Supplements for: Subcellular fractionation enables Assessment of nucleotide sugar donors inside the Golgi apparatus as a prerequisite for unravelling culture impacts on glycoforms of antibodies

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Keywords: Nucleotide sugar donors, subcellular fractionation, Golgi apparatus, antibody glycosylation, metabolomics

Supplementary Materials

Supplementary Table S 1) Digitonin stock solution for digitonin lysis buffer

Compound	Molarity (mM)	
sodium chloride	115	
sodium bicarbonate	0.02	
digitonin	0.81	
EDTA	2	(protein analysis only)

Supplementary Table S 2) Cell lysis buffer for protein extraction

Compound	Molarity (mM)
sodium chloride	115
sodium bicarbonate	25
Triton X-114	1.97
SDS	0.35
EDTA	2
Natriumazid	0.15

Supplementary Table S 3) Dilution buffer for digitonin lysis buffer

Compound	Molarity (mM)	
Sodium chloride	115	
Sodium bicarbonate	0.02	
EDTA	2	(protein analysis only)

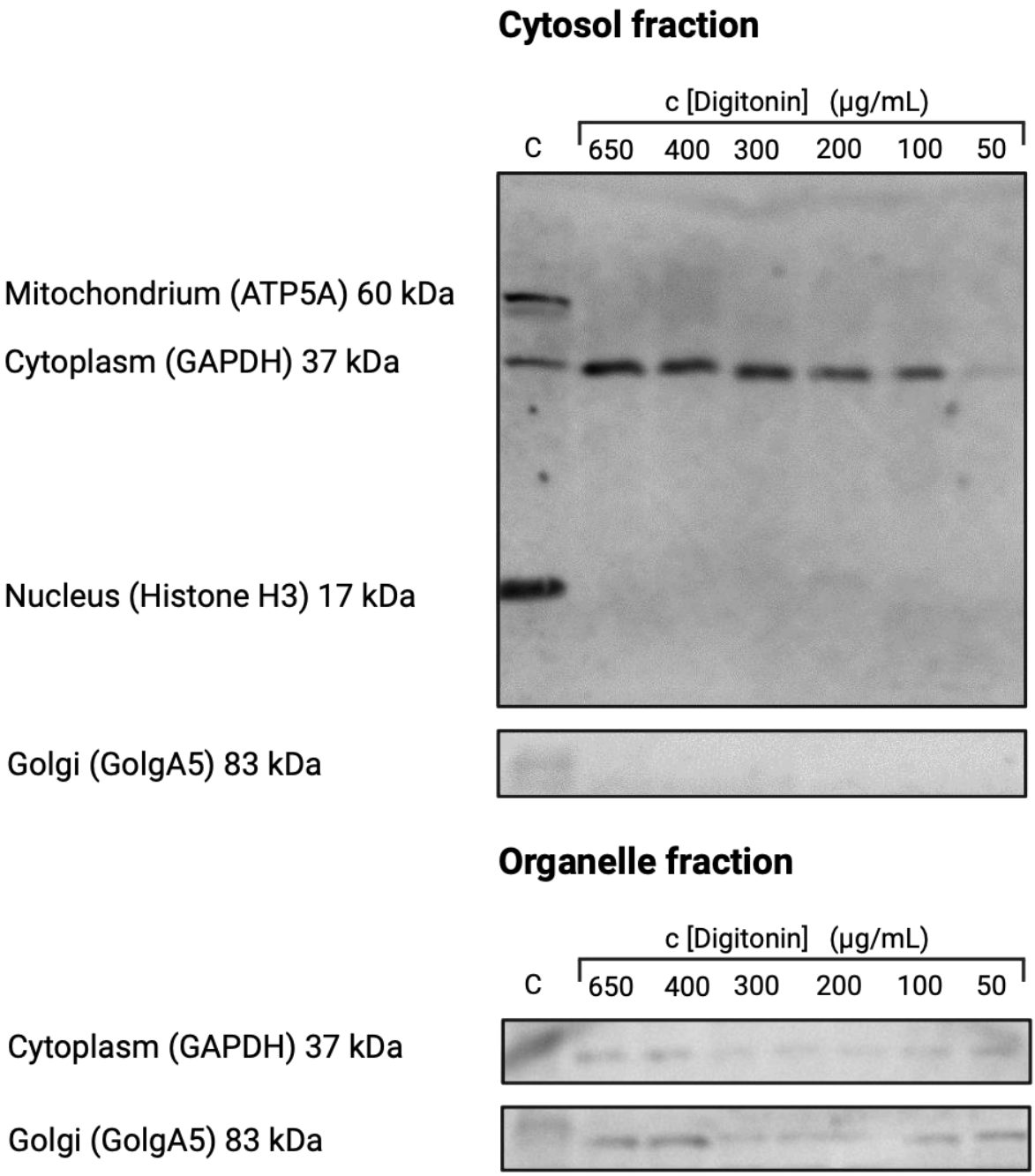
The solutions were adjusted to pH (7.3) and osmolarity (266 mOsmol) to resemble the cultivation medium used according to the manufacturer's recommendations, thereby preventing unnecessary stress on the cells during fractionation.

Supplementary Table S 4) Volumes and compositions of pulsing solutions in NSD-inducing shaking flask pulsings.

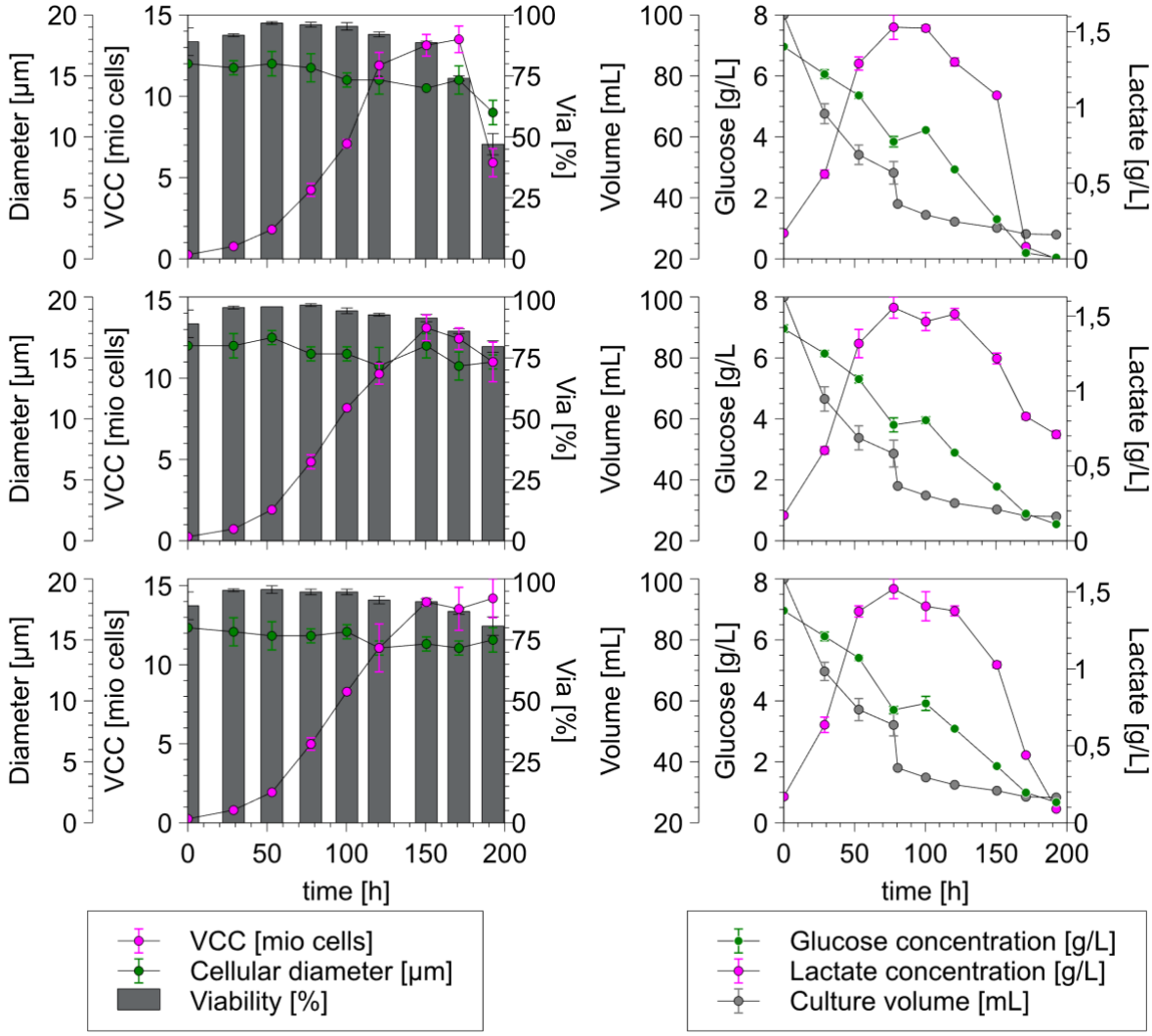
		Medium	Fructose	Galactose	Uridine	Manganese (II) chloride dihydrate (Mn)
Pulsing A	Volume [µL]	3698				
	Final conc. [mM]					
Pulsing B	Volume [µL]		3500		128.21	70
	Final conc. [mM]		100		0.5	0.001
Pulsing C	Volume [µL]			3500	128.21	70
	Final conc. [mM]			100	0.5	0.001

Supplementary Table S 5) Literature data for cell and compartment volumes, as well as molar distributions of NSDs between organelles and CP

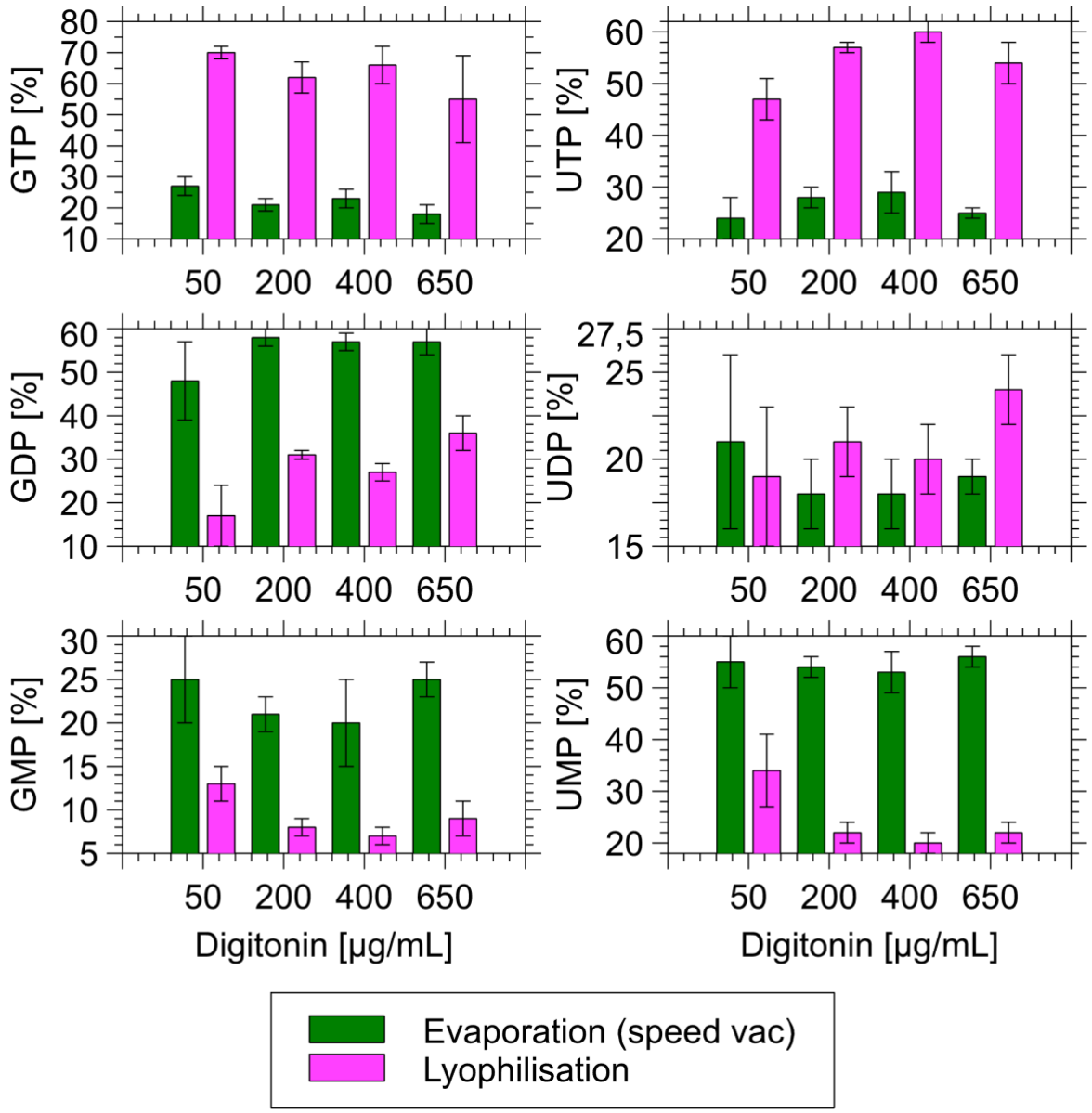
Parameter	**Value**	**Source**
V_{Cell}	1.12×10^{-12} L	[1]
V_{Golgi}	2.50×10^{-14} L (approximately 2.2%)	[1]
$V_{Cytoplasm}^{rel}$	50–60 % (approximately 55%)	[2]
Ratios $[c_{Golgi}]:[c_{Cytoplasm}]$	40:1	
$[c_{Golgi}^{rel}]$	40	[3], [4]
$[c_{Cytoplasm}^{rel}]$	1	



Supplementary Figure S 1) Western blot analysis of protein fractionations. (Top = CP, Bottom= organelle) with marker proteins of different organelles throughout the cell. Created in BioRender. https://BioRender.com/j12b168



Supplementary Figure S 2) Cultivation parameters of CHO shaking flask cultivations. Parameters under three different nutrient-pulsing regimes. Data show condition (A, Top) with only medium spiked, fructose spiked medium with Mn and uridine (B, middle), and galactose spiked medium with Mn and uridine (C, bottom). Left columns show cell vitality in viable cell count (VCC) [10^6 cells /mL] (pink), viability [%] (grey) and average cell diameter [µm] (green). Right side shows nutrient conditions in substrate concentration (glucose)[g/L] (green), byproduct concentration (lactate) [g/L] (pink) and culture volume [mL] (grey). Error bars show standard deviation for mean values of n=3. In rare cases, sample size had to be reduced.



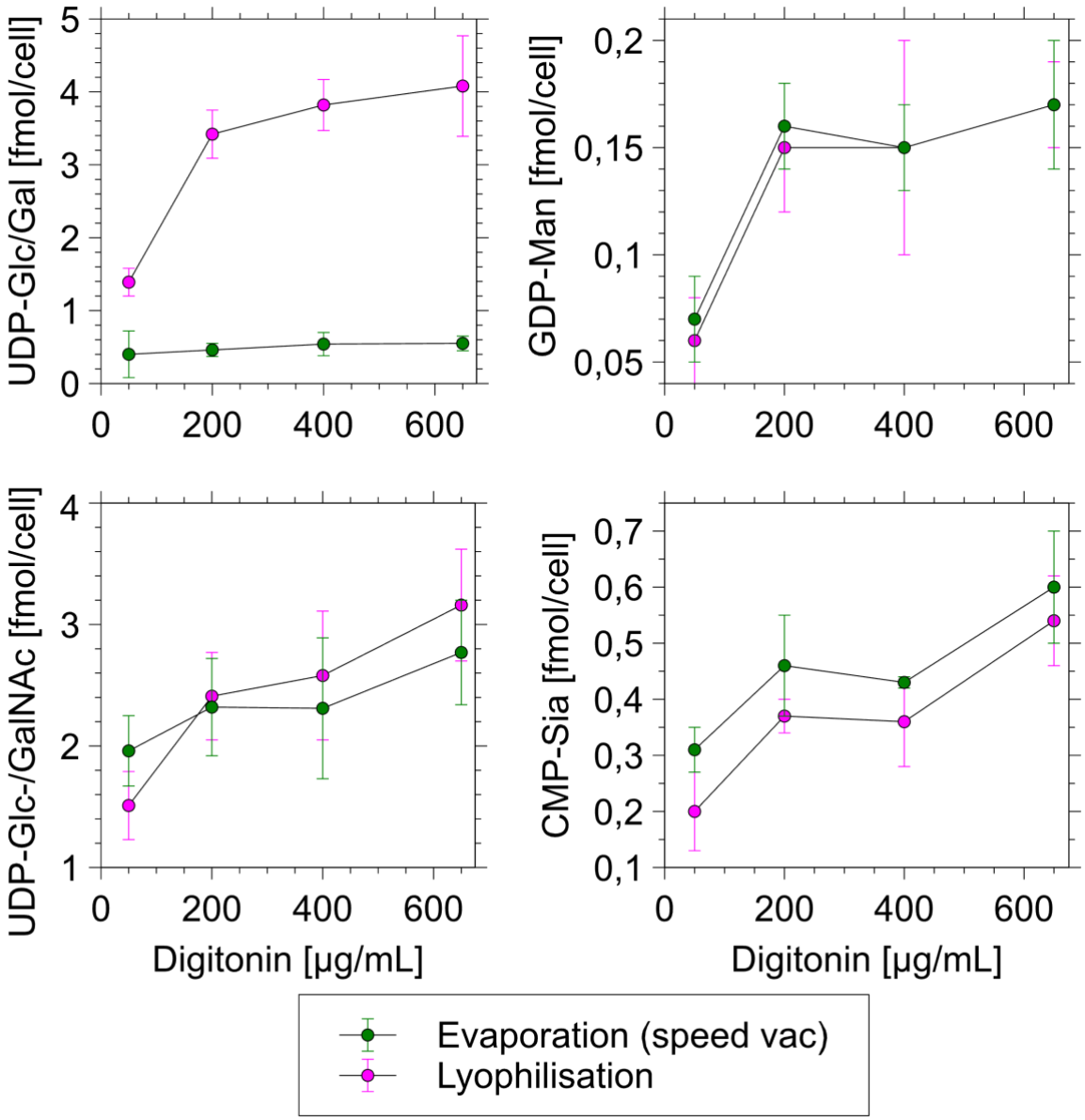
Supplementary Figure S 3) Oligophosphate levels. After concentration of fractions with lyophilization (pink) or evaporation (green). Error bars show standard error for mean values of n=3. In rare cases, sample size had to be reduced to n=2.

Supplementary Table S 6) Mean EC values GEC = guanosine energy charge, UEC = uridine energy charge. Deviations indicate standard deviations of averages of n = 11 with 4 different digitonin concentrations (50 µg/ml, 200 µg/ml, 400 µg/ml and 650 µg/ml) in the fractionation buffer.

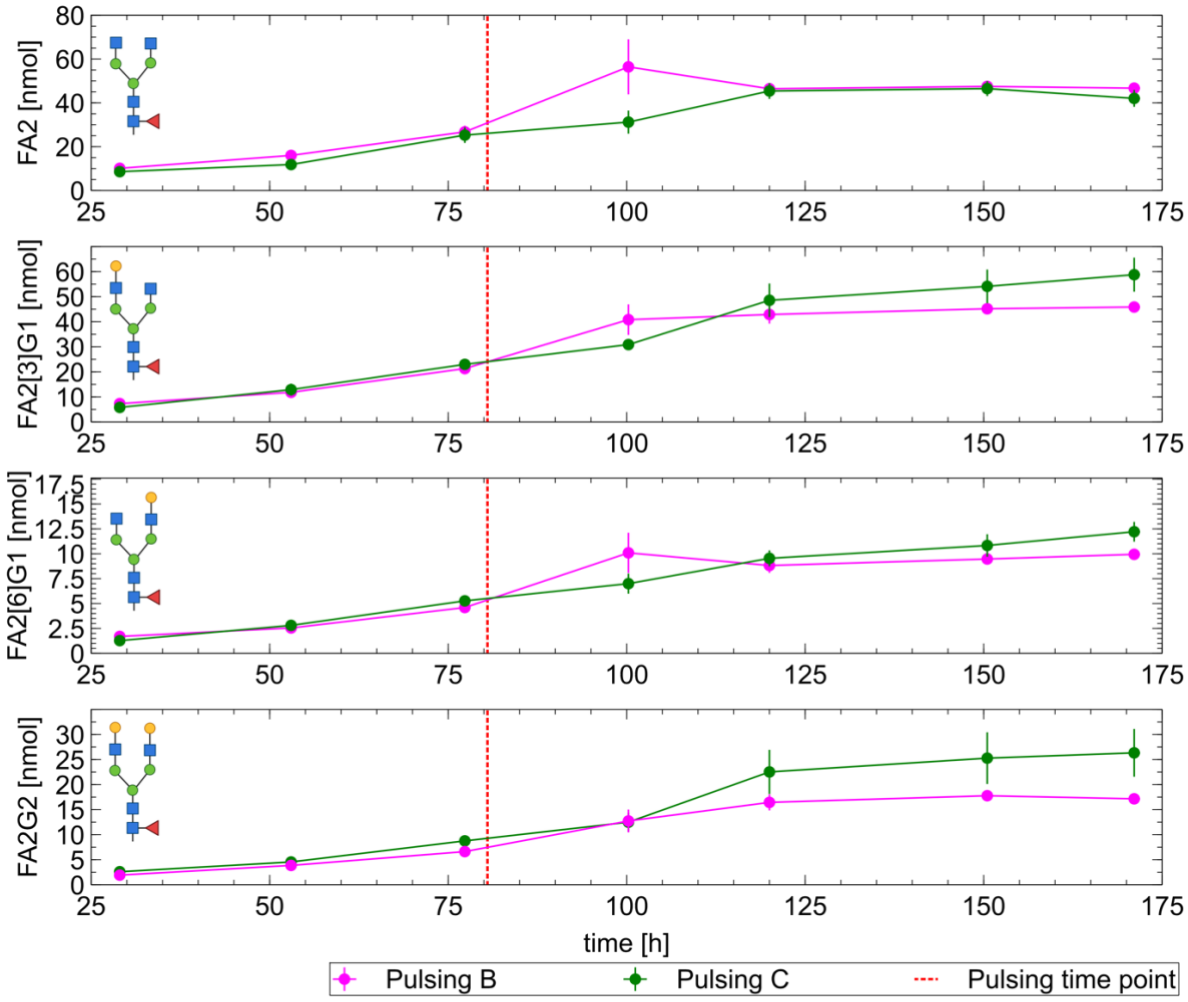
	Lyophilization	**Evaporation**
GEC	0.77 ± 0.03	0.5 ± 0.02
UEC	0.657 ± 0.06	0.4 ± 0.01

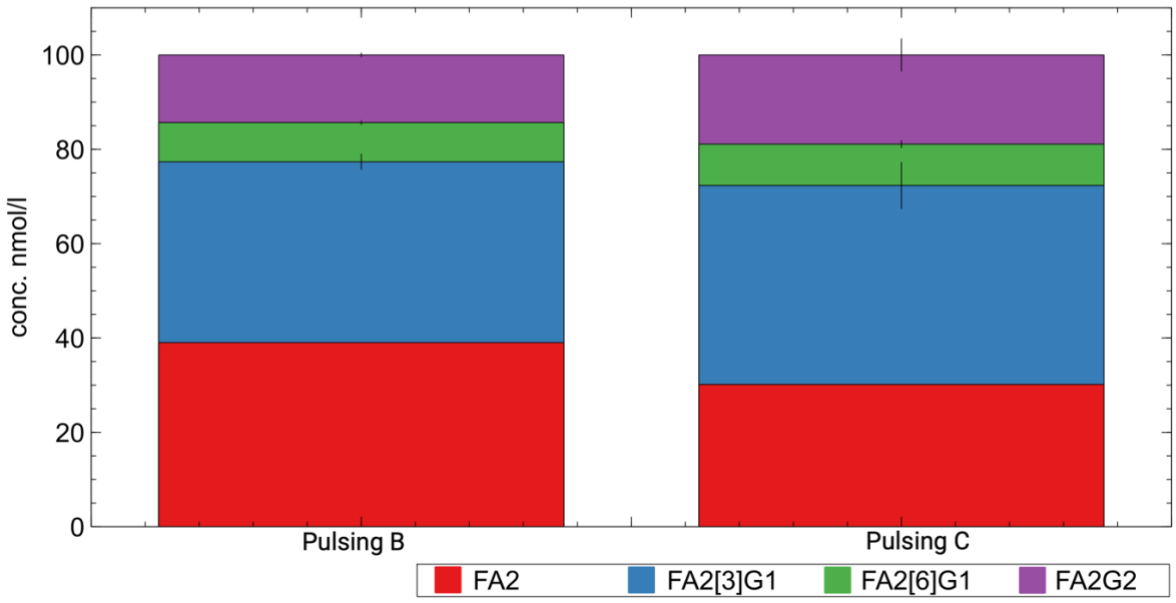
Supplementary Table S 7) Literature values of guanine and uridine energy charges (GEC and UEC) with sources

Energy charge	Literature	Source
GEC	0.82 ± 0.020	[5]
UEC	0.58 ± 0.016	[6]



Supplementary Figure S 4) Comparison of NSD conservation in sample concentration. NSD levels after concentration of fractions with lyophilization (pink) or evaporation (green). Error bars show standard error for mean values of n=3. In rare cases, sample size had to be reduced to n=2.





Supplementary Figure S 5) Pulse dependent Glycosylation patterns. Top: Absolute concentration courses of glycans produced under pulsing condition B (fructose, uridine, and Mn, shown with magenta) and C (galactose, uridine, and Mn, shown with green). Bottom: relative glycan distributions expressed on antibodies at end of cultivations under pulsing condition B (left) and C (right). Error bars show standard error for mean values of n=3. In rare cases, sample size had to be reduced. Created in BioRender, https://BioRender.com/b13d335.

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