Is microbial production a good option for exceptionally low cost monoclonal antibodies for global access?

March 30, 2025

Vijay Yabannavar, Ph.D.

Gates Foundation

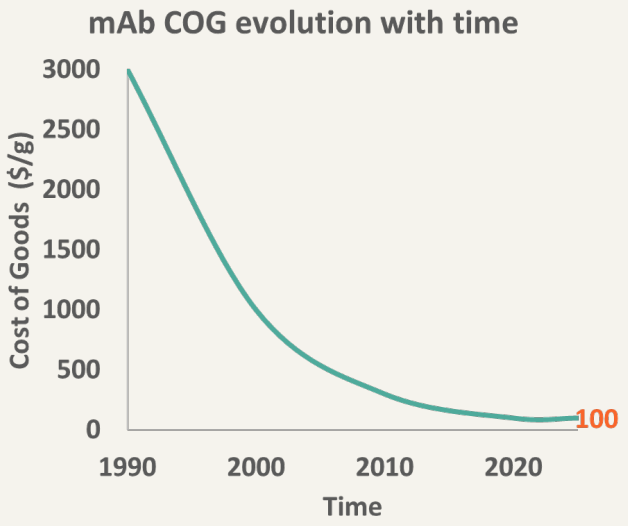
Presentation Outline

1. mAbs COGs Overview
2. N-linked Glycosylation of mAbs
3. Glycosylation Impact on Immunogenicity, Pharmacokinetics and Pharmacodynamics
4. Production of mAbs in E.coli
5. Production of mAbs in Pichia
6. Production of mAbs in Filamentous Fungus ‘C1’
7. Summary of mAbs Production in Microbial Systems
8. mAbs COGs Grand Challenge RFP – Gates Foundation and LifeArc

mAbs COGs Overview

*Looking forward, we anticipate **increased demand** for anti-infectives and high dosing requirements. **Our COGS target is ambitiously ≤$10/g which equates to ~$5/dose, considering other competitive interventions.***

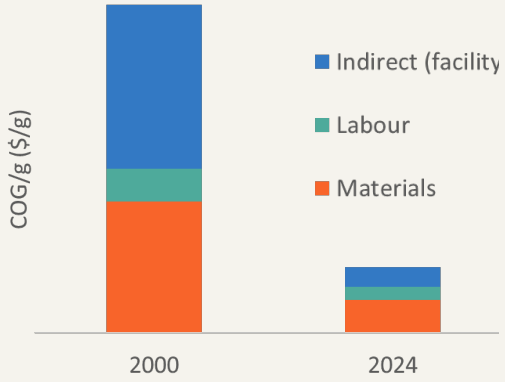
Over the last 30 years, CHO costs have plateaued to around $50-100/g



	1990-1999	2000-2009	2010-2019	2020-
Typical titer	0.5-2 g/L	1-3 g/L	4-8 g/L	8-15 g/L
Typical yield	40-50%	65-70%	70-75%	75-80%
COG ($/g)	$2,000	$500	$200	$100

The table above illustrates published CHO COGS. Lower ranges may be possible, but additional data is needed

Material costs drive a large percentage of total COGS

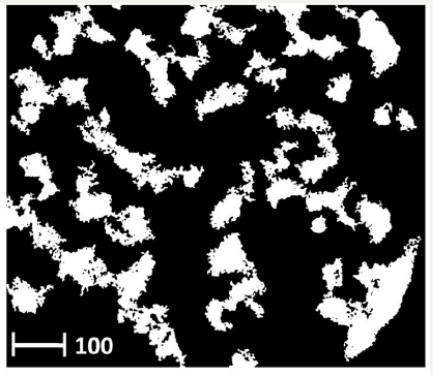


- Increased titers, yields and continuous processing have led to large reductions in facility costs
- Material (i.e. culture media, resins and consumables) have a disproportionate impact on COGS (>60%)
- Investments in more efficient CHO cells and/or alternative hosts and new purification methods are needed

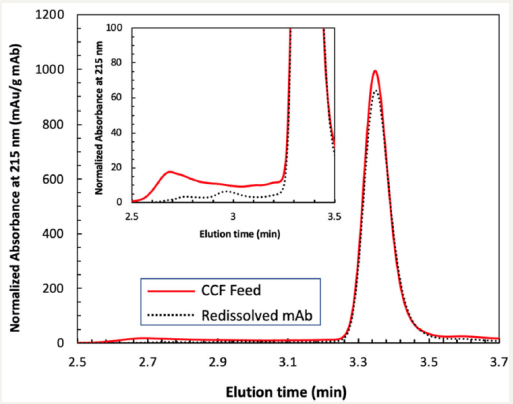
Innovations to drive the costs down

Over the next 1-2 years , our focus is on establishing POC

- Alt hosts may reduce COGS (~30%) due to cheap growth medium, fast upstream process and absence of adventitious agents.
- But pichia, yeast and E. coli have had some challenges with productivity or glycosylation that need to be overcome.
- New downstream processing options like precipitation or membrane chromatography are being explored to reduce costs.



Precipitated mAb by microscopy



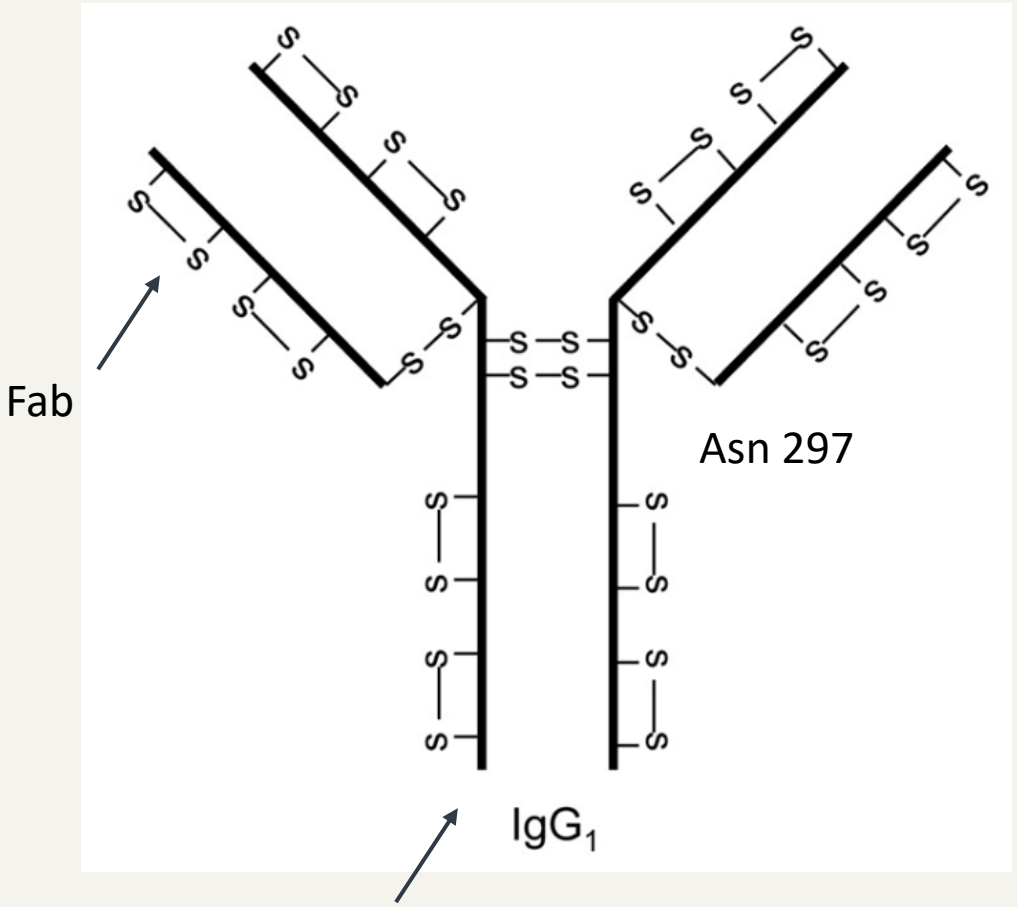
- We have launched a **Grand Challenge RFP** to reduce COGS to ≤$10/g, seeking applications from a broad range of industries (incl. parallel industries such as plasma fractionation)

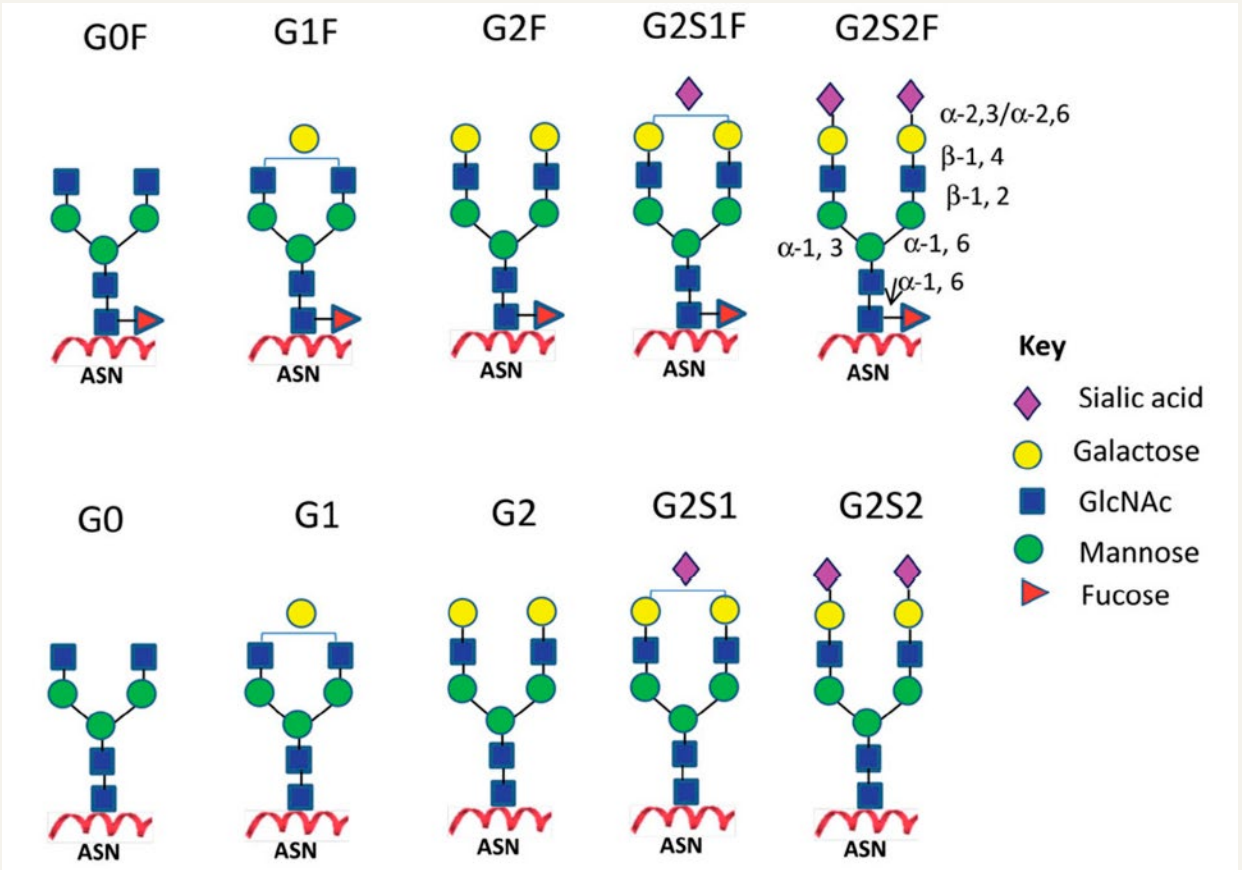
Ultimately, Gates Foundation's plan is to **develop the most successful POC further to achieve our ≤$10/g goal** at scale with additional funding.

Glycosylation Overview

N-Linked Glycosylation of mAbs

Boune S. et. al, Antibodies, 2020, 9(2), 22





Major N-linked glycoforms of therapeutic monoclonal antibodies (mAbs)

Immunogenicity of mAbs

Boune S. et. al, Antibodies, 2020, 9(2), 22

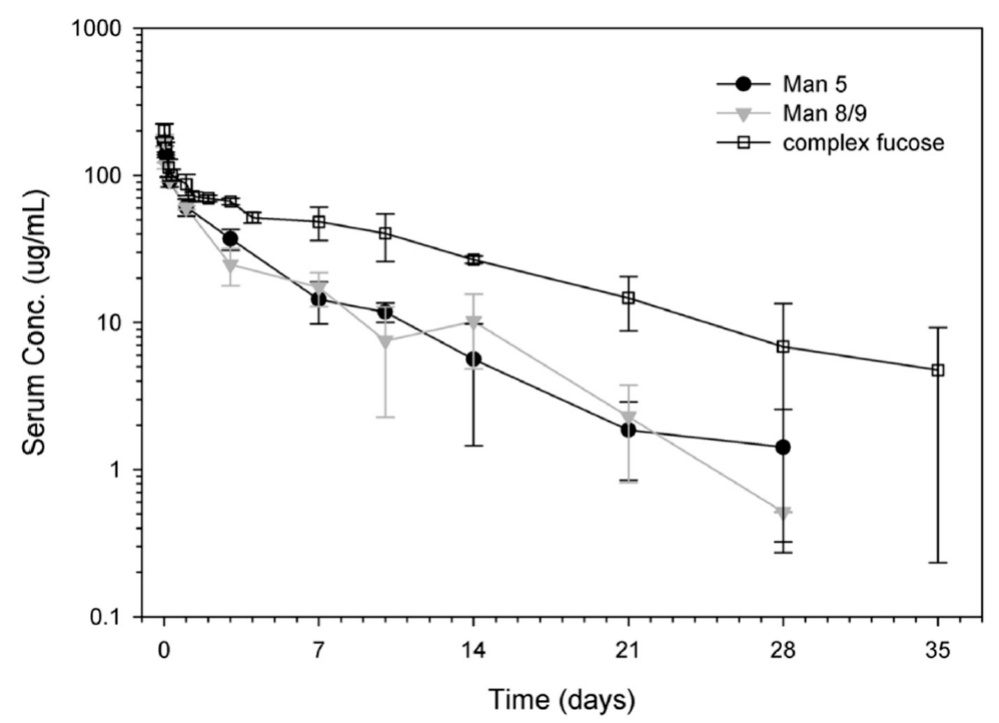
- Terminal-sugar residues in non-human glycoforms not normally found in endogenous serum IgGs could be highly immunogenic in humans.
- Immunogenicity of these therapeutic antibodies can lead to reduced efficacy and safety and cause anti-drug Ab responses (ADA) and hypersensitivity reactions.
- Therefore, the expression system (bacteria, yeast, insect, plant, or mammalian cells) that is used to generate recombinant mAbs is crucial and has tremendous influence on the mAb function in vivo.
- Glycoproteins that are produced in yeasts, plants, and insect cells usually have **high-mannose contents**, which can increase immunogenicity of recombinant mAbs.

Impact of Fc glycosylation on pharmacokinetics

Boune S. et. al, Antibodies, 2020, 9(2), 22

Similar PK properties of glycosylated and non-glycosylated IgGs confirm that antibody clearance in humans and animals is not significantly affected by Fc glycan removal.

In general, glycans that have a major impact on PK of mAbs include mannose, sialic acids, galactose, and fucose.



Pharmacokinetic profiles of mAb variants in athymic nude mice.

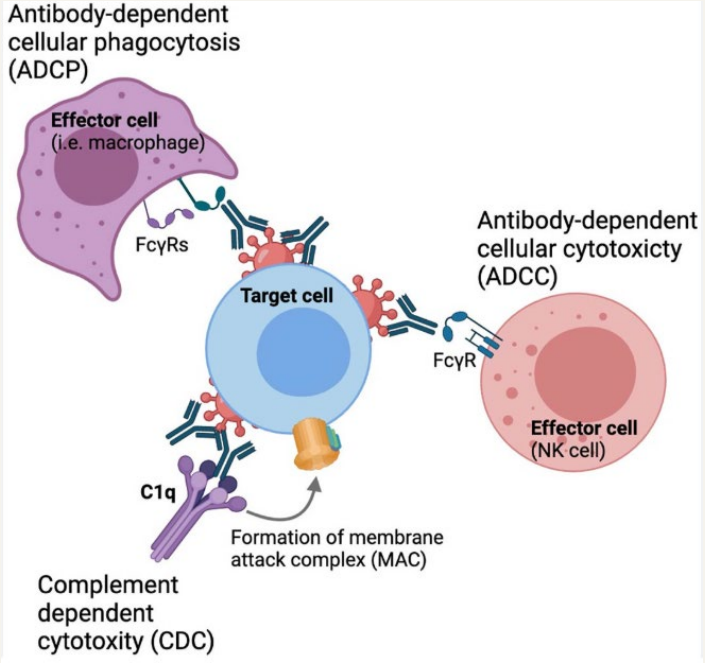
- The two groups conducted in this study were **mAb with >99% Man5 (solid black circle) and mAb with >99% Man8/9 (solid gray triangle).**
- The complex-fucosylated profile (open squares) was from a separate study conducted in a similar fashion to the current study.

Impact of Fc glycosylation on pharmacodynamics

Boune S. et. al, Antibodies, 2020, 9(2), 22

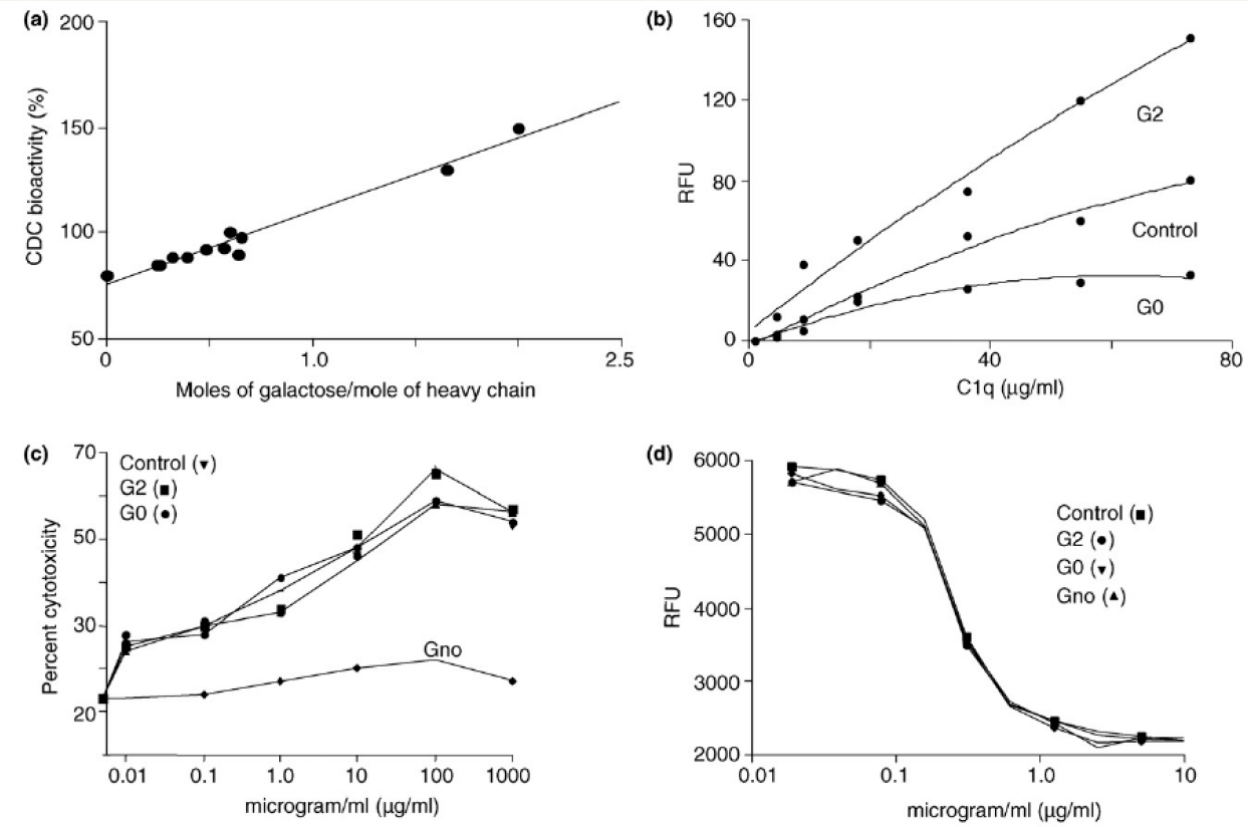
The oligosaccharides of the IgG-Fc play a critical role in activation of FcγRs and complement C1.
- FcγR-mediated effector functions result in the killing of the target cell. FcγRs are responsible for ADCC effector function and, while the receptor C1q mediates CDC.
- Many studies have found that the **lack of glycosylation noticeably decreases the binding affinity** to FcRI and eliminates the binding to FcRII and FcRIII receptors.

Fc sialylation has been attributed to increased anti-inflammatory responses to intravenous Ig (IVIG) for the treatment of autoimmune and inflammatory diseases. However another study showed that remission of rheumatoid arthritis was associated with galactosylation independently of sialylation. In summary, sialylated glycans collectively have both positive and negative influences on IgG effector functions, making it crucial to quantitate the sialylation of mAbs headed for the clinic, especially to treat autoimmune conditions.



Impact of Fc terminal galactose on pharmacodynamics

Boune S. et. al, Antibodies, 2020, 9(2), 22



Increase in terminal Gal content increases complement-dependent cytotoxicity (CDC) activity (**a**) and C1q binding (**b**) of rIgGs, but does not affect antibody-dependent cell-mediated cytotoxicity (ADCC) activity (**c**) and antigen binding expressed as relative fluorescence units (RFU) (**d**).

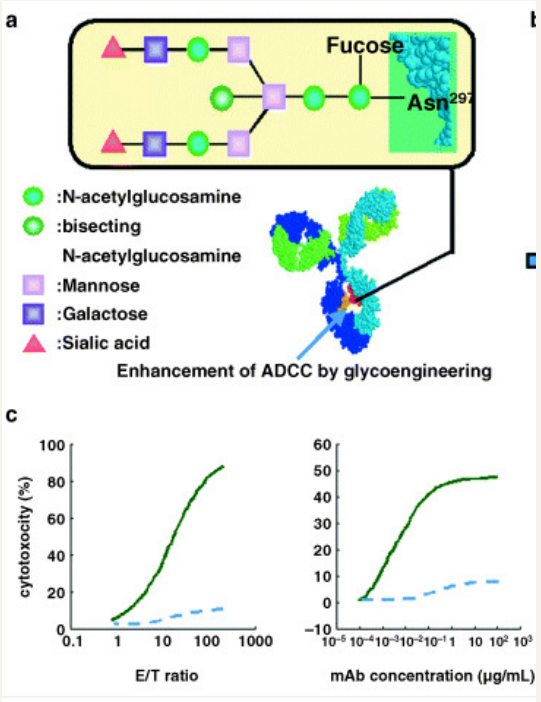
G2, G0, and/or Gno (no glycans) glycoforms of Rituxan and/or Herceptin were prepared by in vitro glycosylation methods. These glycoforms, along with control antibody samples (untreated), were subjected to CDC (Rituxan glycoforms), C1q binding (Rituxan glycoforms), ADCC (Herceptin glycoforms), and antigen binding to HER2-ECD (Herceptin glycoforms).

Rituxan is a chimeric antibody against CD20 and elicits CDC activity but shows very little ADCC activity. Herceptin is a humanized antibody against HER2-neu antigen and elicits ADCC activity but no CDC activity.

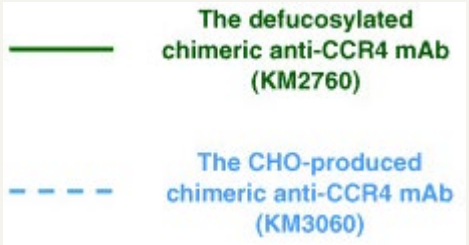
Impact of Fc fucose on pharmacodynamics

Boune S. et. al, Antibodies, 2020, 9(2), 22

- More than 100-fold greater ADCC exhibited by afucosylated anti-HER2 compared to fucosylated recombinant IgG.
- The same engineered cell line was used to produce a nonfucosylated anti-CD20 antibody (mogamulizumab) that showed a 100-fold increase in ADCC activity compared with the nonglycoengineered rituximab.
- mogamulizumab has been approved for the treatment of adult T cell leukemia/lymphoma in Japan.



Ishida T. and R. Ueda, Cancer Sci, 2006 Sep 5: 97(11): 1139-1146



Impact of Fc high mannose on pharmacodynamics

Boune S. et. al, Antibodies, 2020, 9(2), 22

- The presence of **high mannose structures result in enhanced ADCC activity** and increased IgG binding affinity for the FcRIIIa receptor.
- However, it is **unclear whether enhancement in ADCC activity is due to the absence of fucose, since high mannose type glycans possess no core fucose residues.**
- The IgG with high mannose structures have a negative impact on CDC activity because of lower binding affinity for C1q.
- In conclusion, high mannose type Fc glycans have a positive effect on ADCC activity but a negative impact on CDC activity of IgG molecules.

PK and PD of mAbs: Summary of Effects of Glycans

Boune S. et. al, Antibodies, 2020, 9(2), 22

Fc-Glycans	Potential Effects
Fucose	Absence of core fucose enhances: • FcγRIIIa binding • ADCC activity
Galactose	Enhances antibody binding to C1q and CDC
Sialic acid	• Anti-inflammatory activity • NGNA reduces FcγRIIIa binding and ADCC activity • NGNA may be immunogenic in human • Removal of sialic acid decreases half-life
High Mannose	• Decreases half-life • Increases FcγRIIIa binding and ADCC activity • Decreases antibody binding to C1q and CDC

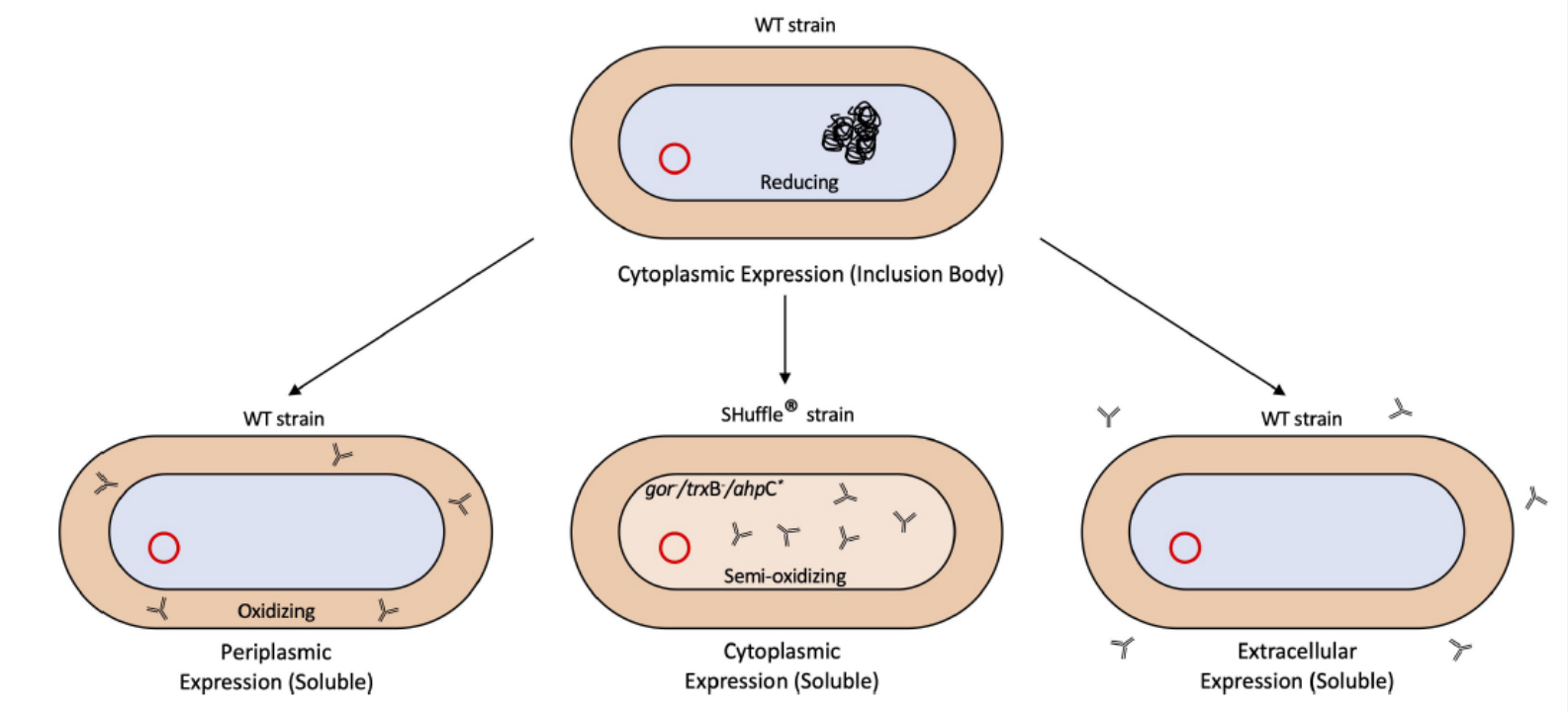
mAbs Production in E.coli

mAbs production in E. coli

Md Harunur Rashid, mAbs. 2022 Aug 26; 14(1): 2111748

- The past decade witnessed substantial progress in expression of full-length antibodies in the *E. coli* cytoplasm and periplasm, as well as in cell-free expression systems.
- The equivalency of *E. coli*-produced aglycosylated antibodies and their mammalian cell-produced counterparts, with respect to biochemical and biophysical properties, including antigen binding, *in vitro* and *in vivo* serum stability, pharmacokinetics, and *in vivo* serum half-life, has been demonstrated.
- Extensive engineering of the Fc domain of aglycosylated antibodies enables recruitment of various effector functions, despite the lack of *N*-linked glycans.

mAbs production in E.coli



Options for antibody expression, unique to *Escherichia coli*. While oxidizing periplasmic compartment initially was the rational choice for soluble expression of antibodies, they are now routinely produced either in engineered semi-oxidizing cytoplasm or are excreted into the culture medium; in addition, a cell-free expression option is also available.

mAbs production in E.coli peripasm

Format	Antibody	Compartment/ System	Titer (mg/ L)	Strategy	Significance
IgG	Anti-TF	Periplasm	150	Two cistrons with a phoA promoter, with balanced HC and LC translational levels	First successful report of IgG production
IgG	Anti-TF	Periplasm	1300	Two cistrons with a phoA promoter, with optimized TIRs for both HC and LCs; co-expression of DsbA and DsbC chaperones	Highest reported yield of IgG production in cell-based system
IgG	Anti-HER2 (Trastuzumab) variants	Periplasm	40–50	IgG1 Fc variants isolated using novel periplasmic display system and FACS sorting; two cistrons with a ptac promoter and a pelB signal peptide	First report of isolation and production of IgG1 effector function variants
IgG	Anti-PA (YMF10)	Periplasm	65	Coexpression of DsbC and SRP-dependent Ffh factor, with an SRP-dependent DsbA signal peptide	Use of engineered and minor SRP secretion pathway to prevent cytoplasmic aggregation
IgG	Anti-PA (YMF10)	Periplasm	362	Coexpression of DsbC and the use of sec-dependent pelB signal peptide, with optimized TIRs	High volumetric productivity with major sec secretion pathway
IgG	Anti-PA (YMF10)	Periplasm	400	Engineered strain with a 16s rRNA gene rrsE mutant, combined with minor SRP-dependent secretion pathway	Secretion limitation of the SRP pathway was relieved, enabling a higher titer
IgG	Anti-EGFR (Cetuximab)	Periplasm	200	First report on sRNA-based screening of chromosomal gene targets utilizing systems-based synthetic biology approach	First successful report of IgG production using systems and synthetic biology approaches
IgG	Anti-EGFR (Cetuximab)	Periplasm	150	Second report on sRNA-based screening of chromosomal gene targets utilizing systems-based synthetic biology approach	Second successful report of IgG production using systems and synthetic biology approaches
BsAb	Fc€RIxFc RIIb	Periplasm	350	Use of Knob-into-Hole (KiH) mutations for Fc HC heterodimerization in a single cell line with a common light chain	First successful report of the production of full-length BsAb using KiH mutations and a common light chain
BsAb	Various BsAbs, including CD3 BsAbs	Periplasm	39–850	Production of full-length BsAb, with natural architecture by coculture instead of separate culture with two HC and two LCs	First report of the production of FL BsAb, with natural antibody architecture

mAbs production in E.coli cytoplasm

IgG-PE38 fusion	Anti-CD30 (T427)	WT Cytoplasm	50	Inclusion body refolding and purification from shake-flask culture	First successful demonstration of the refolding of full-length IgG and IgG-fusion protein into their active forms
IgG	Anti-Dig, -MBP, -Gcn4-bZIP, -gpD, -HAG, -PA63	SHuffle® Cytoplasm	1–25	Full-length IgG expression in soluble and active forms in semi-oxidizing cytoplasm, with cytoplasmic DsbC coexpression	First successful report of the production of soluble and active IgG in semi-oxidizing SHuffle cytoplasm in a shake-flask culture
IgG	Anti-TNF (Humira®)	SHuffle® Cytoplasm	168–427	Coexpression of human PDI-GPx7 fusion protein to use H_2O_2 as an oxidative agent for increased expression	Use of alternate oxidative agent in SHuffle cytoplasm in shake-flask culture

mAbs production in E.coli cell free system

IgG	Anti-CK (MAK33)	CFPS	500	Supplementation with either PDI or DsbC in CFPS reaction mixture	First successful report of the production of soluble IgG in the CFPS system
IgG	Anti-HER2 (Trastuzumab), Anti-CD30 (Brentuximab), Germline IgGs (VH3-7xVK3-20 and VH3-23xVK3-20)	CFPS	1000–1500	Supplementation with DsbC and FkpA with optimized TIRs for both HC and LC	Highest titer of full-length IgG in the CFPS system
BsAb	Various CD3xHER2 & CD3xEpCAM formats	CFPS	200–1000	BsAb production using KiH mutations by manipulating knob and hole plasmid ratio, and further improvement by addition of prefabricated knob or hole	First successful production of BsAb with various formats using KiH mutations in the CFPS system
IgG	IgG1 (Trastuzumab, Adalimumab, Cetuximab), IgG2 (Panitumumab) and IgG4 (Nivolumab)	PURE	33–125	Optimization of DsbC, DnaK, and its cofactors, GSH/GSSH ratio, incubation time and temperature in addition to HC and LC DNA ratio	First successful production of full-length IgGs of various isotypes in the PURE system

Characteristics of mAbs produced in E.coli

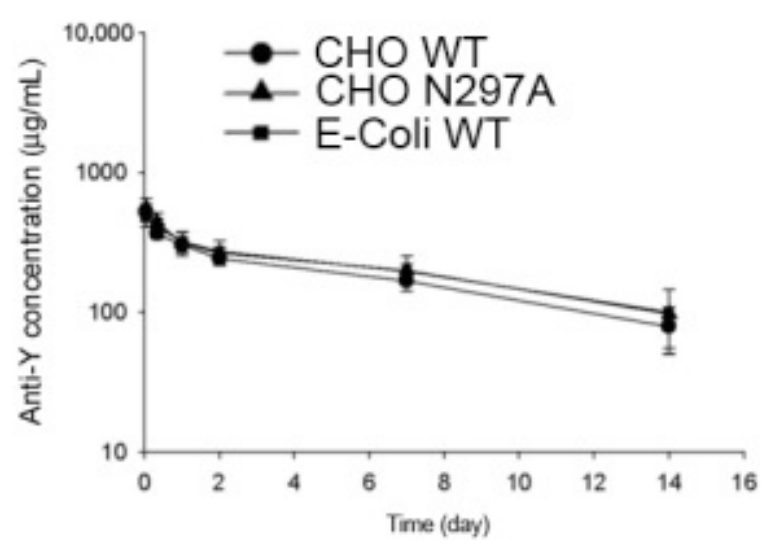
- Many mAbs, BsAbs, and ADCs have been successfully produced, purified, and characterized from either *E. coli* cytoplasm or periplasm or from *in vitro* cell-free systems, such as the CFPS and PURE systems.
- In most cases, similar antigen-binding affinities have been reported with purified antigens and/or *in vitro* cell-based binding assays, inhibition of cell signaling pathways, and/or *in vitro* cell killing potencies compared to their mammalian cell-produced (and glycosylated) counterparts.

Aglycosylated mAbs produced in E.coli

- Both ADCC and CDC activities would be minimal with the aglycosylated antibodies produced in *E. coli*.
- The binding affinity of *E. coli*-produced aglycosylated trastuzumab for FcγR1 was reduced by two orders of magnitude, and its binding to FcγRIIa, FcγRIIb, FcγRIIIa, and complement protein C1q, was undetectable, resulting in the complete loss of ADCC, ADCP, and CDC functions.

PK Characteristics of mAbs produced in E.coli

- The PK properties of many mAbs and BsAbs produced in *E. coli* and mammalian cells were compared in mice, rat, and non-human primates, including cynomolgus monkey.
- Similar PK properties of *E. coli*-produced aglycosylated antibodies were observed in the tested animal models compared with their mammalian cell-produced glycosylated counterparts.



M. Leabman et al., Mabs. 2013 Sep 11; 5 (6): 896-903

Fc mutations of mAbs produced in E.coli to engineer effector functions

Table 3. Engineering of standard and novel effector functions in *Escherichia coli*-produced aglycosylated antibodies.

Fc Variant	Fc mutations	Antibodies tested	Fcγ receptors	Effector functions	Comments
Fc5	E382V/M428I	Trastuzumab	67-fold enhanced binding to Fcγ RI	ADCC by dendritic cells (DC)	Novel mechanism
Fc701	Q295R/L328W/A330V/ P331A/I332Y/E382V/ M428I	Trastuzumab	120-fold enhanced binding to FcγRI	ADCC by dendritic cells (DC)	Novel mechanism
Fc1004	S298G/T299A/E382V/ N390D/M428I	Trastuzumab	160-fold enhanced binding to FcγRIIa; 25-fold enhanced selectivity over Fc RIIb	ADCP by macrophages	Known mechanism
Fc801	K320E/Q386R	Rituximab	231-fold increased affinity for C1q	CDCC and CDCP activities through complement receptors by NK and myeloid cells, respectively	Novel mechanism
Fc-MG48	V264E/S298G/T299A/K326I/ A327Y/L328G/T350A/ E382V/N390D/M428L	Trastuzumab	16-fold and 25-fold increased affinities for FcγRIIIa-V158 and -F158 alleles, respectively	ADCC by NK cells	Known mechanism
Fc-HW86	V264E/S298G/T299A/K326I/ A327Y/L328G/T350A/ E382V/T384A/N390D/ M428L	Trastuzumab	3-fold enhanced binding to FcγRIIIa over glycosylated trastuzumab	2-fold increased ADCC over glycosylated trastuzumab by NK cells	Known mechanism
Fc3aV	V264E/V282M/T299A/ L309Q/S329T/A378V/ P428I	Trastuzumab, Rituximab	Exclusive binding to FcγRIIIa-V158	ADCP by GM-CSF macrophages	Novel mechanism

The Fc mutations conferring various effector functions in aglycosylated antibodies mentioned above might provide a path to retain these important immune functions while sidestepping the common issue of *N*-glycan heterogeneity altogether

mAbs produced in E.coli in clinical trials

Antibody	Target	Diseases	Molecular format	Antibody modification	Clinical trials	Company
Onartuzumab (OA-5D5; RG3638)	HGF	Glioblastoma, NSCLC, mCRC, TNBC, Gastric cancer	One-armed, monovalent mAb	KiH mutations	Phase 3 (NCT02488330, NCT01887886, NCT02031744, NCT01456325, NCT01662869); Phase 2 (NCT01590719, NCT01186991, NCT01418222, NCT01496742, NCT01519804, NCT01632228, NCT01186991, NCT00854308, NCT01418222, NCT02044601); Phase 1 (NCT01897038, NCT02031731, NCT01974258, NCT01068977, NCT02044601, NCT01014936)	Genentech/ Roche
BITS7201A (RG7990)	IL13xIL17	Asthma	BsAb	KiH mutations	Phase 1 (NCT02748642)	Genentech/ Roche
STRO-002	FolRα	Ovarian cancer, Endometrial cancer	ADC	nsAA incorporation	Phase 1 (NCT05200364, NCT03748186)	Sutro Biopharma
STRO-001	CD74	Lymphomas, MM	ADC	nsAA incorporation	Phase 1 (NCT03424603)	Sutro Biopharma
CC99712 (BMS-986352)	BCMA	MM	ADC	nsAA incorporation	Phase 1 (NCT04036461)	Sutro Biopharma/ BMS
M1231	MUC1xEGFR	NSCLC, Esophageal cancer	Bispecific ADC	nsAA incorporation; SEED mutations	Phase 1 (NCT04695847)	Sutro Biopharma/ EMD Serono

Summary of mAbs produced in E.coli

- Due to the absence of glycans, aglycosylated antibodies lack various Fc-mediated effector functions, such as ADCC, ADCP, and CDC.
- However, effector functions conferred by glycosylated antibodies have been engineered into *E. coli*-produced mAbs, in some cases resulting in enhanced effector functions. Moreover, new and unique effector functions (i.e., CDCC and CDCP) as well as improved Fcɣ receptor selectivity have been discovered with *E. coli*-produced aglycosylated antibodies. It will be interesting to see how these new discoveries translate into the clinic.
- Now, both the WT aglycosylated antibodies and mutant aglycosylated antibodies with these unique sets of mutations without and with effector functions, respectively, could be produced in *E. coli* and can be compared with mammalian cell-produced glycosylated antibodies with any drug modality for treating any disease.

mAbs Production in Pichia

Production of mAbs in Pichia

Barone, G.D., Biomolecules 2023, 13, 441

- K. phaffii (old name Pichia pastoris) is an important host that produces different heterologous proteins. K. phaffii rises to the forefront of this area and, probably alongside cell-free protein synthesis, is still a good competitor with mammalian systems in the production of glycosylated proteins.

- This host combines the ability to grow to the point of very high cell densities in minimal medium, typically secreting heterologous proteins into the culture supernatant. The number of companies in this scientific field is increasing, and this trend will continue in the next decades.

Summary of mAbs produced in Pichia

Barone, G.D., Biomolecules 2023, 13, 441

- Glycoengineered K. phaffii (old Pichia pastoris) strains have been optimized during the last decades to synthesize recombinant protein with humanized and homogenous glycosylation patterns, increasing the interest for this host. N-linked glycans of the type $Man_5GlcNAc_2$ are achievable.

- Considering their native post-translational modifications, which can be different from those happening in mammalian cells, some companies specializing in K. phaffii engineering (e.g., BioGrammatics, VALIDOGEN GmbH, and Bisy GmbH) have developed strains to bypass the main differences between higher eukaryotic cells and yeast. In terms of the production of protein with similar glycosylation to those in mammalian cells, different methods have been applied to engineer the N-glycosylation route.

Summary of companies specializing in Pichia

Company	Product	Description	Website
VALIDOGEN GmbH (Trakt, Grambach, Austria)	UNLOCK PICHIA—*Pichia pastoris* protein expression system	Development of strains, bioprocesses, protein purification, and enzyme engineering	validogen.com/pichia-pastoris/applications (accessed on 10 January 2023)
Bisy GmbH (Wünschendorf, Austria)	*Pichia* strains development, vectors, and biocatalysts	Development of vectors, strains, recombinant cytochrome P450 or lipases	bisy.at (accessed on 10 January 2023)
Ginkgo Bioworks (Boston, MA, USA)	*Pichia pastoris* strain and process development, patented methanol-free technology	Generation and development of strains; development of HTS/OMICS methods, workflows, fermentation and scale-up for a wide range of applications and industries	ginkgobioworks.com (accessed on 10 January 2023)
Lonza (Visp, Switzerland)	XS™ *Pichia* 2.0 Expression and Manufacturing Platform	Development of next generation therapeutics	lonza.com/news/2017-11-08-14-20 (accessed on 10 January 2023)
BioGrammatics (Carlsbad, CA, USA)	DIY *Pichia* Strain Construction, and *Pichia* GlycoSwitch Technology	Custom *Pichia* expression strain	biogrammatics.com (accessed on 10 January 2023)

Summary of biologicals produced in Pichia

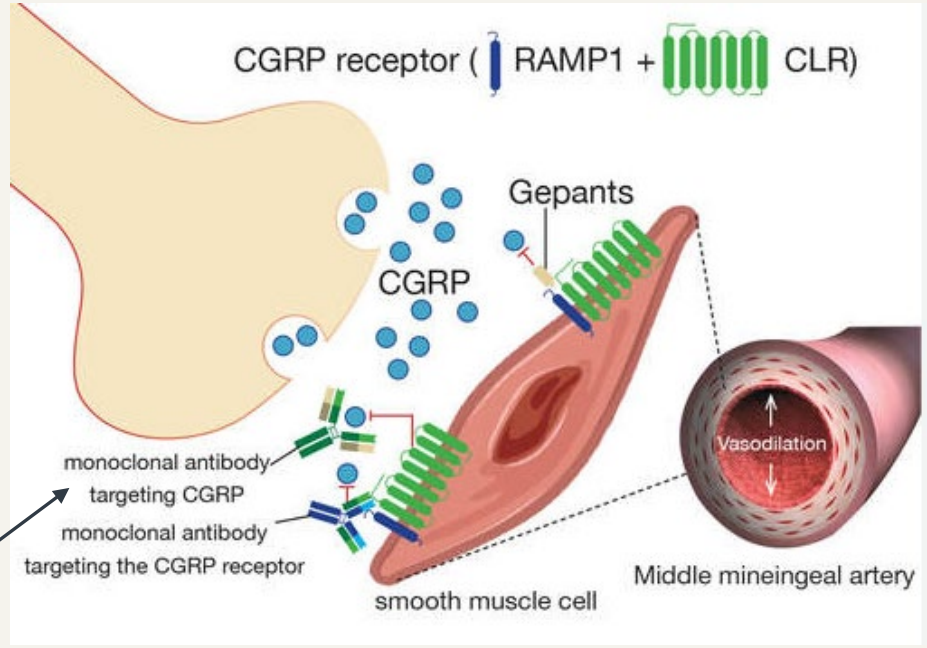
BOLT THREADS (Emeryville, CA, USA)	MICROSILK™	Sustainably produced textile spun from the proteins of the spider web	boltthreads.com (accessed on 10 January 2023)
IMPOSSIBLE™ FOODS (Oakland, CA, USA)	IMPOSSIBLE™ BURGER	Engineering *K. phaffii* to make components for a meat-free burger	impossiblefoods.com (accessed on 10 January 2023)
Dyax/Biotage® (Salem, OR, USA)	Kalbitor® (DX-88 ecallantide: recombinant kallikrein inhibitor protein)	Hereditary angioedema treatment	biotage.com (accessed on 10 January 2023)
Biocon (Bengaluru, India)	Insugen® (recombinant human insulin)	Diabetes therapy	biocon.com/products/key-therapeutic-areas/diabetes/ (accessed on 10 January 2023)
Mitsubishi Tanabe Pharma (Osaka, Japan)	Medway (recombinant human serum albumin)	Expansion of the blood volume	mt-pharma.co.jp/e/ (accessed on 10 January 2023)
Shantha/Sanofi (Telangana, India)	Shanvac ™ (recombinant hepatitis B vaccine)	Hepatitis B prevention	sanofi.com/en/your-health/vaccines/hepatitis-b (accessed on 10 January 2023)
Shantha/Sanofi (Telangana, India)	Shanferon™ (recombinant interferon-alpha 2b)	Hepatitis C and cancer treatment	sanofi.in (indiamart.com/proddetail/shanferon-1700786533.html) (accessed on 10 January 2023)
ThromboGenics/Oxurion (Leuven, Belgium)	Ocriplasmin (recombinant microplasmin)	Vitreomacular adhesion (VMA) treatment	oxurion.com (accessed on 10 January 2023)

Summary of mAbs and biologicals produced in Pichia

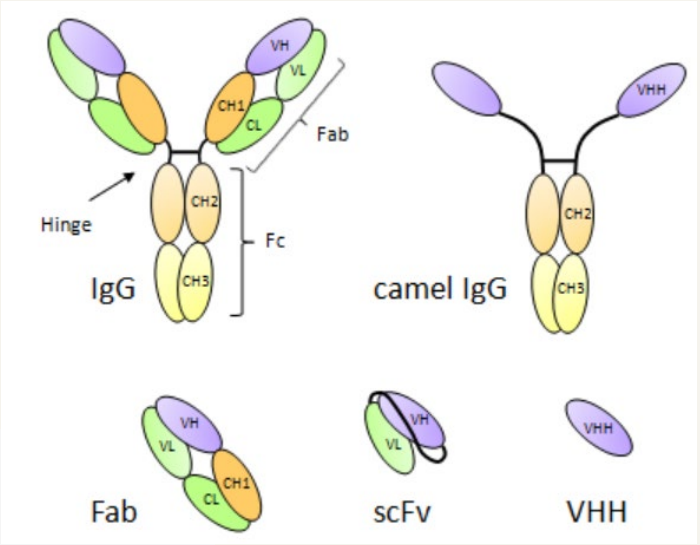
Ablynx/Sanofi (Gent, Belgium)	Nanobody® ALX-0061 (recombinant anti-IL6 receptor single domain antibody fragment)	Rheumatoid arthritis treatment	ablynx.com (sanofi.com/en/science-and-innovation/research-and-development/technology-platforms/nanobody-technology-platform) (accessed on 10 January 2023)
Ablynx/Sanofi (Gent, Belgium)	Nanobody® ALX00171 (recombinant anti-RSV single domain antibody fragment)	Respiratory syncytial virus (RSV) infection treatment	ablynx.com (sanofi.com/en/science-and-innovation/research-and-development/technology-platforms/nanobody-technology-platform) (accessed on 10 January 2023)
Trillium/Pfizer Inc. (Brockville, Canada)	Heparin-binding EGF-like growth factor (HB-EGF)	Treatment of interstitial cystitis/bladder pain syndrome (IC/BPS) treatment	pfizer.com (accessed on 10 January 2023)
Verenium/DSM (Heerlen, Netherlands)	Purifine (recombinant phospholipase C)	Degumming of high phosphorus oils	dsm.com/corporate/home.html (accessed on 10 January 2023)
Roche (Mannheim, Germany)	Recombinant trypsin	Digestion of proteins	lifescience.roche.com (accessed on 10 January 2023)
Fibrogen (San Francisco, CA, USA)	Recombinant collagen	Medical research reagents/dermal filler	fibrogen.com (accessed on 10 January 2023)
Merck/Schering Plough Animal Health (San Francisco, CA, USA)	AQUAVAC IPN (recombinant infectious pancreatic necrosis virus capsid proteins)	Vaccines for infectious pancreatic necrosis in salmon	merck-animal-health.com/contact-us/ (accessed on 10 January 2023)
Phytex, LLC/United Animal Health (Sheridan, IN, USA)	Recombinant phytase	Animal feed additive	unitedanh.com (accessed on 10 January 2023)
The Nitrate Elimination Co. (Lake Linden, MI, USA)	Superior Stock recombinant nitrate reductase	Enzyme-based products for water testing and water treatment	nitrate.com/analytical-enzyme-applications/education (accessed on 10 January 2023)

Examples of mAbs produced in Pichia

eptinezumab (Vyepti) from Alder/Lundbeck AG is a humanized IgG1 mAb approved in several countries as a prophylactic treatment of migraine in adults. It is produced in Pichia pastoris, but lacks N-linked glycosylation as the asparagine residue at position 297 (N297) is replaced by alanine. As a result, it eliminates binding to Fcgamma receptors. The terminal elimination half-life is estimated to be 27 days. The mechanism of action is picomolar affinity to alpha and beta forms of CGRP (calcitonin gene-related peptide) ligand inhibiting its binding to its receptor thus neutralizing its biological activity.



VHH produced in Pichia at Validogen



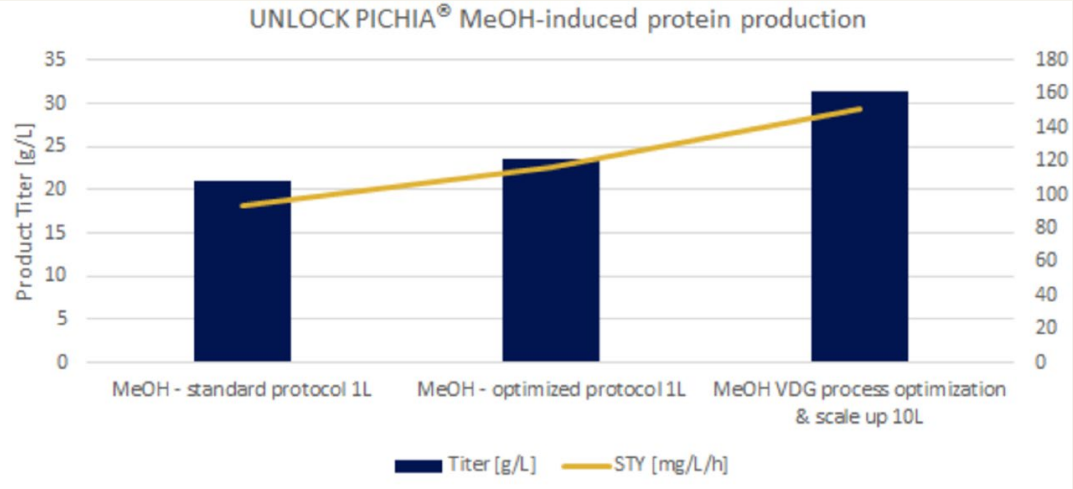
Case Study 1

- 12 g/L of secreted VHH in culture supernatant
- 64-hours process time
- High space-time yield of 96 mg/L/h

Case Study 2

- 19 g/L of secreted VHH in culture supernatant
- 111-hours process time
- High space-time yield of 95 mg/L/h & increase in specific productivity by 14%

Case Study 3



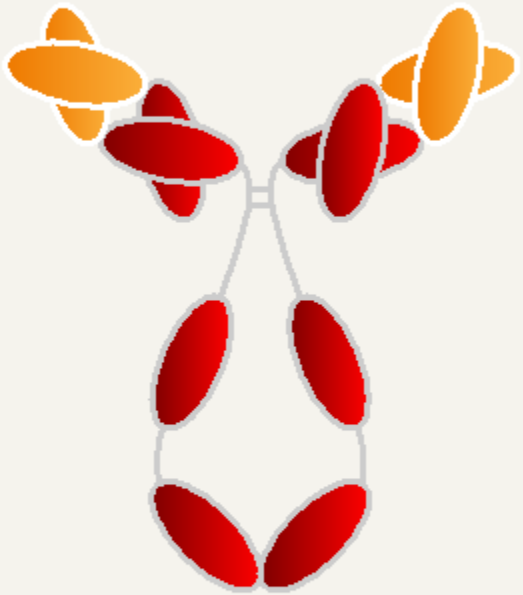
Summary of mAbs produced in Pichia

- Although several biologics are made in Pichia production system, only a few mAbs are made.
- For glycosylated mAbs, Pichia does offer good options to glycol-engineer human-like N-linked glycans of the type $Man_5GlcNAc_2$. Interestingly, the only full length mAb approved in Pichia system is aglycosylated (eptinezumab) as its MOA doesn't require Fc glycosylation.
- Pichia is used extensively to make nanobodies, much smaller format antibodies.

mAbs Production in Filamentous Fungi Thermothelomyces heterothallica

Dyadic's Thermophilic Filamentous Fungus C1 Platform

C1 platform produces comparable therapeutic proteins as CHO while overcoming key production limitations



C1 produces stable and correctly folded mAbs that have binding and neutralizing properties similar to those produced from CHO cells

Lower Cost

- Flexible production scale, single use and/or stainless-steel bioreactors
- C1 media <1/20 of the cost of CHO media
- No viral inactivation required; limited infrastructure investments

Faster Development and Production

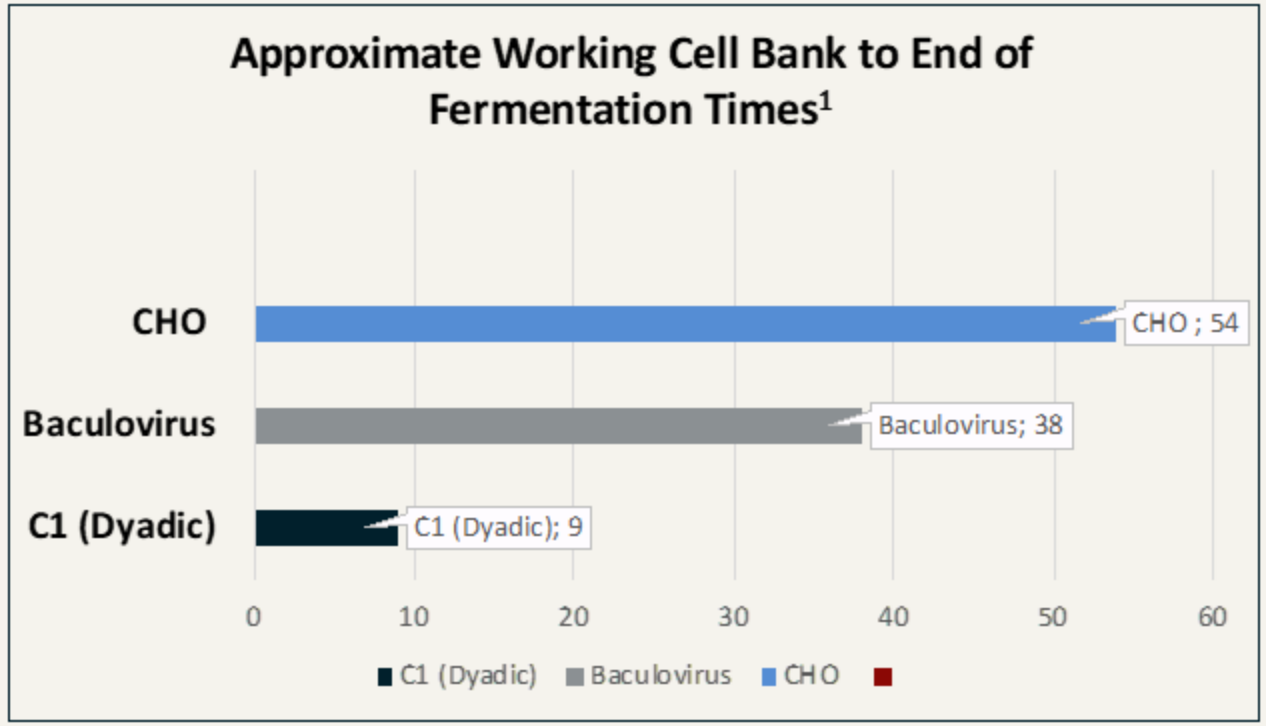
- Stable cell lines in 7 weeks enabling rapid development and response
- C1 produces product significantly faster (12-14 days) than CHO cells (41-54 days); ~ Potential to produce three to four batches using C1 in the same timeframe as one batch using CHO cells

Higher Yields

- C1 has the potential to produce more product per batch and larger overall quantities
- ~ Potential to produce three to four batches using C1 in the same timeframe as one batch using CHO cells

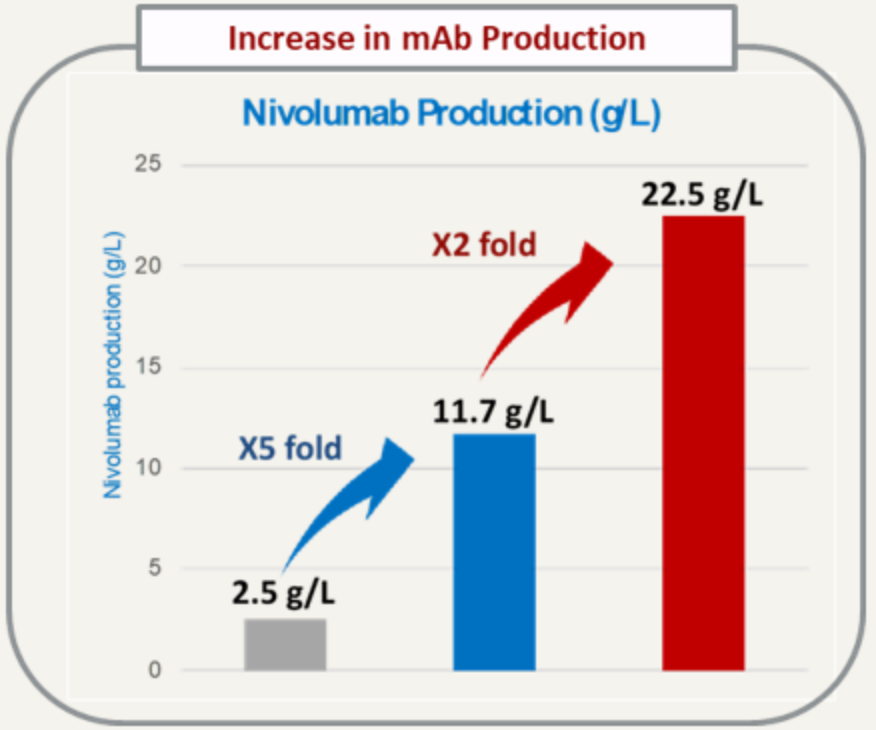
Fermentation Time and Yield Favors C1 Over CHO Cells

Less Time, No Viral Clearing Needed



[1] Estimated Ranges: C1 7-9 days; Baculovirus 28-38 days; CHO 41-54 days

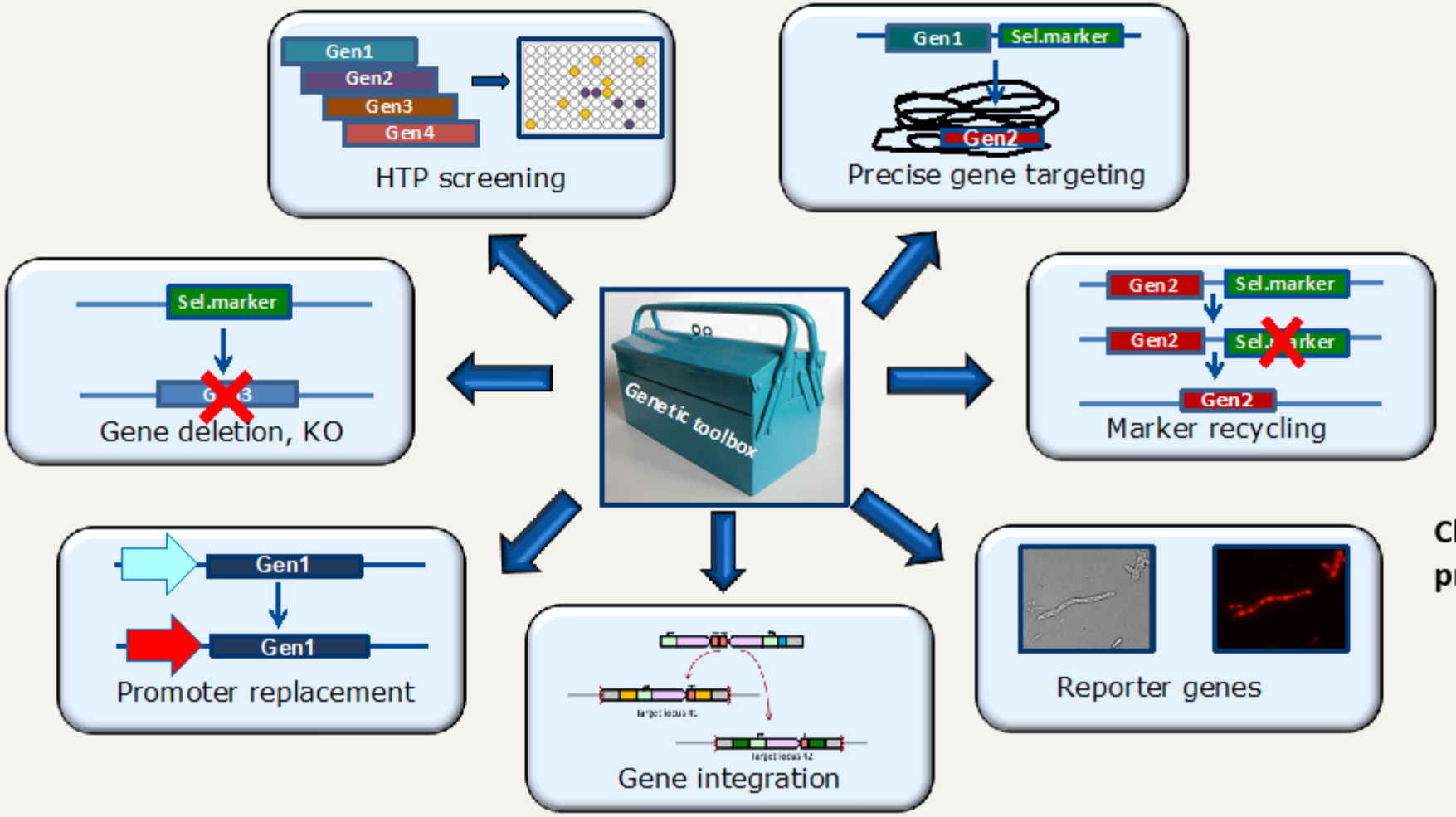
Higher Productivity

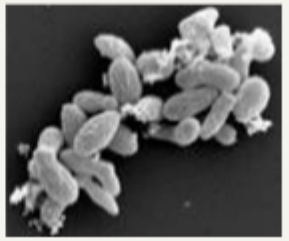


- Nivolumab G2/G1 strain, 9.2 g/l in 7 days
- Good product quality with human N-glycan profile

Genetic toolbox for rapid, stable, high yield C1 strain development

The advanced genetic toolkit for synthetic biology



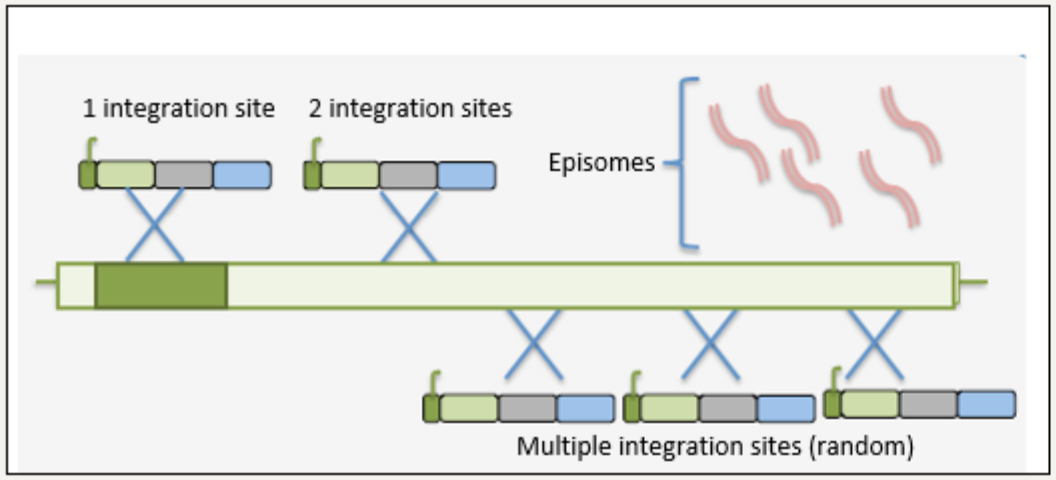


Change in morphology led to hyper-productivity and low viscosity.

C1 Expression Technology

Efficient transformation methods can be applied:

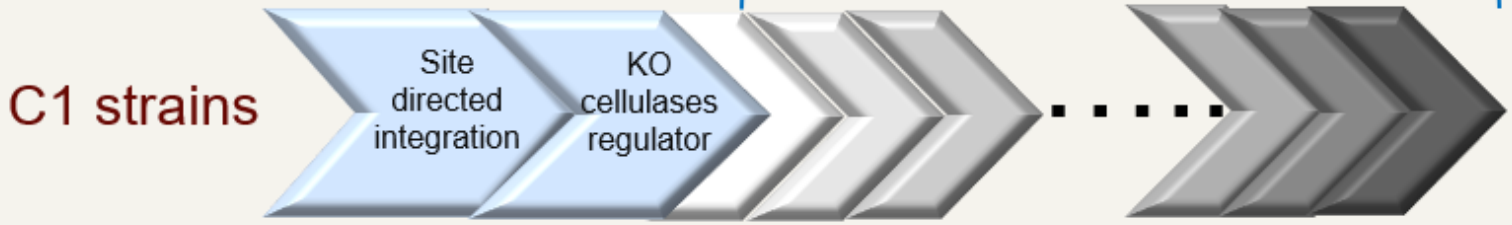
- •Single site directed integration
  - By standard integration: 1 or 2 genes into the same site. Two integration sites can be used
  - By Cas9 technology: site directed integration at multiple sites
- •Random integration: integration at multiple sites
- •Episomal vectors



- •Transformation procedure based on chemical (PEG) method with protoplasts or electroporation
- •Frequencies for 1µg DNA:
  - **20 transformants for site specific integration**
  - **Up to 100 transformants for random integration**
  - **~13,000 transformants for telomeric vector transformation**

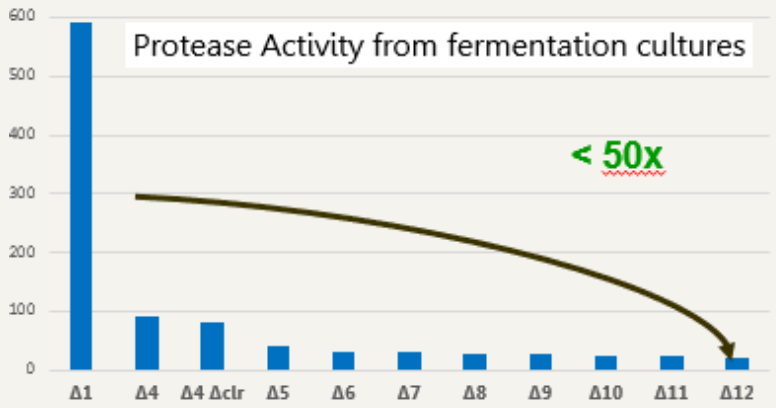
Systematic deletion of active proteases in C1 host to improve stability

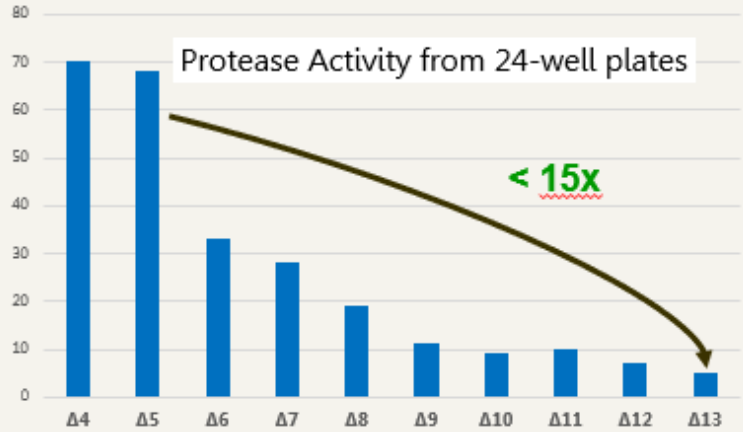
KO proteases to generate 15X protease deletion strain

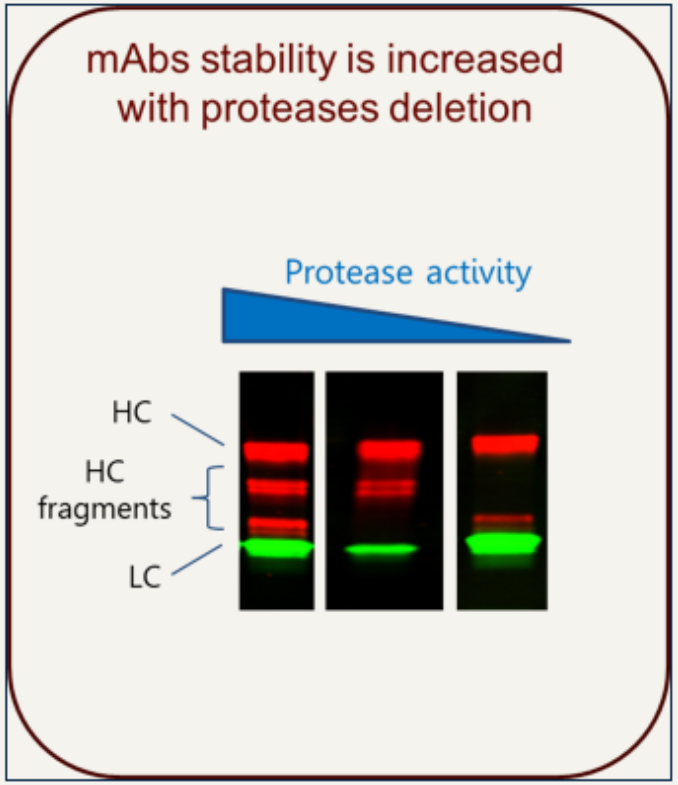


Systematic deletion of proteases genes based on:

- Isolation and identification of extracellular proteases
- C1 protease library in Pichia pastoris that allows to test more than 50 proteases
- Effect of different protease inhibitors on protease activity







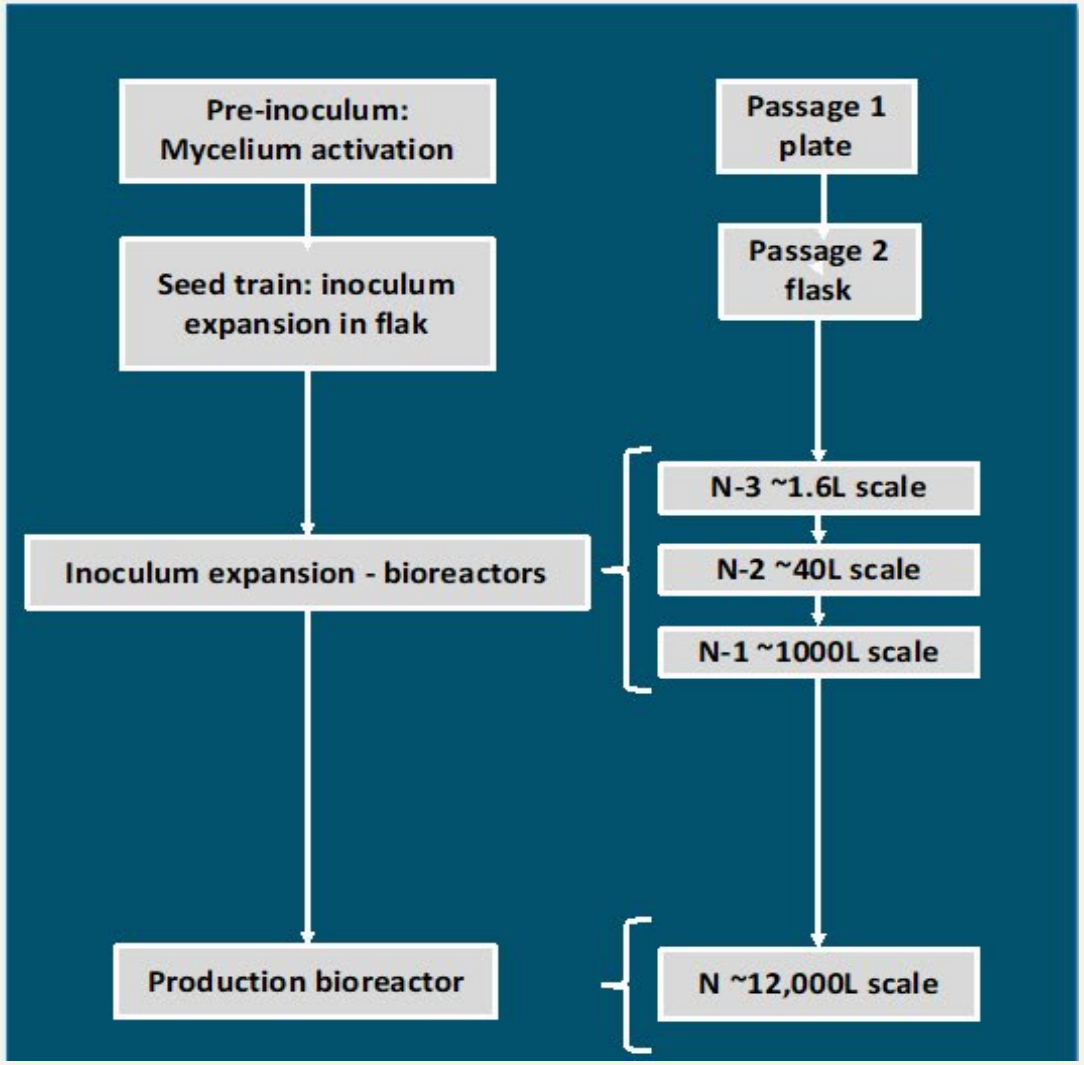
Generic process flow chart for C1 is 7 - 9 days

1 – 1,5 days

0,75 -2,5 days

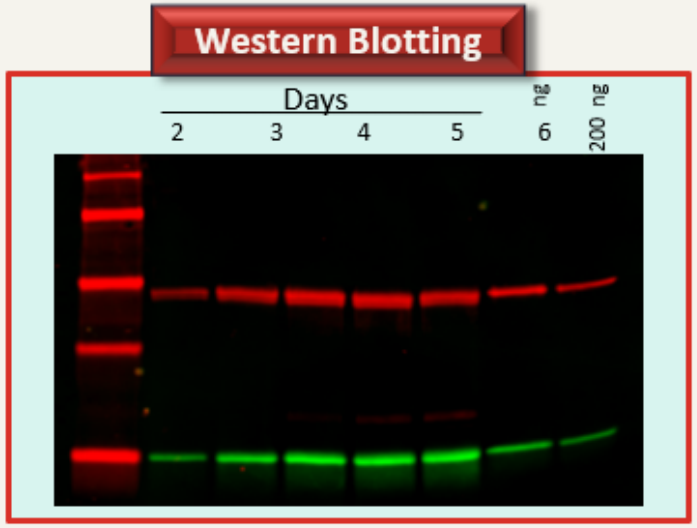
5 days

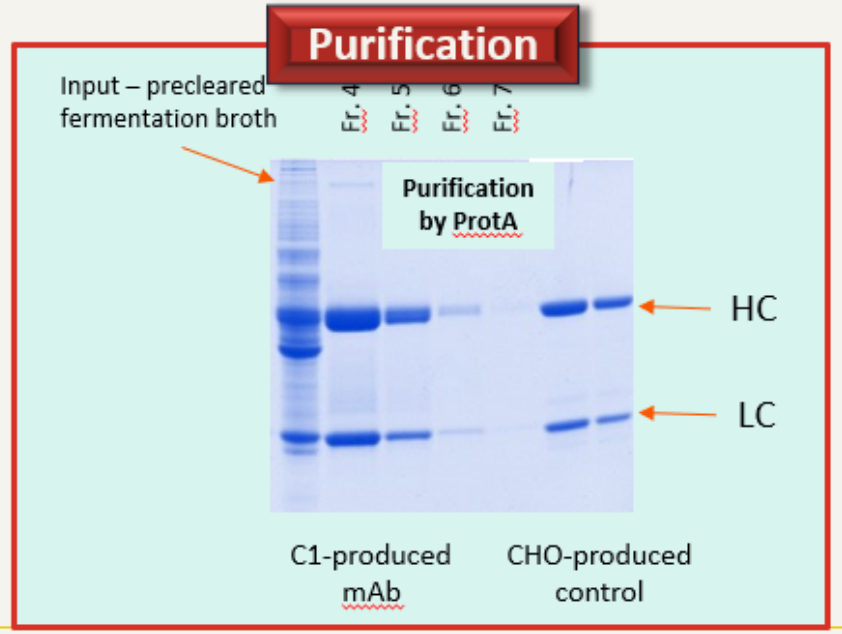
Generic Process Flow Chart for CHO is 41 – 54 days

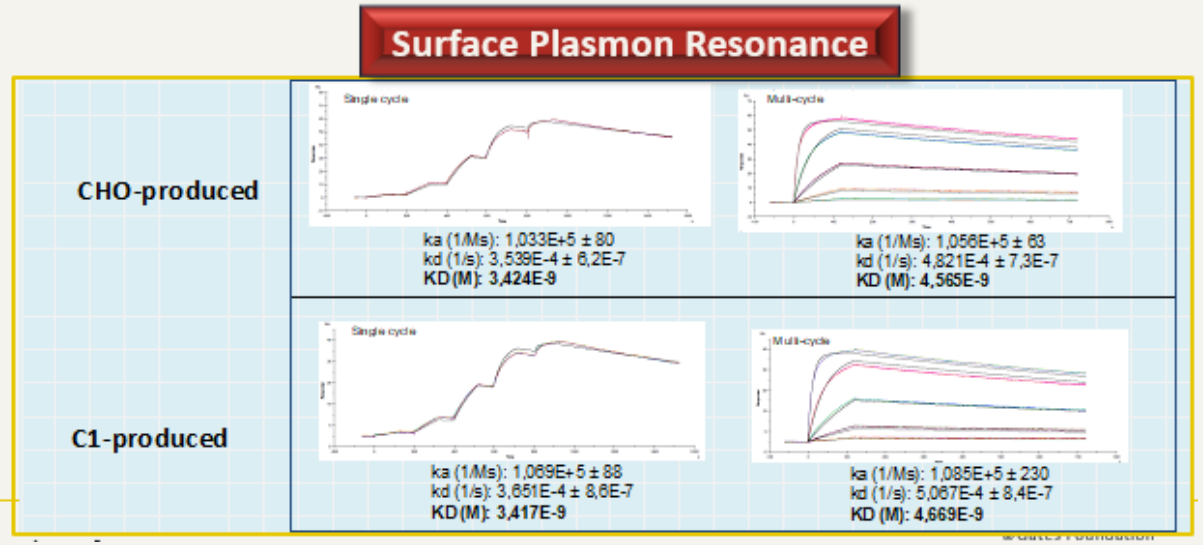


C1 manufactured monoclonal antibodies are stable and correctly folded

- **Typically, stable mAbs production levels are between**
  - **5 – 10 g/L in 5-7 days**
- **Best productivity at Ambr system:**
  - **24.5 g/L in 7 days (3.5 g/l/day)**
- **Best productivity so far at 30L scale:**
  - **20.6 g/L at 157 h (3.1 g/L/day)**

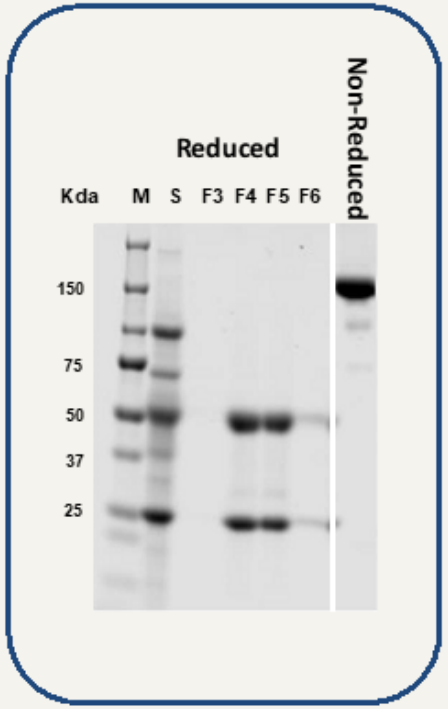




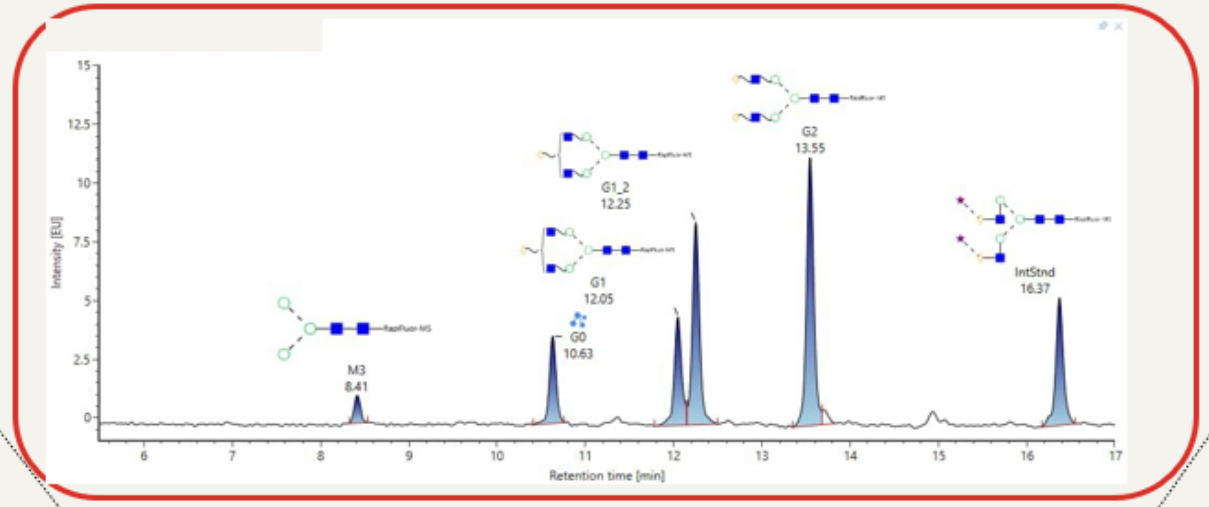


Engineering Nivolumab C1 G2/G1 strain

The use of library of folding factors enhanced the productivity of the Nivolumab G2/G1 strain
New production record obtained: 9.2 g/l Good product quality with excellent N-glycan profile



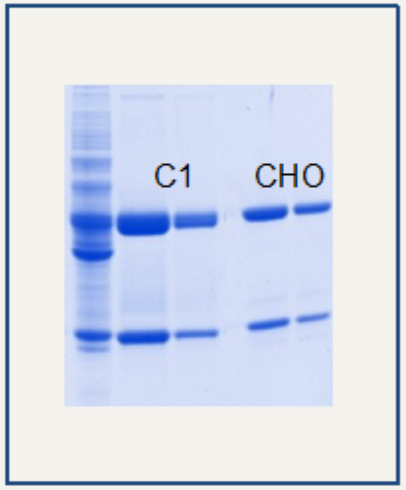
Single-step purification using 1 ml Eshmuno A (Protein A) column



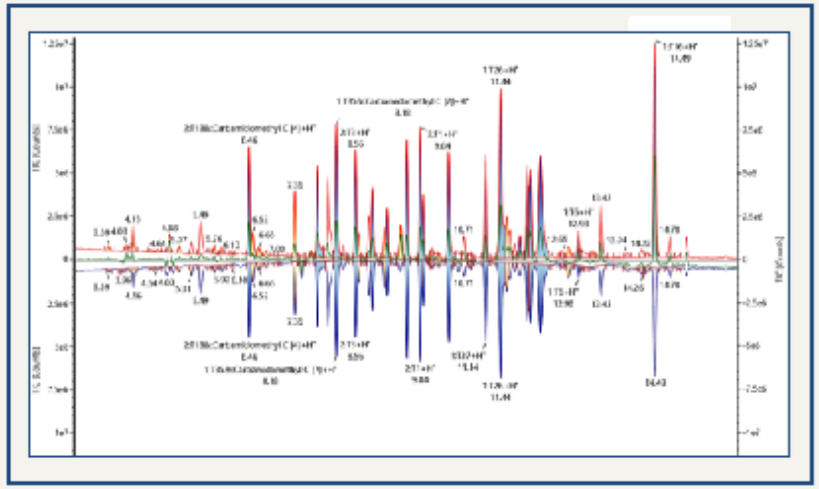
		MT771-6
		M7184
Glycan	Obs. RT (min)	Amount, %
Man2	6,22	-
Man3	8,42	3,51
G0	10,63	12,01
G1	12,05	16,29
G1_2	12,25	29,20
G2	13,55	38,99
% Humanised N-glycans		96,49
Total response		137,9
Nivolumab, g/l		9,23

Similar structure of mAbs produced in C1 to CHO produced mAbs

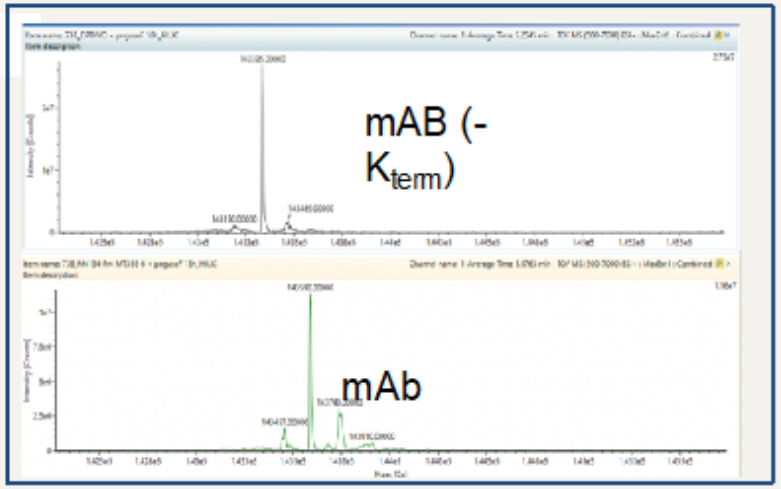
SDS PAGE



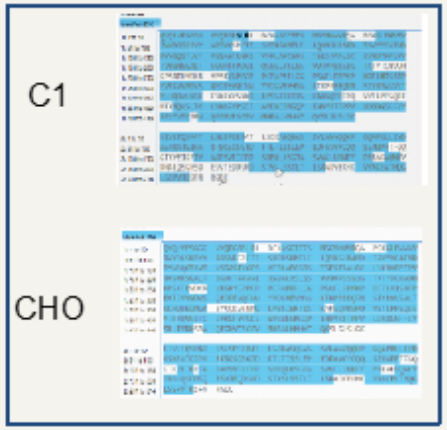
Peptide fingerprint



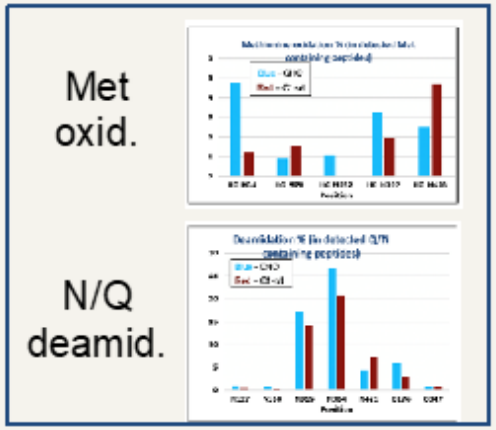
Intact mass analysis (after de-N-glycosylation)



Peptide mapping



Oxidation and deamidation

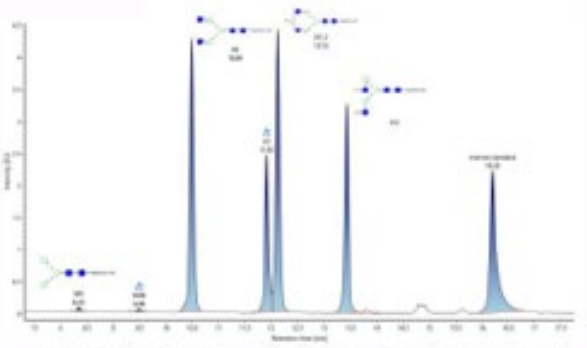


Disulfide bridges

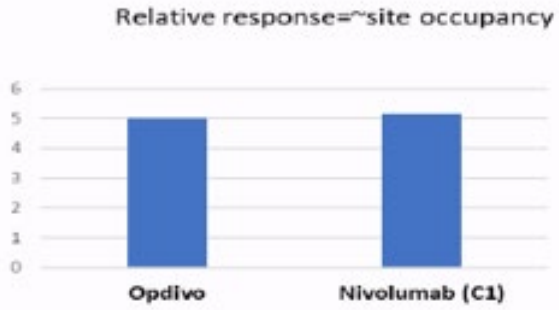
Disulphide bridges	Location
Cys 132-Cys 214	inter chain
Cys 135-Cys 148	intra chain HC
Cys 22-Cys 96	
Cys 227-Cys227	
Cys 230-Cys230	
Cys 261-Cys 321	
Cys 367-Cys 425	
Cys 134-Cys194	intra chain LC
Cys 23-Cys 88	

Dyadic demonstrates human-like glycosylation and activity

C1 Humanized Proteins for Monoclonal Antibodies



Glycan	Observed RT (min)	Amount (%)
M3	8,35	0,35
M3B	9,49	0,45
G0	10,49	32,54
G1	11,92	17,21
G1_2	12,13	29,51
G2	13,43	19,94
	Sum w/o IS	100
	Response w/o IS	101843184
	All human-like	99,2



- Nearly all human-like N-glycans; over 99 %, the remaining percentage is Man3-species
- Site-occupancy: relative response close to the values of reference Opdivo

Summary of mAbs Production in Microbial Production

Summary of Microbial Production of mAbs

- mAbs production in microbial production has advanced significantly
- Microbial production definitely offers cost advantage due to cheap growth medium, fast upstream process and absence of adventitious agents
- Based on the mechanism of action of the mAbs, you need to decide if glycosylation is required or not
- For glycosylated mAbs, Pichia and filamentous fungi offer options with suitable glycoengineering
- E. coli is a good option when glycosylation is not required. However, Fc engineering is advancing to achieve certain functionalities without glycosylation.
- Downstream processing suitable for these specific production systems needs to be designed for low COGs.

Grand Challenge RFP
Gates Foundation and LifeArc

Low-cost mAbs Grand Challenge RFP (Gates Foundation and LifeArc)

Initiative	Low-Cost mAbs Grand Challenge RFP
Objective	Fund innovative and bold ideas that enhance production efficiency and improve overall process economics for mAb production, ultimately reducing COGS toward our ≤$10/g target.
Progress to date	**Option A: Proof of Concept** (RFP closed Jan 31st) • 178 proposals received total • **6 proposals are being selected for funding considerations** **Option B: Operationalization & Economic Viability** • RFP open until May 31st • If you already have strong POC data, please apply.

Thank You

Gates Foundation