



# Parallelization of Paratope Optimization and Antibody Format Screening for Efficient Characterization and Development of Multispecific T Cell Engagers

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Nasdaq: ZYME | [zymeworks.com](https://zymeworks.com)



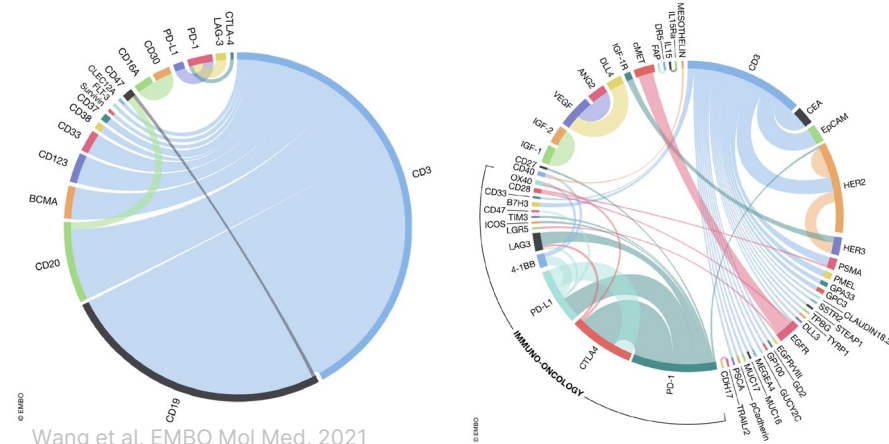
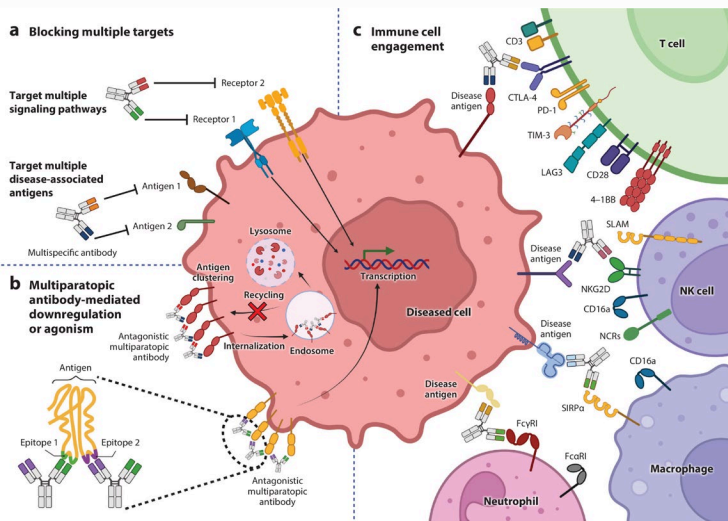
# Multispecific Antibodies have Increased the Therapeutic Design Space for Treatments for Oncology and Autoimmune Diseases

Engaging multiple targets with a single molecule can enable novel therapeutic mechanisms of action that are not possible with mAbs alone or in combination

## Target combinations of bispecific antibodies:

Hematological malignancies

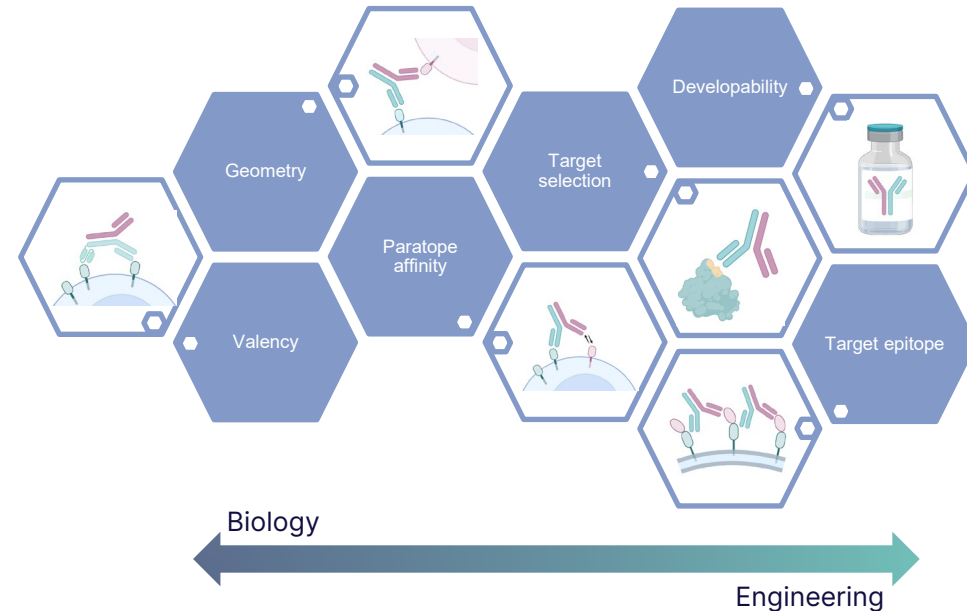
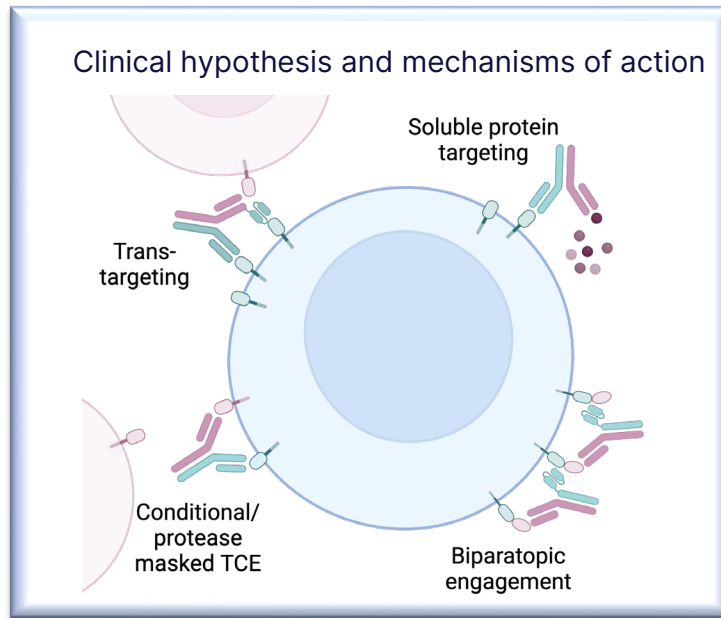
Solid tumors



Fine J, et al. 2024  
Annu. Rev. Chem. Biomol. Eng. 15:105-38

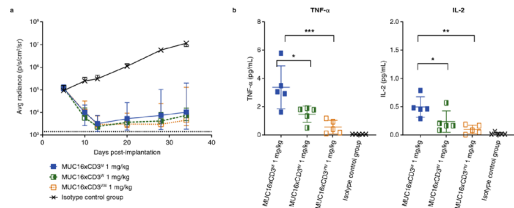
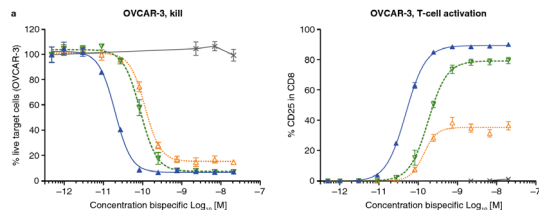
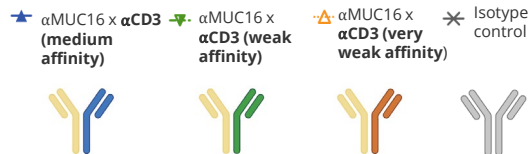
# Multispecific Antibody Development Requires Optimization of Multiple Parameters Specific to the Desired Mechanism of Action

- Understanding the interplay of antibody geometry with optimal paratope affinity, valency, and target epitope is critical to identifying multispecific antibody therapeutics



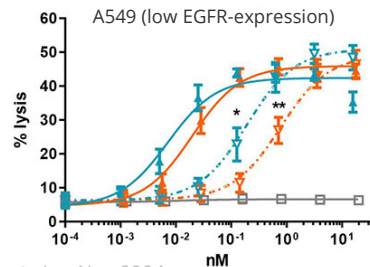
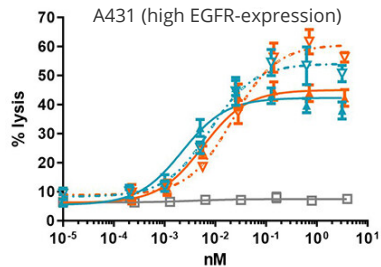
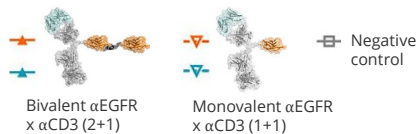
# Optimization of T Cell Engager Properties and Geometry Critical to Driving Intended Biological Effect – No “one-size fits all”

## Affinity



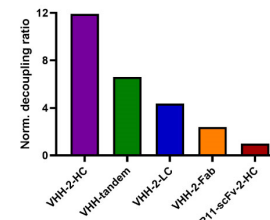
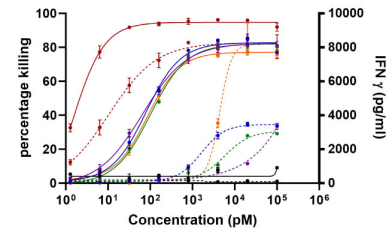
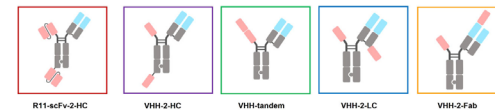
Haber et al, Sci Rep, 2021

## Valency



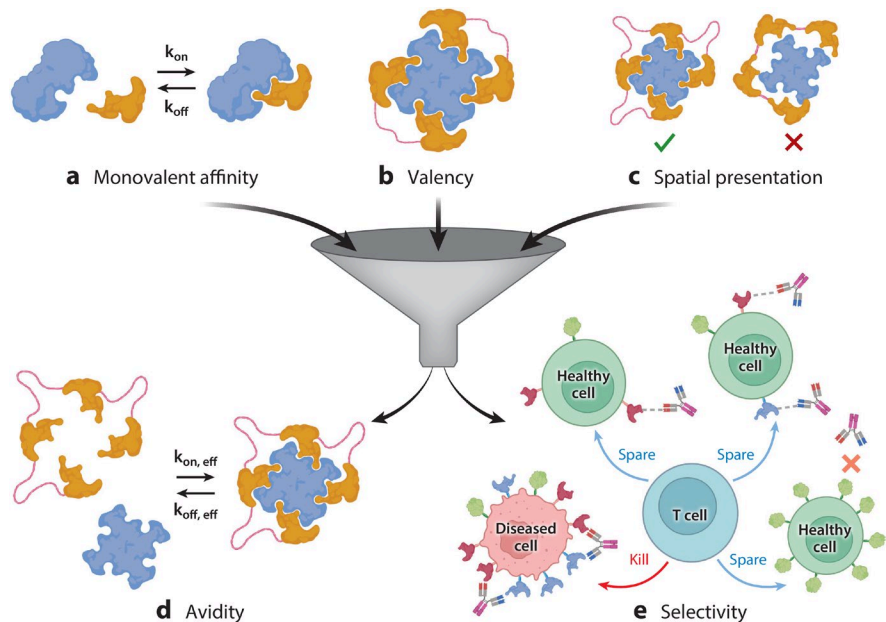
Boje et al, mAbs, 2024

## Geometry



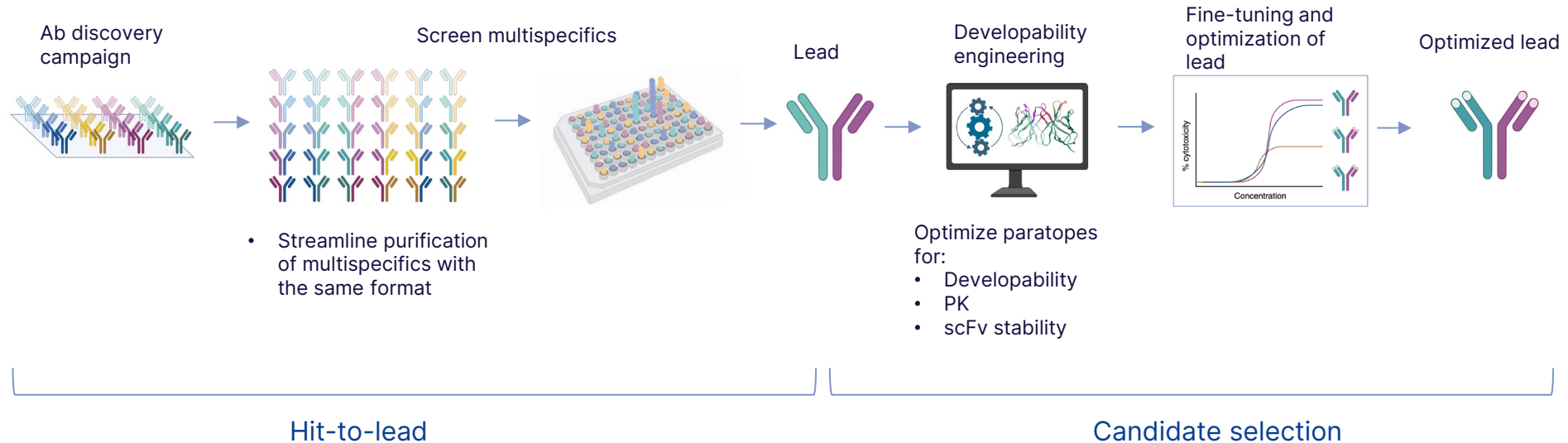
Zhou et al, Front Immunol, 2024

# Understanding How These Tunable Parameters Come Together to Design Mechanistic-driven Multispecific Antibody is Challenging

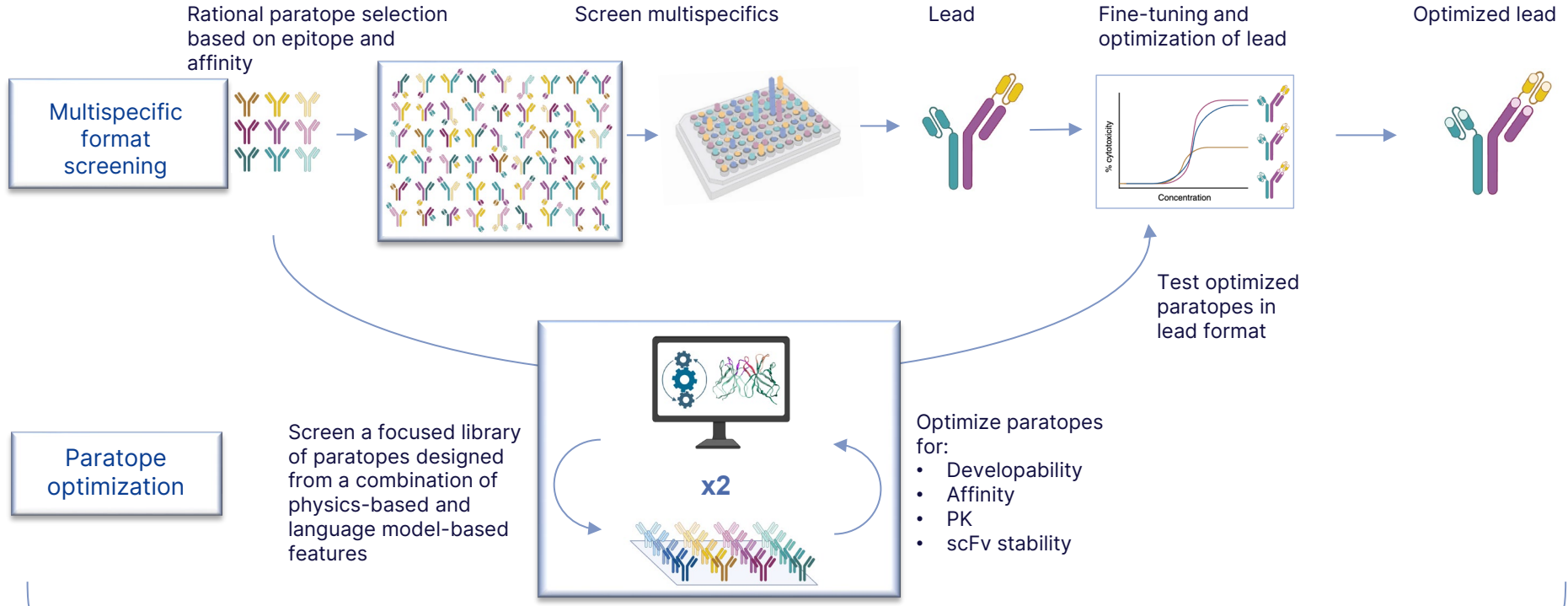


- Goal of creating a molecule that meets both functional and developability requirements necessitates:
  - robust protein engineering strategies
  - empirical format screening
  - biophysical characterization
- Challenges associated with testing multiple parameters comprehensively and quickly
- Will present some of the strategies we are employing to address these challenges

# Traditional Multispecific Antibody Screens are Often Limited in the Number of Formats Tested but Exhaustive in the Number of Paratopes Tested

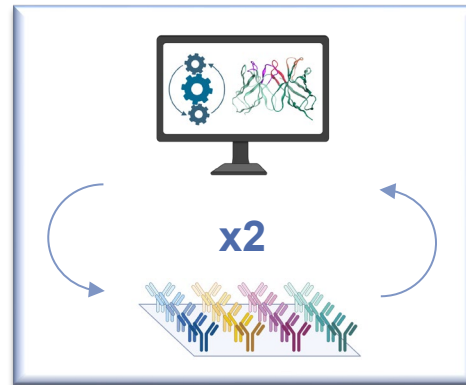


# High Throughput Antibody Format Screening Workflow and Paratope Optimization can be Performed in Parallel to Efficiently Identify and Optimize Lead Format



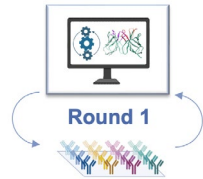
Hit-to-lead + Candidate selection

# Paratope Optimization

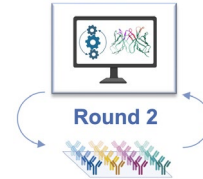




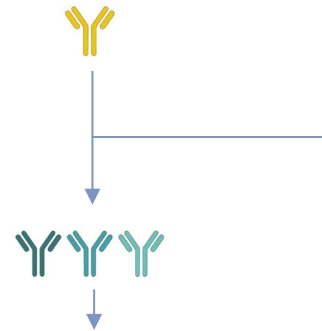
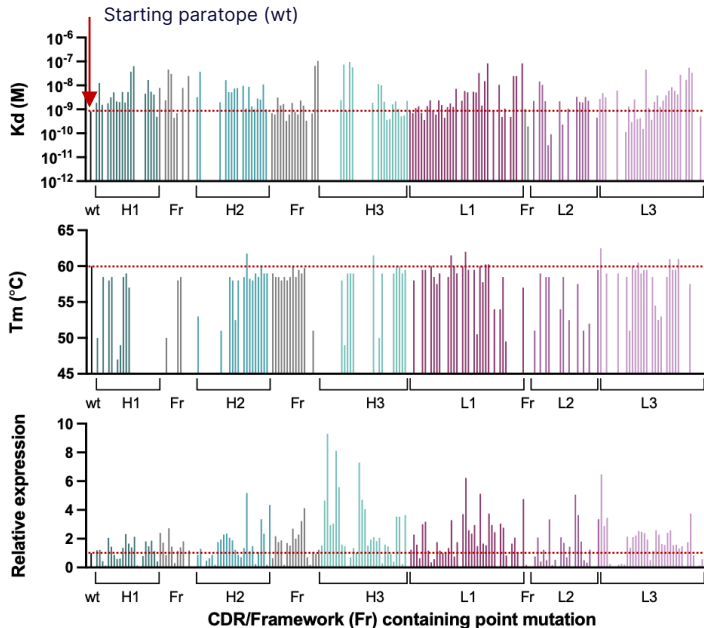
# Paratope Optimization Conducted Using Two Rounds of *In Silico* Engineering and Expression/Characterization of a Focused Library of Antibody Variants



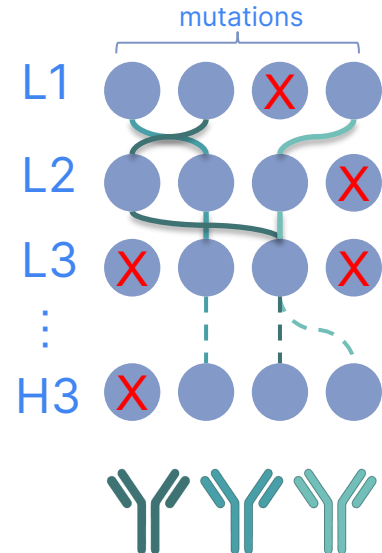
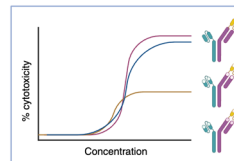
- **Focused 1x screen** to gain information on CDR mutational tolerance, affinity, thermal stability, expression characteristics, etc
- 284 variants tested from  $\sim 10^4$  computationally evaluated mutations



- Identify and **combine best point mutations** from 1x results into optimized paratopes
- 285 combinations selected for testing out of  $\sim 10^7$  computationally evaluated designs

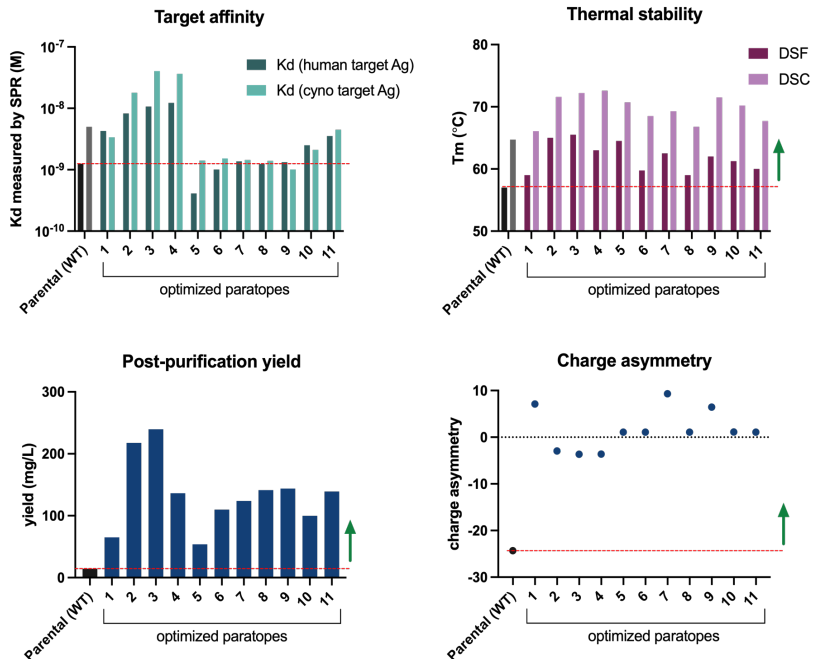


Fine-tuning and optimization of lead

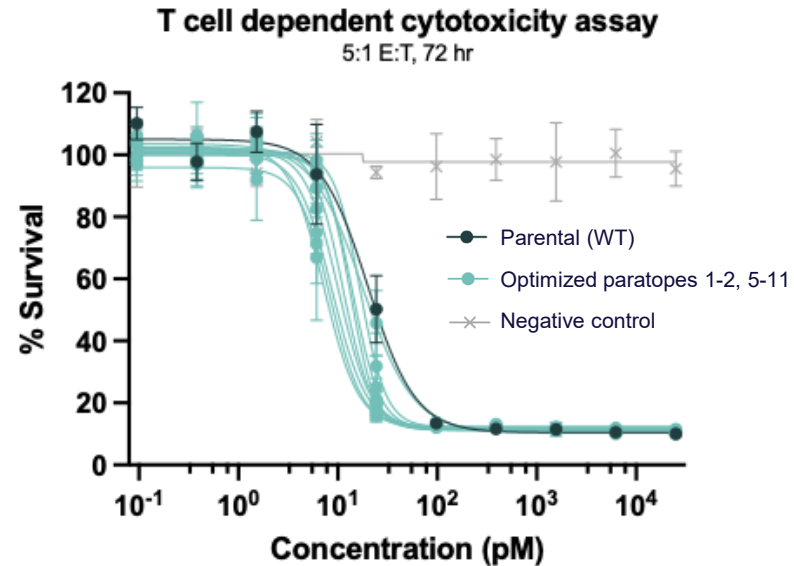


# *In Silico* – designed Paratopes are Screened for Improved Biophysical Properties and Top Paratopes are Tested for Function in Lead Multispecific Format

- Using language models provides an opportunity to optimize multiple parameters in parallel and yields paratopes with improved expression, thermal stability, developability parameters, while maintaining target affinity

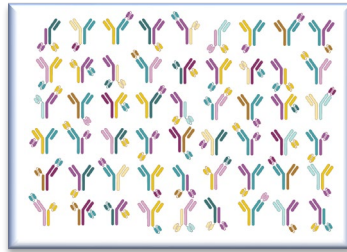


Fine-tuning of lead TCE format with optimized paratopes



# Multispecific Format Screening

Rational paratope selection  
based on epitope and  
affinity



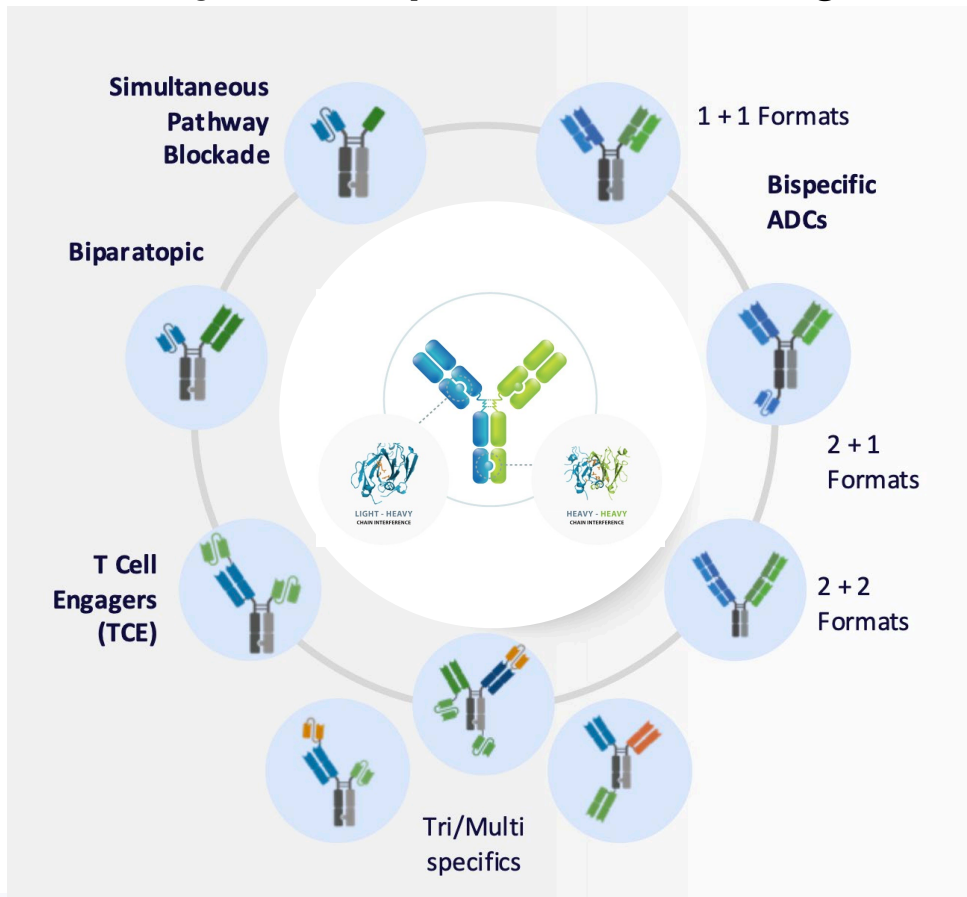
Screen multispecifics



Lead

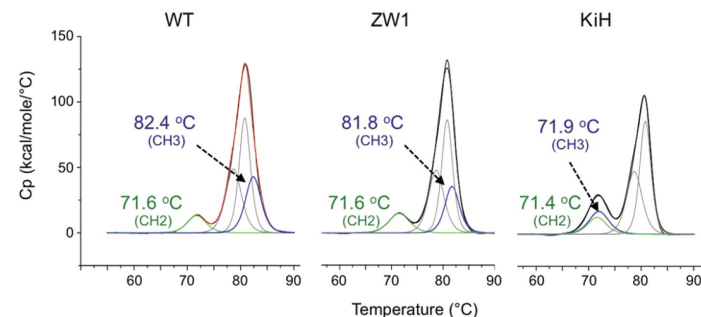


# Azymetric™ Solutions for Heterodimeric Fc and Light Chain Pairing Enable Flexibility in Multispecific Format Design



Highly manufacturable:

- High purity due to strength of CH3 interface and HetFab designs
- Stability of design comparable with WT IgG Fc



Von Kreudenstein et al, mAbs, 2013

Leveraged by multiple pharma/biotech with various clinical stage programs in development

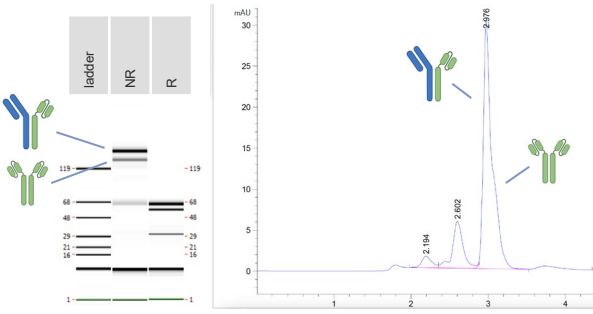
# Optimizing Antibody Heterodimer Expression Can Make Multispecific Screening More Amenable for High Throughput Methods

Product-related impurities due to chain mispairing can be mitigated by:

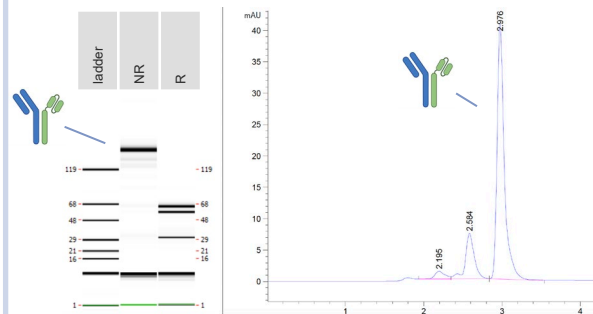
- Optimizing transfection plasmid DNA ratios
- Tailoring purification methods to predominantly purify the heterodimer (eg. CH1 capture, kappa/lambda capture, VH3 capture, prismA)



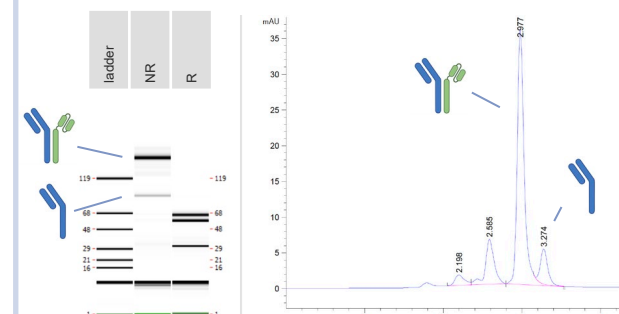
1:1:3



1:1:1.5

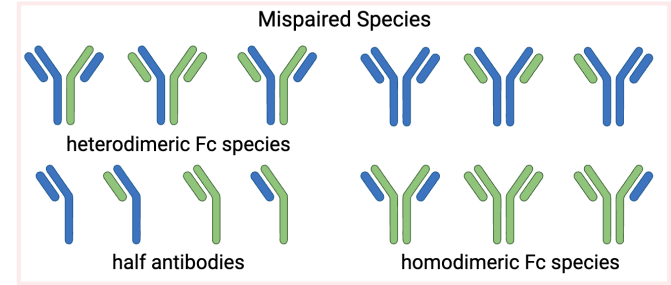
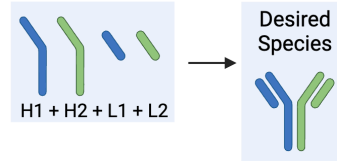


1:1:1



# Azymetric™ Platform Mutations Allow for HTP Screening of HetFab-containing Multispecifics

- Extensive plasmid DNA ratio scouting usually required to optimize for expression of properly paired heavy chains and light chains
- Azymetric™ HetFc and HetFab solutions prevent LC/HC mispairing even when plasmid DNA ratios are not optimal



## Antibody species abundance by LC-MS

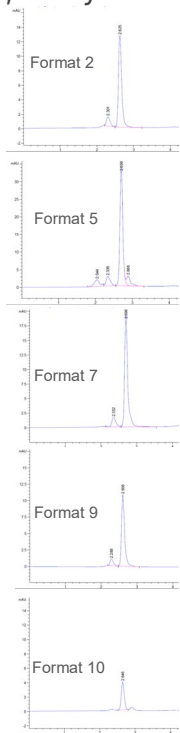
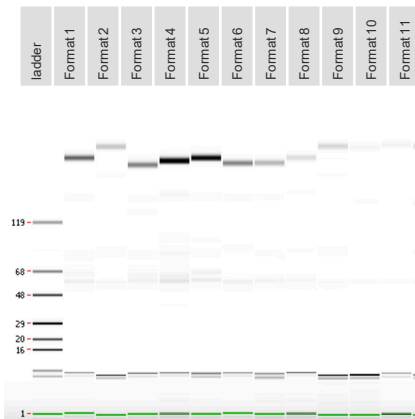


Ratios (H1:H2:L1:L2)	H1+H2+L1+L1	H1+H2+L1+L2	H1+H2+L2+L2	H1+H1+L1+L1	H1+H1+L1+L2	H1+H1+L2+L2	H2+H2+L1+L1	H2+H2+L1+L2	H2+H2+L2+L2	H1+L1	H1+L2	H2+L1	H2+L2	L1+L1	L1+L2	L2+L2
15:15:35:35	<0.1%	22.70%	<0.1%	0.30%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%	74.40%	<0.1%	0.20%	1.60%	<0.1%	<0.1%	0
15:15:53:17	<0.1%	14.10%	<0.1%	0.40%	<0.1%	<0.1%	<0.1%	<0.1%	<0.1%	84.30%	<0.1%	0.10%	0.60%	<0.1%	<0.1%	0
8:22:35:35	<0.1%	63.30%	<0.1%	0.10%	<0.1%	<0.1%	<0.1%	0.20%	0.50%	18.50%	<0.1%	<0.1%	16.40%	<0.1%	0.20%	0
8:22:17:53	<0.1%	53.10%	2.60%	<0.1%	<0.1%	<0.1%	<0.1%	0.10%	1.00%	7.50%	<0.1%	0.10%	34.70%	<0.1%	0.10%	0

# Azymetric™ Platform Enables 1-step Purification of Complex Multispecific Antibodies

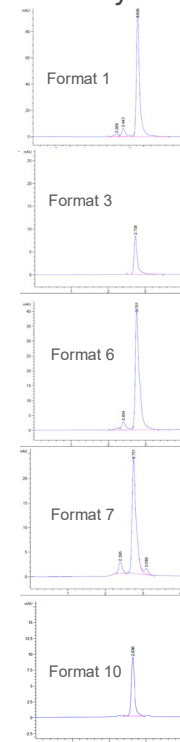
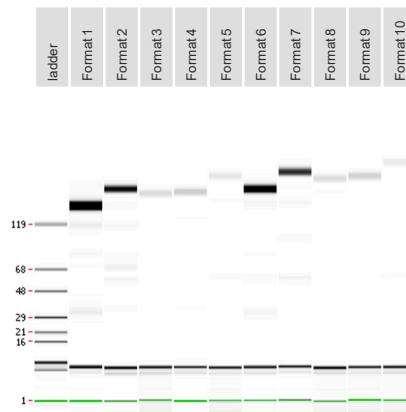
- 1-step purification amenable for HTP automation in 96 deep well plate (1-3 x 0.8 ml)
- Yield ranges from 30 ug – 500 ug
- Variants analyzed by CE-SDS, analytical SEC, A280, thermal stability (DSF), and functionally screened in cytotoxicity assay

1. Post 1-step purification analytics of different dual/triple-TAA TCE formats with  $\alpha$ CD28 co-stim



Representative formats

2. Post 1-step purification analytics of mono- and bivalent-TAA TCE formats with  $\alpha$ CD28 co-stim



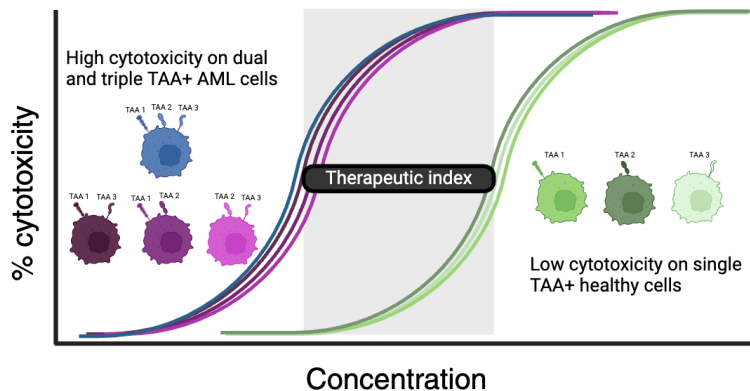
# Overcoming Antigen Escape and T Cell Dysfunction to Help Improve Treatment Responses in AML

Challenges faced with designing therapeutics for AML:

- High inter- and intratumoral target heterogeneity
- No single clean target between AML blasts, LSCs, and healthy cells
- Risk of antigen escape and lack of long-term responses

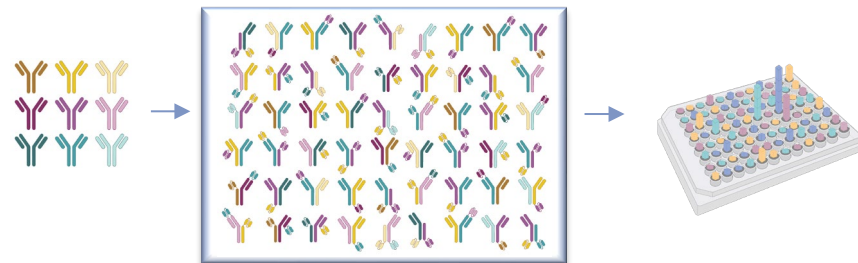


## Selective tumor cytotoxicity in the presence of two or three tumor-associated antigens (TAA)



Dimensions of multispecific antibody library screen:

- Target three AML TAAs
- TAA epitope/domain
- T cell engager format





# Apply Screening Approach to Identify Candidate Molecules with Desired MOA and Differentiated Biology

## T cell-dependent cytotoxicity assay

Triple positive cell

TAA 1 TAA 2 TAA 3



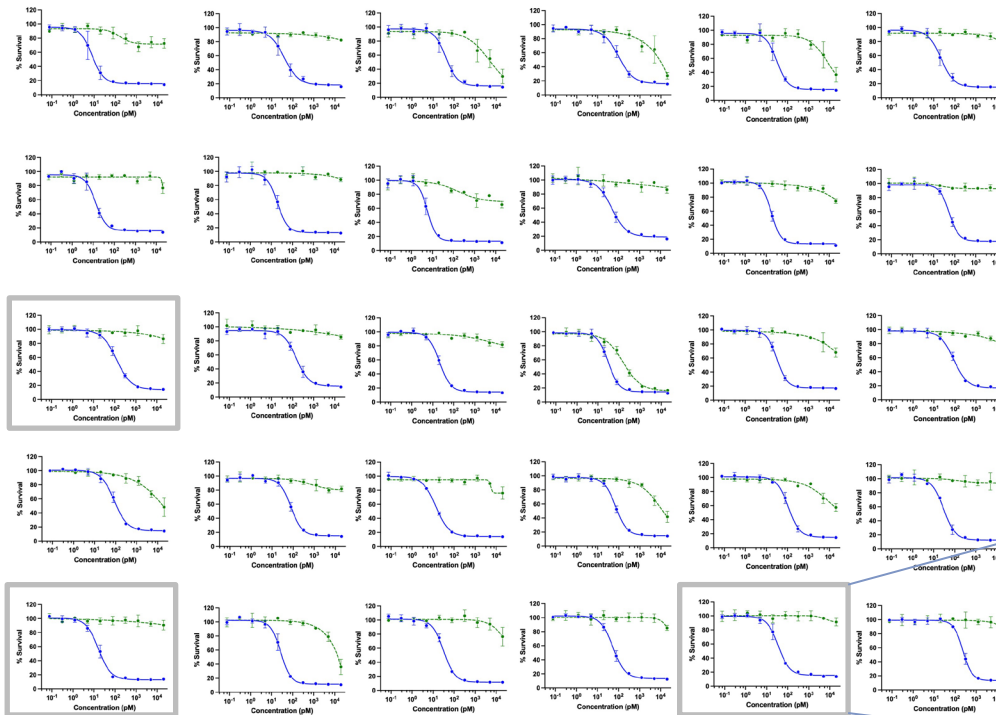
Single positive cell

TAA 2

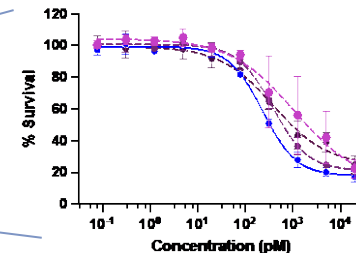
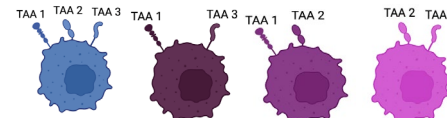


WT (TAA 1<sup>+</sup>TAA 2<sup>+</sup>TAA 3<sup>+</sup>)

TAA 2<sup>+</sup>

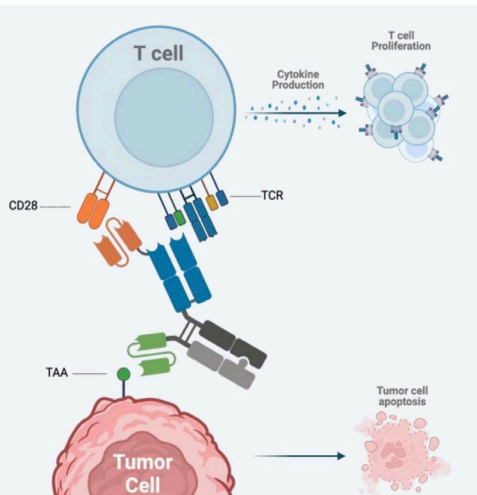


Multiple candidates with cytotoxicity against triple and dual target-expressing cells

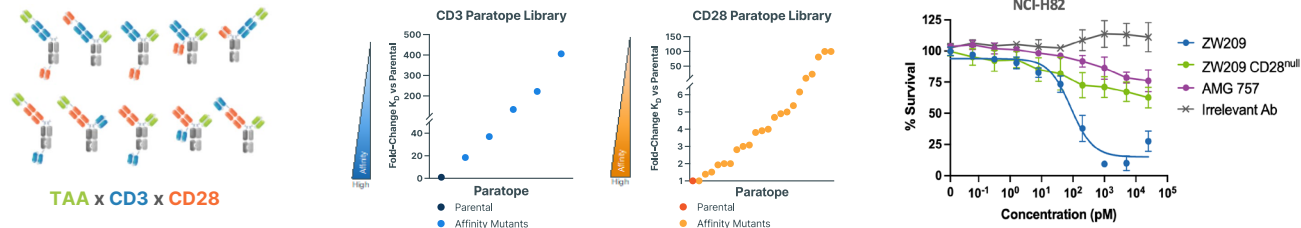


# TriTCE Co-stim: Trispecific T Cell Engager Platform with Integrated CD28 Co-stimulation

Conditional CD28 co-stimulation to enhance T cell activation and proliferation



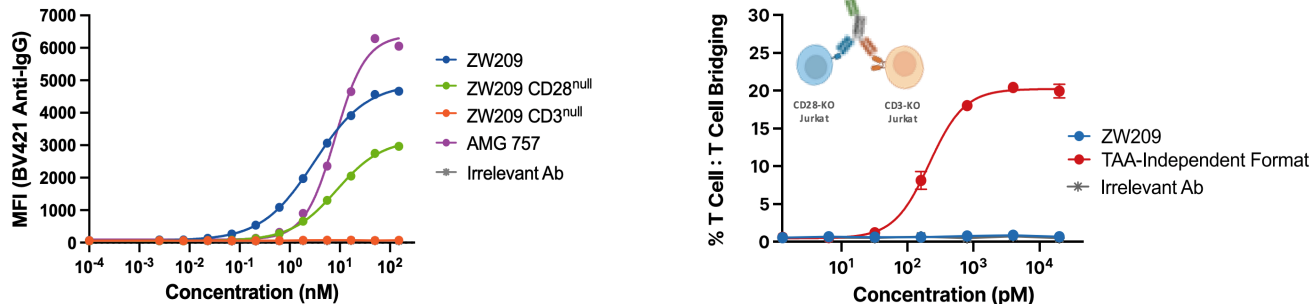
## Lead TriTCE Co-stim selected following CD3 and CD28 paratope and format screening for potent, target dependent T cell activation



## Lead DLL3 TriTCE co-stim (ZW209) unique geometry critical for safety:

Conditional CD28 engagement (requires co-engagement of CD3)

Obligate *cis* binding to T cells (no T cell-to-T cell bridging)



# Summary



## Protein Engineering

**Paratope optimization** driven by combination of **language-model and physics-based metrics** to yield paratopes with favorable developability characteristics



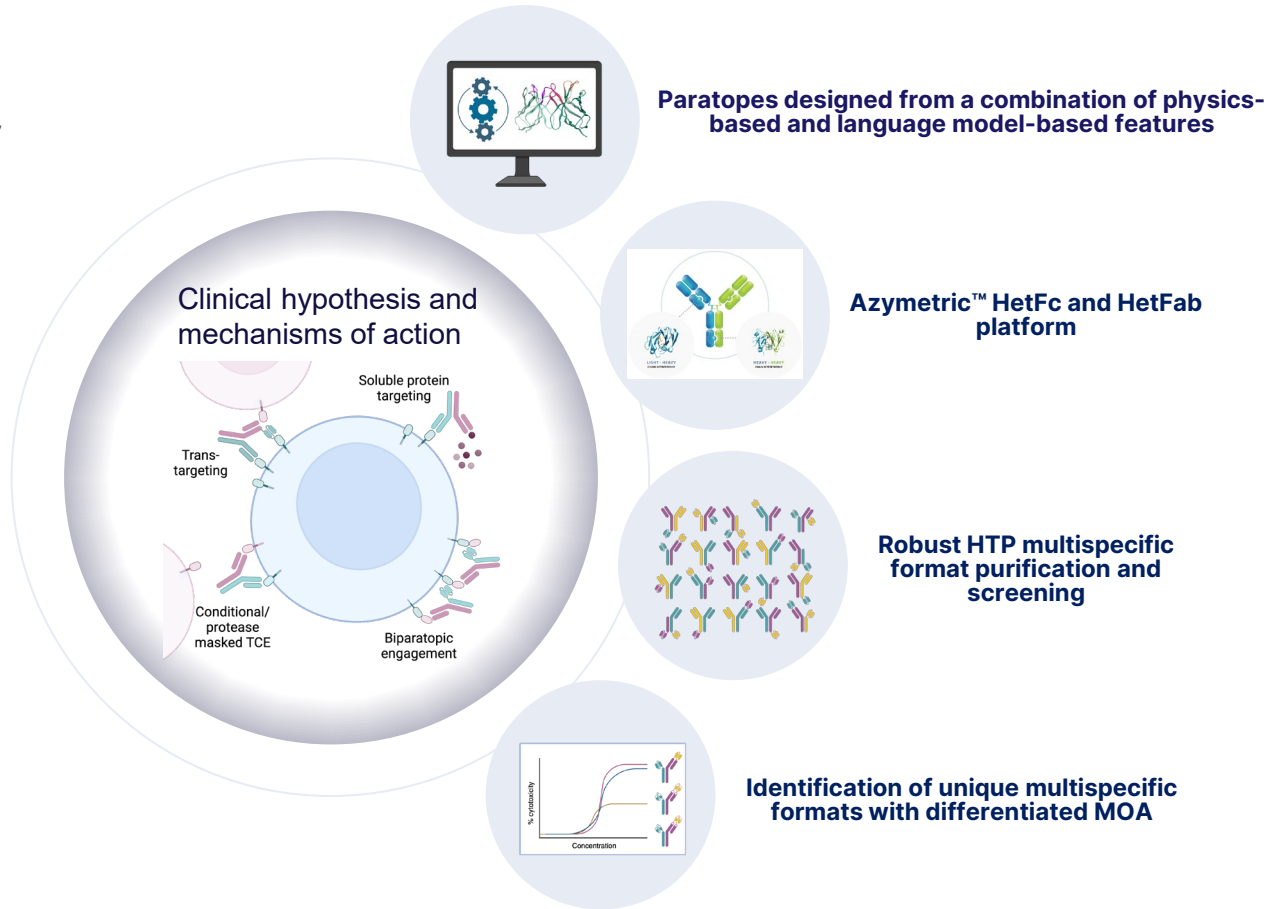
## Screening

Azymetric™ HetFc and HetFab solutions enable HTP screening of **multispecific formats** to interrogate **novel therapeutic mechanisms of action**



## Quality by Design

Parallelization of format screening and paratope optimization can **improve efficiency and promote success** in the development of multispecific antibodies with new functionalities



# Acknowledgements

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Nina Weisser  
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## Zymeworks' Multispecific Antibody Therapeutics Team

