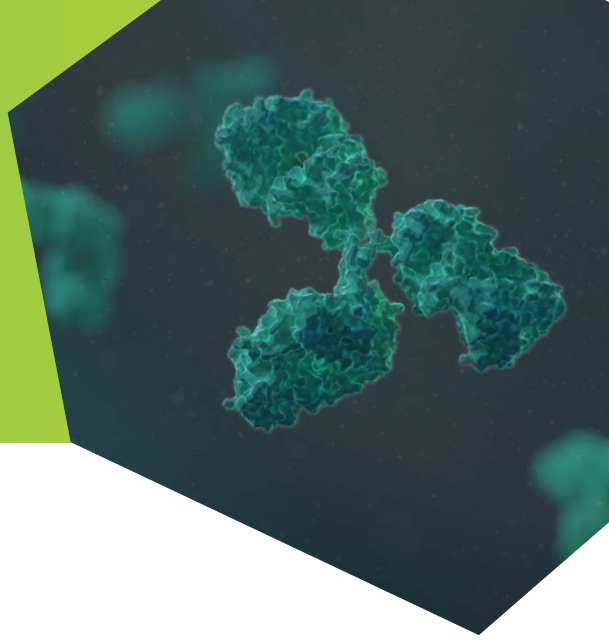
SCIENTIFIC CASE STUDY

Antibody candidates for IND-enabling studies

Deep screening of humanized mice to identify high-quality antibodies with minimal engineering



The following data was presented at the **European Protein & Antibody Engineering Summit 2023**.

INTRODUCTION

Generating antibodies that meet a stringent target candidate profile

Antibody candidates with high likelihood of clinical success need to meet a highly stringent target candidate profile (TCP), with criteria spanning target and epitope specificity, species cross-reactivity, complex functional properties, and developability. Identifying the rare antibodies that meet these criteria is technically challenging and time-intensive.[1,2]

In this case study, we present data from a program in which we leveraged our discovery and development engine to find functional and developable antibodies against a single-pass transmembrane protein with complex biological function. The TCP includes human & cyno cross-reactivity, pH-independent binding, antagonistic activity, favorable developability profile, and suitability for subcutaneous formulation.

Integrating data from over 50 assays to rigorously down-select antibody candidates

We designed immunization and high-throughput single-cell screening campaigns to generate hundreds of antibodies that meet species cross-reactivity criteria. We then expressed and analyzed antibodies at high-throughput and selected those with desired binding and functional properties. Integrated high-throughput and mid-scale antibody assessments — including high-concentration stability, viscosity, and forced degradation studies — combined with structural analyses, were then used to identify molecules with properties suitable for IND-enabling studies.

OUTCOME

Three molecules that are extensively de-risked and ready for IND-enabling studies

These molecules met key properties in the TCP, including:

- Human & cyno cross-reactive, high-affinity binding
- Strong *in vitro* potency
- Favorable developability profile and high stability
- Desirable target-binding geometry
- Expected profile observed in mouse pharmaco-kinetics (PK)
- Suitability for high-concentration and potential subcutaneous administration

With deep screening of humanized mice and integrated antibody assessment technologies, we show a streamlined approach to finding optimal antibody candidates that do not require extensive engineering.

A panel of cross-reactive, target-binding antibodies

To amplify and capture diversity from the natural immune system, we performed immunization campaigns in two strains of humanized mice. We screened 1.3 million single cells and identified 735 target-specific, cross-reactive, fully human antibodies (Figure 1).

We mapped potential sequence liabilities of antibodies *in silico* and overlaid this with sequence diversity data using our proprietary software, Celium™. We prioritized antibodies with low liability scores while maintaining clonal diversity, selecting 184 unique antibodies for high-throughput expression and characterization (Figure 2).

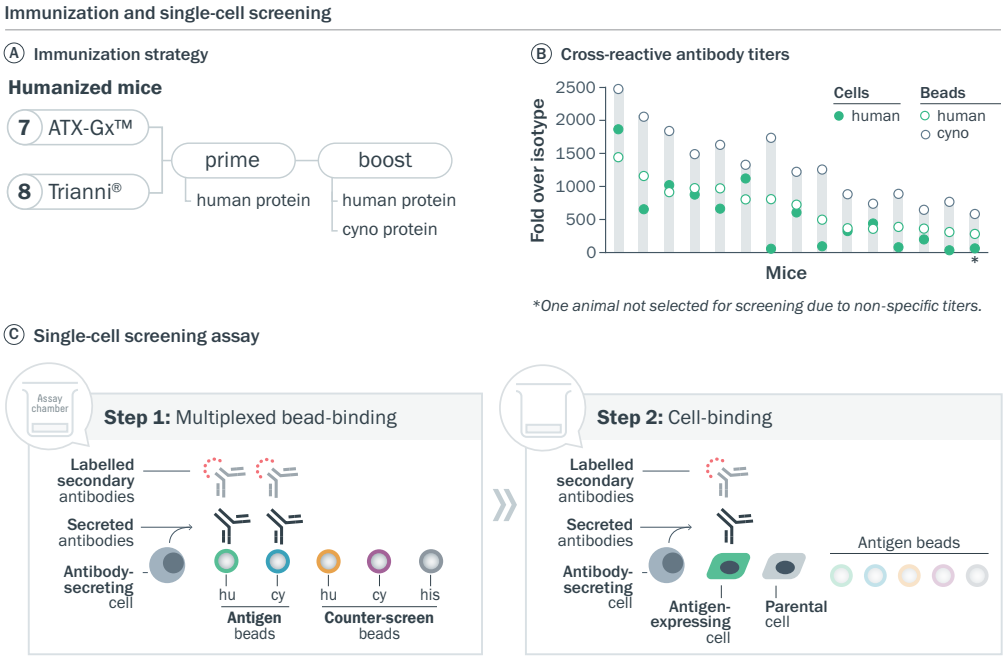


FIGURE 1. Immunization and high-throughput screening strategies were designed to identify diverse, cross-reactive binders

(A) Two strains of humanized mice were immunized using recombinant protein and proprietary protocols. **(B)** Immunized mice were assessed for cross-reactive titers using bead- and cell-based assays at six time points, each at multiple dilutions with counter selection against negative controls. Data shown are from Bleed 5 at 1:100 dilution for cells and 1:500 dilution for beads. Out of 15 immunized mice, 14 showed target-specific, cross-reactive titers and were selected for screening. **(C)** Multiplexed bead-based single-cell screening assays were used to screen 1.3 million single cells and identify target-specific, cross-reactive, fully human antibodies. Counter-screening with beads coated in an undesired target was used as a negative control.

hu: human; cy: cyno; his: histidine tag.

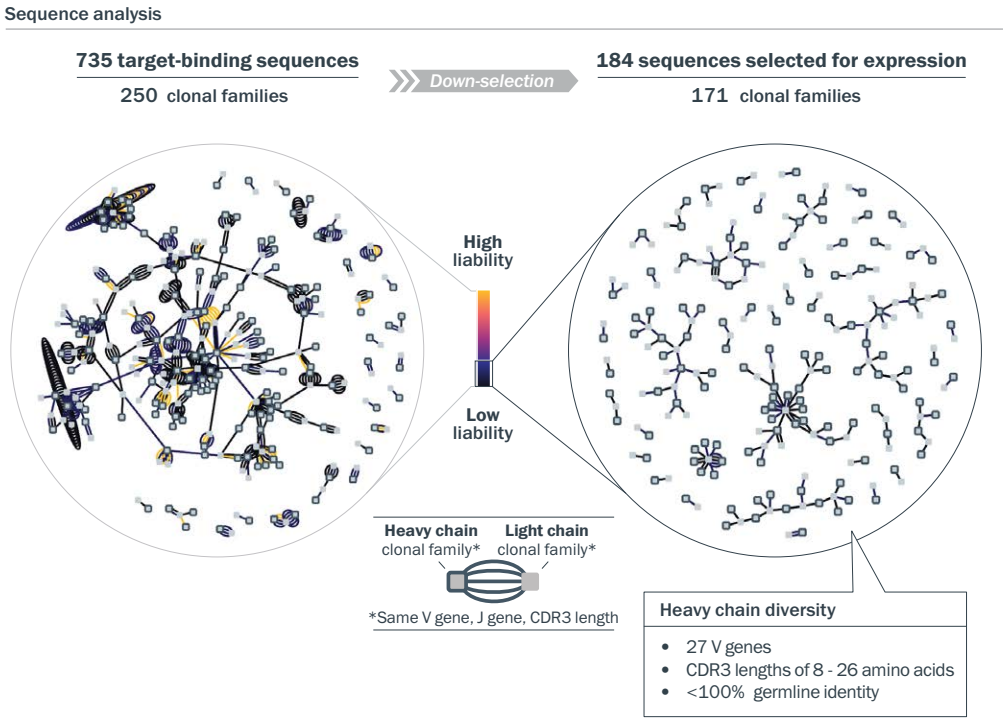


FIGURE 2. From 735 hits, 184 target-binding antibodies were selected for high-throughput expression and characterization based on *in silico*-predicted sequence liabilities and clonal diversity

Potential sequence liabilities were identified *in silico* and mapped using proprietary bioinformatics pipelines. Antibody sequence diversity and *in silico* liabilities were visualized using Celium™, and custom algorithms were used to select antibodies with low liability scores while maintaining high clonal diversity. Clusters represent clonal families based on V genes, J genes, and CDR3 lengths; color represents sequence liability scores.

Potent antibodies with favorable developability properties

We assessed function and binding kinetics using high-throughput, parallel assays. Over 94% of expressed antibodies met the desired potency, shown in a cell-based assay measuring antagonistic activity (Figure 3). We confirmed the species cross-reactivity observed during single-cell screening and identified antibodies with high-affinity across the desired pH range using surface plasmon resonance (SPR) (Figure 4).

To identify high-quality and developable lead molecules, we assessed biophysical properties using high-throughput assays (Figure 5). We selected a lead panel of 12 antibodies that met the TCP for potency and cross-reactivity and showed low developability risk, including high stability and low aggregation, polyspecificity, and self-association (Figure 5).

FIGURE 3. Blocking of a functionally relevant biological pathway revealed over 94% of expressed antibodies met the desired potency

Antibodies were tested for antagonist activity using CHO-K1 cells expressing the target, and activity was quantified using ELISA. Percentage inhibition compared to isotype control was calculated for all test antibodies over a concentration range. Reported values are EC_{50} and the % maximum inhibition reached.

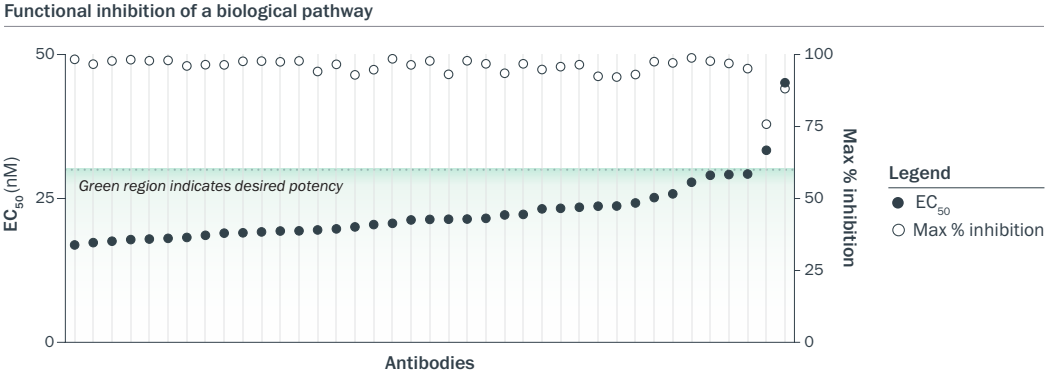


FIGURE 4. High-throughput binding assessment revealed cross-reactive binders with high affinities across the desired pH range

(A) Antibodies bound to human and cyno homologs at low and high pH, assessed by SPR. **(B)** SPR sensorgrams with top concentration of 50 nM and three-fold serial dilutions for an example antibody are shown.

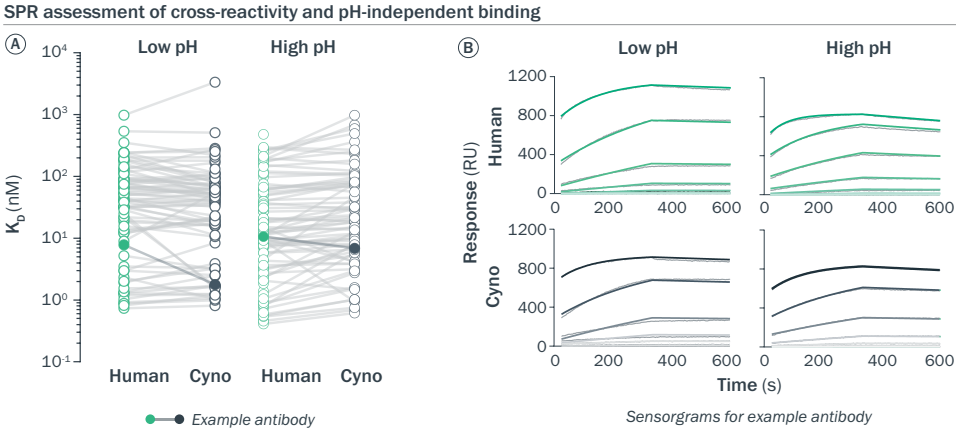
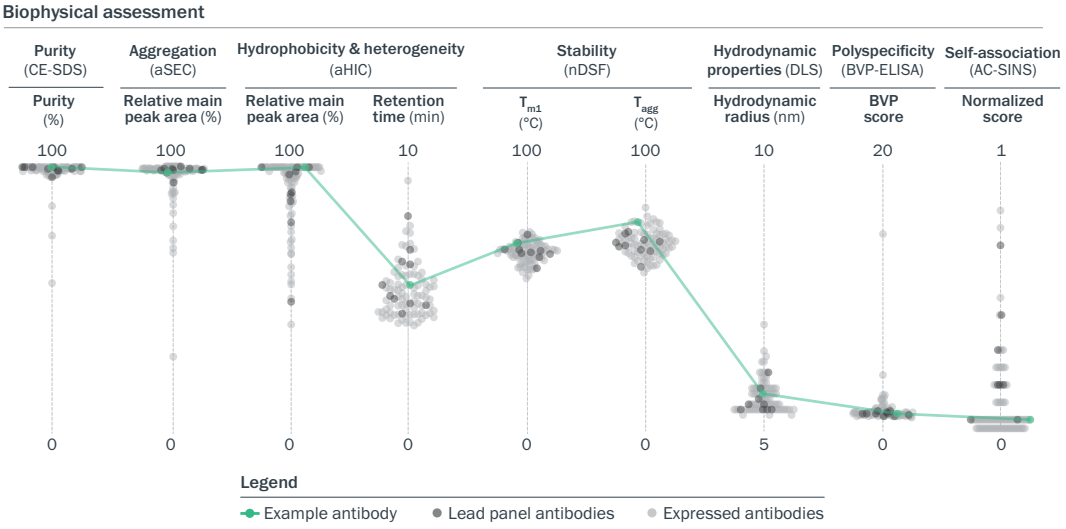


FIGURE 5. Lead panel antibodies showed favorable developability profiles

Selected lead panel antibodies showed high purity and stability, low aggregation, relative surface hydrophobicity, polyspecificity, self-association, and heterogeneity, and typical hydrodynamic properties. Polyspecificity and self-association scores were normalized to high and low controls.



Multiple candidates with desirable stability

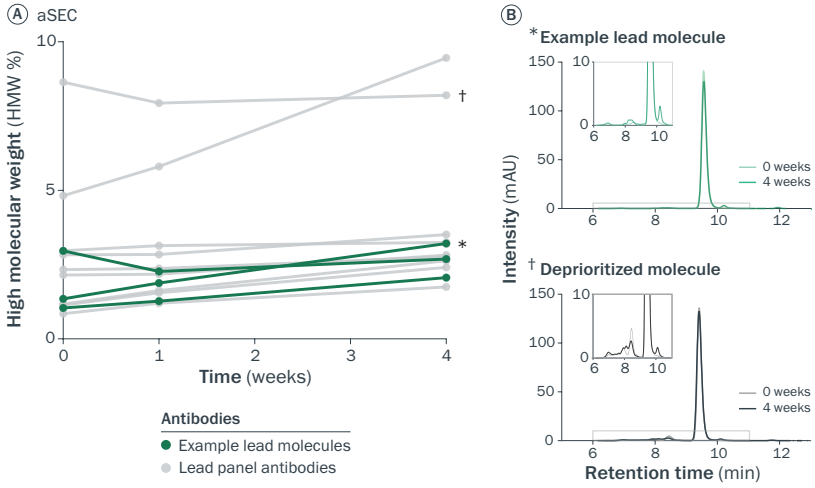
Twelve antibodies were produced at mid-scale for in-depth lead assessment and identification of molecules suitable for IND-enabling studies. We assessed stability of lead panel antibodies under forced degradation conditions, including accelerated stability, freeze/thaw, high-concentration, and low-pH hold. Molecules showed minor changes in aggregation and binding affinity over time, measured under accelerated stability conditions (Figure 6A, B).

Integrating insights across antibody assessments supported confident, data-driven down-selection. In one example, SPR analysis revealed reduced binding affinity following thermal stress. Peptide mapping analysis confirmed that this antibody harbored an unstable post-translational deamidation modification in the critical CDR-H3 domain, making it unsuitable for further development (Figure 6C-E).

FIGURE 6. Multiple antibodies showed high stability under forced degradation conditions (100 mg/mL, 40°C)

(A) The majority of antibodies tested showed minor changes in aggregation over time following thermal stress. Three example molecules from the lead panel with favorable stability profiles are highlighted in green. **(B)** aSEC chromatograms for a lead molecule and a deprioritized molecule are shown. **(C)** Mass spectrometry peptide mapping analysis was used to identify chemical modifications in each lead panel antibody following a 4 week accelerated stability assessment. A subset of the data is shown, including a deamidation event in CDR-H3 of molecule A and a site in CDR-H3 of molecule B that was insensitive to oxidation. **(D)** Detailed SPR assessment demonstrated a decrease in binding affinity for molecule A under accelerated stability conditions, correlated with the deamidation event in CDR-H3. Binding of other lead panel antibodies was largely unchanged. **(E)** Example SPR sensorgrams for molecules A and B are shown, with top concentration of 300 nM.

Aggregation



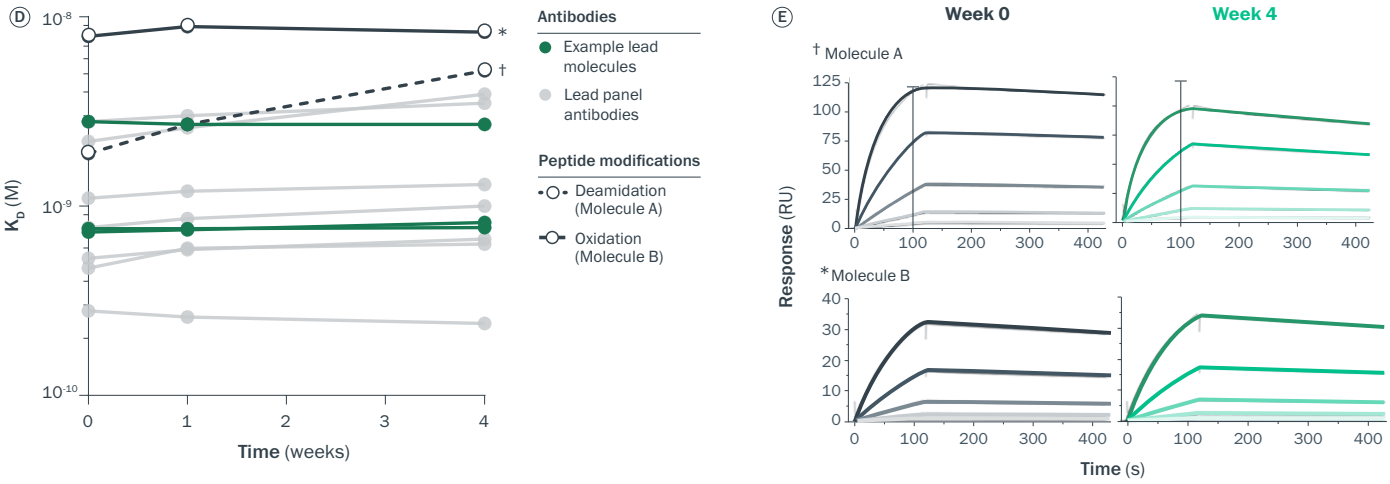
Peptide mapping

C MS

Modification type	Modification location	Modification % Week 0	Week 1	Week 4
Deamidation (Molecule A)	CDR-H3	7.34	11.2	26.9
Oxidation (Molecule B)	CDR-H3	18.0	19.4	18.0

Binding affinity

SPR



LEAD PANEL TO CANDIDATES CONT'D

We assessed *in vivo* PK and viscosity of antibodies compared to clinical comparator molecules. Multiple antibodies showed suitability for subcutaneous delivery with low viscosity at high concentration (Figure 7), and all 12 antibodies had favorable PK properties (Figure 8).

To support selection of antibodies for further assessment, we generated three-dimensional structural models and compared them to four known benchmark molecules. The desired structural binding profile, confirmed in 11 of the 12 lead panel antibodies, did not display an antigen-Fab interaction with known toxicology effects (Figure 9). We further observed broad diversity in the Fab binding site and angle of approach, highlighting that the molecular diversity prioritized during down-selection translated to diversity in antigen-antibody interactions.

FIGURE 7. Potential for subcutaneous delivery was demonstrated for multiple antibodies, with low viscosities at high concentrations

Dynamic viscosity was assessed at concentrations up to 150 mg/mL. Viscosity was compared to a panel of clinical stage comparator antibodies with known viscosity profiles.

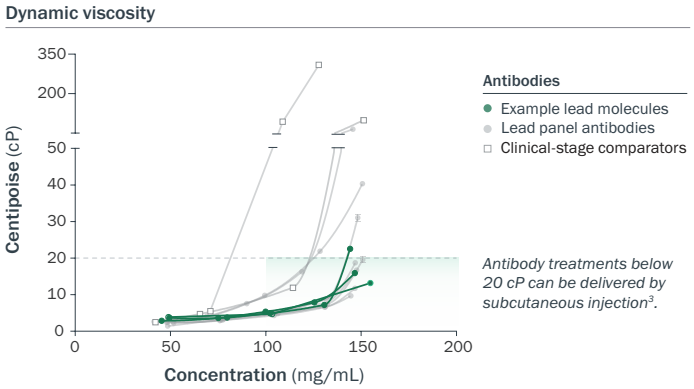


FIGURE 8. All 12 lead panel antibodies showed favorable PK properties

Antibody concentration was quantified following a single 5 mg/kg dose in C57/BL6 mice, measured in an electro-chemiluminescence assay. Values are shown per antibody and are an average of four biological replicates.

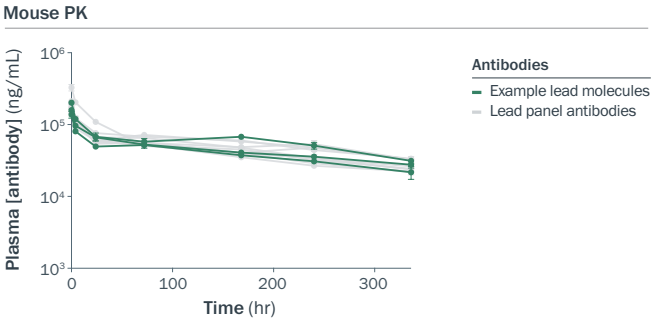
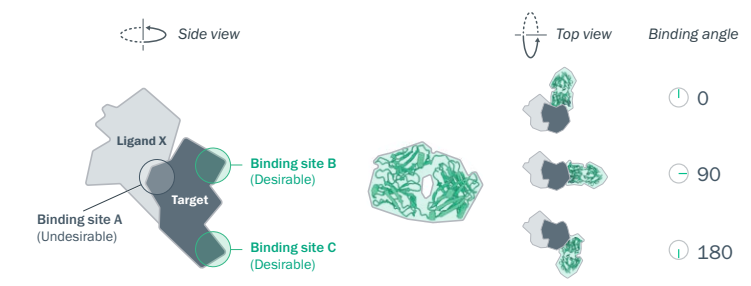


FIGURE 9. 3D modeling revealed desirable structural binding profiles in 11/12 lead panel antibodies, and diversity in binding complex

(A) Structural models were generated using SEC and negative stain electron microscopy. The side view shows an illustrative structure of the Fab and target. The top view shows the range of Fab binding angle of approach. **(B)** 11/12 antibodies did not block Ligand X, and broad diversity was observed in the binding angle of approach. The lead panel was assessed alongside four benchmarks. Benchmarks 1 and 2 had known structures, while structures for benchmarks 3 and 4 were determined in this program.

3D structural modeling



*Protein structure diagram is intentionally blinded to obscure the target and antibodies in this program.

B

Lead panel antibody	Binding site A interaction	Site of Fab binding	Approximate binding angle of approach (degrees)
ab 1	not blocked	B	60
ab 2	not blocked	C	60
ab 3	not blocked	B	60
ab 4	not blocked	B	60
ab 5	not blocked	B	90
ab 6	not blocked	B	90
ab 7	not blocked	C	90
ab 8	not blocked	C	90
ab 9	not blocked	B	120
ab 10	not blocked	B	120
ab 11	not blocked	B	150
ab 12	inconclusive	--	--
Benchmarks			
bm 1	not blocked	B	0
bm 2	not blocked	B	60
bm 4	not blocked	B	60
bm 3	not blocked	B	180

CONCLUSION

Here, we showed how our antibody discovery and development engine is able to identify molecules with properties suitable for IND-enabling studies, including strong *in vitro* potency, favorable developability, and high-affinity, cross-reactive binding. To generate antibodies that meet the TCP without multiple rounds of engineering, we used immunization and deep screening, paired with high-throughput antibody assessments, to amplify diversity from natural immune systems and enrich for critical properties up-front. With integrated structural, stability, PK, and developability assessments, we gained insights into critical TCP parameters and streamlined the identification of candidates with suitability for IND-enabling studies.

REFERENCES

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2. Bailly M, et al. (2020). mAbs. 12 (1). 1743053.
3. Berteau C, et al. (2015). Med Devices (Auckl). 8. 473-84.