

Operating 100 L Moss Bioreactors Secreting mAbs with Defined Mammalian-like Glycosylation

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Presentation Outline

1. Bryotechnology
2. Designer Glycosylation
3. Moss Bioreactors: USP & DSP

Company Facts – 10 Years of Successful Operations

Company

Founded in 1999: University of Freiburg spin-off

Venture Capital financed

25 members of staff

Headquartered in Heilbronn, Germany

Technology Development in Freiburg, Germany



Bryotechnology

Production of biologicals (glyco-engineered where applicable)

Bryo-Speed™ - transient screening platform

Bryo-Master™ - stable, transgenic production platform

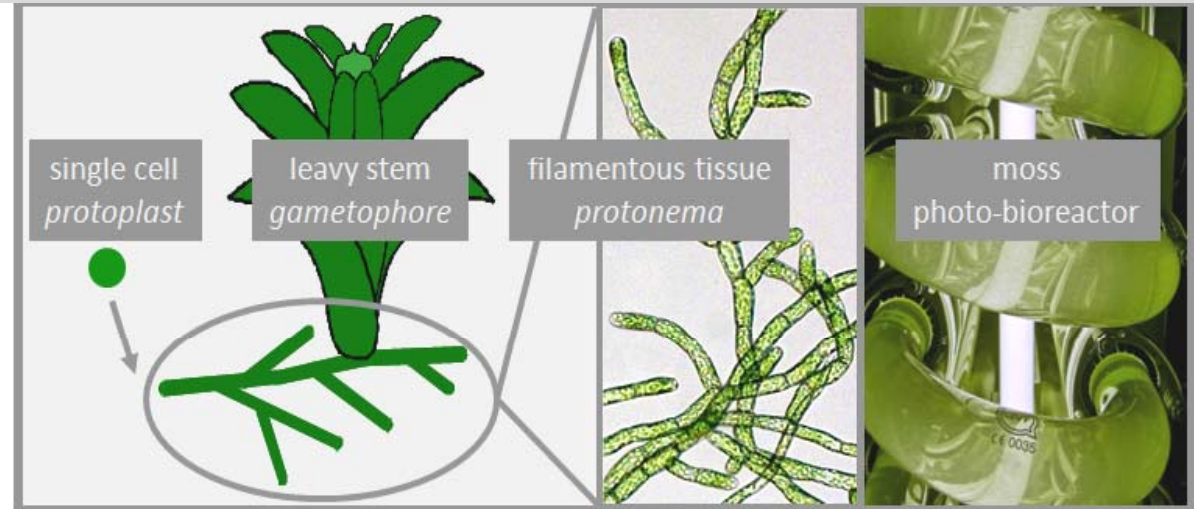
Numerous granted patents out of six patent families
(expression technology and glyco-engineering)

Additional patent applications filed



Moss (*Physcomitrella patens*) as a novel expression platform for pharmaceutical proteins

Biological Advantages

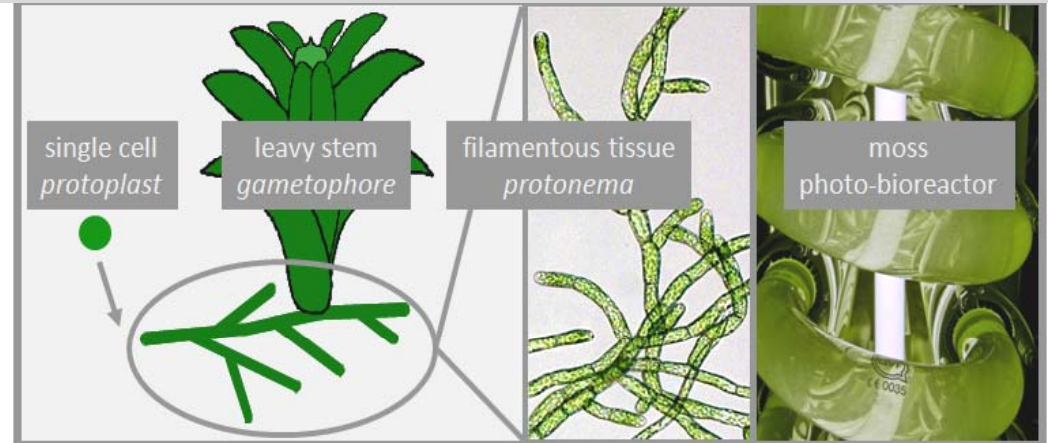


- Haploid during most of ontogenetic development
- High homologous recombination frequency
- homologous recombination => gene integration into targeted site of genome
- Genetically stable transgenes
- Clonal propagation of transgenes, no sexual propagation
- Genome is fully sequenced and largely annotated

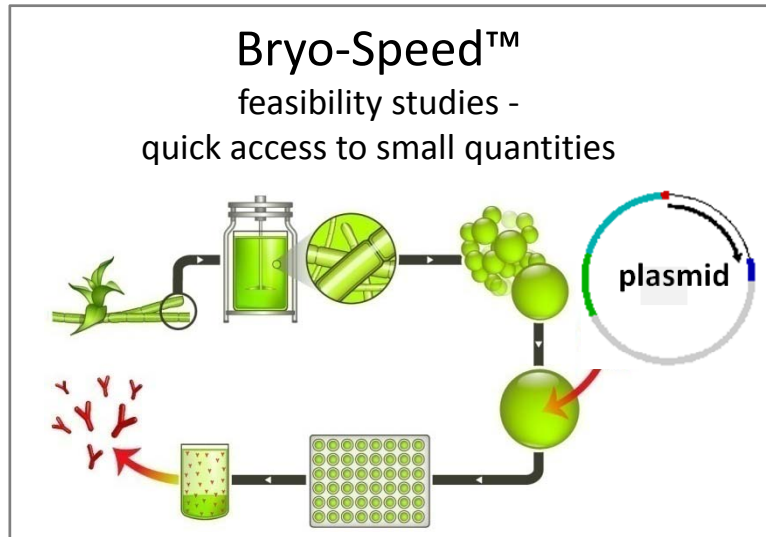
Moss (*Physcomitrella patens*) as a novel expression platform for pharmaceutical proteins

System Advantages:

- No protease issue
- Wide range of process pH
- Wide range of process temperature
- no need for codon optimization for mammalian genes
- PEG-mediated DNA transfer; marker-free transgenic plants achievable
- robust tissue in culture (protonema)
- no virus or mycoplasma issue
- photoautotrophic => light & CO₂ & minerals in water (no animal-derived products)
- fully contained production system
- product secreted into media resulting in high initial purity of greater 80%



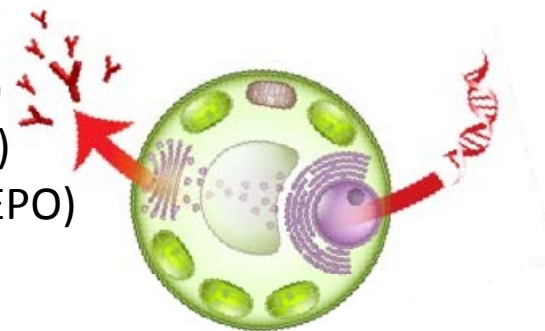
Secretion-based Expression Technologies



linearized expression construct - integrated

Secreted glyco-proteins tested:

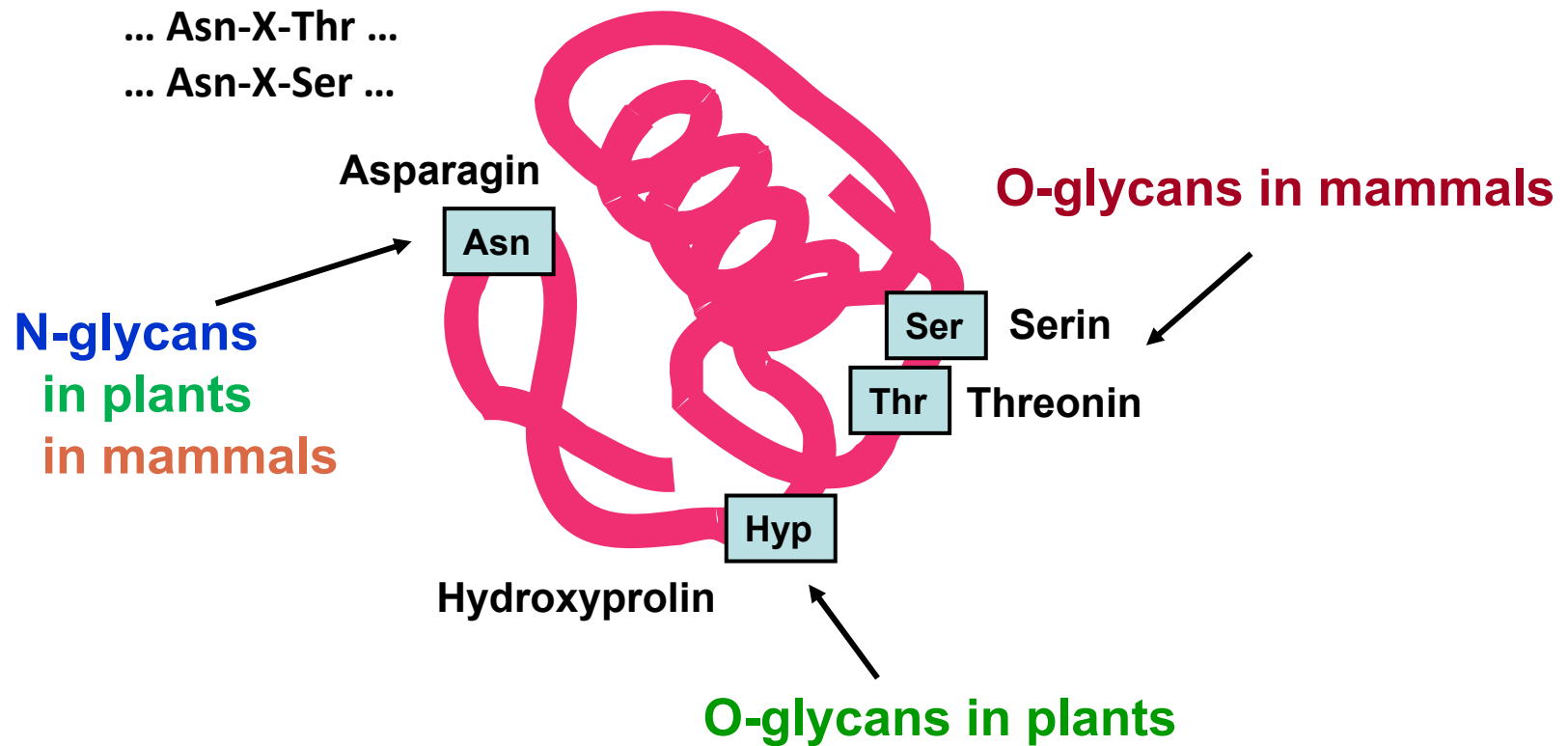
- growth factor (VEGF)
- serum proteins (HSA)
- peptide hormones (EPO)
- enzymes
- vaccines
- mAbs



Moss Promotor
Moss 5' UTR (incl. 5' Intron)
Plant Signalpeptid
Gene of Interest
Moss 3' UTR / Term

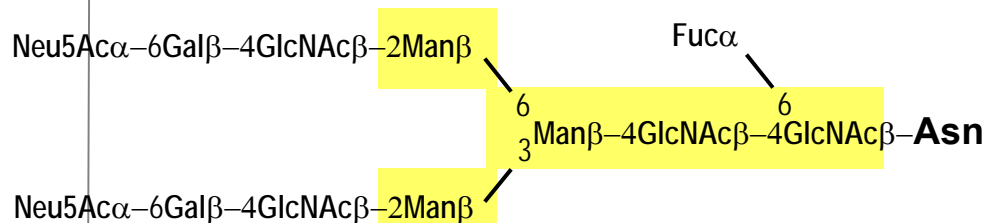
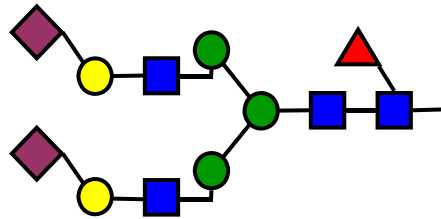
Therapeutic Proteins are Often Glycosylated

Consensus Sequence for N-Glycosylation
(provided spatial accesibility):



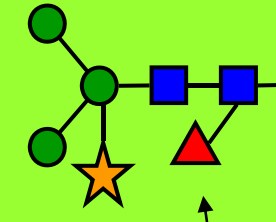
Basic Differences: Mammalian vs. Plant N-Glycans

A mammalian N-glycan

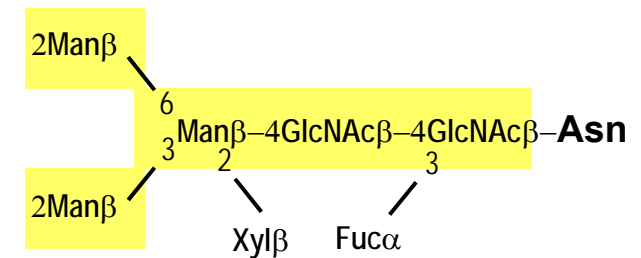


- GlcNAc *N*-acetylglucosamin
- Man mannose
- Gal galactose
- ▲ Fuc fucose
- ★ Xyl xylose
- ◆ Neu5Ac *N*-acetylneuraminic acid

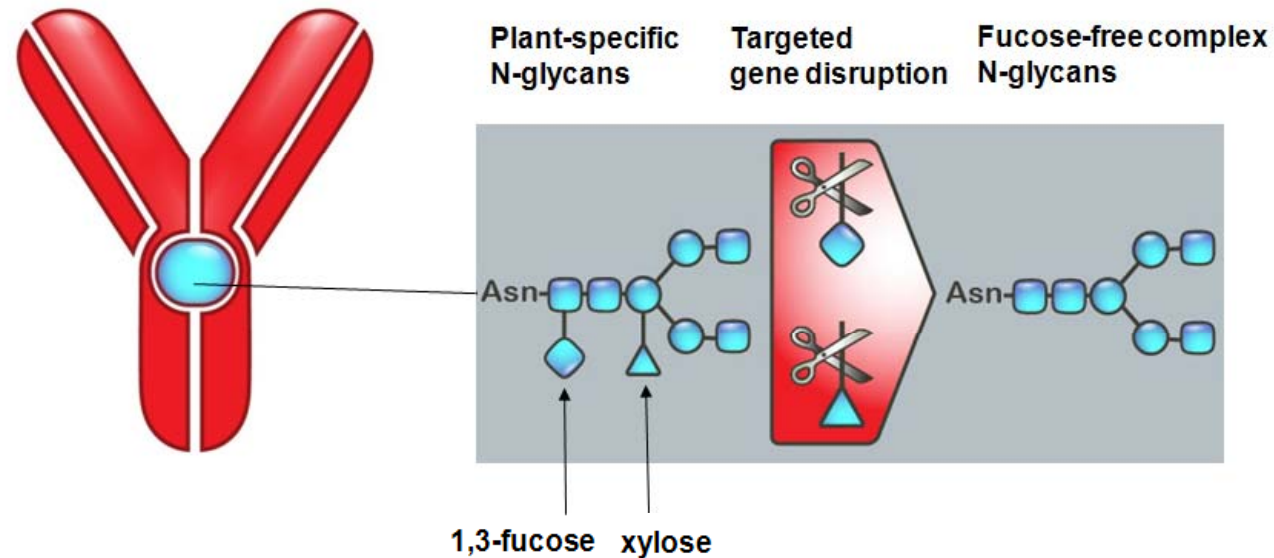
A plant N-glycan



β -1,2-Xylose and core α -1,3-fucose are foreign to mammals = immunogenic potential



Designer Glycosylation, 1: Elimination of Immunogenicity

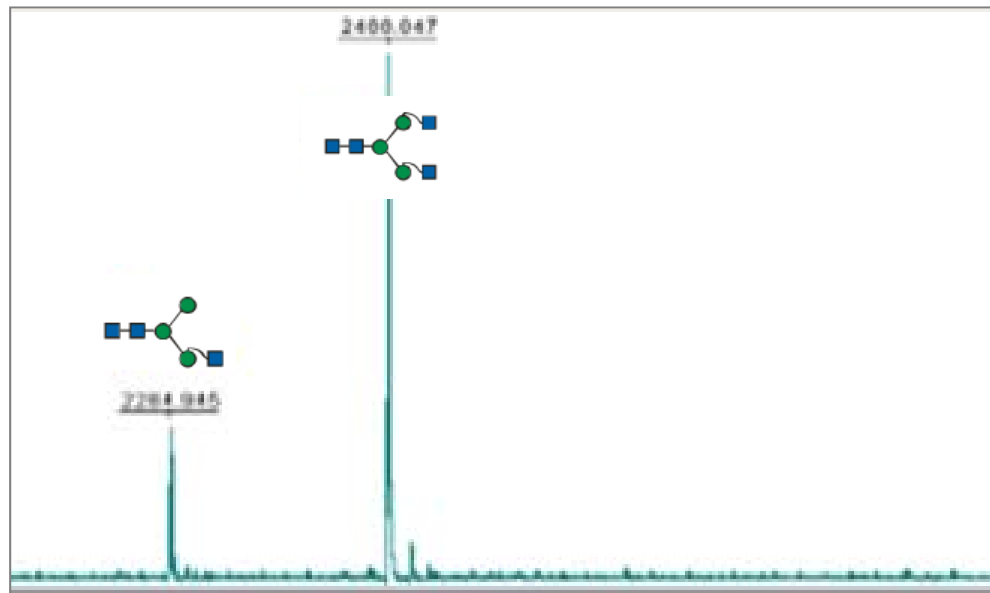


We specifically knocked out two genetic loci that encoded for plant-specific glycosylation:
 β -1,2-XylT and α -1,3-FucT

Result: Near Homogeneous Glycan Structure

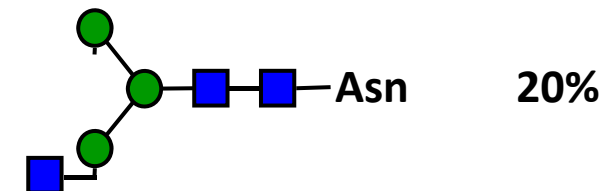
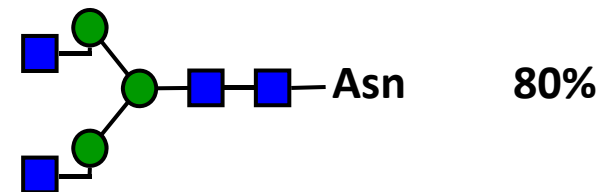
Mass Spectroscopy (MALDI TOF)

Peptide mapping



■ GlcNAc *N*-acetylglucosamin
● Man mannose

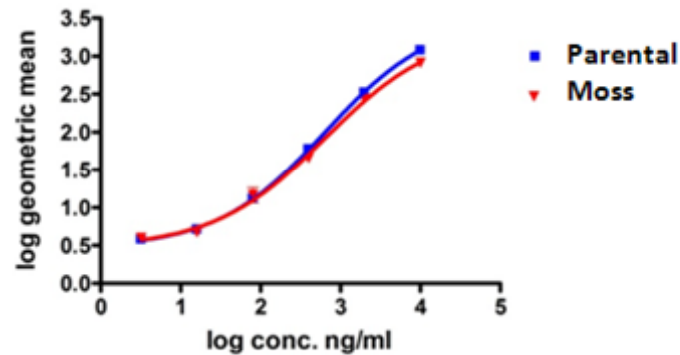
- High homogeneity
- Sequence integrity
- Correct signal peptide cleavage
- C-terminus without Lysine
- Confirmed for several mAb



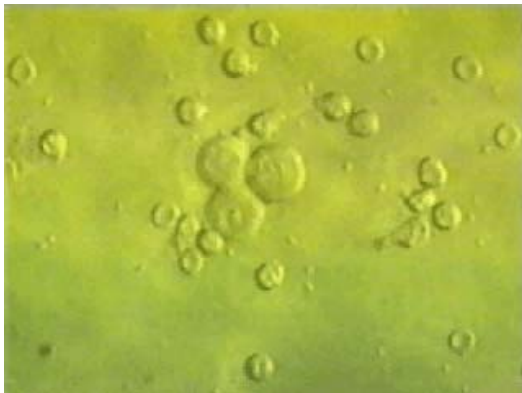
Designer Glycosylation, 2: Better ADCC

FACS

Binding to target cancer cells



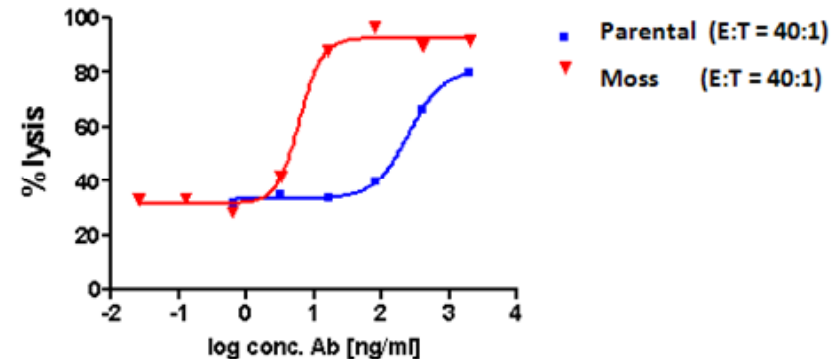
Fluorescence Activated Cell Sorting



Confirmed for several mAbs

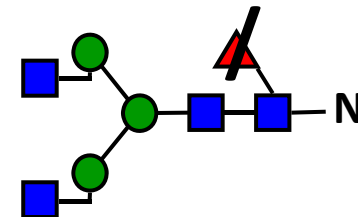
ADCC

Lysis of target cancer cells



Antibody Dependent Cellular Cytotoxicity

Core α -1,6-fucosylation intrinsically not carried out in plants
=> lack on mAbs increases effector functions (enhanced ADCC)

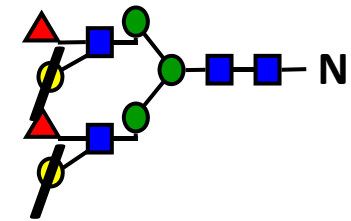


Designer Glycosylation, 3: Elimination of Immunogenicity

Target: Elimination of Lewis A Antigenic Epitope

Targeted Gene

Knock out of β -1.3 galactosyltransferase gene



Protein Example

Erythropoietin (EPO)

Result

Lack of β -1.3 galactose prevents the addition of α -1,4-Fucose

Hence:

No Lewis A glycan structure formation, an important blood group antigen

■ GlcNAc *N*-acetylglucosamin

● Man mannose

● Gal galactose

▲ Fuc fucose

Designer Glycosylation, 4: Adding Novel Glycan Structures

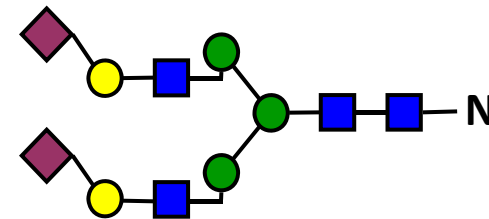
Target:

Complex, mammalian-like glycan structure

Targeted Genes

Transfer of:

β -1,4-galactosyltransferase, and of five additional genes of the *N*-acetylneuraminic acid pathway

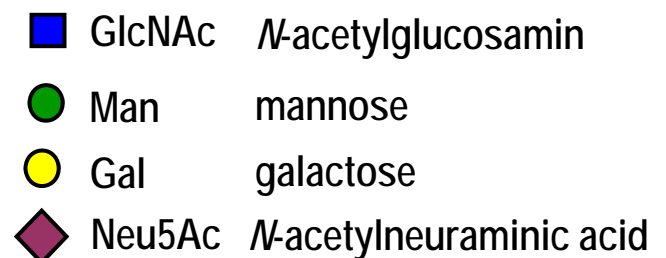


Protein Example

mAb

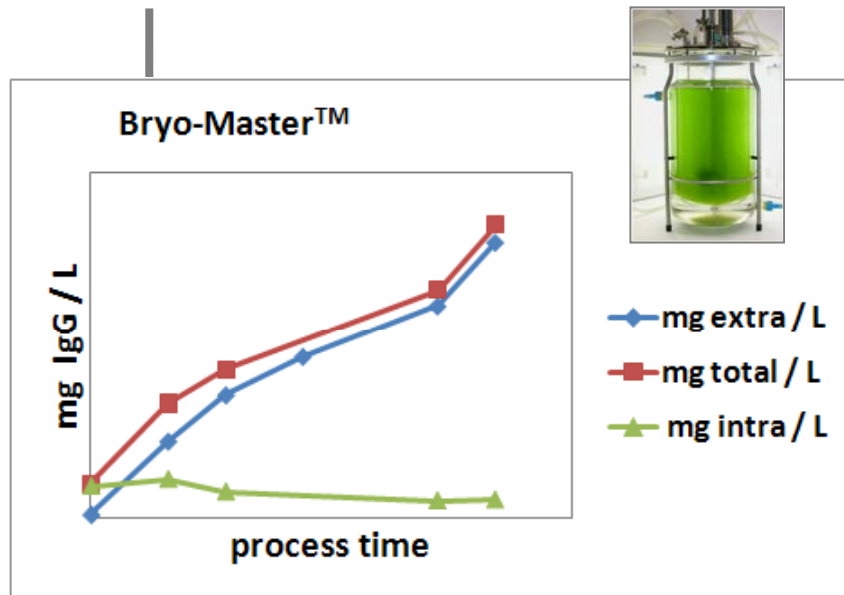
Result

Optimization of expression is under way



Case Study – Secretion of Therapeutic mAbs

Actual Production Data
(5 L stirred tank photo-bioreactors)



Typical Titres upon harvest
~ 50 mg / L

Specific Productivities
~ 2 mg mAb / (L x Days)

Transferred to Industrial Scale (≥ 100 L)
Systematic Optimization

Development of Pilot Scale Production Platforms

Two Types of Photo Bioreactors -PBR-

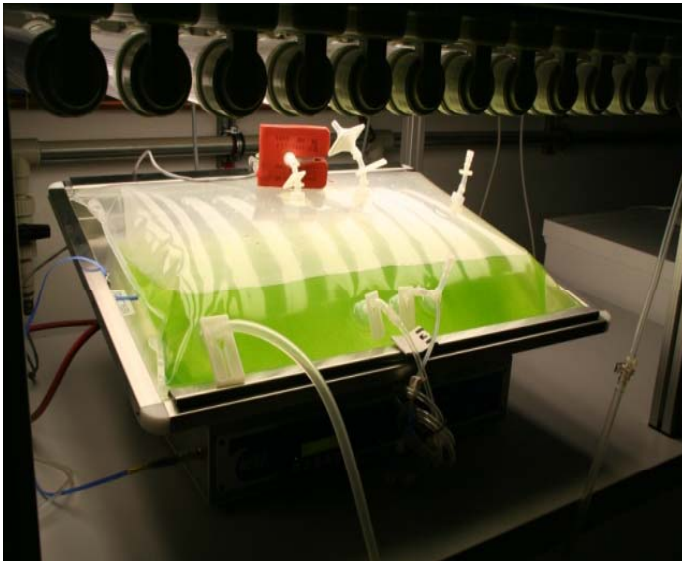
Glass Tube PBR (100 L)



Development of Pilot Scale Production Platforms

Two Types of Photo Bioreactors -PBR-

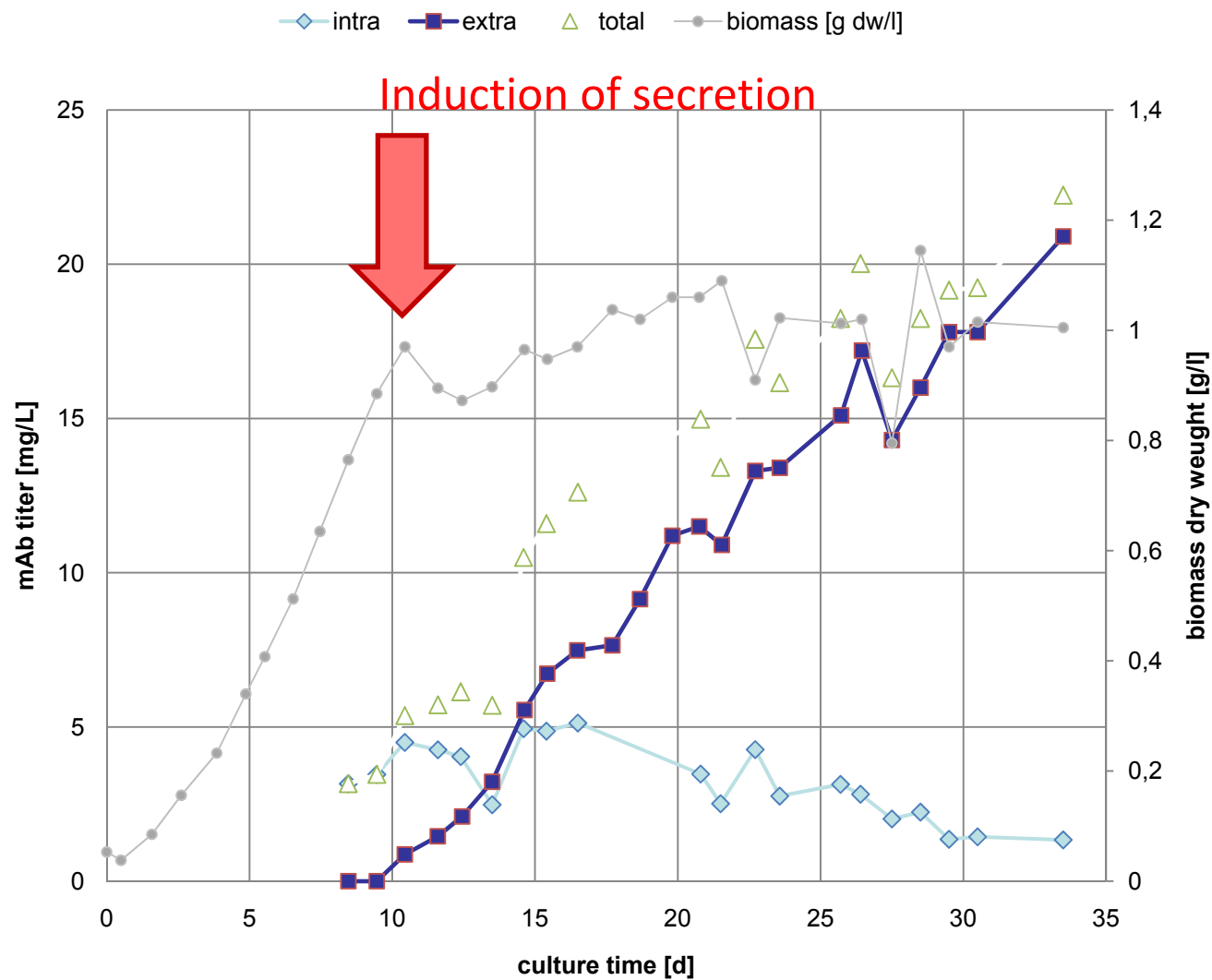
Disposable Bag (10 L)



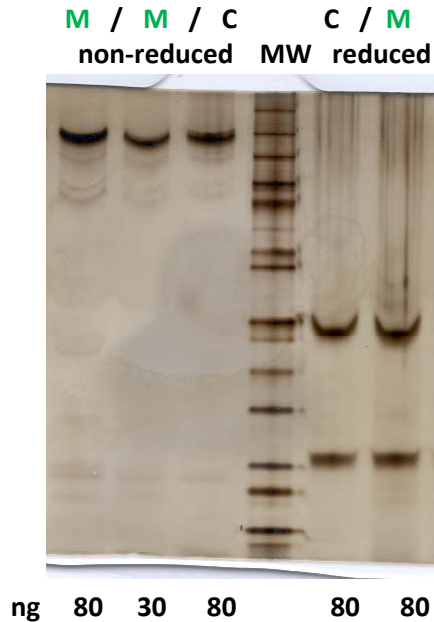
Disposable Bag (2 x 50 L)



Pilot Production Run 100 L Tubular PBR mAb Production



Case Study – Therapeutic mAbs



Bryo-Master™ mAbs SDS-PAGE, 80 ng, - Silver-stained -

M: crude moss photo-bioreactor supernatant

C: CHO-produced mAb and purified

=> low background of host cell contaminants

- secretion => high initial purity
- simple salt medium
- broad pH-range possible
- no animal derived products
- no virus or mycoplasma issue
- no proteolytic problem

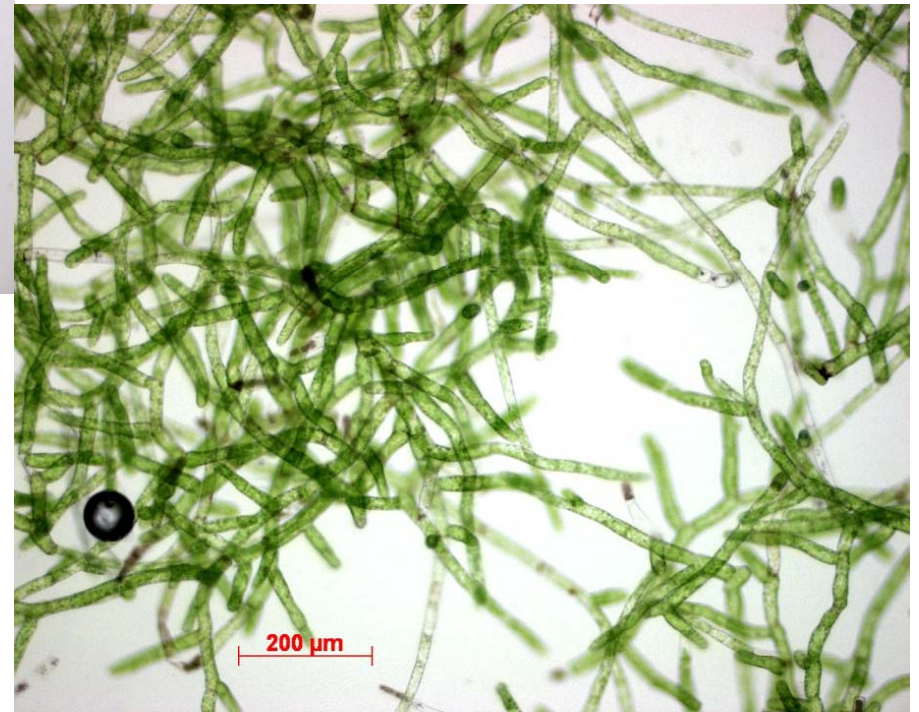
**Advantageous
downstream processing**

Pilot Production Run, 100 L Tubular PBR

Defined cell morphology during cultivation



Doc 5:
five days before induction of secretion



Doc 14:
four days after induction of secretion

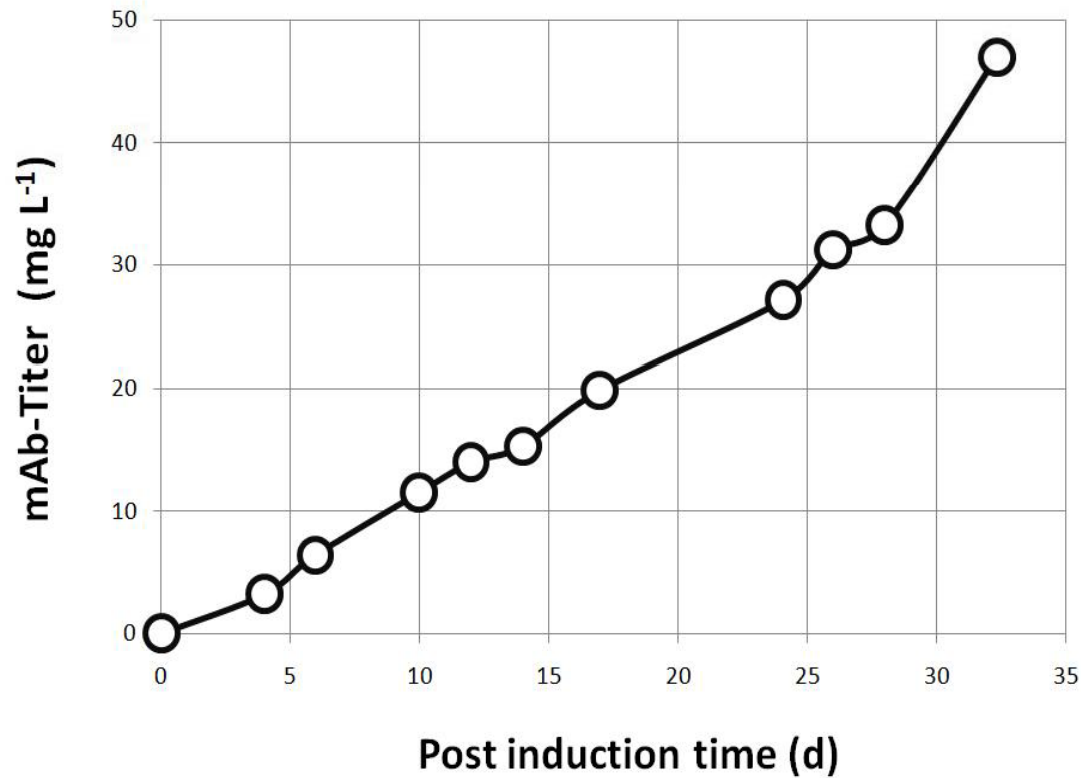
Pilot Production Run

100 L Disposable Bag “Wave” PBR



Wave Reactor mAb-Production After Secretion Induction (20L+200L)

 **Induction of Secretion**



Biomasse:

4 g dw/l

Titer:

>60mg/l

Summary Disposable Bag PBR

- Transferability to 100 L pilot scale is achieved
- Volumetric secretion rates up to $3,1 \text{ mg L}^{-1} \text{ d}^{-1}$
- Next step: Testing 500 L disposable bio-bag reaktor
- Flexibility and speed of process design
- Customized process design
- Easy transfer to CMO



Downstream Process Design (DSP)

From harvest of reactor to purified product

- Separation of biomass from supernatant
- Cascade of depth filtration steps
- Aseptic filtration
- TFF: Buffer exchange and/or Concentration step
- Capture chromatography (Membrane chromatography, EBC, Mixed mode resin)
- Principal purification step
- Polishing purification step (Membranes)
- Fill-finish

DSP Design Criteria

- Scalability
- Efficiency in downstream processing (DSP): Newest materials and concepts
- Simplicity of process design: Capital vs. disposables
- Least amount of process steps: Minimizing potential for errors
- Continuous process design
- Shortest amount of process time: Production time is very costly
- Hence: Most economical design of process



Thank You! Questions?