A Rational Affinity Maturation Platform (RAMP™)

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Fusion Antibodies have developed a **Rational Affinity Maturation Platform (RAMP™)** by creating a small library of variants of a given antibody sequence with a relatively high proportion of variants having increased affinity, contrasting existing technologies.

We have demonstrated the success of this platform with Cathepsin S antibody (Fsn0503h) and have generated a small library of 66 variants. After one round of RAMP™, 50% of the 66 variants have shown increased affinity and some have a 10-fold increase. The variants showed excellent expression yield, low aggregation and fragmentation in CHO cells. This is a significant improvement to existing technologies such as phage-display which may produce variants with poor developability profiles and require further engineering.

Methods

The RAMP™ Process

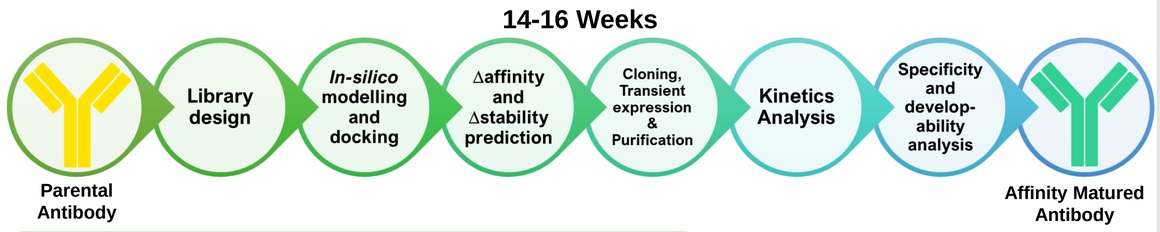

Figure 1. Schematic diagram of one round of the RAMP™ process.

Library Design

An antibody library was generated for Fsn0503h by mimicking the natural somatic hypermutation in humans. A computer algorithm was designed to identify possible naturally occurring mutations within an antibody DNA coding sequence and generated a library of variants, as shown in **Figure 2.**

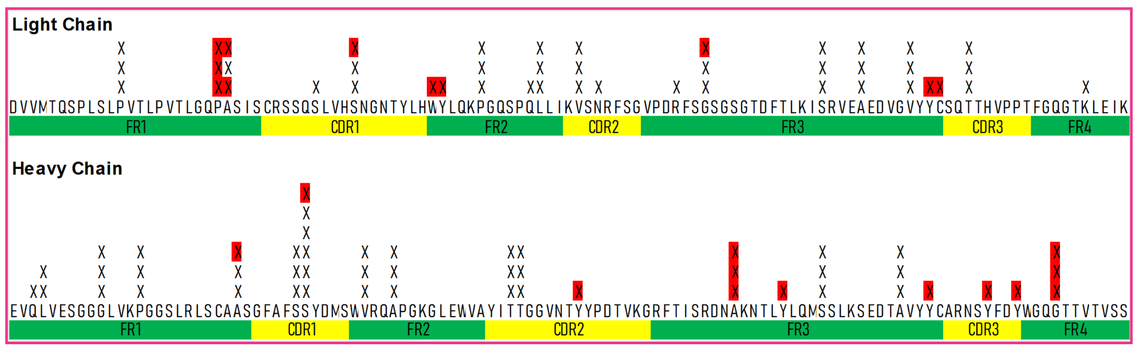

Figure 2. Amino acid sequences of the Fsn0503h antibody light chain and heavy chain showing potential mutations above each chain. Amino acids shown in red were not permitted in the library due to their uncommon usage at that position.

Combinational Mutation Analysis and Molecular Docking

Analysis of the effect of each mutation in an increasing combinatorial fashion was performed using molecular docking algorithm, as shown in **Figure 3**. Cathepsin S antigen (Cat S) was docked to the surface of the antibody model using the protein-protein docking tool. Residue scanning was performed on the docked pose and 66 variants that had a predicted improvement in both affinity and stability, as shown in **Table 1**.

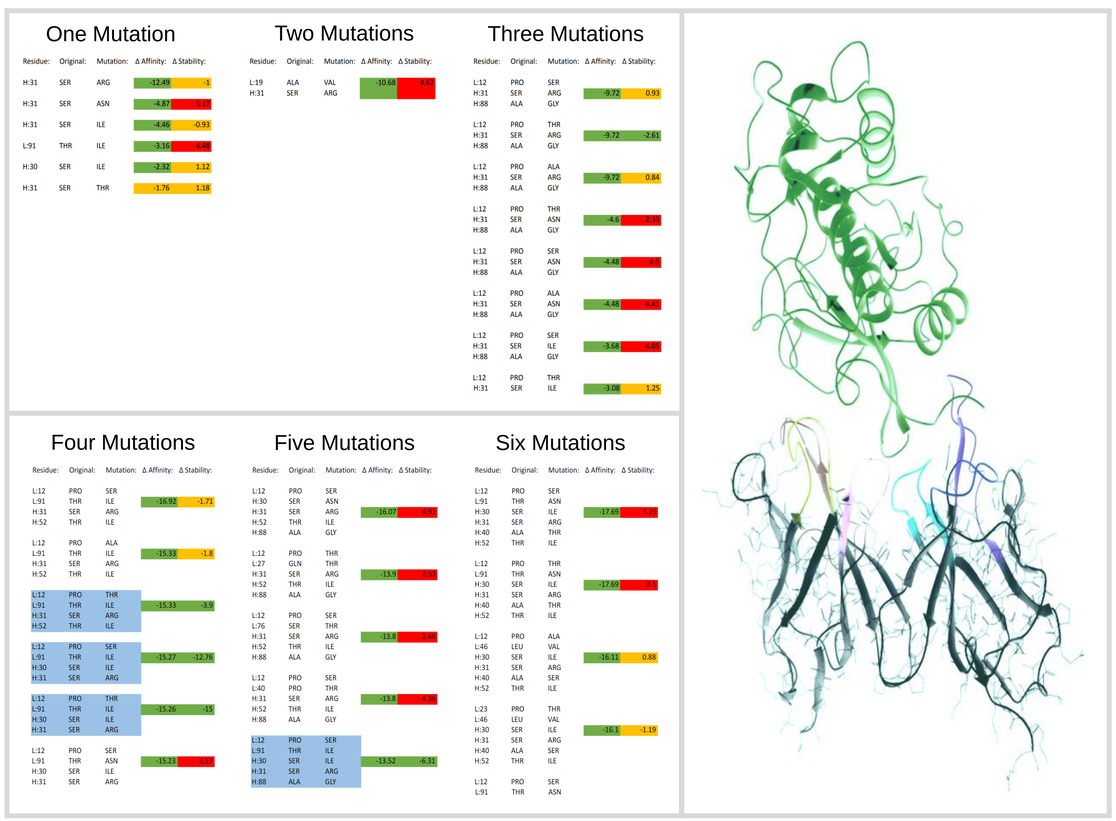

Table 1.
Affinity and stability predictions of amino acid mutations in a combinatorial fashion for 1 to 6 mutations. Groups of mutations highlighted in blue represent variants with both improved stability and affinity where the change is greater than -2.

Figure 3.
Virtual molecular docking: predicted protein-protein interaction between a Fsn0503h variant and Cat S.

Antibody Synthesis

Transient transfection and purification: For each of the Fsn0503h variants, 25ml of ExpiCHO cells were transfected with 1µg/ml of plasmid DNA. The supernatant was purified using automated two-Step purification method. Following basic purity and quantity assessment, analytical SEC was performed under standard conditions with appropriate controls. Protein peaks was monitored at 214 nm and spectra analysed.

Antibody Ranking

BLI: Affinity ranking assays were performed by using anti-human biosensors using Sartorius Octet system and dissociation rate constants (koff) were calculated using Data Analysis software.

Results

BLI Binding Analysis

Approximately 50% of the variants have improved affinity when measured against the average reading for the Fsn0503h antibody (**Figure 4**).

The lead candidate improvement in affinity is about 10-fold (K_D from 4.45nM to 0.4nM).

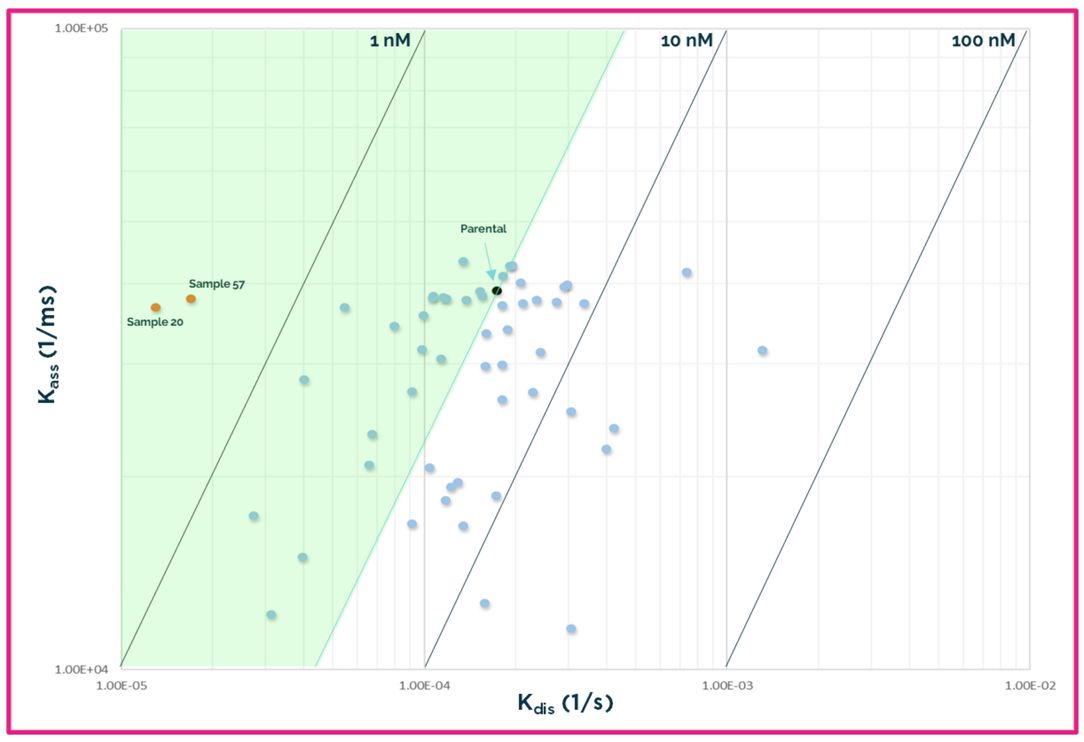

Figure 4. Affinity on-off rate map of the variants of Fsn0503h. The parental antibody is shown as the black dot. The green line represents equal affinity of 4.45nM to the parental antibody, and any variants with an increased affinity (<4.45nM) are within the shaded green area.

Expression Yield and Analytical SEC Analysis

Transient expression in CHO cells showed that all the variants expressed, with the average expression yield of 214.8mg (**Figure 5**). Analytical SEC analysis showed that most variants have >90% monodispersity (**Figure 6**). Six variants including variant 57 (affinity improvement ~10-folds) demonstrated further improvement of monodispersity from the parental antibody, from a very high starting point. The robust expression yield and high monodispersity (low aggregation and fragmentation) show that the variants are highly developable and amenable for downstream manufacturing.

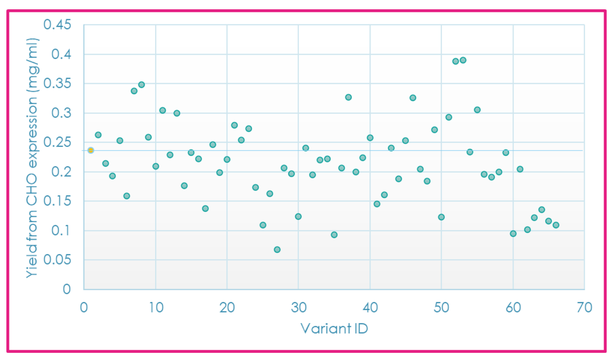

Figure 5. Expression yield plot of the 66 variants. The expression level of the parental antibody is indicated by the yellow dot and the horizontal line.

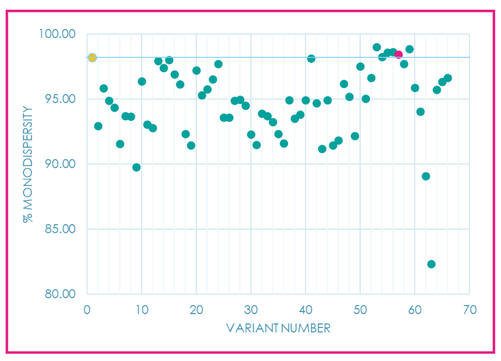

Figure 6. Monodispersity% by aSEC of the 66 variants. Yellow dot and horizontal line: the parental antibody; pink dot: variant 57.

Conclusion and Discussion

- Using a process of rational library design, combinatorial mutations, prediction ranking for affinity and stability, and synthesis of a small library of variants, we have successfully demonstrated that **the affinity of an antibody for its target can be improved without the need to generate a very large physical library of antibody variants and subsequent selection/screening process.**
- Given the high starting point of the parental antibody (K_D=4.45nM, monodispersity of 98%), the RAMP™ process can still achieve 10-folds increase in affinity and improvements in developability. **Affinity improvement of >50 folds can be achievable if the parental antibody has a lower binding affinity (e.g. K_D 50nM - 1µM).**

Application

- **Binding affinity improvement:** Improvement on the potency of the lead molecule by enhancing its binding to the target.
- **Developability improvement:** Generation of backup molecules / biobetters with better developability profiles.
- **IP protection:** There are finite structures that can bind to the target and exert biological functions. By identifying diverse binding sequences for patent protection, it increases the difficulty for competition to develop antibodies against similar epitopes.

Acknowledgements Thank you to the entire Fusion Antibodies team who have been a part of the development of the RAMP™ rational affinity maturation platform to date. Thank you to the Fusion Antibodies Scientific Advisory Panel for their advice.

Learn More For more information, please contact **Richard Ma (richard.ma@fusionantibodies.com)** or visit **Fusion Antibodies' website (https://fusionantibodies.com/antibody-engineering/affinity-maturation/)**