Multivariate profiling as a pivotal tool in identifying critical raw materials that rescue N-linked glycosylation profiles

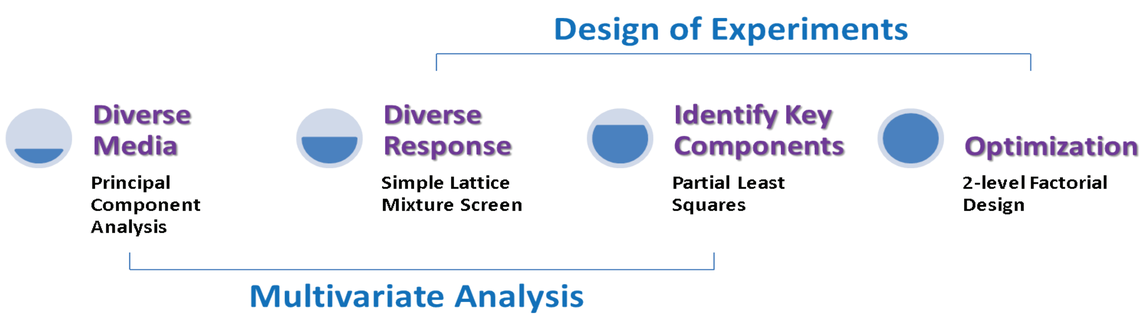
Steven B. Richardson[1], Omar Wahab[2], Christopher Kornfeld[1], James S. Ross[1], and Kevin Kayser[1]

1 Cell Sciences and Development, SAFC, 2909 Laclede Avenue, Saint Louis, MO 63103, USA
2 SAFC Irvine, Second Avenue, Heatherhouse Industrial Estate, Irvine, Ayrshire, KA12 8NB

Introduction

Over the past decade, notable advances in the characterization and control of post translational modifications (PTMs) to enhance therapeutic properties have continued to drive the development of many next-generation biopharmaceuticals. It has been widely reported that the quality of secreted therapeutic proteins is dependent on the consistency of attached glycan moieties. Insufficient glycosylation potentially impairs efficacy and safety. Our particular interest lies in identifying critical raw materials that rescue N-linked glycosylation profiles of therapeutic proteins from selected high-producing clones. This study represents an important step towards establishing cGMP ready chemically defined supplements that significantly and reproducibly adjust glycan moieties. In addition, the multivariate profiling process described here represents a pivotal tool in the upstream characterization of biopharmaceutical platforms.

Technical Approach



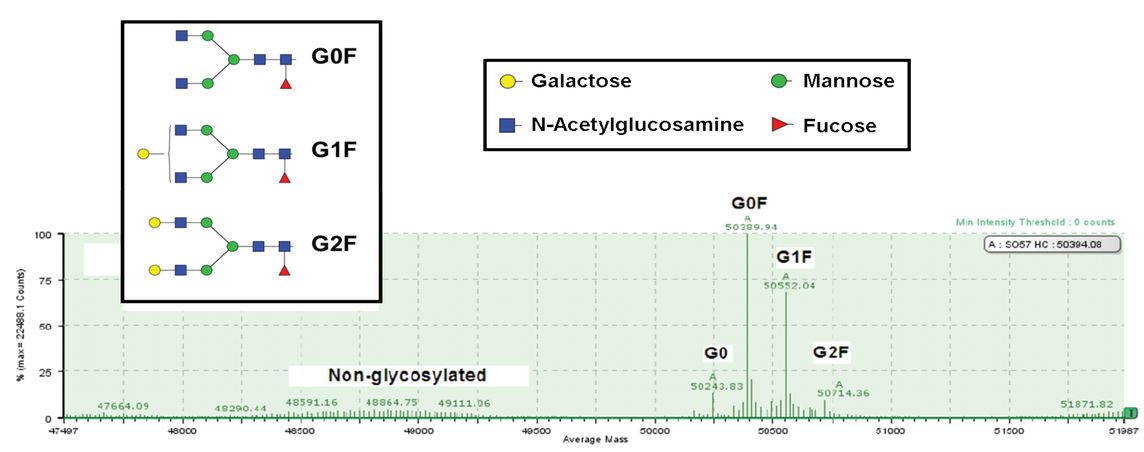
Materials & Methods

Fed-Batch Culture

Glutamine Synthetase (-/-) recombinant IgG producing CHOZN™ cell line. Duplicate 30 mL cultures in 125 mL vented shake flasks at 37°C and 140 rpm with 80% Rh and 5% CO2. Glucose supplementation as needed. Cultures maintained in Ex-CELL CHO CD Fusion with 5% additions of imMEDIAte ADVANTAGE™ manufactured chemically defined feeds on days 3, 5, 7, 9, and 11.

Intact Protein Accurate Mass Spectrometry

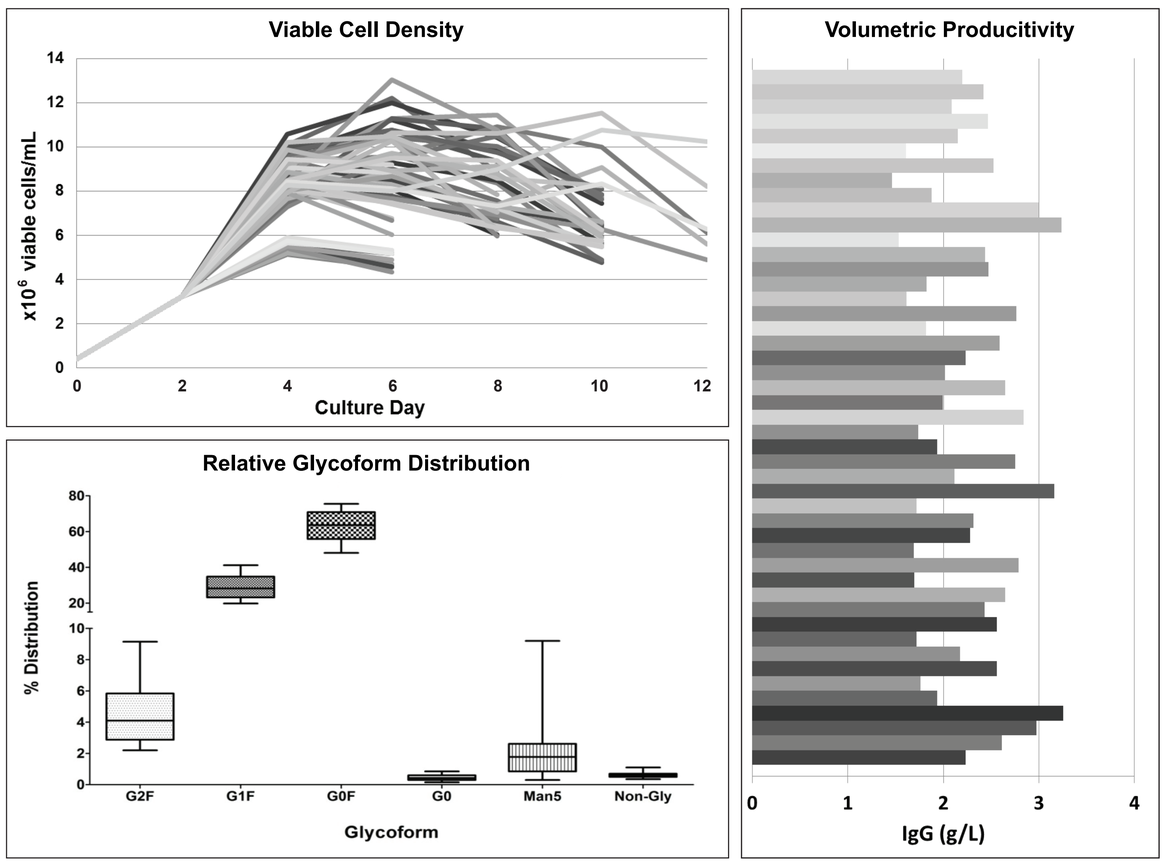
A high-throughput SEC-MS (Waters Acquity UPLC®/Q-Tof Premier™) workflow was developed. Protein-A purification of day 14 culture supernatants followed by intact mass analysis of antibody heavy chain constituents. Automated deconvolution and data processing performed using Waters BiopharmaLynx™ software.



Representative intact protein mass deconvoluted data of IgG-producing CHOZN™ GS clone in chemically defined fed-batch process.

Results

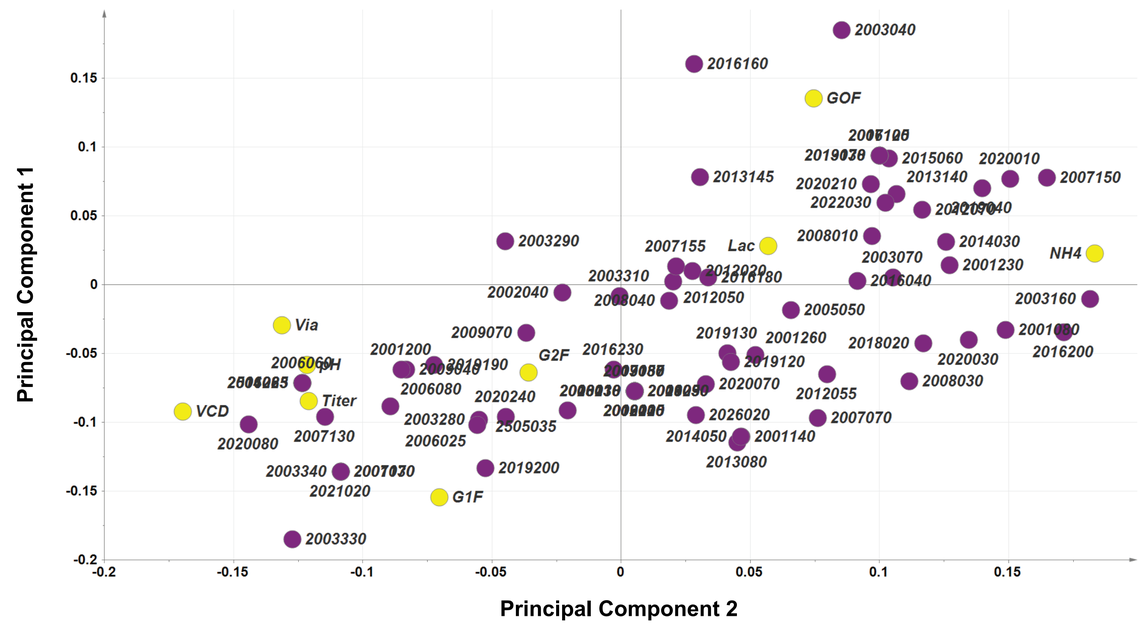
Proprietary Feed Screen



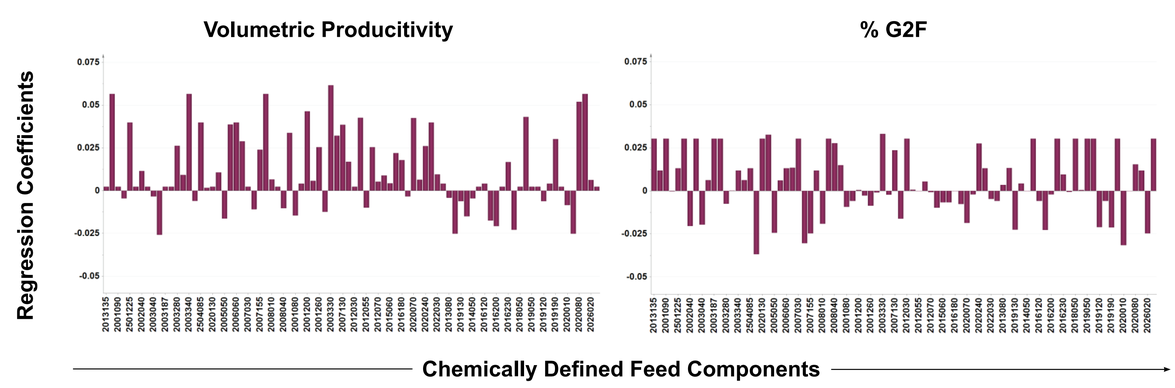
Variable culture performance of SAFC chemically defined feeds pre-selected for biochemical diversity by principal component analysis (SIMCA 13.0).

Results (cont.)

Multivariate Identification of Critical Raw Materials

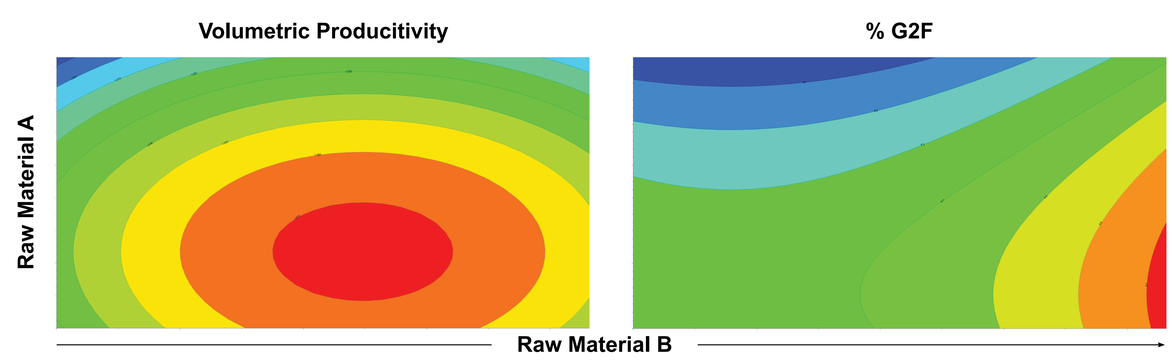


Loading Plot demonstrating correlations of critical raw materials to overall cell culture performance (SIMCA 13.0).



Coefficient Plots demonstrating positive and negative correlations of critical raw materials to specific cell culture performances (SIMCA 13.0).

Optimization



Response Contour Plots based on 2-Level Factorial DoE model illustrating local optimums (mg/L) of critical raw materials for selected cell culture performance (Design Expert 9.0).

Conclusion

Ex Cell Glycosylation Adjust (Gal+)

14 Day Fed- Batch Culture	G2F (%)	G1F (%)	G0F (%)	G0 (%)	Man5 (%)	IVCD (vc/mL/day)	IgG (g/L)
No Supplement	n.d.	11.1	82.7	5.9	n.d.	1.4E+08	3.1
1/2x Supplement	6.2	28.9	58.4	5.9	n.d.	1.4E+08	3.0
1x Supplement	9.8	41.5	42.3	6.0	n.d.	1.4E+08	3.1

- Identified critical raw materials that demonstrate 2-4 fold increases in relative G1F and G2F distributions.
- Optimized supplement component ranges that minimally affect volumetric productivity and viable cell density.
- Established culture supplementation protocol at 0.2% (v/v) beginning on day 2 and then every other day.
- Demonstrated scalable performance over a wide variety of bioproduction cell origins including CHO-GS, CHO-M, DuxB11, and NS0 lines (data not shown).
- Established multivariate profiling process as an important tool in upstream biopharmaceutical development.