# Glycoengineered Therapeutic Proteins:

## *In vivo* production of *O*-glycosylated proteins in *E. coli*

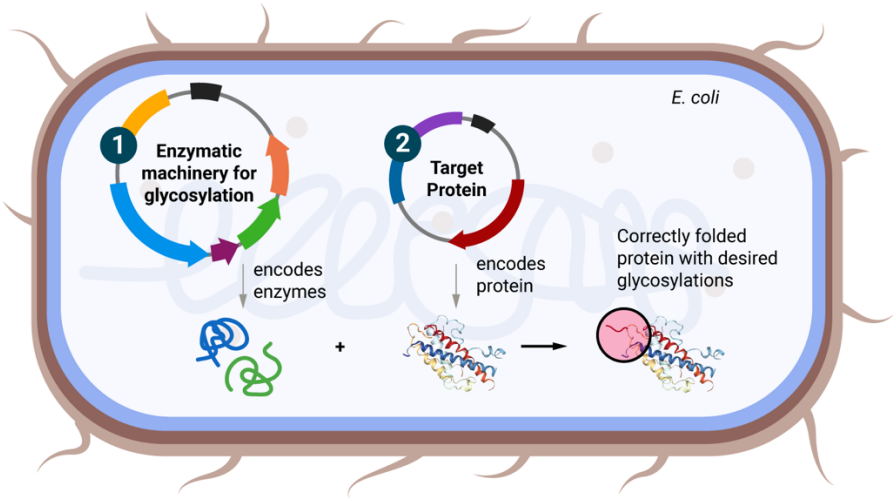
Industrial production of therapeutic glycoproteins often relies on mammalian cellular hosts to add *O*-linked or *N*-linked glycans to the target protein. However, this system is limited by the cells' slow growth, costly media, and difficulty in controlling the homogeneity of glycosylation due to endogenous glycotransferases. GlycoNet's technology uses an *E. coli* expression system that can efficiently add authentic *O*-glycans to cytokine type proteins at > 80 mg/L. The expression system can also be easily extended to produce a variety of therapeutic glycoproteins in a controlled and cost-effective manner.

### Key Benefits

- Rapid growth of host strain in simple bioreactors
- High yield of folded glycoprotein
- Flexible platform to produce different target proteins

### Global Impact & Market

In 2018, the overall therapeutic proteins market was estimated at $179 billion and is expected to grow at compound annual growth rate of 6.5%. The cytokine segment of this market that can immediately benefit from this platform, was estimated at $16.5 billion.




### Current Production Systems

Eukaryotic production systems remain the preferred approach for large-scale production of human therapeutic proteins since they produce correctly folded products with natural glycosylation. But the production processes are expensive, time-consuming, and still of relatively low productivity. On the other hand, prokaryotic expression systems offer fast growth, well-characterized genetics, confirmed safety, and high protein yield. However, they do not naturally produce *N*- or *O*-glycosylated products, limiting their utility in industrial process.

### The Technology

The expression system leverages a dual-plasmid system consisting of:

① **Plasmid encoding enzymatic machinery required for *O*-glycosylation,** including human transferases, epimerase, and isomerase.

② **Plasmid encoding the therapeutic protein target**

The system can produce full *O*-glycosylation that incorporates sialic acids, which are residues essential in prolonging serum half-life and providing immunogenic properties.

### Application

The technology provides a more efficient method to produce therapeutic proteins with tunable glycosylations and longer circulatory half-life, which is directly applicable to industrial production of hormone fusion proteins, and proteins of anti-viral, anti-bacterial, and cytostatic properties.