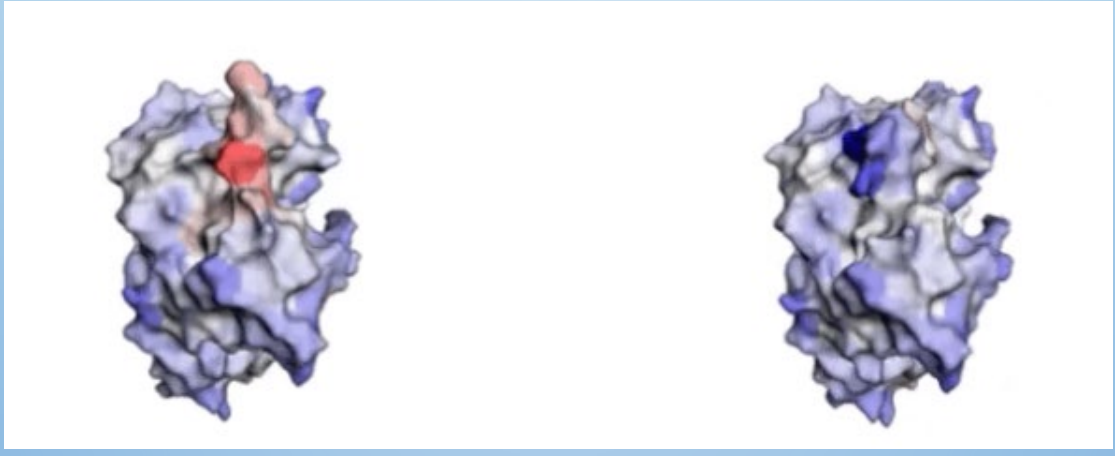
AlfaDAX: AI-Enabled Developability Assessment and Optimization Platform for Generating Clinical-Grade Candidate Molecules

Introduction

Great Bay Bio's independently developed AI-enabled developability assessment and optimization platform, AlfaDAX, leverages AI technology to enable molecular developability assessment and optimization. It provides a comprehensive "sequence input – intelligent assessment – precise optimization" workflow, covering mainstream large-molecule drug types such as monoclonal antibodies, bispecific antibodies, ScFvs, and recombinant proteins. This empowers clients to shorten development timelines, reduce the risk of failure, and generate clinical-grade candidate molecules.

The AlfaDAX platform rapidly predicts key developability metrics of molecules—such as isoelectric point, humanization score, immunogenicity, stability, and aggregation and precipitation risk—using AI models. It intelligently generates predictive results and outputs targeted optimization strategies. Whether it is enhancing humanization to reduce immunogenicity, improving affinity to increase efficacy, or optimizing physicochemical properties to minimize production and storage risks, the platform delivers "on-demand customization," simultaneously providing optimization across three dimensions, empowering clients to develop clinical-grade candidate molecules.



AI-based Developability Prediction AI-driven Sequence Optimization

▲ Comparison Before and After Molecular Optimization on the AlfaDAX Platform: The red regions indicate hydrophobic amino acid aggregation zones before optimization (which can lead to precipitation and poor molecular stability). The blue regions represent the hydrophilic areas after optimization.

The above pics demonstrate how the platform redesigns amino acid mutations to transform hydrophobic risk regions into hydrophilic, stable regions. This provides structural-level evidence of improved developability.

Developability Assessment of Bispecific Drugs

The AlfaDAX platform has demonstrated significant effectiveness in evaluating the developability of bispecific drug molecules. Using AI, we analyzed key developability metrics of multiple drug candidates, covering different bispecific formats and domains (VHH/Fab/ScFv, etc.) and eight critical physicochemical and functional parameters: **isoelectric point, humanization score, immunogenicity, stability, aggregation and precipitation, viscosity, non-specific binding, and BsAb aggregation**.

×Bispecifics With Identified Issues

BsAb-1: Precipitation
BsAb-2: Precipitation
BsAb-3: Aggregation
BsAb-7 / BsAb-8: HIC multi-peak profiles

√Well-Performing Molecules

BsAb-4: No aggregation or precipitation
BsAb-5: No aggregation or precipitation
Fusion Protein: No aggregation or precipitation

➢ Conclusion

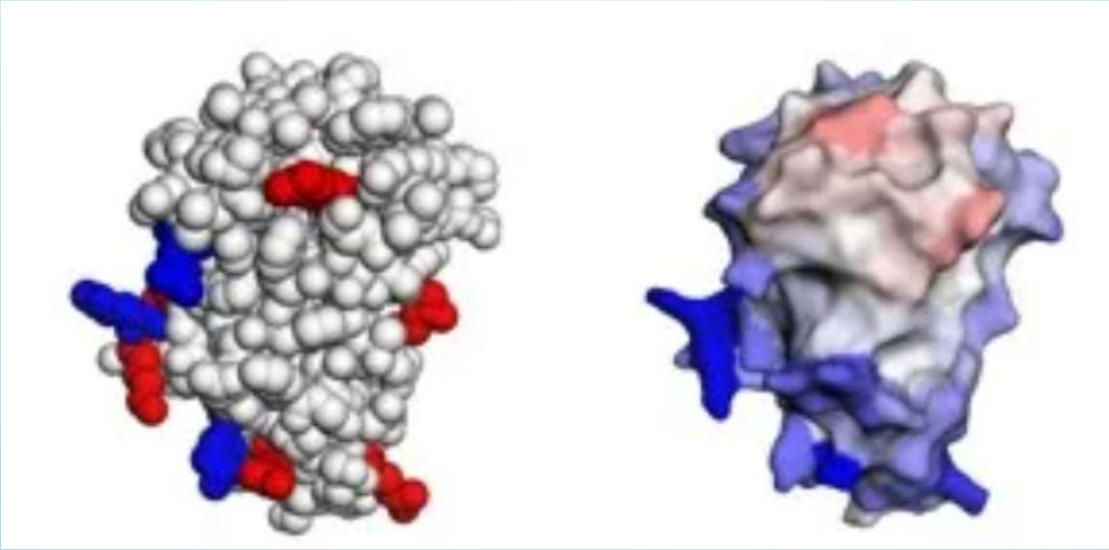
The predictions showed 100% consistency with the final experimental validation.

ID	Domain	pI	Humanisation score	Immunogenicity	Stability	Aggregation & Precipitation	Viscosity	Non-specific binding	BsAb Aggregation	Experimental results
BsAb-1	VHH	8.86	0.09	1.52	-31.38	1.13	0.89	0.66	1.49	Precipitation
	Fab	7.31	0.41	0.81	-46.69	0.42	0.47	0.92		
BsAb-2	VHH	9.52	0.82	0.52	-24.06	0.75	0.63	0.63	2.03	Precipitation
	Fab	5.59	0.62	0.43	-32.48	0.6	0.96	0.39		
BsAb-3	Fab	7.07	0.42	0.43	-47.15	0.46	0.32	1.37	1.91	Aggregation
	scFV	8.25	0.77	0.32	-47.06	0.47	0.32	0.61		
BsAb-4	Fab	8.15	0.23	3.18	-68.41	0.48	1.2	0.63	0.86	Normal
	scFV	8.92	0.19	2.48	-46.88	0.28	0.79	0.38		
BsAb-5	Fab1	7.02	0.93	0.13	-54.35	0.94	1.05	0.8	0.59	Normal
	Fab2	6.58	0.24	0.59	-57.33	0.46	0.52	0.92		
BsAb-6	Fab	7.11	0.42	0.43	-49.56	0.47	0.35	1.19	1.23	-
	scFV	8.82	0.26	3.1	-64.43	0.38	0.94	0.88		
BsAb-7	Fab	7.78	0.47	1.62	-46.64	0.21	0.75	0.26	1.34	HIC Multiple Peaks
	scFV	5.46	0.42	2.11	-54.96	0.4	2.2	0.53		
BsAb-8	Fab	5.46	0.42	2.11	-54.96	0.4	2.2	0.53	1.48	HIC Multiple Peaks
	scFV	8.66	0	3.14	-32.23	0.67	0.93	0.48		
Fusion Protein	Fab	8.69	0.52	0.76	-54.52	0.5	0.84	0.63	0.33	Normal
	Peptide	9.18	-	-	11.03	0.19	0.75	0.75		

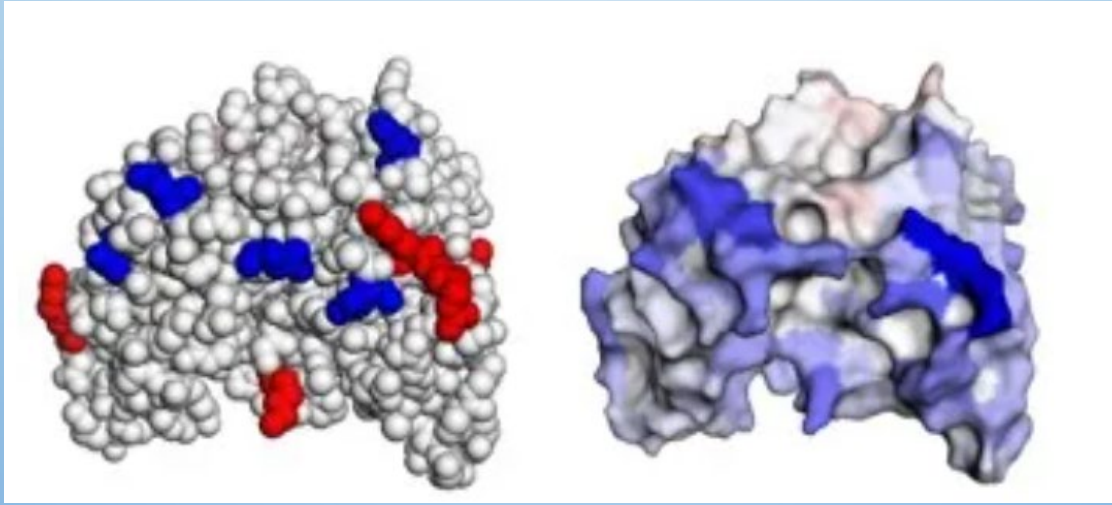
▲ 100% concordance between bispecific drug developability assessment and experimental validation.

Relationship Between Structure and Aggregation and Precipitation

We performed structural visualization analysis of the typical case BsAb-1 and interpreted the causes of precipitation using two types of structural diagrams. AI-driven analysis suggested that the precipitation of BsAb-1 may result from “hydrophobic amino acid clustering in the VHH domain” or “electrostatic attraction between VHH and Fab.” Based on these insights, the molecule can be further engineered and optimized.



▲ BsAb-1 VHH: Charge & Hydrophobicity Distribution



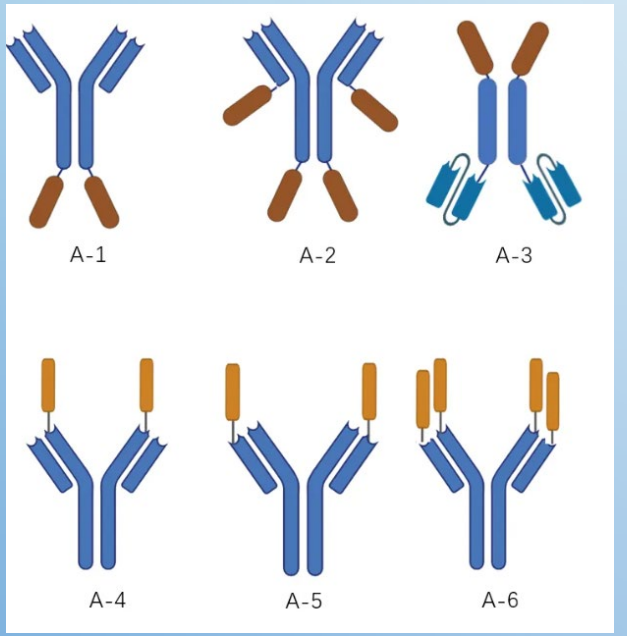
▲ BsAb-1 Fab: Charge & Hydrophobicity Distribution

Note: For charge distribution (spherical structure), red = positive, blue = negative, white =neutral.
For hydrophobic/hydrophilic distribution (smoothed surface), red = hydrophobic, blue = hydrophilic.

Relationship Between Conformation and Activity

The AlfaDAX platform has also made clear progress in elucidating the logical relationship between drug activity and molecular conformation, facilitating subsequent engineering and optimization of different conformers. Using AI, we analyzed the "α-GIPR antibody + GLP1" molecule and quantified the activity differences among six antibody/GLP1 conformations, leading to the following core findings:

- The reference group (antibody/GLP1) exhibited normal dual activity (α-GIPR binding 1.64 nM; GLP1 binding 1.36 nM).
- Partially inactivated conformations include A-1/2 (no GLP1 binding), A-3 (α-GIPR not detectable; no GLP1 binding), and A-6 (no α-GIPR binding).
- Conformations with reduced activity include A-4/5 (both activities decreased: A-4 α-GIPR binding 6.24 nM, GLP1 binding 10.68 nM; A-5 α-GIPR binding 5.01 nM, GLP1 binding 11.58 nM).



	α-GIPR Antibody-Cell Binding (nM)	GLP1 Cell Binding (nM)
Antibody/GLP1	1.64	1.36
A-1	2.39	Non-binding
A-2	2.95	Non-binding
A-3	N/A	Non-binding
A-4	6.24	10.68
A-5	5.01	11.58
A-6	Non-binding	8.69

▲ Six Antibody/GLP1 Conformations: α-GIPR & GLP1 Cell Binding (nM)
(Lower values indicate stronger binding; "Non-Binding" = no activity, "N/A" = not tested)

Inactivation Mechanisms of Inactive Conformations:

Conformations 1, 2, and 3 can be explained from a structural steric hindrance perspective.

- When GLP1 is located at the C-terminus, steric hindrance between the antibody and the GLP1 receptor (GLP1R) directly blocks GLP1 from binding to GLP1R, resulting in complete loss of GLP1 cellular activity. Thus, conformations 1/2 correspond to the C-terminal inactivation mechanism of GLP1.

- When GLP1 is buried between the antibody's Fc domains, it cannot extend outward and lacks sufficient space to engage the binding pocket of GLP1R, leading to loss of cellular activity. Therefore, conformation 3 represents the Fc-encapsulated inactivation mechanism.

Key Factors for Reduced Activity:

Conformations 4, 5, and 6, which show reduced activity, feature GLP1 connected to the N-terminus of the antibody variable regions (VH/VL). This produces a dual effect:

- Positive aspect: Reduced steric hindrance allows GLP1 to retain its binding activity with GLP1R partially.

- Negative aspect: Connection at the variable region N-terminus can obstruct the antibody's CDR regions (critical for antigen binding), resulting in decreased α-GIPR binding activity.

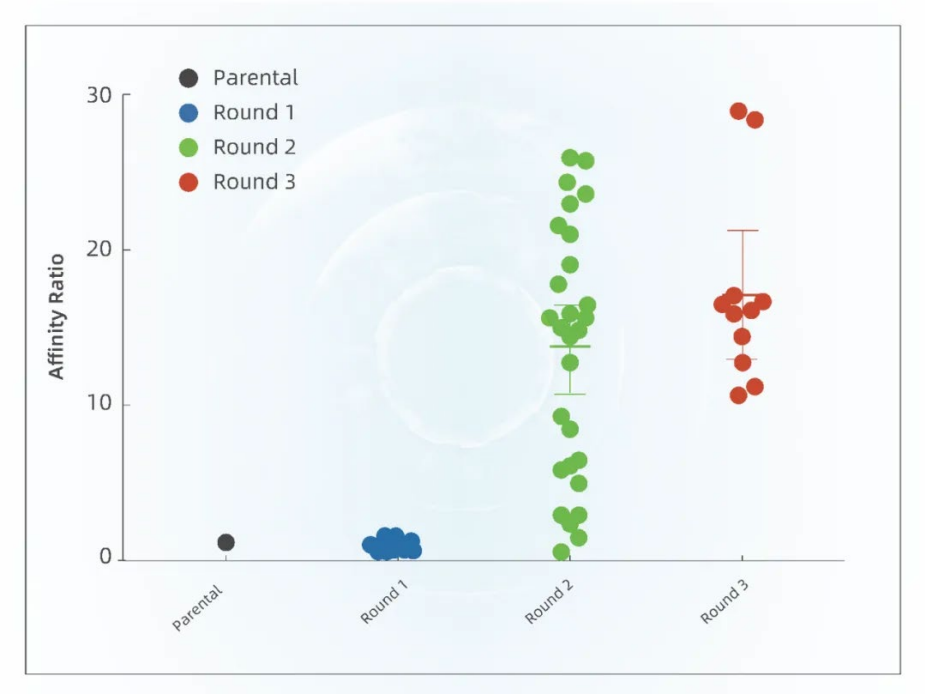
Antibody Molecule Optimization

Leveraging AI-driven precision design and multi-dimensional coordinated optimization, the AlfaDAX platform has accumulated extensive practical experience in antibody molecule optimization. It enables simultaneous breakthroughs across three core dimensions—**affinity, humanization, and developability**—ultimately delivering clinical-grade candidate molecules that are "highly active, low-risk, and manufacturable." This provides efficient and reliable core support for translating antibody drugs from laboratory research to clinical application.

1. Affinity Enhancement

Affinity is the core indicator of an antibody's ability to bind its antigen, directly determining the drug's target specificity and efficacy. The AlfaDAX platform predicts key residues at the antigen-antibody interface using AI, designs efficient mutation libraries, and achieves stepwise affinity improvement through multiple rounds of optimization.

Case 1: Monoclonal Antibody Affinity Increased 29-Fold



▲ 29-Fold Increase in Monoclonal Antibody Affinity

From the Parent (baseline) sample through Rounds 1, 2, and 3, the platform shows a clear upward trend in affinity (y-axis): the baseline molecule has a high KD due to an imprecise binding-site conformation.

- **Round 1:** Initial adjustments to key amino acids lead to a noticeable improvement in affinity.
- **Round 2:** Further optimization of the binding interface enhances spatial complementarity, resulting in a continued decrease in KD.
- **Round 3:** AI-selected optimal mutation combinations **achieve a 29-fold affinity improvement over the baseline**, fully meeting clinical pharmacodynamic requirements.

Case 2: 43-Fold Affinity Improvement with Multi-Metric Validation

AlfaDAX	Sample ID	K_D (M)	k_a (1/Ms)	k_{dis} (1/s)	Ratio
Round 1	000	1.53E-07	4.80E+04	7.60E-03	1
	001	7.71E-08	6.83E+04	5.46E-03	1.99
	003	4.31E-08	8.15E+04	3.65E-03	3.56
	004	2.72E-08	1.31E+05	3.72E-03	5.64
	006	3.90E-08	1.41E+05	5.70E-03	3.94
	002	5.55E-08	1.19E+05	6.82E-03	2.77
	007	6.94E-08	8.70E+04	6.26E-03	2.21
	008	5.31E-08	6.95E+04	3.83E-03	2.89
Round 2	000	2.49E-07	9.19E+04	2.38E-02	1
	041	6.92E-09	1.79E+05	1.29E-03	36.03
	038	1.29E-08	8.35E+04	1.11E-03	19.36
	022	1.35E-08	2.01E+05	2.82E-03	18.45
	040	1.77E-08	1.75E+05	3.22E-03	14.06
	039	1.88E-08	1.36E+05	2.65E-03	13.24
	029	2.05E-08	1.58E+05	3.37E-03	12.17
	022	2.05E-08	1.58E+05	3.37E-03	12.17
Round 3	000	4.04E-08	2.97E+05	1.24E-02	1
	043	9.31E-10	1.57E+06	1.52E-03	43.44
	048	9.39E-10	1.29E+06	1.26E-03	13.06
	047	1.10E-09	1.53E+06	1.74E-03	36.84
	055	1.11E-09	1.58E+06	1.81E-03	36.55
	057	1.13E-09	1.74E+06	2.05E-03	35.62
	056	1.18E-09	1.56E+06	1.91E-03	34.25
	046	1.22E-09	1.60E+06	2.02E-03	33.11

▲43-Fold Affinity Improvement with Multi-Parameter Quantitative Validation

- **Round 1:** The best sample 004 showed KD decreasing from **1.53E-07 M (baseline) to 2.72E-08 M, Ratio = 0.178 (5.64-fold increase).** The ka value rose from **4.80E+04 1/(Ms) to 1.31E+05 1/(Ms), nearly a 3-fold** increase in association rate.
- **Round 2:** Sample 041 performed best, KD dropped to **6.92E-09 M, Ratio = 0.028 (36.03-fold increase).** The kdis value decreased from **2.38E-02 1/s to 1.29E-03 1/s**, reducing the dissociation rate nearly 18-fold and greatly improving binding stability.
- **Round 3:** Sample 043 reached a new level, KD decreased to **9.31E-10 M, Ratio = 0.023 (43.44-fold increase)**, and ka reached **1.57E+06 1/(Ms), 32-fold** higher than the baseline.

➢ Conclusion

These multi-round data confirm that AlfaDAX not only enhances affinity but also optimizes association and dissociation kinetics, achieving the ideal "fast-on, slow-off" binding pattern.

Case 3: Nanobody Affinity Increased 19.6-Fold

Nanobodies, with small molecular weight and high tissue penetration, are widely used in cancer and autoimmune disease therapy, but their natural affinity often fails to meet clinical requirements. AlfaDAX designed targeted optimization strategies based on nanobody structural features (VH domain only, with concentrated binding sites):

AlfaDAX	ID	K_D (M)	Ratio
Round 1	Control	5.12E-07	1.0
	24	6.53E-08	7.5
	49	1.27E-07	4.5
	30	1.25E-07	4.4
	42	1.65E-07	3.8
	26	1.59E-07	3.4
Round 2	Control	9.36E-08	1.0
	59	4.82E-09	19.6

▲19.6-Fold Improvement in Nanobody Affinity

- **Round 1:** AI predicted key mutation sites in the nanobody CDR regions (complementarity-determining regions) and screened sample 24. **KD = 6.53E-08 M, Ratio = 0.133, a 7.5-fold** improvement over the Control (KD = 5.12E-07 M).
- **Round 2:** Further optimization of CDR side-chain orientations yielded sample 59, **KD = 4.82E-09 M, a 19.6-fold** increase in affinity. The KD values were highly consistent across repeated experiments, demonstrating excellent structural stability and providing a solid foundation for subsequent production and clinical applications.

Antibody Molecule Optimization

2. Humanization Enhancement

Non-human antibodies (e.g., mouse-derived antibodies) can be recognized as "foreign" by the human immune system, triggering anti-drug antibody (ADA) responses that reduce efficacy or cause adverse effects. **The AlfaDAX platform uses AI-driven structural prediction to precisely distinguish "framework regions requiring modification" from "binding regions to retain," overcoming the common issue in traditional humanization where excessive mutations lead to loss of affinity, thereby achieving dual assurance of humanization and function.**

Case 1: Triple Optimization of Monoclonal Antibody Humanization

For a mouse-derived monoclonal antibody, AlfaDAX innovatively combined three optimization strategies—**humanization, developability optimization, and additional CDR disulfide bond introduction**—avoiding single-dimension shortcomings. Comparing the template with four humanized samples (Hu-003, Hu-004, Hu-006, Hu-008):

Sample	K_D (M)	k_a (1/Ms)	k_{dis} (1/s)	Ratio	pI	Hu	AP	Vis	NSB	VH Identity	VL Identity
Control	4.52E-09	5.75E+05	2.58E-03	1.01	6.01	0.07	2.49	0.75	0.28	65.45%	83.02%
Hu-003	4.12E-09	5.69E+05	2.32E-03	1.11	7.70	0.47	0.27	0.80	0.20	91.00%	91.91%
Hu-004	4.41E-09	6.02E+05	2.63E-03	1.01	8.57	0.37	0.26	0.75	0.53	91.00%	91.91%
Hu-006	3.80E-09	6.28E+05	2.36E-03	1.21	8.24	0.37	0.28	0.77	0.26	92.11%	91.91%
Hu-008	4.64E-09	5.65E+05	2.60E-03	1.01	8.32	0.40	0.26	0.80	0.21	92.11%	91.91%

▲Triple Optimization Enhances Monoclonal Antibody Humanization

- **Significant humanization improvement:** The template's humanization score (Hu) was only 0.07 (typical mouse antibody), with VH/VL germline identity at 65.45% / 83.02%. After optimization, Hu-003 **reached Hu = 0.47 (meeting humanized antibody standard), with VH/VL identity >91%; Hu-006 VH identity reached 92.11%**, greatly reducing immunogenicity risk.
- **Developability improvement:** Template AP score was 2.49 (>1, indicating aggregation risk), while all four humanized samples had AP <0.3, eliminating aggregation risk. Viscosity, non-specific binding, and other metrics remained within safe ranges, meeting large-scale production requirements.
- **Affinity retention:** KD values for all four humanized samples remained around 4E-09 M, similar to the template (4.52E-09 M), avoiding the common affinity loss seen in traditional humanization. Additionally, extra disulfide bonds in the CDRs stabilized the binding site conformation, maintaining affinity under simulated in vivo pH and temperature changes.

Case 2: Breakthrough in Challenging Monoclonal Antibody Humanization

A client's monoclonal antibody experienced orders-of-magnitude affinity loss after multiple rounds of traditional germline substitution during humanization attempts. Using AI-driven structural modeling, the AlfaDAX platform identified four critical residues that should not be mutated and avoided them:

- **Site 1:** Prone to generating electrostatic repulsion, disrupting the binding interface.
- **Site 2:** May alter the CDR2 conformation, affecting antigen binding.
- **Site 3:** Could convert an existing salt bridge into a repulsive charge interaction, reducing binding stability.
- **Site 4:** May distort the non-CDR loop structure in FW3, indirectly impacting binding.

By avoiding these non-permissible sites, AlfaDAX achieved **simultaneous optimization in humanization, functionality, and expression** ("triple compliance").

The final optimized results showed···

ID	K_D (M)	Ratio	Title (mg/l) @ day 5	FW Identity (%)	Human EC_{50} (nM)	Ratio	Cynomolgus EC_{50} (nM)	Ratio
Control	2.11E-08	1.0	293.0	77.6	0.047	1	0.069101	1
064	2.43E-08	1.2	359.0	94.1	0.05483	1.31	0.09422	1.42
070	2.38E-08	1.1	387.0	94.1	0.04313	1.07	0.07501	1.06
076	1.51E-08	0.7	378.3	91.9	0.03886	1.08	0.11087	1.67

▲Breakthrough in Challenging Monoclonal Antibody Humanization

This achieved simultaneous compliance in humanization, function, and expression, with final results:

- **Humanization:** Framework identity improved from **77.6% to 91.9%–94.1%,** achieving a high level of humanization.
- **Affinity & Function:** Optimized variants (064, 070, 076) maintained KD values of **1.5E-08 –2.5E-08 M**, comparable to the control (2.11E-08 M). EC50 values in both human and cynomolgus systems showed no notable reduction, confirming preserved functional activity.
- **Production Performance:** Expression levels increased **from 293 mg/L (baseline) to as high as 387 mg/L** (sample 070), reducing overall manufacturing costs.

Case 3: Nanobody Humanization Enhancement

Nanobody humanization requires improving sequence identity while considering small molecular size, optimizing charge distribution and specificity to avoid off-target risks. AlfaDAX addressed two types of nanobody needs, combining "**humanization + charge optimization" and "humanization + non-specific binding optimization**".

✓ *Humanization + Charge Optimization*

ID	K_D (M)	k_a (1/Ms)	k_{dis} (1/s)	Ratio	vh_fw_ identity	PTM	PI	vHH PI
000	1.28E-10	4.74E+05	5.95E-05	1.00	76.8%	W57	6.72	6.87
032	2.75E-11	7.09E+05	5.31E-07	4.63	92.4%	W57Y	8.21	8.36
047	1.74E-10	6.78E+05	1.16E-04	0.73	92.7%	W57Y	8.14	8.23

- Baseline sample 000 showed low humanization (VH_FW_identity = 76.8%), a PTM risk at W57 (prone to oxidation, affecting stability), and a PI of 6.72, which deviates from physiological pH and may lead to accelerated clearance in vivo.
- Optimized sample 032 introduced an AI-designed W57Y mutation that eliminated the PTM risk, **increased the humanization score to 92.4%, and adjusted the PI to 8.21 (closer to physiological conditions)**. Its KD improved from 1.28E-10 M to 2.75E-11 M, **achieving a 4.63-fold enhancement and simultaneously improving humanization, stability, and efficacy**.
- Sample 047 achieved an even higher humanization score (92.7%) but showed **a slightly higher KD (Ratio = 0.73).** Based on this, the platform provided recommendations prioritizing either stability or affinity, demonstrating the flexibility of the optimization strategy.

Case 3: Nanobody Humanization Enhancement

✓ *Humanization + Non-Specific Binding Optimization*

Baseline nanobody 000 specifically bound Target1 but cross-reacted with homologous Target2/3, posing an off-target toxicity risk. AlfaDAX used a three-step approach:

Sample	pI Value	Humanization (>0.2)	Heat Stability (<-15)	Aggregation (<1)	Viscosity (<1)	Non-specific Binding (<1)
000	9.10	0.09	-27.60	0.16	0.66	1.00

Antibody (000)	abPTM	Energy
000 - Target 1	0.86	-10.55
000 - Target 2	0.89	-6.78
000 - Target 3	0.84	-4.73

ID	K_D (M)	k_a (1/Ms)	k_{dis} (1/s)	Target 1 Cell Binding EC_{50} (nM)	Target 1 ELISA Binding EC_{50} (nM)	Target 2 OD_{450} (300 nM)	Target 3 OD_{450} (300 nM)
000	2.99E-09	5.38E+04	1.56E-04	0.4236	0.03576	1.9967	2.0457
086	5.33E-09	5.58E+04	2.88E-04	0.5824	0.05258	0.5664	1.3153
094	7.50E-09	5.04E+04	3.66E-04	0.5424	0.06676	0.4352	0.5089

Step 1 Structural Prediction: Binding energies were evaluated: **Target1 = –10.55 (<–5, indicating strong and stable binding), Target2/3 = –6.78 and –4.73 (Target3 close to –5**, suggesting potential non-specific binding risk). Key residues responsible for cross-binding were identified.

Step 2 Metric Evaluation: Baseline sample 000 had a non-specific binding score of 1.00 (critical risk) and a humanization score (Hu) of 0.09 (non-human).

Step 3 Functional Validation: Optimized samples 086 and 094 showed significant improvement in humanization and substantial reduction in non-specific binding. OD450 values for Target2 **decreased from 1.9967 to 0.4352, and for Target3 from 2.0457 to 0.5089 (>70% reduction).** KD values **increased slightly from 2.99E-09 M to a maximum of 7.50E-09 M (<2-fold),** meeting clinical requirements for improved specificity without compromising efficacy.

Notes:

- **Isoelectric Point (pI):** The pH at which the antibody variable region carries no net surface charge. At the pI, the protein is highly unstable and has minimal solubility due to the lack of electrostatic repulsion, making it prone to aggregation and precipitation.
- **Humanization Score:** The higher the score, the greater the degree of humanization. Scores <0.2 indicate non-human antibodies, 0.2–0.6 indicate humanized antibodies, and >0.6 indicate fully human antibodies.
- **Stability Score:** A score > –15 indicates a potential risk of reduced molecular stability.
- **Aggregation Score:** A score >1 indicates a higher risk of aggregation or precipitation.
- **Viscosity Score:** A score >1 indicates a risk of high viscosity at high concentrations (>150 mg/mL).
- **Non-specific Binding Score:** A score >1 indicates a risk of non-specific binding.

Antibody Molecule Optimization

3. Triple-Dimension Optimization: Affinity, Humanization, and Developability

In practical drug development, optimizing a single dimension is insufficient to meet clinical requirements. For example, a high-affinity molecule may be unsuitable for manufacturing due to aggregation risk, while a highly humanized molecule may lose functional activity.

The core advantage of the AlfaDAX platform lies in its ability to **simultaneously optimize affinity, humanization, and developability** through AI-driven coordination, ultimately generating clinical-grade candidate molecules with no performance trade-offs.

Case 1: Multidimensional Optimization of a Bispecific Antibody

For a bispecific antibody designed to bind both Target A and Target B, the AlfaDAX platform predicted the compatibility of the two binding interfaces and designed non-conflicting mutation schemes. This resulted in simultaneous improvements in affinity, function, and developability:

ID		K_D (M)	Ratio	Cell Function EC_{50} (nM)	Ratio	IC_{50} (nM)	Ratio	EC_{50} (nM)	Ratio	Cell binding (nM)	Ratio
Target A	000	1.52E-08	1	40.37	1	0.57	1	0.26	1	5.18	1
	083	9.56E-09	1.64	4.62	11.3	0.30	0.92	0.30	0.92	2.02	2.56
	124	8.00E-09	1.95	5.31	7.2	0.25	1.02	0.25	1.02	1.81	2.87
	126	7.32E-09	2.15	6.04	7.2	0.30	1.23	0.33	1.23	2.38	2.15
Target B	000	5.08E-09	1	5.69	1	0.27	1	0.24	1	0.78	1
	083	2.80E-09	1.82	2.46	2.31	0.16	1.74	0.24	1.43	0.48	1.64
	124	4.44E-09	1.15	2.27	2.50	0.19	2.05	0.20	1.23	0.62	1.23
	126	3.55E-09	1.43	1.43	3.98	0.14	2.15	0.19	1.43	0.60	1.33

▲Three-Dimensional Optimization of a Bispecific Antibody

- **Affinity Improvement**

Target A KD **decreased from 1.52E-08 M to 7.32E-09 M** (Ratio = 0.48, 2.15-fold improvement); Target B KD **decreased from 5.08E-09 M to 2.80E-09 M** (Ratio = 0.55, 1.82-fold improvement), significantly enhancing dual-target binding capability.

- **Functional Enhancement**

Target A cell function IC50 **decreased from 40.37 nM to 4.62 nM** (11.3-fold improvement); Target B IC50 **decreased from 5.69 nM to 1.43 nM** (3.98-fold improvement), demonstrating a substantial increase in cellular potency.

- **Developability Achieved**

Aggregation score <0.3, viscosity <0.8, and non-specific binding <0.5. All key metrics met preclinical standards, successfully producing a "dual-target, high-potency, low-risk" candidate molecule.

Case 2: 77-Fold Affinity Improvement with Concurrent Functional and Stability Enhancement

For a monoclonal antibody project requiring high affinity, the AlfaDAX platform performed multiple rounds of AI screening to achieve breakthrough-level affinity enhancement. The baseline clone 000 showed poor pocket matching, with KD = 2.27E-08 M, ELISA EC50 = 2.19 nM, and cell-blocking IC50 = 19.03 nM, falling short of clinical potency needs. After optimization, clone 090 achieved comprehensive improvements through precise mutations at key residues:

ID	K_D (M)	k_a (1/Ms)	k_{dis} (1/s)	Ratio	ELISA EC_{50} (nM)	Cell Blockade IC_{50} (nM)
000	2.27E-08	5.61E+04	1.30E-03	1.00	2.19	19.03
054	4.15E-09	4.56E+04	1.93E-04	5.38	0.55	4.03
083	3.09E-09	5.14E+04	1.62E-04	7.21	0.74	4.70
090	2.86E-10	5.68E+04	1.66E-05	77.87	0.56	3.95

▲ 77-Fold Affinity Enhancement of the Monoclonal Antibody with Maintained Function and Stability.

- KD improved to 2.86E-10 M, making the affinity **77.87-fold** better than the benchmark.
- ELISA EC50 decreased to 0.56 nM (**3.9-fold improvement**), showing much stronger binding activity.
- Cell-based blocking IC50 decreased to 3.95 nM (**4.8-fold improvement**), indicating a significantly enhanced inhibitory effect.
- Kinetic parameters improved: **ka reached 5.68E+04 1/(Ms) for stable association**, while **kdis dropped to 1.66E-05 1/s (78-fold lower),** allowing longer antigen engagement and extended drug action.

Case 3: Nanobody Optimization Achieving Both High Affinity and High Humanization (94.32% Germline Identity)

For a nanobody intended for clinical translation, the AlfaDAX platform first optimized affinity and then performed humanization, achieving both key criteria simultaneously:

AlfaDAX	ID	K_D (M)	Ratio
Round 1	Control	7.36E-08	1.0
	Mut-16	6.53E-08	6.5
	Mut-19	1.27E-07	3.2
	Mut-5	1.25E-07	1.5
	Mut-13	1.65E-07	1.2
	Mut-22	1.59E-07	1.1
Round 2	Control	9.36E-08	1.0
	Mut-62	4.82E-09	11.61
	Mut-66	7.36E-08	11.2
	Mut-63	6.53E-08	11.04
	Mut-64	1.27E-07	10.52
	Mut-66	1.25E-07	8.7
	Mut-60	1.65E-07	5.7
	Mut-58	1.59E-07	4.57
	Mut-69	9.36E-08	2.2

▲Nanobody Achieving Both High Affinity and High Humanization

- **Affinity optimization**: the first-round of Mut-16 **achieved KD = 1.23E-08 M (6.5-fold improvement)**, and the second-round of Mut-62 **further improved KD to 8.44E-09 M (11.61-fold).**
- **Humanization**: using AI germline matching, the platform replaced framework regions with the closest human sequences, resulting in Mut-74 showing **94.32% germline identity (near fully human) while maintaining KD = 8.63E-09 M**, achieving both high affinity and high humanization to reduce clinical risk.