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# Up and down scale considerations for the continuous production of glycooptimized biopharmaceuticals

Steffen Kreye

*Glycotope GmbH*, [Steffen.Kreye@glycotope.com](mailto:Steffen.Kreye@glycotope.com)

Rainer Stahn

*Glycotope GmbH*

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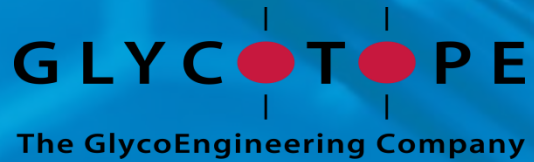
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Applying world-leading glyco-biology  
to immuno-oncology

**Up and down scale  
considerations for  
the continuous  
production  
of glycooptimized  
biopharmaceuticals**  
September 18<sup>th</sup> 2017



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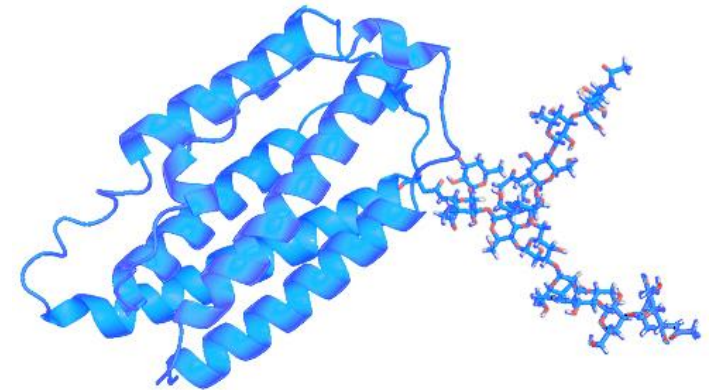
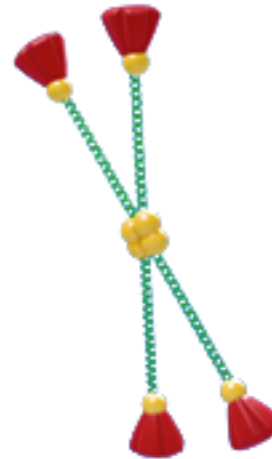
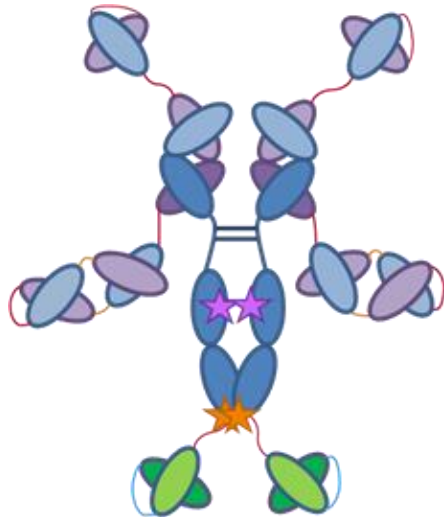
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# Biopharmaceutical expertise at Glycotope

- ▶ Experts in perfusion production of biopharmaceuticals
- ▶ Proprietary human GEX™ cell line
- ▶ well suited for glyco-optimized and hard-to-express proteins



★ Glycosylation

Source: <http://www.airwaytherapeutics.com/at-100/>

Source: <http://www.nyhf.org/wp-content/uploads/2017/05/glycoprotein.jpg>

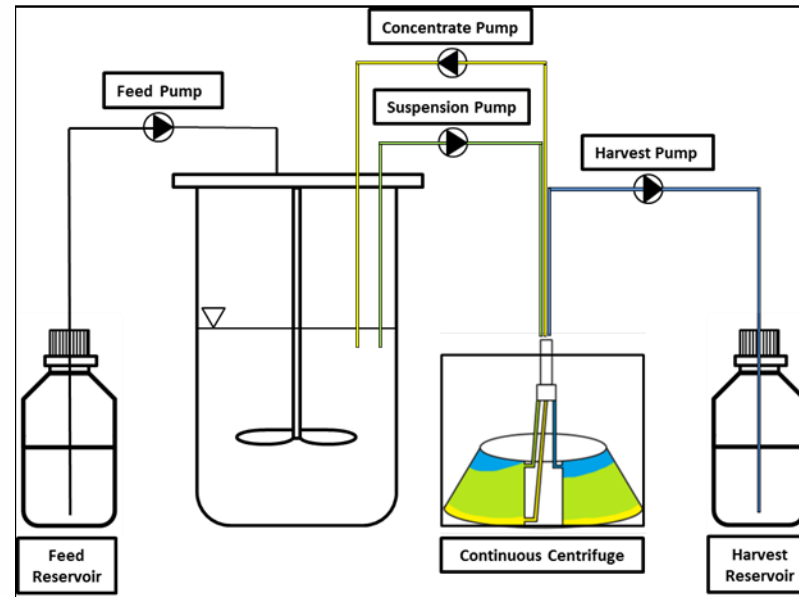
# SAM-perfusion, Up-Scaling and Down-Scaling

Down-Scale 12mL



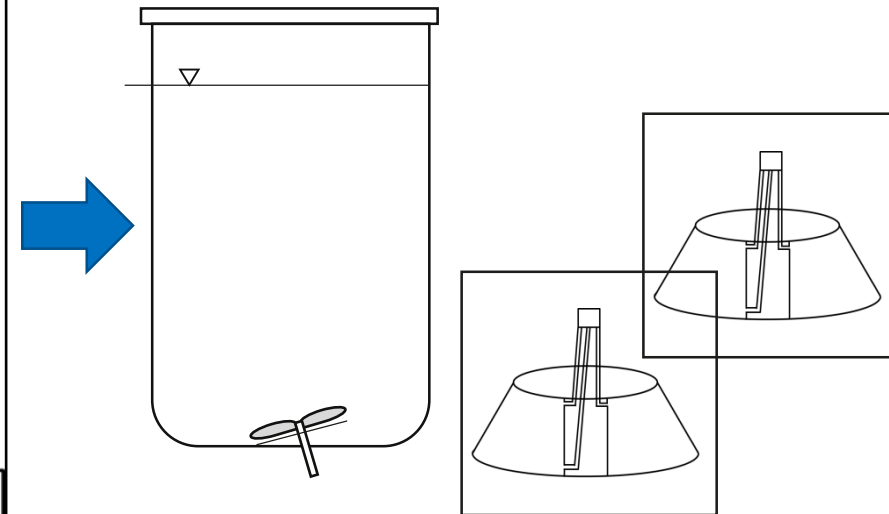
SAM-Perfusion  
DOE  
SUB

Lab-Scale 1L



Centritech  
ATF  
Stainless steel / SUB

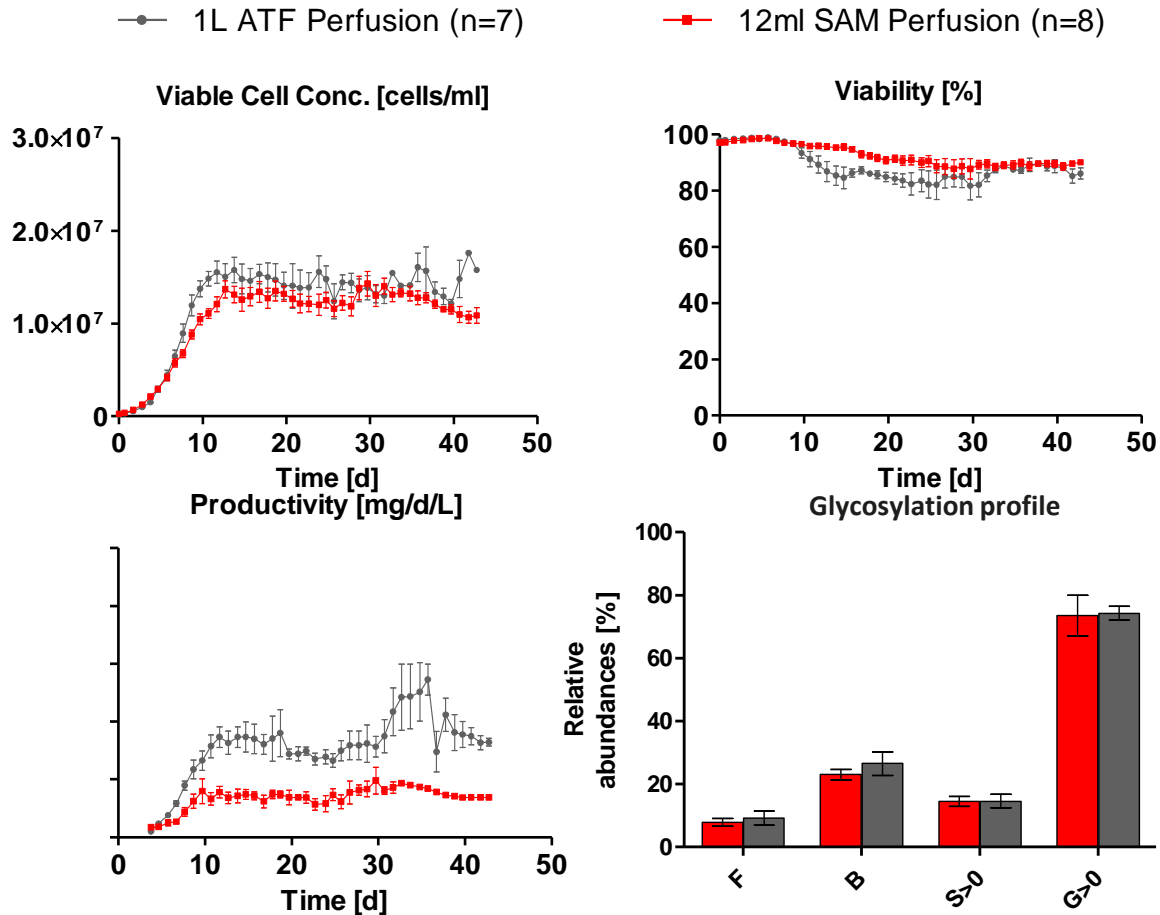
Up-Scale 1000L



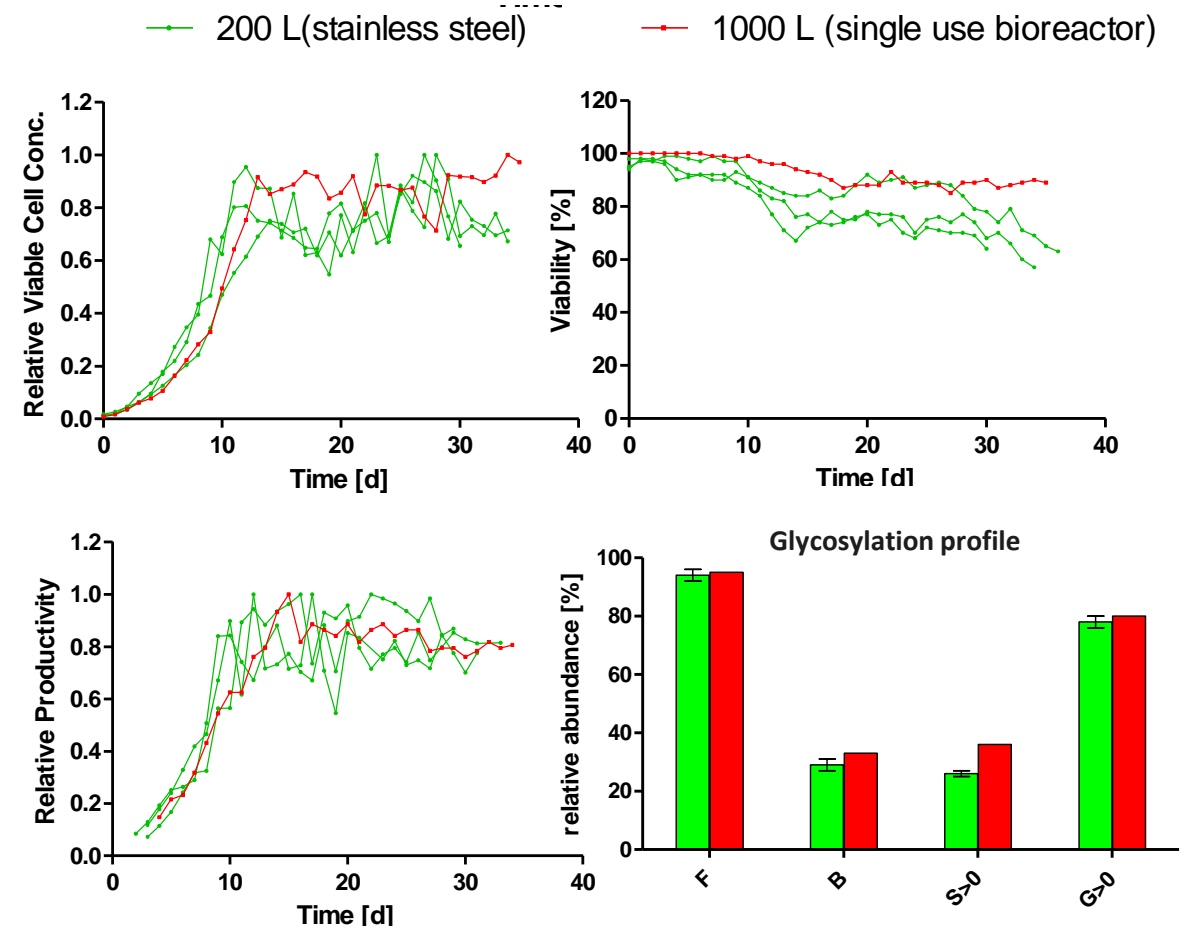
Centritech  
ATF  
SUB

# Results confirm approaches

## Downscale feasible using SAM-Perfusion in AMBR15



## Up-Scale feasible to 1000L SUB





**GLYCOTIPE**

The GlycoEngineering Company

Applying world-leading glyco-biology  
to immuno-oncology

**Glycotope GmbH**

Robert-Roessle-Str.10  
13125 Berlin  
Germany

[Vicky.Goralczyk@Glycotope.com](mailto:Vicky.Goralczyk@Glycotope.com)

**Thank you!**

September 18<sup>th</sup> 2017



## Up and down scale considerations for the continuous production of

### glyco-optimized biopharmaceuticals

**V. Goralczyk, S. Kreys, R. Stahn, A. Danielczyk**

Glycotope GmbH, Berlin, Germany

Vicky.Goralczyk@glycotope.com

Backgrund

Product quality especially with respect to glycosylation is a critical product attribute which can have an immense influence on the activity of glycoproteins and should be closely monitored during process development and manufacturing. GlycoExpress® cells (GEX) have been developed in this context to provide human host cell lines with robust and optimized glycosylation machineries. This work will address challenges in two case studies for up and down scaling of production processes with focus on product quality and continuous processing.

**GLYCOTOP**

The GlycoEngineering Company

A 10 mL down-scale system for perfusion cultivations in a microbioreactor is introduced and its application for process development is evaluated.

#### Down-Scale

#### Development of downscale SAM 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 suitable system. Here, a small scale perfusion system is described which is based on sedimentation in a micro-bioreactor system (SAM). For cell retention, stirring and pH/O<sub>2</sub> control is stopped allowing cells to settle (Figure 1 A). After a specific time cell free harvest is removed (Figure 1 B) 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 automatically by programmable pipetting robot.

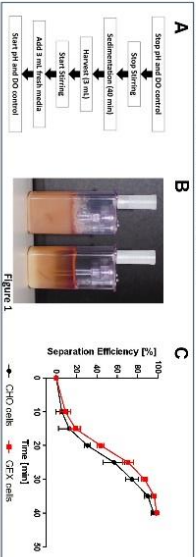


Figure 1

40 minutes are sufficient to achieve a separation efficiency of >95% for GEX and CHO cells (Figure 1 C). Shorter sedimentation time can be used to implement automatic bleeding.

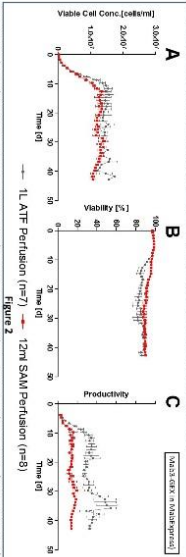


Figure 2

Down scale cultivations are in very good agreement with 1 L ATF cultivations (Figure 2 A,B). Viability for sedimentation based perfusion is higher than for ATF cultivations (Figure 2 B). Only productivity is lower for SAM perfusion, probably due to the stop of the stirrer (Figure 2 C).

#### Fully Comparable Product Quality for SAM-Perfusion

Glycan structures (Figure 4) as well as other product quality attributes (Table 1) are highly reproducible and comparable to larger scale cultivations (in this case to 1 L ATF)

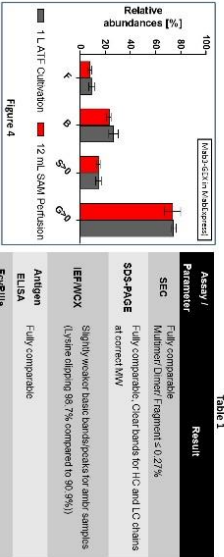


Figure 4

Further product quality attributes (Table 1) are also comparable between SAM perfusion and ATF perfusion. Only bioreactor focusing (EF) and weak cation exchange (WCA) chromatography show deviation due to differences in lysine clipping.

**SAM Perfusion correctly displays the glycosylation pattern of larger scale cultivations**



Glycosylation profile of material obtained at the 1000 L scale in SUB is comparable to other scales

A GlycoExpress-based Phase II GMP perfusion process for mAb production at the 200 L stainless steel scale was successfully transferred to a 1000 L single-use bioreactor. Growth, productivity and stable glycosylation were maintained during scale up. The developed perfusion down-scale system SAM shows very good comparability to larger scale ATF runs not only for USP characteristics but also for product quality.

#### Up-Scale

Data of a process transfer from a 200 L stainless steel to a 1000 L single-use bioreactor for a continuous cell culture application is discussed. The impact of engineering aspects for the bioreactor and the cell retention device as well as product quality considerations are addressed

#### Transforming a perfusion process from stainless steel to a 1000 L single-use bioreactor

An established GMP GEX-based perfusion process is transferred from the 200 L stainless steel scale to 1000 L single use bioreactor. Cell retention is achieved by continuous centrifugation (Centrect®) intermediate steps in the seed train are replaced with single-use technology as well (Figure A).

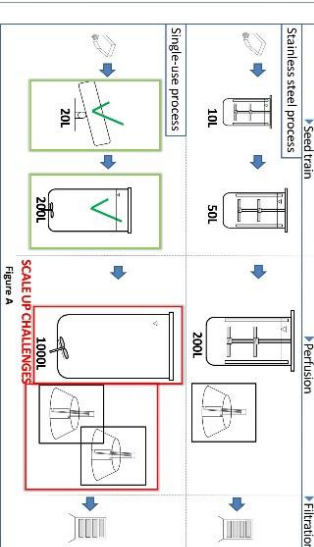


Figure A

Design space for specific power input, impeller tip speed, impeller tip shear and mixing time was defined at the 1 L scale. Scale-up was based on constant power input which resulted in critical mixing times for the 1000 L scale. An increase in specific power input for the 1000 L (and to a smaller extent for the 200 L scale) decreased mixing time to an acceptable value while also keeping tip speed and tip shear within specification.

For scale-up of the continuous centrifuge (Centrect®) residence time in the centrifuge and centrifugal forces should be kept constant (pump rates, cell densities in concentrate, separation efficiency will change).

Upstream data for 1000 L SUB (red) is comparable to 200 L (green) (Figure B). Viability is slightly increased while cell growth and productivity are comparable.

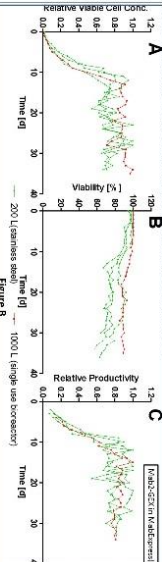


Figure B

Results of glycan profiling (Rapifluor labeling + UPLC/MS/MS (Figure C) show full comparability for 1000 L material to 200 L scale material. Additional assay e.g. PAE, AG Binding, ADCC and further PTMs are within specification as well.

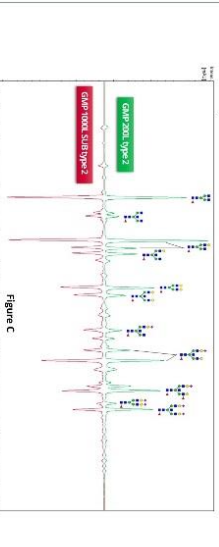


Figure C

Glycosylation profile of material obtained at the 1000 L scale in SUB is comparable to other scales

F: Focussation; S: Staining; C: Colocalization