Screen, Select, Enhance: An Integrated Workflow for ADCC-Enhanced Antibody Development and Validation

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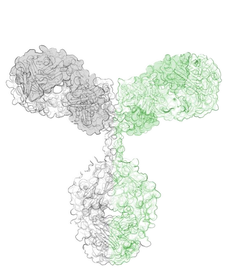
From high-throughput screening to validated ADCC-enhanced lead candidates

1 Introduction

This study focuses on evitria's high-throughput (HTP) antibody expression pipeline from candidate selection and scale-up of ADCC-enhanced variants to mechanistic characterization by our partner Biofidus. Leveraging robust, standardized processes with high flexibility, evitria offers expertise in bispecific development, high-throughput expression, and Fc-engineered antibodies. We also in-licence technologies such as ProBioGen's GlymaxX™ system, which afucosylates the antibody Fc to enhance ADCC effector function.

Biofidus AG, a leading (bio)analytical laboratory, provides comprehensive bioanalytical services as a dynamic one-stop partner for biologics. As a member of the Midas Pharma Group, we are embedded in a global network, expanding our capabilities and international reach to deliver even greater value to our partners. At Biofidus, we use our Biacore 1K surface plasmon resonance (SPR) platform to deliver fast, label-free binding data for an in-depth characterization of biologics.

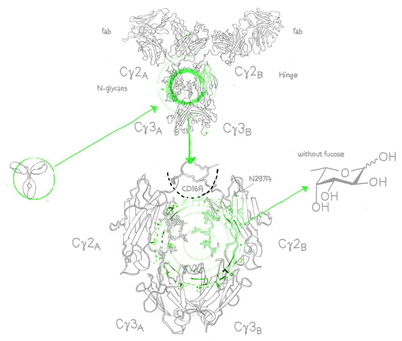


2 GlymaxX™ technology

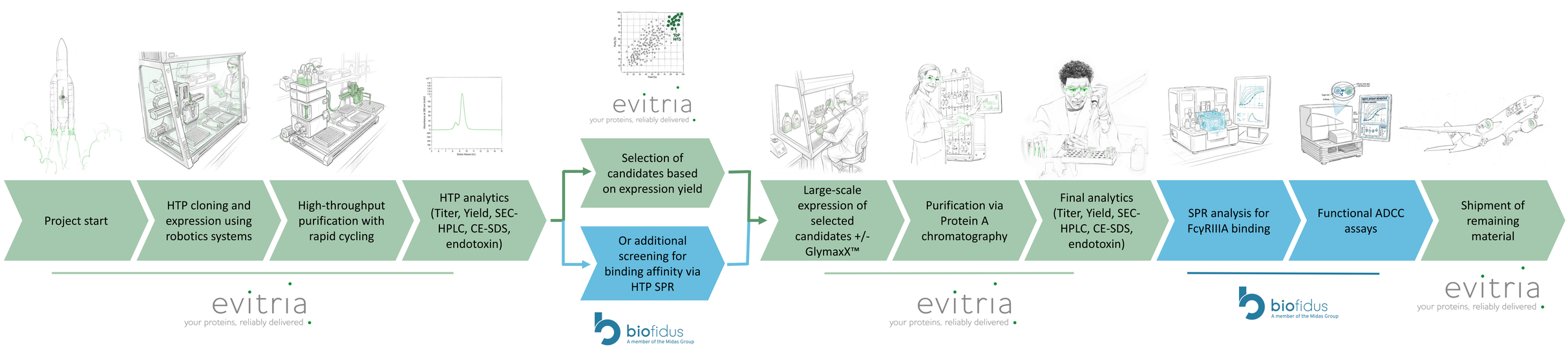
GlymaxX™ redirects fucose synthesis using a heterologous enzyme that depletes the intracellular fucose pool. This enables production of both fucosylated and afucosylated antibodies from the same cell line, with no amino acid sequence change.

Afucosylated antibodies show up to 40-fold increased affinity to FcγRIIIA/B, resulting in up to 100x higher ADCC activity. A unique glycan at Asn162 in FcγRIIIA/B (not present in other FcγR subtypes) collides with the core fucose on IgG-Fc.

Afucosylation therefore specifically boosts ADCC via FcγRIIIA-expressing NK cells, without altering other receptor interactions.



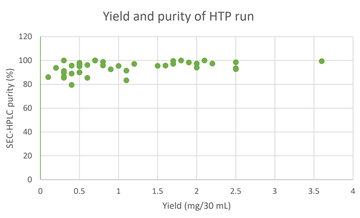
3 End-to-end workflow from HTP protein expression and screening to functional data with evitria and Biofidus



4 Selection of top candidates and expression +/- GlymaxX™

After initial HTP expression of a 48-constructs panel of different binder variants (Figure A), the best expressing and most stable construct was selected and up-scaled to 250 mL expression (+/- GlymaxX™, +/- LALAPG) followed by protein A purification (Table B)

A

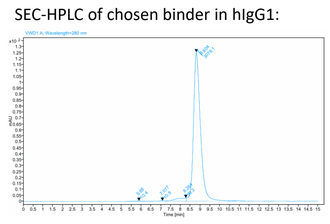


B

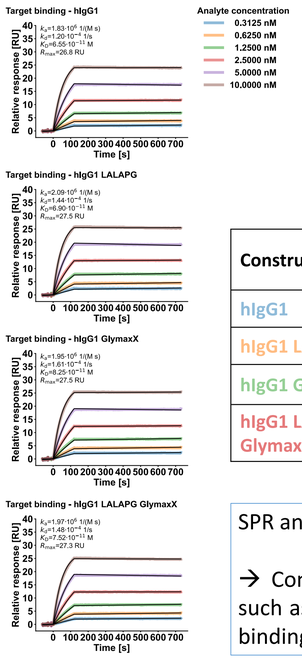
QC data for scale-up expression after Protein A			
Backbone	**Yield (mg /250 mL)**	**HPLC purity (%)**	**CE-SDS purity (%)**
hIgG1	135,5	99,2	98,3
hIgG1 LALAPG	104,4	97,5	92,4
afucosylated hIgG1	118,2	92,7	96,2
afucosylated hIgG1 LALAPG	98,4	94,6	97,5

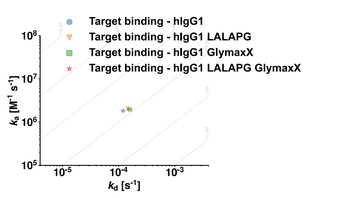
→ High expression levels achieved across constructs without need for polishing
→ Final candidate rapidly identified for downstream characterization

SEC-HPLC of chosen binder in hIgG1:



5 Validating target binding of final lead candidates via SPR



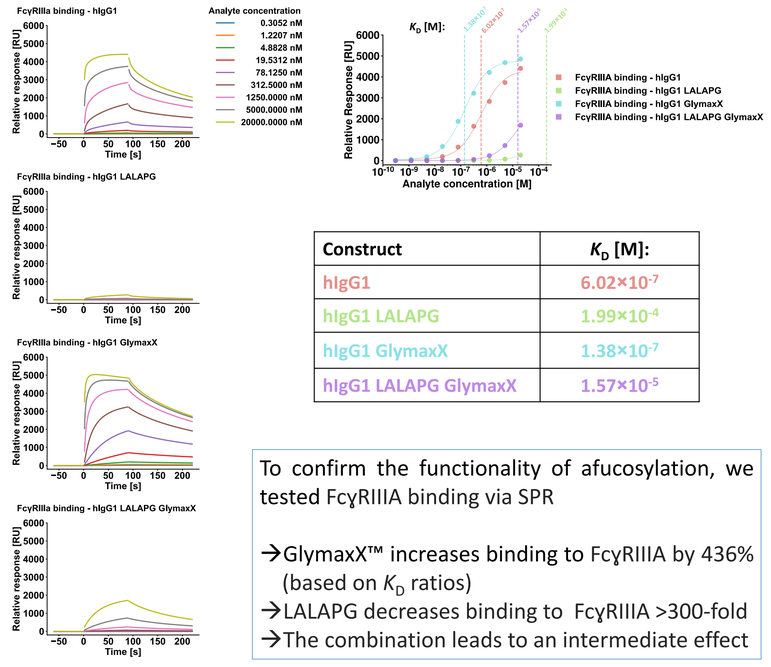


Construct	k_a [M^{-1} s^{-1}]:	k_d [s^{-1}]:	K_D [M]:
hIgG1	1.83×10^{6}	1.20×10^{-4}	6.55×10^{-11}
hIgG1 LALAPG	2.09×10^{6}	1.44×10^{-4}	6.90×10^{-11}
hIgG1 GlymaxX	1.95×10^{6}	1.61×10^{-4}	8.25×10^{-11}
hIgG1 LALAPG GlymaxX	1.97×10^{6}	1.48×10^{-4}	7.52×10^{-11}

SPR analysis against the target was performed

→ Confirmation that backbone modifications, such as LALAPG or afucosylation, did not affect binding to the target

6 GlymaxX™ leads to increased binding to FcγRIIIA-176V

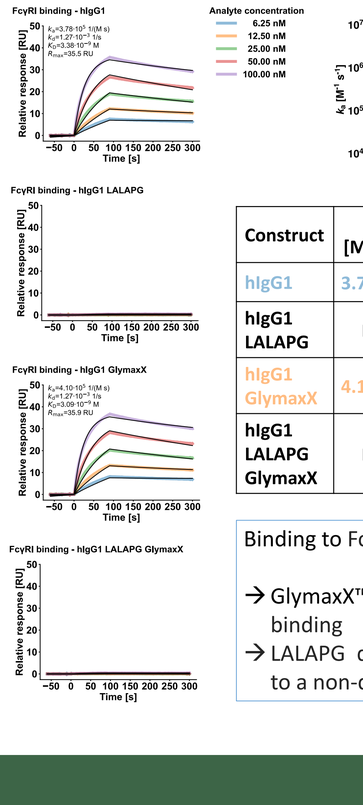


Construct	K_D [M]:
hIgG1	6.02×10^{-7}
hIgG1 LALAPG	1.99×10^{-4}
hIgG1 GlymaxX	1.38×10^{-7}
hIgG1 LALAPG GlymaxX	1.57×10^{-5}

To confirm the functionality of afucosylation, we tested FcγRIIIA binding via SPR

→GlymaxX™ increases binding to FcγRIIIA by 436% (based on K_D ratios)
→LALAPG decreases binding to FcγRIIIA >300-fold
→The combination leads to an intermediate effect

7 GlymaxX™ does not affect binding to FcγRI

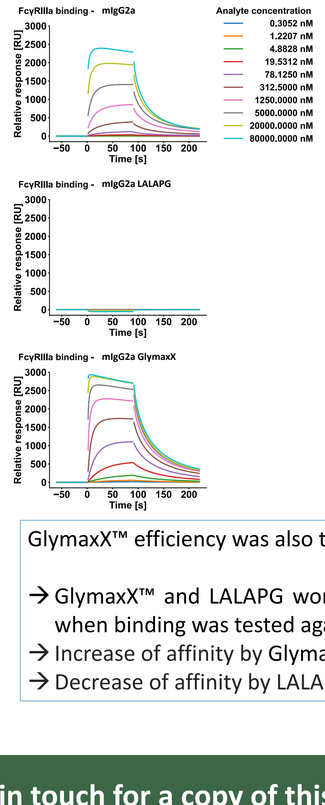


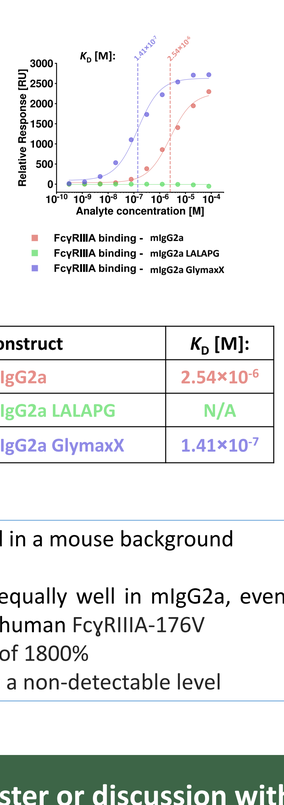
Construct	k_a [M^{-1} s^{-1}]:	k_d [s^{-1}]:	K_D [M]:
hIgG1	3.78×10^{5}	1.27×10^{-3}	3.38×10^{-9}
hIgG1 LALAPG	N/A	N/A	N/A
hIgG1 GlymaxX	4.10×10^{5}	1.27×10^{-3}	3.09×10^{-9}
hIgG1 LALAPG GlymaxX	N/A	N/A	N/A

Binding to FcγRI was tested via SPR

→ GlymaxX™ did not affect FcγRI binding
→ LALAPG decreased FcγRI binding to a non-detectable level

8 GlymaxX™ effect can be recapitulated with mouse IgG2a





Construct	K_D [M]:
mIgG2a	2.54×10^{-6}
mIgG2a LALAPG	N/A
mIgG2a GlymaxX	1.41×10^{-7}

GlymaxX™ efficiency was also tested in a mouse background

→ GlymaxX™ and LALAPG worked equally well in mIgG2a, even when binding was tested against human FcγRIIIA-176V
→ Increase of affinity by GlymaxX™ of 1800%
→ Decrease of affinity by LALAPG to a non-detectable level

9 Conclusions

- ✓ evitria's integrated HTP-to-lead pipeline compresses ADCC antibody development timelines without sacrificing analytical rigor by screening constructs directly in CHO
- ✓ GlymaxX™-mediated afucosylation selectively increases FcγRIIIA binding (4.4-fold K_D decrease) without affecting FcγRI binding or target engagement
- ✓ LALAPG and GlymaxX are orthogonal, stackable Fc engineering tools: their opposing effects on FcγRIIIA binding allow fine-tuned effector function across a wide dynamic range
- ✓ Comparable results in a mouse background confirm cross-species applicability of GlymaxX™ , de-risking translational studies
- ✓ evitria–Biofidus partnership closes the loop from sequence to functional ADCC data under a single coordinated workflow

10 Material & Methods

Protein expression and purification
cDNA was cloned into evitria's vector system. Suspension-adapted CHO K1 cells were transfected using eviFect in serum-free media. Supernatants were harvested by centrifugation, and antibodies were purified using MabSelect™ SuRe™ Prot A columns.

SPR spectroscopy
Samples were analyzed on a Biacore 1K system (Cytiva). His-tagged human FcγRIIIa-176V, target antigen, or FcγRI were captured on a CM5 chip via immobilized anti-histidine antibody. Double-referenced sensorgrams were evaluated using Biacore Insight Evaluation Software with a 1:1 binding model for kinetic constants and steady-state analysis for equilibrium dissociation constants (K_D).

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