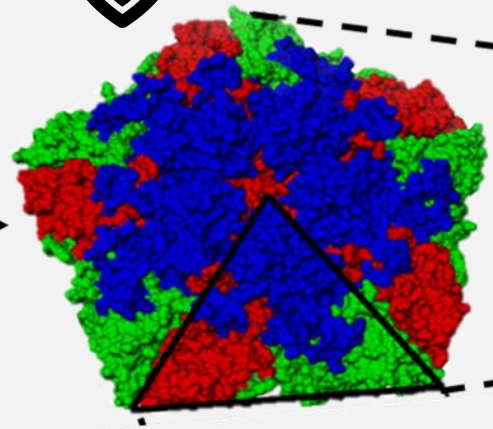


VLPs

- Capsid structure with no infectious ability
- Preserves WT epitopes
- Highly stable through engineering

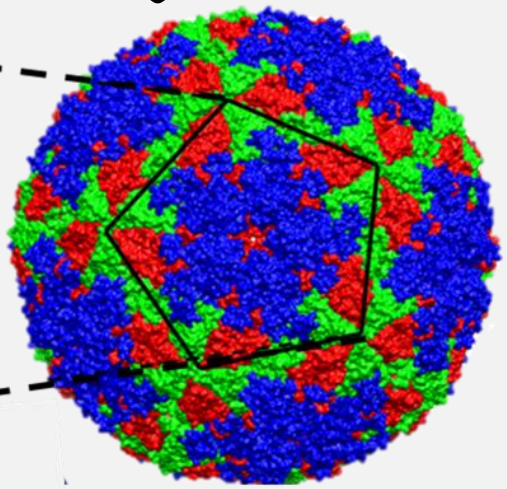
~25kDa





~475kDa

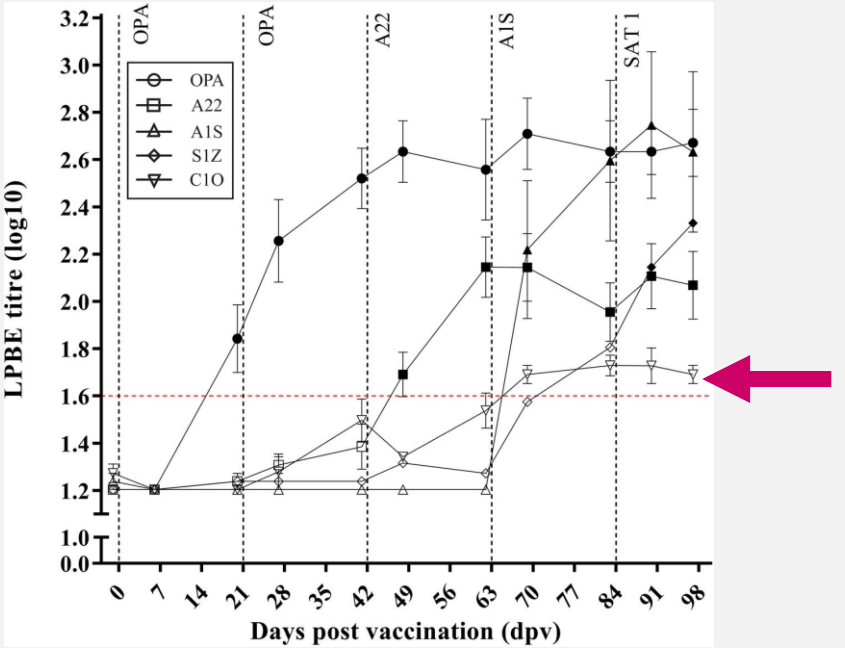
~5700kDa
25-30nm



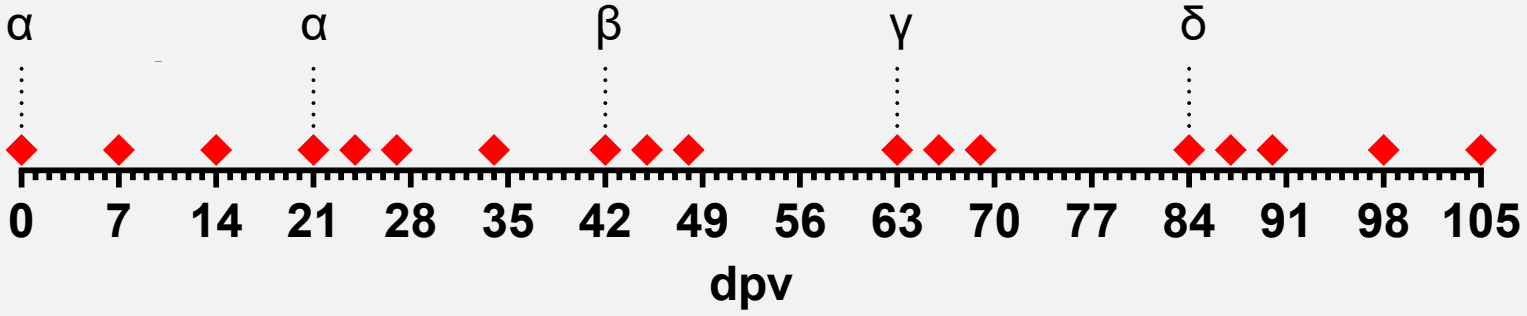
Sequential vaccination promotes cross specific responses to FMDV

- Known to boost cross specific response *(Grant et al., 2017)*

  C serotype naïve in this study but response generated by vaccination with others



- Vaccination study, different serotypes sequentially



Predicting and selecting FMDV specific antibodies

Antibody Sequencing Platforms

Whole Repertoire

Antibody transcript enrichment from PBMC

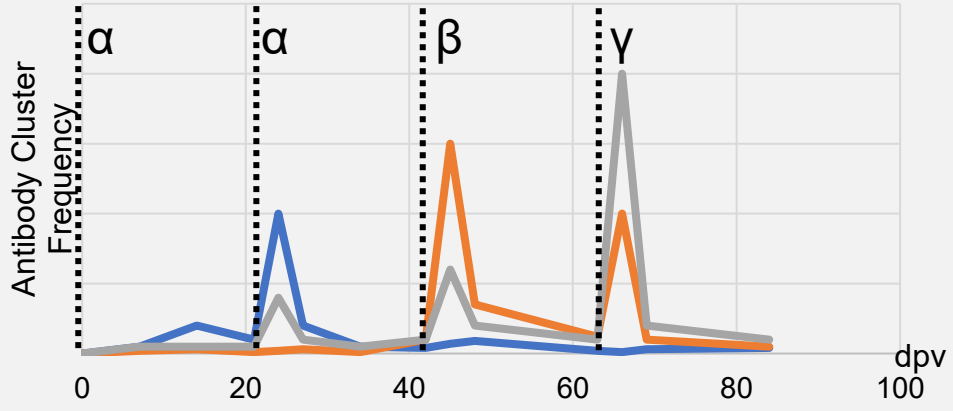
10x GENOMICS

Sorting of FMDV specific B cells & single cell antibody transcript enrichment

AbCellera

Microfluidic screening with FMDV conjugated beads

Antibody Cluster Frequencies



15 **40** **89**

Selected Abs produced with thanks to Pirbright Immunological Toolbox and Twist Biosciences

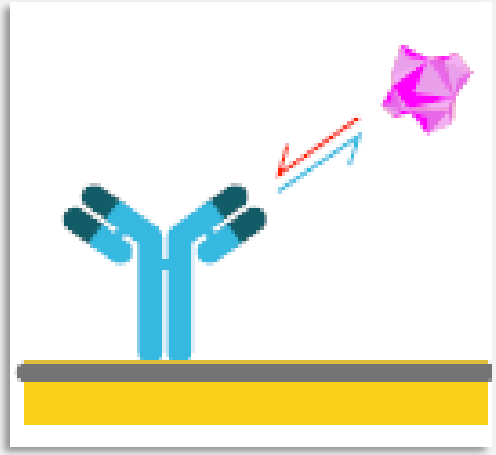
Project Overview

Aims

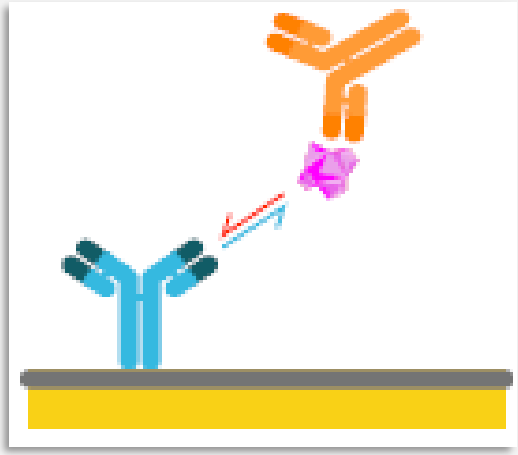
- To characterise specificity of selected antibodies for serotypes of interest
- To identify distinct epitopes relating to cross reactivity

Assays

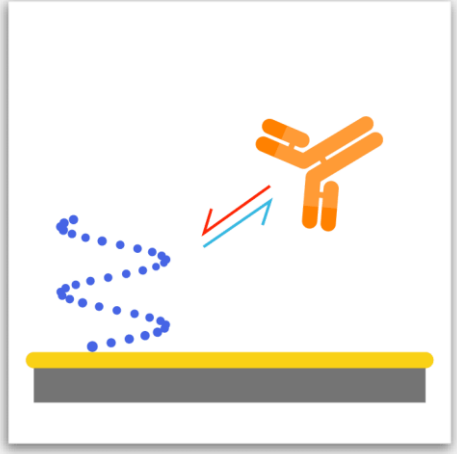
Screening + Kinetics



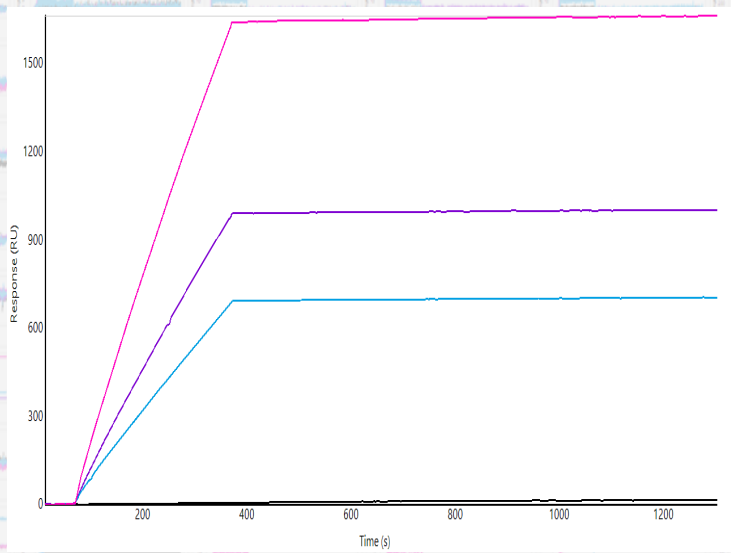
Pre-Mix Epitope Binning



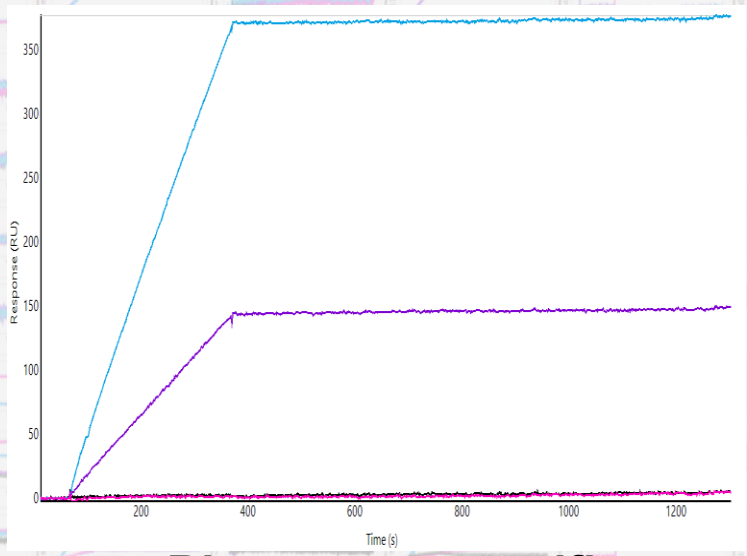
Peptide Mapping



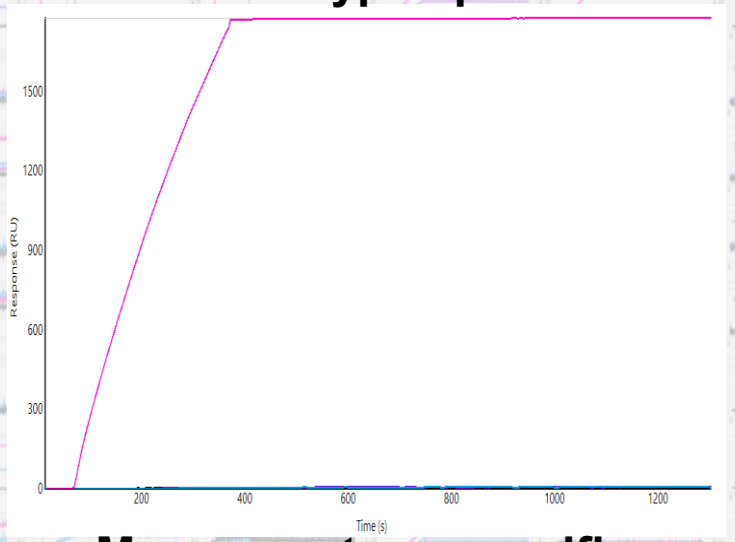
Capsid specificity screening reveals multiple combinations of cross-specificity



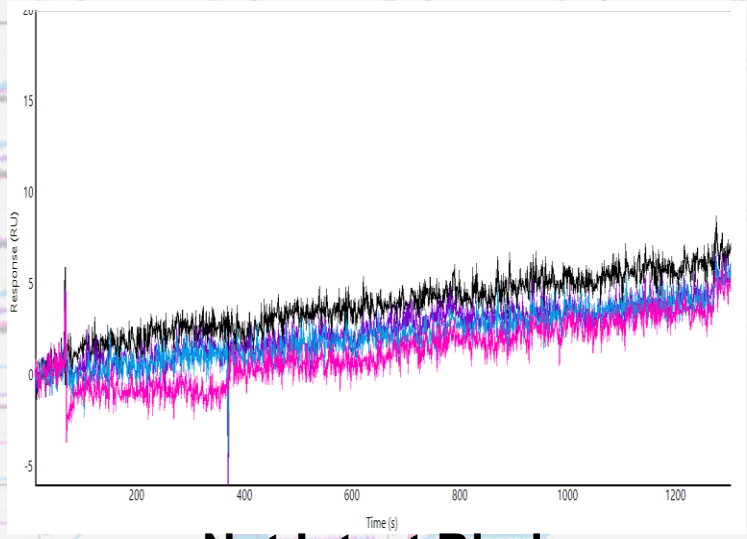
Tri-serotype specific



Bi-serotype specific



Mono-serotype specific



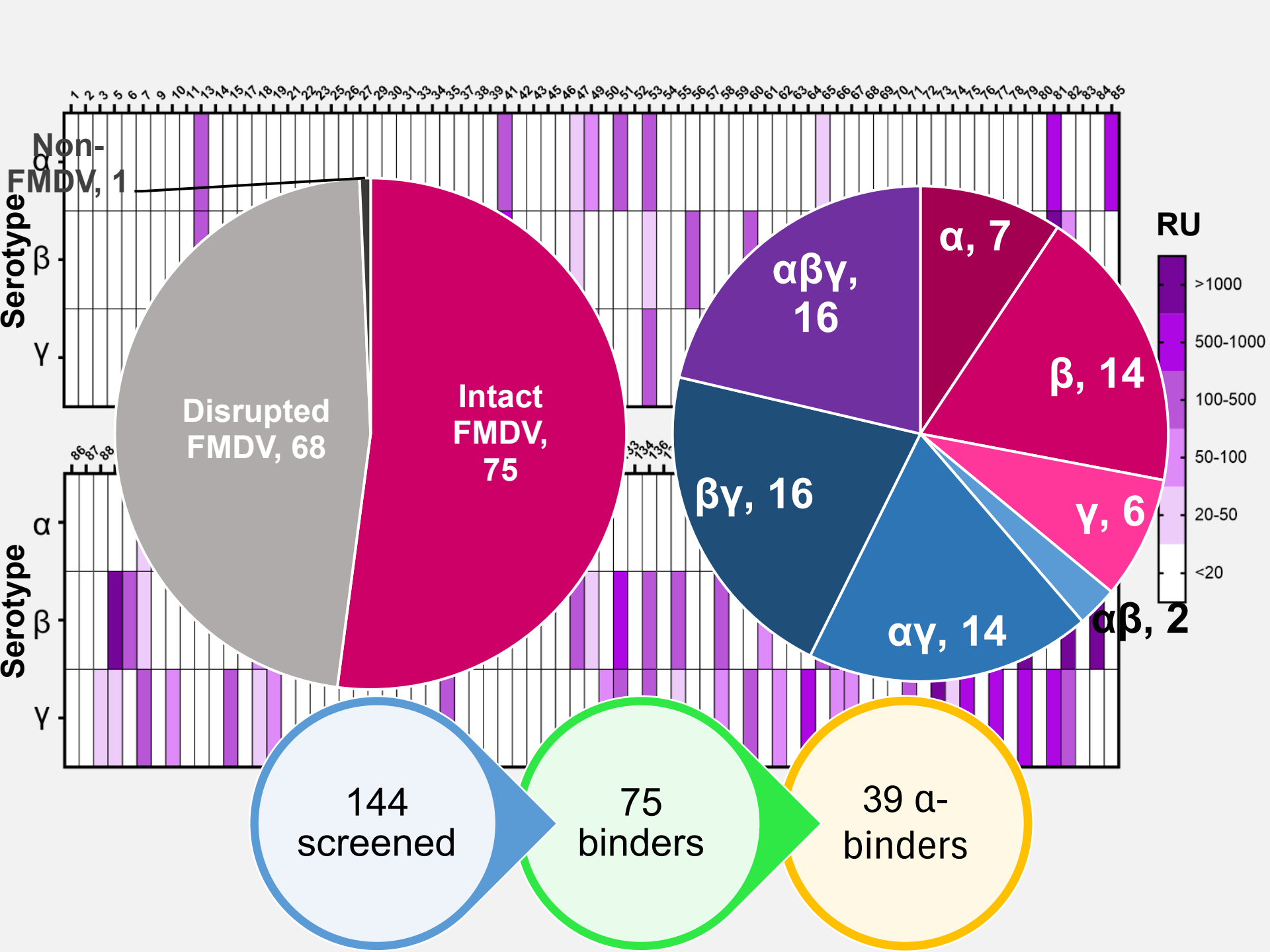
Not Intact Binder

KEY

α

β

γ


Non-FMDV, 1
Serotype
α
β
γ
Disrupted FMDV, 68
Intact FMDV, 75
αβγ, 16
α, 7
β, 14
βγ, 16
γ, 6
αγ, 14
αβ, 2
RU
>1000
500-1000
100-500
50-100
20-50
<20
144 screened
75 binders
39 α-binders

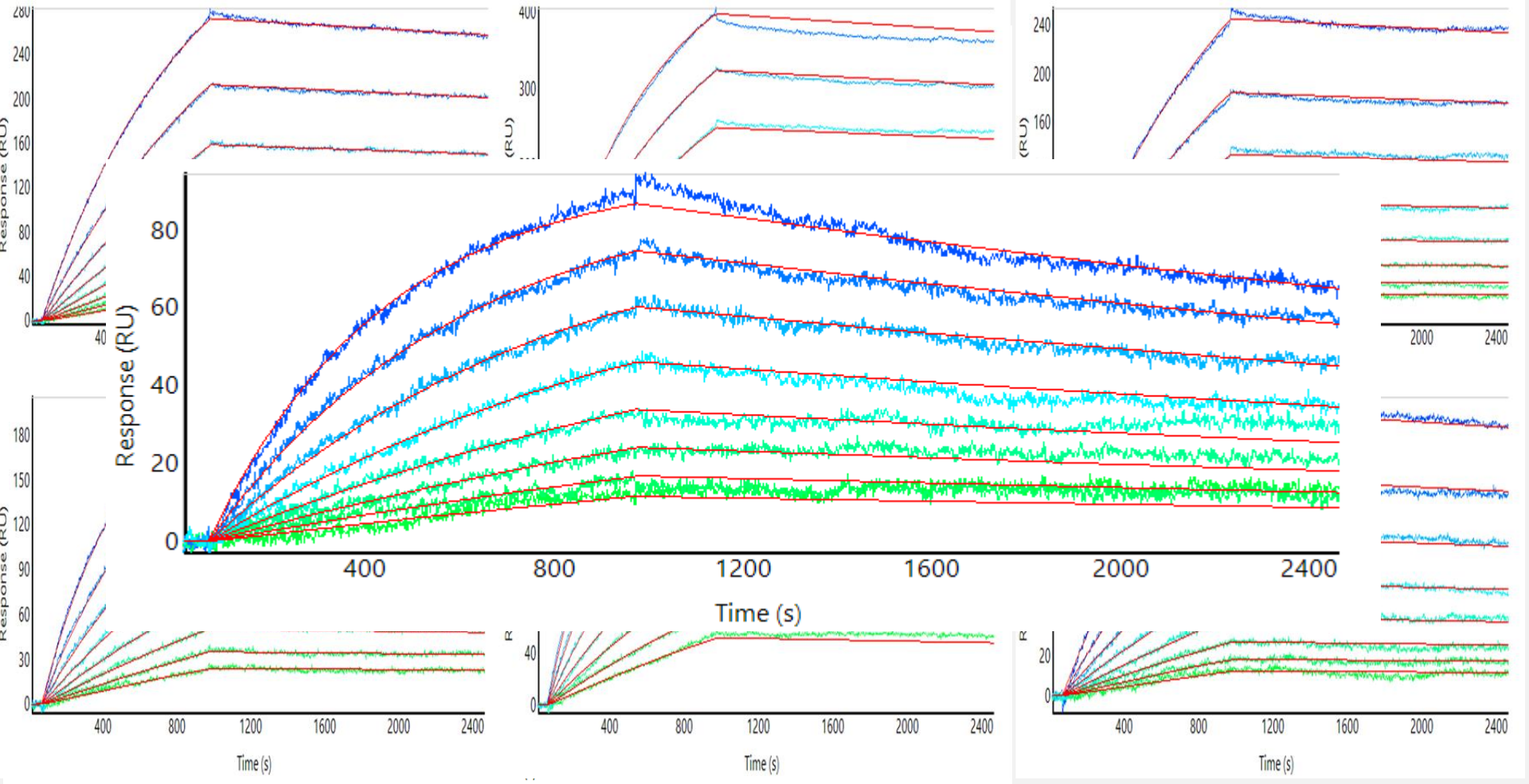
Optimising Kinetics Analysis for VLPs

Challenges

- Multivalency
- Relatively large

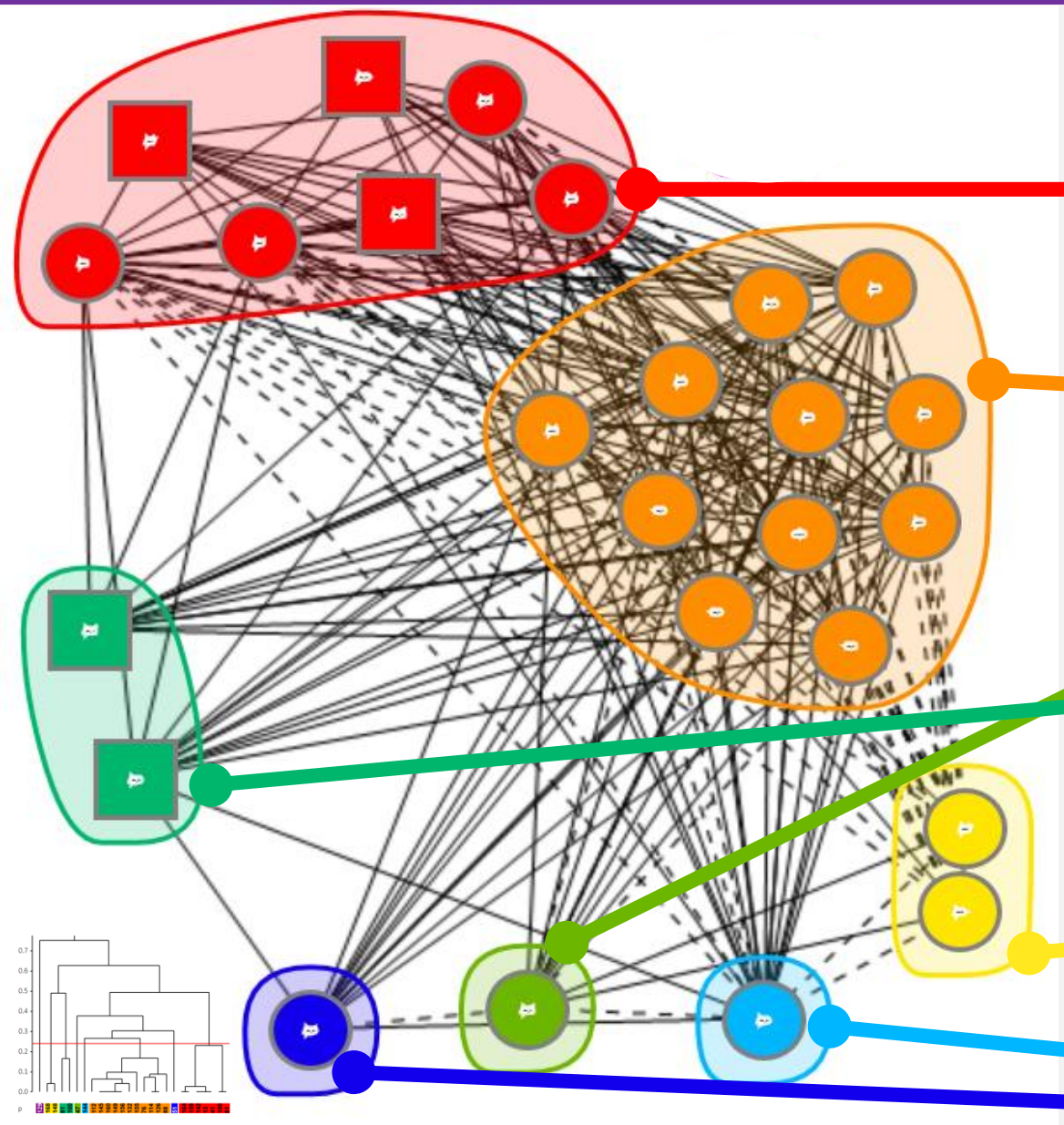
Solutions

- CMDP Chip, Low couple of Ab
- Titrated VLP [ ]



Distinct mono-, bi-, and tri-serotype specific epitopes revealed through epitope binning

39 α-binders



All binders of all THREE serotypes

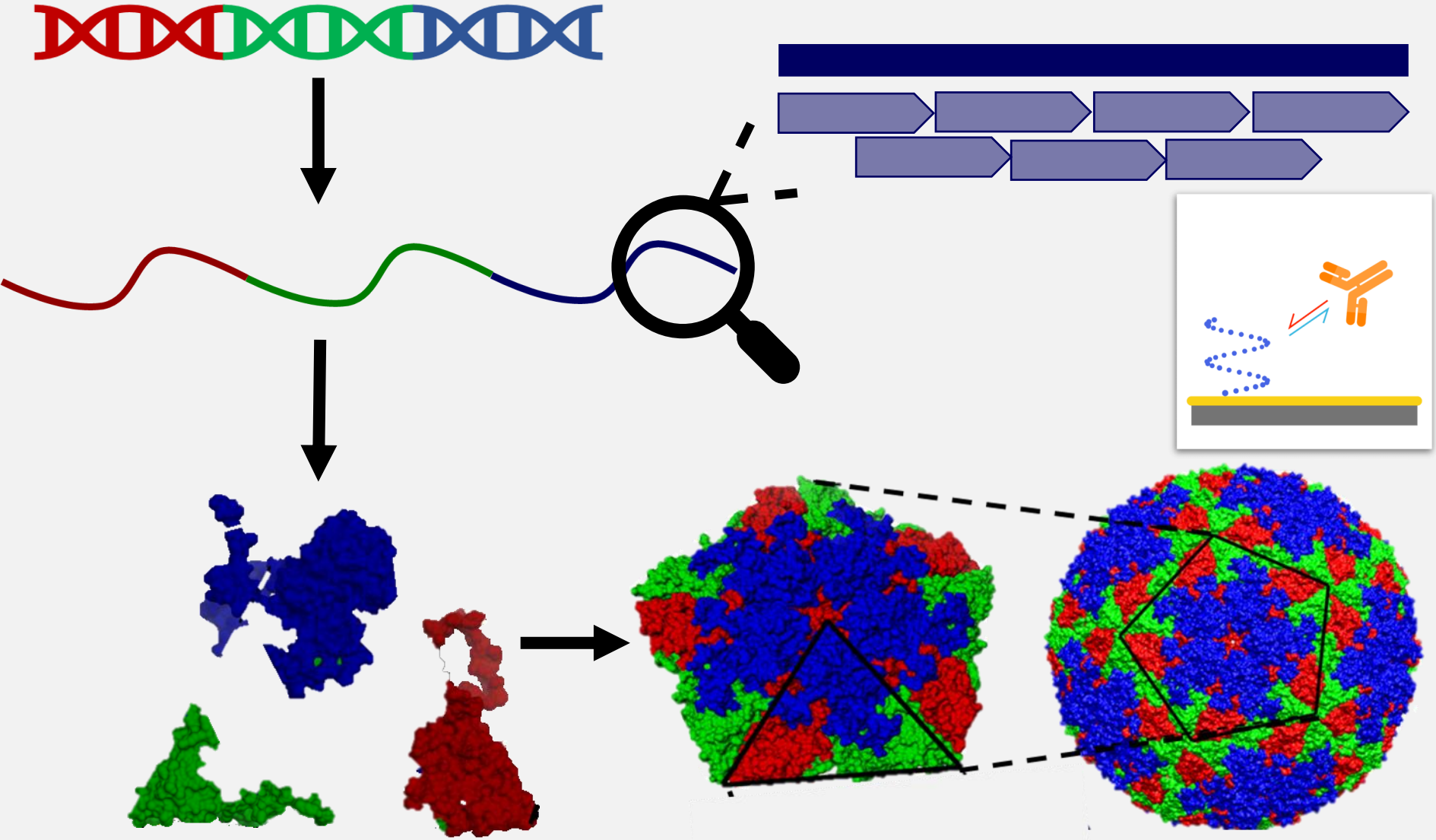
All bind α and γ

Less clear? Distinct Functions?

Non-neutralising binders of α

Only bind α

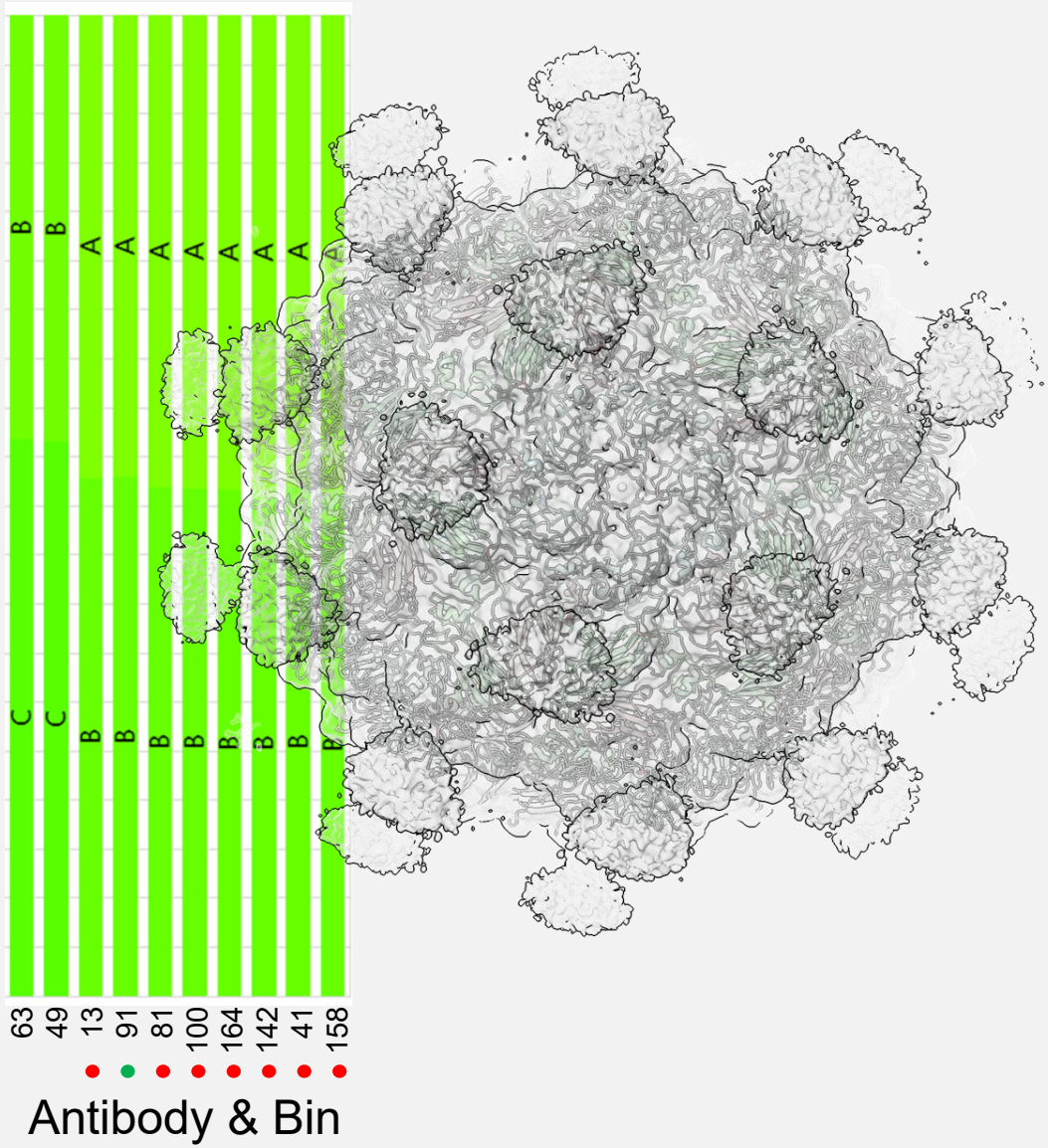
Epitope discovery: Linear mapping



Multi-serotype specific linear epitopes

39 α-binders

- Two linear epitopes recognised by antibodies binding three FMDV serotypes
  - Leaves 6 tri-serotype specific antibodies with conformational epitopes
- Peptide A confirmed as epitope by crystal structure/CryoEM



Summary

Aim 1: To characterise specificity of selected antibodies for serotypes of interest

- ~52% of antibodies bind intact FMDV with range of specificity combinations

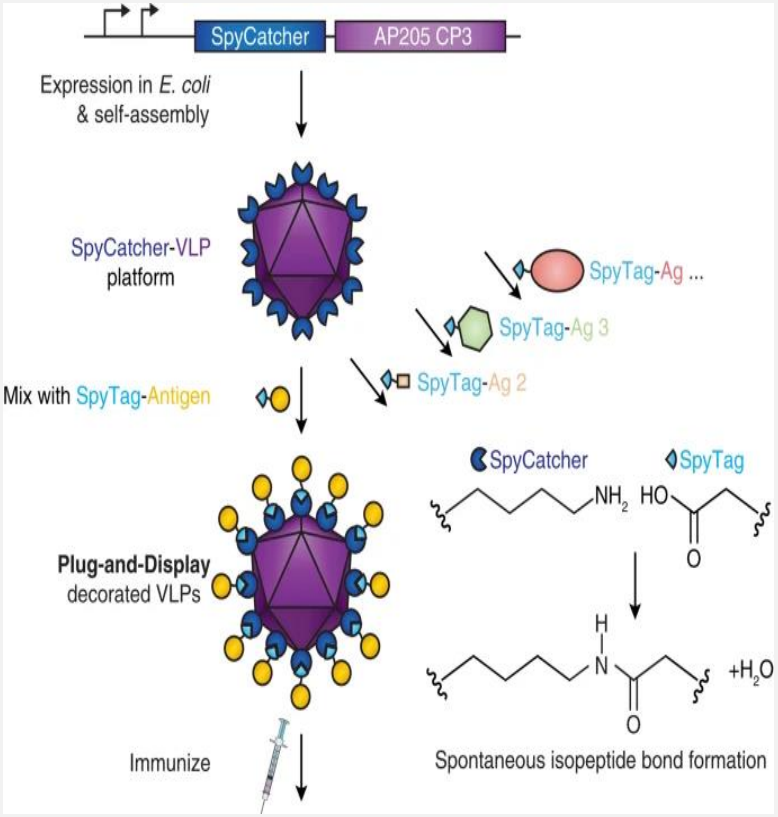
Aim 2: To identify distinct epitopes relating to cross reactivity

- Epitope binning reveals relatively few epitope communities
- Mapping highlights existence of linear and conformational cross-specific epitopes
  - Further characterisation to be performed

Future Work

Expand workflow to fully characterise the FMDV epitope landscape for a serotype

- Epitopes discovered (particularly linear) taken forward to develop peptide display vaccines or engineer capsids for enhanced cross protection.



Revolutionising antibody discovery and characterisation at Pirbright

For the FMDV project alone in our first year

- Without LSA-XT using systems we had before…
  - >220 plates of BLI costing >£120,000!
  - almost 2mg of each VLP
  - 6 MONTHS of continuous BLI runs

- What we actually used…
  - 8 chips (Total cost < £10,000)
  - <100ug of each capsid
  - <4 weeks of LSA-XT runs, largely hands off

Also at Pirbright…

- Systemic Antibody Response to Methanogenic Archaea
- Somamers for next generation COVID LFTs
- HT characterisation of broadly reactive anti-COVID mAbs
- Understanding residue importance in affinity of bovine mAbs

…with interest continuing to come from groups working on a diverse range of animal viruses, and wider immune system interests

Acknowledgements

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