



Strategies for Optimized Medium Design

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Imagination at work

Outline

Medium development

Glycosylation as an aspect of product quality

Case studies

Conclusions



Medium development



Media

Serum

Supplements

Buffers & Process liquids



Custom medium design



Collaborations

Full collaboration

Prototype design
Spent media analysis
Data evaluation
Cell culture performed at GE

Customization kit

Prototype sampling

Medium collaboration

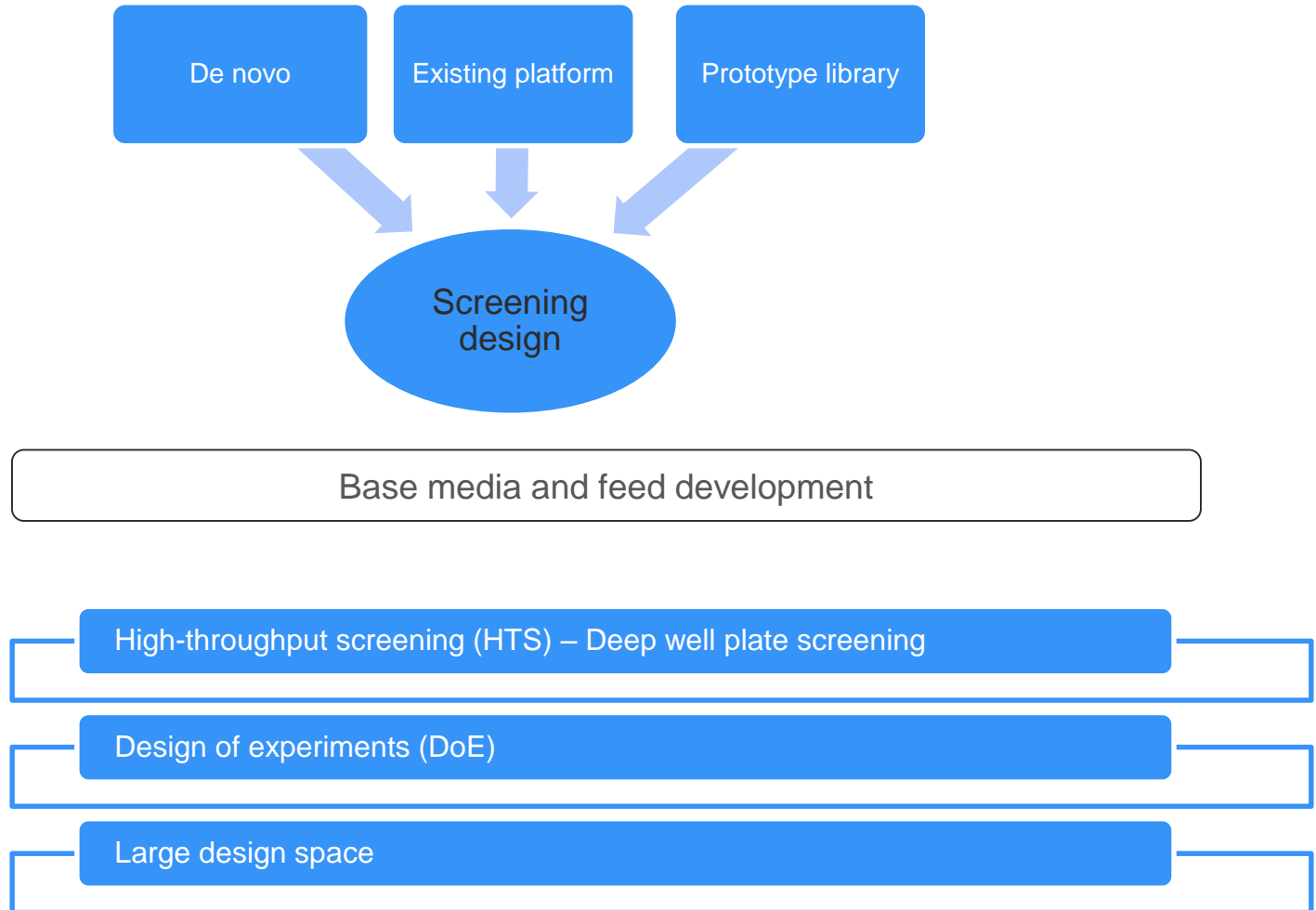
Prototype design
Spent media analysis
Data evaluation
Cell culture performed by customer

Spent media analysis

Amino acids
Vitamins
Trace elements



Development approach



High-throughput workflow summary



Automated 96-deep well format set-up

Hamilton Robotics Microlab™ STAR™ Line



Cell counting and analysis

IntelliCyt™ iQue™ Screener
(High-throughput flow cytometer)



Product quantitation

Valitacell Valita™ TITER HS kit



Select design type based on objectives, number of factors/levels, and resolution

Common design types and subtypes

Screening designs

Full factorials

Fractional factorials

Definitive Screening

Response surface designs

Central composite
(CCC, CCI, and CCF)

Box-Behnken

Optimal designs
(D,I,A,G-criteria)

Mixture designs

Simplex-lattice

Simplex-centroid

Extreme-vertices



Recent medium development projects

Chinese hamster ovary (CHO) programs	Average of ~ 15 cell lines
Average titer increase	380%
Range of titer increase	150% to 1000%
Average VCD increase	250%
Average q_p increase	> 200%

q_p = cell specific productivity



Glycosylation as an aspect of product quality



Key impacts of glycosylation on the PK and PD of mAb and Fc-fusion proteins

Glycan	Impacts
Mannose	<ul style="list-style-type: none"> Increases the clearance of mAb Enhances FcγRIIIa binding/ADCC of mAb Reduces C1q binding/CDC of mAb
Fucose	<ul style="list-style-type: none"> Interferes with binding to FcγRIIIa Defucosylation enhances FcγRIIIa binding/ADCC activity
Galactose	<ul style="list-style-type: none"> Exposed galactose can increase the clearance of mAb Enhances CDC of mAb
GlcNAc	<ul style="list-style-type: none"> Bisecting GlcNAc enhance FcγRIIIa binding/ADCC Increases the clearance of Fc-fusion proteins
Sialic acid NANA	<ul style="list-style-type: none"> Critical for reducing the clearance of Fc-fusion proteins Anti-inflammatory activity
Sialic acid NGNA	<ul style="list-style-type: none"> Interferes with FcγRIIIa binding and reduces ADCC activity of mAb Can be immunogenic in humans
Gal α 1–3Gal β 1–4GlcNAc-	<ul style="list-style-type: none"> Immunogenic in humans and can induce anaphylaxes

ADCC = antibody-dependent cellular cytotoxicity, CDC = complement-dependent cytotoxicity, GlcNAc = N-acetylglucosamine, NANA = N-acetylneuraminic acid, NGNA = N-glycolylneuraminic acid, PD = pharmacodynamics, PK = pharmacokinetics



Manipulation of glycan profile

Cell engineering

Medium and feeds

Cell culture process conditions

- Duration
- Harvest viability
- Fed-batch or perfusion
- Temperature shift
- Other culture parameters



Case studies



Increase antennary
structures and terminal
sialylation (rProtein)



Improve product quality and support high titer expression for a non-mAb product

Cell line

- CHO DG44 cell line, recombinant highly sialylated non-mAb biosimilar

Objective

- Develop optimized basal media and feed media

Goals

- Improve product quality
 - Match sialic acid profiles of innovator
 - Match antennary structures
- Support titer expression of 500 mg/L



Experimental design

Base medium development

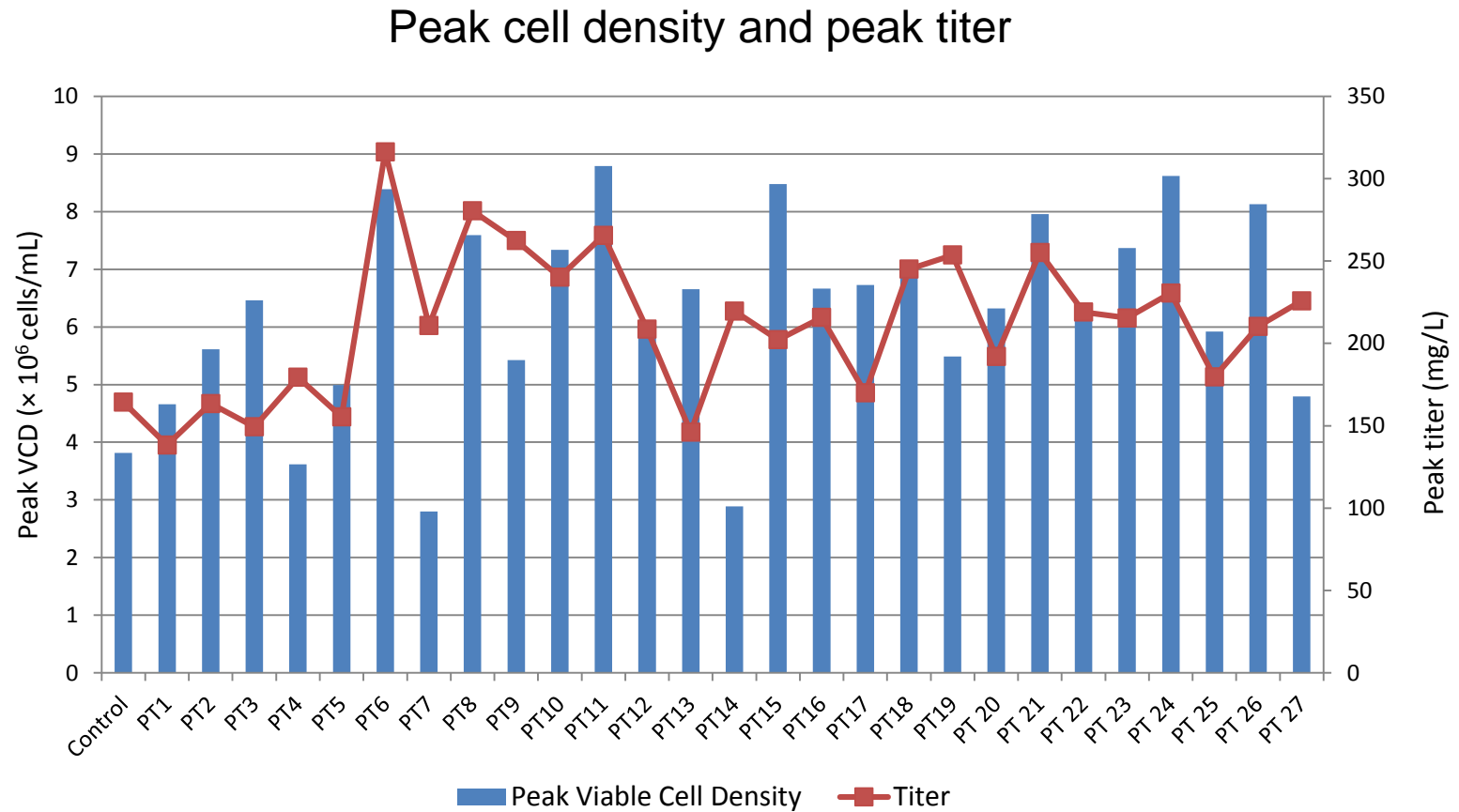
- Simplex-lattice design based on top medium prototypes from initial screen

Feed development

- Partial factorial design using specific components intended to increase sialylation



Growth and productivity base media

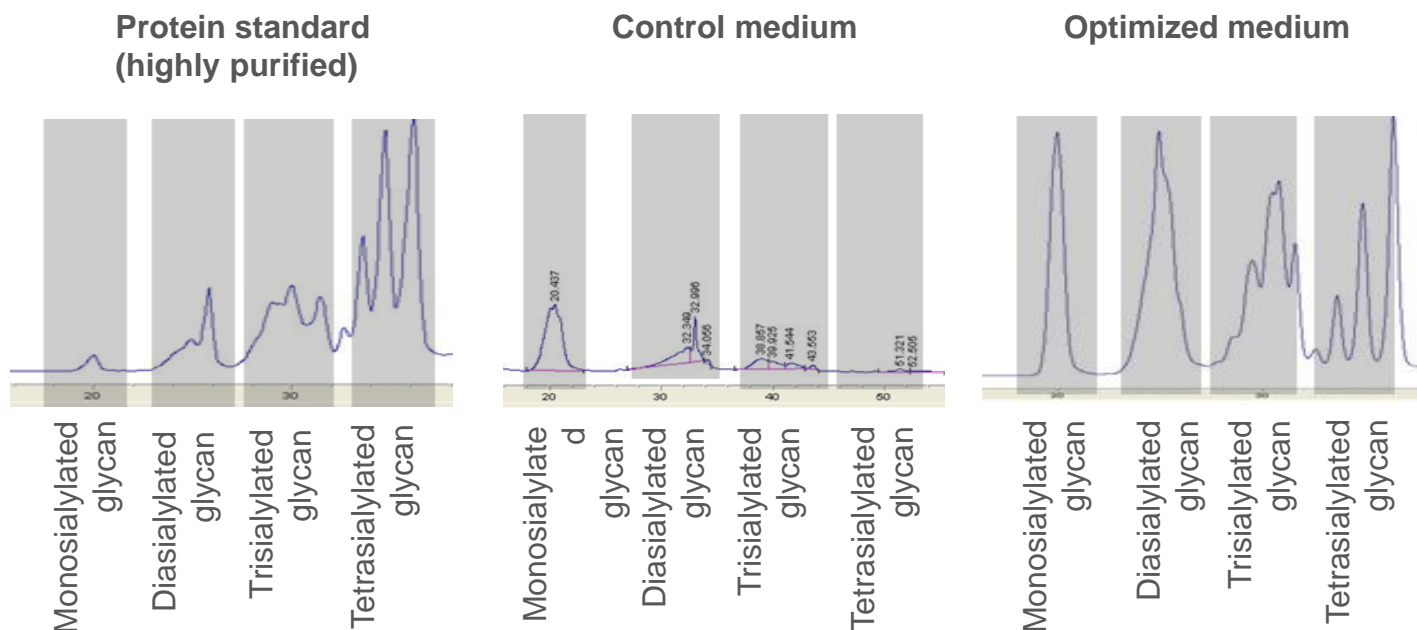


Product quality: tri- and tetra-sialylated glycans

Results

- The control medium lacked tri- and tetra-sialylated glycans
- Optimized formulation providing highest product quality
- Modified formulation showed improved tri- and tetra-sialylated glycans

Protein glycosylation profile comparison



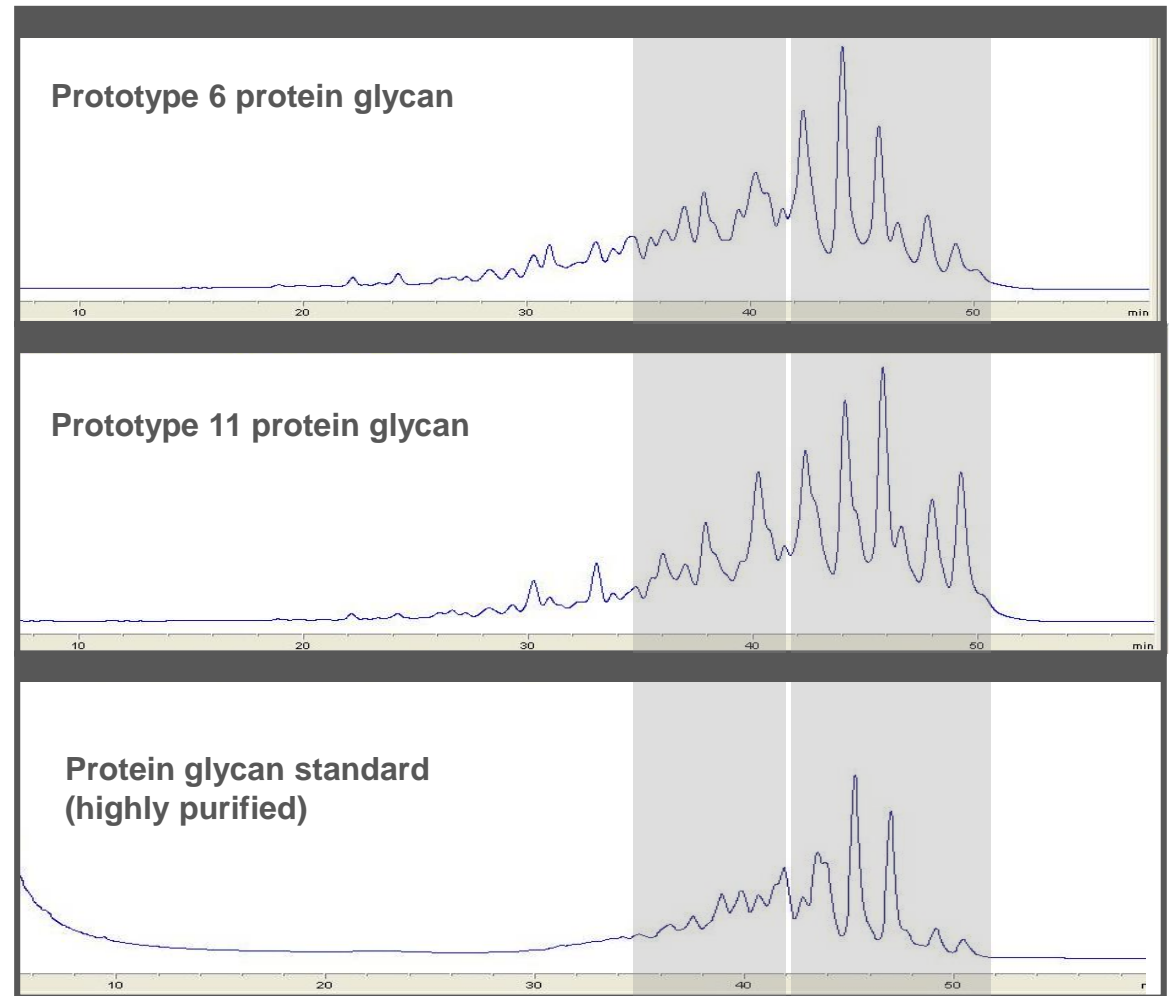
WAXHPLC analysis of protein expressed in CHO cells grown in the selected basal media compared with control and protein glycan standard



Improvement of antennary structure

Results

- Improvement of antennary structure
- NP-HPLC analysis of protein glycan standard, prototype 6 protein glycan and prototype 11 protein glycan.
- Note similarity of the tri-antennary and tetra-antennary profiles of the prototype media compared to the protein glycan standard



Tri-antennary
structures

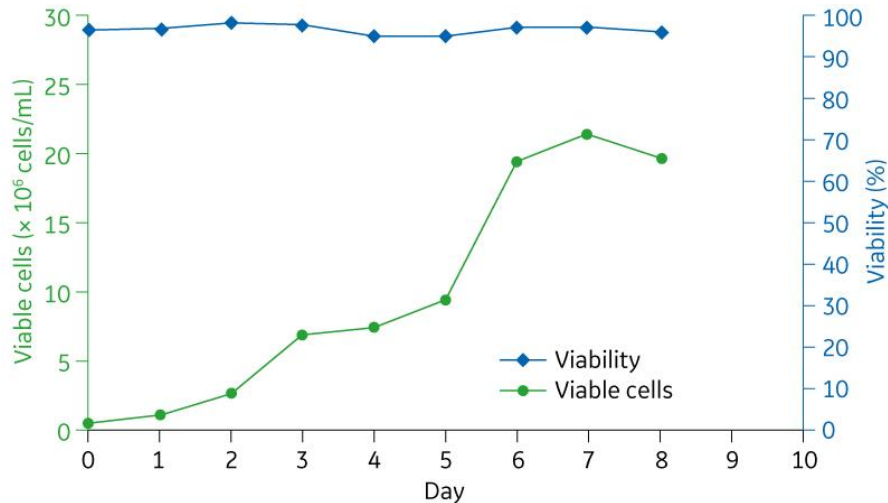
Tetra-antennary
structures



Increase antennary structures and terminal sialylation (rProtein)—summary

- Improved antennary structures
- Improved sialylation
- Improved growth and titer

Bioreactor fed-batch



Condition	Titer
Baseline control	~ 164 mg/L
Optimized medium	~ 316 mg/L
Optimized medium and feed	~ 550 mg/L



Adjust charged variants (mAb)



Improve product quality and develop feeds that support high cell densities and mAb expression

Cell line

- CHO DG44 (mAb)

Objective

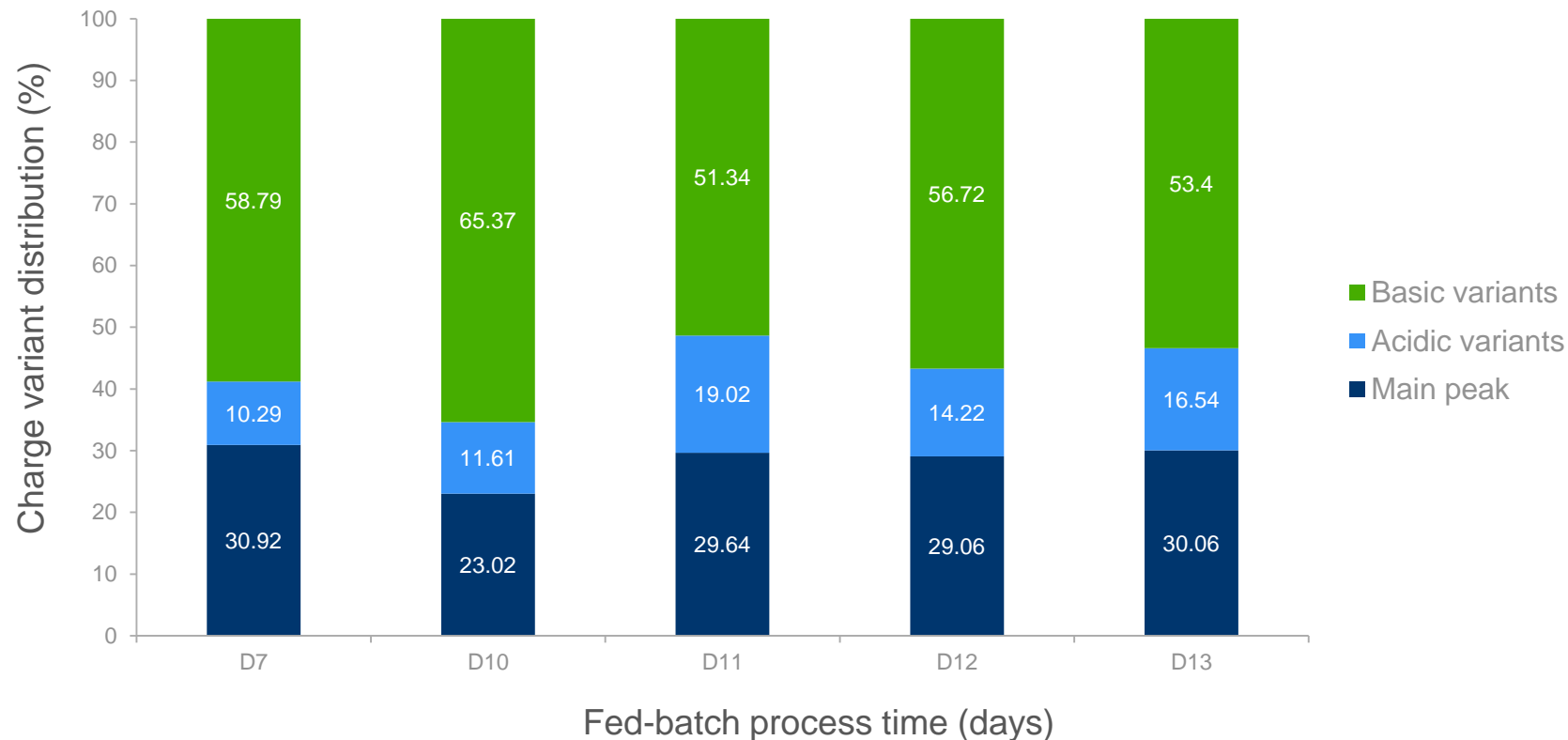
- Optimize feed medium and keep current basal medium

Goals

- Improve product quality
 - Lower acidic isoforms < 20% in bioreactor production
 - Lower basic isoforms < 30% in bioreactor production
- Develop feed/-s capable of supporting the expression of 1.6 g/L
- Develop feed/-s that support high cell densities of $> 20 \times 10^6$ cells/mL



Historical data for charged variants



Experimental design

Two-part screening design for initial feed development

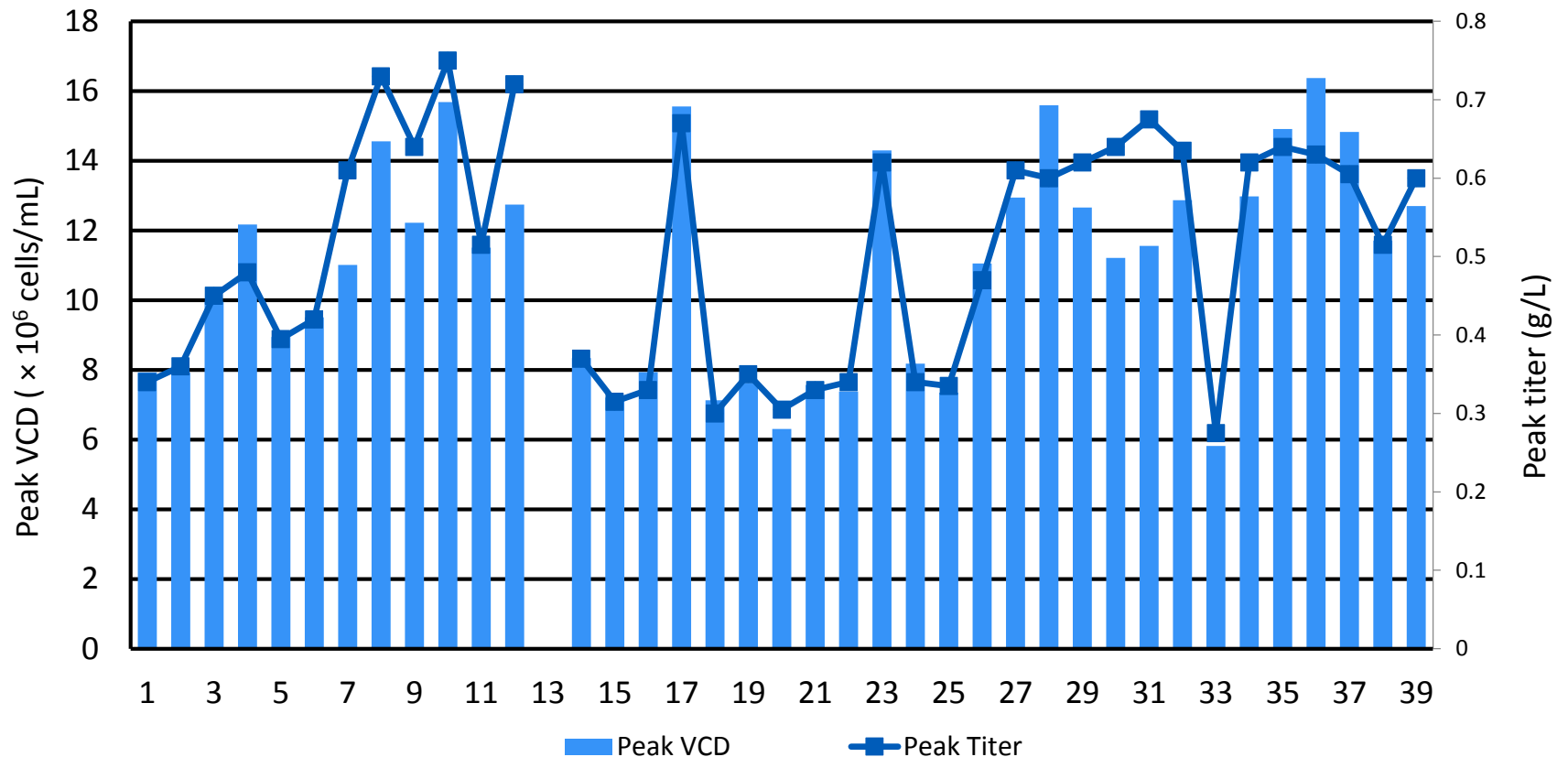
- Part 1: Simple screening, six feed prototypes at two concentrations
- Part 2: D-optimal, six factor/groups varied in two base feeds
 - reduced basic and acidic variants
 - increase main variant expression

Response variables

- VCD, viability, titer, and charge profile

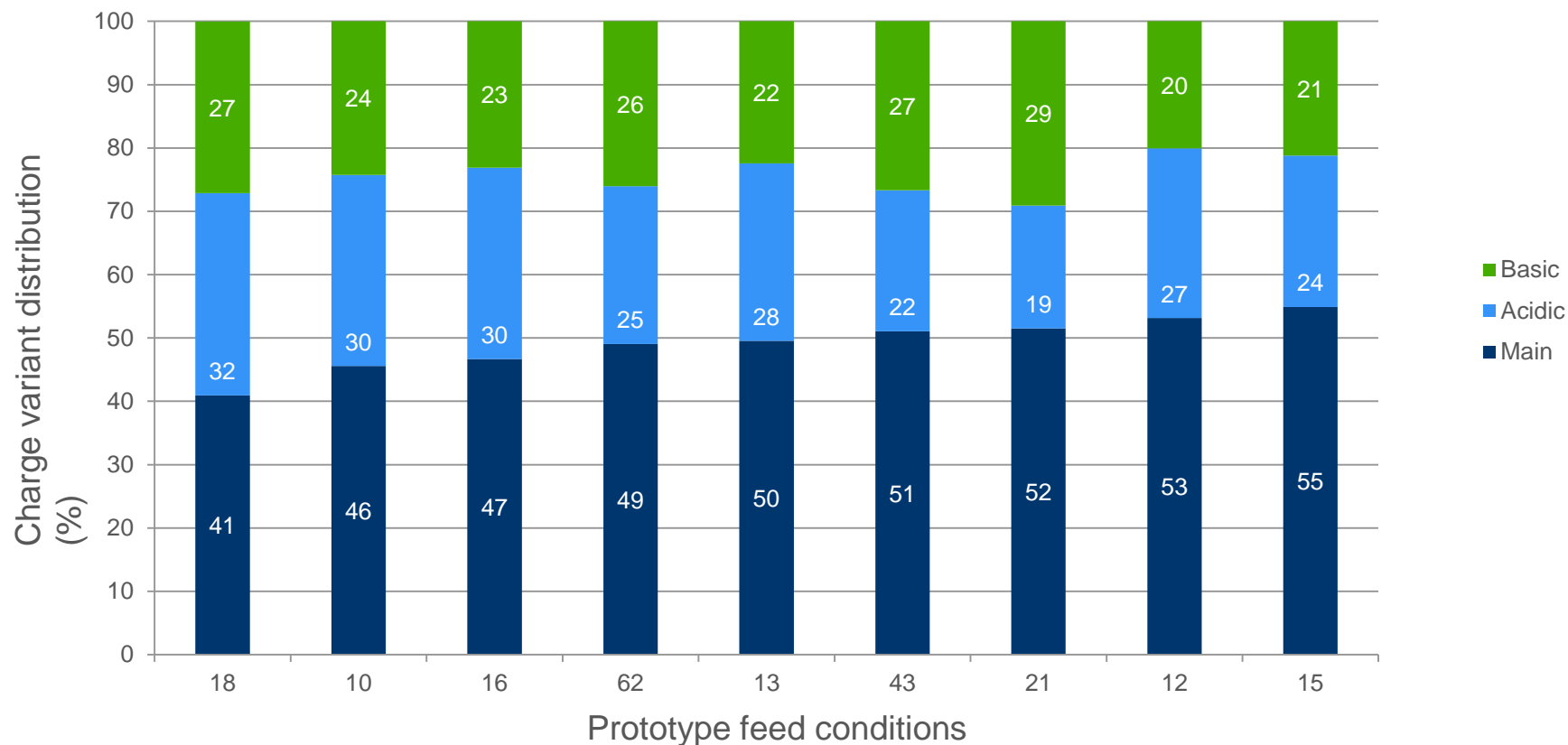


Growth and productivity



Part 2 results

Phase II: charge profile of selected prototypes



Adjust charged variants (mAb)—summary and next steps

Screening campaign successful

- Increasing main charge variant > 50%
- Lowering basic charge variant < 30%
- Maintaining low acidic charge variants < 20%
- Identifying key charge variant drivers

Next steps (ongoing)

- Further optimize key components
- Increase titer



Conclusions



A cell culture medium development toolbox

- Flexibility in many different formats and methodologies
- Large library of base media and feeds
- Large and manageable design space
 - HTS DoE synergy
- Different approaches for your different needs

Great success in modulating protein quality

- Improving sialylation and antennary structures
- Improving mAb charge profiles

Offering a set of diverse and proven tools for increased product quality in your cell culture

DoE = design of experiments, HTS = high-throughput screening



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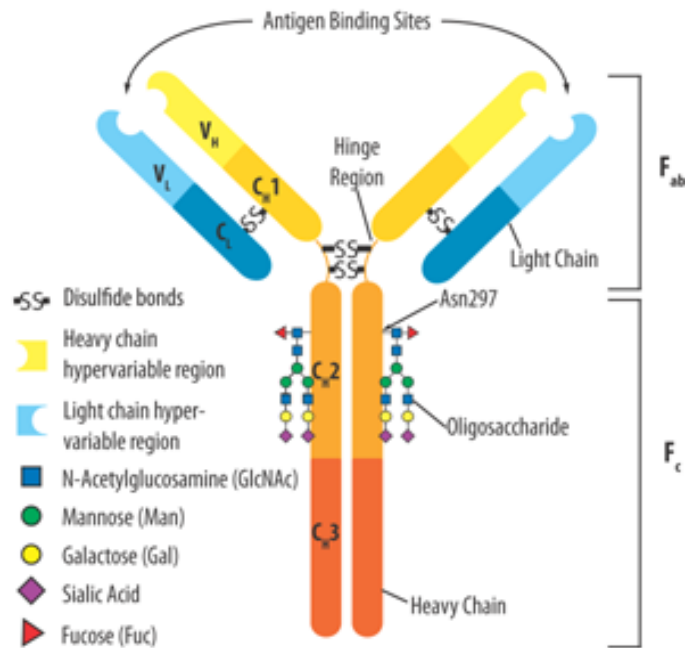
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mAb glycosylation forms



Shi H.H. and Goudar C.T. Recent advances in the understanding of biological implications and modulation methodologies of monoclonal antibody N-linked high mannose glycans. *Biotechnol. Bioeng.* **111**(10), 1907–1919 (2014).

Liu L. Antibody glycosylation and its impact on the pharmacokinetics and pharmacodynamics of monoclonal antibodies and Fc-fusion proteins. *J. Pharm. Sci.* **104**(6), 1866–1884 (2015).

