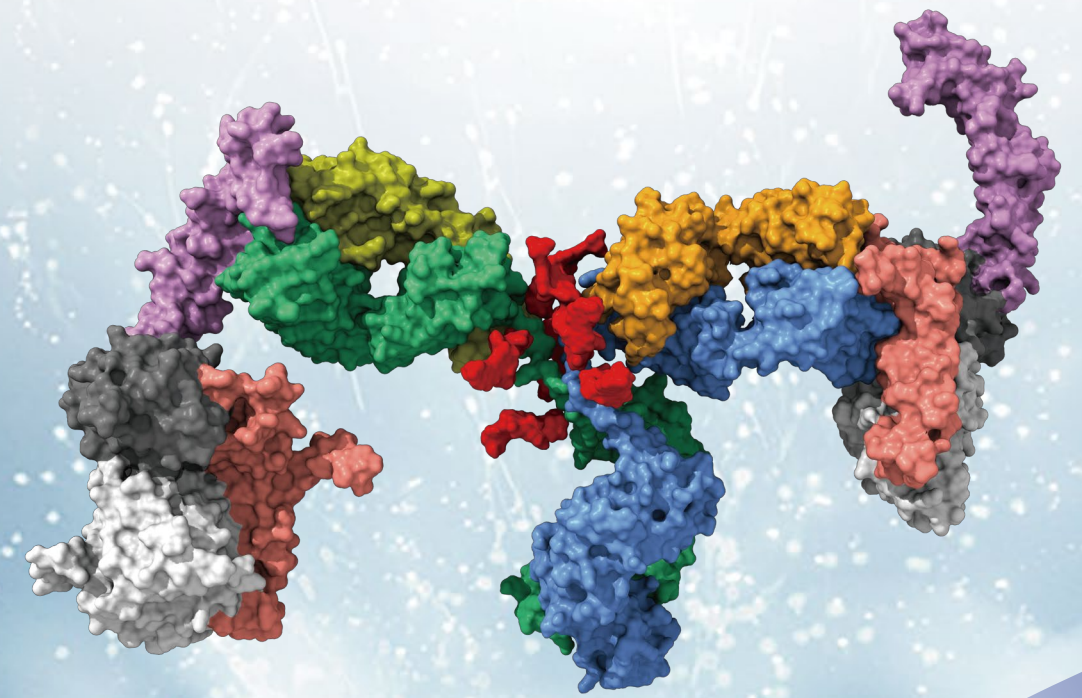


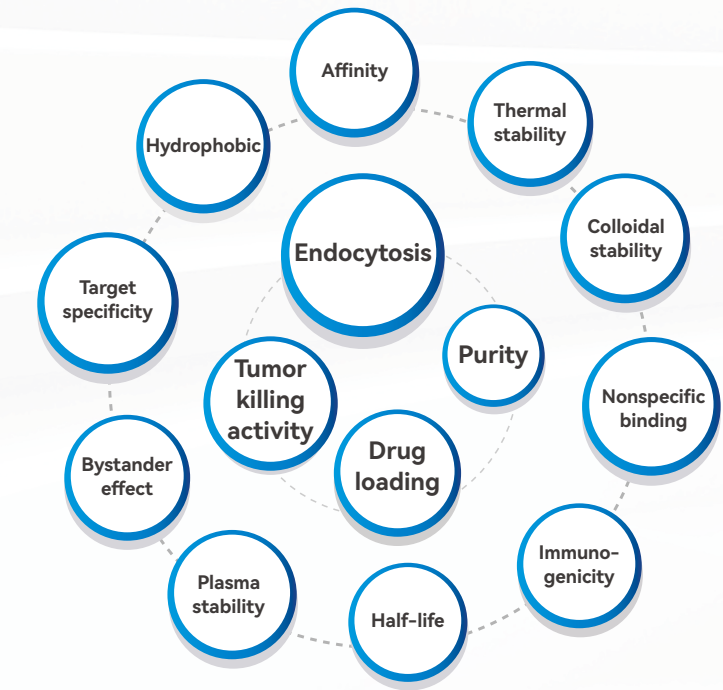
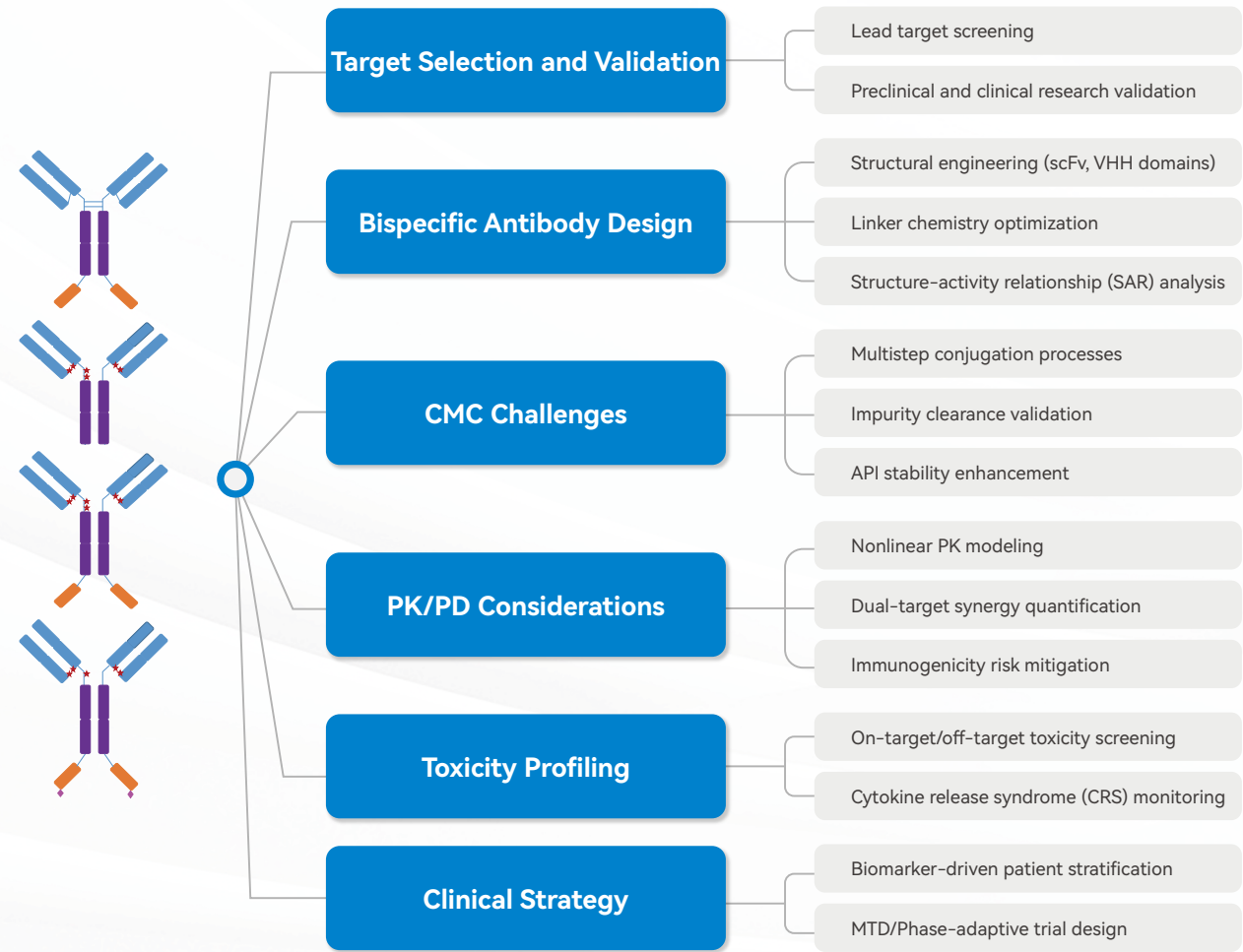


**Empowering Biologics,
Uniting to Heal Humanity!**

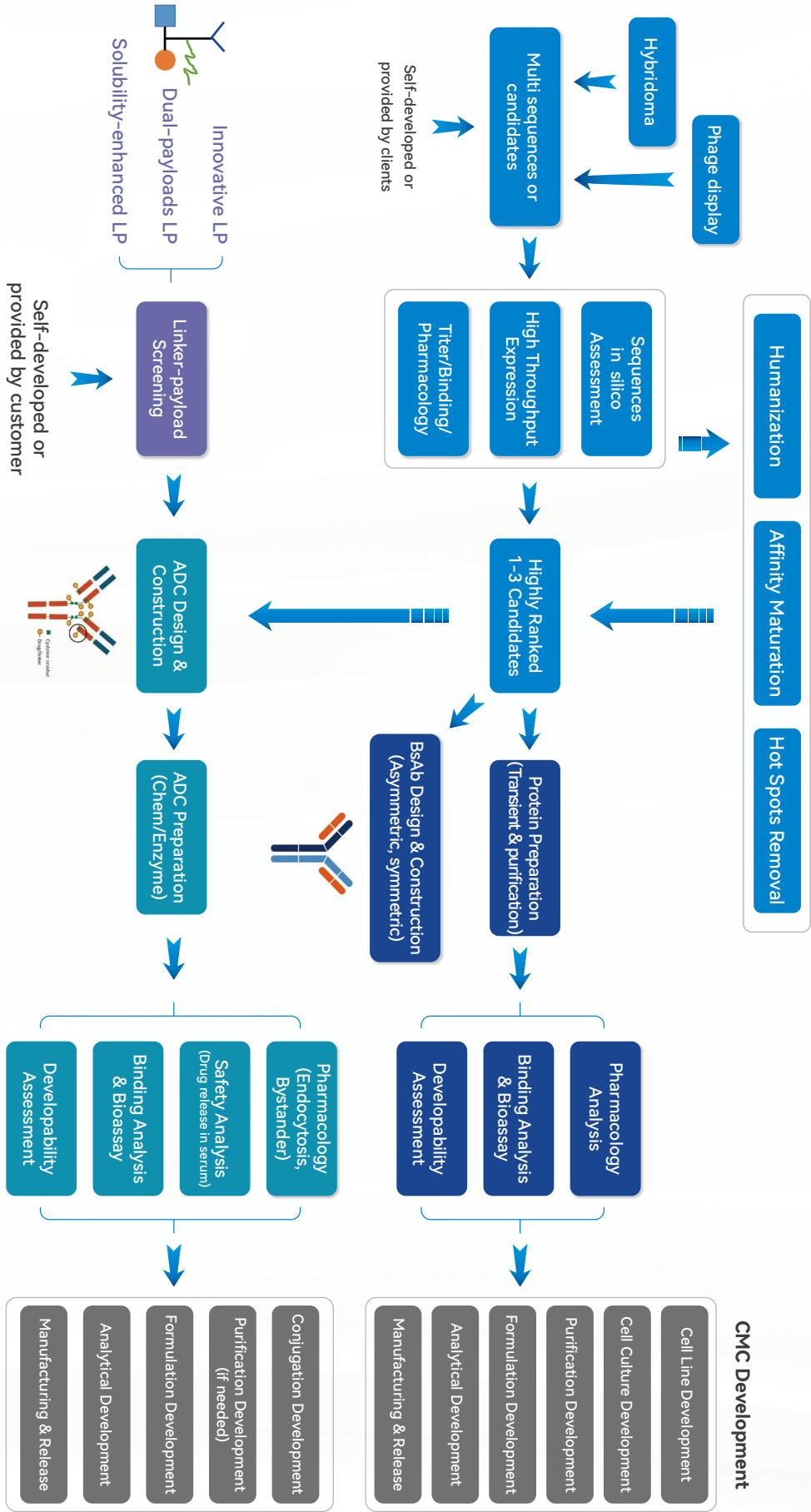


Ginspire Biologics Introduction

The Development Challenges of Innovative Complex Molecules



Consider all aspects of the design/screening/evaluation/op-timization/development of inno-vative complex molecules
Source intervention to improve the development efficiency of innovative complex molecules!



- Ginspire Biologics, an early research services subsidiary of Thousand Oaks Biologics.
- Located in the core area of Zhangjiang High-tech Park, covering over 1,000 square meters.
- Currently employs 18 people, with over 60% holding master's or doctoral degrees. Average tenure exceeds 6 years.
- Offers molecular design and optimization, linker-payload and antibody screening, pharmacological efficacy and drug evaluation, and other services.
- Collaborates with clients on demand to develop differentiated innovative molecules and licenses self-developed molecules.

Technology platform of Ginspire



AI prediction and development platform

Sequence analysis

EVQLVESGGGLVQPGGSLRLSCAASGFNIK-
DTYIHVWRQAPGKGLEWVARIYPTNGYTRY-
ADSVKGRFTISADTSKNTAYLQMNSLRAEDTA
VYYCSRWGGDGFYAMDYWGQGTLVTVSS

Sequence analysis

The variable region of heavy and light chain (VH/VL)

Deamidation sites (NG, NS, QG)
Isomerization sites (DG, DS)
Cleavage sites (DP, TS)

Amino acids containing benzene ring in the VH/VL

WYF clusters in the CDR region
Tryptophan in heavy chain CDR3

Variable region and isoelectric point of intact protein

CDR length

Extra N-glycosylation sites

Asn-Xaa-Ser/Thr (Xaa cannot be proline)

Unpaired sulfhydryl groups

Non-germline frame zone sequence in the variable zone

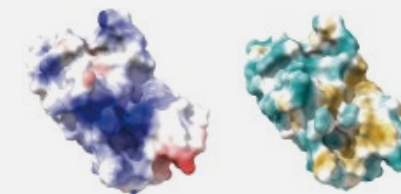
Total hydrophilic average (GRAVY)

High Viscosity Index (HVI)

Solubility (CamSol)

.....

In silico analysis



In silico analysis

Hydrophobic cluster

Total hydrophobic cluster
Near CDR hydrophobic cluster

Charge cluster

Total charge cluster
Near CDR positive charge cluster
Near CDR negative charge cluster

Variable zone net charge

Solubility (CamSol - after structural correction)

Developability index (DI)

Aggregation-prone regions

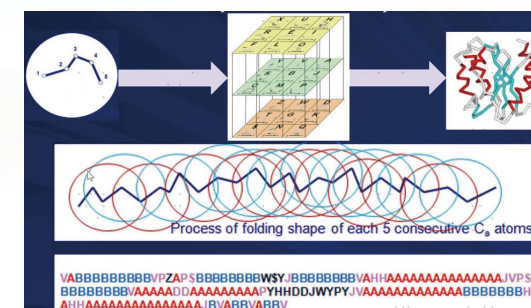
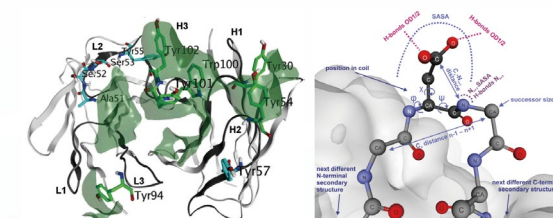
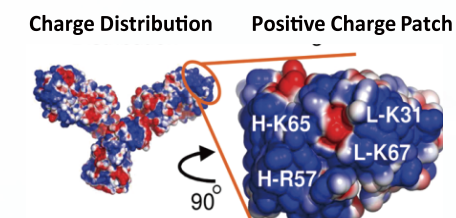
Therapeutic Protein Indicator

Viscosity (SCM/ Deep SCM)

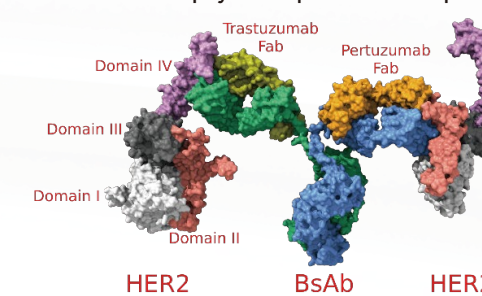
Protein structure and antibody-antigen structure prediction (AlphaFold3/IgFold)

Protein Fingerprint map (FiveFold)

.....



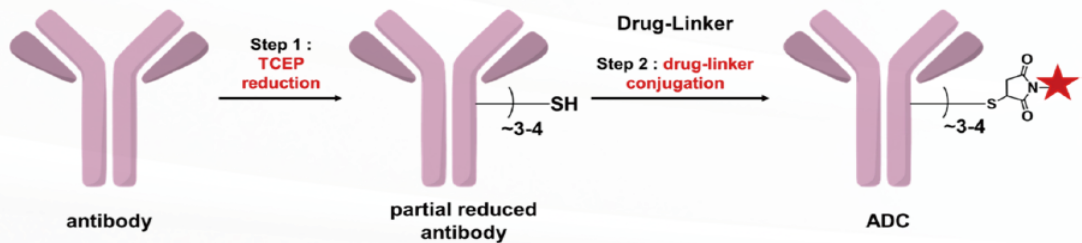
Localization and deployment of protein structure prediction



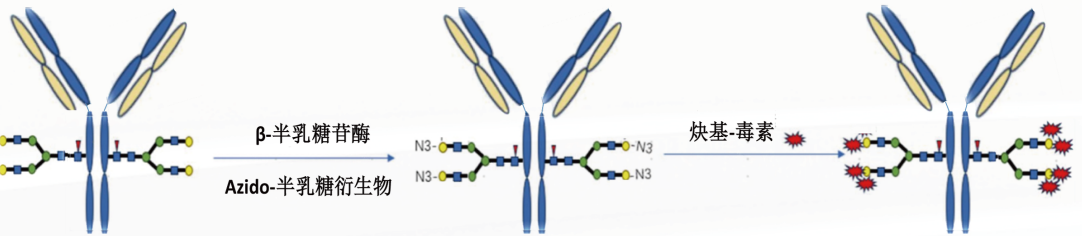
Innovative linker-payload and conjugation development platforms

Serial number	Linker-payload Structural formula	Coupling mode	Suggested DAR values	Serial number	Linker-payload Structural formula	Coupling mode	Suggested DAR values
1	MC-VC-PAB-MMAE	Sulfhydryl chemical coupling	2-4	6	Mal-Gly-PAB-Exatecan-D-glucuronic acid	Sulfhydryl chemical coupling	4-8
2	MC-VC-PAB-Eribulin	Sulfhydryl chemical coupling	2-4	7	BiM-GLPY-Exatecan	Sulfhydryl sulfur bridge coupling	4-8
3	Mal-PEG2-VC-PAB-Eribulin	Sulfhydryl chemical coupling	2-4	8	BrAcAm-VA-A-1743332	Mercaptobromine coupling	4-8
4	MC-GGFG-DXd	Sulfhydryl chemical coupling	6-8	9	GSLP0001, TOP1i-based, highly hydrophilic	Sulfhydryl chemical coupling	6-8
5	Mal-PEG8-VA-PAB-Exatecan	Sulfhydryl chemical coupling	4-8	10	GSLP0002, TOP1i-based, highly hydrophilic	Sulfhydryl sulfur bridge coupling	4
				11	...	...	...

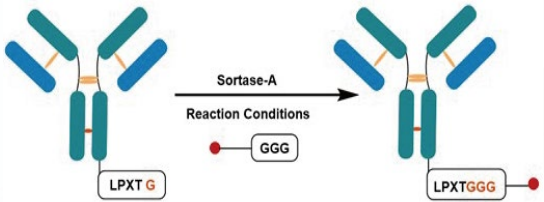
Sulfhydryl chemical coupling



Glucosidase induces coupling



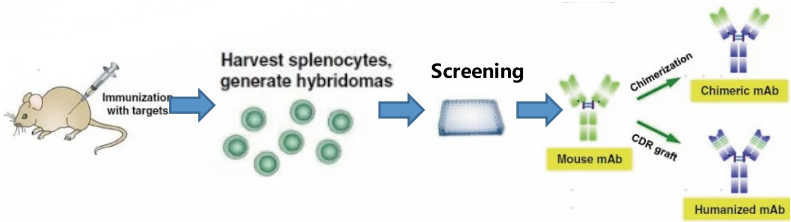
Transpeptidase promotes coupling



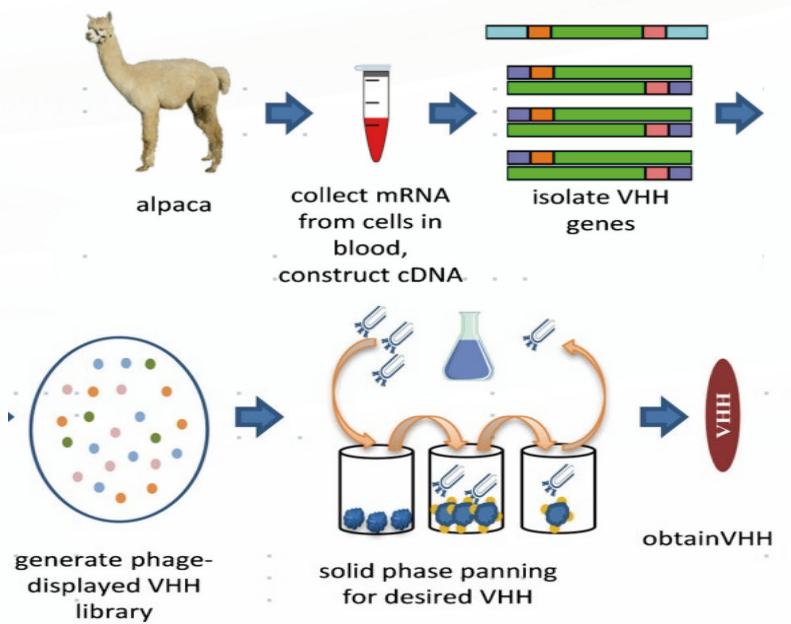
Antibody discovery and engineering platform

Antibody discovery and engineering modification strategy

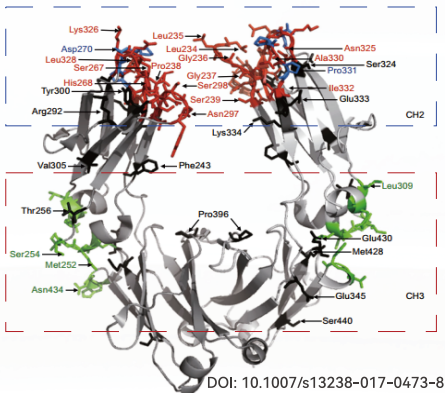
Hybridoma screening
Phage screening
Humanization
Affinity maturation
Fc Silence Modification
Enhanced ADCC modification
Enhanced ADCP modification
Enhanced CDC modification
Extended Half-life modification (Fc amino acid mutation)
Extended half-life modification (with Anti-HSA VHH)
Fc heteropairing modification (Fc mutation)
Conjugate site modification (new Cys mutation)
Conjugate site modification (Sortase recognition peptide)
N-glycosylation site removal
Hot spots removal
Hydrophobic cluster modification
Charge cluster modification
Aggregation property modification
High Viscosity Property Modification



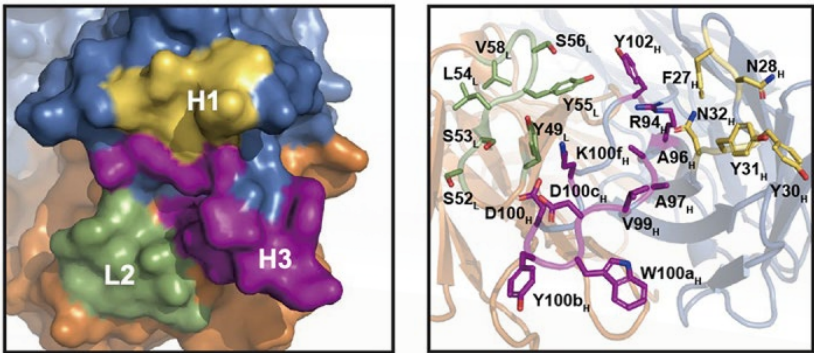
Hybridoma screening and humanization of mouse antibodies



Phage display screening and mutation library screening modification



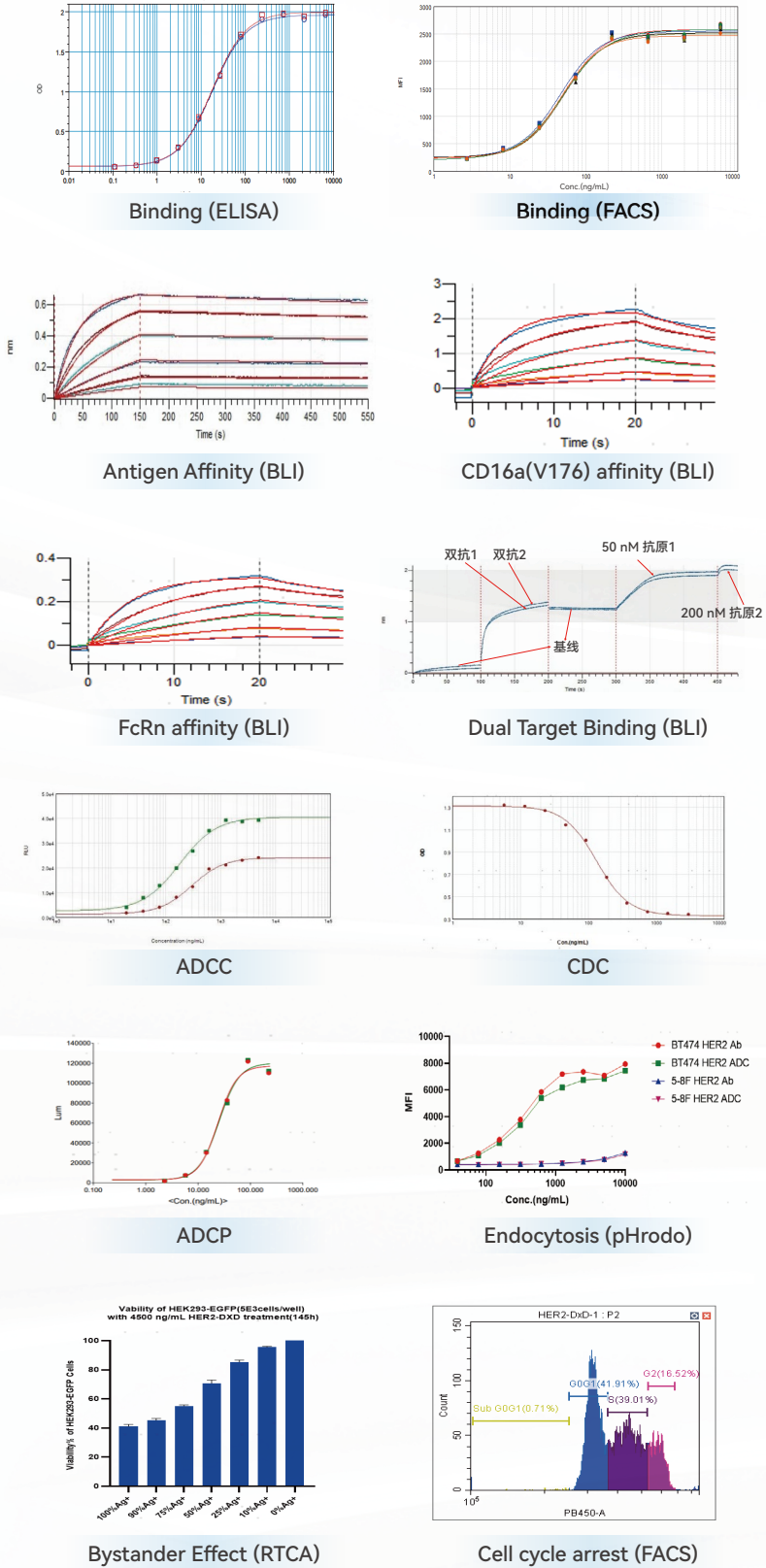
DOI: 10.1007/s13238-017-0473-8



FC Engineering

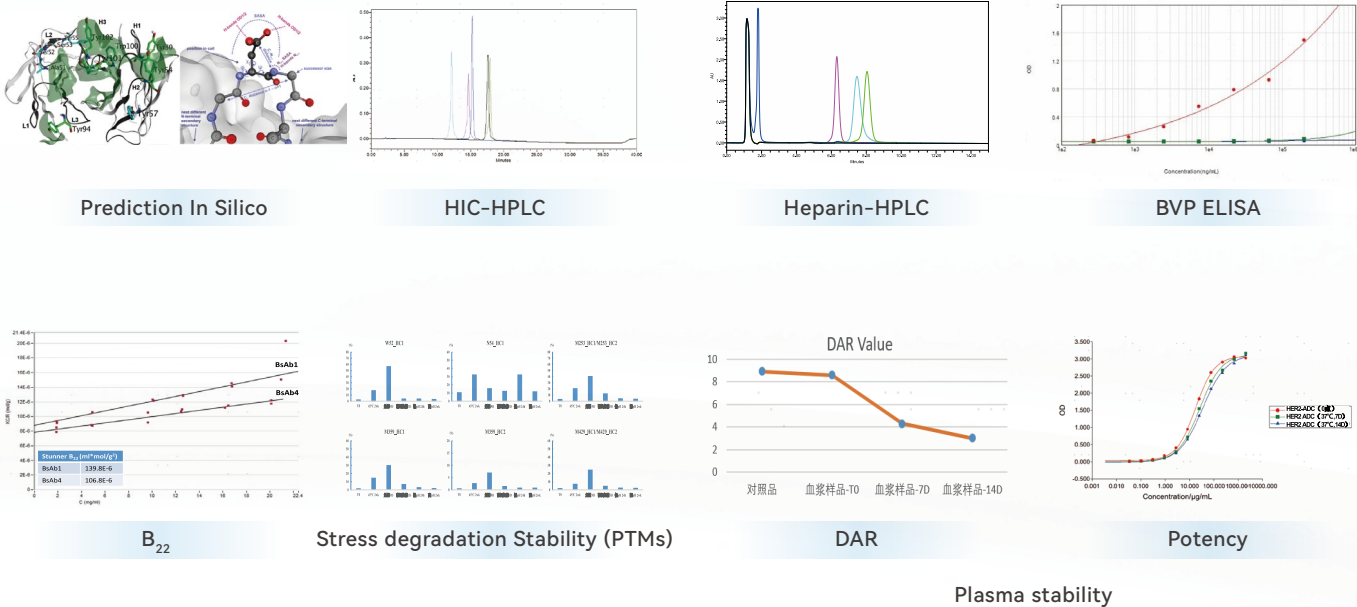
Pharmacological efficacy and pharmacokinetic evaluation platform

Pharmacological efficacy and pharmacokinetic evaluation	Analytical methods
Antigen-specific binding activity	BLI/ELISA
Antigen cross binding activity (human, mouse, monkey)	BLI/ELISA
Epitope analysis	BLI/HDX
Receptor occupancy rate	FACS
FcRn/FcγR/C1q binding activity	BLI/ELISA
Tumor cell surface antigen detection	FACS
Biological activity (proliferation inhibition)	Cell-based assay
Biological activity (cell killing)	Cell-based assay
Biological activity (reporter gene method)	Cell-based assay
Cytokine release	ELISA
Endocytosis	Cell-based assay
ADCC/CDC/ADCP	Cell-based assay
Bystander effect	Cell-based assay
Cell Cycle	Cell-based assay
Plasma stability (human, mouse, monkey)	ELISA & MS
CDX efficacy	Animal experiments
PDX efficacy	Animal experiments
mAb/BsAb blood concentration detection	ELISA
Exfoliating small molecule (Free drug) blood concentration detection	LC-MS/ MS
ADC blood concentration detection	ELISA
Total antibody blood concentration detection	ELISA



Druggability evaluation platform

In Silico predictive characteristics evaluation	Molecular physicochemical and structural properties	Mimicking in vivo properties	Preliminary stability properties
pI _{ProtParam} _Fv	Molecular size purity (SEC-HPLC)	Nonspecific binding (PSP)	High temperature stability (45 °C, T0, 1 wk, 2 wks)
pI _{ProtParam} _VH	Molecular Size Purity (rCE-SDS)	Nonspecific binding (BVP)	Light stability (1.2*10 ⁶ Lux.h, T0/D5/D10)
pI _{ProtParam} _VL	Molecular Size Purity (nrCE-SDS)	Nonspecific binding (CHO cell binding)	High oxidizer stability (0.02%BHP, T0/D3/D5)
pI _{ProtParam} _Complete protein	Isoelectric point (iCIEF)	Nonspecific binding (293 T cell binding)	Low pH stability (pH3.5, T0, 2h, 4h)
Total CDR length	Purity of charge (iCIEF)	PK related properties (Heparin column binding)	High pH stability (pH8.5, T0, D5, D10)
CDR variable sites	Solubility (PEG method)	PK related properties (FcRn affinity with BLI method)	Freeze-thaw stability (-80 °C/RT, 3, 5 reps)
Near CDR PSH score	Solubility (Ultrafiltration)	PK related properties (pH6.0/7.4 FcRn binding)	Shake stability (200 rpm, T0, D3, D5)
Near CDR PPC score	Viscosity	PK characteristics analysis (ELISA or MS)	
Near CDR PNC score	Colloidal stability (k _D)	Immunogenicity (PBMIC)	
SFvCSP score	Colloidal stability (B ₂₂)	Plasma stability analysis (ELISA&MS)	
Excess sulfhydryl	Thermal stability (T _m)		
Excess glycosylation sites	Thermal stability (T _{agg})		
GRAVY (Hydrophilic)	Particle size (DLS)		
CamSol_VL (Solubility)	Mass spectrometric Molecular weight (RP-MS)		
CamSol_VH (Solubility)	Mass spectrum peptide map (RP-MS/MS)		
	Self-interaction (AC-SINS)		
	Hydrophobicity (HIC-HPLC)		



Early research sample preparation & Cell line construction platform

Preparation of early-stage samples and cell lines

Transient Transfection

Protein A affinity purification

Low pH treatment conditions

Cation exchange chromatography

Hydrophobic chromatography

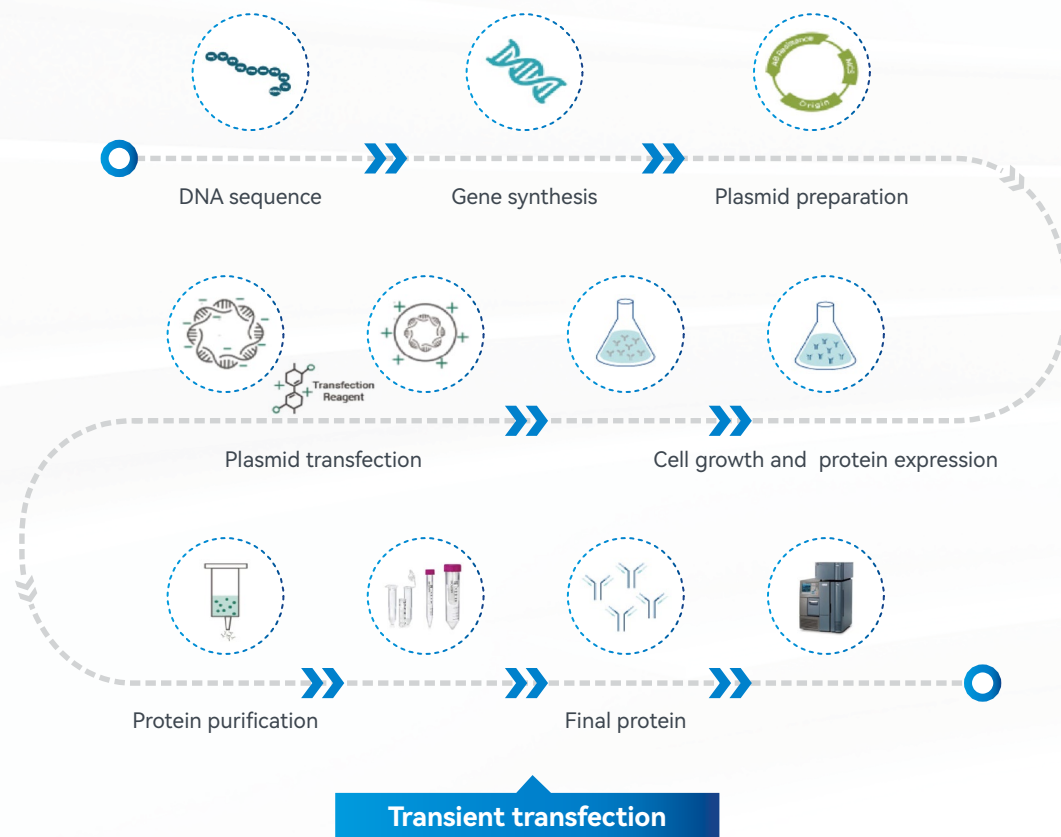
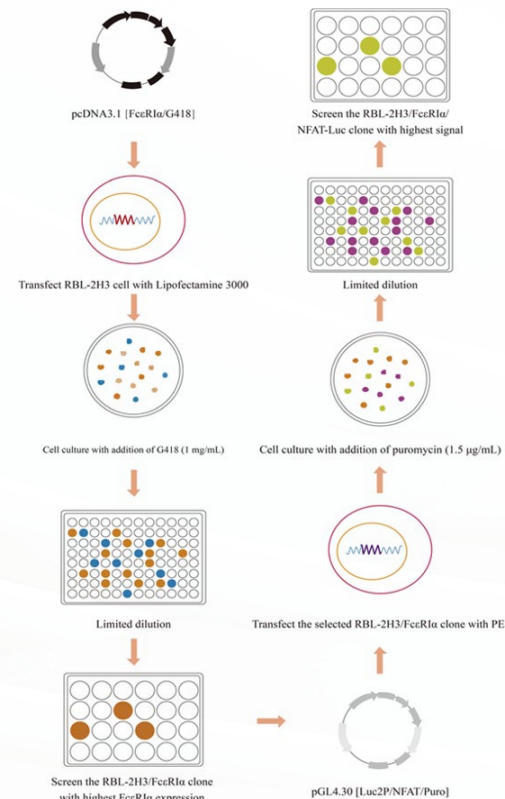
Formulation prescription substitution

Overexpressed cell lines construction

Reporter gene cell line construction

Enhanced ADCC protein expression
(Fut8 KO cell line)

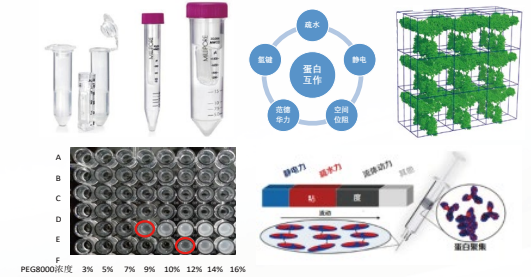
Cell line construction



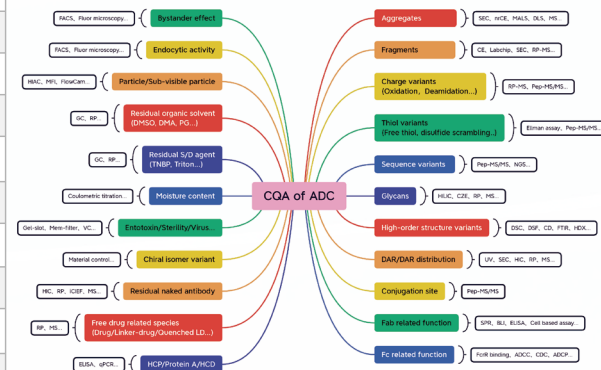
Structure characterization and novel formulation development platform

Structural characterization and development of novel formulation	Methods
Denaturing MS molecular weight analysis	RP-MS/ SEC-MS
Native MS molecular weight analysis	Native SEC-MS
Amino acid complete sequence analysis	RP-MS/MS
Post-translational modification analysis	RP-MS/MS
Extinction coefficient analysis	RP-HPLC-FLD
N-glycosylation/O-glycosylation site identification	RP-MS/MS (conventional or H ₂ O ¹⁸ method)
N-glycosylation/O-glycosylation composition analysis	RP-MS/MS
Sialic acid content analysis	RP-HPLC-FLD
CEX-MS analysis	CEX-MS
Sequence variant analysis	RP-MS/MS
Acid-base peak identification analysis	CEX-HPLC/ RP-MS, MS/MS
Polymeric fragment identification analysis	SEC-HPLC/ RP-MS, MS/MS
BsAb mismatch analysis	(iCIEF or HIC) /CE/ RP-MS
HCP qualitative analysis	RP-MS/MS (DDA)
Qualitative and quantitative analysis of high-risk HCP	RP-MS/MS (DDA&MRM)
HCP coverage rate analysis	2D SDS-PAGE/Western Blot
Analysis of Tween degradation products	RP-HPLC-CAD/QDA
Epitope analysis	Epitope competition, HDX-MS, AI prediction
Advanced structure analysis	HDX-MS
High concentration preformulation development	Buffer/protectant/viscosifier screening
PFS preformulation development	Buffer/protectant/package screening
Co-prescription preformulation development	Buffer/protectant etc screening

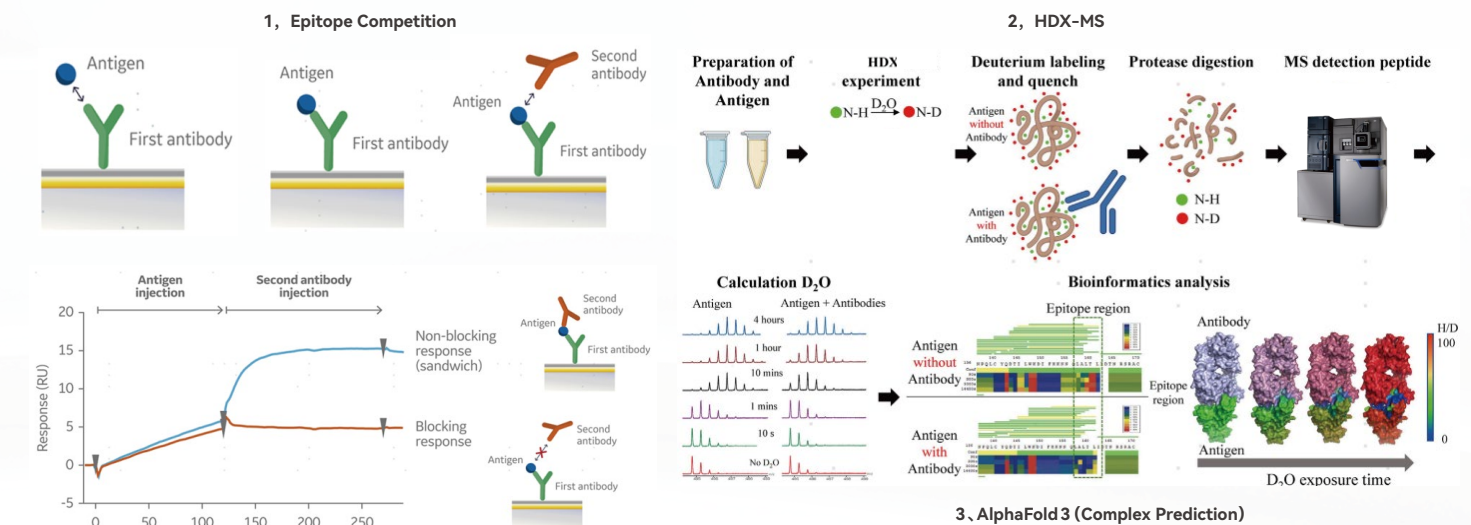
Evaluation and development of high concentration formulations



Comprehensive characterization study



Multidimensional epitope analysis



<https://alphafoldserver.com/welcome>