Machine Learning via Sequence Diversification for the Co-optimisation of NANOBODY® Properties

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RESEARCH PARTNERSHIP INTRODUCTION

LabGenius and Sanofi initiated a research partnership to demonstrate that LabGenius' machine learning (ML)-driven antibody engineering platform, **EVA™**, is able to co-optimise 2 pre-determined properties of a monovalent NANOBODY® protein, whilst also maintaining favourable production characteristics. The success criteria were defined as LabGenius' ability to deliver 10 NANOBODY® variants with a more than 2-fold improvement in property 1 compared to the Sanofi starting molecule, whilst maintaining potency (property 2) and maintaining acceptable yield and minimal tendency for aggregation. The starting molecule was a single-domain antibody (VHH) that binds to and blocks a pro-inflammatory cytokine.

APPROACH: FROM START MOLECULE TO FINAL PANEL

The starting parent NANOBODY® molecule was subjected to a deep mutational scan and phage-based selection, followed by the construction of a combinatorial library with subsequent selection. The NGS enrichment data after each round allowed the development of an ML-based model to predict both protein affinity (as a proxy for potency) and NANOBODY® property 1. The top 85 predicted variants were then produced and characterised in high-throughput format to assess both potency and NANOBODY® property 1.

100% of the predicted variants showed varying degrees of improvement in NANOBODY® property 1 and maintained potency (property 2) when compared with the starting protein.

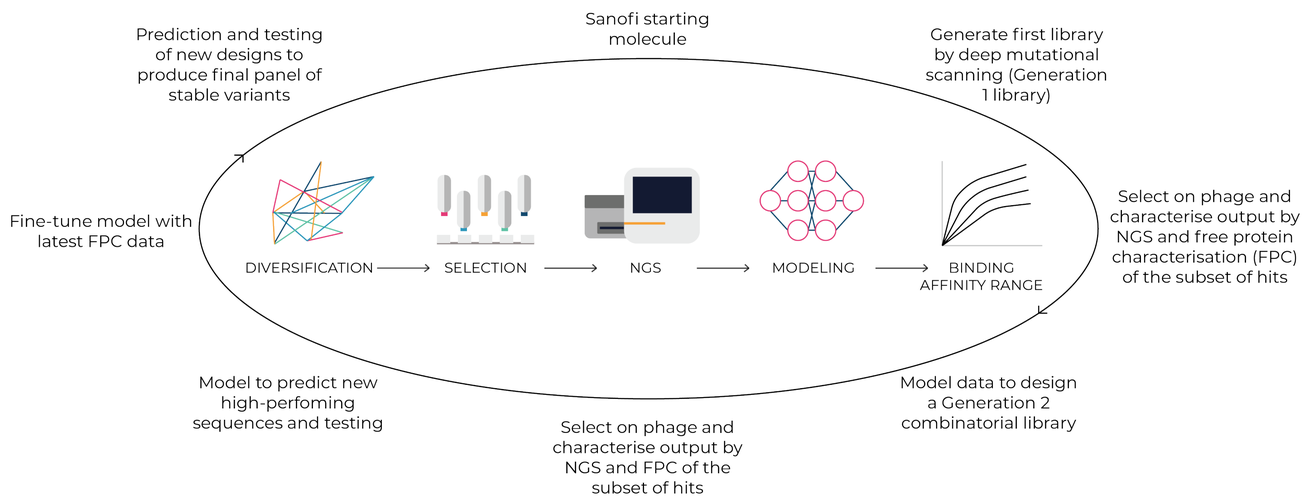

Figure 1: LabGenius' ML-driven process. Shown for affinity optimisation example.

CHARACTERISATION OF TOP 24 VARIANTS

16/24 molecules showed >2-fold improvement in the desired NANOBODY® property 1 compared with the starting molecule.

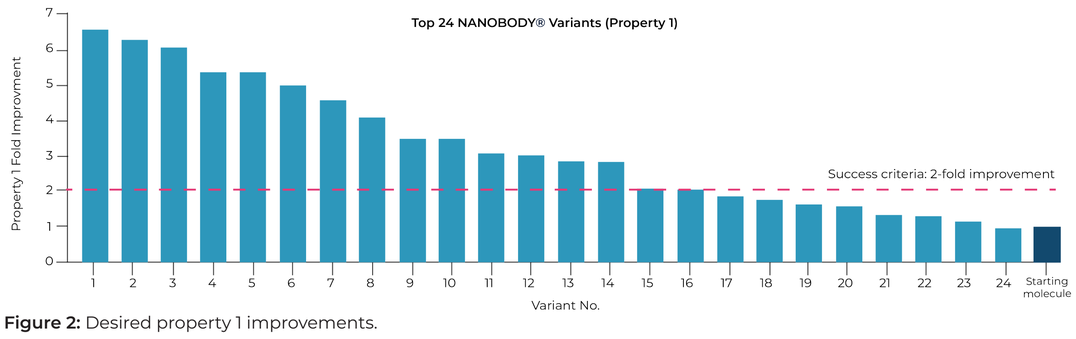

Figure 2: Desired property 1 improvements.

100% of top 24 variants with improved property 1 characteristics maintained an acceptable potency within 2-fold of the starting molecule.

Figure 3: Potency improvements.

TOP 10 VARIANTS DEMONSTRATED 7-FOLD IMPROVEMENT IN THE DESIRED PROPERTIES

The top 10 improved NANOBODY® variants were then selected for extended characterisation. All variants expressed well in *E. coli* with yields varying from 8.1 to 64.8 mg/L and all proteins showed minimal tendency for aggregation (98-100% monomer).

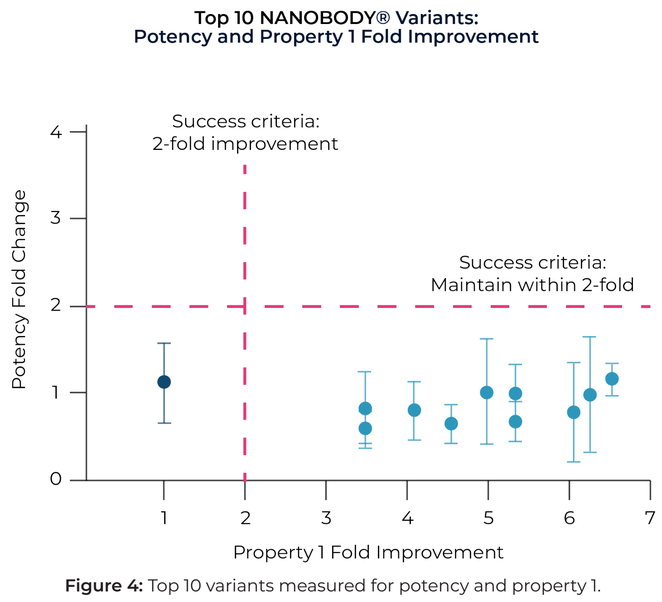

Figure 4: Top 10 variants measured for potency and property 1.

Shown in the bottom right corner of the graph, the top 10 predicted variants:

- Maintain the potency of the starting molecule
- Show a 3.5 to 6.6-fold improvement in the desired NANOBODY® property when compared to the starting molecule

VARIANTS CONTAINED NON-INTUITIVE DESIGNS

The top 10 improved NANOBODY® variants were selected for extended characterisation. These variants had between 4 and 9 mutations compared to the starting molecule. Interestingly, analysis of the mutations showed that of all the different mutations predicted, 2 were common to all 10 selected variants, both of which were non-intuitive.

Introduction of the 2 mutations into the starting molecule resulted in a 2-fold improvement in the NANOBODY® property being optimised. Their removal from the optimised variants resulted in loss of activity previously observed, showing that the mutations are essential but not sufficient for the improved NANOBODY® molecule property 1 and the combination with the other 2 to 7 ML-predicted mutations was essential for the improved NANOBODY® molecule performance.

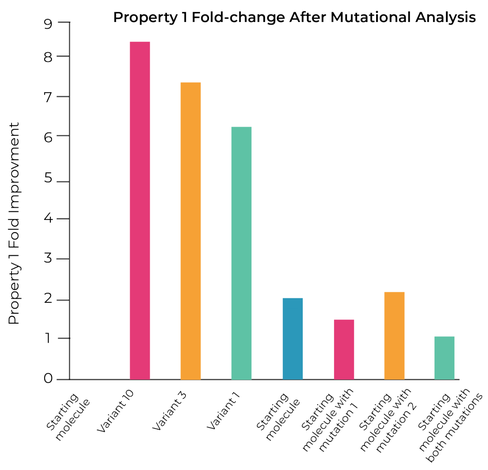

Figure 5: Mutational analysis of property 1.

CONCLUSION

Through two rounds of selection, LabGenius were able to build an ML-based model to predict protein potency and improve a second NANOBODY® property. The output of this model was the discovery of more than 10 NANOBODY® variants that showed up to nearly 7-fold improvement of the key desired property compared to the starting protein, and maintained potency within the 2-fold of the starting protein. These variants had between 4 and 9 mutations compared to the starting molecule.

Overall, the **EVA™** platform's use of sequence diversification and ML proved a novel and effective method to co-optimise NANOBODY® properties.

Acknowledgements: K. Boukra, G, Bucaite, A. Kovaltsuk, A. Ling, B. MacKrow, M. Martin, B. Rajeenthira, E. Wong.

References: 1. LabGenius, London, United Kingdom. **2.** Ablynx, a Sanofi Company, Zwijnaarde, Belgium. **3.** Sanofi, Frankfurt, Germany.