Breaking the Expression Bottleneck: Achieving a 2.4x Increased Enzyme Yield after Reaching the Limits of Traditional Optimization

Summary

In the rapidly evolving landscape of biotechnology, the transition from lab-scale discovery to industrial-scale production is often hindered by a single, persistent obstacle: protein expression yield. Conventional codon optimization tools, which have served the industry for decades, are increasingly proving insufficient for complex proteins or high-titer requirements.

This white paper details a collaboration between MNDL Bio and an innovative biotechnology partner seeking to commercialize a healthier, low-calorie sugar alternative: ***allulose***. After more than two years of internal efforts utilizing standard optimization protocols, the client reached a production plateau that threatened the economic viability of the product. By applying MNDL Bio's proprietary AI-driven biophysical algorithms, the client was finally able to break through this plateau, achieving a 240% increased enzyme yield.

The Challenge: The Quest for Affordable Allulose

Our client is at the forefront of the food manufacturing revolution, focusing on scalable and affordable solutions to global health challenges. Their primary target is the production of allulose, a rare sugar found in nature that is FDA-approved, tastes like sugar, but contains only 10% of the calories. It is a critical tool for reducing sugar content in consumer products without compromising taste.

Natural enzymes responsible for the conversion of fructose to allulose typically lack industrial applicability due to their low efficacy and low robustness. Our biotechnology partner developed a proprietary design of the natural enzyme, Allulose Epimerase, engineered for maximal activity in the catalysis of ordinary sugar feedstocks to allulose.

However, to make allulose commercially viable, the enzyme must be produced in massive quantities at a very low cost. For over two years, the client utilized their internal expertise and multiple open-source and commercial codon optimization tools to improve the expression of this proprietary Allulose Epimerase design. Despite these extensive efforts, they were unable to achieve the expression levels required to move

from the research phase into full-scale bioconversion. The project had reached a technical plateau where traditional methods could no longer provide incremental gains.

The MNDL Bio Approach: Beyond Codon Frequency

The failure of standard tools to solve this bottleneck stems from a fundamental limitation: most optimization software focuses on "Codon Adaptation Index" (CAI), simply matching the frequency of codons to the host's preference. While useful, this ignores the complex biophysical reality of the cell.

MNDL Bio's platform is built upon 15 years of academic research, supported by over 200 peer-reviewed publications. Our engine does not just "swap codons"; it performs deep biophysical simulations of the translation process.

Key differentiators of our optimization included:

- **Ribosomal Trafficking:** Modeling the speed and movement of ribosomes along the mRNA to prevent traffic jams that lead to truncated proteins or cellular stress.
- **Co-translational Folding:** Ensuring the speed of translation is synchronized with the protein's folding requirements, preventing the formation of insoluble inclusion bodies.
- **mRNA Stability and Structure:** Designing the transcript to avoid stable secondary structures near the start codon that inhibit translation initiation.
- **Contextual Awareness:** Optimizing the coding sequence (CDS) in the full context of the client's specific genomic construct and *Escherichia coli* (E. coli) host.

For this project, we implemented our engine to optimize specific regions of the transcript that were identified as high-impact expression bottlenecks. MNDL Bio produced 15 unique variants, each designed to resolve different biophysical constraints identified by our algorithms.

Results: Data-Driven Validation

The optimization process followed a structured screening protocol to ensure accuracy and reproducibility.

Phase 1: Initial Screen

All 15 variants were synthesized and expressed at a small scale. Following this initial screen, the three best-performing variants were selected for in-depth quantitative analysis.

Phase 2: Comparative Analysis

Each selected variant was expressed in *E. coli* and induced using IPTG. To ensure a true "apples-to-apples" comparison, we measured these against the client's previous best-performing sequence (the baseline).

Phase 3: Quantitative Measurement

SDS-PAGE analysis provided visual confirmation of the improvement, as seen in Figure 1.0 below. The bands corresponding to the Allulose Epimerase molecular weight were significantly thicker for all MNDL-optimized variants compared to the baseline. Following protein purification, fully quantitative levels were calculated to determine the final titer.

Key Findings:

- **Baseline Yield:** 7.5 mg / 50ml culture
- **MNDL Bio Yield:** 17.5+ mg / 50ml culture
- **Total Improvement:** 240% increased enzyme yield

The results demonstrated that every single variant designed by MNDL Bio outperformed the client's "gold standard" sequence, which had been the result of two years of traditional optimization.

Future Perspectives: The Start of the Story

The 240% increase achieved in this project is remarkable, particularly given that it was achieved in a single iteration. This initial round focused primarily on the coding sequence (CDS) using MNDL Bio's online platform.

However, MNDL Bio's full capabilities extend far beyond the coding region. In a full-scale partnership, we typically propose a multi-iteration process that includes:

1. **Non-Coding Region Engineering:** Optimizing UTRs, promoters, and signal peptides to further fine-tune the initiation and secretion of the protein.
2. **Custom System Calibration:** Training our AI models on the client's specific industrial strain to identify unique cellular constraints that general models might miss.
3. **Genomic Integration Optimization:** Identifying the optimal chromosomal locations and copy numbers for maximum stable production.

By moving into these deeper levels of optimization, we expect to push titers significantly beyond the 240% mark, providing our partners with the high-performance strains necessary for global industrial scale.

Conclusion

The results of this collaboration prove that the expression "ceiling" often encountered in biotech is frequently a limitation of the tools, not the biology. By replacing trial-and-error with predictive biophysical modeling, MNDL Bio helps companies move from stalled research to commercial reality. We have established a clear pathway for the industrial-scale production of allulose, paving the way toward a healthier world.

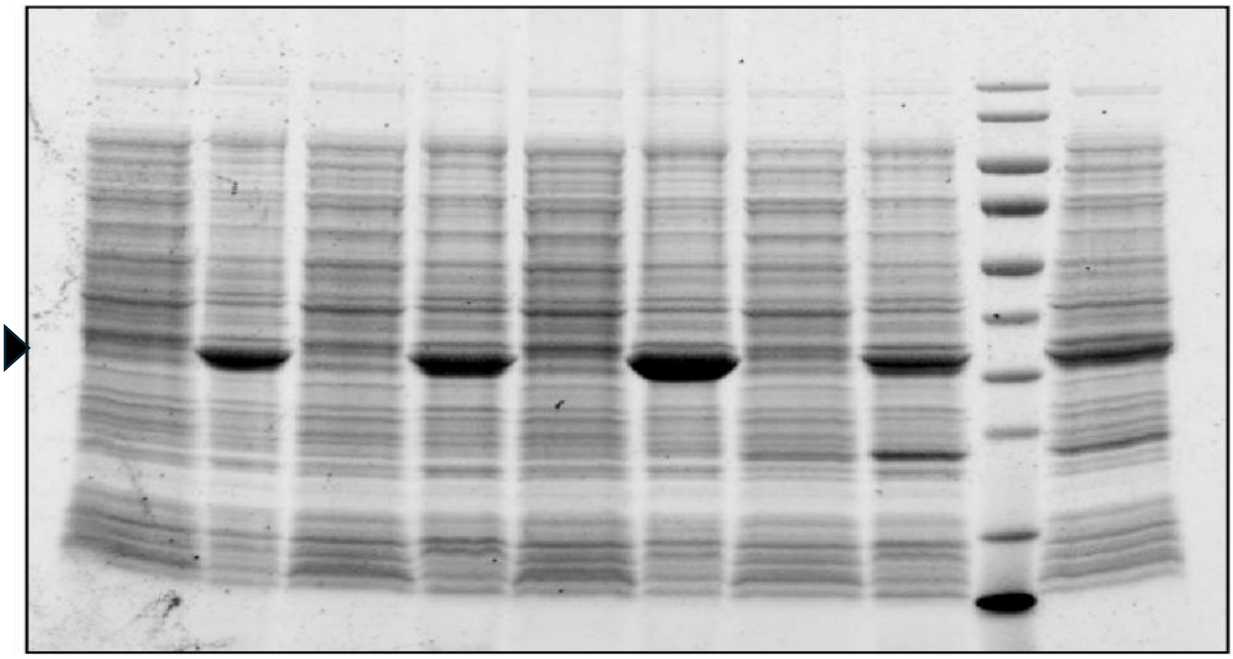
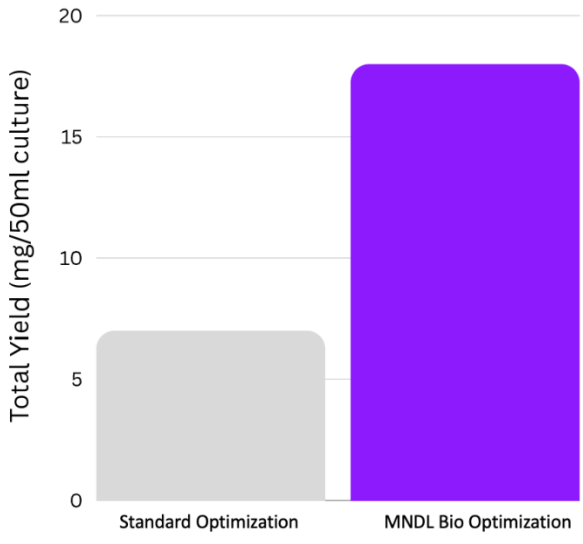


Figure 1.0. Greater expression yield across all MNDL Bio sequence variants can be visualized in this gel. A thicker band is produced at the expected molecular weight for Allulose Epimerase (see arrow) for the 3 best performing gene variants expressed following MNDL Bio sequence optimization compared to before MNDL Bio optimization (baseline).



Graph 1.0. A 240% increased yield was achieved with MNDL Bio optimization following prolonged attempts by our client to improve Allulose Epimerase expression. They were able to see expression yields go from approximately 7.5 mg/50ml culture to over 17 mg/50ml culture, demonstrating a significant improvement.