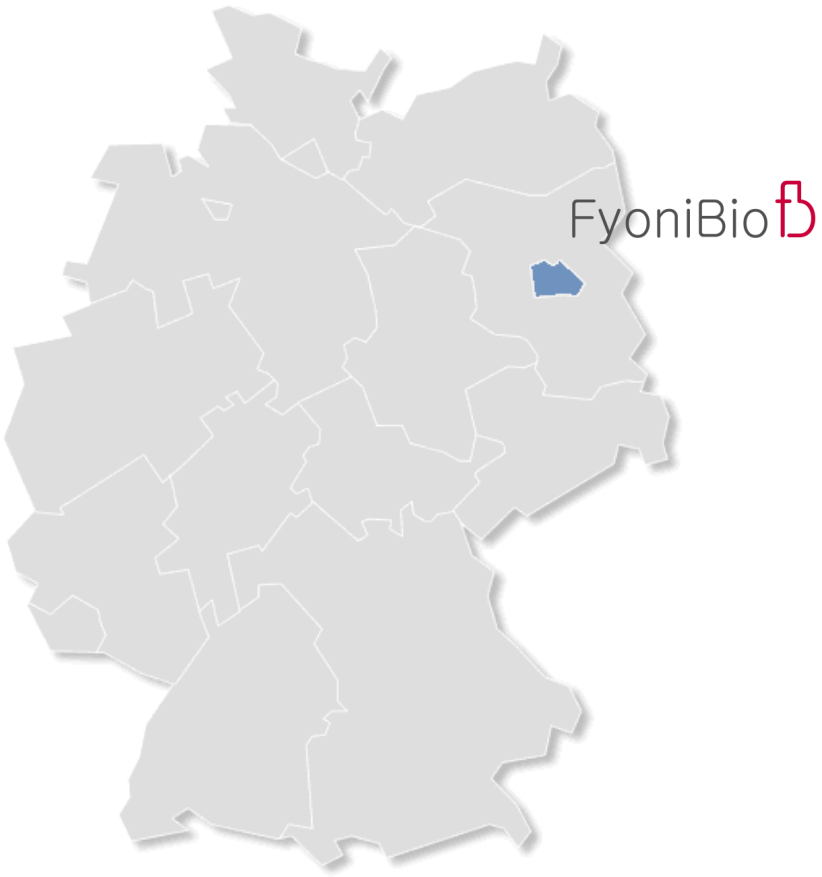
FyoniBio

GlycoExpress® - An alternative host for difficult to express proteins

30 March 2022

R&D Competence from Berlin



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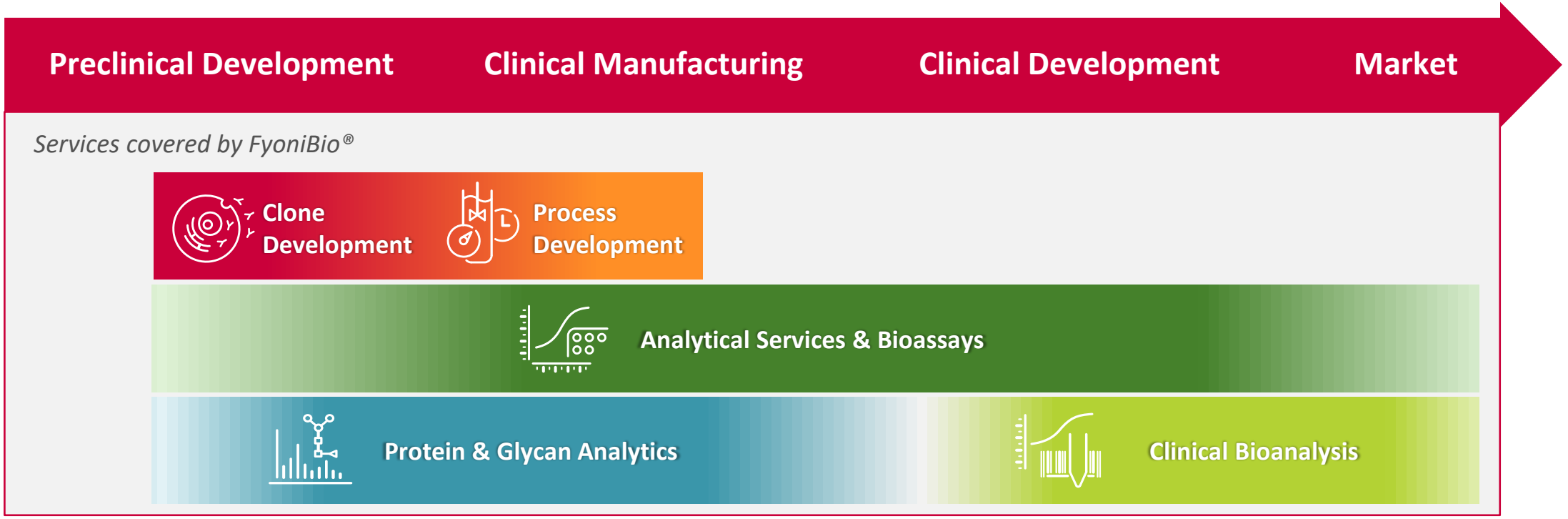
ISO 9001 Certified Services

Contract Development since 2005

Successful spin-out from Glycotope GmbH in February 2022

Development steps of a biopharmaceutical



We provide all necessary services to develop biotherapeutics from gene of interest to transfer to a GMP facility and we act as specialty laboratory to analyze clinical samples

Cell Line Development

GlycoExpress® and CHO host cells

With almost **20 years experience** in cell line development FyoniBio has gained experience with **CHO cell lines** as well as the in-house developed **GlycoExpress® (GEX®) cell lines** to provide the customer with the perfect cell clone for your clinical development.

CHOnamite® (CHO-DG44, CHO-K1)

- Proven well known expression hosts for uncounted biopharma projects
- High-yield production in fed-batch processes
- Applications: standard mAbs, biosimilar development, complex mammalian proteins
- Glyco-optimizing strategies can be applied

GlycoExpress® (GEX®)

- Genuine human glycosylation
- High productivity for glyco-optimized products
- Toolbox of glycoengineered cell lines: adjustment of sialylation, fucosylation, and mannose-6-phosphate
- Established expression platform for difficult-to-express, complex glycoproteins, and defucosylated proteins

GlycoExpress® - (GEX®)

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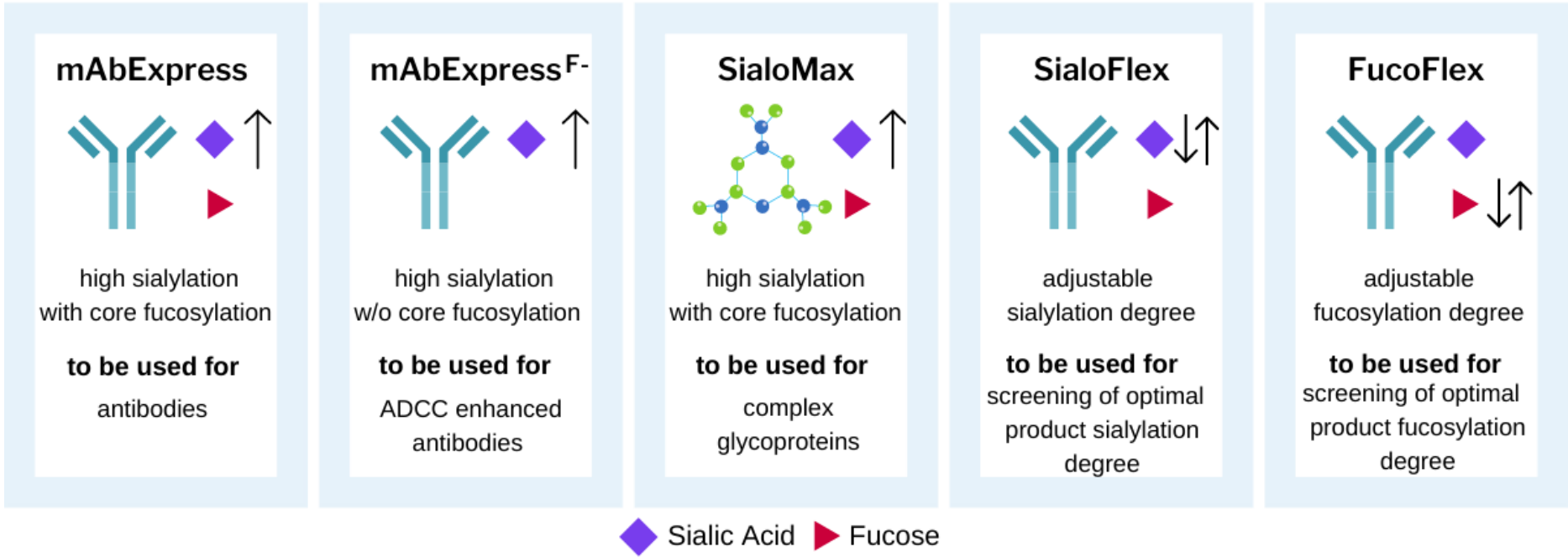
- Established **toolbox** of proprietary **human** glycoengineered cells for high titer production of complex glycoproteins
- Optimized proprietary vector and chemically defined media system
- Glycosylation features: Presence of all human glycosylation features



Regulatory Status of GlycoExpress®

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- ✓ **All cell lines of the GEX® platform are derived from one parental cell line.**
- ✓ **Products manufactured using different cell lines of the GEX® platform where approved in human clinical trials.**
- ✓ **GlycoExpress® system was accepted to date by regulatory authorities throughout the world:**

- Countries with aproved clinical trials with GEX derived products coloured red

Regulatories stated that the cell line characterization performed is in accordance to appropriate guidance and is adequate for production of biopharmaceuticals

Biopharmaceutical Production using GEX®

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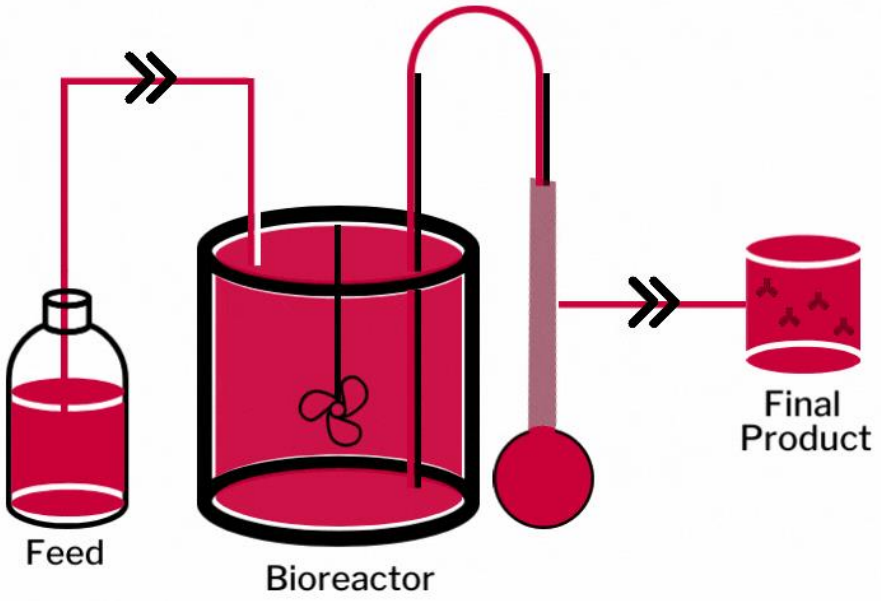
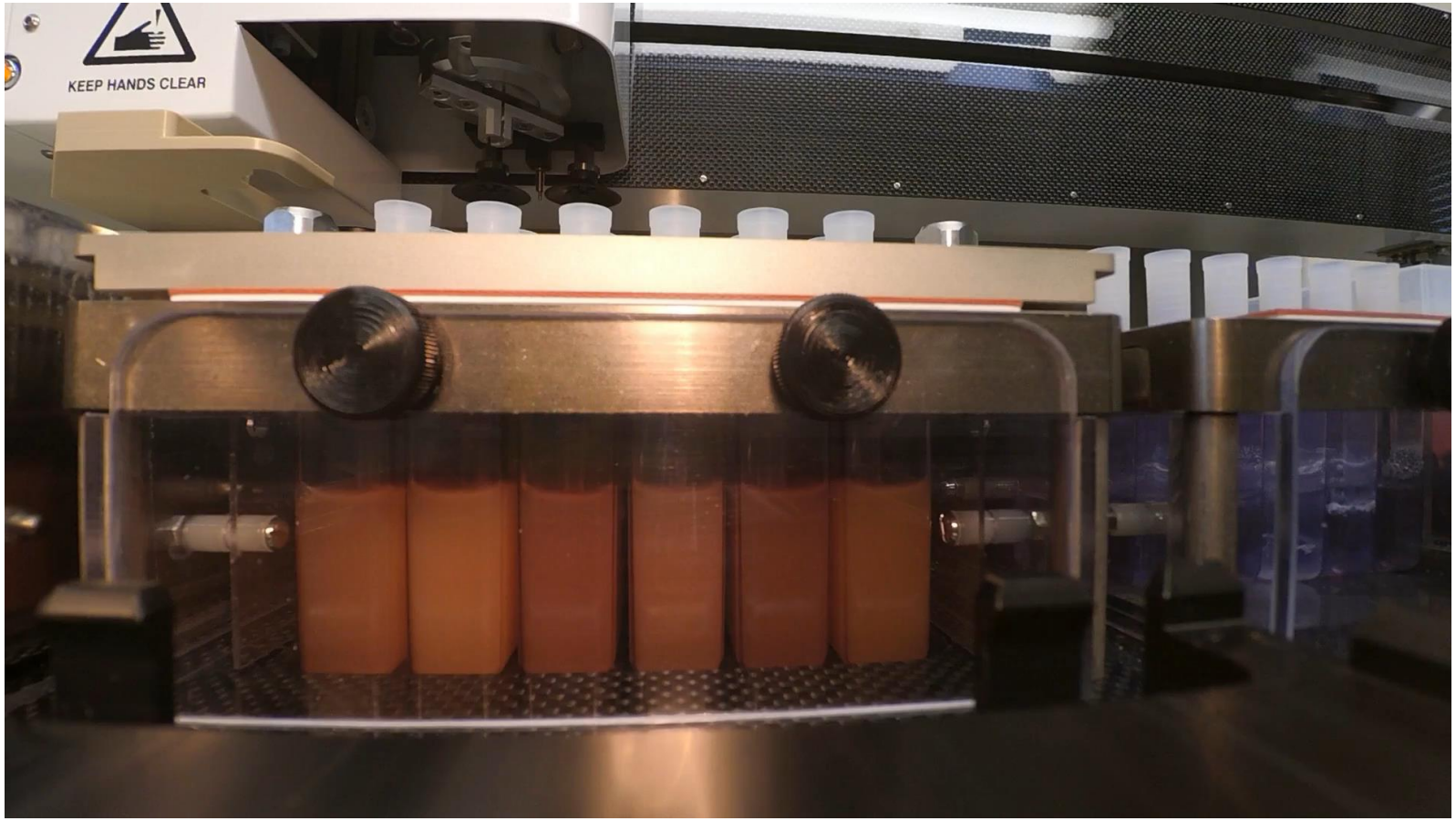
Optimized Perfusion process with high reproducibility & scability

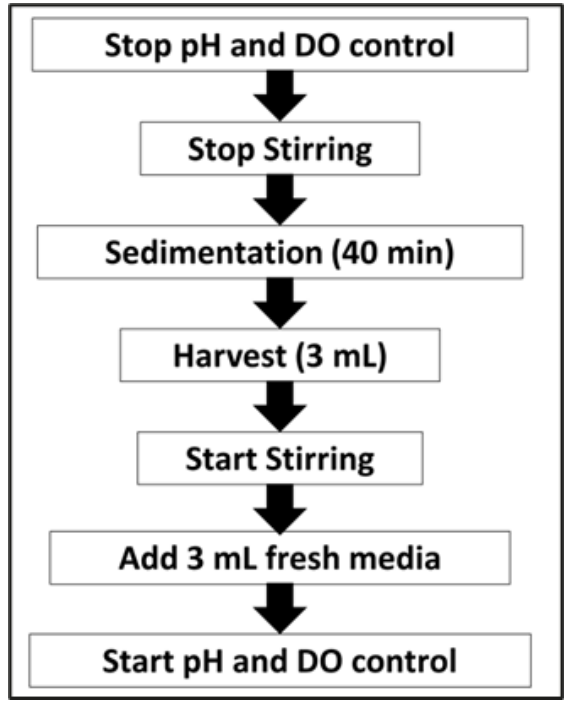
- Continuous feed and product harvest
- Consistent product quality
- Possibility to produce unstable products
- Long operation times, high productivities



- Small scale models development for CLD and USP development and optimization

SAM Perfusion:





Case I:
Complex glycoprotein Surfactant Protein-D

Case Study I - Surfactant Protein -D

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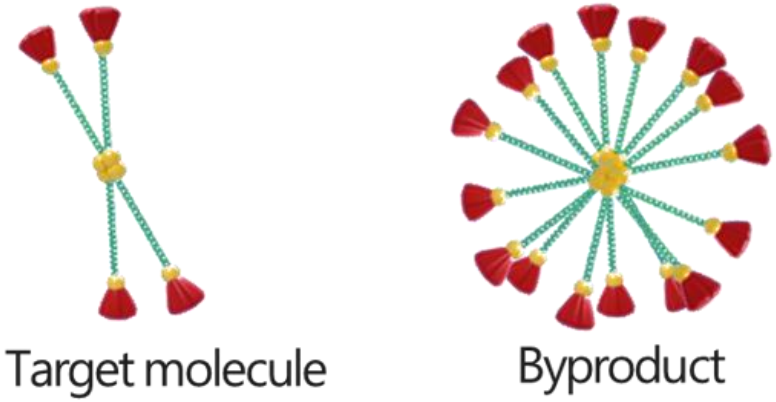
Therapeutic protein:

Surfactant Protein-D (SP-D) has a role in the pulmonary innate immune system by providing anti-inflammatory and antimicrobial/antiviral activities that address chronic pulmonary diseases.

Task:

Airway Therapeutics reached out to us with a set of CHO production clones and asked us to

- Identify the best clone and develop a CHO based production process
- Test in parallel in a **feasibility study** the usability of GEX® for the production of the protein
- Optimize the process to obtain high quality SP-D



AIRWAY THERAPEUTICS

Case Study I - Surfactant Protein -D

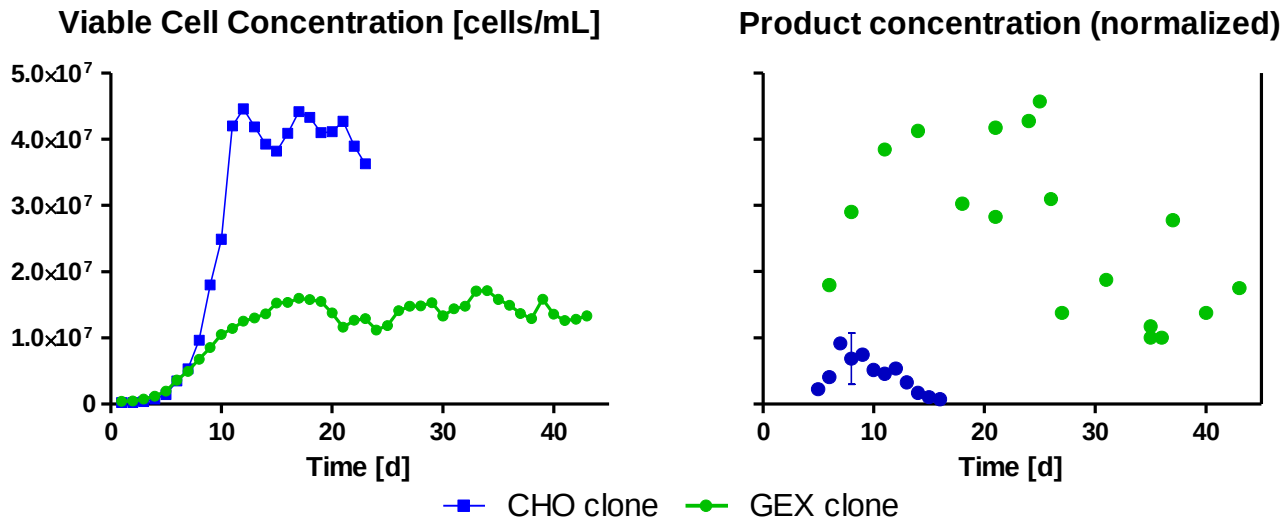
- Cultivation of the final CHO **clone** resulted in ~10 µg/ml protein in supernatant in T-flasks
- Cultivation of the stable GEX® **cell pool** resulted in ~80-120 µg/ml protein in supernatant in T-flasks

CHO process:

- Yield: < 0.1-0.5 mg/L after purification

GEX process:

- Yield: 10 mg/L after purification



Case Study I - Surfactant Protein -D

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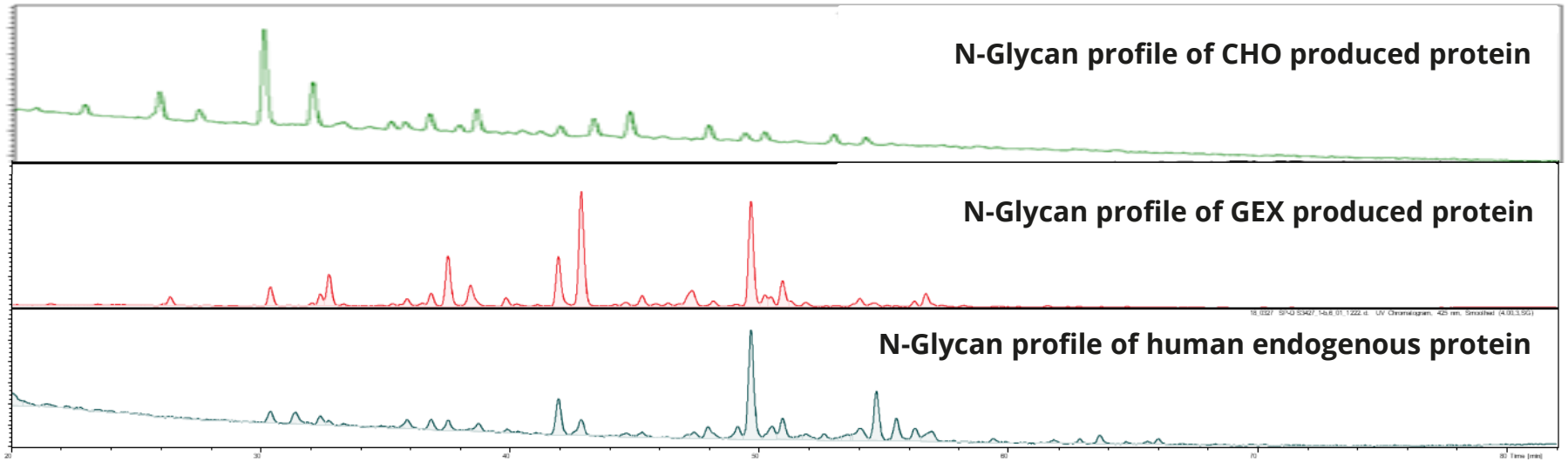
- N-glycan profile



- **N-glycan highly comparable to the human endogenous counterpart**

AIRWAY THERAPEUTICS

Case Study I - Surfactant Protein -D

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- **Current status:**
  - Process successfully transferred to a US based and a EU based GMP manufacturer
  - Clinical studies initiated in US :
    - AT-100 Intervention (rhSP-D) in Preterm Neonates at High Risk for Development of Bronchopulmonary Dysplasia (BPD)
    - A Clinical Safety Study on AT-100 in Treating Adults With Severe COVID-19 Infection

AIRWAY THERAPEUTICS

Case II:
Blood Factor

Case Study II: Expressing of Blood Factor VII

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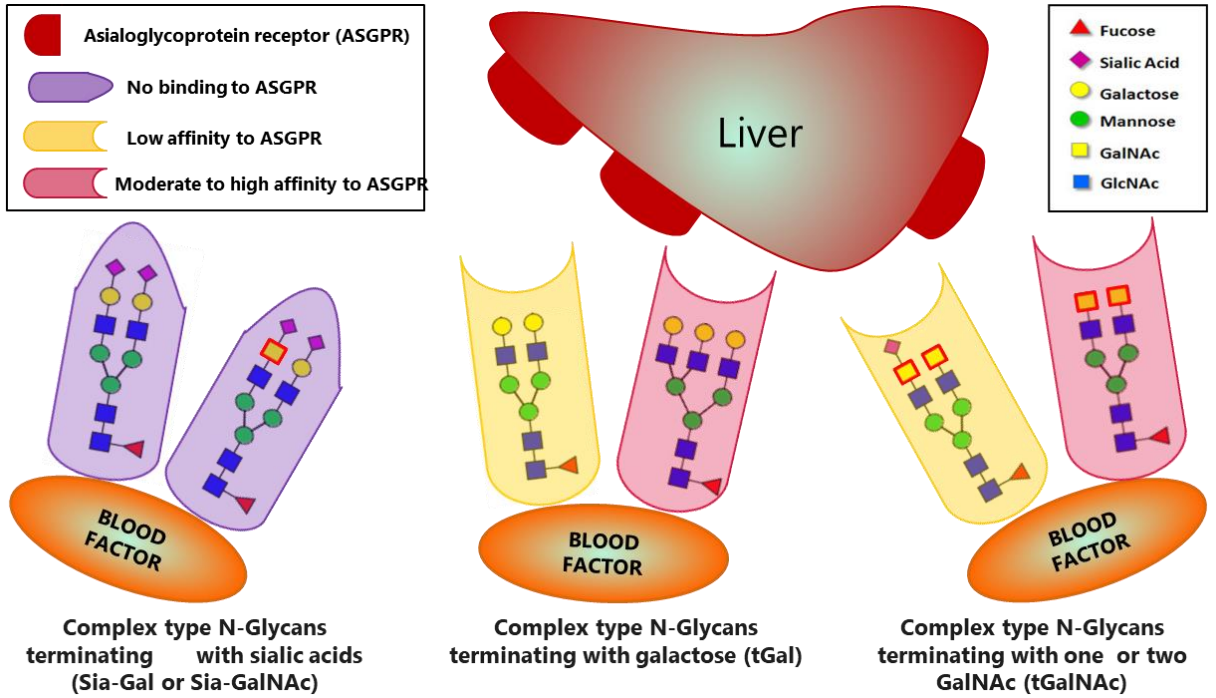
Task:

- Development of expression system for the production of coagulation relevant blood factor VII
- Dependent on terminal Glycan structure the blood factor can be bound to Asialoglycoprotein receptor (ASGPR) leading to receptor mediated endocytosis
- ASGPR binds terminal N-acetylgalactosamine (tGalNAc) ~50 fold stronger than terminal Galactose tGal
- Increase of sialylated amount of protein desired



Case Study II: Glycoengineering of GalNT

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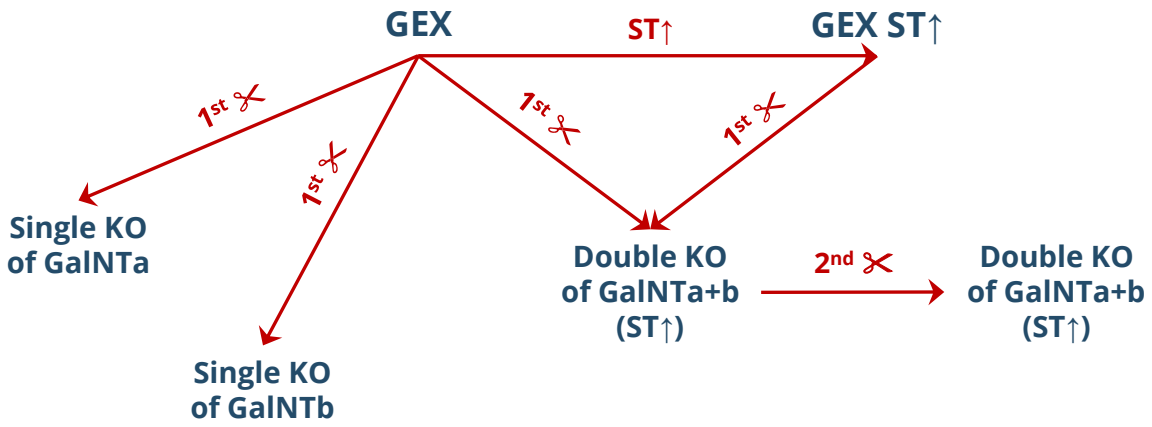
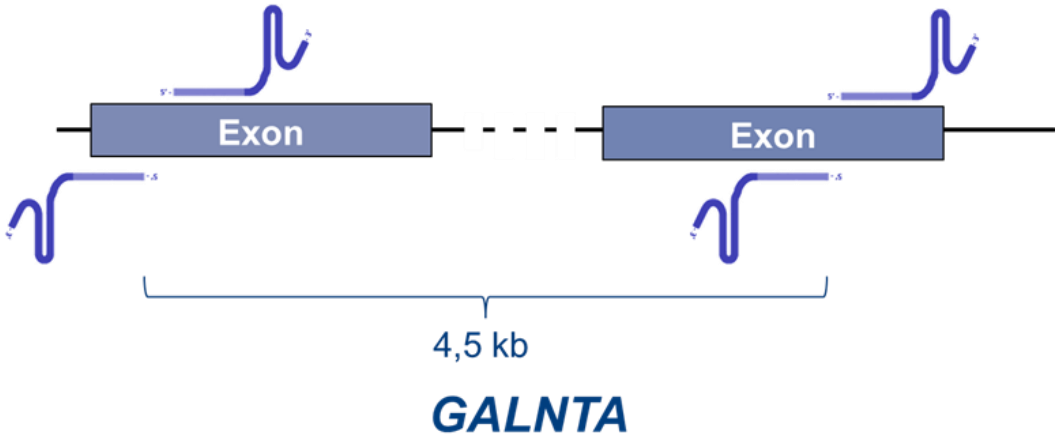
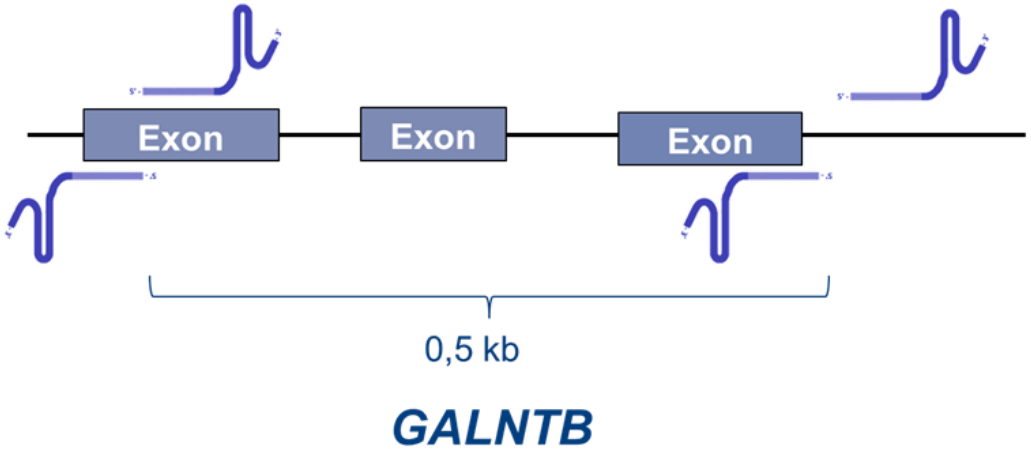
- Single KO and double KO of GalNAc transferases
- GalNAc transferases were targeted simultaneously in two exons to enhance the event of premature termination codon formation







Case Study II: Glycoengineered GEX Cells expressing Factor VII

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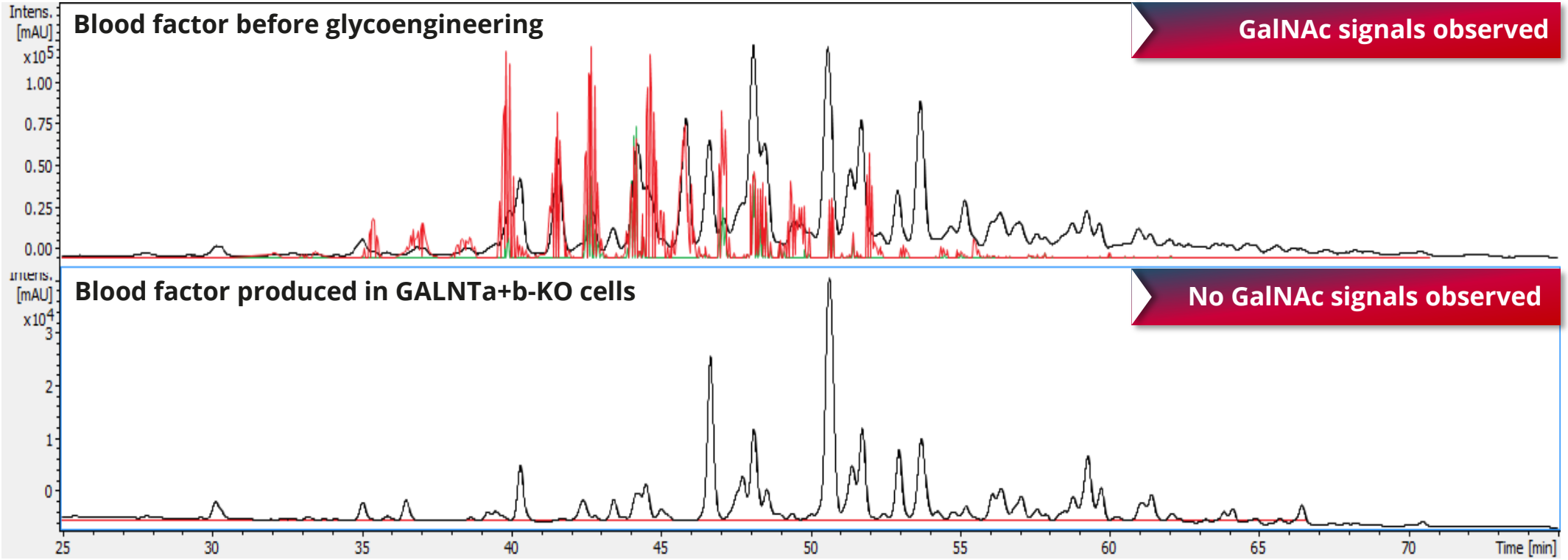
Fluorescence & GalNAc indicating EIC signal for N-glycans before & after GalNAc-KO



Red trace: M/Z 407.16; characteristic fragment for GalNAc, GlcNAC

Green trace: M/Z 698.27; characteristic fragment for NeuAc, GalNAc, GlcNAC

Case Study II: Glycoengineered GEX Cells expressing Factor VII

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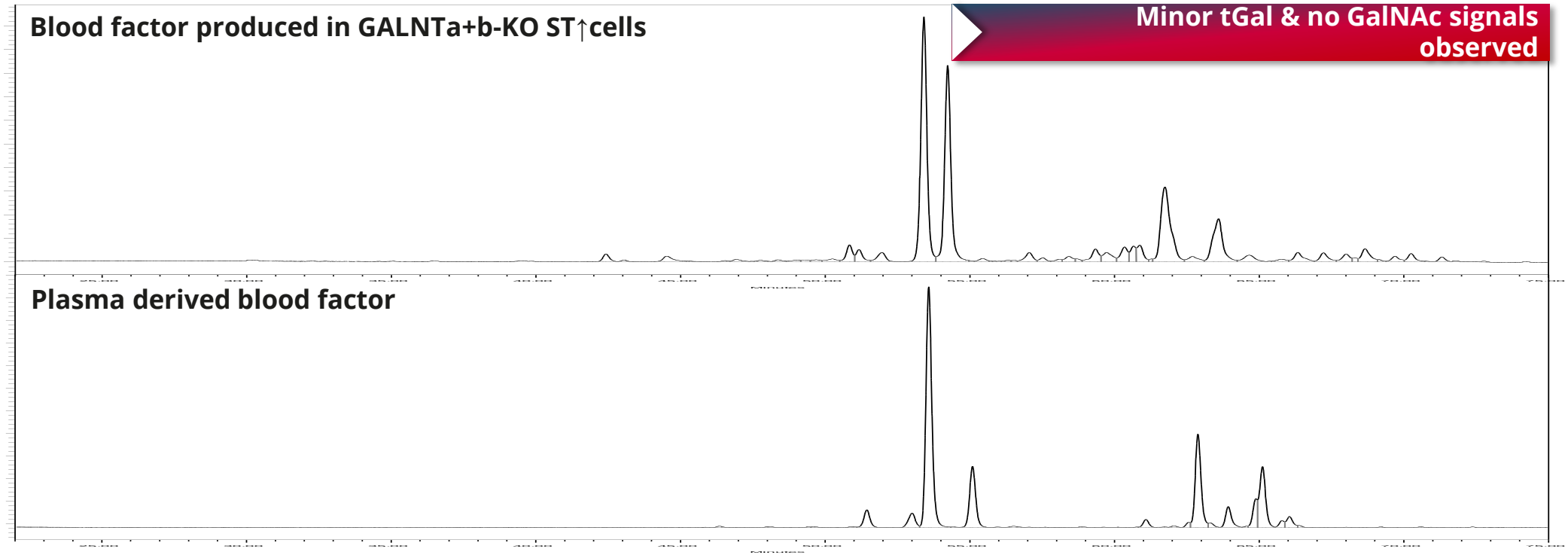
Fluorescence signal for glycoengineered and plasma derived blood factor



Blood factor expressed in glycoengineered GEX cells highly resembles human plasma derived glycoprotein

Thank you and Acknowledgments

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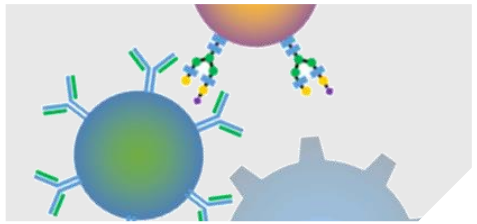
Process development



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