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GlycoExpress: A toolbox for the high yield production of glycooptimized fully human biopharmaceuticals in perfusion bioreactors at different scales

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GlycoExpress™: A toolbox for the high yield production of glycooptimized fully human biopharmaceuticals in perfusion bioreactors at different scales

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GLYCOTOPE
The GlycoEngineering Company

Background

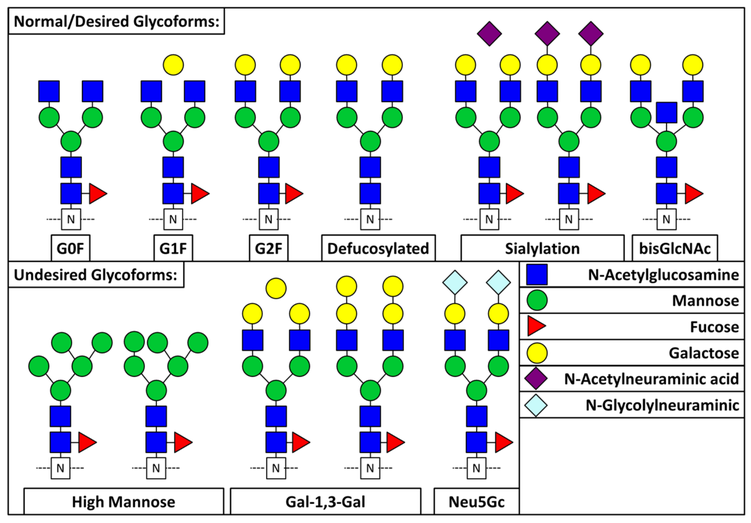
Glycosylation is one of the major post-translational modifications of biotherapeutics important for bioactivity, bioavailability, immunogenicity and patient coverage. By establishment of the **GlycoExpress™ toolbox (GEX™)** we have generated a set of **glycoengineered human cell lines** for the **high yield production** of **fully human glycoproteins** to optimize the glycosylation of antibodies and non-antibody biotherapeutics for improvement of the clinical efficacy and side effects. The system is biotechnologically superior in quality, reproducibility and yield compared to other including conventional production systems. All four clinical products derived from GlycoExpress™ cells are produced using a **perfusion bioreactor** system in order to assure **highest possible product quality** and reproducibility combined with high yield production.

Experimental approach:

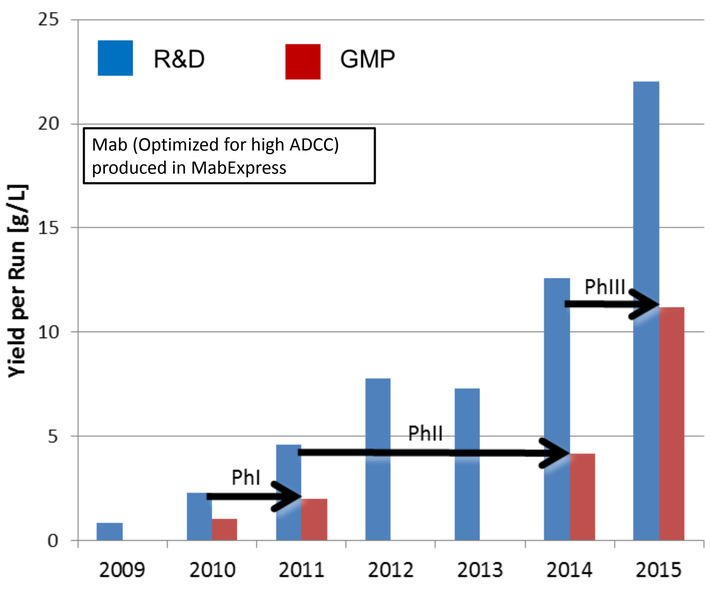
GlycoExpress™ cells producing mAb were cultivated with perfusion bioreactor systems applying different cell retention mechanisms such as centrifugation (centritech™) or alternating tangential flow (ATF™) filtration at different scales. A 10 mL based down-scale perfusion system with good comparability to larger scale cultivations was established. Growth, product yield and product quality, especially glycosylation, are evaluated during the cultivations.

Superior glycan structures and yield

GlycoExpress™ cells produce only high class human glycans. Glycosylation can have a huge impact on the bioactivity of recombinant proteins. For example, defucosylated antibodies as well as bisecting N-acetylglucosamine structures are known to increase ADCC activity and terminal sialic acids have an anti-inflammatory function and decreased cytoxicity. The figure on the right shows typical N-glycans for mAbs. Standard analytics at Glycotope cover other PTMs such oxidation, lysine clipping or deamidation as well.

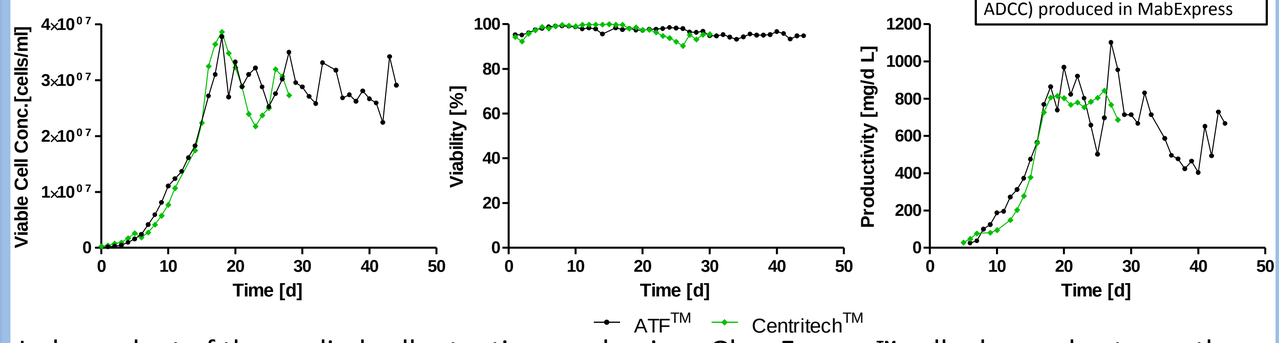


High productivity for glycooptimized products:
(total yield per 40 day perfusion run per bioreactor volume, R&D data)
~15-20 g/L IgG antibody
~10-15 g/L IgA antibody
~ 3 g/L IgM antibody
~0.2 - 2g/L for complex glycoproteins
(e.g.FSH, bloodfactors, hormones etc.)
- Hybrid process:
continuous USP, discontinuous DSP
- USP conditions
- optimized for ATF™ or Centritech™
- perfusion length: approx. 40 -100 day

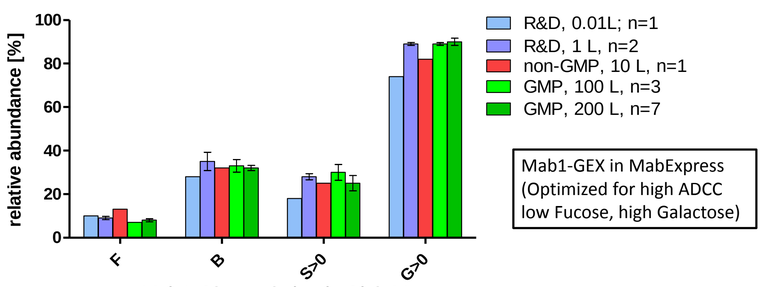


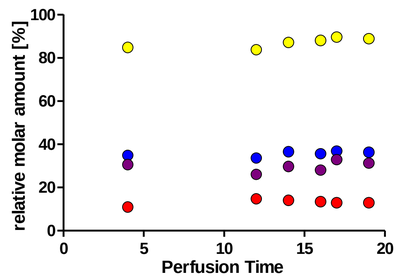
Production of IgG using GlycoExpress™

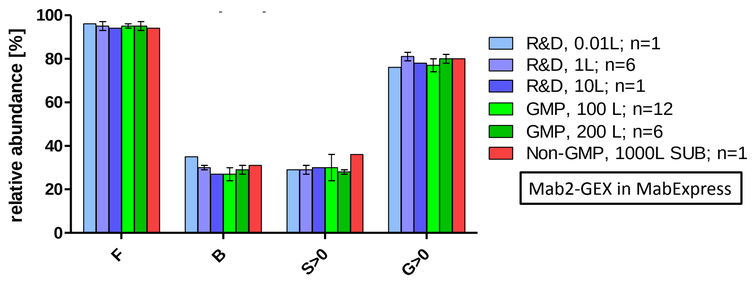
Glycoexpress™ is well suited to produce monoclonal antibodies (mAbs) in large quantities with tailor made glycosylation (e.g. defucosylated for high ADCC)

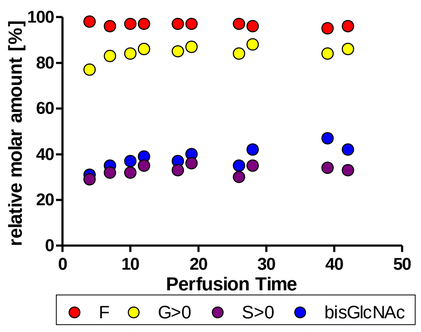


Independent of the applied cell retention mechanism, GlycoExpress™ cells show robust growth and consistent productivity.









No measurable differences in product quality between batches, batch sizes, reactor sizes, process control strategies, DSP scales and production site. Glycan structure analysis was performed by Instant AB™ labelling and HPLC-MS.

GlycoExpress™: A toolbox of glycoengineered proprietary human cell lines

Toolbox of proprietary human glycoengineered cell lines allows the **adjustment of various glycosylation parameters**

Glycooptimized Anti-Tumor BioSuperiors

CetuGEX™
(Colorectal, Head & Neck Cancers, and other new indications)

TrasGEX™
(Breast, Gastric Cancers, & other new indications)

Novel Glycooptimized Biotherapeutics

FSH-GEX™
(IVF and other indications)

Anti Tumor GlycoBodies

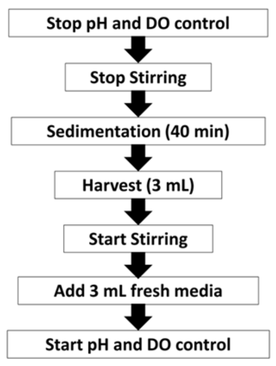
PankoMab-GEX™
(Ovarian, Lung, Breast & Colorectal Cancers)

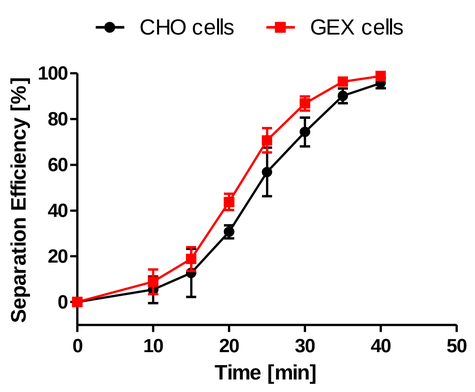
		Potential areas of improvement
1	mAbExpress	for **optimized glycosylation of antibodies** including optimization of bisGlcNAc, de-fucosylation, galactosylation and sialylation
2	SialoMax	for **maximal sialylation** Maximal sialylation potential, incl. α2-3 and α2-6 sialylation
3	SialoFlex	for **optimization of** the **sialylation degree** Allows generation of various sialylation degrees
4	FucoFlex	for **optimization of** the **fucosylation degree**
5	Other (non-disclosed)	

Fast & high yield expression of higher active antibodies & glycoprotein biopharmaceuticals
Gradual adjustment and combination of glycosylation parameters possible

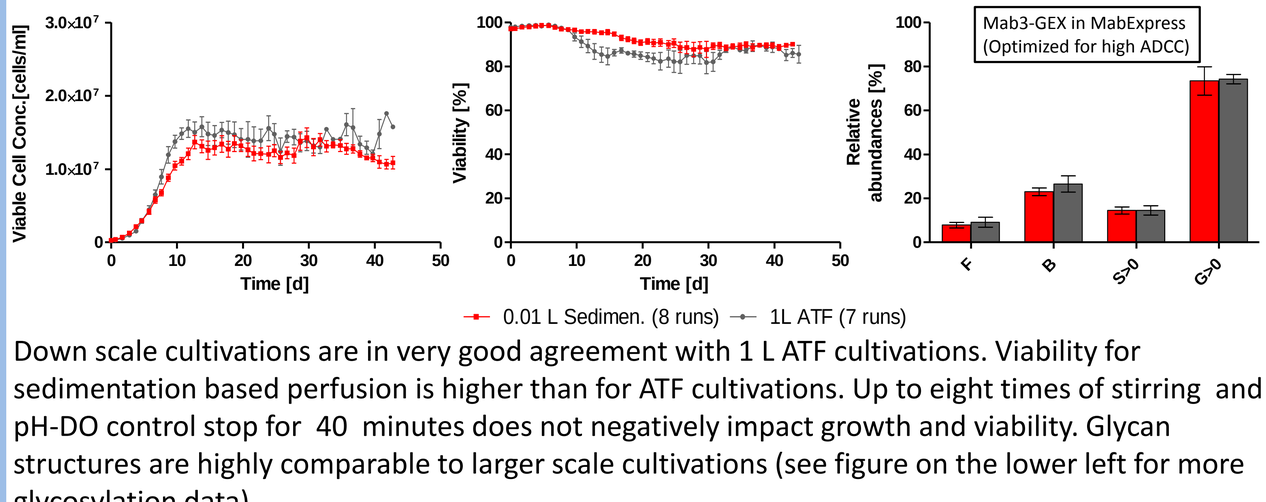
Development of downscale ambr perfusion system

For the efficient development of a perfusion process a good downscale system is necessary to evaluate different clones at an early stage as well as to perform process optimization with a system as close as possible to the final production scale. Here, we describe first data of small scale perfusion system based on sedimentation in a micro-bioreactor system. For cell retention , stirring and pH/DO control is stopped allowing cells to settle. After a specific time cell free harvest is removed from the top of the bioreactor and replaced by fresh medium. This procedure is then repeated several times to enable a perfusion rate of up to 2 reactor volumes a day. All steps are done autonomously by programmable pipetting robot.

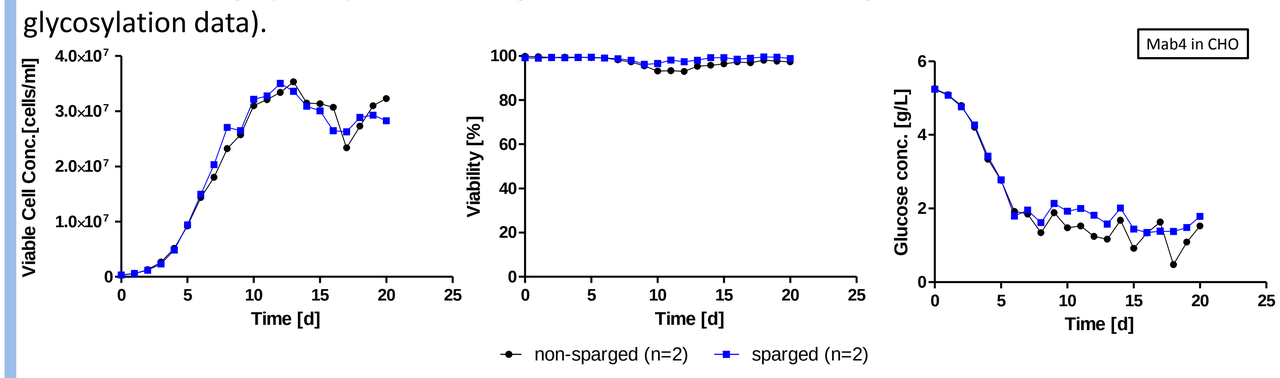




40 minutes are sufficient to achieve a separation efficiency of >99% for GEX and CHO cells. Shorter sedimentation time can be used to implement automatic bleeding.



Down scale cultivations are in very good agreement with 1 L ATF cultivations. Viability for sedimentation based perfusion is higher than for ATF cultivations. Up to eight times of stirring and pH-DO control stop for 40 minutes does not negatively impact growth and viability. Glycan structures are highly comparable to larger scale cultivations (see figure on the lower left for more glycosylation data).



The sedimentation based ambr perfusion is applicable to CHO as well. CHO cells show good growth and high viability independent of the aeration strategy for a non-optimized perfusion process. Data for larger scale CHO perfusion is not available yet.

Conclusion

The GlycoExpress™ technology is a platform for the high yield production of recombinant proteins with fully human glycosylation. In combination with perfusion culture cells are kept in the optimal growing and production phase over the production process which leads to highly stable product quality allowing a flexible duration of the run in one batch size in combination with stable high productivity of the cells over time. Furthermore the product qualities produced in different scales ranging from 10 mL to 1000 L cultivation vessels are highly comparable. A small scale perfusion system was successfully established for GEX and CHO cells with good comparability to 1L ATF perfusion cultures.