Ailux
Biologics from XtalPi

XupremAb™

Supreme Antibody from AI-Powered Discovery Platform

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Convergence of biology, AI & automation: all under one roof.

CONTENT

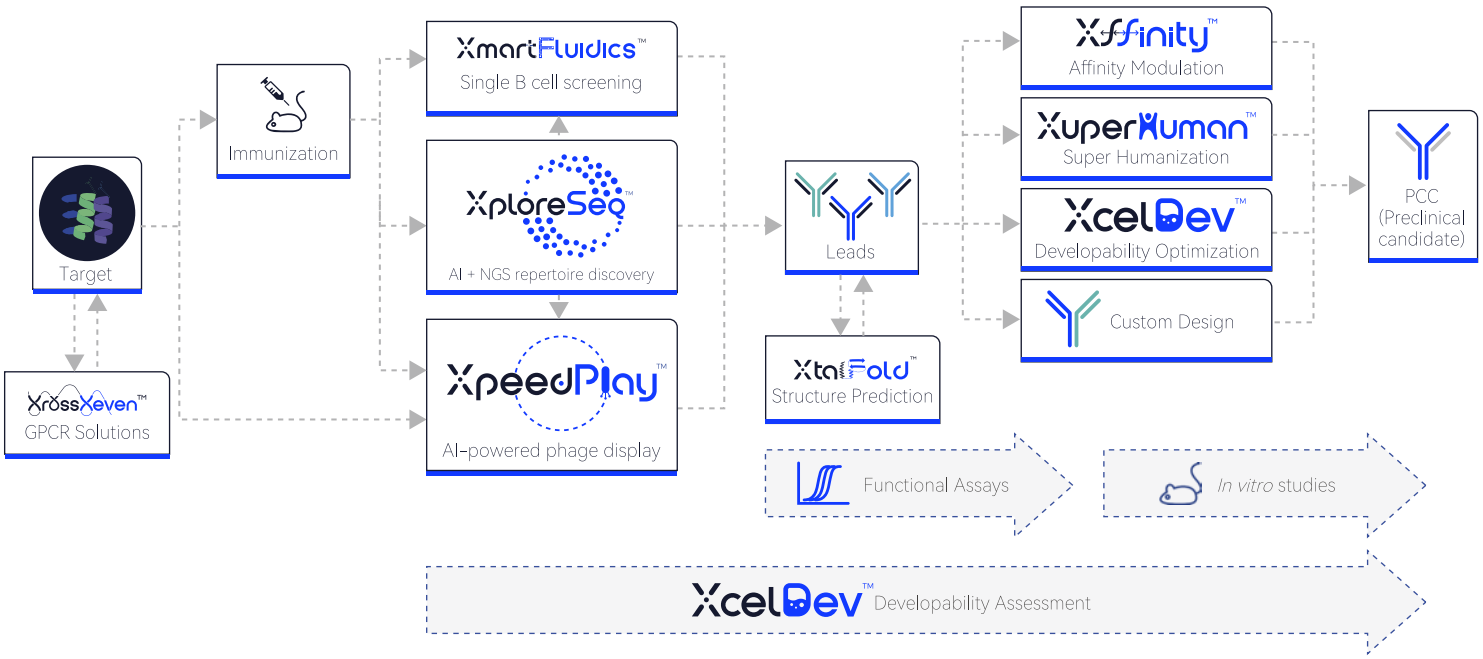
XupremAb™

XupremAb™ is Ailux's antibody discovery platform that combines the best of wet-lab techniques and cutting-edge AI technology.

As a sub-brand from Xtalpi, which is a global pioneer in AI drug discovery, Ailux Biologics targets on leveraging our proprietary AI algorithms in combination with wet-lab methods to find solutions to the most challenging bottlenecks in biologics discovery.

XupremAb™ is our one-stop solution for antibody discovery that incorporates AI throughout the whole process. The platform can be accessed as a whole, where we can take a partner's project all the way from target to preclinical candidates, or it can be accessed as individual sub-platforms.

Overview of Our Antibody Discovery Platforms



Development of XupremAb™ was guided by the following principles

CAST A WIDER NET

- Larger search space = Better candidates
- Orthogonal & complementary screening approaches
- High-throughput experimentation + AI-powered repertoire mining

DESIGN, NOT GUESS

- Engineering as design work, not guesswork
- AI-guided multi-parametric optimization
- Minimize random mutagenesis & trial-and-error

EXCEL, IN ALL DIMENSIONS

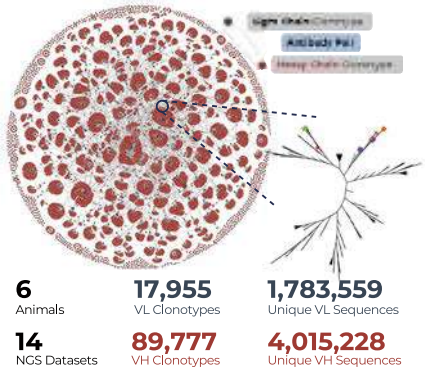
- Deliver candidates that excel across function, developability and immunogenicity
- Comprehensive developability assessment covering 15+ properties
- Predicted *in silico*, validated *in vitro*

BEND THE RULES OF NATURE

- Redefine what's possible with cutting-edge AI
- Remove constraints of legacy experimental methods
- Tackle the most unthinkable problems in discovery

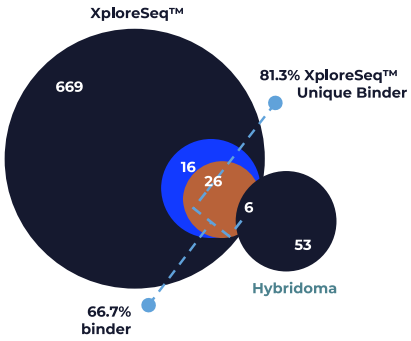
Tap into greater search space for more quality hits

Case 1 | *In silico* screening of millions of antibody sequences in the immune repertoire



6 Animals | **17,955** VL Clonotypes | **1,783,559** Unique VL Sequences

14 NGS Datasets | **89,777** VH Clonotypes | **4,015,228** Unique VH Sequences



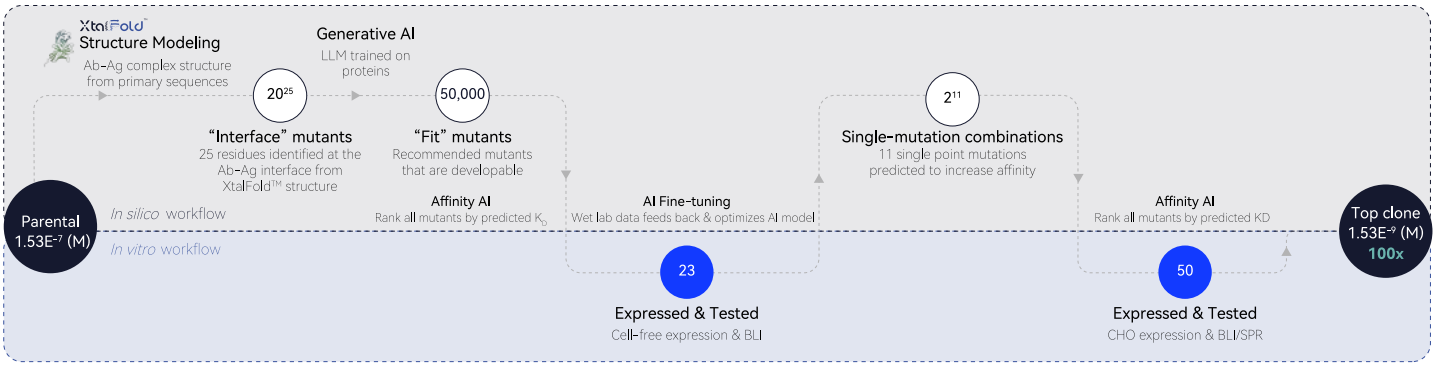
XploreSeq™ routinely sequences millions of BCRs and identifies hits with high confidence (left). In this project, XploreSeq™ predicted 717 binders, from which 48 were randomly chosen for expression and testing. 66.7% (32/48) were confirmed to be binders, and only 18.6% (6/32) binders overlap with hybridoma hits (right).

Utilize AI to make antibody engineering more efficient, enabling multi-objective optimization

Case 2 | Affinity 100x↑ in 3.5 weeks – no structure information

- **Goal**: Improve VHH KD from hundred nM to single-digit nM in a short timeframe
- Ultra-fast iteration enables active learning

2 rounds of optimization | **73** variants expressed | **3.5** weeks | **100x** increase in affinity



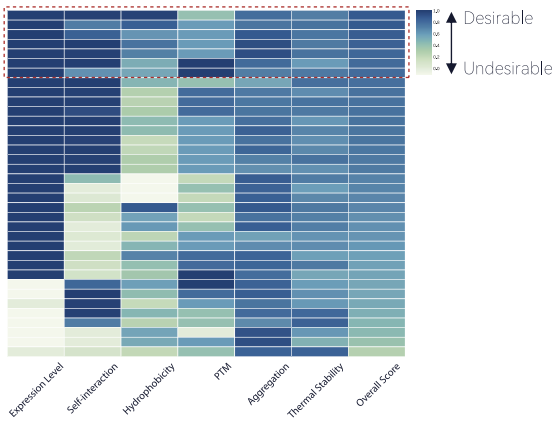
Instill excellent developability into every molecule

Case 3 | XcelDev™ Silico evaluates and ranks 30+ candidates in terms of developability

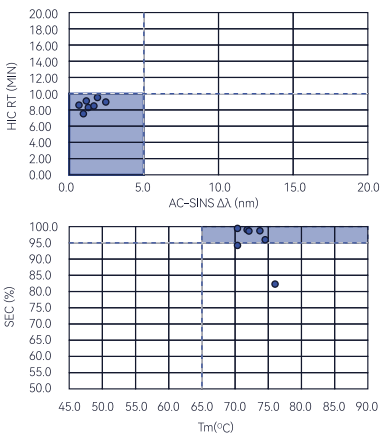
36 hits that had passed binding and functional screening were analyzed by XcelDev™ Silico. 6 properties of each antibody was predicted and scored on a scale of 0-1 (displayed in color gradient).

An overall developability score was calculated to rank all 36 hits. To verify the effectiveness of the ranking, the top 7 (in the red box) were expressed and subject to a battery of *in vitro* developability assays. All 7 performed well in these assays, their Tm, SEC, AC-SINS and HIC results shown on the right.

XcelDev™ Silico results for 36 antibodies



Wet lab results for top 7 candidates



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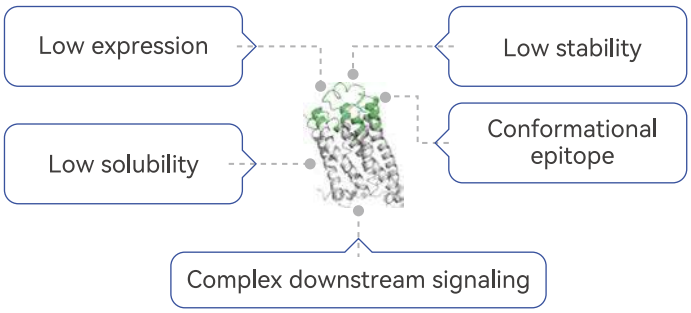
XrossXeven™ | AI-Powered One-Stop Solution for GPCR Antibody Discovery

GPCR antibody discovery requires specialized solutions

GPCRs, while being an important class of therapeutic targets, present challenges for therapeutic antibody development. Their inherent structural complexity, conformational adaptability, and hydrophobicity create significant obstacles for antibody discovery.

To obtain high-quality, functional antibodies that specifically target GPCRs, specialized solutions are needed to address two key challenges:

1. Preparing stable GPCR antigens for immunization;
2. Screening for rare candidates that meet all the criteria.



XrossXeven™ is an integrated and comprehensive solution tailored for GPCR antibody discovery at every stage

1 Antigen Preparation

Format		GPCR protein Engineering	Expression System	Solubilizer
Protein	DNA	Expertise-guided	Insect	**Nano-disc**
Cell	mRNA	Small-scale Large-Scale **AI-guided**	Mammal	Detergent

2 Immunization

Proprietary immunization protocols to consistently achieve **high titer**, even for high-homology targets.

3 Hit Generation

XupremeHit™ | XtraDoma™ | XploreSeq™ | XpeedPlay™ | ...

- Multi-platform approach to deliver a high-quality panel of hits
- Select candidates with desired **affinity**, **specificity**, and **species-cross-reactivity**

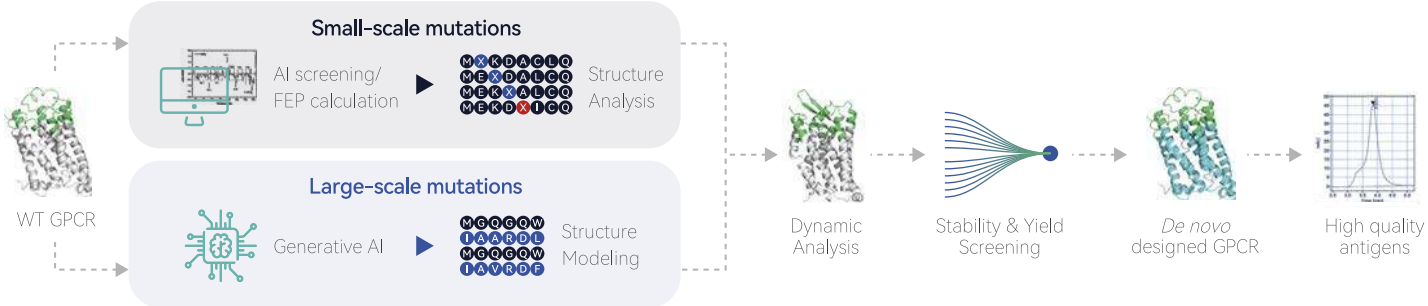
4 Lead Identification

Established assays for GPCR targets:

- Receptor blocking
- Calcium flow
- IP-1/3
- Primary cell assays
- ...

Biotin

Rapid high-quality antigen readiness powered by AI-guided GPCR sequence design workflow



Full-length protein antigens

- High & specific immune titer
- High antibody screening efficiency

Obtaining sufficient and stable GPCRs

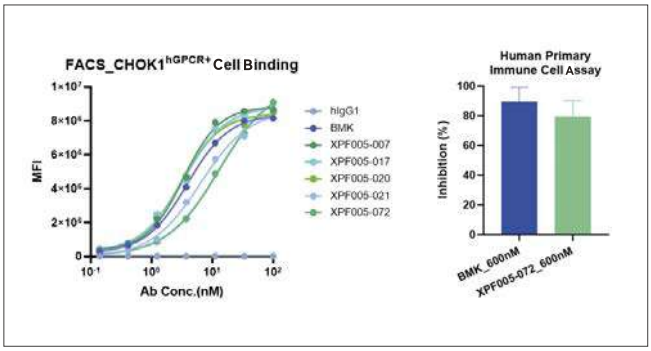
- Traditional Engineering >6 months
- XrossXeven™ 2-3 months

Case 1 | AI-guided GPCR antigen design with large-scale mutations

Goal

1. **Rapid** identification of mutations in TM and ICL;
2. **Maintain** conformation of ECL;
3. **Enhance** stability and expression level

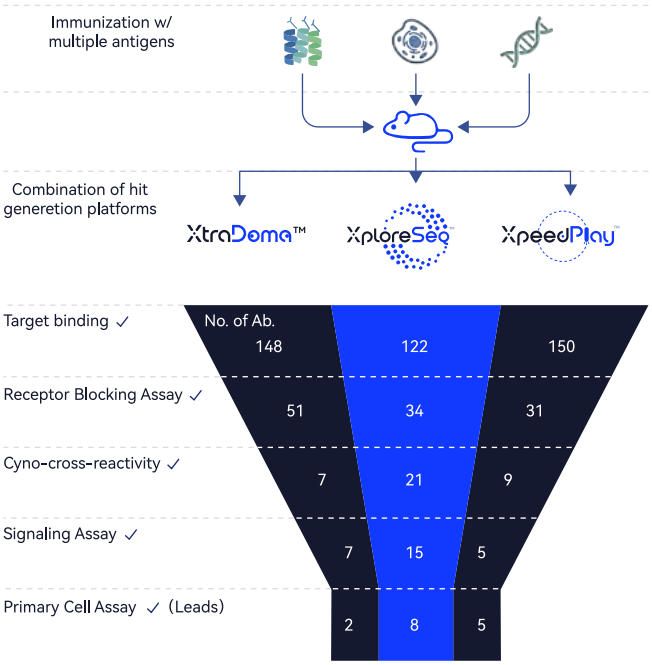
Mutants (**>30%** pt) meeting the goal were obtained in **2-3 months**. After immunization, **13 hits** and **1 lead** were identified (results partially shown on the right)

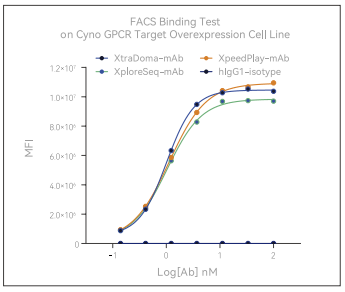


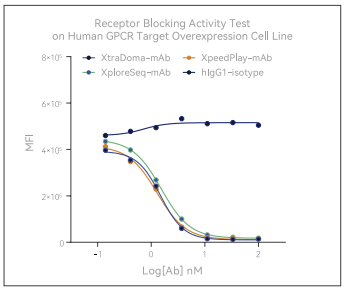
WT GPCR	Generative AI	Predictive AI	MD Modeling	Expression	Validation	AI-designed Mutants
	Proprietary LLM AI generated "fit" mutants	Narrowed down by stability and ECL conformation	Fine-grained assessment of stability	Expression of top mutants	Bound to BMK antibody & cleared stability test	• 120-160 pt. mutations in TM & ICL • ECL conformation maintained

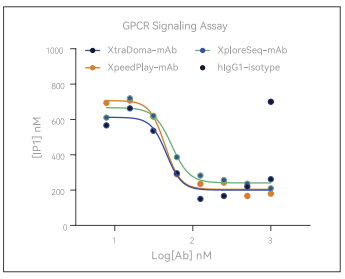
Case 2 | Generating cyno-cross leads for a challenging, low-homology GPCR target

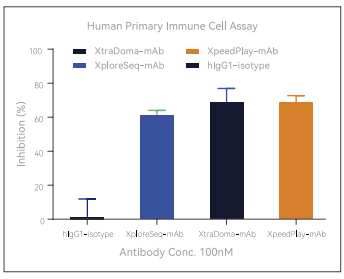
In a typical GPCR antibody discovery campaign powered by XrossXeven™, different antigens and screening platforms were combined to deliver high-quality candidates that meet all requirements. Top lead candidates from each platform performed well in a variety of assays shown below.











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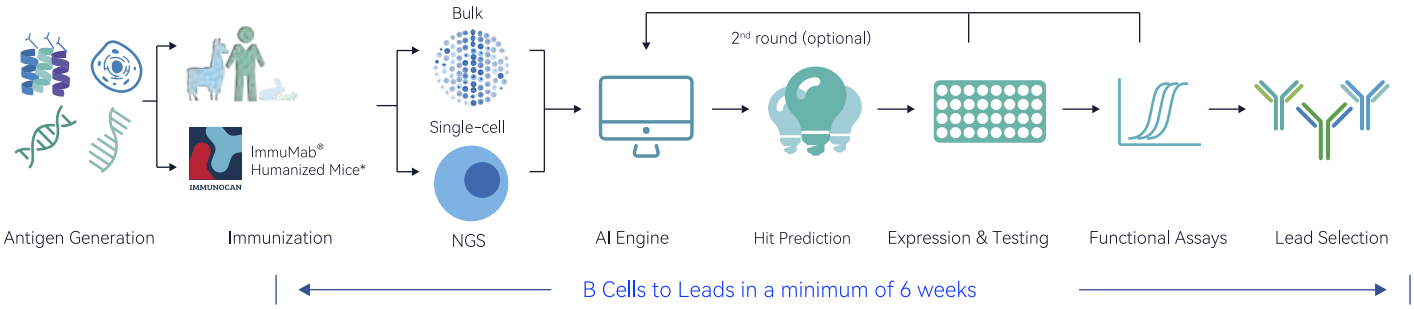
XploreSeq™

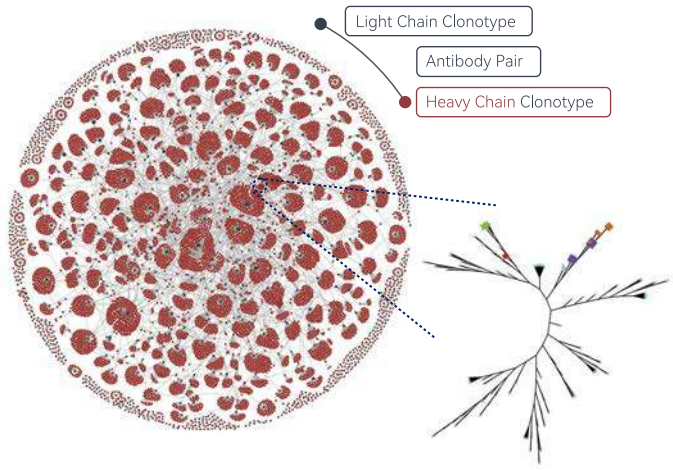
Ultra High-throughput, Rapid Screening of Millions of B Cells Powered by NGS & AI

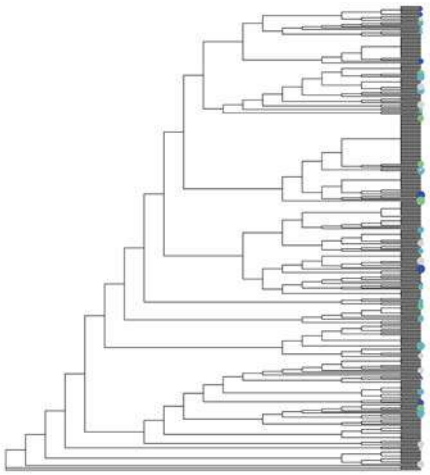
XploreSeq™ is our next-gen antibody discovery platform that utilizes NGS & AI to mine the BCR repertoire with ultra-fast turnaround



* Partnered with Immunocan®

XploreSeq™ analyzes millions of antibody sequences and recommends a highly-diverse panel of candidates with >50% hit rate





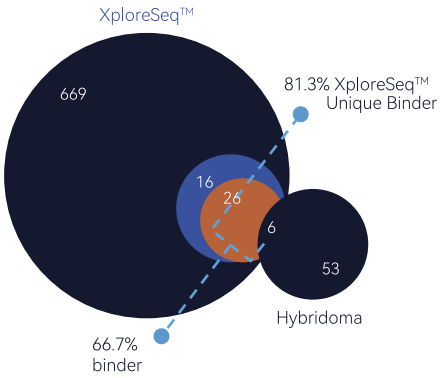
6 Animals	17,955 VL Clonotypes	1,783,559 Unique VL Sequences
14 NGS Datasets	89,777 VH Clonotypes	4,015,228 Unique VH Sequences

The immunized BCR repertoire is huge and contains numerous sequences with therapeutic potential. XploreSeq™ screens through this repertoire to identify hits, powered by our AI engine that has been trained and refined over the years. A typical discovery program could generate millions of BCR sequences (shown above) and poses tremendous challenges for finding the needle in the haystack.

Reconstructed antibody lineage tree. Visualization from real program.

We believe in both the quality & diversity of candidates. The objective is to pick antibodies from different lineages with high confidence of target-binding and excellent developability right out of the box. This gives us a diverse panel of developable hits that could cover a maximum number of epitopes, leading to higher success rate in downstream assays.

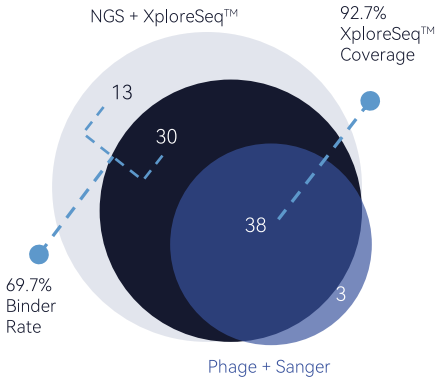
Case 1 | XploreSeq™ identifies hits with high accuracy



For this campaign, XploreSeq™ predicted 717 binders, from which 48 were randomly chosen for expression and testing. 66.7% (32/48) were confirmed to be binders, and only 18.6% (6/32) binders overlap with hybridoma hits.

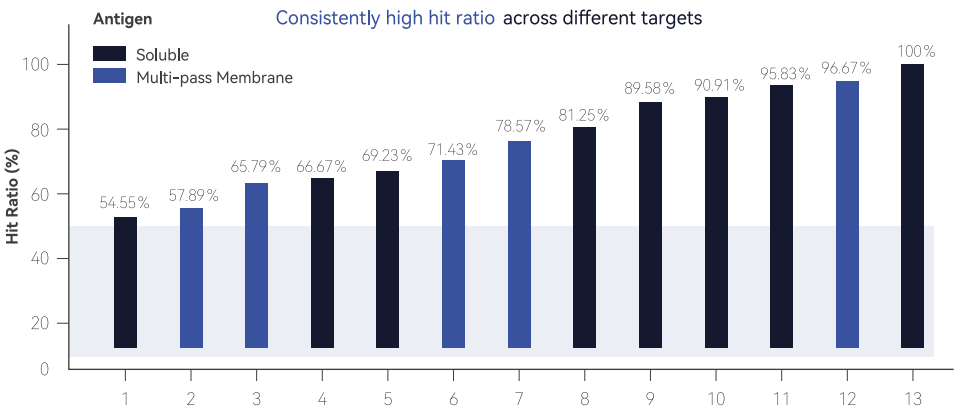
Case 2 | Applying XploreSeq™ in VHH discovery

For this campaign, a total of 41 unique VHH hits were identified by immune phage library through Sanger sequencing.
The library was then NGS sequenced and subject to XploreSeq™ analysis. NGS results include 38 of the 41 hits identified previously. In addition, XploreSeq™ predicted 43 new VHH binders. When expressed and tested, 30 of these 43 (69.7%) were confirmed to be binders, which significantly expanded the diversity of hits obtained by phage display alone.



Case 3 | Xploreseq™ for Robust & Reliable Hit Generation

XploreSeq™ as our standard antibody discovery platform has been verified by over a dozen of projects with varied targets. The results summarized that XploreSeq™ consistently achieved a >50% hit rate for both soluble and membrane protein targets, with the highest hit rate of 100%.



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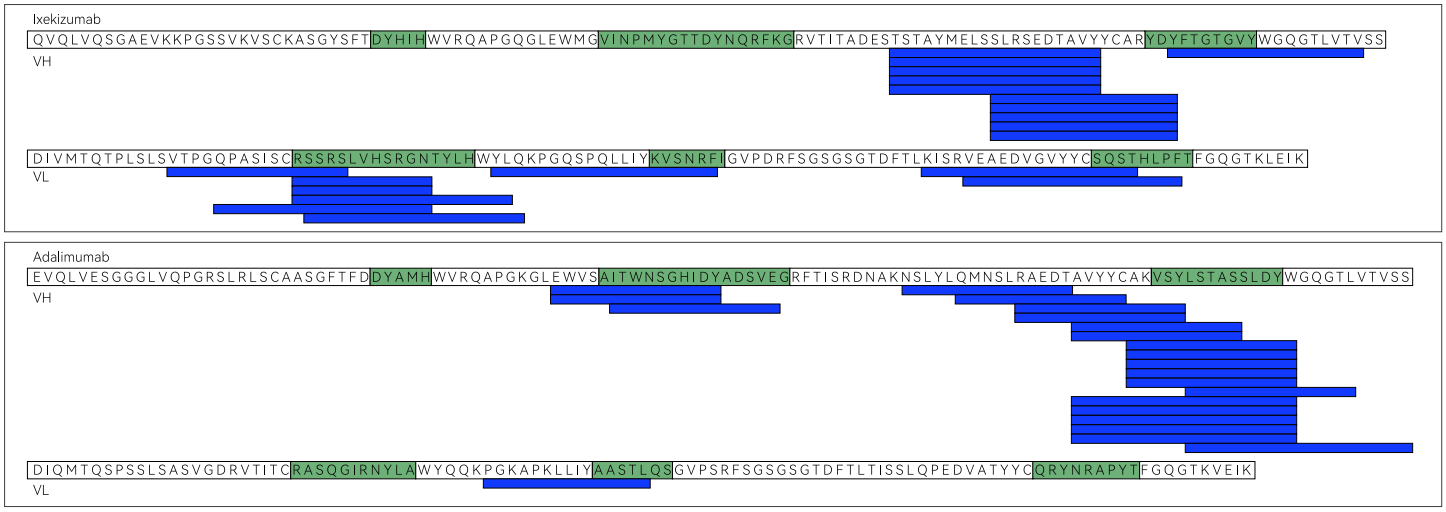
XuperHuman™ | Pushing the Limit for Fully Human Biologics

Humanized & fully human antibodies can still harbor "unhuman" motifs in their CDRs, eliciting ADAs in patients

Humanized & fully human antibodies, albeit an advancement from chimeric antibodies, can still elicit an immune response in the clinic, resulting in the production of Anti-Drug Antibodies (ADAs). This immunogenicity is primarily attributed to T-cell epitopes present in the CDRs (as shown below).

Traditional "humanness scores" cannot fully capture the immunogenicity risk in antibody CDRs. Even when such a hotspot is identified, there exists no established method to remove it with high confidence, for a single point mutation in the CDRs could abrogate antibody binding to its target. This presents a multi-objective optimization problem that is challenging to solve using traditional techniques.



A humanized antibody[1] (top) and a fully human antibody[2] (bottom) were found to contain T-cell epitopes (blue bars), primarily in their CDRs (green).

Reference:

1.Spindeldreher S, Karle A, Correia E, et al. T cell epitope mapping of secukinumab and ixekizumab in healthy donors[C]//MAbs. Taylor & Francis, 2020, 12(1): 1707418.

2.Meunier S, Hamze M, Karle A, et al. Impact of human sequences in variable domains of therapeutic antibodies on the location of CD4 T-cell epitopes[J]. Cellular & Molecular Immunology, 2020, 17(6): 656-658.

AI models can predict immunogenicity in alignment with clinical data

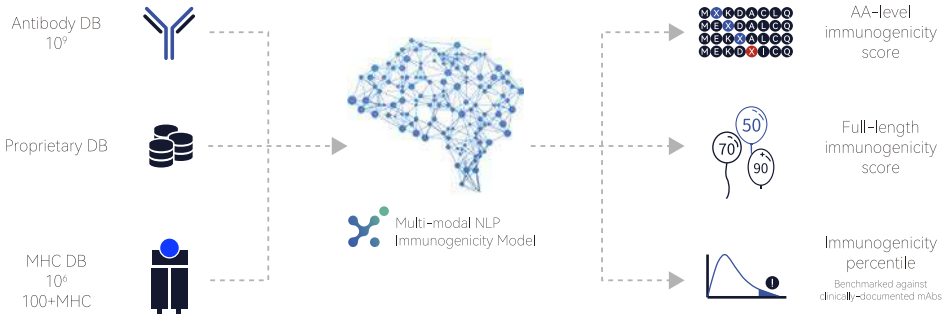
Advances in AI have enhanced our capacity to evaluate and mitigate immunogenicity. AI models trained on vast datasets of natural antibody sequences from various species can discern subtle differences that may not be detectable using traditional sequence-identity-based algorithms.

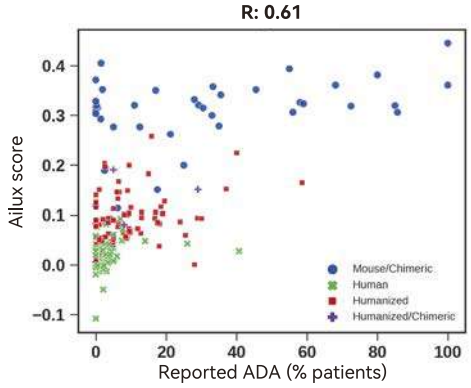
We developed a multi-modal NLP (Natural Language Processing) model that is trained on multiple types of public and proprietary datasets. The model can predict antibody immunogenicity at both whole sequence and amino acid level. Additionally, it can assign a percentile ranking to the candidate amidst benchmark mAbs in the clinic.

We used the model to predict 217 clinical-stage antibodies with documented ADAs. The result shows good correlation (R=0.61) between predicted ADA & actual ADA.

The model can also identify the individual amino acids that are causing immunogenicity, including those in the CDRs. (Shown in Case 1)

This AI model forms the basis of our XuperHuman™ platform, which aims to identify and eliminate immunogenicity in antibodies and other protein-based therapeutics.



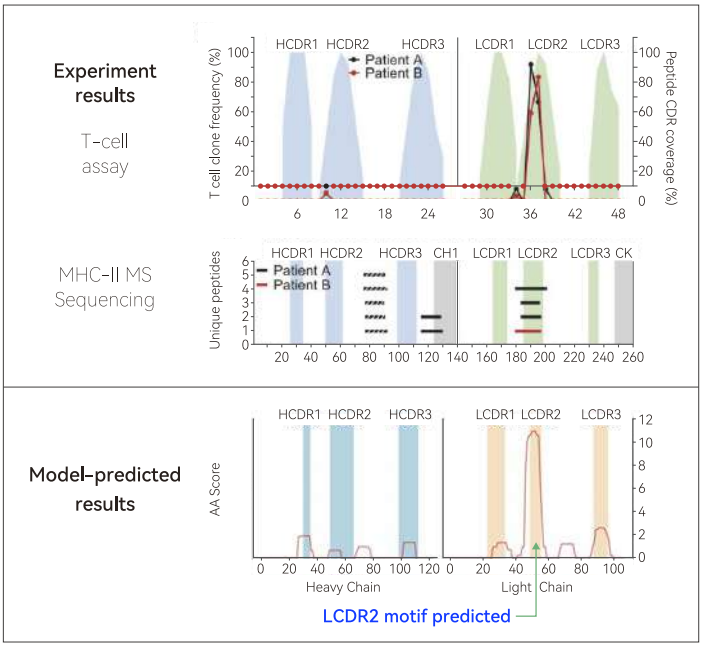


Case 1 | Predicting ADA for a clinical antibody & pinpointing the motif

We used our XuperHuman™ AI model to predict immunogenicity for Natalizumab, an approved antibody with mild-to-high observed ADA in patients. The model not only predicted the ADA % in line with clinical records (shown below), but also successfully pinpointed the underlying motif which was previously identified through *in vitro* assays (shown on the right).

Predicted ADA Occurrence	Actual ADA Occurrence
9.65%	9% (PMID17761550)
	12% (PMID17761550)
	4% – 28% (PMID27806057)

Predicted & actual ADA occurrence of Natalizumab



Case 2 | Removing the motif in CDR while maintaining binding

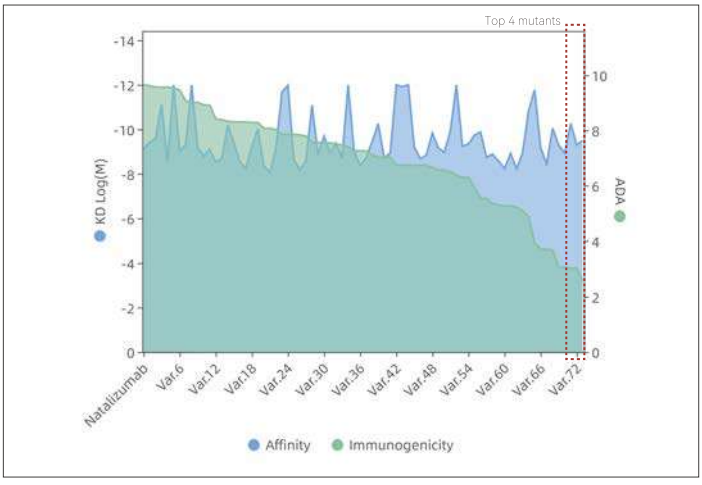
Based on previous findings, we set out to engineer out the immunogenicity motif in the CDR of Natalizumab while preserving its binding to the target.

AI model explored an ultra-large mutation space (1E23) and recommended 14000+ mutants that are likely to retain affinity and posses acceptable developability profiles.

688 candidates passed both immunogenicity and developability assessment.

Top 88 mutants were expressed and characterized for binding.

Of the top 88 mutants that passed our *in silico* workflow, 72 maintained binding (shown below). The top 4 mutants in terms of immunogenicity reduction are now in the first 50% percentile of clinical benchmarks (ADA of ~3%) as compared to WT in the last 25% (ADA of ~10%). All 4 mutants maintained binding affinity at the same level with WT. Further cell-based immunogenicity assays are underway.



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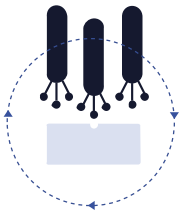
Affinity™ | AI-Driven Affinity Modulation

Tuning the binding affinities of biotherapeutics remains a challenge



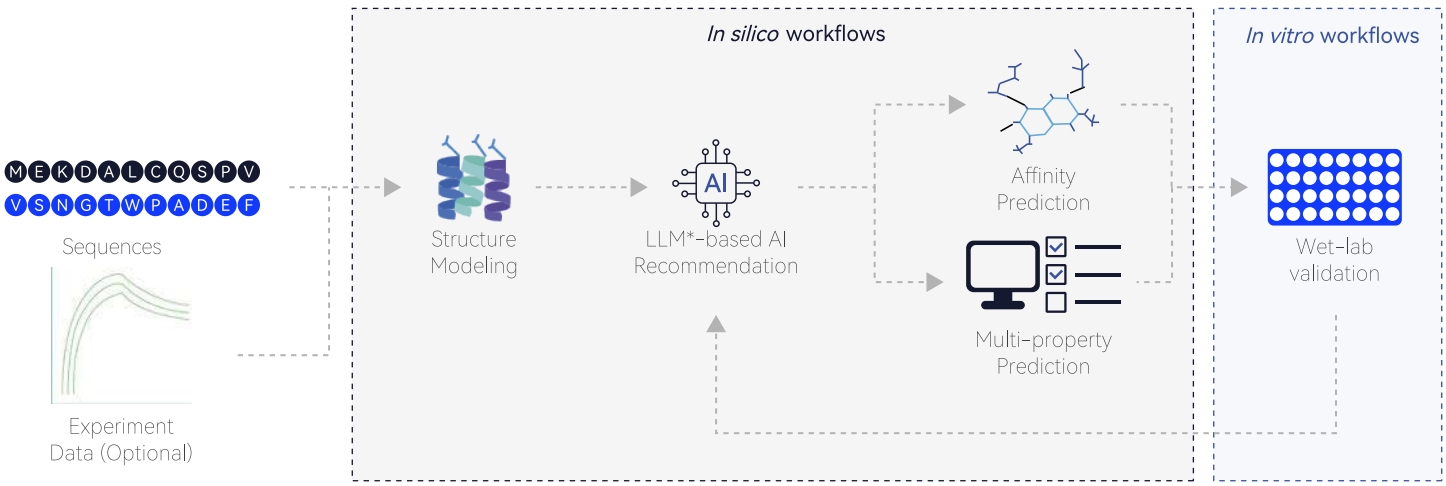
Display Platforms

- Time-consuming and cost-intensive
- Difficult to optimize multiple properties simultaneously
- Limitations in generating "natural" variants

Expert-driven Mutagenesis

- Human bias involved
- Difficult to optimize multiple properties simultaneously
- Inconsistent and less reliable

Affinity on-demand: hitting your desired affinity while maintaining crucial properties – now possible with our AI-centric workflow



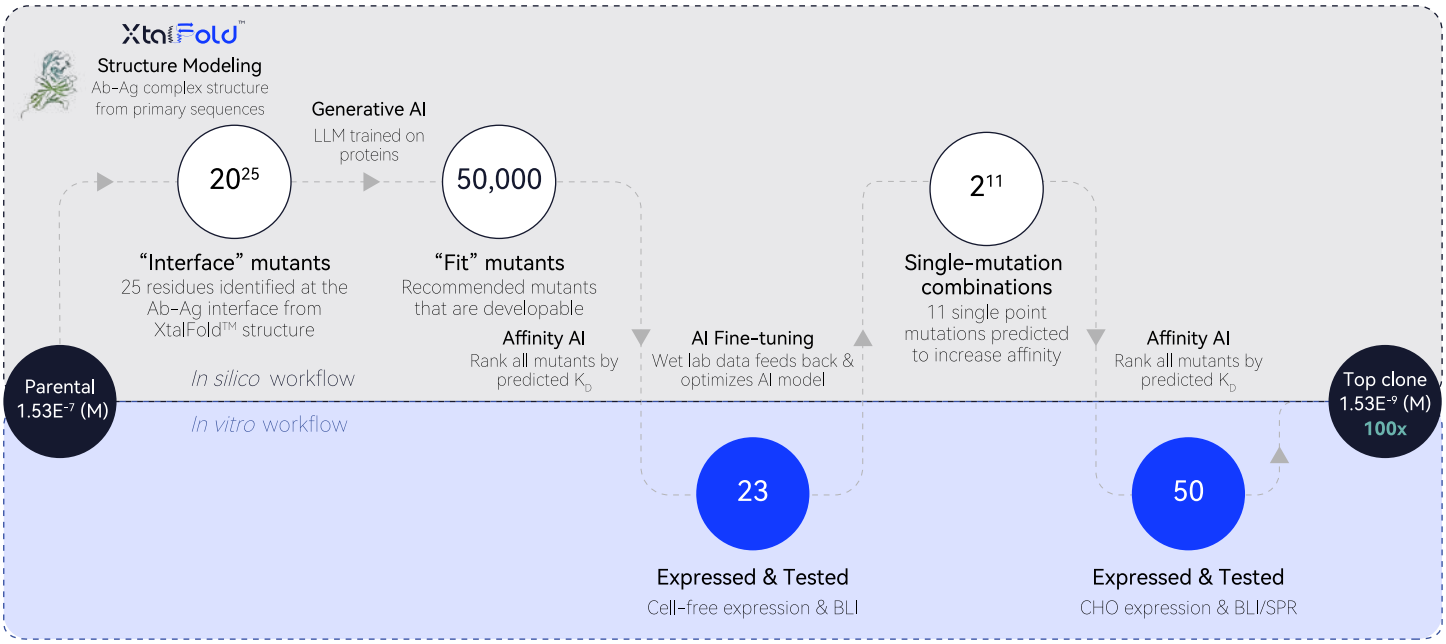
*LLM: Large Language Model, a deep learning technique that also powers ChatGPT

- Get to any affinity you want -- up or down from the parental molecule
- Maintain/optimize developability/immunogenicity profiles
- Fast turn-around
- Proprietary AI model that can search an ultra-large mutant space in the 10^{25}
- High-accuracy affinity & developability prediction algorithms
- High-throughput make & test system that gives quick feedback

Case 1 | Affinity 100x↑ in 3.5 weeks – no st

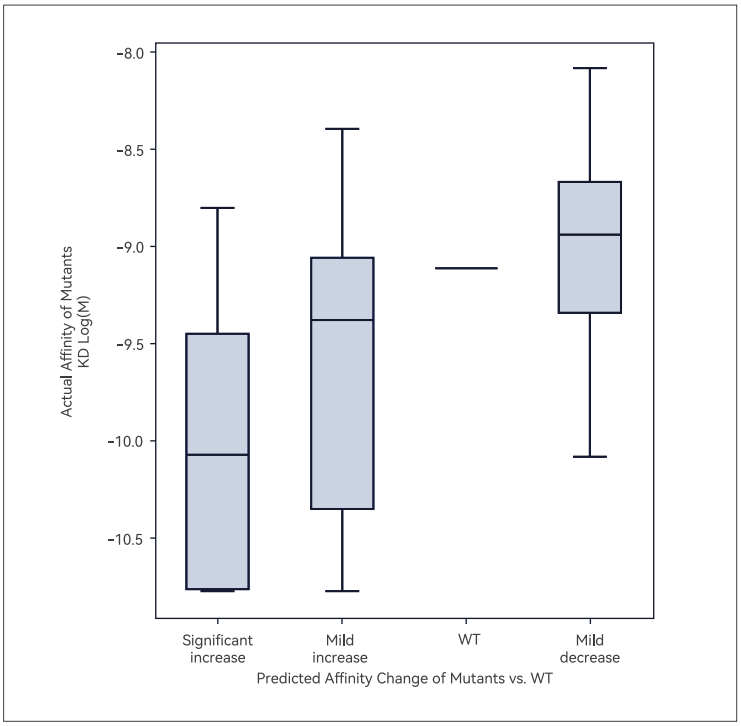
- **Goal:** Improve VHH KD from hundred nM to single-dig
- Ultra-fast iteration enables active learning

2 rounds of optimization **73** variants expressed **3.5** weeks **100x** increase in affinity



Case 2 | Bi-directional affinity tuning of a lead antibody

- Goal: tuning the affinity of a lead antibody both upwards and downwards to provide more options for bispecifics development
- Our AI model generated 3 buckets of designed mutants with predicted affinities of:
  1) significant increase (target: 10x),
  2) mild increase (target: 3x) and
  3) mild decrease (target: -3x) vs. WT
- When expressed and tested, these mutants show affinities that correspond to their targeted ranges. (Shown on the right)
- 1 round of design-make-test of 96 mutants to arrive at a panel of highly optimized leads with KD that span 3 orders of magnitude and optimal developability profiles



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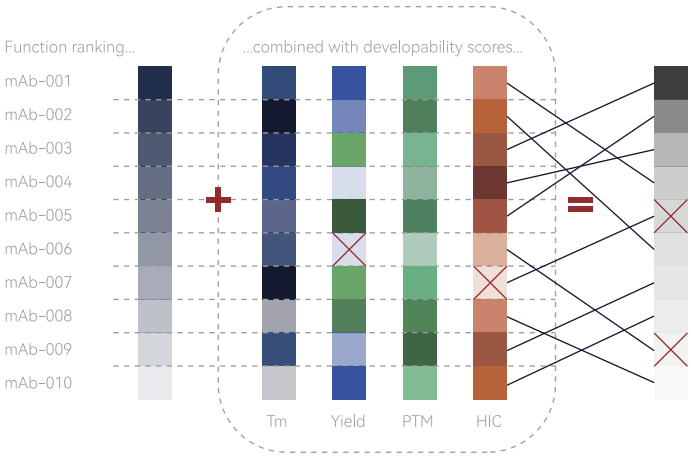
XcelDev™ Silico

Developability Assessment at Your Fingertips

Incorporating developability considerations earlier in antibody discovery

Developability has been recognized as a crucial driver for clinical success of antibodies. The traditional "function first, developability second" screening paradigm is sequential and not optimal, which frequently results in less developable candidates that require additional engineering.

We advocate a parallel approach where developability is considered **alongside** function when selecting candidates. This is made possible by our *in silico* developability assessment platform, with fast turnaround for minimal cost.



...gives a more holistic ranking, which differs from functional ranking alone.

Sequences with liabilities are recognized early to minimize downstream risk.

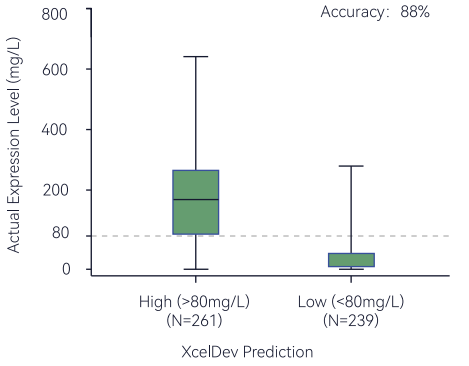
Comprehensive assessment with our validated *in silico* models, offered in two versions

- Comprehensive suite of computational models, including both physics-based and AI-based
- AI models trained on thousands of internal data points; achieved state-of-the-art (SOTA) performance on multiple developability prediction tasks
- Thoroughly validated and optimized in numerous internal projects
- Quick turnaround (minimum of 1 week); only antibody sequences needed
- High-throughput (HTP) version for fast evaluation of up to 1000 candidates
- Low-throughput (LTP) version for detailed, fine-grained analysis of selected candidates

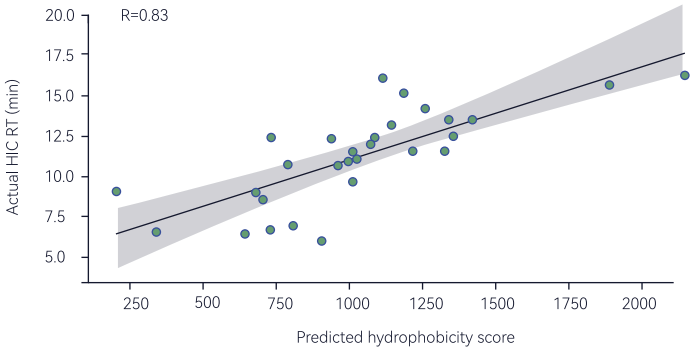
Property \ Throughput	HTP: 10s to 100s	LTP: Up to 10
pI	✓	✓
PTM: Deamidation, Isomerization, Free Cysteine, N/O-Glycosylation	✓	✓
Expression Level	✓	✓
Hydrophobicity	✓	✓
Self-Interaction	✓	✓
Aggregation	✓	✓
Viscosity	✓	✓
Solubility	✓	✓
Thermal Stability	✓	✓
Poly-Specificity		✓
Hotspot Diagnosis (Deep-dive into the identified issues)		✓

XcelDev™ Silico predicts developability with high accuracy, using only sequence as input

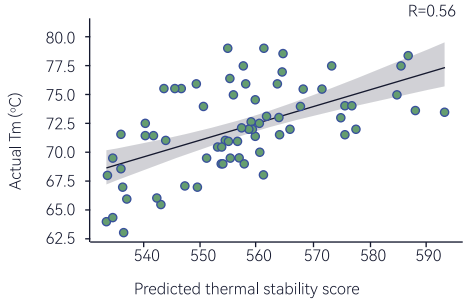
Expression level (transient CHO expression)



Hydrophobicity (HIC RT)

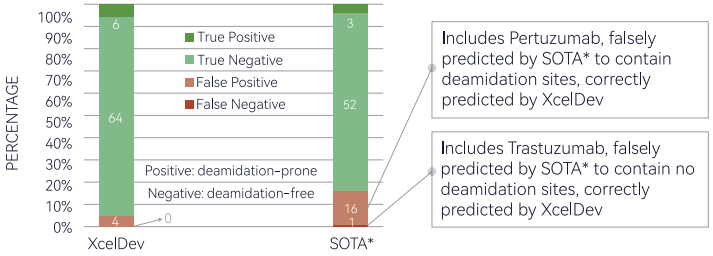


Thermal Stability (Tm)



PTM (Deamidation)

Prediction for 72 Clinical mAbs Benchmarked Against Actual Results

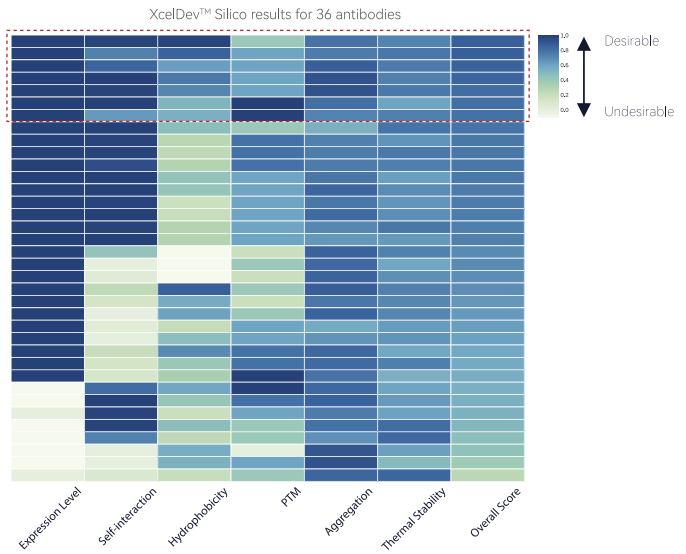


SOTA* : another widely-used model that's considered industry benchmark (state-of-the-art)

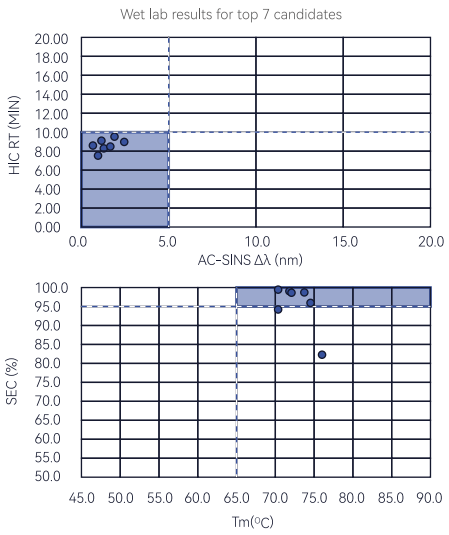
Case Study: XcelDev™ Silico evaluates and ranks 30+ candidates in terms of developability

36 hits that had passed binding and functional screening were analyzed by XcelDev™ Silico. 6 properties of each antibody were predicted and scored on a scale of 0–1 (displayed in color gradient shown below).
An overall developability score was calculated to rank all 36 hits. To verify the effectiveness of the ranking, the top 7 (in the red box) were expressed and subject to a battery of *in vitro* developability assays. All 7 performed well in these assays, their Tm, SEC, AC-SINS and HIC results shown below.

XcelDev™ Silico results for 36 antibodies



Wet lab results for top 7 candidates



Ailux
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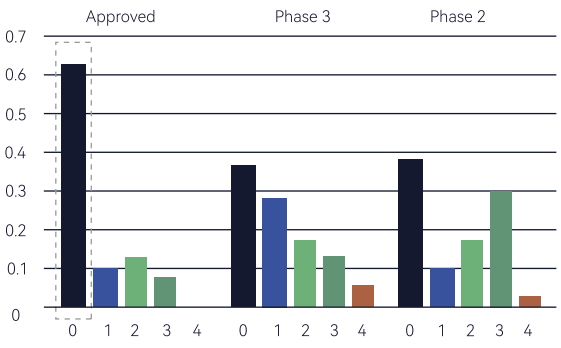
XcelDev™ Plus | One-Stop Antibody Developability Assessment

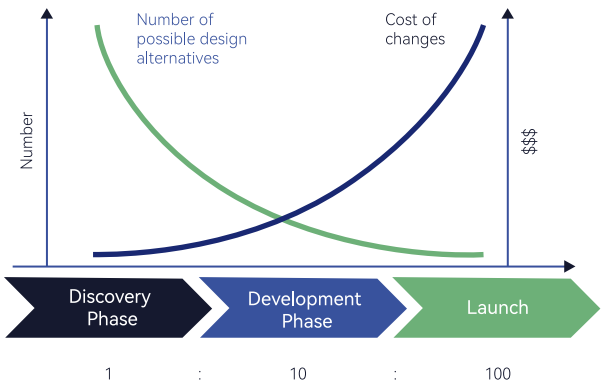
Developability assessment in discovery is crucial and requires different combinations of tools

The importance of developability was brought to full light in a study of 137 clinical-stage & approved mAbs. 12 biophysical properties were found to correlate with clinical success. Approved mAbs have the lowest number of warning flags (left).

It makes the most economical sense to incorporate developability assessment early, preferably in the discovery stage (right). Meanwhile, the varying number of candidates throughout the discovery phase calls for a staged assessment paradigm, where different combinations of *in silico* or *in vitro* methods are needed to meet timeline and budget requirements.



Jain T, et al. PNAS. 2017;114(5):944-949.



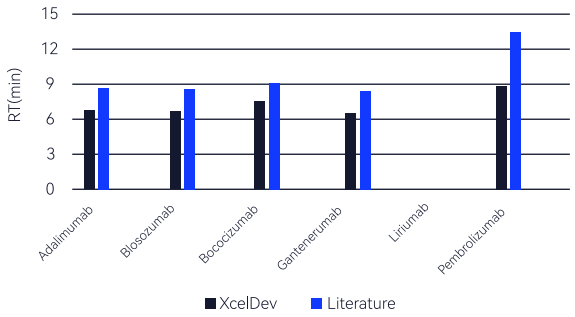
XcelDev™ Plus: one-stop solution for developability assessment

XcelDev™ Plus offers you exclusive access to all the gold-standard *in vitro* assays and cutting-edge *in silico* models for developability assessment. Different combinations of tools are tailored to guide you through every step of the discovery process. All tests are performed in house to ensure fast turnaround and consistent results.

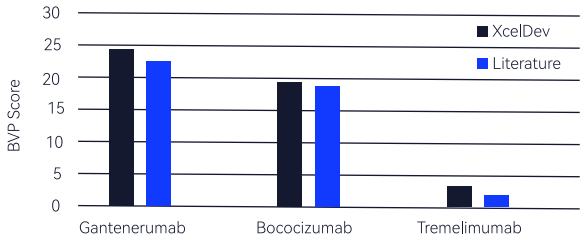
Scenarios		Hit screening >100	Hit screening <100	Lead Identification <20	Lead selection <5
TAT (includes antibody expression)		1-2 Weeks	5 Weeks	5 Weeks	10 Weeks
XcelDev™ Silico *in silico* assessment		✓	✓	✓	✓
Expression & Purification	CHO S, Protein A 1-step purification		3mL	20mL	100mL
Expression level	BLI		✓	✓	✓
Purity	SEC (NR/R), CE(NR/R)		✓	✓	✓
Non-specific Binding	BVP score, DNA		✓	✓	✓
Hydrophobicity	HIC, SMAC		✓	✓	✓
Thermal stability	Tm		✓	✓	✓
Colloidal stability	Tagg, AC-SINS, B22, DLS			✓	✓
Charge distribution	iCIEF			✓	✓
Solubility	PEGs				✓
Accelerated Stability (pH: 3.2, 7.0, 8.0; 40°C: T0, 1d, 3d, 7d, 2w; -80°C/RT, 5×)	SEC(NR/R), CE(NR/R), iCIEF, SPR				✓
Analysis Report		✓	✓	✓	✓

XcelDev™ assays are reliable and consistent, achieved through continuous calibration, optimization, and automation

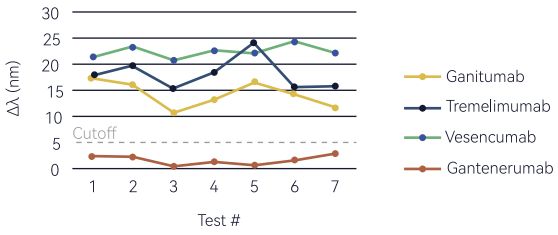
In-house vs. literature SMAC results of benchmark mAbs
(SMAC: Standup Monolayer Absorption Chromatography)



In-house vs. literature BVP assay results of benchmark mAbs

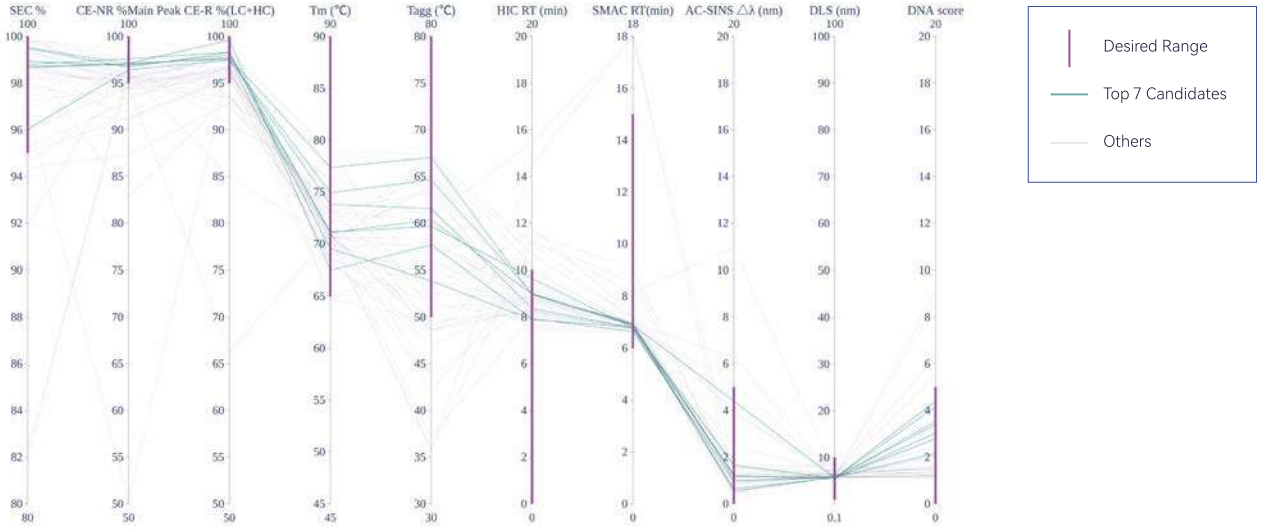


In-house AC-SINS assay results of benchmark mAbs



Case Study

In a hybridoma campaign, 43 hits were identified through binding and functional screening. They were then recombinantly expressed and subject to a high-throughput assessment from XcelDev™ Plus. A total of 7 candidates were recommended because they met all the desired ranges.



Developability assay results of the 43 hits (Top 7 highlighted)

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XcelDev™ Enhance

Elevate Your Candidate's Potential with Developability Enhancements

There are multiple reasons to enhance developability for an existing candidate.

Salvage Candidate

Developability issues can surface late in the discovery process. Salvaging an otherwise qualified candidate is more practical than starting over.

Differentiate Product

Superior developability can serve as a central differentiator in developing best-in-class therapeutics.

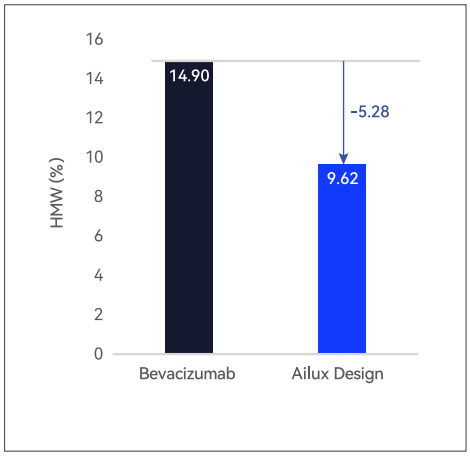
Design New Formats

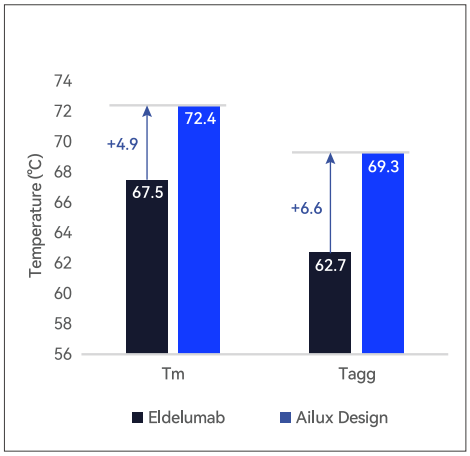
For highly engineered formats (e.g., multi-specifics, ADCs), overcoming developability hurdles is a major part of the engineering process.

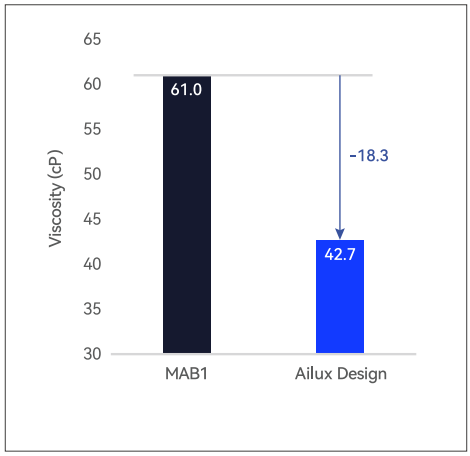
Our AI-powered workflow can enhance antibody developability while keeping other crucial properties intact.

- AI-driven exploration of ultra-large mutant space, up to 10^{25}.
- Multiple properties considered: optimization at no expense to others.
- Expert knowledge combined synergistically with AI.
- Powered by XcelDev™ *in silico* & *in vitro* platforms to rapidly characterize variants.

Case 1-3 | Enhancing antibody developability to acceptable levels







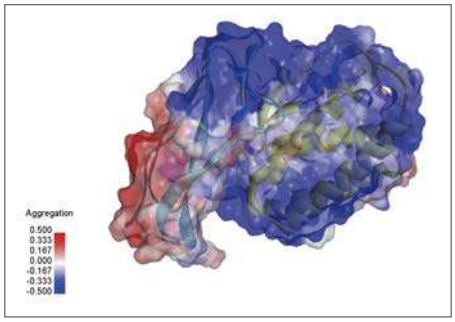
Case 1: Aggregation | **Case 2: Thermostability** | **Case 3: Viscosity**

We utilized our AI-driven workflow to enhance 3 antibodies with known developability risks. All 3 resulted in variants with substantial improvement. Fewer than 15 variants of each were expressed and tested to achieve the above results.
Case 1: Aggregation reduction of Bevacizumab (Formulated in 50 mM PB buffer, incubated at 52 ℃)
Case 2: Improvement of both Tm & Tagg for Eldelumab
Case 3: Viscosity reduction for a highly-viscous antibody

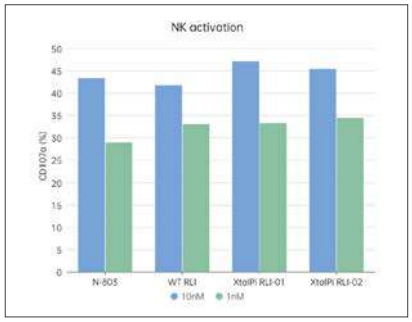
Case 4 | Enhancing recombinant cytokine developability for BIC profile

IL-15 is an immunostimulatory cytokine that has shown therapeutic potential in immuno-oncology. IL-15 (often used in complex with its proprietary receptor IL-15Rα, aka receptor-linker-IL-15 or **RLI**) is prone to aggregation, which poses a major hindrance for therapeutic development.

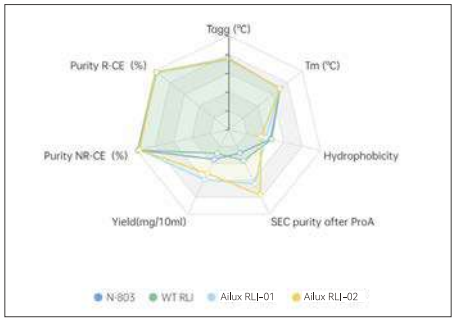
We enhanced the developability of WT RLI to obtain two candidates with potential as best-in-class IL-15 super agonists (Ailux RLIs). They achieved similar levels of binding and potency vs. WT RLI & a clinical benchmark (N-803), but exhibited significantly optimized purity, yield and stability.



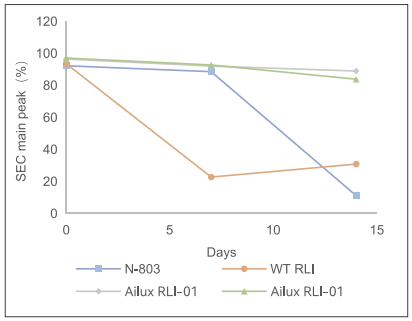
Modeling of IL-15



Optimized RLIs show comparable NK cell activation



Optimized RLIs show better overall developability



Optimized RLIs show less aggregation in stress test

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