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THE ANTIBODY COMPANY

Antibody Optimization by Mammalian Display

Optimization of enzyme inhibitor to generate antibody with best-in-class profile including exquisite specificity, potency and developability properties

Dr. Marc Van Dijk, Ph.D., CSO Discovery and Engineering

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CASE STUDY

ANTIBODY OPTIMIZATION · MAMMALIAN DISPLAY

Multiparametric optimization of an enzyme-inhibitor antibody for subcutaneous delivery

>1,000-fold	< 15 pM	> 10^6-fold	< 12 months
AFFINITY IMPROVEMENT OVER PARENT	LEAD K_D AT PH 7.4 BY SPR	SPECIFICITY VS. PROENZYME	TO IND FROM PROJECT INITIATION

BACKGROUND

Engineering an enzyme inhibitor away from binding to a near-identical, highly abundant pro-form

For an antibody whose mechanism of action is inhibition of an active enzyme, the dominant pharmacology problem is binding to the pro-enzyme. The pro-form circulates at far higher concentration than the mature target and shares a near-identical interface – any meaningful binding to it acts as an antibody sink that erodes free-drug exposure, compromises pharmacokinetics, and, in a subcutaneous dosing regimen, makes it impossible to reach a therapeutic dose within an acceptable injection volume. Affinity must improve, but it must improve without binding the pro-form. In parallel, the molecule must be a developable candidate for subcutaneous administration: low aggregation, low viscosity, high solubility, cynomolgus cross-reactivity for toxicology, and pH-dependent target release, facilitating target lysosomal degradation and avoiding antibody-mediated antigen accumulation. Conventional sequential optimization – affinity first, specificity second, developability last – trades gains in each axis against the next.

STRATEGY

HCDR3/LCDR3 targeted mutagenesis with multi-parametric Mammalian Display selection

A double-digit nM parental lead clone, with confirmed enzyme-inhibitory function and residual pro-enzyme binding, was chosen to build a bespoke optimization library targeting HCDR3 and LCDR3 – the CDRs most likely to drive both affinity and pro-form discrimination. A >10^9-clone phage display library was constructed and enriched for binder populations against the active enzyme. Output was transitioned into Mammalian Display library, where >10^6 IgGs were screened in their final full therapeutic format by FACS – gating sequentially for superior binding to the target while deselecting for binding to the pro-form, and for developability by Display level. Display level on the mammalian cell surface tracks closely with downstream CMC behaviour, so the developability axis is read out at the selection stage, in parallel with all other targeted properties targeted rather than retrospectively at the end. IgG characterization included binding assays, SPR kinetics, ex vivo enzyme-inhibition assays, and a full developability panel (Figure 1).

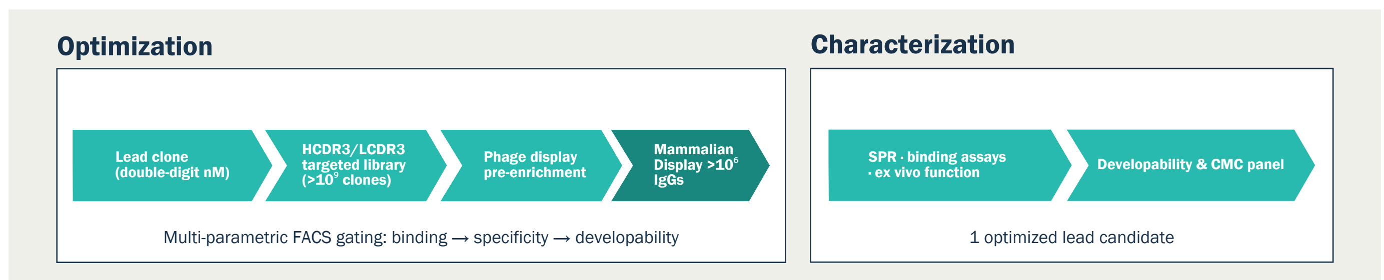

Figure 1. Showing the different processes to select the lead candidate.

RESULTS

Mammalian Display Enriches For Pro-Enzyme-Discriminating Clones

Analytical flow cytometry of the Mammalian Display library in IgG format, discriminated the optimized population from the parent: augmented binding to the target, simultaneously reduced binding to the pro-enzyme, and better expression display levels. FACS gating identified IgGs combining superior active-enzyme binding with suppressed pro-enzyme engagement (Figure 2, left). Monoclonal IgG screening confirmed a discrete population of optimized clones with markedly elevated target binding and pro-enzyme binding reduced to background; the parent IgG fell outside this cluster (Figure 2, right). The advantage of the platform is that the selection for different multiple critical features, including developability, occurs in a single step.

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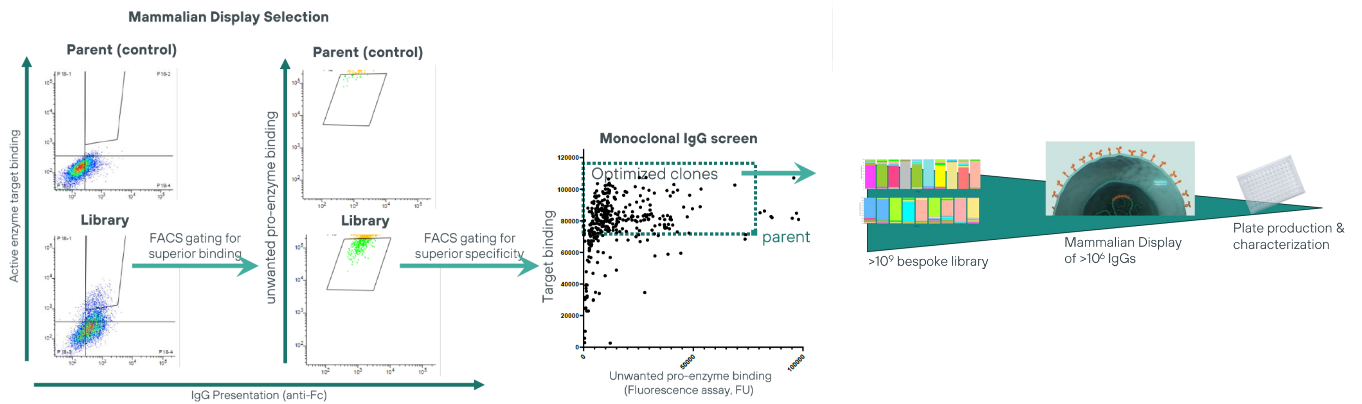

Figure 2. Mammalian Display selection. Left dot plots: Analytical FACS of the library vs. parent control. Top dot plots active-enzyme target binding against IgG presentation (anti-Fc); left dot plots represents pro-enzyme binding against IgG presentation. Library populations are clearly separated into a high target / low-pro-enzyme sub-population, which is captured by the FACS gates (dark blue boxes). Right: Monoclonal IgG screen — active-enzyme binding plotted against undesired pro-enzyme binding (fluorescence assay). A discrete cluster of optimized clones (dashed turquoise box) is recovered, separated from the parent (turquoise dot).

Lead Clone Profile — Picomolar Affinity, $>10^6$-fold Specificity, Excellent Developability

The optimized lead clones were characterised in IgG format. Affinity to the active enzyme by SPR at pH 7.4 (37 °C) returned KD <15 pM – a >1,000-fold improvement over the parental antibody. Pro-enzyme binding was undetectable at 10 µM antibody concentration, corresponding to a specificity differential exceeding 10^6-fold. Affinity at pH 6.0 was reduced >100-fold, favouring recycling of antigen-free antibody via FcRn. Cynomolgus orthologue cross-reactivity was preserved (equipotent), supporting the standard non-human primate toxicology model. Developability properties – solubility, viscosity, aggregation propensity – improved materially over the parent, consistent with the subcutaneous dosing intent. Ex vivo enzyme-inhibition assays confirmed substantial potency gains: three independent optimized clones each delivered low-nanomolar IC50 values, compared with high-nanomolar potency for the parent (Figure 3, Table 1).

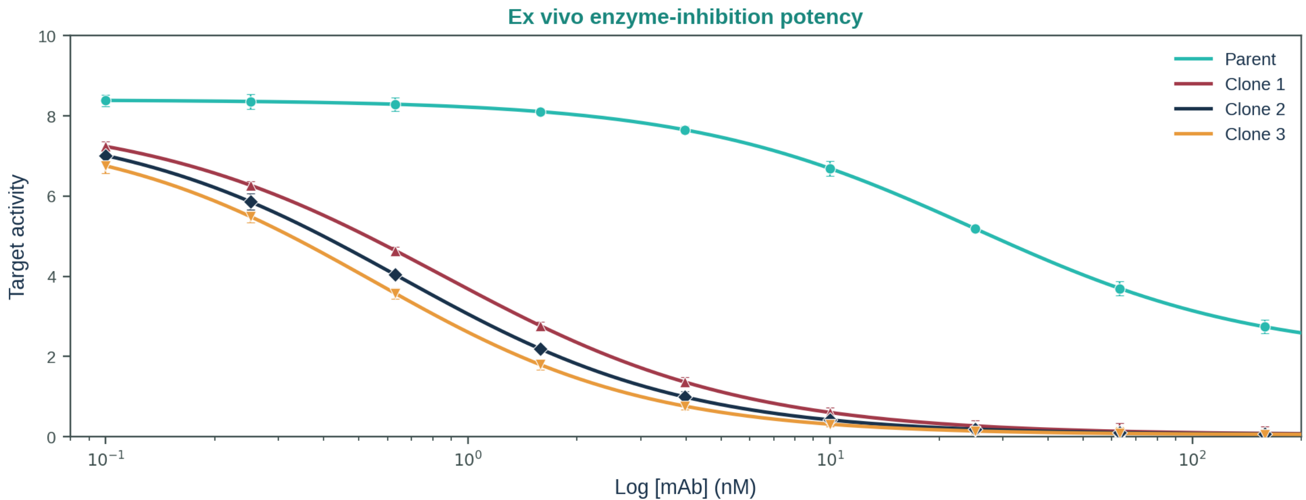

Figure 3. Ex vivo enzyme-inhibition dose-response curves for three optimized clones vs. parent IgG. The optimized clones share consistent low-nM IC_{50} values and approach complete target inhibition at the top of the dose range; the parent retains substantial residual target activity across the full dose range tested.

Table 1 · Optimized Lead Profile	
Affinity (K_D) at pH 7.4	<15 pM — >1,000-fold improvement over parent
Pro-enzyme specificity	No detectable binding at 10 µM — specificity > 10^6-fold
pH dependence	>100-fold affinity reduction at pH 6.0 (compatible with endosomal recycling)
Cynomolgus cross-reactivity	Equipotent on cyno orthologue
Developability	Improved solubility and reduced viscosity; low propensity to aggregate
Developability	IND in <12 months from candidate selection

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DISCUSSION

Why multi-parametric selection is the only credible route for this molecule class

For an enzyme-inhibitor antibody intended for subcutaneous delivery, the parameters that define the therapeutic profile – affinity, proenzyme discrimination, pH dependence, species cross-reactivity, viscosity, aggregation – are not independent. Conventional sequential workflows typically collapse one axis to recover another, lengthening optimization by months and producing molecules that miss the target product profile at the last stage. Mammalian Display imposes the selection for optimal overall fitness in a single experimental step, on full IgG molecules in their final therapeutic format, in the same mammalian system that will eventually express them at scale – which means developability is selected for directly, rather than screened for after the fact.

Deselecting against the pro-enzyme at the same step as selecting for active-enzyme affinity removed the pro-form sink risk before commitment to a candidate. Mammalian Display selects variants in final IgG format with developability prioritised alongside affinity and specificity; FullSpectrum Characterization then closes the loop on the full biophysical and biochemical profile. Together, they compress the path to clinical-grade candidates.

BOTTOM LINE

Multi-parametric Mammalian Display selection – simultaneous engineering of affinity, pro-enzyme specificity, pHdependence, species cross-reactivity, and developability – converted a double-digit nM parent into a sub-15 pM lead with >10^6-fold pro-form specificity and a developability profile compatible with subcutaneous dosing, on a <12-month timeline to IND.

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info@fjbio.com | fjbio.com
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www.fjbio.com
info@fjbio.com