Enabling Rapid Bispecific Antibody Discovery with Invenra's B-Body Platform

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Introduction

Bispecific antibodies represent a transformative approach in next-generation biologics, offering enhanced therapeutic efficacy through dual-targeting capabilities. Despite their promise, the efficient and rapid identification of optimal lead candidates remains a significant challenge in drug discovery. The Invenra B-Body® Platform addresses these obstacles by providing an integrated, end-to-end solution that accelerates the discovery process. This platform combines rapid de novo antibody identification, seamless bispecific production, and comprehensive functional screening within a streamlined workflow.

Central to workflow success are our proprietary antibody libraries, engineered for exceptional human-like characteristics and developability. These libraries facilitate the high-throughput generation of hundreds of bispecific candidates across a variety of formats, including 1x1, 2x1, and 2x2 configurations. Notably, the B-Body Platform consistently delivers assay-ready, high-quality bispecific molecules in under four months. Moreover, these molecules exhibit developability and stability metrics comparable to monoclonal antibodies, minimizing downstream development risks.

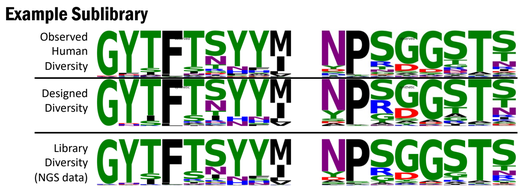
By integrating data-driven diversity and robust expression technologies, the B-Body Platform sets new standards for speed and reliability in bispecific antibody discovery. Here, we describe capabilities to streamline lead selection, reduce development timelines, and enhance the probability of clinical success, positioning the platform as a cornerstone of next-generation biotherapeutic development.

Phase Library Design

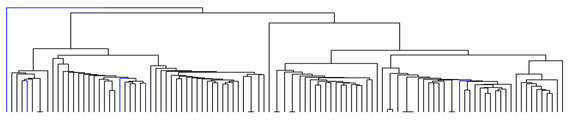
Structured to Enhance Natural Diversity

- **Comprehensive Germline Representation:** Libraries encompass a diverse array of human germline sequences, carefully chosen to maximize clinical relevance and therapeutic potential.
- **Preservation of Native Pairings:** Natural complementarity-determining region (CDR) pairings are maintained, ensuring minimal liabilities and enhanced developability.
- **Optimized Germline Frameworks:** Four IGHV germline frameworks are utilized, each selected for their superior biophysical behavior, clinical success rates, and diverse representation across the antibody repertoire.
- **Natural Diversification:** Libraries are engineered to retain native CDR-framework pairings, avoiding artificial shuffling and preserving the integrity of natural antibody structure.
- **Extensive Diversity Coverage:** Over 30 specialized sub-libraries expand discoverable diversity, providing a robust platform for identifying high-affinity, developable leads.

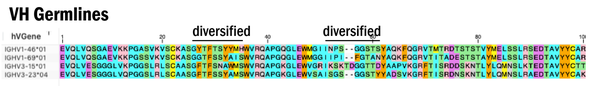
Example Sublibrary



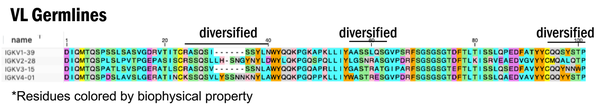
Example Selection Campaign



VH Germlines

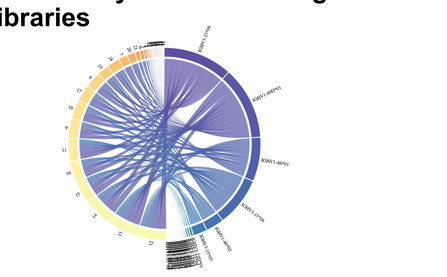


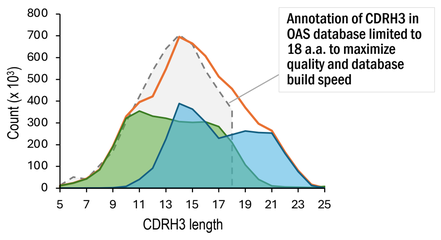
VL Germlines



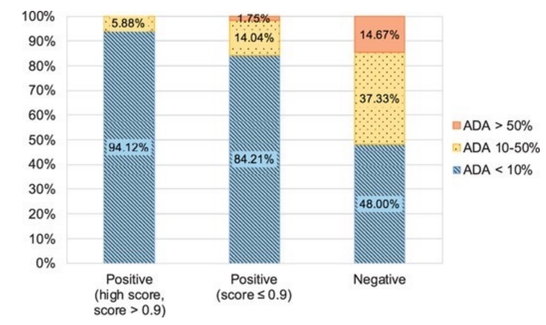
*Residues colored by biophysical property

Natural Diversity of CDRH3 Length Across Sub-Libraries





Humanness Scoring of Library Derived New Binders vs. Benchmarks



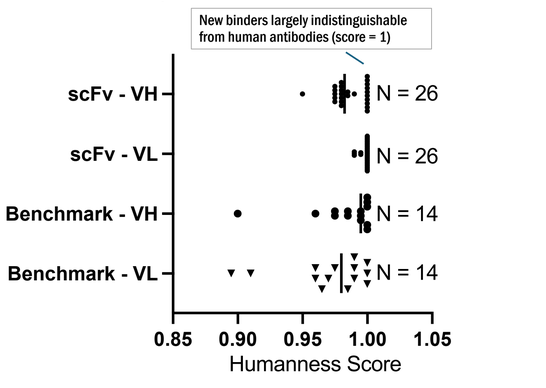


Figure 1. LSTM-Based Model for Evaluating Binder Humanness

- **Correlation with Clinical Outcomes:** Analysis of clinical antibody datasets reveals a strong correlation between humanness scores and reduced anti-drug antibody (ADA) rates, highlighting the importance of humanness in therapeutic antibody development.
- **High Humanness Across Binders:** Both benchmark and newly identified binders surpass the established humanness threshold, with many new binders achieving scores indistinguishable from fully human antibodies (score = 1).

Invenra's Rapid Bispecific Antibody Discovery: Antigens to Novel Lead Bispecifics in 4 Months

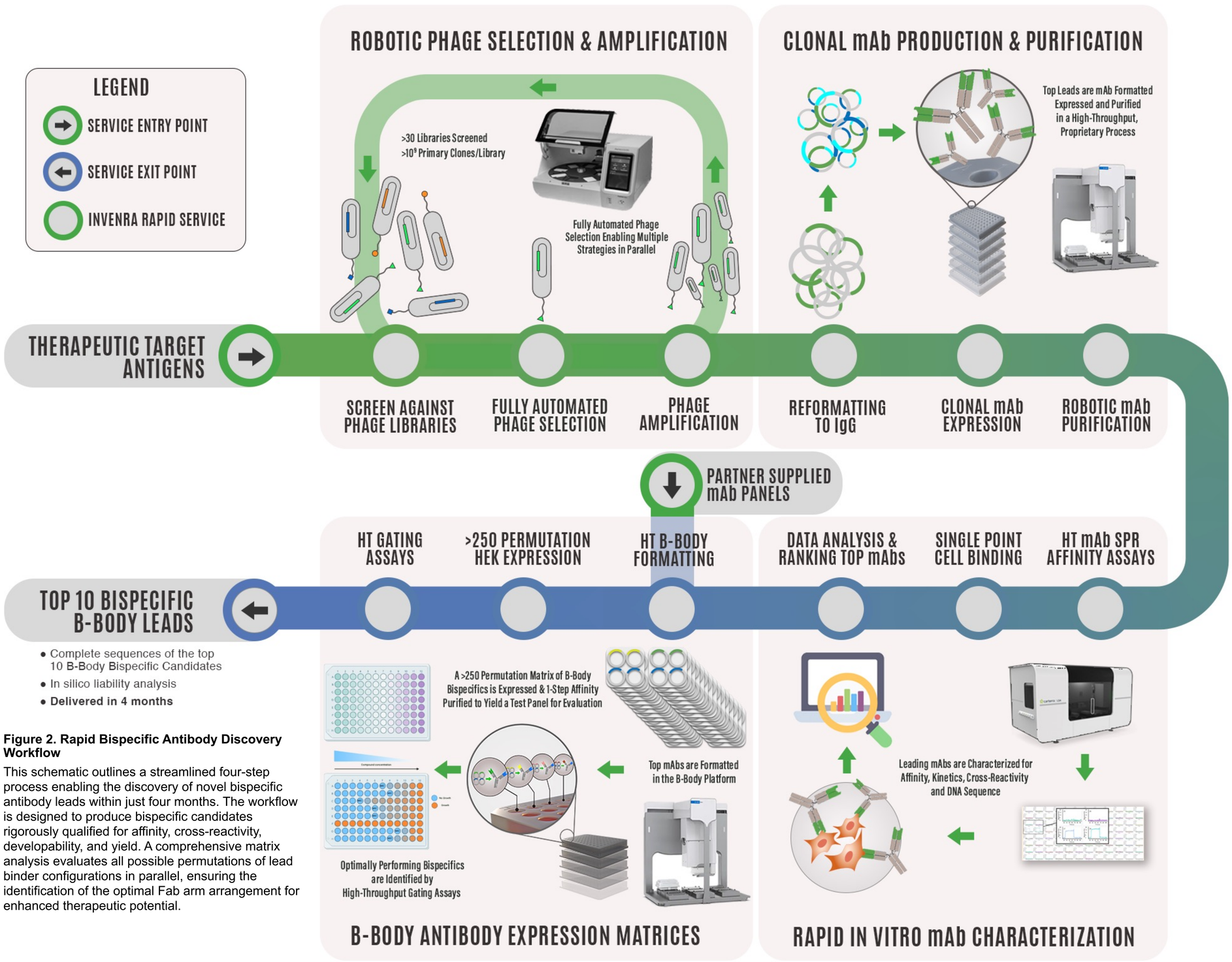


Figure 2. Rapid Bispecific Antibody Discovery Workflow

This schematic outlines a streamlined four-step process enabling the discovery of novel bispecific antibody leads within just four months. The workflow is designed to produce bispecific candidates rigorously qualified for affinity, cross-reactivity, developability, and yield. A comprehensive matrix analysis evaluates all possible permutations of lead binder configurations in parallel, ensuring the identification of the optimal Fab arm arrangement for enhanced therapeutic potential.

The B-Body Platform: Enabling Rapid Bispecific Antibody Discovery

Discovery Stage Innovations

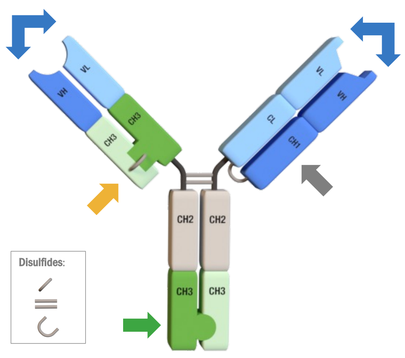
Proprietary Symmetrical Heavy & Light Chain Inversions in Fab Arms
- Enhances expression, yield and stability
- Facilitates one-step purification in discovery phase

Sole CH1 Domain Allows Another Level of Purifications Beyond Protein A and IEX
- One-step purification with anti-CH1 resin
- Simplifies and streamlines in format discovery
- Eliminates knob-know homodimers and other impurities

CMC Innovations

Fc Region
- Clinically validated Knobs-Into-Holes
- Drives heavy chain heterodimerization

Fab Arm – Proprietary CH3 Domains
- Substitutes for CH1/CL in one Fab arm
- Assures proper light chain pairing
- Novel CH3 mutations, without knobs-into-holes or charge pairs
- pI asymmetry streamlines CMC polishing processes



A Top Performance Bispecific Platform
- Exceptional stability and expression performance
- Enables multiple formats including 1x1, 2x1, 2x2, etc.
- Incorporate mAbs from any source

Facile HT Expression & Testing
- Easy expression and purification
- Screen for function in final format
- Compatible with plate-based assays
- ADC killing, internalization, cell killing, agonism, antagonism

Best-in-Class Yield, CMC Ready
- 6-11 g/L yield from developed cell lines
- Manufacturing timelines comparable to mAbs
- High Stability, with concentration up to 200 mg/L for sub-q compatibility
- Compatible with typical mAb modifications

Figure 3. Overview of the B-Body Platform for Accelerated Bispecific Discovery

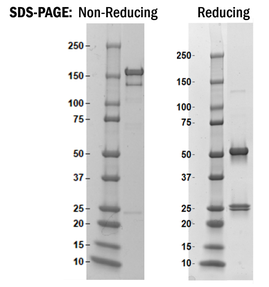
The B-Body Platform streamlines bispecific antibody discovery and development by addressing common challenges encountered in the production pipeline. Candidate bispecific antibodies are expressed at high yields and purified efficiently in a single step, enabling rapid evaluation. The platform facilitates a seamless transition from early-stage discovery to cell line development and high-yield CMC (chemistry, manufacturing, and controls), ensuring continuity and scalability within a unified system.

Example Transient Yield of a B-Body Lead

Expression Titer	442.8 mg/L
Purification Resin	MabSelect Sure
Molecular Weight	146270 Da
Isoelectric Point	7.8

Biophysical Properties

PDI	Z-Ave (nm)	Tm (°C)	Tagg (°C)
0.410	71.6	68.7	66.2



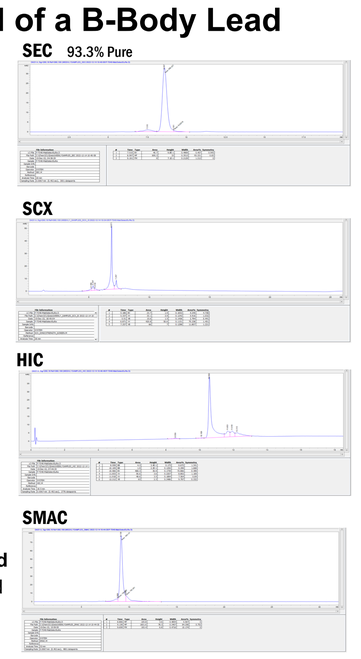


Figure 4. High-Yield of a B-Body Bispecific Lead

A B-Body Bispecific lead was transiently expressed in 100 mL of CHO cells and purified using a single-step Protein A chromatography process. The resulting antibody achieved a purity level exceeding 93%, as confirmed by both SDS-PAGE and HPLC analysis, demonstrating the platform's efficiency in generating high-quality bispecific candidates.

Validation of the B-Body Bispecific Platform Matrix Approach to Antibody Discovery & Optimization

Name	hVGene	lVGene	HEK Titer (mg/L)	Fab Tm DSF (°C)	SGAC-SINS AS100 ($(NH_4)_2SO_4$, mM)	HIC Retention Time (Min)	SMAC Retention Time (Min)	Poly-Specific. Reag. SMP Score (0-1)	AC-SINS^ ΔAmax (nm) Average
Adalimumab (ADA)	IGHV3-9*01	IGKV1-27*01	134.9	71.0	900.0	8.8	8.7	0.00	1.1
Atezolizumab (ATE)	IGHV3-23*04	IGKV1-NL1*01	164.1	73.5	300.0	13.4	19.3	0.07	15.0
Canakinumab (CAN)	IGHV3-33*01	IGKV6D-21*02	45.7	72.0	800.0	9.3	8.7	0.00	0.7
Daclizumab (DAC)	IGHV1-46*01	IGKV1-5*01	245.1	74.0	900.0	9.3	8.8	0.00	-0.1
Elotuzumab (ELO)	IGHV3-7*05	IGKV1-27*01	213.2	83.5	700.0	10.3	9.3	0.00	-0.2
Evolocumab (EVO)	IGHV1-18*01	IGLV2-14*01	260.7	65.0	700.0	10.4	9.1	0.20	2.2
Farletuzumab (FAR)	IGHV3-30*03	IGKV1D-33*01	220.8	75.5	800.0	9.5	9.1	0.00	-0.5
Golimumab (GOL)	IGHV3-30*01	IGKV3-11*01	163.2	70.0	0.0	11.4	12.7	0.23	23.0
Guselkumab (GUS)	IGHV5-10-1*04	IGLV1-40*01	167.3	69.5	700.0	11.4	9.2	0.47	3.4
Trastuzumab (HER)	IGHV3-11*05	IGKV1-5*05	159.5	78.5	800.0	9.7	8.8	0.00	2.0
Ipilimumab (IPI)	IGHV3-30*01	IGKV3-20*01	169.6	73.0	400.0	11.6	13.0	0.23	10.4
Ixekizumab (IXE)	IGHV1-46*01	IGKV2D-29*02	97.3	83.0	500.0	10.9	9.1	0.81	20.0
Mepolizumab (MEP)	IGHV2-70*20	IGKV4-1*02	221.5	78.5	900.0	9.2	8.8	0.00	-1.0
Ramucirumab (RAM)	IGHV3-21*01	IGKV1-12*01	90.7	66.0	900.0	9.4	8.7	0.00	0.0
Ustekinumab (UST)	IGHV5-51*01	IGKV1D-16*01	152.7	69.5	1000.0	8.8	8.6	0.15	0.4

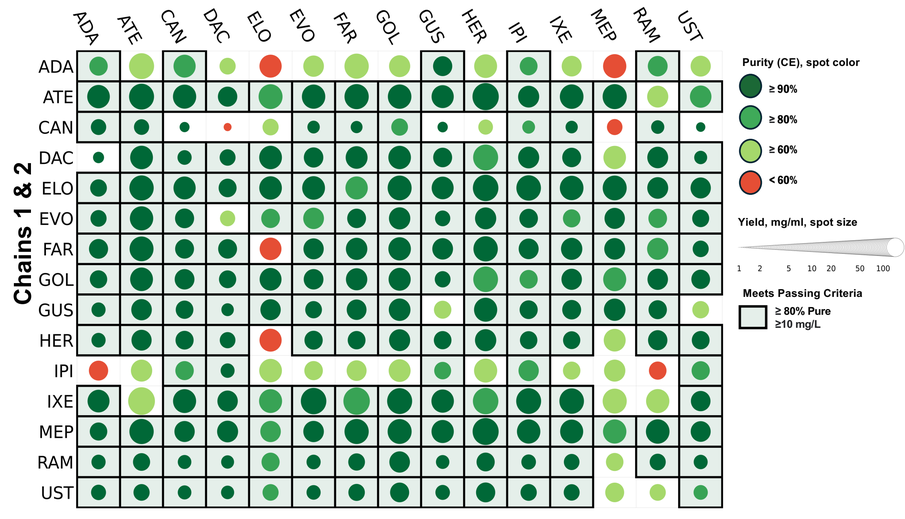


Figure 5. Matrix Evaluation of Clinical-Stage Antibodies Expression, Yield and Purity in the B-Body Platform[2]

A 15x15 matrix of clinical-stage antibodies shown in the table (above, left) was expressed in 1 mL volumes in deep well plates and purified in a single-step using Anti-CH1 resin. The selected set of antibodies represent a variety of heavy chain and light chain germlines and exhibit a broad range of biophysical properties as shown. The produced matrix of bispecific antibodies was characterized by capillary electrophoresis (purity, represented by spot color), Octet® BLI (binding, *data not shown*, trademark Sartorius AG) and mass spectrometry (assembly, *data not shown*). The yield for each well is represented by the spot size, and wells that passed minimum purity and yield were represented by shaded boxes. It was determined that >99% of bispecific combinations passed purity (>80%) and yield (>10mg/L) specifications in at least one orientation.

Summary

- **Rapid Discovery Workflow:** Invenra has developed an accelerated process for discovering novel bispecific antibodies, delivering comprehensive lead panels within just four months.
- **Optimized Phage Libraries:** The process begins with phage libraries engineered to maximize diversity while maintaining exceptional humanness and developability.
- **Seamless Lead Development:** Identified lead binders are rapidly expressed, characterized, and formatted into the B-Body Platform for further evaluation.
- **Streamlined Evaluation:** The B-Body Platform enables high-throughput assessment of all binder permutations, achieving high antibody yields and single-pass purity through an efficient workflow.
- **Scalable Production:** The platform supports high-yield production in both transient and stable engineered cell lines, with observed yields reaching up to 11 g/L.

References

1 Protein Engineering, Design and Selection, Volume 32, Issue 7, July 2019, Pages 347–354 Bioinformatics, Volume 37, Issue 22, November 2021, Pages 4041–4047
2 Jain T, Sun T, Durand S, *et al.* Biophysical properties of the clinical-stage antibody landscape. *Proc Natl Acad Sci U S A*. 2017;114(5):944-949.