Case Study - SdAb Humanization by HuSdL™ Platform

Introduction

In the field of biotechnology, the humanization of antibodies has become a crucial aspect of drug development. One such case study involves the humanization of a single domain antibody (sdAb, V_HH) derived from a camelid species. These antibodies, known for their small size and high affinity, offer unique advantages in therapeutic applications.

Creative Biolabs is a leading service provider in the field of sdAb development and downstream engineering. Based on our extensive experience with sdAb humanization and the novel HuSdL™ platform, our scientists provide the best-fit solutions to generate novel humanized sdAbs for our customers all over the world.

Project Objective & Achievement

For this case study, the sequence of the single domain antibody was screened from a phage display library constructed by Creative Biolabs. Creative Biolabs is entrusted to perform humanization to engineer this single domain antibody to meet further therapeutic applications.

Based on the bioinformatics analysis results of the parental sdAb, computational modeling for the variable regions was performed, and the most appropriate human VH framework acceptor was searched. After the CDR grafting *in silico*, putative back mutations in the acceptor framework were designed, as a result, a series of sdAb variants were obtained.

The TOP 5 designed humanized sdAb variants and the parental sdAb were expressed and tested for their binding ability. All the TOP 5 variants were expressed successfully and showed similar binding ability against the target when compared to the parental sdAb.

The HuV_HHv1 was selected as the top hit, which has the highest humanization percentage. By using HuV_HHv1 as a typical example of humanized single domain antibody designs, we conducted the T-cell epitope, B-cell epitope, and MHC II epitope study. Then, the *in silico* post-translational modifications (PTMs) assessment and aggregation assessment of parental sdAb and HuV_HHv1 were performed.

Milestone Overview

Stage 1: Bioinformatics Analysis & Computational Modeling

The bioinformatics analysis and computational modeling for the sdAb were performed (Figure 1), and the most appropriate human VH framework acceptor for parental sdAb was identified. *In silico* CDR-grafting was conducted based on this human VH framework acceptor. Based on the IMGT® humanization percentage data, HuV_HHv1 is the top hit with the highest humanization percentage (Table 1).

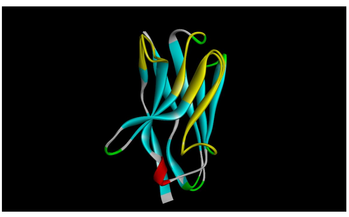

Figure 1. CDR determination and computational modeling

Table 1. Humanization percentage

Design	IMGT® Humanization Percentage
Parental sdAb	70%
HuV_HHv1	>80% Top design of humanized V_HH

Stage 2: Immunogenicity Assessment

The T-cell epitope, B-cell epitope, MHC II epitope, and antigenicity epitope prediction were conducted. The backmutations in all the epitopes were adjusted accordingly to avoid potential loss of affinity and/or developability.

Stage 3: Post-translational modification (PTM) Assessment

The *in silico* post-translational modifications (PTMs) assessment of parental sdAb and HuV_HHv1 were performed. Only typical PTM motifs with side chain has > 50% accessibility to the 3D surface (Average SAS) are ranked as “high risk” residues. The main PTMs in HuV_HHv1 are isomerization and deamidation. There are two high-risk isomerization sites in the CDR2 region and one deamidation site in the FR region.

Table 2. PTM analysis for both parental sdAb and humanized varients

Seq_Name	Type of PTM	Residues	Risk	Seq_Name	Type of PTM	Residues	Risk
Parental sdAb	Isomerization	X1	High risk	HuV_HHv1	Isomerization	X5	High risk
	Isomerization	X2	High risk		Isomerization	X6	High risk
	Deamidation	X3	Low risk		Deamidation	X7	High risk
	Deamidation	X4	High risk		Deamidation	X8	Low risk

Stage 4: Aggregation Assessment

The aggregation analysis of parental sdAb and HuV_HHv1 was predicted. The parental sdAb and HuV_HHv1 have similar aggregation regions in the CDR3. These sites with a high aggregation propensity score on antibody surface are prone to aggregation. The aggregation tendency of HuV_HHv1 is stronger than that of parental sdAb, which is caused by germline humanization.

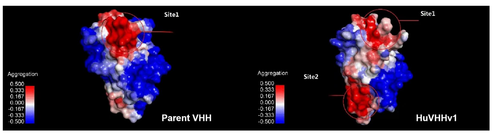

Figure 2. Aggregation analysis of parental sdAb and HuV_HHv1

The atom having a high protein aggregation score is displayed in red, and the atom having a low protein aggregation score is displayed in blue.

Stage 5: TOP 5 Humanized Variants Expression

The expression and purification for the TOP 5 humanized variants and parental sdAb were performed. No obvious aggregation and precipitation were found during the expression and purification process, indicating that the 5 variants were stable under present conditions. All 5 variants were of good quality and can be used for further affinity measurement (Figure 3).

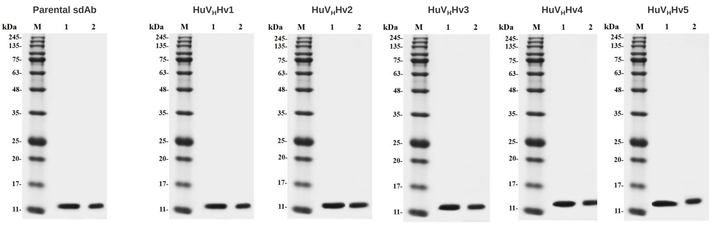

Figure 3. SDS-PAGE result of TOP 5 humanized V_HH candidates and parental sdAb

Lane 1: Reducing. Lane 2: No-Reducing

Stage 6: Affinity Measurement for TOP 5 Humanized Variants

The binding affinity between the target and sdAbs was detected by BLI system. The ligands (sdAbs) were immobilized onto the biosensor chip surface and different concentrations of the analyte were injected into the sensor surface for interaction with the sdAbs. As a results, all the TOP 5 humanized V_HH candidates showed similar binding ability against target protein when compared to the parental sdAb (Table 3).

Table 3. BLI detection of the binding affinity between the target and six sdAbs

Ligand	Analyte	KD (M)	Fit Method
Parental sdAb	Target	10^{-9}	1:1 binding
HuV_HHv1	Target	10^{-9}	1:1 binding
HuV_HHv2	Target	10^{-9}	1:1 binding
HuV_HHv3	Target	10^{-9}	1:1 binding
HuV_HHv4	Target	10^{-9}	1:1 binding
HuV_HHv5	Target	10^{-9}	1:1 binding

Conclusion & Key Words

- ✓ **HuSdL™ Platform** - Scientists at Creative Biolabs have over ten years experience of humanization specialty and are capable of designing the most suitable humanized V_HH candidates that almost eliminate the immunogenicity of an sdAb to humans while retaining its specificity and affinity.
- ✓ **One-Stop Solution** - Extensive experience and integrated procedure enable our scientists to shorten the overall lead time from discovery to integrated single domain antibody engineering.

Contact Us

USA

SUITE 203, 17 Ramsey Road, Shirley, NY 11967, USA

Tel: 1-631-416-1478

Fax: 1-631-207-8356

Email: info@creative-biolabs.com

UK

Tel: 44-207-097-1828