

# An Innovative AI-Based Platform for Antibody Stability Improvement and Affinity Optimization

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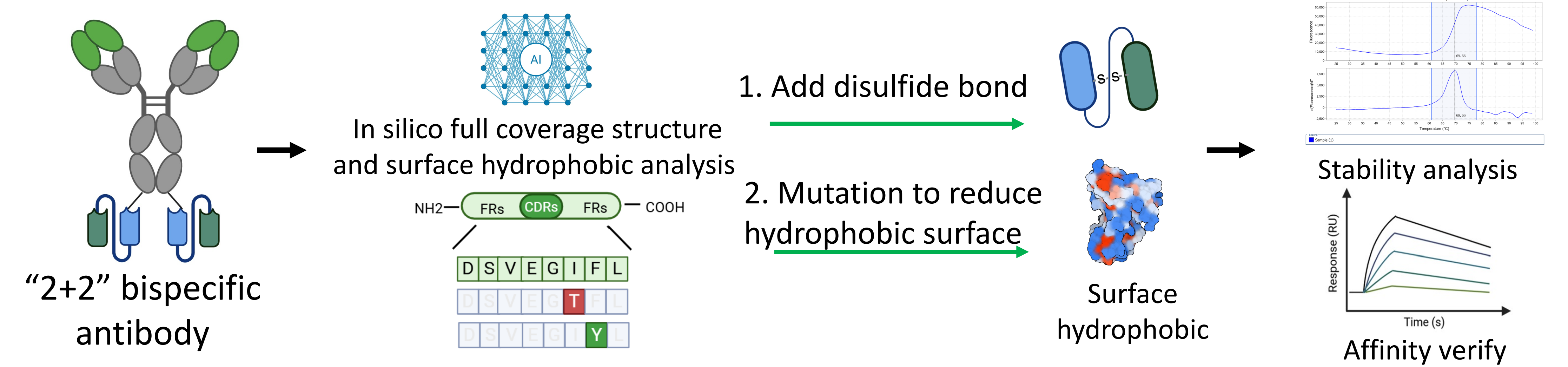
## Abstract

Bispecific antibodies (bsAbs), which target two distinct antigens, have enhanced specificity, efficacy, and reduced resistance, making them an increasingly mainstream approach in therapeutic antibody development. However, their complex formats impose stringent requirements on affinity and stability. Here, we present an AI-driven approach to predict affinity-enhancing mutations, significantly reducing screening efforts and accelerating antibody optimization.

**Methods:** a deep learning-based AI model for antibody engineering that simulates antigen-antibody docking and predicts affinity changes to guide targeted mutations. It improves stability by introducing disulfide bonds or CDR/framework mutations to modulate hydrophobicity while preserving affinity, and enhances function by identifying key CDR residues and generating combinatorial multi-site variants with improved activity.

**Results:** For a symmetric scFv bispecific antibody, we applied AI-driven design to generate 50 variants, followed by binding-based screening, expression, purification, and stability evaluation. This approach identified a mutant with aggregation reduced from 100% to <5% after one-week accelerated thermal stress and a Tm increase of ~10 °C. For nanobody optimization, three rounds of combinatorial design (260 variants, ~1000-fold fewer than traditional libraries) yielded a nine-site mutant with ~2× improved blocking activity.

## ScFv-Stability Improvement



Before

Treatment/conditions	Concentrating	Solubility	High-temperature accelerated stability (40°C)		Tm
	20mg/ml	20mg/ml	1 week	2 weeks	
Sample	20mg/ml	20mg/ml	1 week	2 weeks	
Parental	~4% aggregation	Stability	~100% aggregation	~100% aggregation	50.35°C

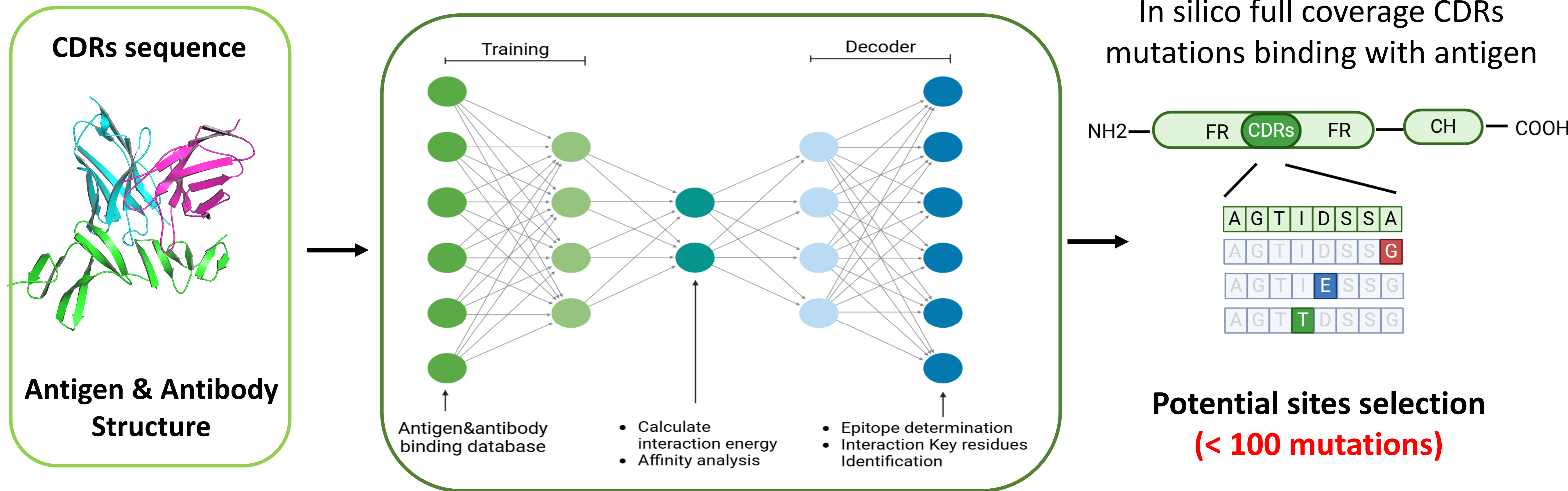
After

Treatment/conditions	Concentrating	Solubility	High-temperature accelerated stability (40°C)		Tm
	20mg/ml	20mg/ml	1 week	2 weeks	
Sample	20mg/ml	20mg/ml	1 week	2 weeks	
Mutant-1	~5% aggregation	Stability	~12% aggregation	~23% aggregation	59.28°C
Mutant-2	Stability	Stability	~7% aggregation	~13% aggregation	54.32°C

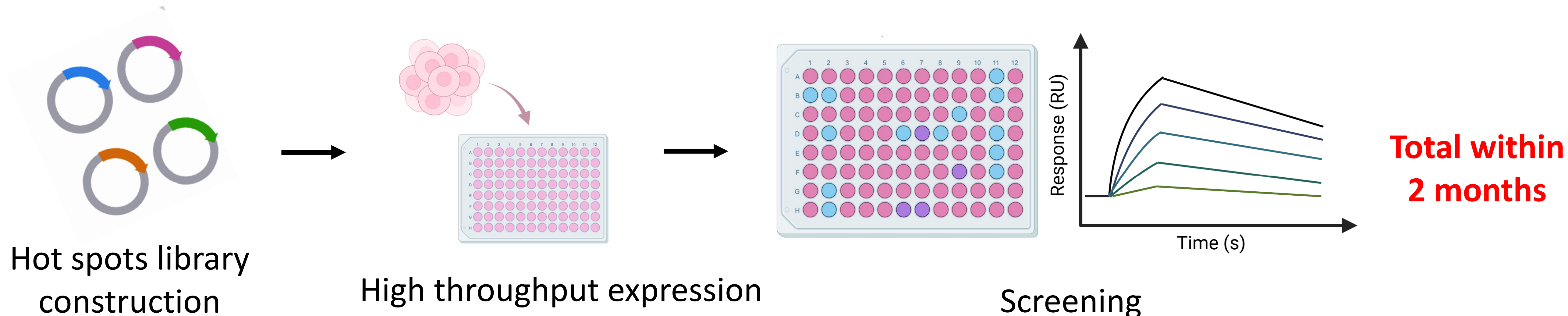
Buffer: 20mM citrate-sodium citrate, 150mM NaCl pH=5.5

## AI-powered Hotspot Identification

Deep learning model training

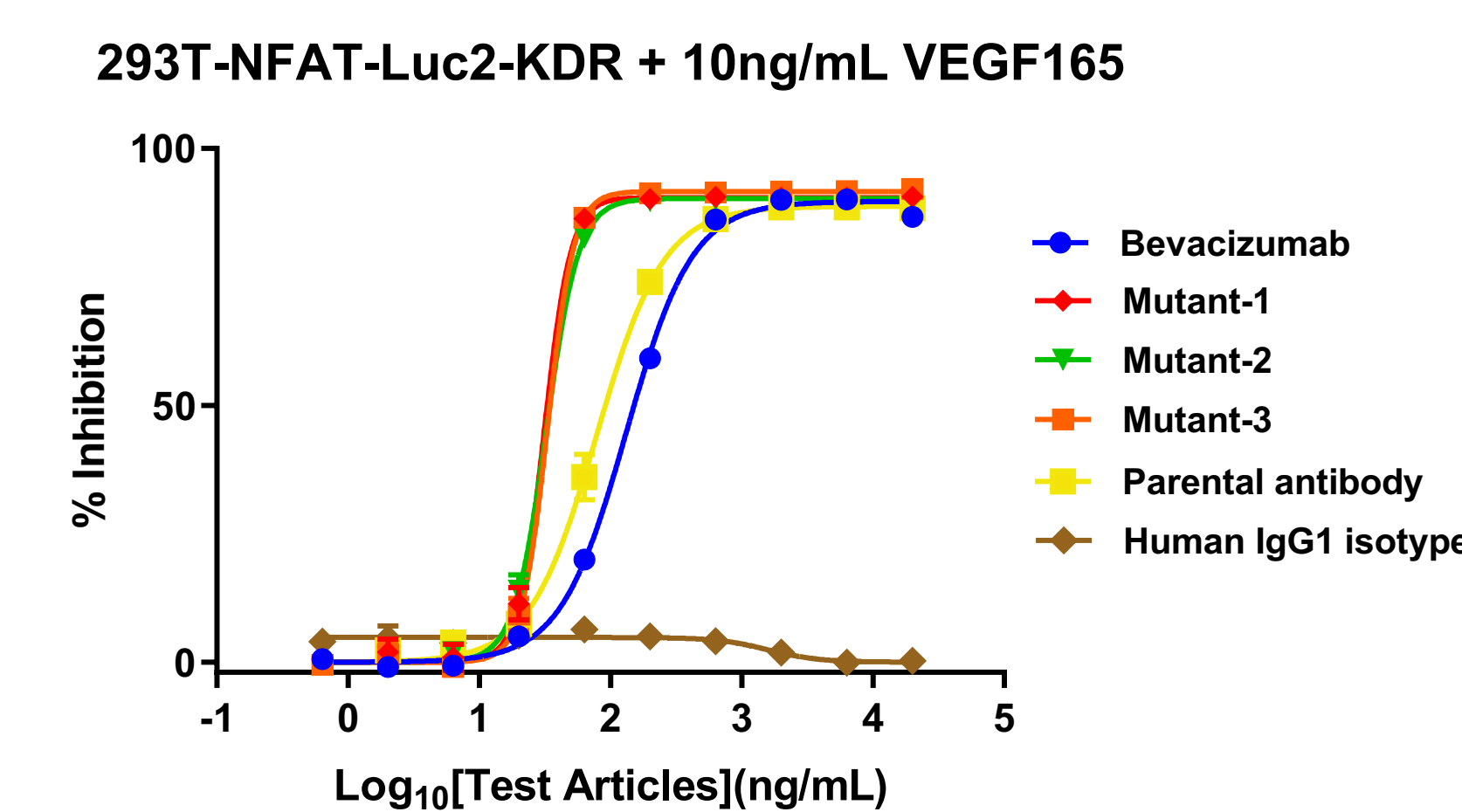


All mutations are performed at these sites and affinity with antigens is calculated by ELISA/FACS and KD.



## Antibody Optimization via Multisite Mutations

A VEGF-targeting nanobody was engineered by introducing single or multiple point mutations across all three CDRs, followed by combinatorial variant design. A total of 260 mutants were generated and screened using a reporter-based blocking assay, leading to the identification of three top candidates with two-fold enhanced blocking activity, each harboring 4–7 mutations within the CDRs.



Sample	Number of CDRs mutations	IC50(ng/mL)	Inhibition. max
Parental	/	78.8	88.72
Mutant-1	4	31.41	90.47
Mutant-2	5	33.18	91.65
Mutant-3	7	32.42	90.28

A TSLP-targeting nanobody underwent affinity maturation through the design of 53 multi-point mutants, from which two candidates were identified with over 20-fold improved affinity.

Sample	Number of CDRs mutations	KD (M)	kon(1/Ms)	kdis(1/s)	Full R <sup>2</sup>	Fold change
Parental	/	2.59E-10	1.15E+06	2.97E-04	0.9927	/
Mutant-1	6	9.23E-12	5.72E+05	5.28E-06	0.9968	28.06
Mutant-2	6	6.15E-12	7.24E+05	4.45E-06	0.9961	42.11