High-throughput antibody discovery and screening

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Carterra Symposium Utrecht

The translational gap

Discovery

Early research

- Strong scientific rationale
- Novel target, mechanism
- Biomarkers

Translation

- Designing molecules/ assays
- Navigating development
- Protecting innovation

Development

- Therapeutics suitable for further development
- Diagnostics ready for clinical trials

Market approval

LifeArc

01

Neuro-degeneration

Translational Challenge:

Motor Neuron Disease

02

Respiratory Health

Translational Challenge:

Chronic Respiratory Infection

03

Global Health

Translational Challenge:

Anti-Microbial Resistance

Neglected Tropical Diseases

Emerging Viral Threats

04

Rare Disease

Translational Challenge:

Centers of excellence

EB repurposing call

05

Childhood Cancer

Translational Challenge:

Currently defining strategy

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LifeArc's translational centres

Edinburgh

- Molecular diagnostics
- Platform and biomarker development
- Full ISO accreditation

Stevenage

- Focus on early therapeutics discovery
- Small molecule and antibody modalities
- Biologics discovery and development, Chemical Biology and Molecular Cellular Pharmacology teams in one place

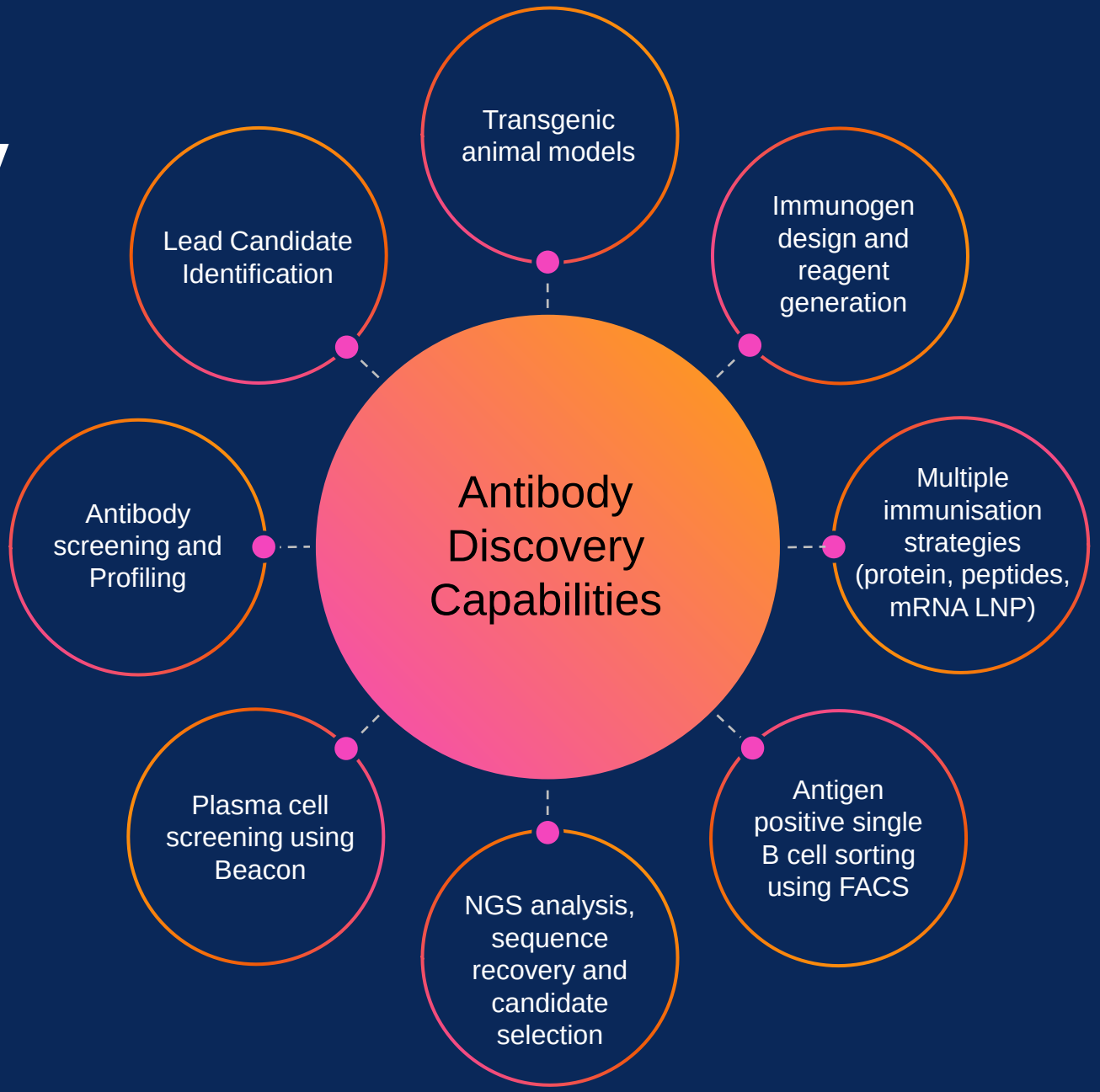
London

- Fully-human antibody discovery platform
- Based at the Francis Crick Institute

LifeArc

Antibody discovery and humanisation

Antibody discovery



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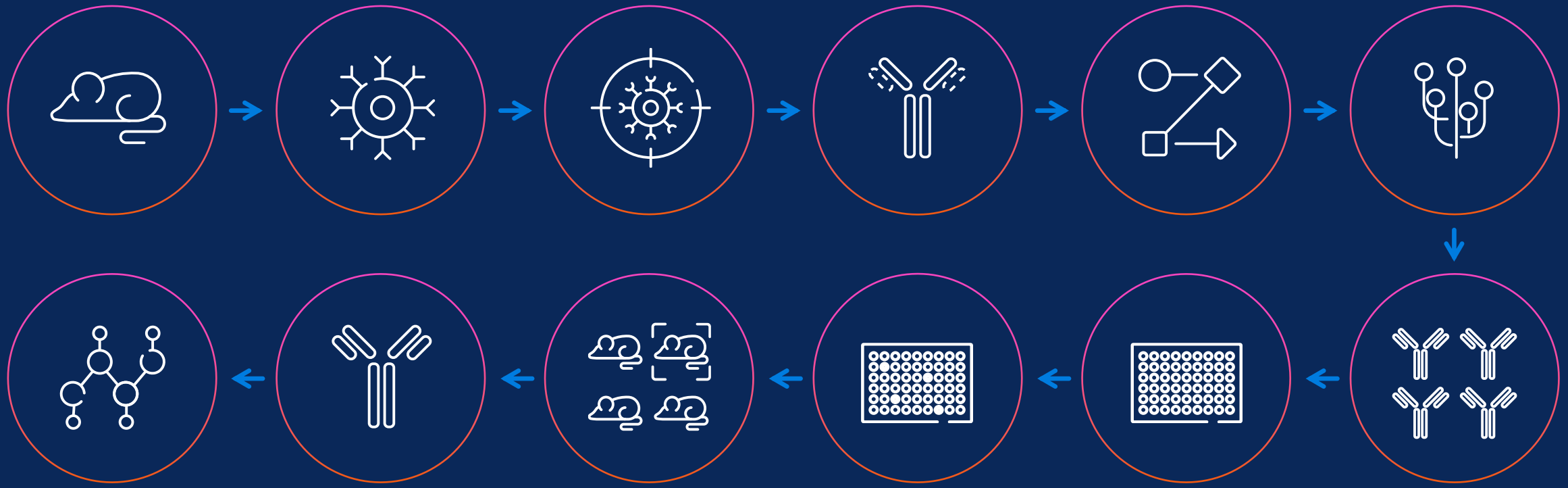
Antibody discovery



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Antibody discovery capabilities:
Single B-cell platform and HT Ab production for screening

Stevenage | **Francis Crick Institute** | **Stevenage**

Reagent generation (mRNA/Cells / Protein) → Transgenic colony management → Immunisation and tissue harvesting → B cell sorting → RT-PCR → Paired H/L recovery and NGS sequencing → B cells lineages clustering & selection → HT candidate production → Hit to Lead screening → Lead generation and characterisation

Access to further transgenic animals

B cell sorting → PCR Ab recovery → Single B cells NGS → Cluster analysis and candidate selection → Small-scale expression → Purification (option) → Quantification → Screening


FACSAria Fusion (BD)


Beacon


Robotic platform (PAA)


MiSeq (Illumina)

Sequencing software

Microlab STAR (Hamilton)

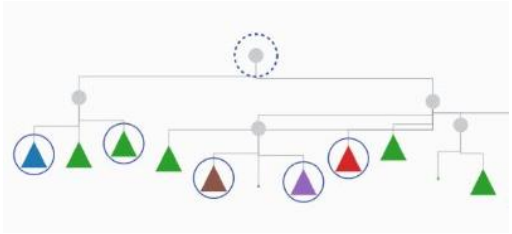

ISF-1X shakers (Kuhner)


Octet RED384 (ForteBio)


Cedex Bio HT Analyzer (Roche)

Streamlining the screening cascade

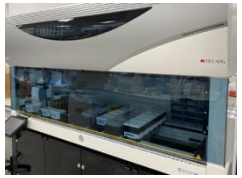
Antibody panel
(~1500s of Antibodies)



geneious biologics

ENPICOM
DECODING THE IMMUNE SYSTEM

Phase 1:
High throughput characterisation
(100s of Antibodies)

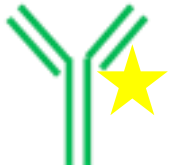


Phase 2:
Lead triaging
(<50 of Antibodies)

	Candidate			
	1	2	3	4
Aggregation				
Thermal stability				
Accelerated stability				
Serum stability				
Solubility				
Charge profile				

Most promising hits are produced in larger amount and comprehensively assessed to identify the lead candidate with the best therapeutic and developability potential.

Lead selection
1-10 antibody



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Antibody humanisation

We can work with parent antibodies from any species: mice, hamsters, rats, rabbits, chickens, camelids...

We have experience of humanising antibodies against different target classes

The therapeutic candidate molecules can be of various modalities: IgG of different subclasses or with tailored effector function or Fab, VHH, VHH-Fc, VH-Fc...

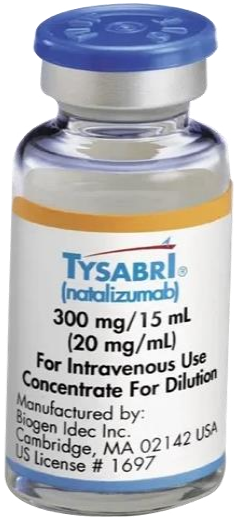
Our humanisation projects focus on reproducing the binding and functional properties of the parent molecule whilst maximising developability

LifeArc

Our Success

Entyvio
(Crohn's Disease)

Actemra
(Rheumatoid Arthritis)



Tysabri
(Multiple Sclerosis)

Keytruda
K(Cancer)

Leqembi
(Alzheimer's)

- We have over 30 years of experience
- 98% success rate
- ~20 antibodies, humanised by us are currently in clinical pipelines

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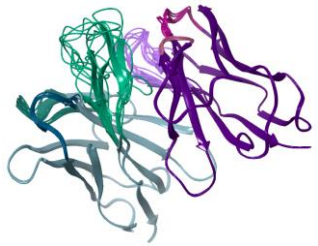
Humanisation design for an IgG



Parent VK and VH sequences

In silico modelling (X-ray crystallography facilities)

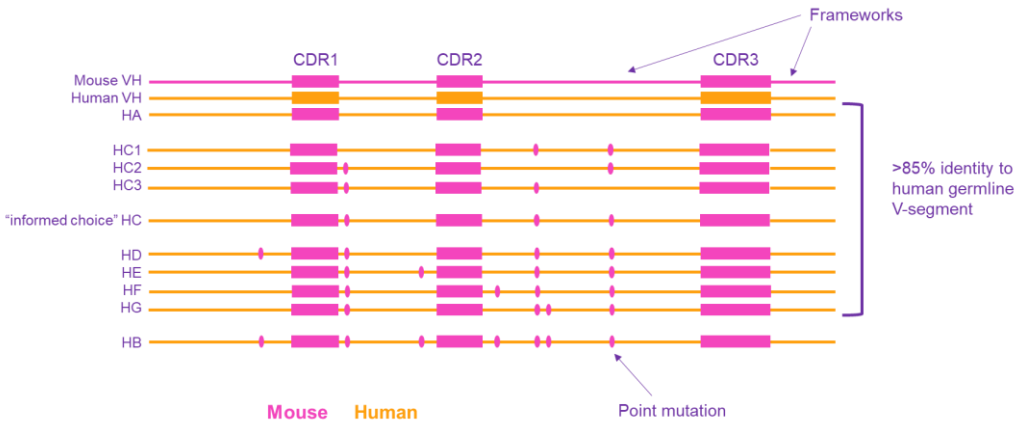
Possible human frameworks

Filtering parameters

- Matching key residues
- Overall similarity
- CDR length
- % human

Best frameworks

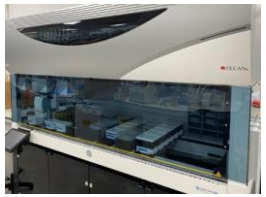
Design of humanised variants

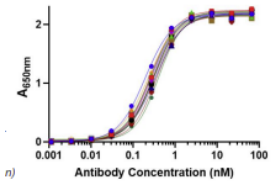


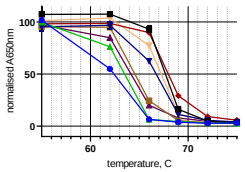
Screening of humanised variants

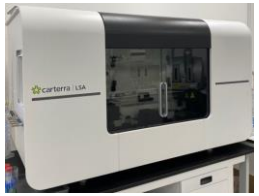
~300 Variants

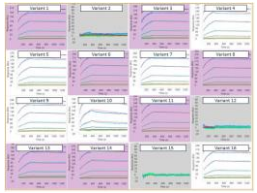
Automated high throughput expression for screening











4-6 variants

Lead candidate panel assessment

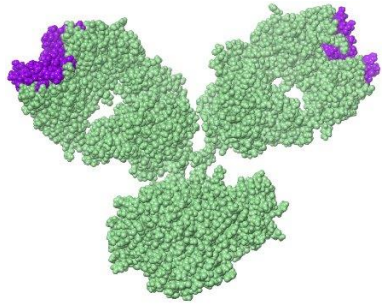
Developability assessment with Pharma –standard suite of assays

Collaborator's functional assays (10 mg of each antibody supplied)

1-2 variants

Lead and backup antibodies

Delivery of lead antibody with binding and functional properties of the parent molecule and maximised developability



- ✓ Comprehensive humanisation report to assist with patenting and publications
- ✓ Ongoing translation Advise
- ✓ Collaborator retains IP ownership and onward commercialisation control

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Developability Assessment

Tm
Tagg
Intrinsic fluorescence, SLS (Uncle)

Non-specific protein-protein interactions
Cross-interaction chromatography

Solubility
Solvent absorption

Glycan profiling
Mass spec

QC
Mass spectrometry SEC-MALS / DLS / cIEF / CE-SDS

Accelerated stability:
Heat, Freeze/thaw and pH stress

Propensity for Asp isomerisation or Asn deamidation
Mass spectrometry

Serum stability
Human, mouse, cyno: retention of binding

Pharma-standard assays to de-risk clinical development of antibodies

LifeArc

Making more informed decisions quicker

Triaging Required for Kinetics and Affinities

- Throughput of the System
- Sample consumption

Improvements in automations, reduced sample consumption, and optimisation in Data workflow

Quantification: Octet, Bioanalyser HT	**Binding - ELISA** Binding off-rate ranking - Octet Binding to cells Flowcytometry	**HIT Specificity -** ELISA and Flow	**Kinetics -** Biacore	**Epitope binning -** Biacore

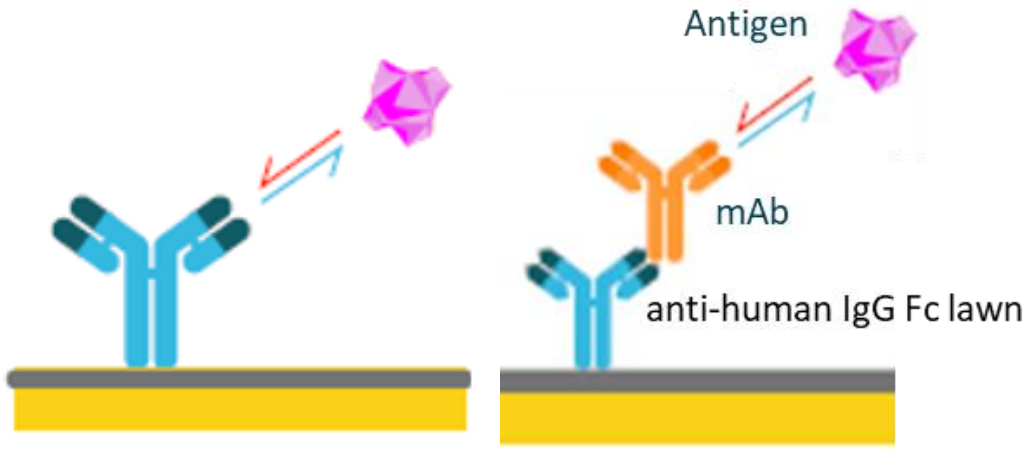
- Carterra LSA allows Kinetics, Specificity and Epitope binning on larger number of Candidate

- More data collected
- More informed decisions on which candidates to carry forward

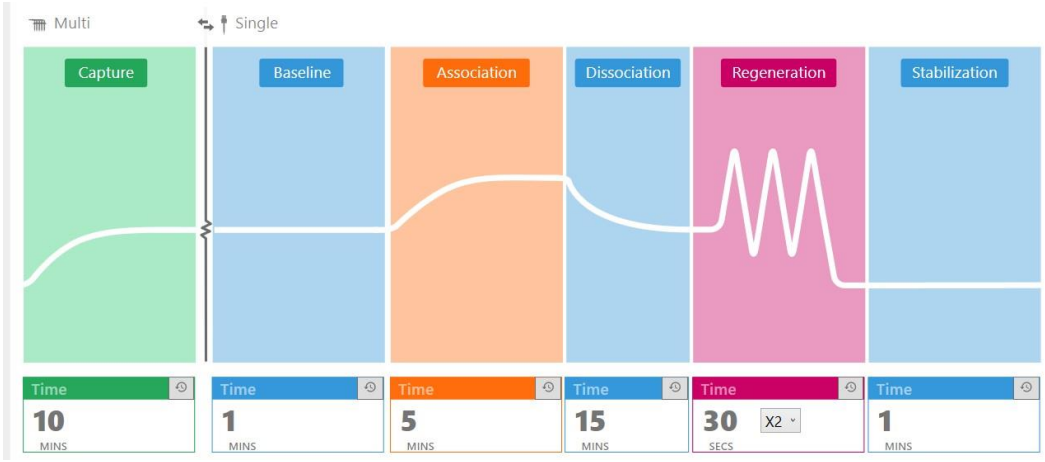
Kinetics

Kinetics

- Detailed kinetic analysis (ka/kd) and/steady state affinity
- Covalent or non-covalent attachment
- Crude or purified sources



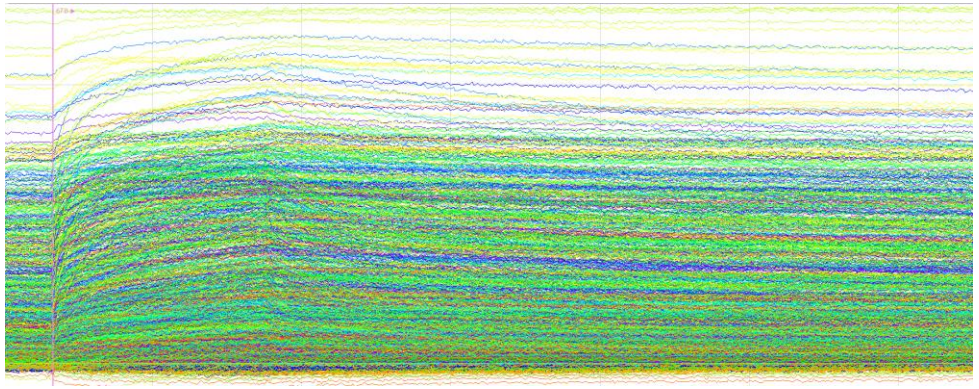
Assay set up

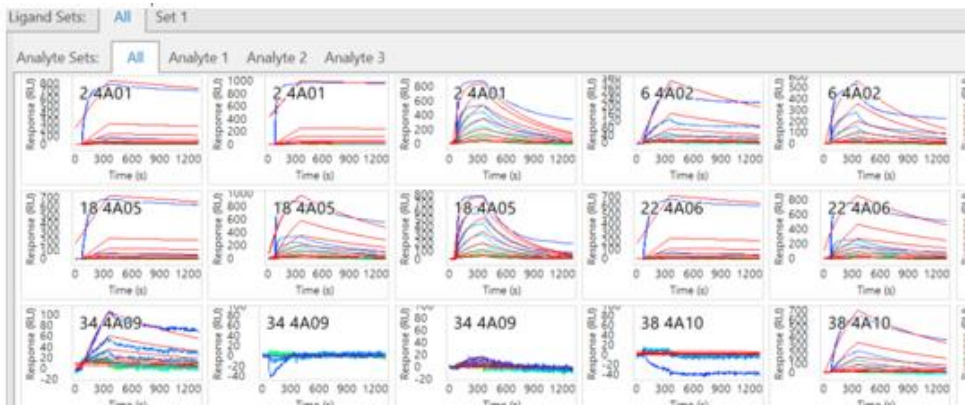


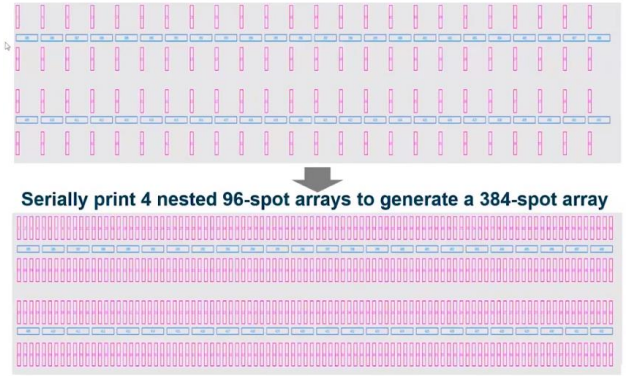
Data processing

Raw data

- Reference data
- Y-off set
- Double reference
- Y align serial view
- Apply baseline correction
- Crop
- Fitted using *ka*/*kd* model

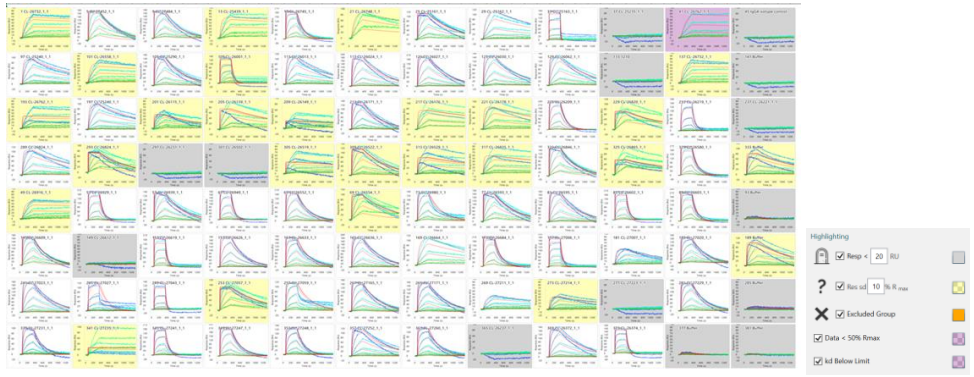




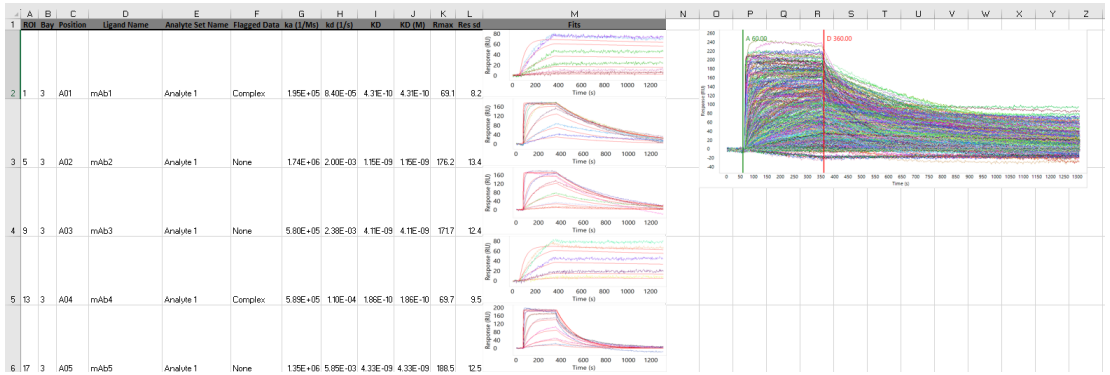


Kinetics Overview

Array view



Kinetics snapshot

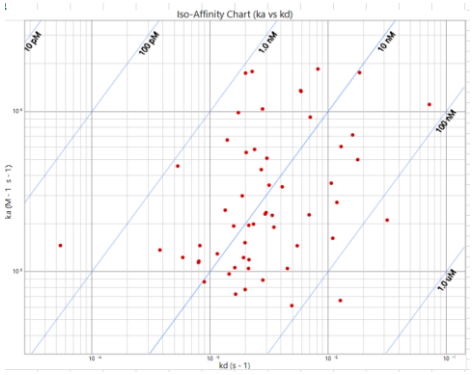


Kinetics table

	A	B	C	D	E	F	G	H	I	J	K	L
1		Link Group 1										
2					Analyte 1							
3		ROI ID	Name	Group	k_a (M-1 s-1)		k_d (s-1)		k_D (M)	Rmax (RU)		
4					Value	Error	Value	Error		Value	Error	Res SD
5		1	mAb1	Set 1	1.95E+05	± 1.1e4	8.40E-05	± 7.4e-6	4.31E-10	6.91E+01	± 1.2	8.24E+00
6		5	mAb2	Set 1	1.74E+06	± 7.8e4	2.00E-03	± 2.2e-5	1.15E-09	1.76E+02	± 1.4	1.34E+01
7		9	mAb3	Set 1	5.80E+05	± 2.7e4	2.38E-03	± 3.1e-5	4.11E-09	1.72E+02	± 1.6	1.24E+01
8		13	mAb4	Set 1	1.10E-04	± 7.9e-6	1.86E-10	6.97E+01	± 1.2	9.51E+00		
9		17	mAb5	Set 1	1.35E+06	± 6.7e4	5.85E-03	± 1.1e-4	4.33E-09	1.88E+02	± 1.6	1.25E+01
10		21	mAb6	Set 1	2.91E+06	± 2.3e5	3.93E-04	± 1.2e-5	1.35E-10	9.35E+01	± 2.8	1.17E+01
11		25	mAb7	Set 1	6.65E+05	± 4.0e4	1.40E-03	± 1.9e-5	2.11E-09	1.14E+02	± 1.2	1.14E+01
12		29	mAb8	Set 1	1.23E+05	± 5.1e3	5.88E-04	± 7.9e-6	4.79E-09	1.20E+02	± 1.8	6.13E+00
13		33	mAb9	Set 1	1.11E+06	± 1.6e5	7.21E-02	± 9.2e-3	6.51E-08	1.72E+02	± 3.6	1.43E+01
14		37	mAb10	Set 1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
15		41	mAb11	Set 1	1.46E+05	± 1.0e4	5.41E-05	± 5.2e-6	3.71E-10	9.77E+01	± 3.8	5.03E+00
16		45	IgG4 isotype control	Set 1	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A

Name	k_D (M)
mAb1	4.31E-10
mAb1	3.48E-10
mAb23	6.91E-09
mAb23	6.99E-09
IgG4 isotype control	N/A
-ve control	N/A
Buffer	N/A

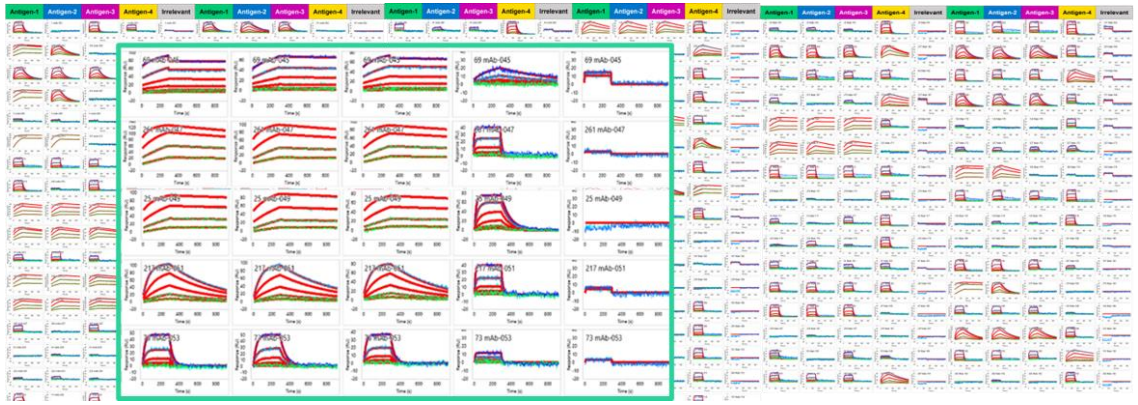
Iso-affinity plot

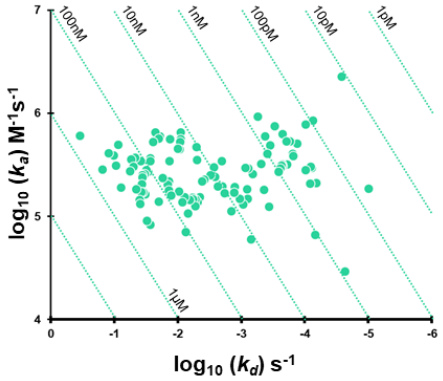


Affinity and Cross Reactivity

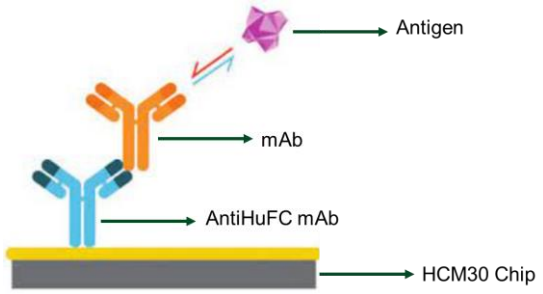
- 192 mAb supernatants tested at 10ug/ml
- Screened against 5 antigens
- HC30M chip used
- Same screen via ELISA with analysis took 1.5 months

Cross-reactivity	No candidates
Hu Antigen-1/cyno	89
Hu Antigen-1/mo/cyno	48





- 123 mAbs bound & 27 mAbs bound with sub-nanomolar affinity
- 35 mAbs bound to Antigen-1
- 88 mAbs bound to Antigen-2



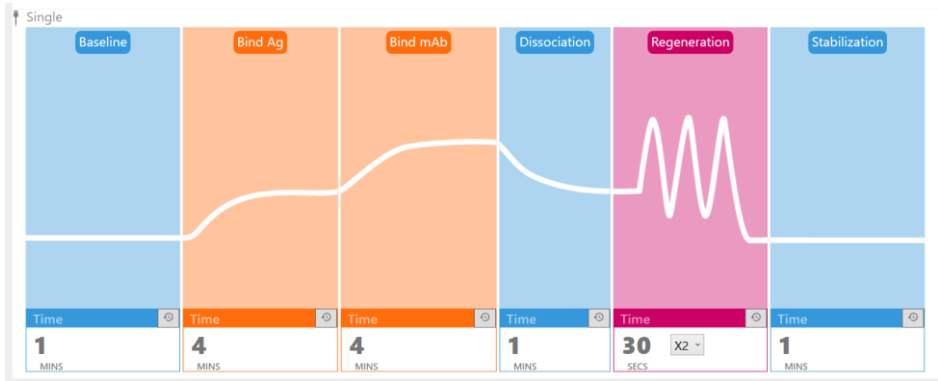
Epitope Binning

High Throughput Epitope Binning

Purified Antibodies

- Purified mAb are covalently coupled to the chip surface
- Antigen is first injected over the covalently coupled mAb-surfaces followed by the injection of the second mAb
- After the end of each cycle, the covalently coupled mAb surfaces are regenerated
- Cycles are repeated, 49x49 mAbs were tested

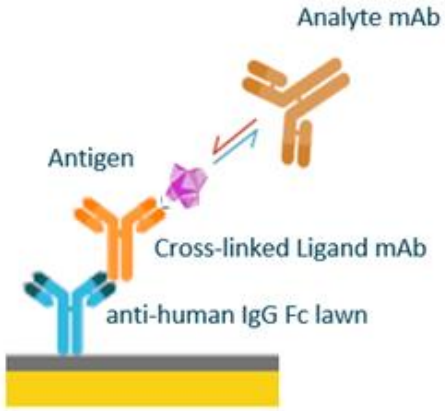
Monovalent Antigen: Classical Binning



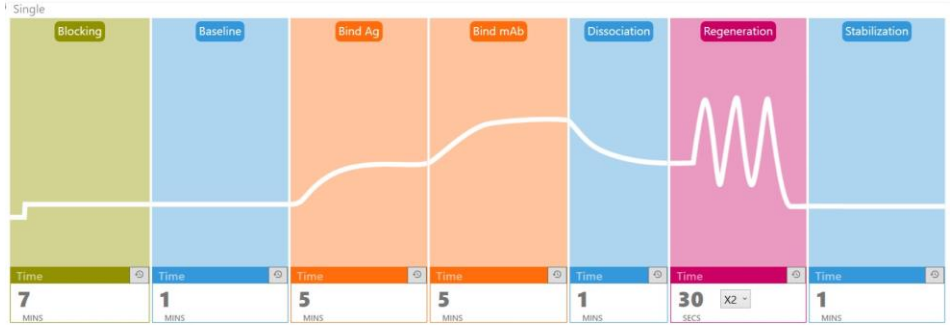
High Throughput Epitope Binning

Supernatants

- An Anti-human IgG FC surface using the standard EDC/NHS surface chemistry is made
- mAbs are captured Anti-human IgG FC surface then crosslinked using BS3
- Following a blocking injection of irrelevant human IgG, Antigen is first injected over the covalently coupled mAb-surfaces followed by the injection of the second mAb
- After the end of each cycle, the covalently coupled mAb surfaces are regenerated
- Cycles are repeated, 56x56 mAbs were tested



Monovalent Antigen:
Classical Supernatant Binning



Epitope Binning with Sups

- When we had a well-behaved antigen this worked well and we able to screen 56x56 antibodies
- Issues with unstable antigen, or when the antigen is not monovalent some further optimisation required.
- If the Antigen is unstable the experiment can be split into smaller number of cycles (20-30 mabs a day), the data can then be merged and analysed together. Temperature can also be reduced
- If the antigen is not monomeric the antigen and second antibody can be pre-mixed before flowing on the chip
- Stickiness to the of antigen or sups – include 0.1-0.5% BSA into the buffer
- The experiment is cleaner with purified antibodies, we have introduced HT plate-based purification
- Important to include both positive and negative controls within the panel

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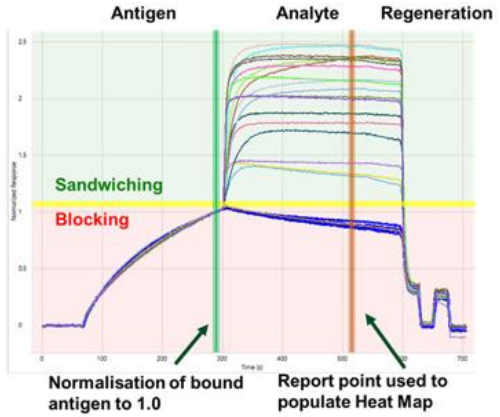
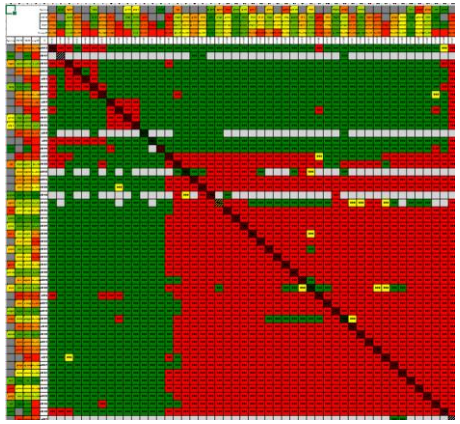
Epitope Binning Software User Interface

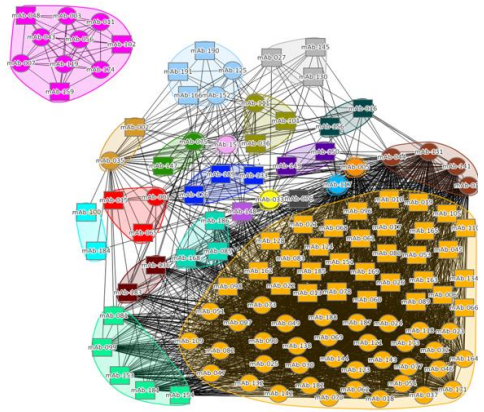
Data linked across 3 visualisation panels



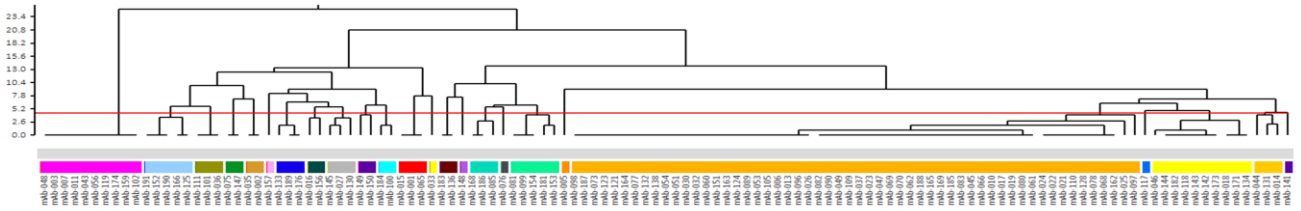
Senograms



Heat map



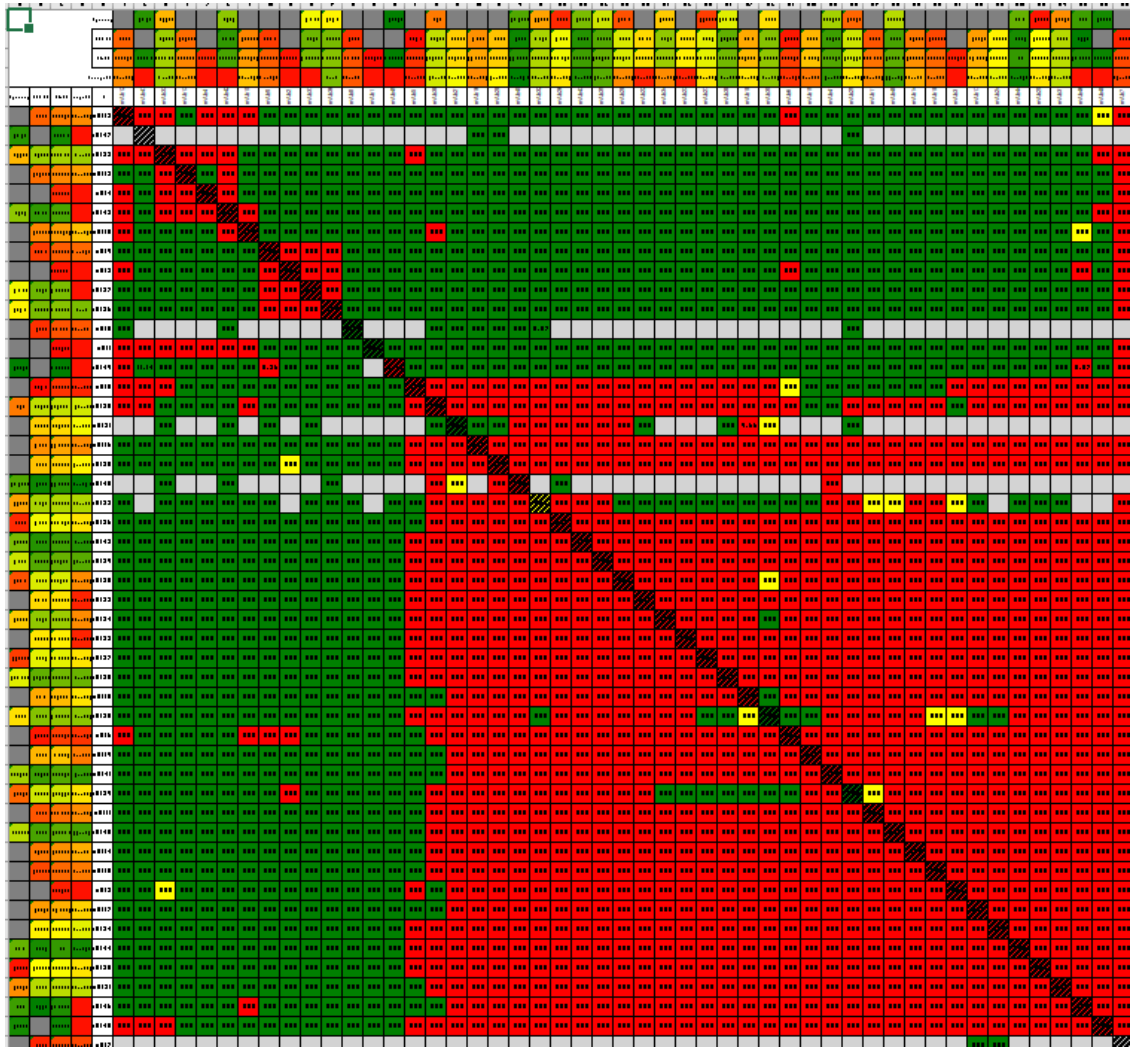
Networks plots



Combined Dendrogram

Heat map generation

- A feature of the software is that it allows you to add additional data such binding and sequence data
- This can be then visualised on the heat map and network plots



Carterra Color Key	
	Self-Self Interaction
	Competitor
	Non-Competitor
	Asymmetric

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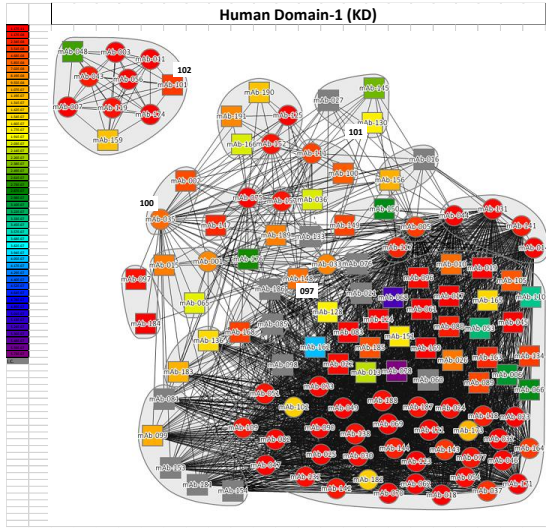
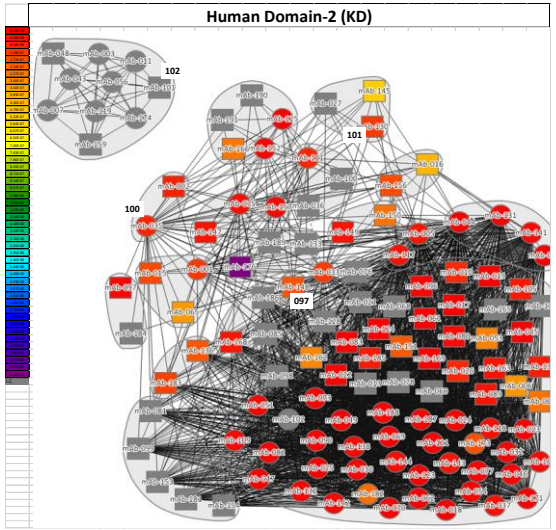
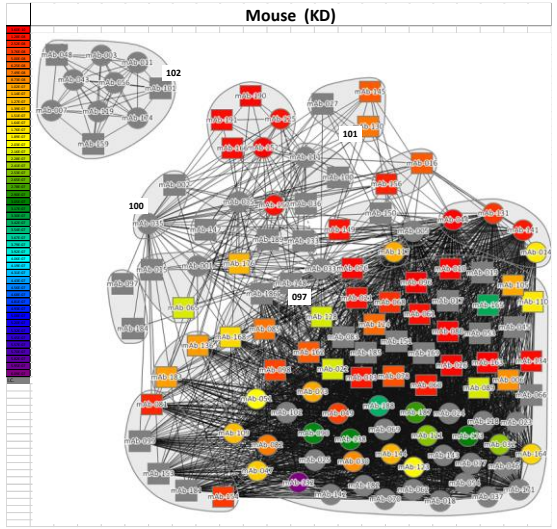
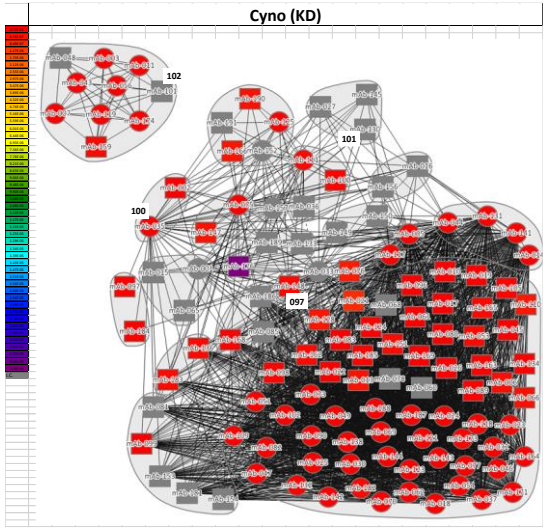
Linking Binning data with Kinetics Data

- We were able to separate out the data for each antigen
- Kinetics data can be linked (shown below as heat map on the left-hand side of each plot)









Summary

- The Carterra LSA has been a great asset in our efforts to streamline our screening process
- It allows for lower sample consumption, reduced timelines and complete data package for a diverse range of antibodies.
- SPR has always been carried out on smaller number of down selected clones, the LSA has allowed us to shift SPR upstream to screening
- It has enabled easier triaging of candidates which we struggled with in our traditional ELISA approach
- Epitope binning allows us differentiate from the prior art in order to have the best chance of securing IP and maintaining epitope diversity. We are now able to get this data earlier in the screening process
- Certain epitopes can relate to function so we can now select candidates from different pools to increase our chances of identifying a functionally active lead candidate


LifeArc
Thank you