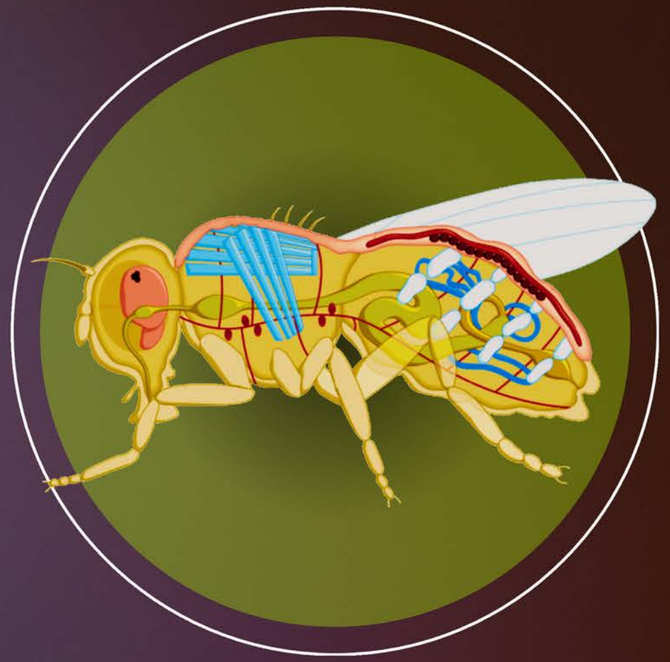
FUTURE FIELDS

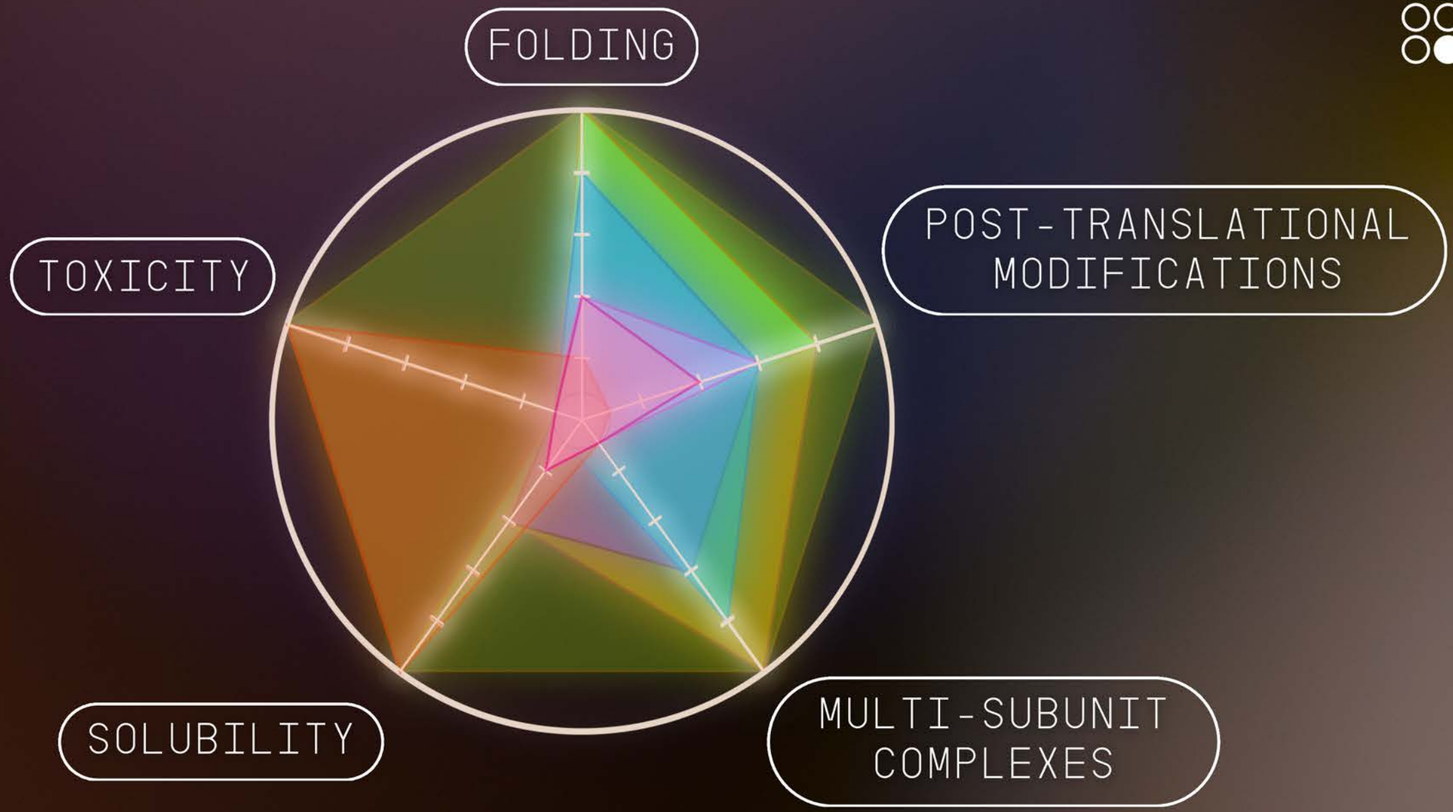
EntoEngine™ in Action

SOLVING PROTEIN EXPRESSION WITH TRANSGENIC *DROSOPHILA MELANOGASTER*

FUTURE FIELDS

Protein Expression Challenges

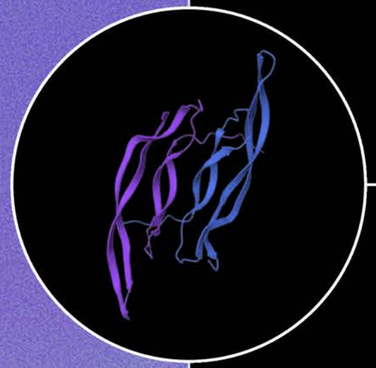




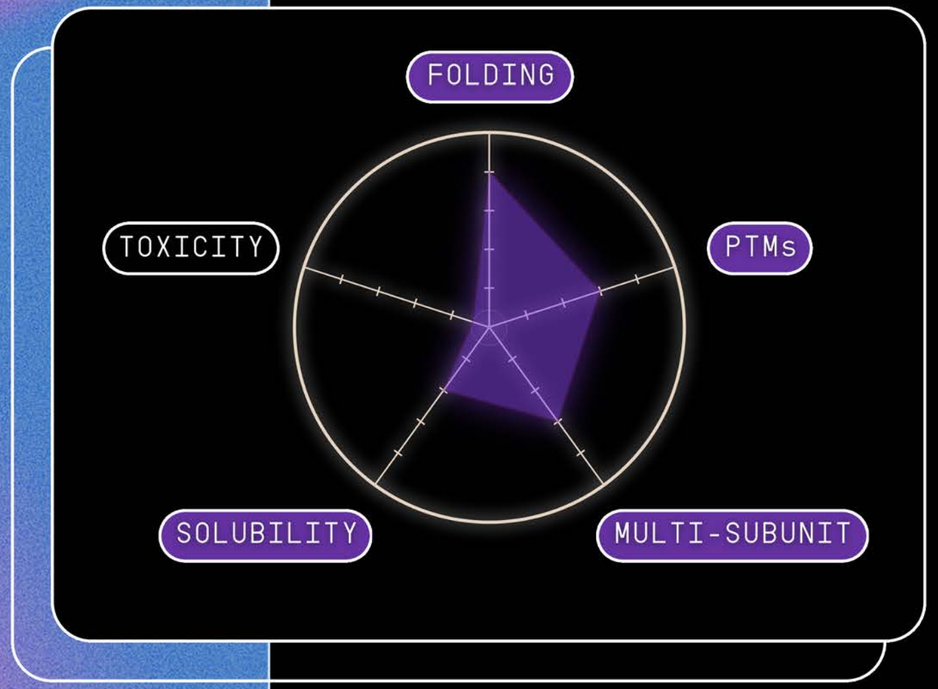
DTEPs have multiple challenging properties.
Fly by design, the EntoEngine™ platform is robust and customizable to effectively address multiple challenges.

Case Study: PDGF-BB

Homodimer Formation



Platelet-derived growth factor BB (PDGF-BB) is a homodimeric growth factor composed of two identical subunits, which are linked by disulfide bonds.



FOLDING SOLUBILITY

PDGF-BB is prone to aggregation due to its hydrophobic nature.

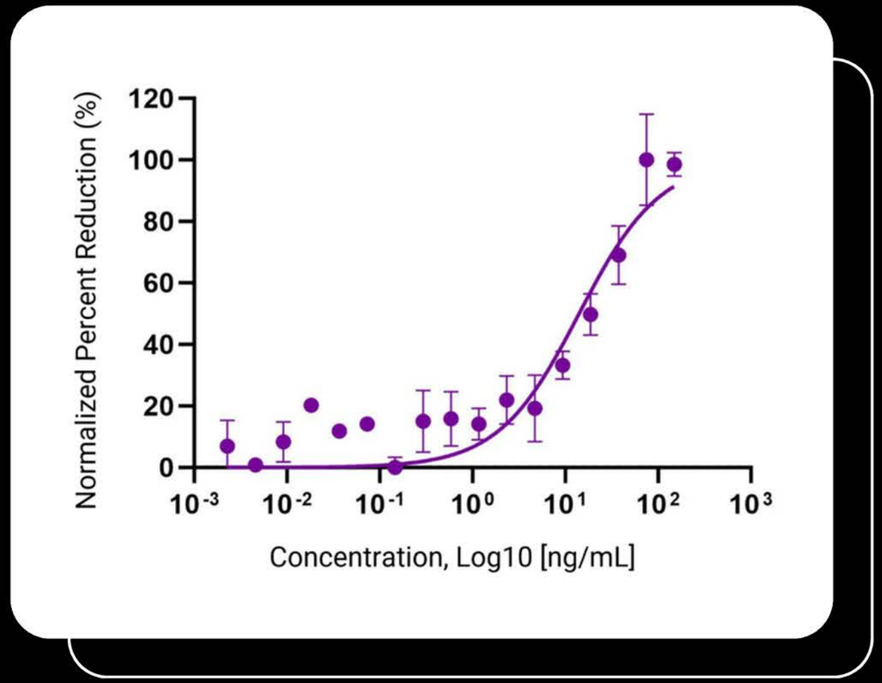
MULTI-SUBUNIT PTMs

PDGF-BB presents challenges in recombinant expression due to its requirement for correct homodimerization and disulfide bond formation between its identical subunits.

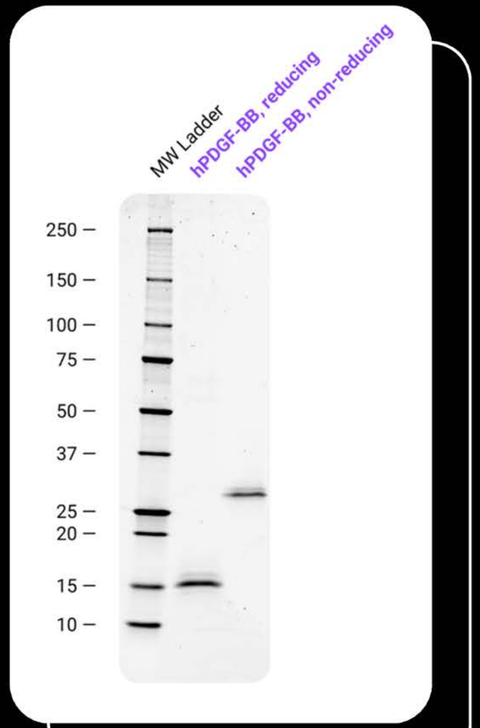
Case Study: PDGF-BB

FOLDING SOLUBILITY PTMs MULTI-SUBUNIT

Future Fields' EntoEngine™ successfully expresses the dimeric complex of PDGF-BB and achieves > 95% purity.



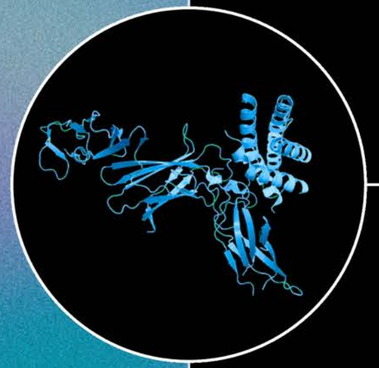
Activity: EC50 = 14.1 ng/mL
NIH-3T3 cells cultured with PDGF-BB produced in the EntoEngine™. Activity was assessed with an alamarBlue assay.



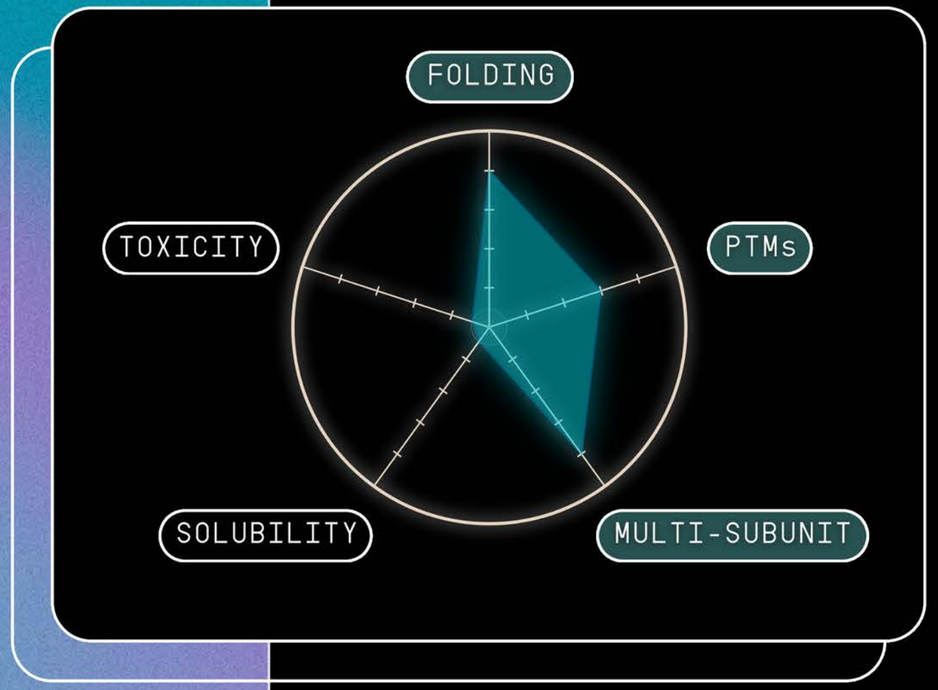
Purity: 96%
PDGF-BB purified to > 95% from the EntoEngine™ shown under reducing and non-reducing conditions.

Case Study: IL-12

Heterodimer Formation



Interleukin-12 (IL-12) is a heterodimeric cytokine composed of two subunits, p35 and p40, linked by a disulfide bond.



FOLDING PTMs

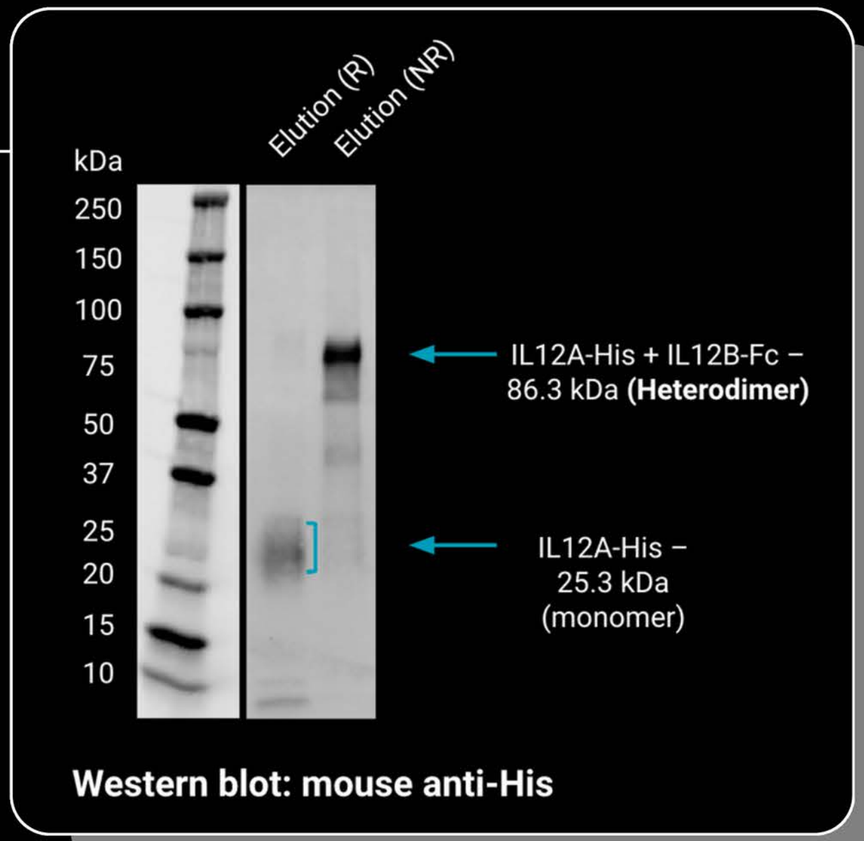
Correct disulfide bond formation is essential for proper IL-12 folding and its biological activity.

MULTI-SUBUNIT

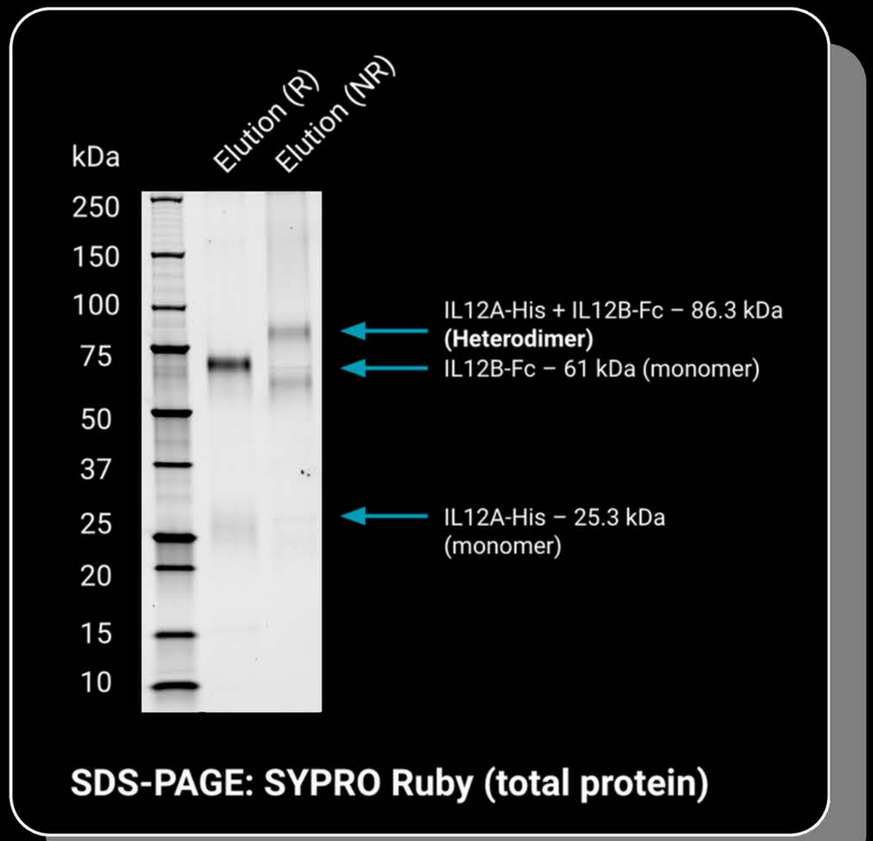
IL-12 is difficult to express due to the strict stoichiometric balance required between its p35 and p40 subunits for proper heterodimerization and bioactivity. Imbalances in subunit expression often lead to excess of free p40, which can form homodimers that act as antagonists to the IL-12 heterodimer.

Case Study: IL-12

FOLDING PTMs MULTI-SUBUNIT



Western blot: mouse anti-His



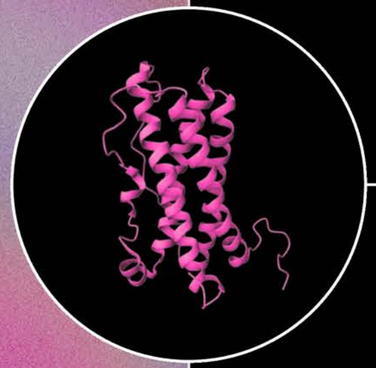
SDS-PAGE: SYPRO Ruby (total protein)

Images of IL-12 in development.

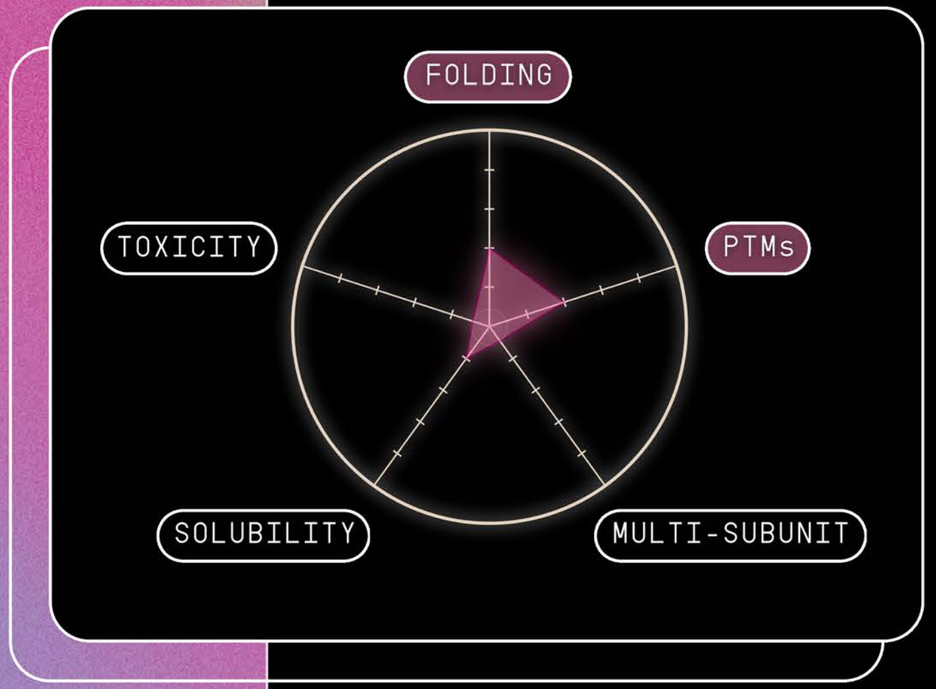
Future Fields' EntoEngine™ successfully expresses heterodimeric complex of IL-12.

Case Study: Prolactin

Glycoprotein Production



Prolactin is a multifunctional hormone that plays a key role in lactation, immune modulation, reproduction, and cellular growth. Its structural, functional, and biological properties are influenced by its glycosylation state.



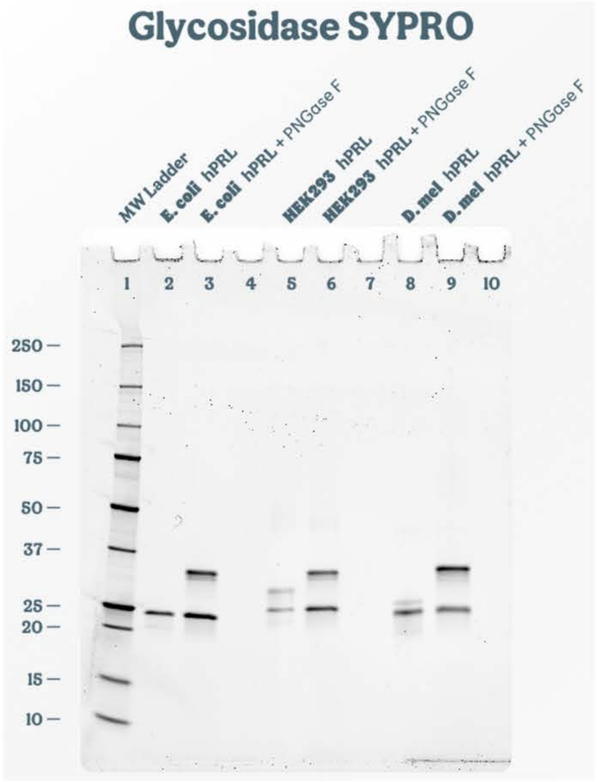
FOLDING PTMs

Glycosylation is crucial for prolactin’s activity, stability, and secretion, as it affects receptor binding, protects against degradation, and ensures proper conformation and solubility.

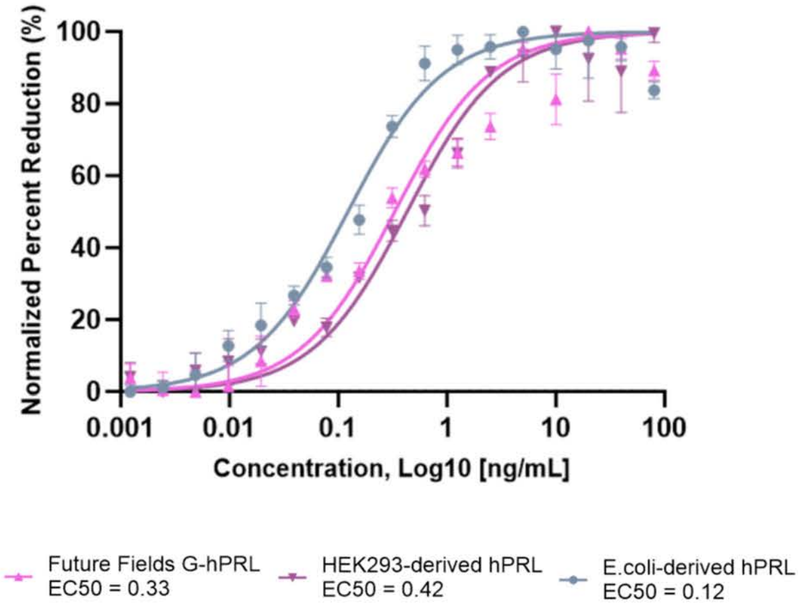
Case Study: Prolactin

FOLDING PTMs

Future Fields' EntoEngine™ successfully expresses glycosylated recombinant human Prolactin (hPRL) and achieves ≥ 90% purity.



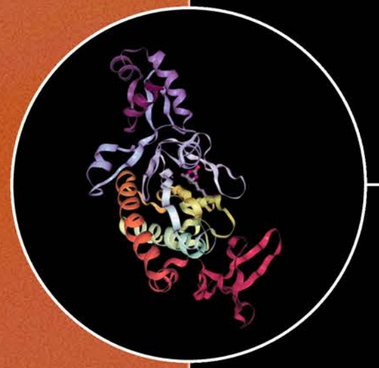
Glycosidase Assay
E. coli-produced hPRL appears as a single non-glycosylated band, while HEK293- and EntoEngine™-produced hPRL show a larger, glycosylated band that reduces to a single band with glycosidase treatment.



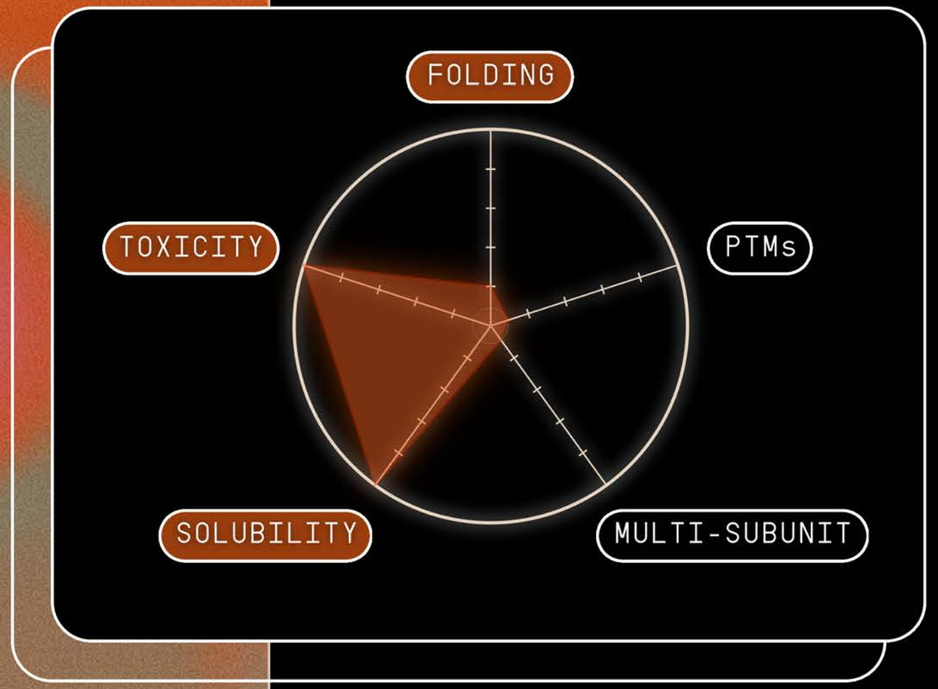
Activity: EC50 = 0.33 ng/mL
EntoEngine™-produced hPRL, EC50 = 0.33 ng/mL, functions similarly to HEK293-produced hPRL, EC50 = 0.42 ng/mL.

Case Study: BirA

Insolubility + Host Cell Toxicity



BirA is the *E. coli* biotin ligase that site-specifically biotinylates biotin-accepting proteins.



FOLDING SOLUBILITY

BirA tends to form insoluble aggregates and accumulate in inclusion bodies when overexpressed in bacterial systems.

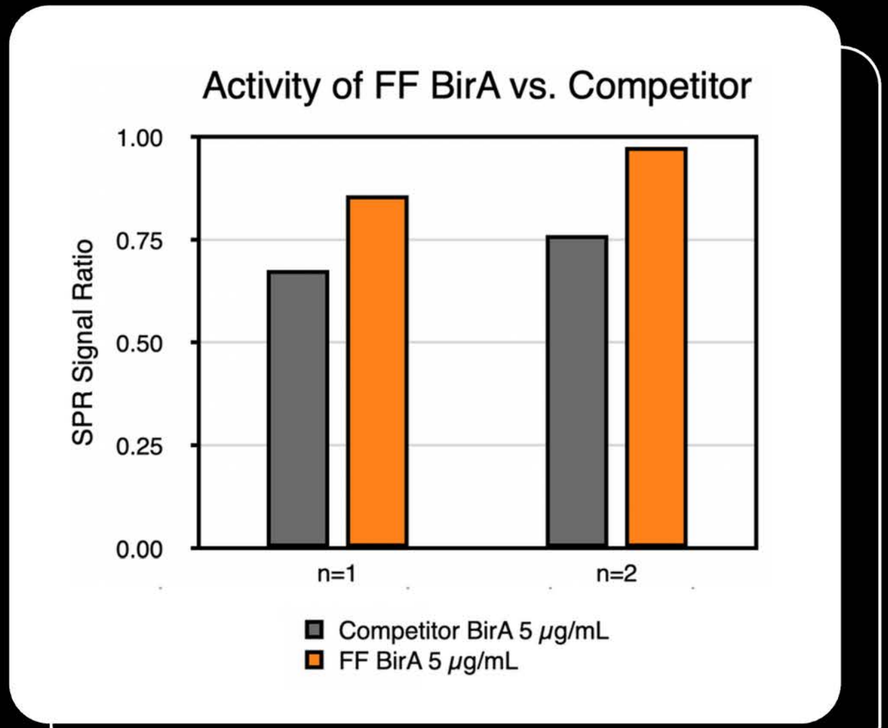
TOXICITY

Overexpression of BirA in *E. coli* can lead to cellular toxicity, likely due to its dual role as an enzyme and a transcriptional repressor of the biotin biosynthesis pathway.

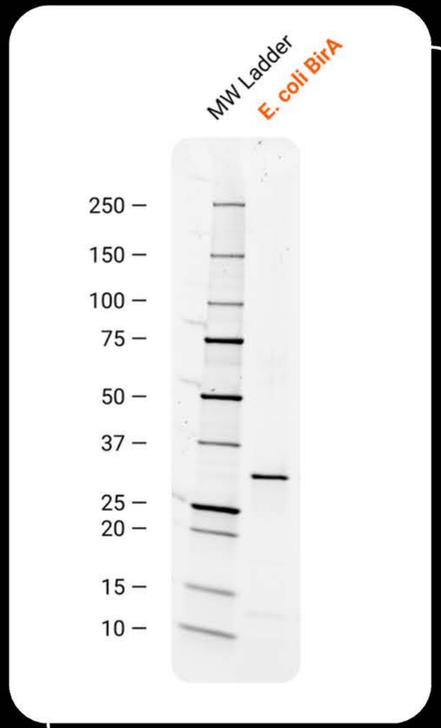
Case Study: BirA

FOLDING SOLUBILITY TOXICITY

Future Fields' EntoEngine™ successfully expresses high quantities of active, high-purity BirA without compromising the host system.



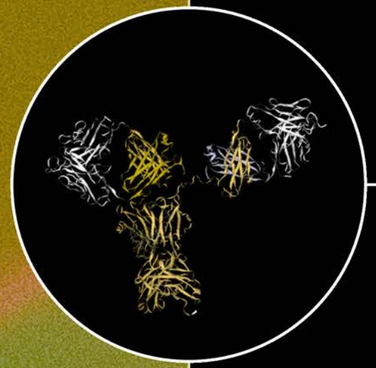
Activity: 500 Units/µg of BirA
Definition of Activity: One Unit (U) of BirA is the amount of enzyme that will biotinylate 1 pmol of Avi Tag substrate in solution at 2.3 µM within 30 minutes at room temperature.



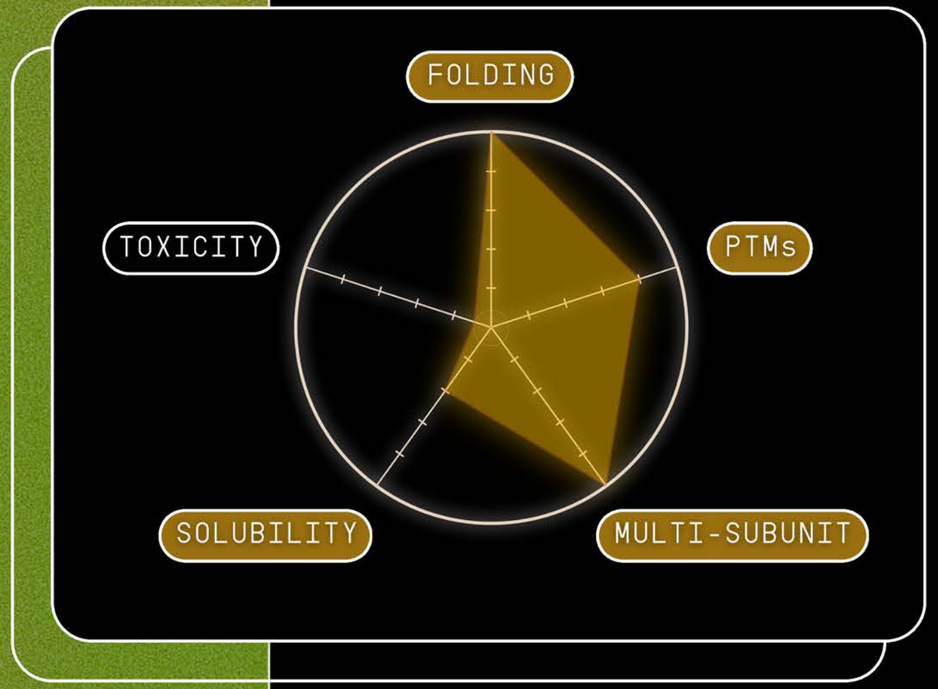
Purity: 91%
BirA purified to > 90% from the EntoEngine™.

Case Study: Trastuzumab

Monoclonal Antibody (mAb) Production



Trastuzumab is a humanized monoclonal antibody that selectively targets the extracellular domain of the human epidermal growth factor receptor 2 protein (HER2). It is a tetrameric glycoprotein composed of two identical heavy and light chains, linked to each other by disulfide bonds.



FOLDING PTMs SOLUBILITY

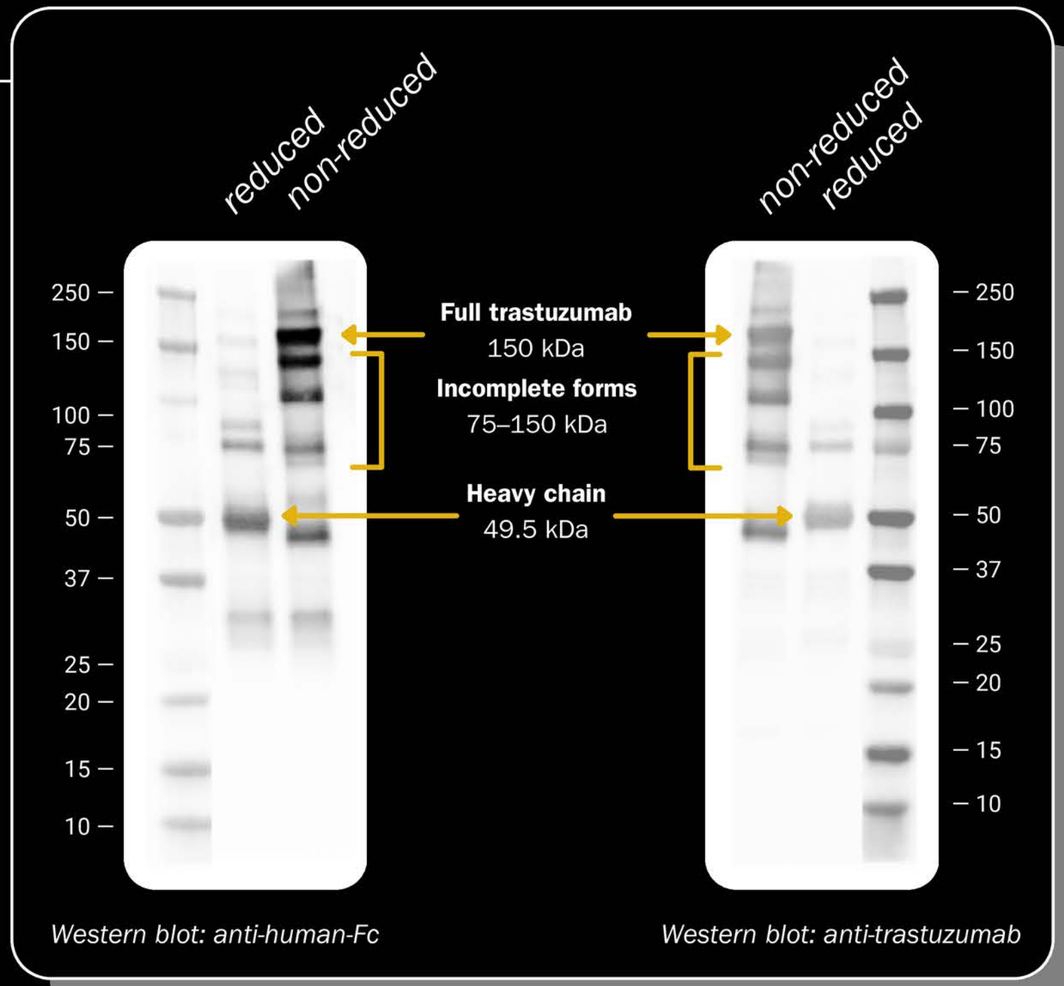
Glycosylation, a critical PTM, affects stability, solubility, and effector functions. Variability in glycosylation patterns can impact therapeutic efficacy and immunogenicity.
Disulfide bond formation is essential for proper folding of a fully functional monoclonal antibody (mAb). Partially folded or improperly assembled mAbs can aggregate.

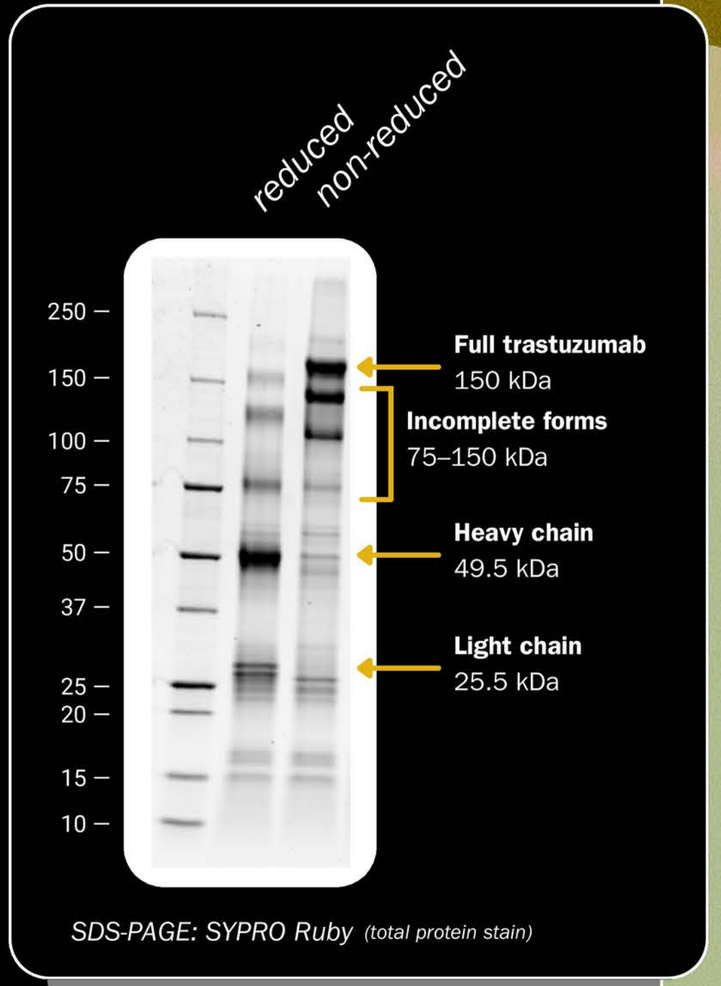
MULTI-SUBUNIT

Correct stoichiometric balance of heavy and light chains is critical–both chains must be expressed in the correct ratio to ensure efficient assembly into a functional heterotetrameric structure. Imbalances can result in the accumulation of free heavy or light chains, leading to poor yield and heterogeneity.

Case Study: Trastuzumab

FOLDING PTMs SOLUBILITY MULTI-SUBUNIT





Images of Trastuzumab in development.

Future Fields' EntoEngine™ successfully expresses mAb.

The heavy and light chains of Trastuzumab were co-expressed from a single open reading frame using the 2A self-cleaving peptide system.

FUTURE FIELDS

Questions?

support@futurefields.io