curia

Rapid VHH Antibody Discovery by Single B-Cell Platform at Curia

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June 24, 2025

Hayward Discovery Biologics

Site

We welcome you at Curia's Hayward facility, located in California, US. Here, we support you across all of your early drug discovery needs.

Capacity

- 38,000 ft²
- 300+ DNA Molecular Constructions per week
- Gram scale DNA plasmid production
- 300+ L transient mammalian protein production per week
- 40-60+ annual Discovery & Engineering programs

Contact

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Core technologies

- PentaMice® platform
- Beacon® optofluidic system for human, mouse, rat, and llama single B cell Ab discovery
- Tuna293^{SM} and TunaCHO^{SM} expression systems
- XOMA® fully human scFv and FAB libraries
- High-throughput (HTP) protein production
- Carterra® LSA® and Octet® BLI systems
- HTP flow cytometry

Quality & regulatory

- AAALAC vivarium accreditation
- Hayward is a non-GMP site.

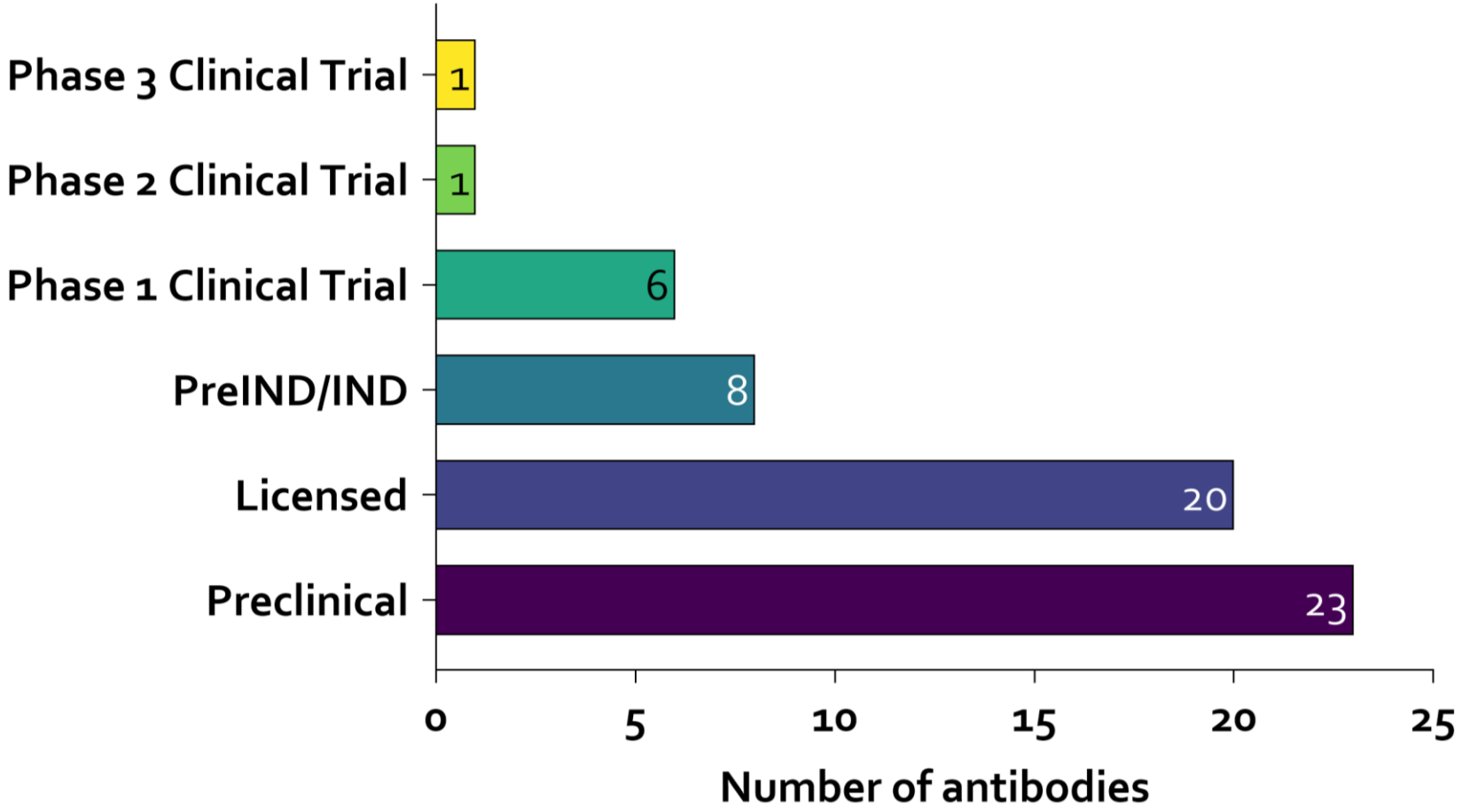
PROUDLY, AN aaalac INTERNATIONAL ACCREDITED PROGRAM

Products & services

- Recombinant DNA production
- Mammalian protein expression (Ab/BsAb, Ag, AAV)
- Rodent, llama, and rabbit immunizations
- Ab discovery: Hybridoma, Single B cell, Phage- and Yeast-Display
- Affinity maturation
- Antibody humanization
- Ab characterizations (kinetics, epitope binning, and binding potency)
- Developability analysis

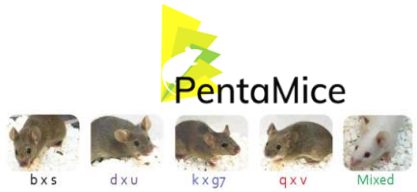
Curia's Antibody Discovery, Engineering & Characterization (ADEC) Team & Services

Discovering high quality antibodies!
200+ successful discovery campaigns



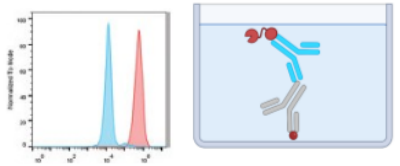
Antibody Discovery Workflows Utilize State-of-the-Art Hybridoma, Single B Cell, and Display Technologies to Deliver Quality Leads

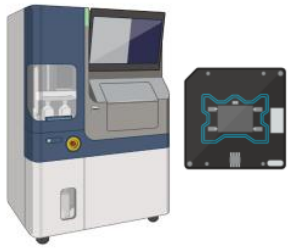
Upfront Strategy Design & Reagent QC



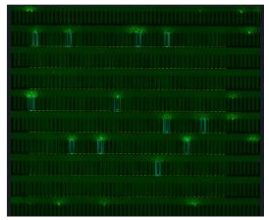
Immunizations with DNA, protein, cells

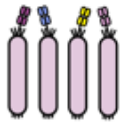
Hybridoma





Single B cell Bruker (Berkeley Lights) Beacon





Phage or yeast display

scFv, Fab, VHH

Antibody Production

3-tier developability assessment package

Quality Leads

Turnaround Time
3 to 6+ months depending on platform
Licensing Terms
***None*[1]**

[1] assumes use of in-house PentaMice®

- ❑ Hybridoma biology and Discovery workflow
- ❑ Display-based Library Discovery
- ❑ Single B cell Bruker (Berkeley Lights) Beacon®
- ❑ PentaMice

Curia's State-of-the-art Single B Cell Screening Workflow

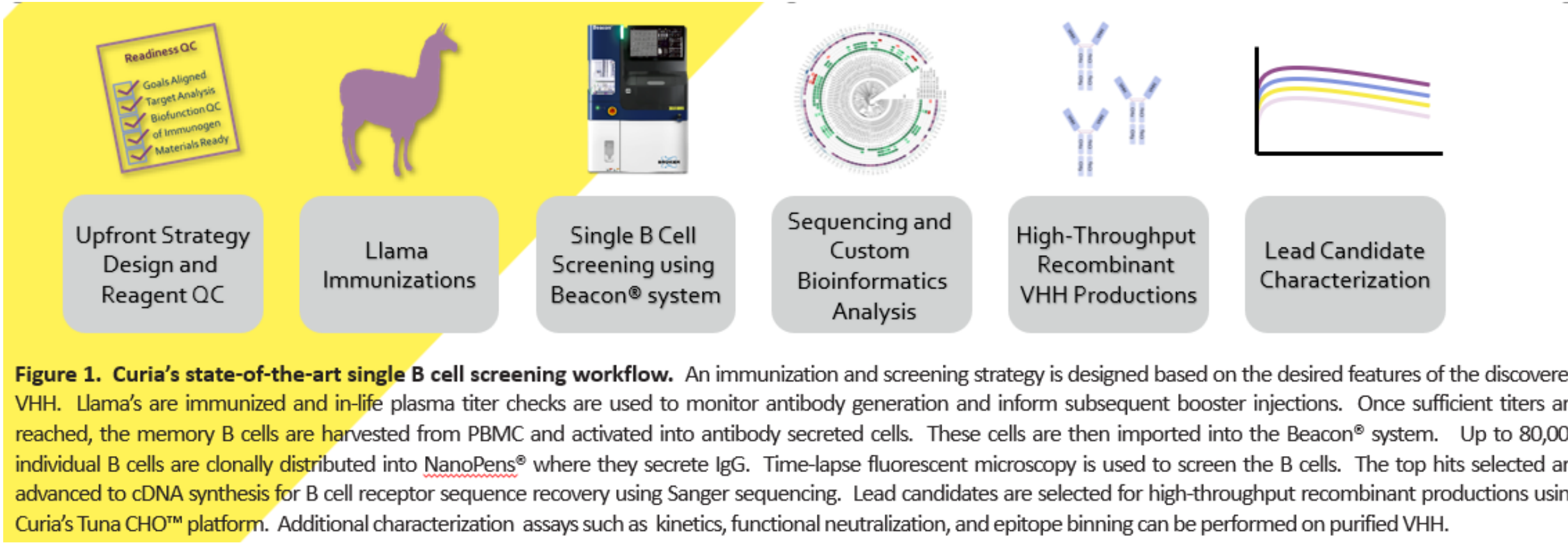
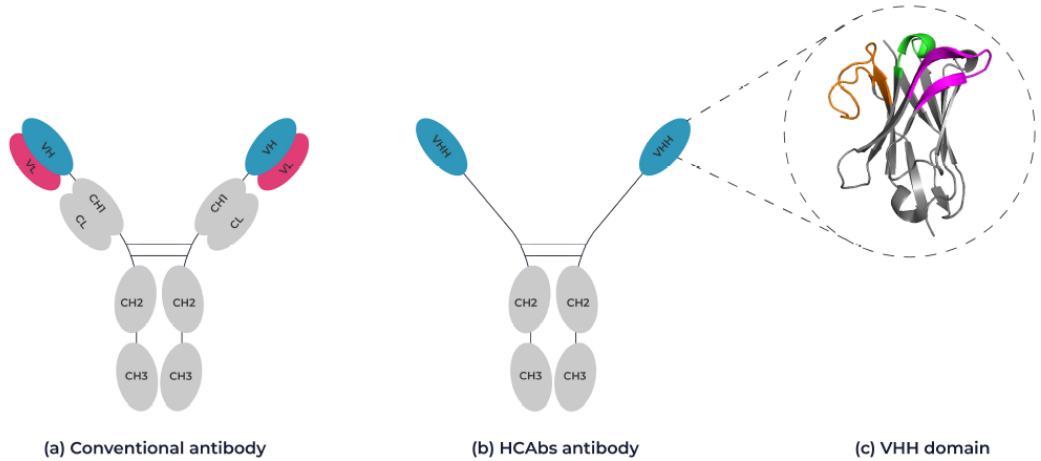


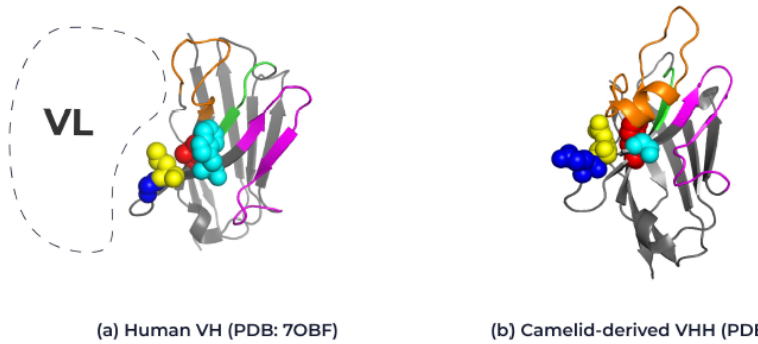
Figure 1. Curia's state-of-the-art single B cell screening workflow. An immunization and screening strategy is designed based on the desired features of the discovered VHH. Llama's are immunized and in-life plasma titer checks are used to monitor antibody generation and inform subsequent booster injections. Once sufficient titers are reached, the memory B cells are harvested from PBMC and activated into antibody secreted cells. These cells are then imported into the Beacon® system. Up to 80,000 individual B cells are clonally distributed into NanoPens® where they secrete IgG. Time-lapse fluorescent microscopy is used to screen the B cells. The top hits selected are advanced to cDNA synthesis for B cell receptor sequence recovery using Sanger sequencing. Lead candidates are selected for high-throughput recombinant productions using Curia's Tuna CHO™ platform. Additional characterization assays such as kinetics, functional neutralization, and epitope binning can be performed on purified VHH.

Reasons for VHH antibody

Advantages

- VHH antibodies are heavy chain only antibodies.
- Easy to penetrate tumor tissues
- Easy to access small cavities or epitopes otherwise not accessible by conventional Ab
- Easy to clone and express
- Easy to be paired with other modality for bispecific





MABS
2024, VOL. 16, NO. 1, 2341443
https://doi.org/10.1080/19420862.2024.2341443

Case Study – anti-CD200R1 VHH Antibody Discovery by Single B Cell

Poster by Curia and Bruker presented at PEGS May 2025

Highlights:

- Strong plasma titer reached 57 days following initial animal immunization
- Rapid discovery: < 1 month from Beacon assays to unique hits sequencing results
- High-throughput screening: 20k single B cells screened in one day (scaleable up to 80k single B cells for a multiple-day workflow)
- Diverse antibody hits recognizing different epitopes on target
- hu/mu/cyno cross-reactive hits
- ~60% hits are CD200R1 neutralizing Abs (*i.e.* blocking CD200 binding to CD200R1)

Background and Scope

Background

- CD200R1 is a cell surface receptor that plays a key role in regulating immune responses.
- The interaction between CD200R1 and its ligand, CD200, delivers an inhibitory signal leading to the suppression of immune responses.
- The CD200/CD200R1 interaction has autoimmune, inflammatory, and oncology implications.

Scope

- Discover diverse antagonistic antibody hits against CD200R1
- Improve the antibody discovery timeline by using the single B-cell technology
- Comprehensive antibody hits characterizations including Carterra Kinetics and Epitope binning analyses

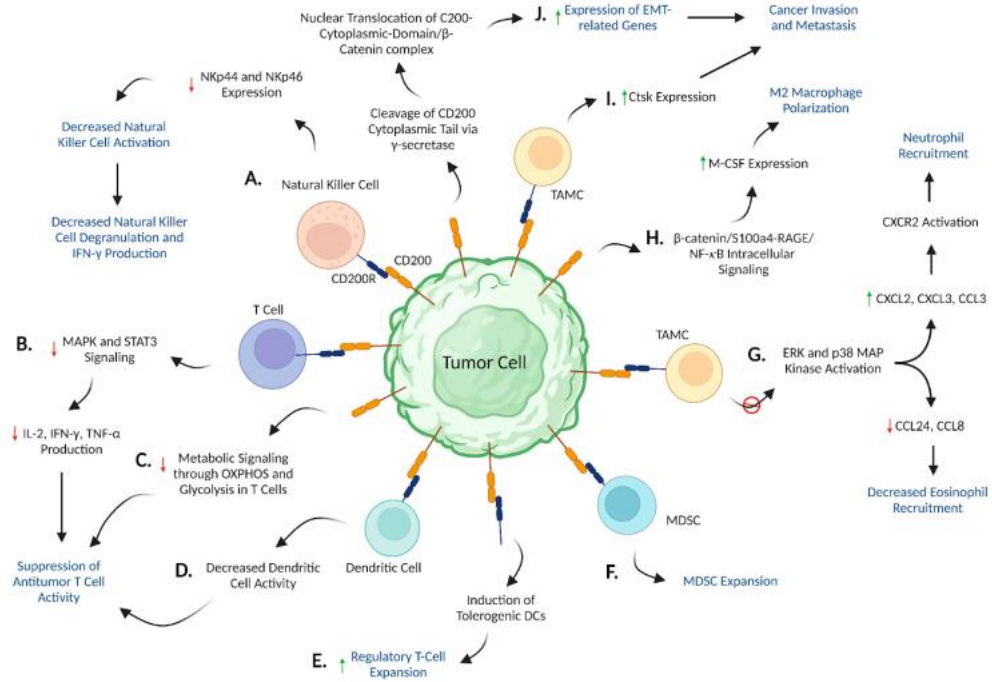


Figure 2. Proposed mechanisms elucidating the pro-tumorigenic role of CD200/CD200R signaling in cancer progression. CD200/CD200R signaling promotes an immunosuppressive TME by (**A**) inhibiting NK cell activation through decreased NKp44 and NKp46 expression, (**B**) reducing MAPK/STAT3-mediated Th1 cytokine production, (**C**) hampering metabolic signaling within T cells leading to an inactive T cell phenotype, (**D**) suppressing DC-mediated T cell activation, facilitating expansion of (**E**) Tregs and (**F**) MDSCs, (**G**) differentially regulating production of chemokines involved in immune cell recruitment, for example, CXCL2, CXCL3, CCL3, CCL24, and CCL8 by inhibiting ERK and p38 MAP kinases, and (**H**) driving M-CSF-mediated M2 macrophage polarization through increased β-catenin/S100a4-RAGE/NF-*k*B/M-CSF signaling. Furthermore, CD200/CD200R signaling stimulates cancer invasion and metastasis by increasing the expression of (**I**) Ctsk and (**J**) EMT-related genes. "https://www.biorender.com/ (accessed on 22 September 2023)".

Biomedicines **2023**, *11*, 3326. https://doi.org/10.3390/biomedicines11123326

In-life Plasma Titer Checks of Immunized llamas

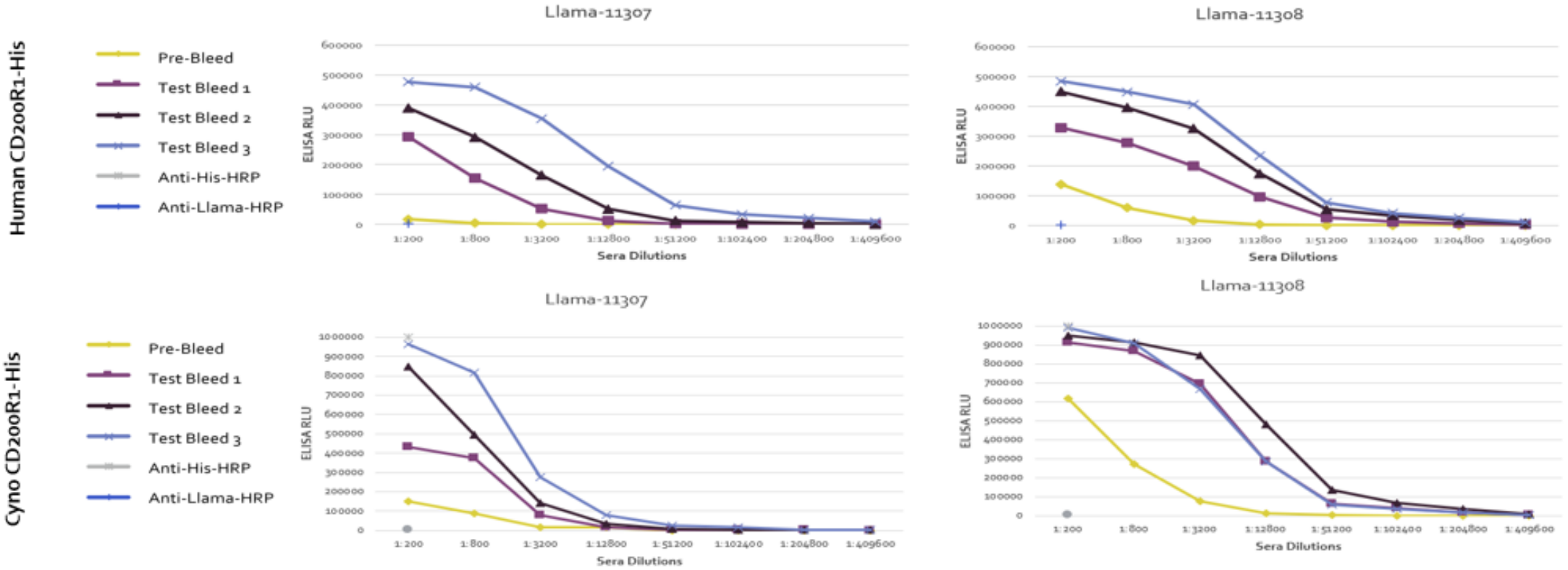


Figure 2. In-life plasma titer checks guide selection of llamas with highest huCD200R1 titer. Two Llamas were immunized with a mixture of human and cyno CD200R1-Fc. Blood plasma was collected at three time points (D16, D30, D57), diluted as indicated and tested for human and cyno CD200R1-HIS binding by ELISA. RLU: relative light unit.

On-chip Screening Assays for Llama IgG Secretion and Antigen Binding

Assay #1	Assay #2	Assay #3
7885 VHH (+)	305 huCD200R1 (+)	169 cynoCD200R1 (+)

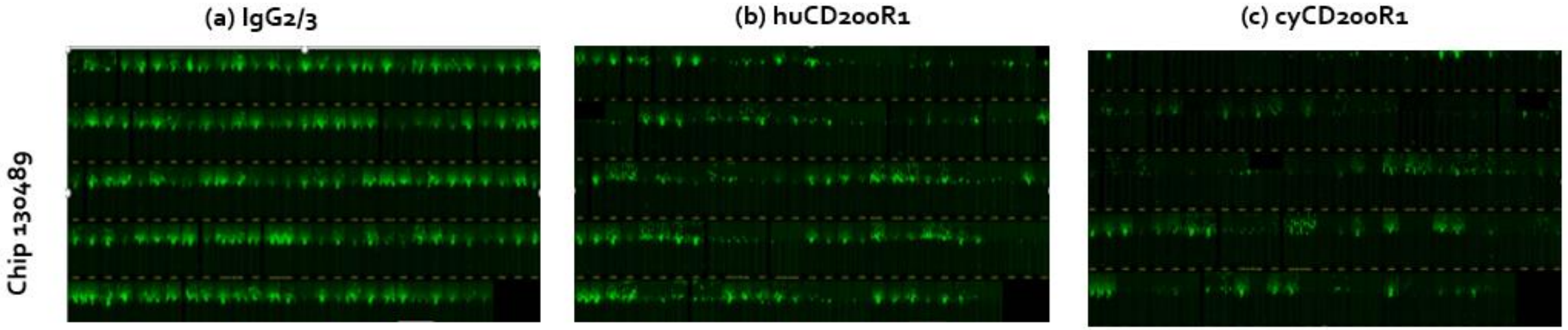


Figure 3. On-chip screening assays for Llama IgG and antigen binding B cells from hu/cy CD200R1-Fc immunized llama were isolated and imported into 40,000 NanoPen® chambers and screened on-chip for **(a)** VHH (Llama IgG 2/3) secretion, **(b)** antigen-binding to human CD200R1-HIS antigen and **(c)** cyno CD200R1-HIS antigen. Pictured are representative field-of-view images. In an automated manner, accompanied by manual verification of hits, the Beacon® system identified hits by analyzing Alexa Fluor 488-positive blooms.

Analysis of CDR3 Length and Extent of Affinity Maturation in vivo

- Hundreds of single B cells were sequenced, and 31 unique clones were identified.

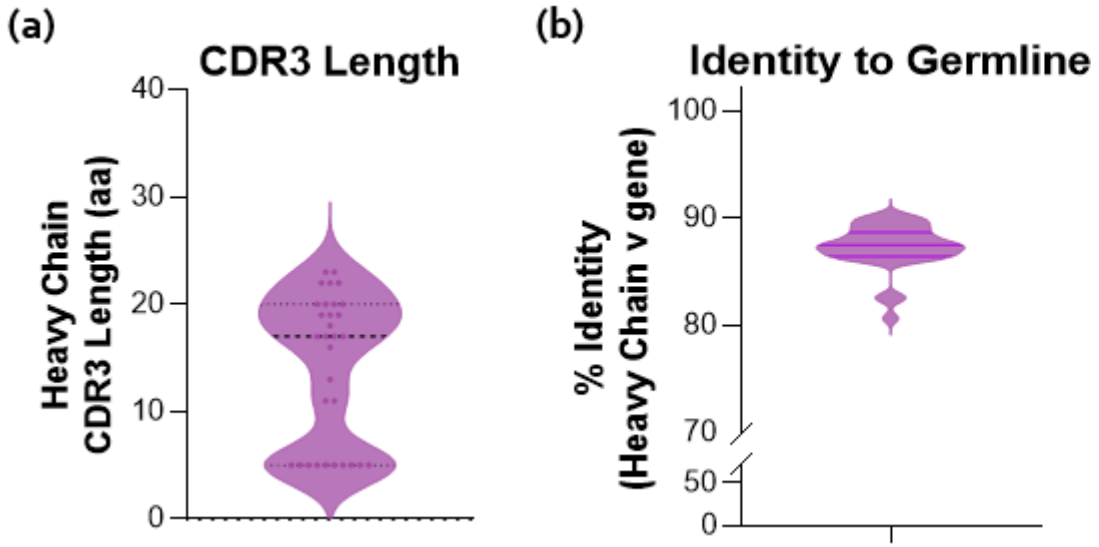


Figure 5. Analysis of CDR3 length and extent of affinity maturation in vivo. **(a)** CDR3 length ranged from 5 to 23 amino acids, 14.1 ± 1.2, mean ± SEM, n=31. **(b)** Percent identity to germline: 87.3 ± 0.4, mean ± SEM, n=31.

Recombinant Production of VHH-huFc Leads and Assessment of Binding Potency against Human and Cyno CD200R1

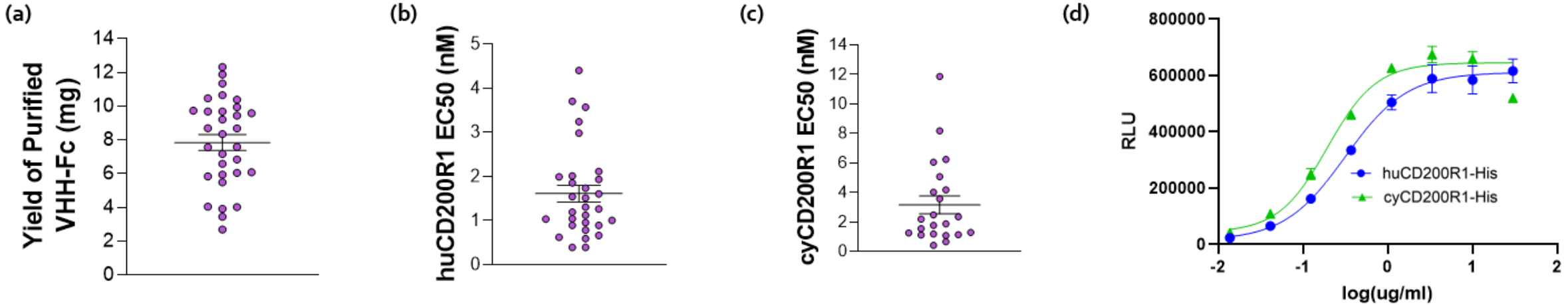
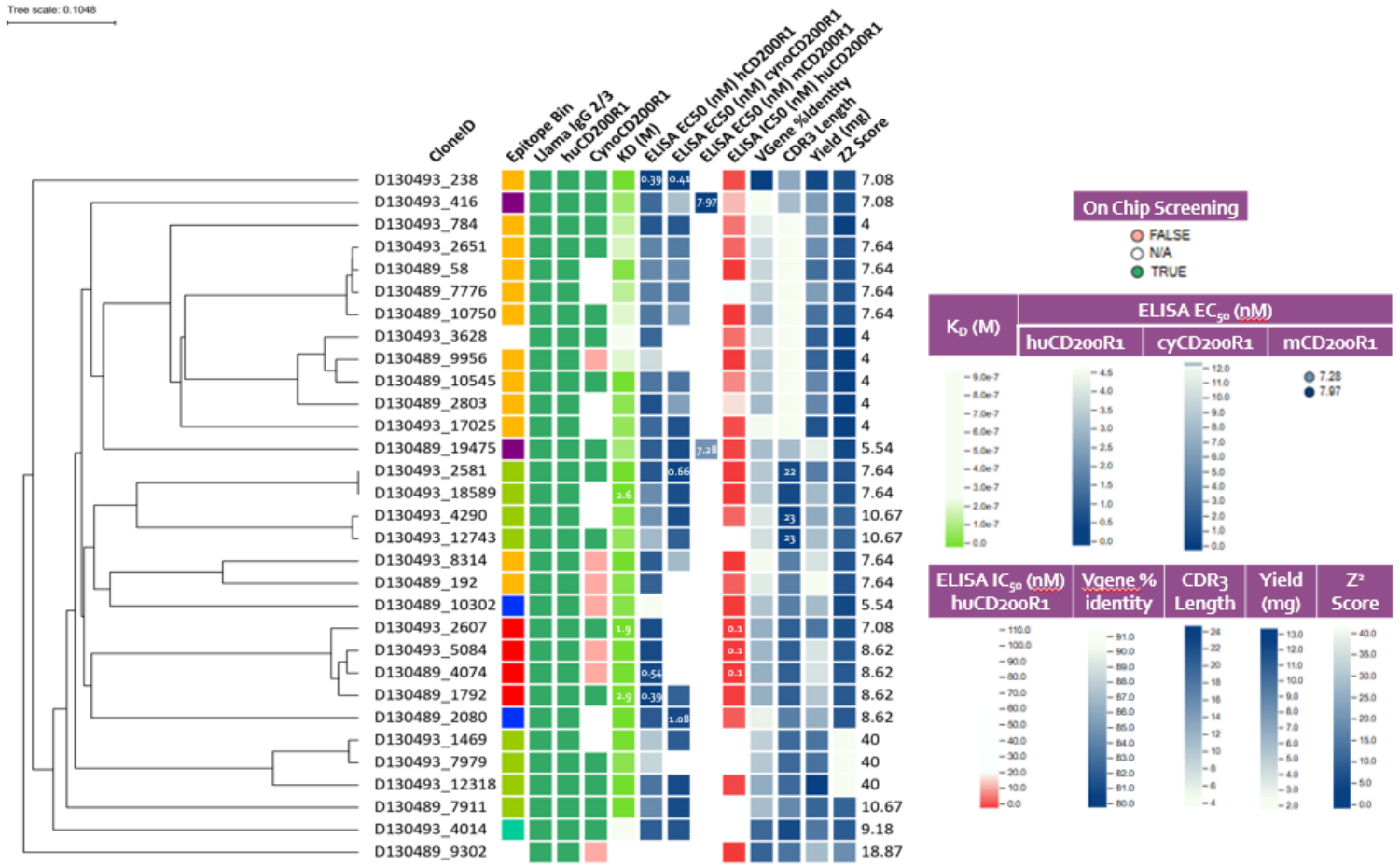


Figure 6. Recombinant production of VHH-huFc leads and assessment of binding potency against human and cyno CD200R1. **(a)** Recombinant antibody yields following small-scale (10 mL) high throughput production using Curia's TunaCHO℠ platform: 7.84 ± 2.63 mg, n=31. **(b)** huCD200R1 EC_{50} by ELISA: 1.6 ± 0.2 nM, n=30. **(c)** cyCD200R1 EC_{50} by ELISA, 3.2 ± 0.6 nM. **(a-c):** mean ± SEM **(d)** example EC_{50} curves for anti-CD200R1 VHH-Fc D130493_4290.

Phylogenetic Tree of 31 VHH-hFc Variants with Assay Data



Notes

- A informatics tool was used to visualize the phylogenetic tree of the unique hits along with the data from different assays .

Carterra HTP Kinetics Results

Methods

- Kinetics Analysis with Carterra LSA (SPR)
- Ligands: (32) Llama VHH-Fcs
  - 31 Purified VHH Fcs from CHO production
  - 1 purified hIgG4 Fc Isotype Control
- Analyte: (1) hu-CD200R1(27-266)-His6
- Chip: HC30M
- Capture Kinetics

Results

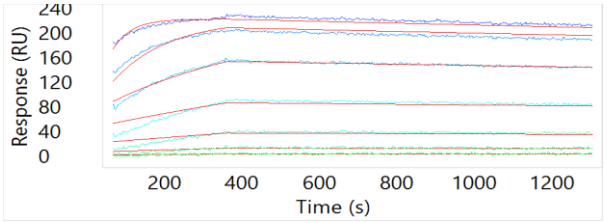
- Sensorgrams were picked for each interaction based on best fit.
- A response was observed for 30 out of 32 test articles.
  - (30) Binders with nM affinity: 13 single-digit, 10 double-digit, and 7 triple-digit nM binders
  - (2) non-binders (Including an Fc Isotype Control)
    - Isotype control is not expected to bind to huCD200R1
- The affinities for all antibodies are summarized in the table to the right. The results (including k_a, k_{dis}, and K_D) and sensorgrams are shown in subsequent slides

Ligand Name	K_D (M)
D130493_238	3.30E-09
D130489_2803	5.00E-08
D130493_784	1.50E-07
D130493_12743	6.10E-08
D130493_4014	4.60E-07
D130489_10750	2.10E-07
D130489_2080	3.60E-09
D130489_9302	n.d.
D130493_12318	4.10E-09
D130489_58	3.70E-08
D130489_9956	2.10E-07
D130489_7911	5.30E-09
D130493_4290	3.30E-08
D130489_10545	6.70E-09
D130493_2581	5.20E-09
D130489_19475	9.40E-08
D130489_7776	1.40E-07
D130489_192	3.70E-08
D130493_8314	5.20E-09
D130493_18589	2.60E-09
D130493_5084	4.90E-09
D130489_10302	4.00E-08
D130493_2607	1.90E-09
D130489_4074	6.20E-09
D130493_2651	1.90E-07
D130489_1792	2.90E-09
D130493_3628	8.50E-07
D130493_17025	6.60E-08
D130493_416	8.20E-08
D130493_1469	7.50E-09
D130493_7979	4.80E-08
hIgG4_S228P/L235A_Fc_Isotype_Control	n.d.

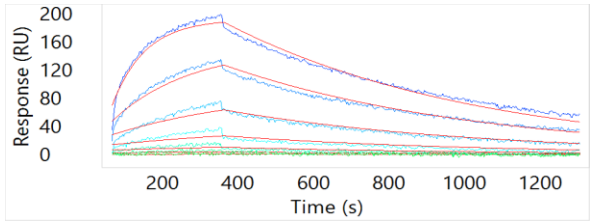
n.d.	No Binding Observed

Sensorgrams and Kinetic Constants [Set 1]

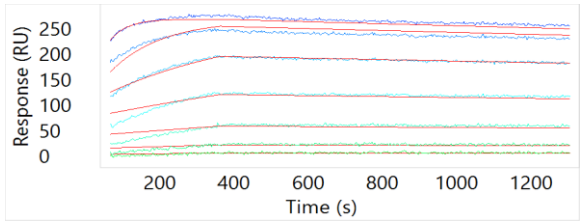
D130493_238



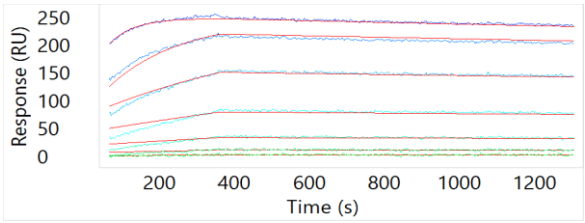
D130493_784



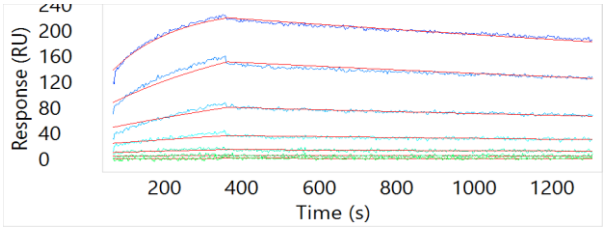
D130489_2080



D130493_12318



D130493_4290



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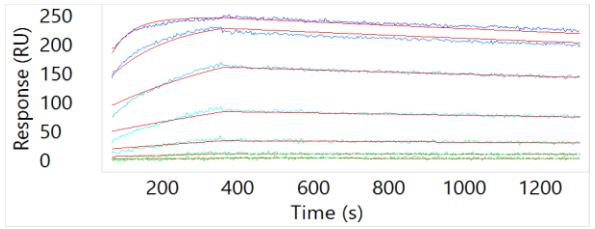
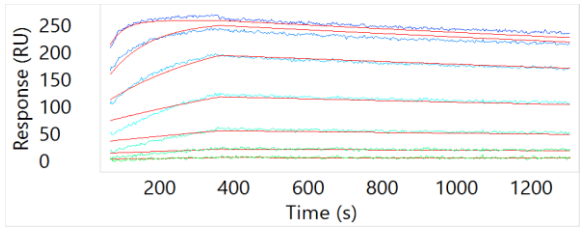


D130493_2581



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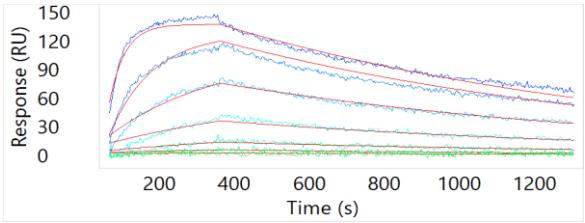

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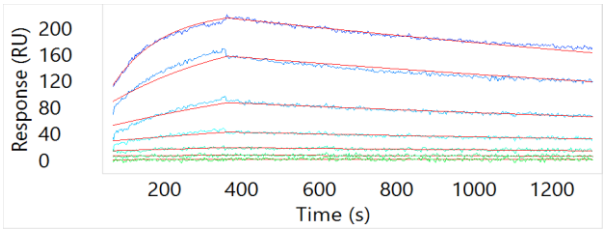
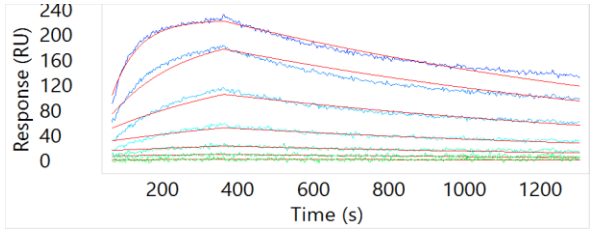


D130493_7979

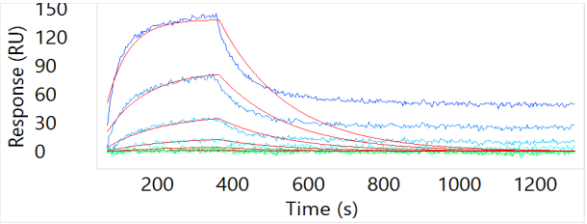


ROI	Ligand Name	K_D (M)	k_a (1/Ms)	k_{dis} (1/s)
1	D130493_238	3.3E-09	2.1E+04	6.8E-05
193	D130493_784	1.5E-07	1.0E+04	1.5E-03
241	D130489_2080	3.6E-09	2.2E+04	7.6E-05
13	D130493_12318	4.1E-09	1.5E+04	6.2E-05
61	D130493_4290	3.3E-08	6.0E+03	2.0E-04
157	D130489_10545	6.7E-09	1.8E+04	1.2E-04
253	D130493_2581	5.2E-09	2.7E+04	1.4E-04
121	D130489_192	3.7E-08	2.3E+04	8.7E-04
169	D130489_10302	4.0E-08	7.5E+03	3.0E-04
277	D130493_7979	4.8E-08	1.4E+04	6.7E-04

Some data processing parameters were changed to achieve best fit for all test articles
Set 1: All concentrations included, full dissociation fit
Set 2: All concentrations included, shortened dissociation fit
Set 3: Top concentration excluded, full dissociation fit
Set 4: Top concentration excluded, shortened dissociation fit

Sensorgrams and Kinetic Constants [Set 2]

D130493_4014



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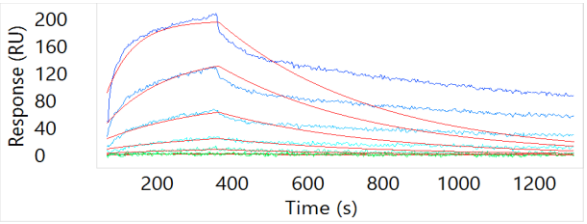

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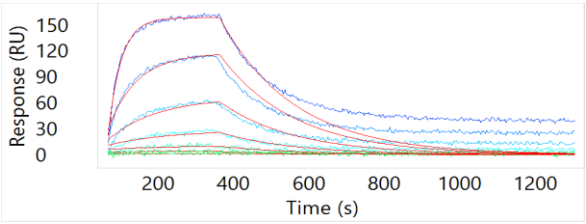

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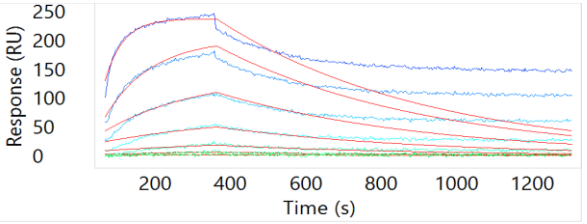

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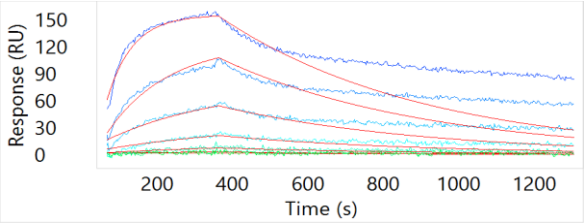

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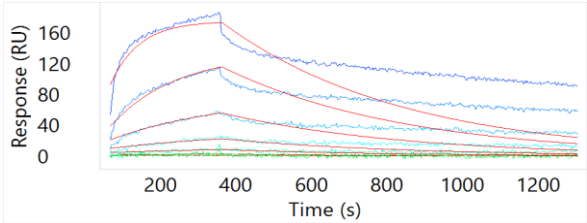

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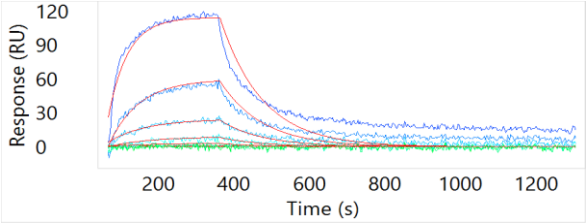

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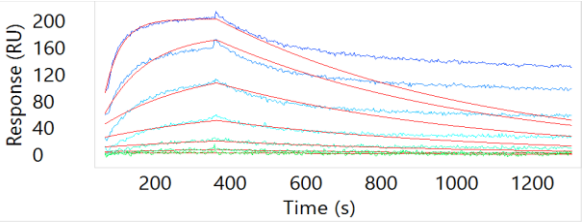
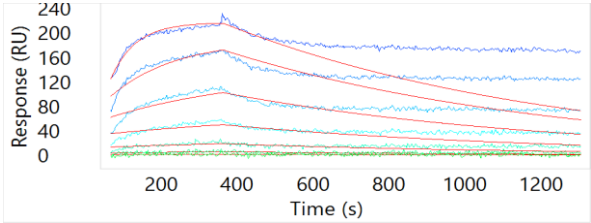


D130493_416

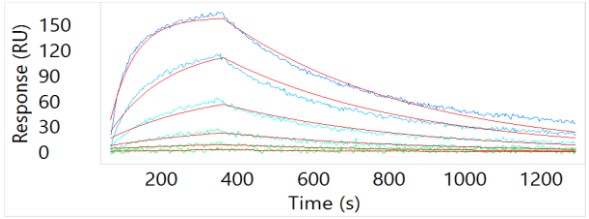


ROI	Ligand Name	K_D (M)	k_a (1/Ms)	k_{dis} (1/s)
49	D130493_4014	4.6E-07	1.2E+04	5.5E-03
145	D130489_10750	2.1E-07	1.2E+04	2.4E-03
205	D130489_9956	2.1E-07	2.4E+04	5.1E-03
351	D130489_19475	9.4E-08	1.9E+04	1.8E-03
25	D130489_7776	1.4E-07	1.3E+04	1.8E-03
37	D130493_2651	1.9E-07	1.1E+04	2.1E-03
229	D130493_3628	8.5E-07	1.1E+04	9.0E-03
325	D130493_17025	6.6E-08	2.2E+04	1.4E-03
85	D130493_416	8.2E-08	1.4E+04	1.1E-03

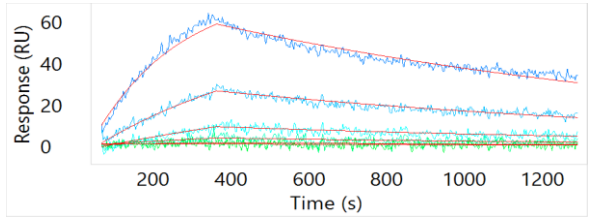
Some data processing parameters were changed to achieve best fit for all test articles
Set 1: All concentrations included, full dissociation fit
Set 2: All concentrations included, shortened dissociation fit
Set 3: Top concentration excluded, full dissociation fit
Set 4: Top concentration excluded, shortened dissociation fit

Sensorgrams and Kinetic Constants [Set 3]

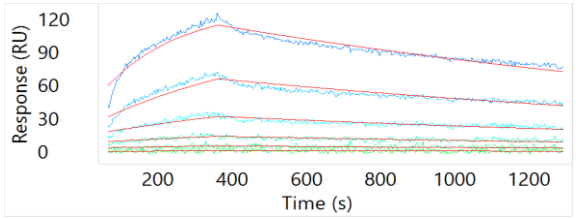
D130489_2803



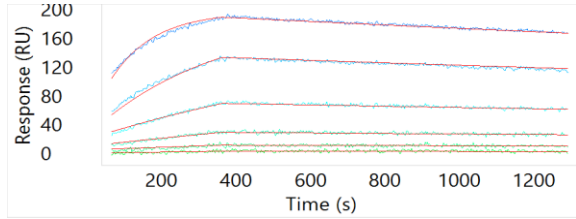
D130493_12743



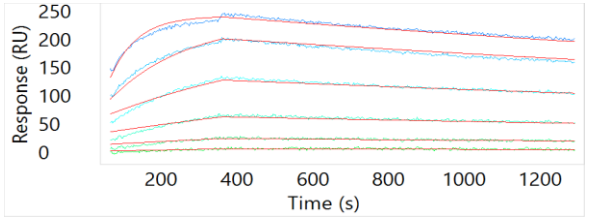
D130489_58



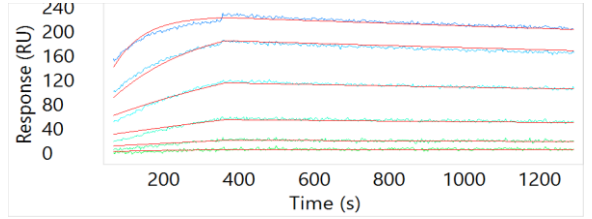
D130489_7911



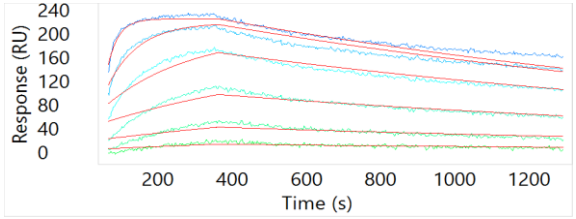
D130493_8314



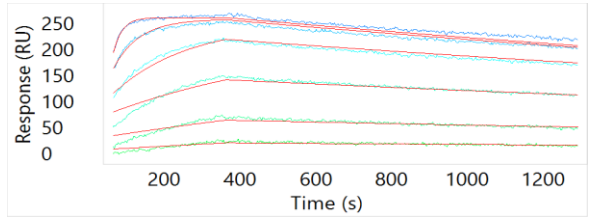
D130493_18589



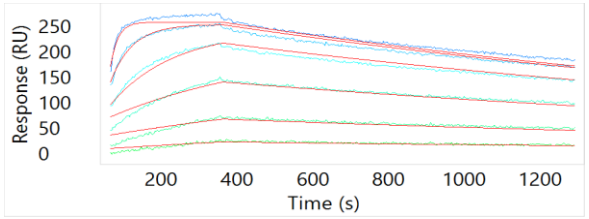
D130493_5084



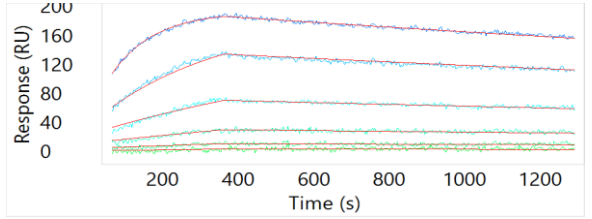
D130493_2607



D130489_1792



D130493_1469

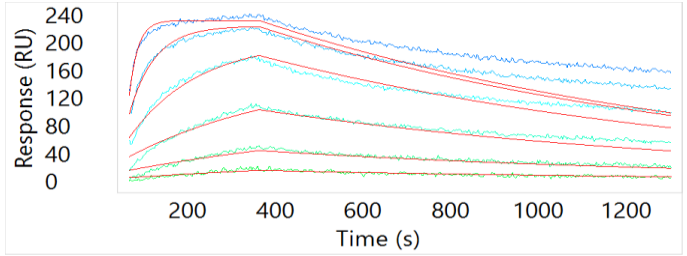


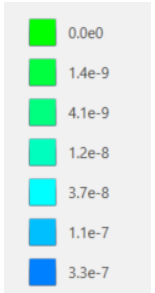
ROI	Ligand Name	K_D (M)	k_a (1/Ms)	k_{dis} (1/s)
99	D130489_2803	5.0E-08	4.1E+04	2.0E-03
289	D130493_12743	6.1E-08	1.2E+04	7.0E-04
111	D130489_58	3.7E-08	1.3E+04	4.9E-04
301	D130489_7911	5.3E-09	2.5E+04	1.3E-04
217	D130493_8314	5.2E-09	4.2E+04	2.2E-04
313	D130493_18589	2.6E-09	3.8E+04	9.9E-05
73	D130493_5084	4.9E-09	1.0E+05	5.0E-04
265	D130493_2607	1.9E-09	1.3E+05	2.5E-04
133	D130489_1792	2.9E-09	1.5E+05	4.4E-04
181	D130493_1469	7.5E-09	2.5E+04	1.9E-04

Some data processing parameters were changed to achieve best fit for all test articles
Set 1: All concentrations included, full dissociation fit
Set 2: All concentrations included, shortened dissociation fit
Set 3: Top concentration excluded, full dissociation fit
Set 4: Top concentration excluded, shortened dissociation fit

Sensorgrams and Kinetic Constants [Set 4]

D130489_4074



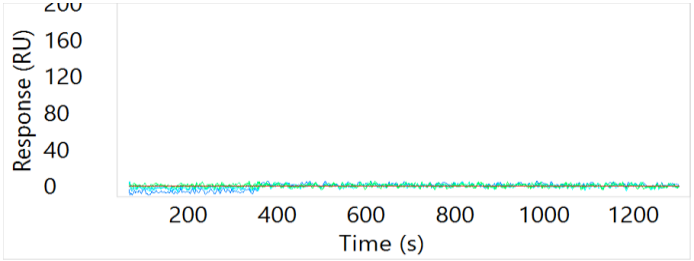


ROI	Ligand Name	K_D (M)	k_a (1/Ms)	k_{dis} (1/s)
361	D130489_4074	6.2E-09	1.5E+05	9.1E-04

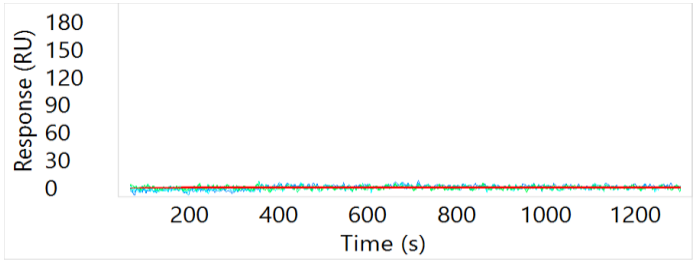
Some data processing parameters were changed to achieve best fit for all test articles
Set 1: All concentrations included, full dissociation fit
Set 2: All concentrations included, shortened dissociation fit
Set 3: Top concentration excluded, full dissociation fit
Set 4: Top concentration excluded, shortened dissociation fit

Sensorgrams and Kinetic Constants [Non-Binders]

D130489_9302



hIgG4_S228P/L235A_Fc_Isotype_Control



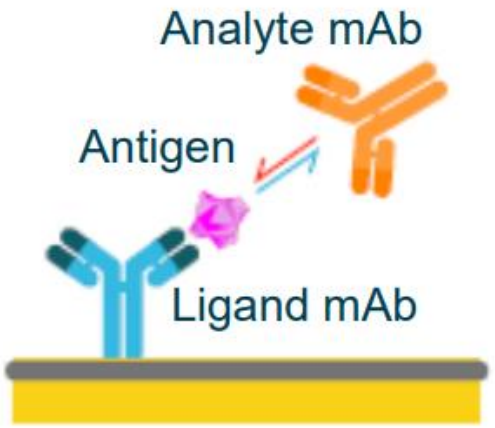
ROI	Ligand Name	K_D (M)	k_a (1/Ms)	k_{dis} (1/s)
337	D130489_9302	n.d.	n.d.	n.d.
373	hIgG4_S228P/L235A_Fc_Isotype_Control	n.d.	n.d.	n.d.

Key	n.d. = Not determined; No binding observed

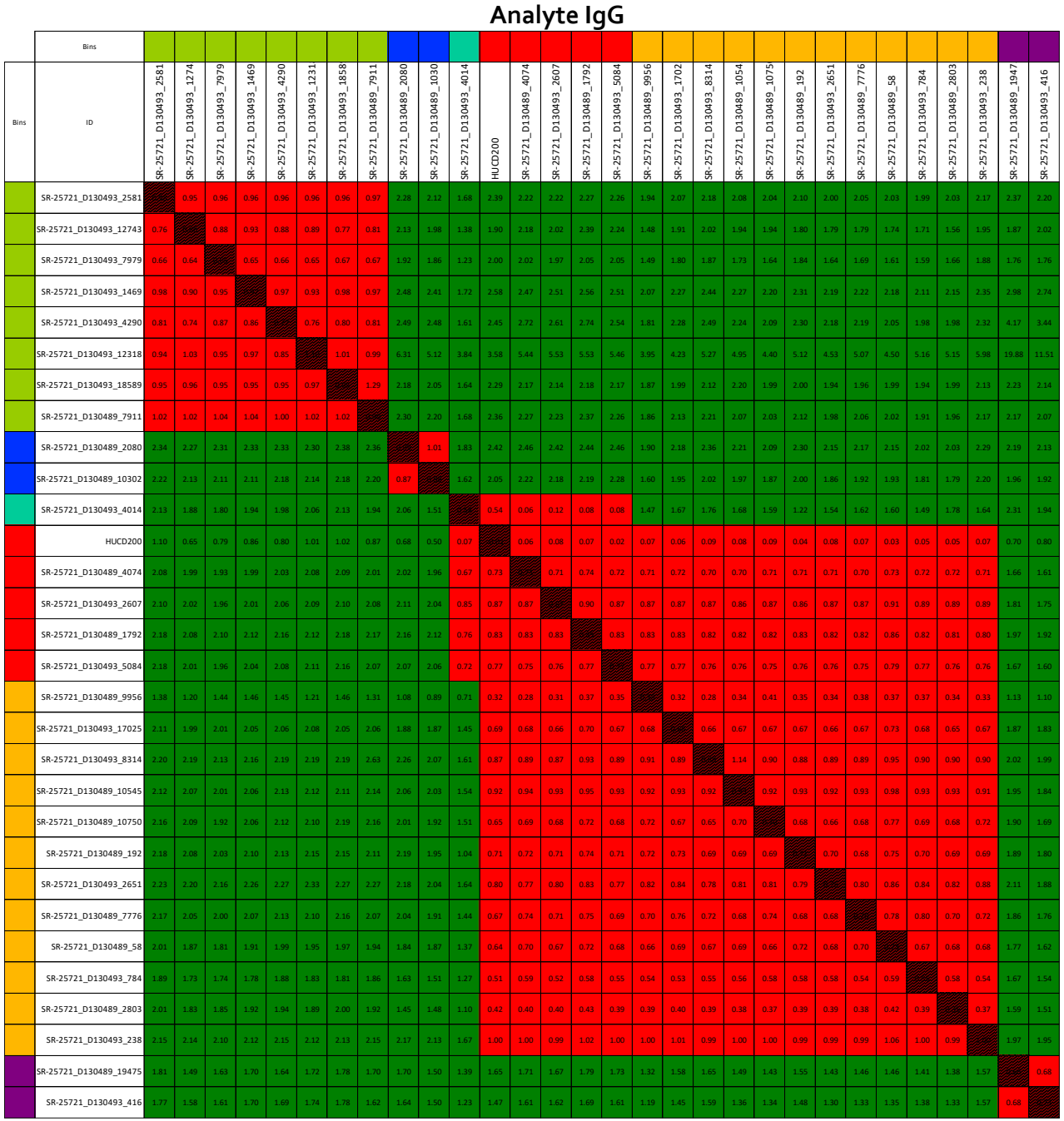
Epitope Binning Methods (Briefly)

1. **Array preparation.** 32 Ligands (31 Ab, and CD200) were coupled to an HC30M chip via Sulpho-NHS/EDC coupling chemistry and blocked with ethanolamine. Samples were run in replicates at three concentrations: 10 µg/mL, 2 µg/mL, and 0.33 µg/mL].
2. 150 nM of antigen in HBSTE+BSA was injected to saturate the ligand binding sites for 10 min
3. 30 µg/mL IgG (analyte) sample in HBSTE+BSA was injected for 7 min to form Ab-Ag-Ab' sandwich.
4. At the end of each cycle, the chip was regenerated with 10 mM Glycine pH 2.0 to remove bound antigen and IgG.
5. Steps 2-4 were repeated for all analyte IgGs.
6. Epitope analysis was performed with Carterra's Epitope© software.

Classical Sandwich



Normalized Heat Map



Heat Map Legend

- Sandwiching
- Blocking/Competition
- Self-self competition

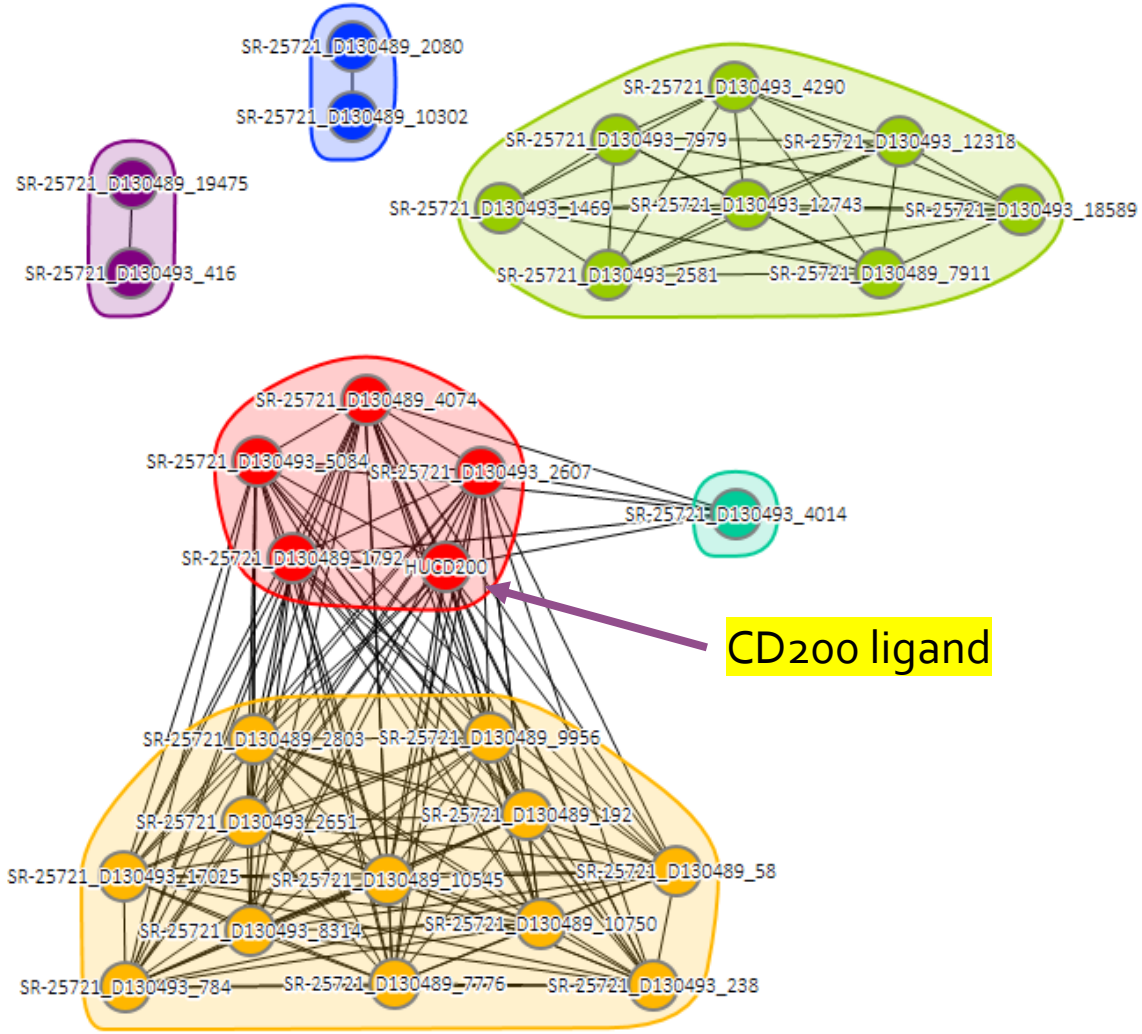
Notes

- The diagonal line in the chart indicates self interaction between the ligand form and analyte form of the same antibody

Notes:

- 6 distinct bins, are unambiguously identified.
- Members of each bin are colored in the same color on the left and on the top.

Network Plot of Bins from Epitope Analysis



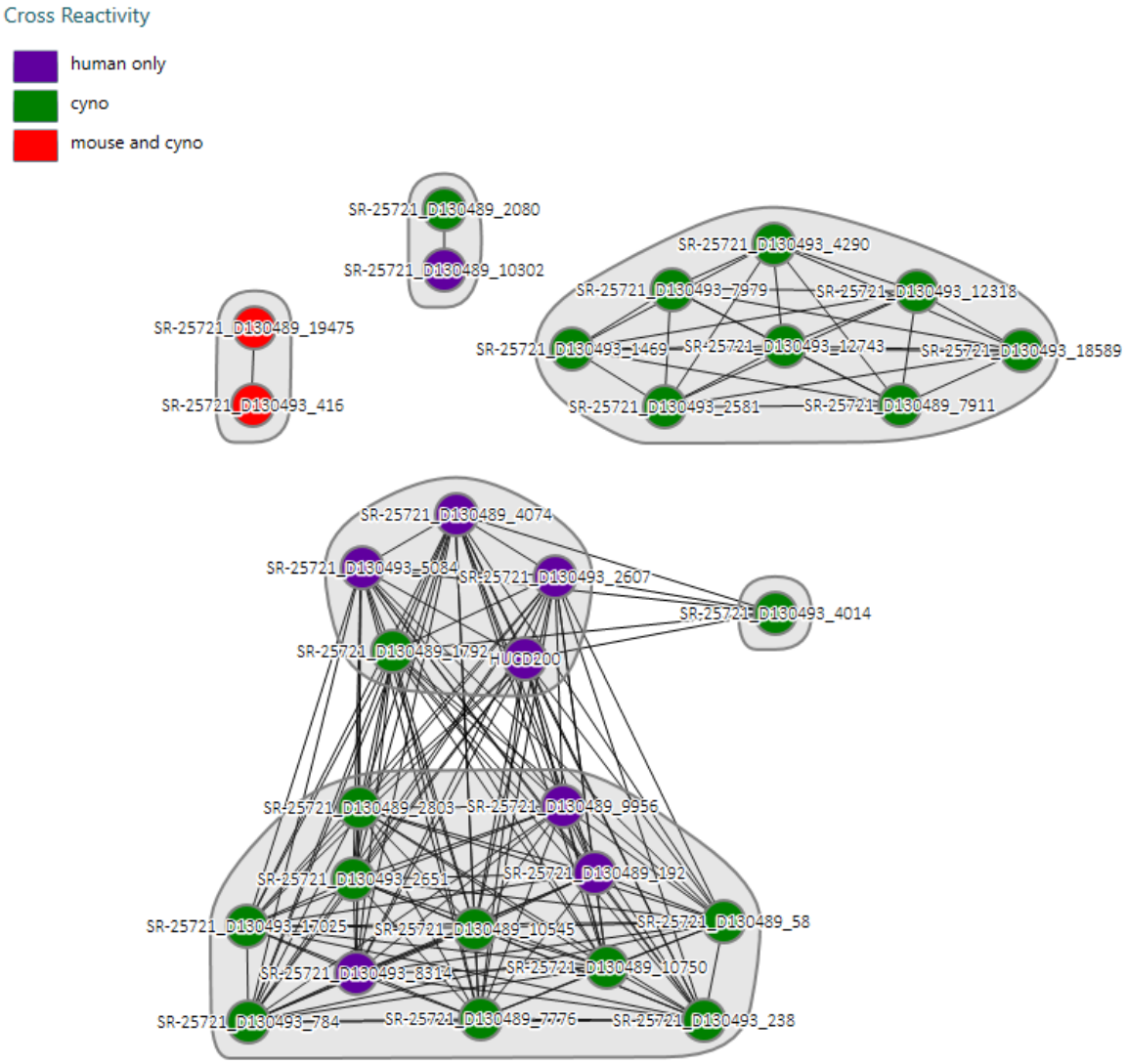
Community Network Plot

- The community network plot is used to explore clustering of mAbs that share similar but not necessarily identical competition profiles.
- Solid lines indicate a symmetrical blocking relationship, where both mAbs in the pairing blocked as analytes.
- Any interactions between Abs that do not have a line connecting them are sandwiching interactions.

Notes:

- 6 distinct bins are unambiguously identified.
- The 6 bins are treated as 6 communities without further grouping.
- CD200 ligand is in the bin colored in red. It is blocked by antibodies in the red, teal, and orange bins.

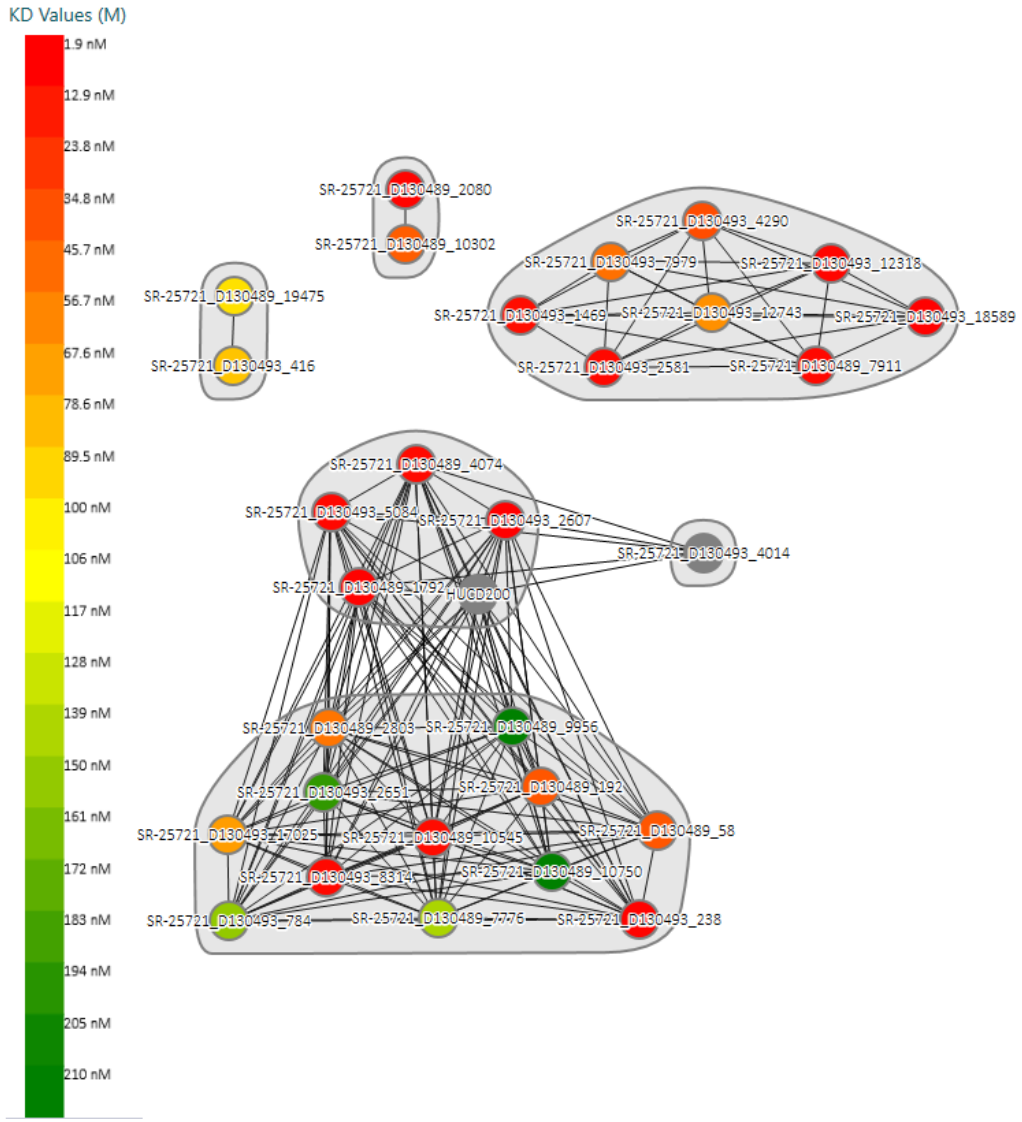
Network Plot of Bins from Epitope Analysis – Cross Reactivity



Community Network Plot

- The community network plot is used to explore clustering of mAbs that share similar but not necessarily identical competition profiles.
- Solid lines indicate a symmetrical blocking relationship, where both mAbs in the pairing blocked as analytes.
- Any interactions between Abs that do not have a line connecting them are sandwiching interactions.
- Colors show cross reactivity to mouse and cyno CD200 based on ELISA

Network Plot of Bins from Epitope Analysis – KD values



Community Network Plot

- The community network plot is used to explore clustering of mAbs that share similar but not necessarily identical competition profiles.
- Solid lines indicate a symmetrical blocking relationship, where both mAbs in the pairing blocked as analytes.
- Any interactions between Abs that do not have a line connecting them are sandwiching interactions.
- Colors show KD values from Carterra Kinetics
  - D130493_4014 has a KD value of 460 nM.
    - Coloring was skewed when this value was included, so it was excluded in this figure

Conclusions

- Curia's HTP single B cell workflow enables the rapid discovery of diverse llama derived VHH antibody hits within one month after immunization, significantly accelerating the discovery timeline.
- This platform identified diverse, high-affinity, functionally blocking anti-human and cyno CD200R1 VHH candidates, providing a robust platform for therapeutic development.
- The CD200R1-targeting VHH antibodies are potential checkpoint inhibitors and are available for licensing. Please contact antibody@curiaglobal.com to learn more.