

Engineering Humanisation of Antibodies with Good Developability Profiles

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Summary

Protein stability and aggregation are major, and still largely unsolved, issues affecting the development and production of biopharmaceuticals. Besides their impact on development costs, the safety of biopharmaceuticals is also affected. Lonza's aggregation prediction platform (AggreSolve™) can be applied to overcome protein stability and aggregation issues, improving the manufacturability of polypeptide drugs.

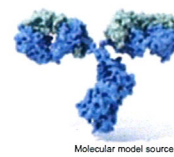
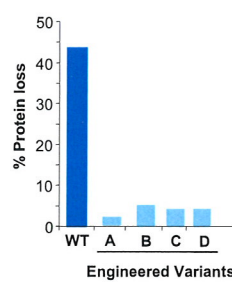
Antibody humanisation can potentially introduce structural alterations in the antibody molecule that frequently result in a reduction of antigen binding activity and can also affect its stability. Additional affinity maturation steps, frequently used to reach the levels of affinity required for therapy, may introduce uncontrolled modifications, with unforeseeable effects on antibody aggregation and stability. The incorporation of developability assessment tools during the engineering process can assist in selecting humanised antibodies with optimal properties.

We have previously shown that AggreSolve™ can be successfully applied to reduce aggregation (PEPTALK, San Diego, 2009), enhance thermal stability and improve productivity of antibodies (PEGS, Boston 2009). Here we show how AggreSolve™ has been used to engineer two humanised antibodies with good stability and productivity properties. Importantly, activity is shown to be retained at therapeutic levels without the need for additional affinity maturation steps.

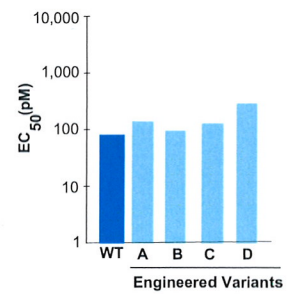
The AggreSolve™ Platform

The AggreSolve platform has been previously shown to reduce aggregation and enhance thermal stability whilst retaining activity. Protein loss, a measure of aggregation, has been used to show thermal stability of samples after incubation at 60°C, 2h. Activity of variants are shown to be comparable to that of wild-type, indicating retention of binding affinity.

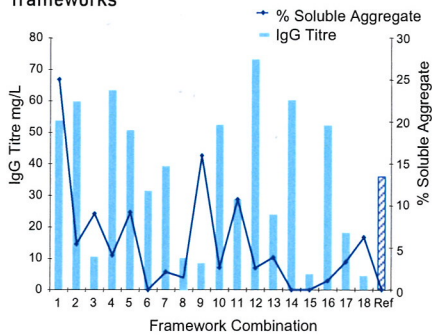
Protein Loss due to Aggregation: heated samples



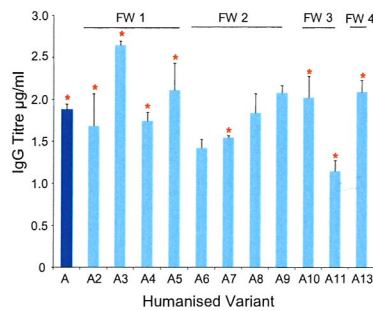
Activity of native samples



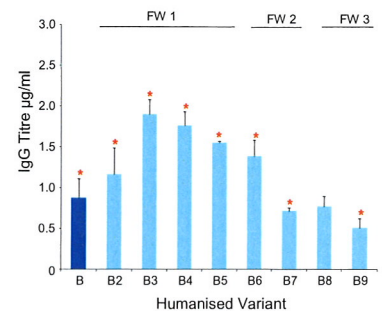
A. Assessment of optimal frameworks



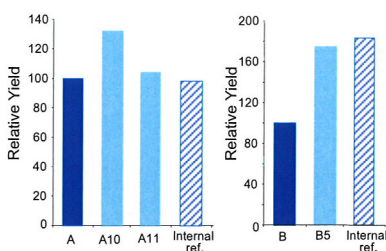
B. Antibody A – Selection of optimal humanised variants



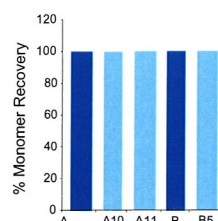
C. Antibody B – Selection of optimal humanised variants



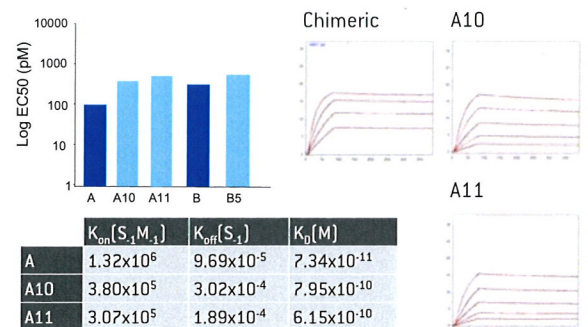
D. Increased Productivity



E. Humanised variants are stable at high temperature



F. Humanised variants retain activity



	$K_{on}(S_1, M_1)$	$K_{off}(S_1)$	$K_D[M]$
A	1.32×10^6	9.69×10^{-5}	7.34×10^{-11}
A10	3.80×10^5	3.02×10^{-4}	7.95×10^{-10}
A11	3.07×10^5	1.89×10^{-4}	6.15×10^{-10}

Panel A shows IgG titre and soluble aggregate levels for a selection of frameworks studied, in order to identify the best framework combination for humanisation. Panels B and C show IgG titre and activity for humanised variants of two antibodies.

* indicate active variants. Bars across the top highlight different frameworks used. It should be noted these numbers do not correlate with the framework combinations in panel A. Panels D-F show variants selected based on titre and activity and expressed at larger scale. Panel D shows that humanised variants have increased productivity. Panel E shows that variants are stable after incubation at 60°C, 2h, with 100% monomer recovery (0% soluble aggregate). Panel F shows that activity of humanised variants is comparable to that of parental chimerics (pM), indicating binding affinity has been retained. In all panels, parental chimerics are shown in dark blue.

The results show how the AggreSolve™ platform has been expanded and successfully applied to engineer humanised antibodies with good developability profiles; such antibodies have good stability, productivity and retain activity at therapeutic levels, eliminating the need for further, potentially de-stabilising, affinity maturation.