Supplementary Material

Identification of Cell Culture Factors Influencing Afucosylation Levels in Monoclonal Antibodies by Partial Least-Squares Regression and Variable Importance Metrics

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Table S1. List of definitions for upstream culture levers considered in the PLSR models.

INDEPENDENT FACTOR	DEFINITION	MOLECULES
ADDITIVE 1 BOLUS CONC.	The concentration of the additive 1 bolus addition.	mAb-γ
ADDITIVE 1 BOLUS DAY	The day of the addition of the additive 1 bolus to the bioreactor.	mAb-γ
ADDITIVE 1 FEED	The concentration of the additive 1 feed.	mAb-γ
ADDITIVE 2 CONC.	The concentration of the additive 2 supplementation.	mAb-δ
BASAL AMMONIA	The real concentration of ammonia in the basal media prior to inoculation.	mAb-γ
BASAL GLUCOSE CONC.	The real concentration of glucose in the basal media prior to inoculation.	mAb-α, mAb-β, mAb-γ, mAb-δ
BASAL GLUTAMATE CONC.	The real concentration of glutamate in the basal media prior to inoculation.	mAb-γ
BASAL GLUTAMINE CONC.	The real concentration of glutamine in the basal media prior to inoculation.	mAb-γ
BASAL NA^+ CONC.	The real concentration of sodium ions in the basal media prior to inoculation.	mAb-γ
BASAL OSMOLALITY	The real osmolality of the basal media prior to inoculation.	mAb-α, mAb-β, mAb-γ, mAb-δ
COPPER SUPP. CONC.	The concentration of the copper sulfate supplementation.	mAb-δ
DAILY FEED %	The percent of the feed added each day.	mAb-α, mAb-β, mAb-γ
FEED AMMONIA	The real concentration of ammonia in the feed media prior to addition to the bioreactor.	mAb-γ
FEED GLUCOSE CONC.	The real concentration of glucose in the feed media prior to addition to the bioreactor.	mAb-α, mAb-β, mAb-γ, mAb-δ
FEED GLUTAMATE CONC.	The real concentration of glutamate in the feed media prior to addition to the bioreactor.	mAb-γ

FEED GLUTAMINE CONC.	The real concentration of glutamine in the feed media prior to addition to the bioreactor.	mAb-γ
FEED NA^+ CONC.	The real concentration of sodium ions in the feed media prior to addition to the bioreactor.	mAb-γ
FEED OSMOLALITY	The real osmolality of the feed media prior to addition to the bioreactor.	mAb-α, mAb-β, mAb-γ, mAb-δ
FEED PH	The real pH of the feed media prior to addition to the bioreactor.	mAb-α, mAb-β, mAb-γ, mAb-δ
FEED START DAY	The start day to begin feeding the bioreactor after the initiation of the culture.	mAb-δ
GLUCOSE SETPOINT	The midpoint between the glucose action limit and glucose supplement limit.	mAb-δ
PH LOWER BOUND	The lower bound for the pH control range of the bioreactor.	mAb-γ
PH SETPOINT	The target pH at the outset of the bioreactor culture that the pH control range can be defined relative to as ± pH units.	mAb-α, mAb-β, mAb-γ
PH UPPER BOUND	The upper bound for the pH control range of the bioreactor.	
SEEDING DENSITY	The cell density of the bioreactor culture following inoculation.	mAb-α, mAb-β, mAb-γ, mAb-δ
TEMPERATURE SETPOINT	The initial target temperature at the outset of the bioreactor culture.	mAb-α, mAb-β
TEMPERATURE SHIFT DIFFERENCE	The difference between the initial culture temperature setpoint and the new culture temperature setpoint following the application of the temperature shift.	mAb-α

Table S2. Summary of the final PLSR model metrics for each of the molecules (Afuc refers to afucosylation, Mann refers to mannose).

Model ID	LVs	R^2	RMSEC (% afuc)	Relative RMSEC (%)	RMSECV (% afuc)
mAb-α Total Afuc	2	0.63	0.60	9.38	0.81
mAb-α High Mann	2	0.72	0.53	8.20	0.70
mAb-α Complex Afuc	2	0.79	0.46	12.56	0.66
mAb-β Total Afuc	3	0.66	0.58	8.82	0.95
mAb-β High Mann	4	0.66	0.58	8.26	1.56
mAb-β Complex Afuc	4	0.70	0.54	12.45	1.09
mAb-γ Total Afuc	4	0.82	0.42	4.07	0.83
mAb-γ High Mann	4	0.84	0.40	5.15	0.83
mAb-γ	6	0.76	0.48	12.74	0.96

Complex Afuc					
mAb-δ Total Afuc	3	0.32	0.82	20.45	0.96
mAb-δ High Mann	3	0.78	0.46	17.21	0.55
mAb-δ Complex Afuc	3	0.75	0.49	12.36	0.59

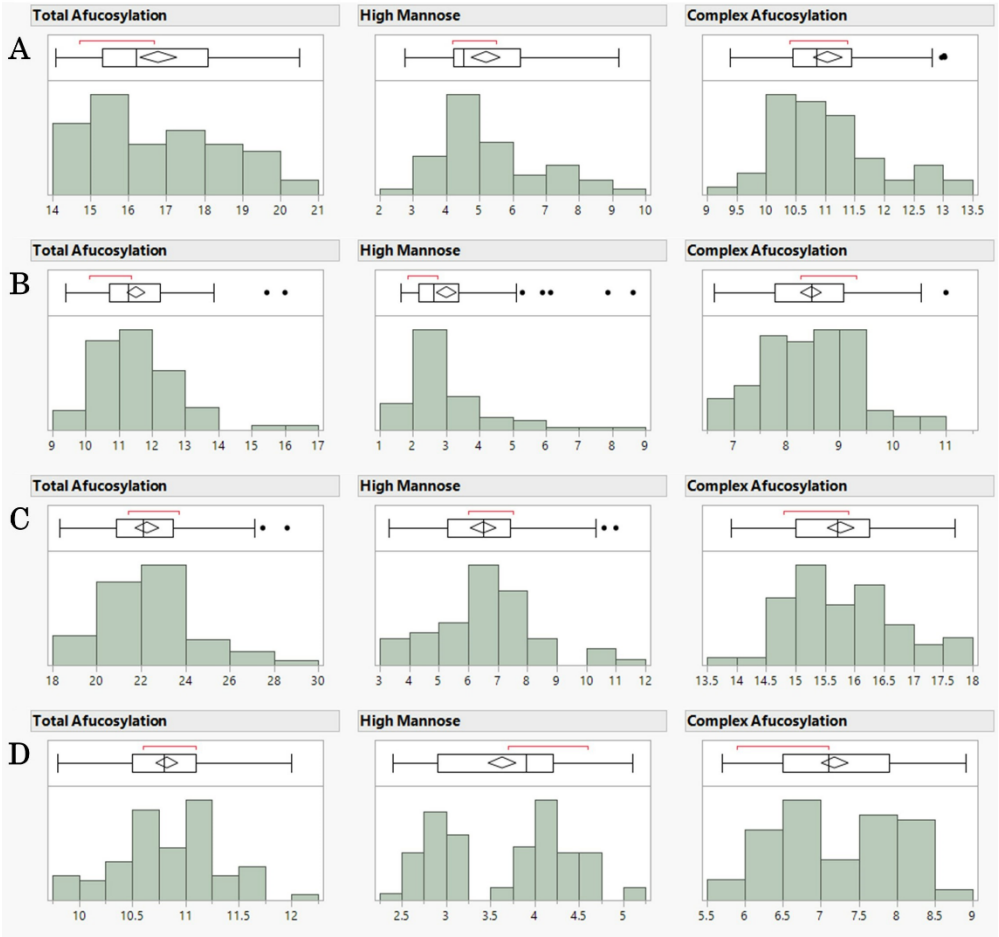

Figure S1. Distribution for the different afucosylation categories for the molecules (**A**) mAb-α, (**B**), mAb-β, (**C**) mAb-γ, and (**D**) mAb-δ. All figures were generated from JMP (Version 15.2.0, SAS Institute Inc., Cary, NC).