Direct Quality Control of Glycoengineered Erythropoietin Variants

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Biotherapeutics

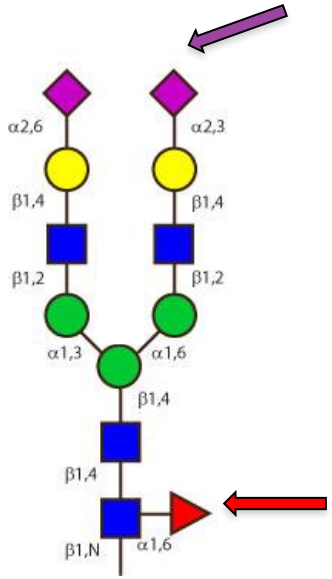
	Drug	Generic name	Sales in 2017 (Billions)	Glycosylation
1	Humira	Adalimumab	$18.94	2 N-glycans
2	Enbrel	Etanercept	$8.34	6 N-glycans, 26 O-glycans
3	Rituxan	Rituximab	$7.78	2 N-glycans
4	Remicade	Infliximab	$7.77	2 N-glycans
5	Herceptin	Trastuzumab	$7.39	2 N-glycans
6	Avastin	Bevacizumab	$7.04	2 N-glycans
7	Lantus	Insulin glargine	$6.72	/
8	Eylea	Aflibercept	$5.93	12 N-glycans
9	Opdivo	Nivolumab	$5.79	2 N-glycans
10	Neulasta	Pegfilgrastim	$4.50	/
11	Stelara	Ustekinumab	$4.01	2 N-glycans
..	….	….	…	…
20	Aranesep/Nesp	Darbepoetin Alfa	$2.62	5 N-glycans, 1 O-glycan

+80% of biotherapeutics are glycosylated

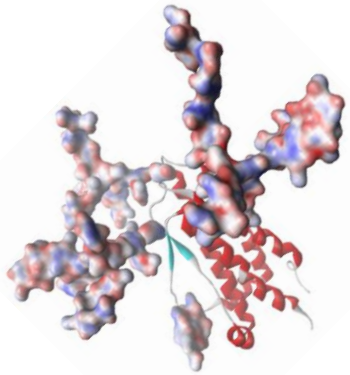
Walsh G. Biopharmacutical benchmarks 2018. Nature Biotechnology

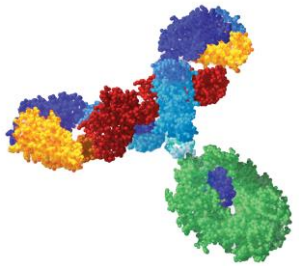
Biotherapeutics

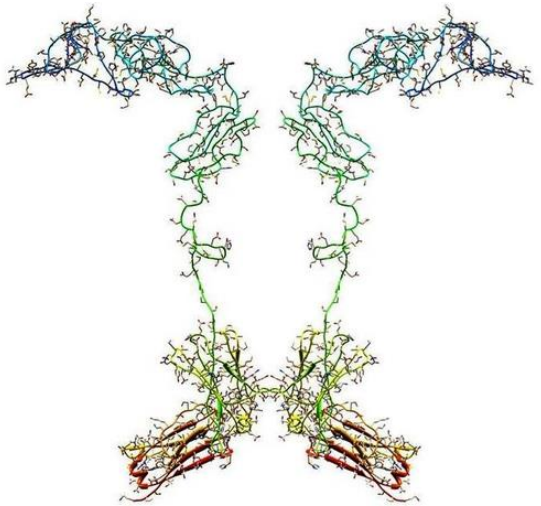
Anti-inflammatory properties
serum half-life increase



Core fucose -ADCC







Control of glycosylation = control immune system activation/deactivation

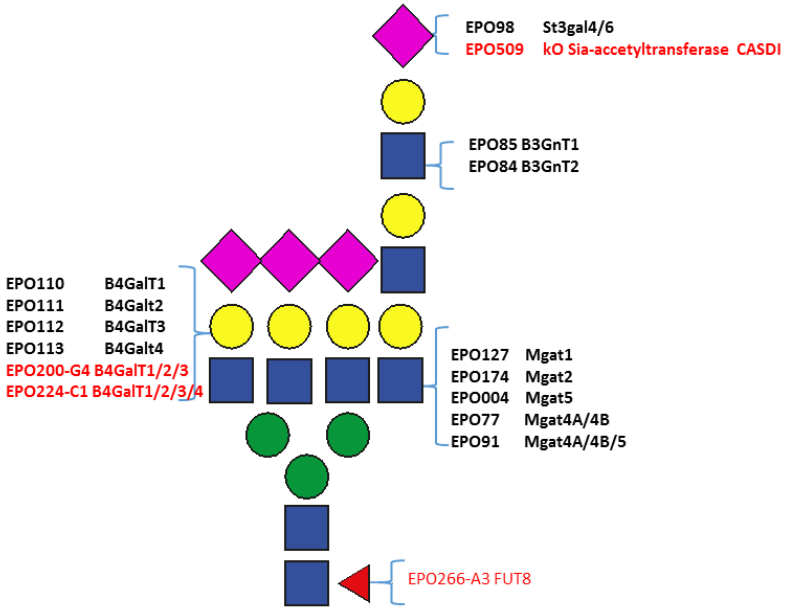
Major issues : 1. Controlling the glycosylation
2. Product characterization

Controlling the glycosylation

NATURE BIOTECHNOLOGY | RESEARCH | BRIEF COMMUNICATIONS

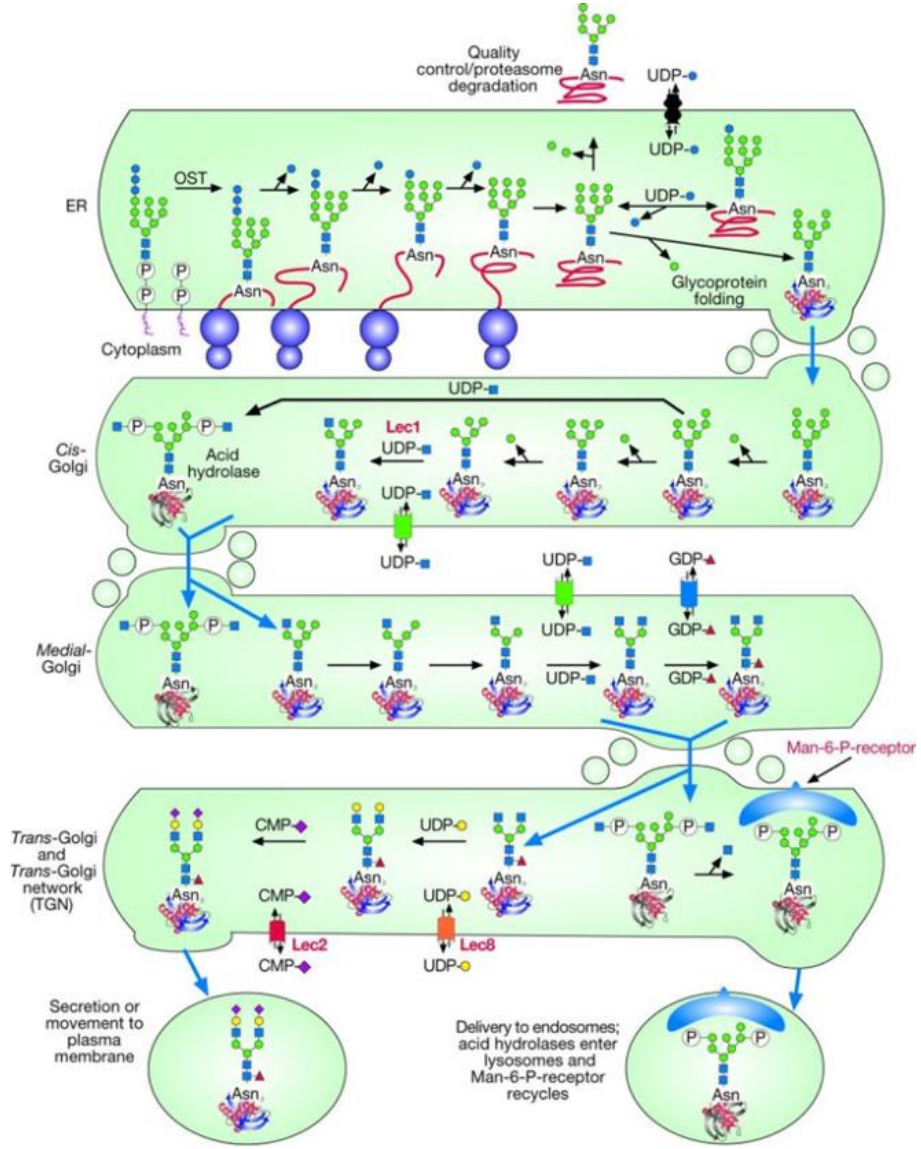
Engineered CHO cells for production of diverse, homogeneous glycoproteins

Zhang Yang, Shengjun Wang, Adnan Halim, Morten Alder Schulz, Morten Frodin, Shamim H Rahman, Malene B Vester-Christensen, Carsten Behrens, Claus Kristensen, Sergey Y Vakhrushev, Eric Paul Bennett, Hans H Wandall & Henrik Clausen

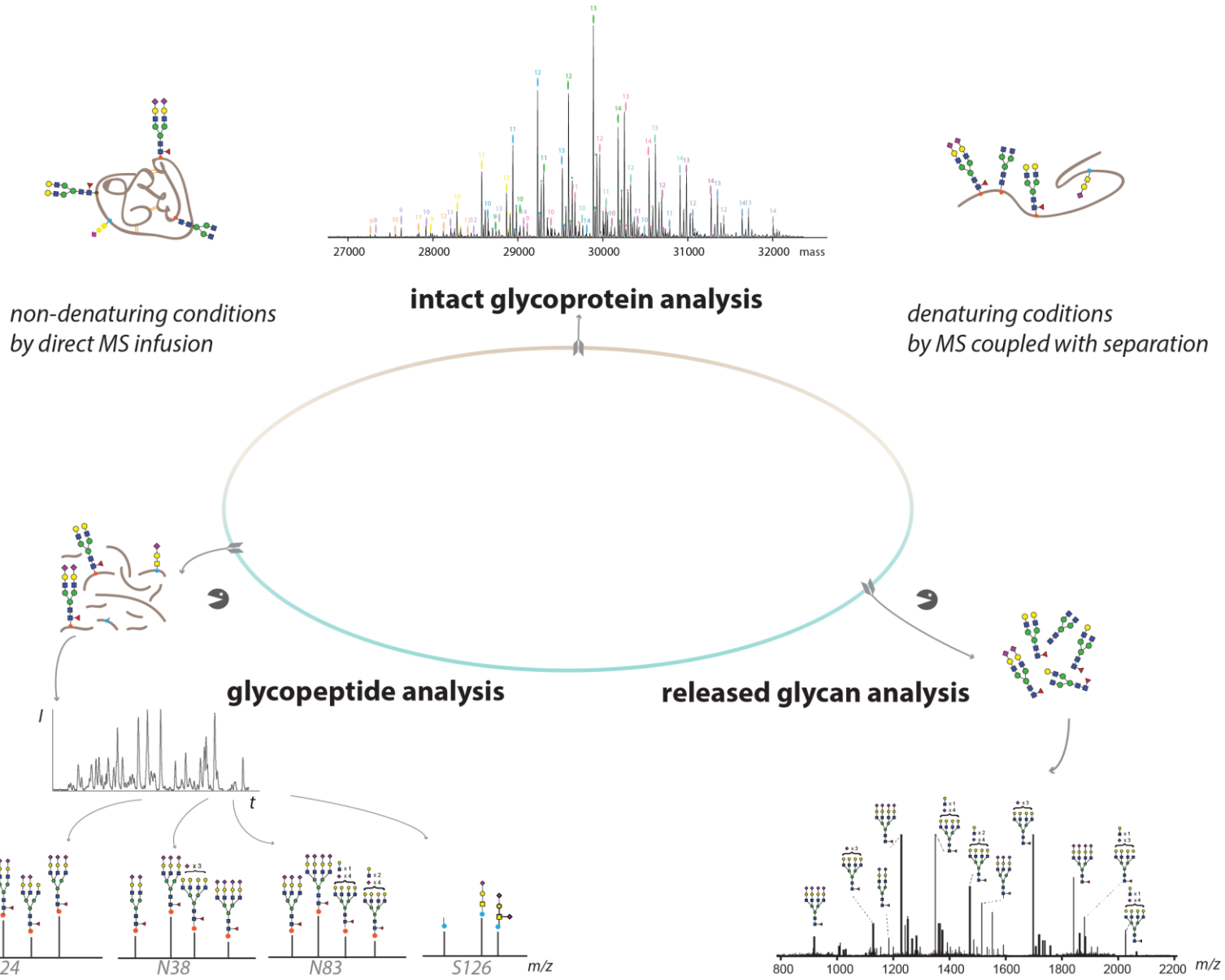


Additional stacked KO/KI

Epo-wt-1c2
EPO167 KO:B3gnt2/mgat4A/4B/5
EPO236 KI ST6Gal1_Mgat4a/4b/5_ST3_4/6/B3GnT2

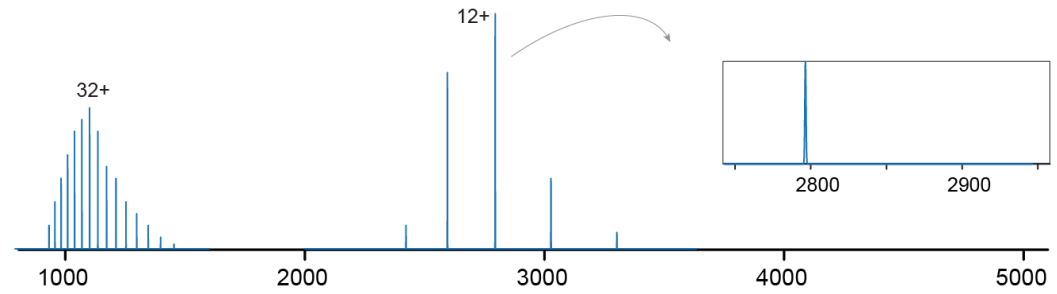


Characterizing the glycosylation

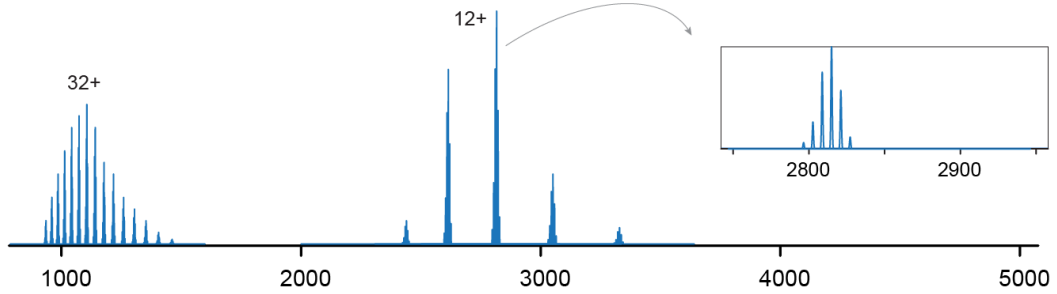


Yang, Y.; Franc, V.; Heck, A. J. R. Trends Biotechnol. 2017

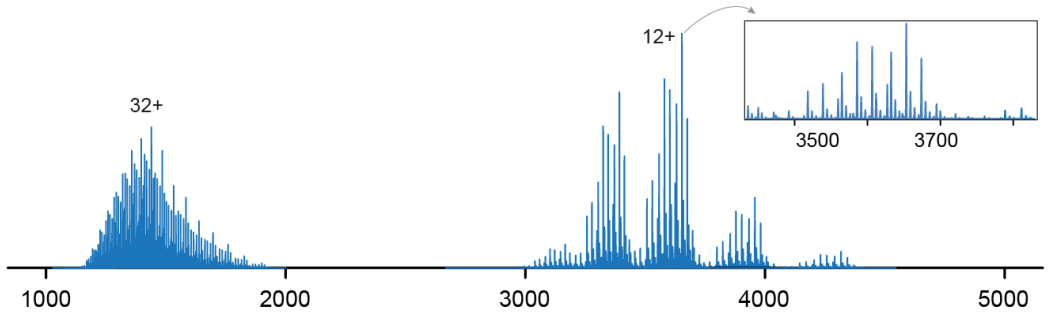
Why Native MS?



Unmodified Protein
35 kDa



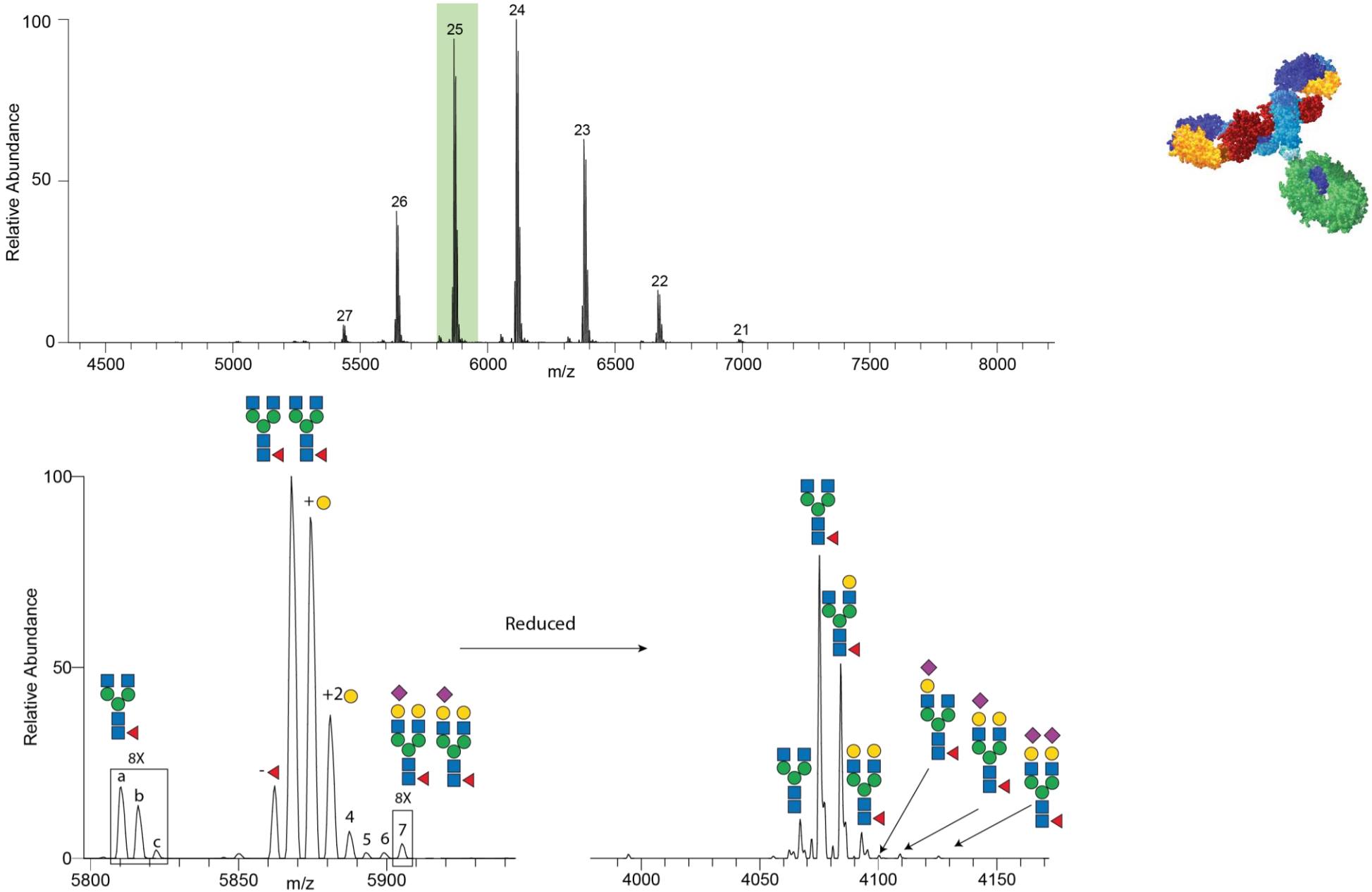
Phosphorylation
induced
heterogeneity



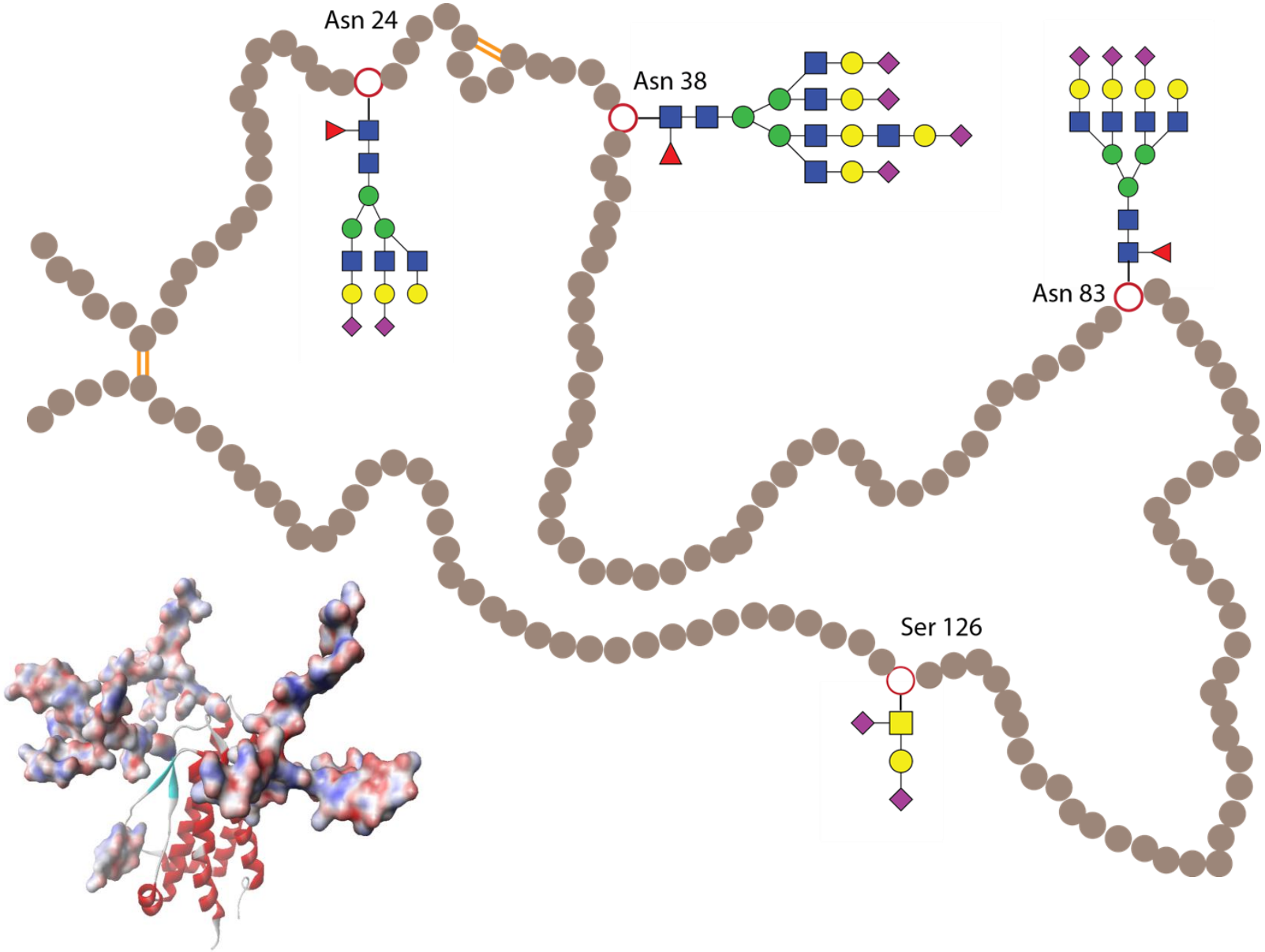
Glycosylation
induced
heterogeneity

Yang, Y.; Franc, V.; Heck, A. J. R. Trends Biotechnol. 2017

Native MS of antibodies

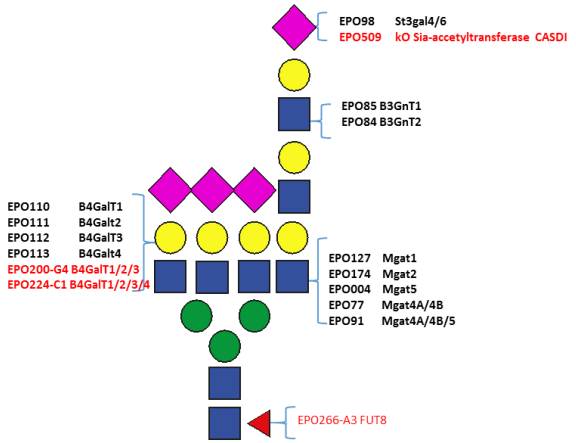


Erythropoietin



Erythropoietin Glycoengineering (Controlling the glycosylation)

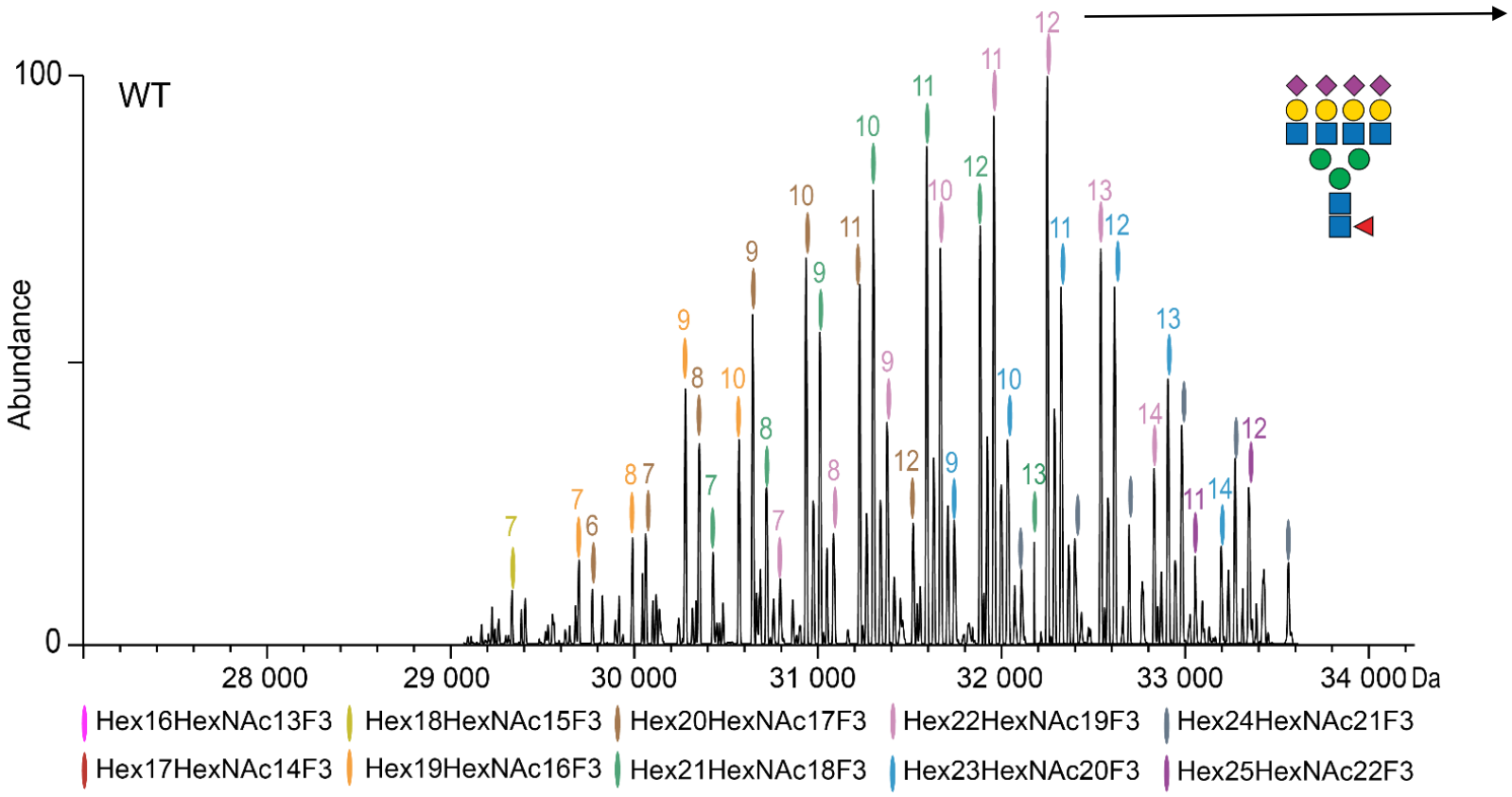
EPO CLONE	Gene engineering	Effect
C 01	*mgat1* KO	High mannose glycans
C 02	*mgat3/4A/4B/5 cosmc* KO	Biantennary glycans + Tn antigen
C 03	*mgat2* KO	Loss of β2-Branch
C 04	KI *st6gal1* in *mgat4A/4B/5 st3gal4/6 -/-*	Homogenous biantennary α2,6-NeuAc sialylated glycans
C 05	KI *st6gal1* in *mgat4A/4B/5 st3gal4/6 -/-*	Biological replicate
C 06	*mgat4A/4B/5 st3gal4/6 B3gnt2* KO + *st6Gal-I* KI	Homogenous biantennary α2,6-NeuAc sialylated glycans
C 07	*B3gnt2/mgat4A/4B/5* KO	Biantennary glycans with eliminated polyLacNAc
C 08	*mgat4A/4B/5* KO	Biantennary sialylated glycans
C 09	*st3gal6* KO in *mgat4a/4b/5/ST3Gal4 -/-*	Biantennary glycans with decreased sialylation
C 10	*st3gal6* KO in *mgat4A/4B/5/st3gal4 -/-*	Biological replicate
C 11	*B3gnt2* KO in *mgat4A/4B/5 st3gal4/6 -/-*	Biantennary glycans with decreased sialylation and no polyLacNAc
C 12	*B3gnt2* KO in *mgat4A/4B/5 st3galt4/6 -/-*	Biological replicate
C 13	*fut8* KO	No fucosylation
C 14	*B4galt3* KO	No effect
C 15	*B3gnt1* KO	No effect
C 16	*B3gnt2* KO	Eliminated polyLacNAc
WT	/	Wild type
C 17	*B4galt4* KO	No effect
C 18	*st3gal4/6* KO	Decreased sialylation on N-glycans
C 19	*st3gal4/6* KO	Biological replicate
C 20	*mgat3/4A/5 cosmc* KO	Eliminates β6-branch of N-glycans, O-glycans all in the form of Tn
C 21	*mgat 5* KO	Eliminates β6-branch of N-glycans
C 22	*mgat4A/4B/5/st3gal4/6 cosmc* KO	UNKNOWN (sample mislabeled), native data indicate mgat4b
C 23	*mgat4A/4B* KO	Eliminates β4-branch of N-glycans
C 24	*mgat4B* KO	Eliminates β4-branch of N-glycans

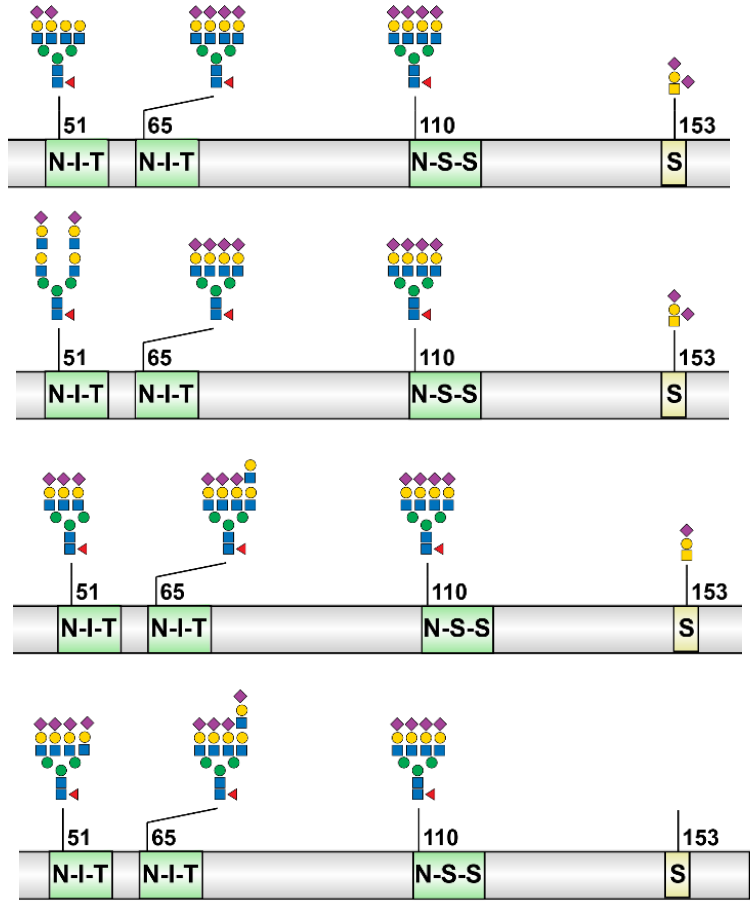


1. Characterization of glycosyltransferases with native MS

2. Can we create homogeneous EPO

Wild type EPO



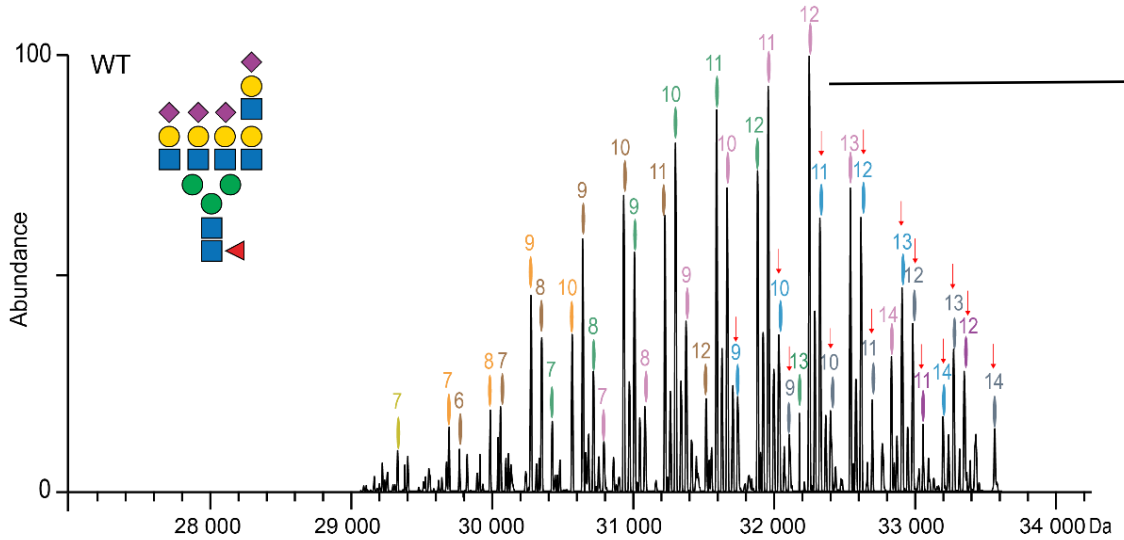


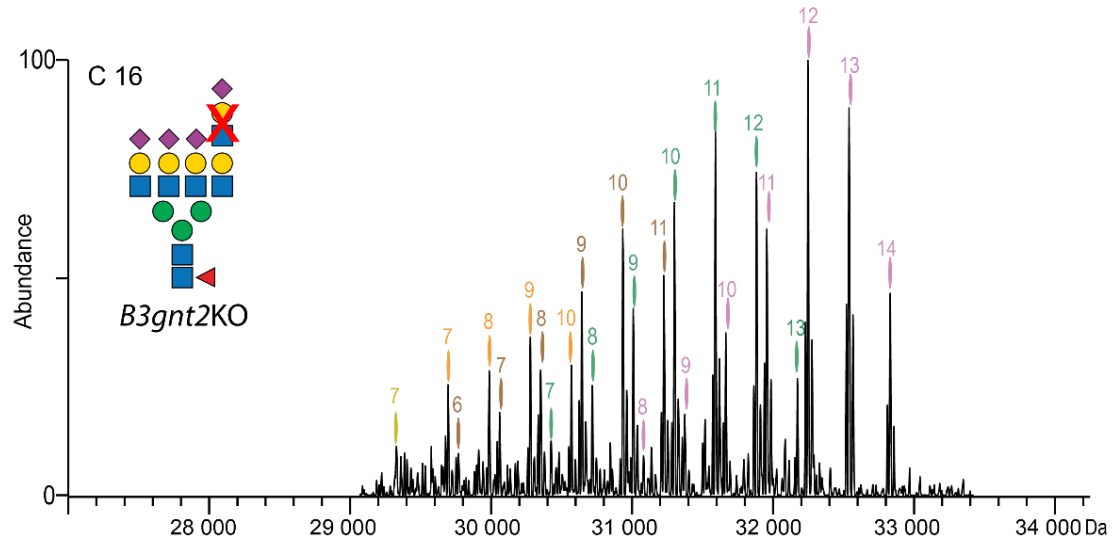
PMI Intact Mass Deconvolution

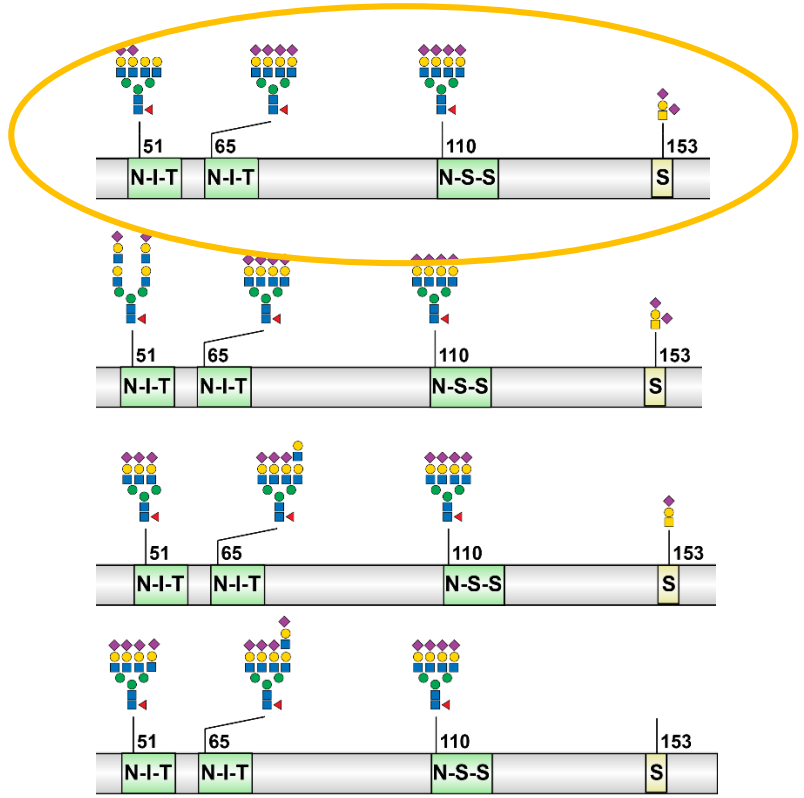
Bern M., Caval T. et al. Journal of proteome research 2018.

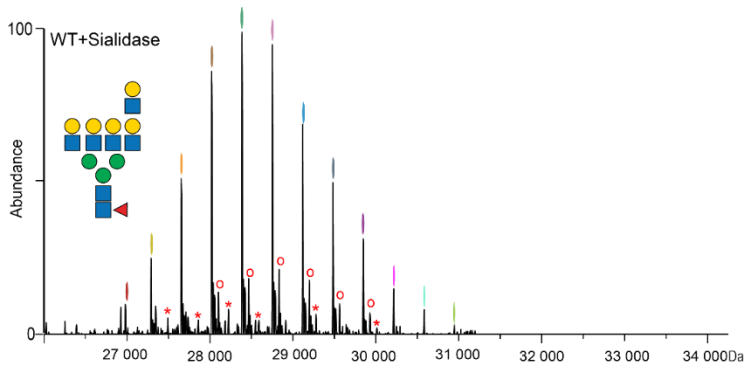
Čaval et al. Nature Communications 2018

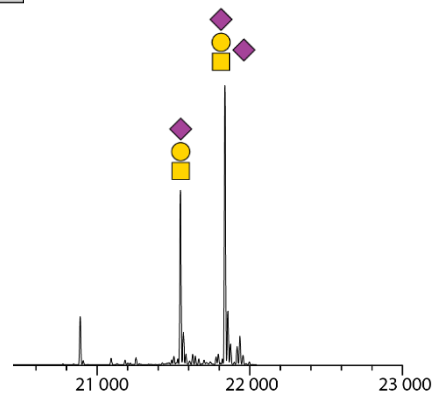
Wild type EPO (II)











Hex17HexNAc14F3 Hex18HexNAc15F3 Hex19HexNAc16F3 Hex20HexNAc17F3 Hex21HexNAc18F3
Hex22HexNAc19F3 Hex23HexNAc20F3 Hex24HexNAc21F3 Hex25HexNAc22F3 Hex26HexNAc23F3
Hex27HexNAc24F3 Hex28HexNAc25F3

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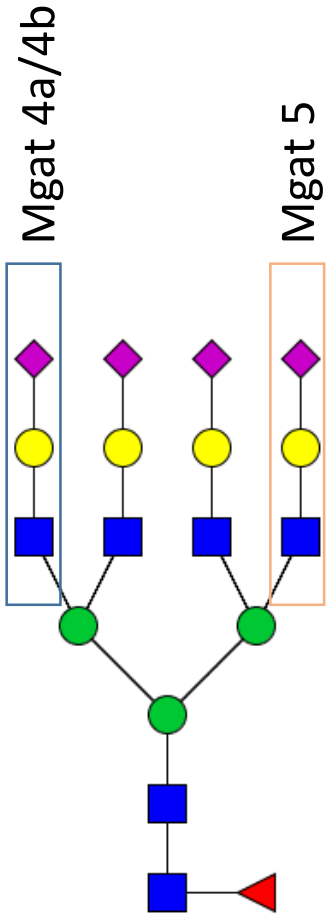
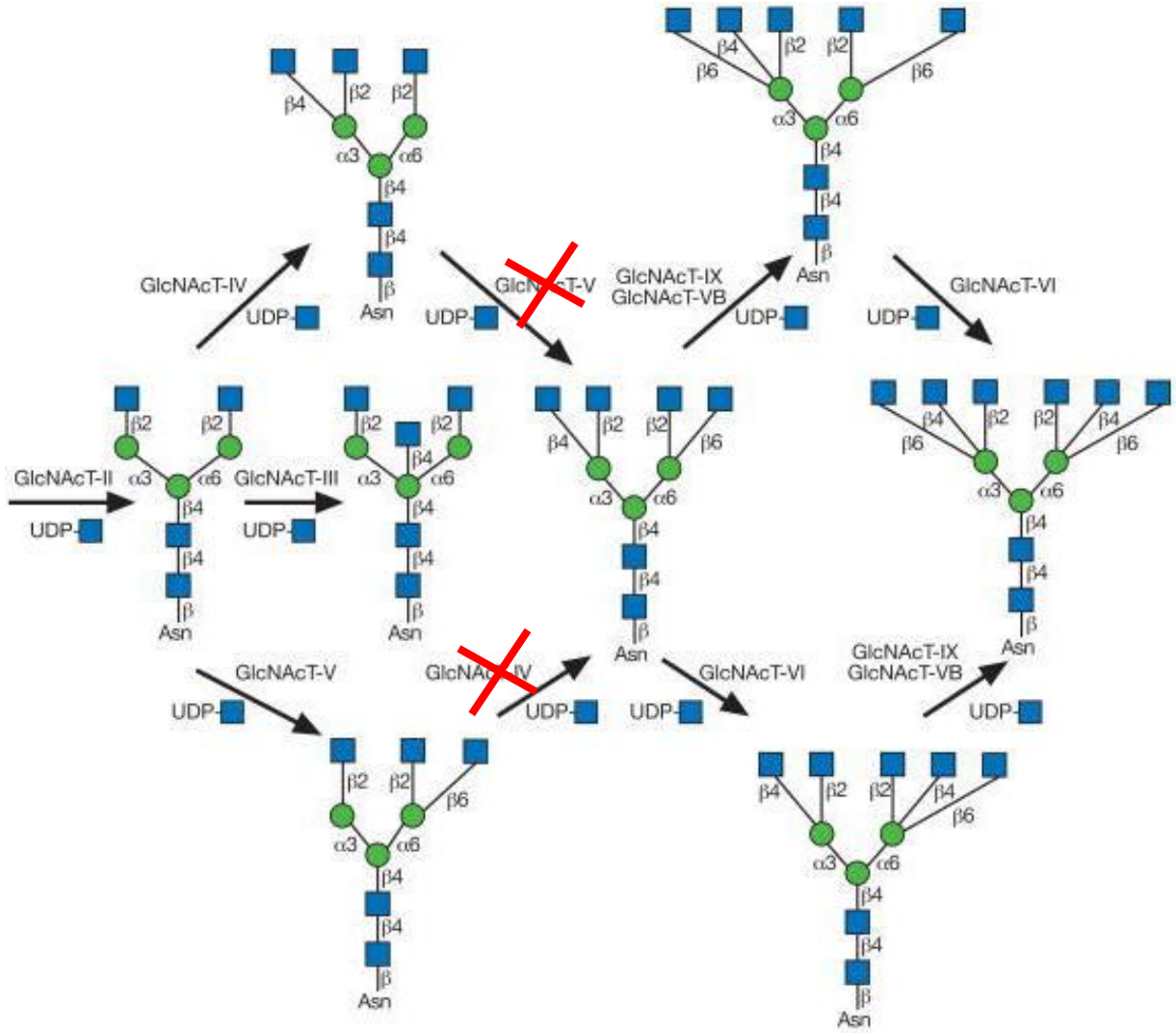
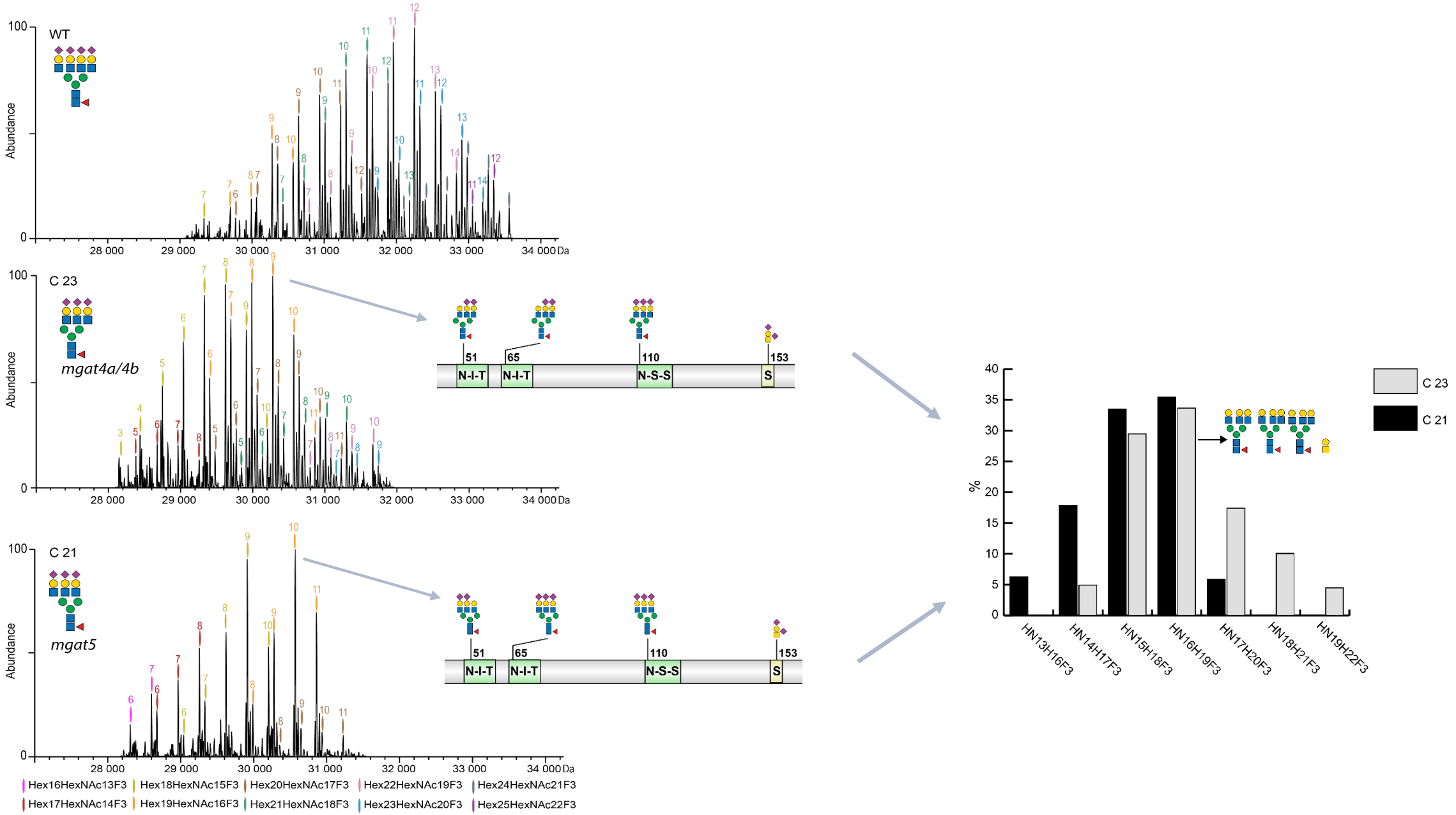
Custom-engineered EPO: Mgat5 versus Mgat 4a/4b example



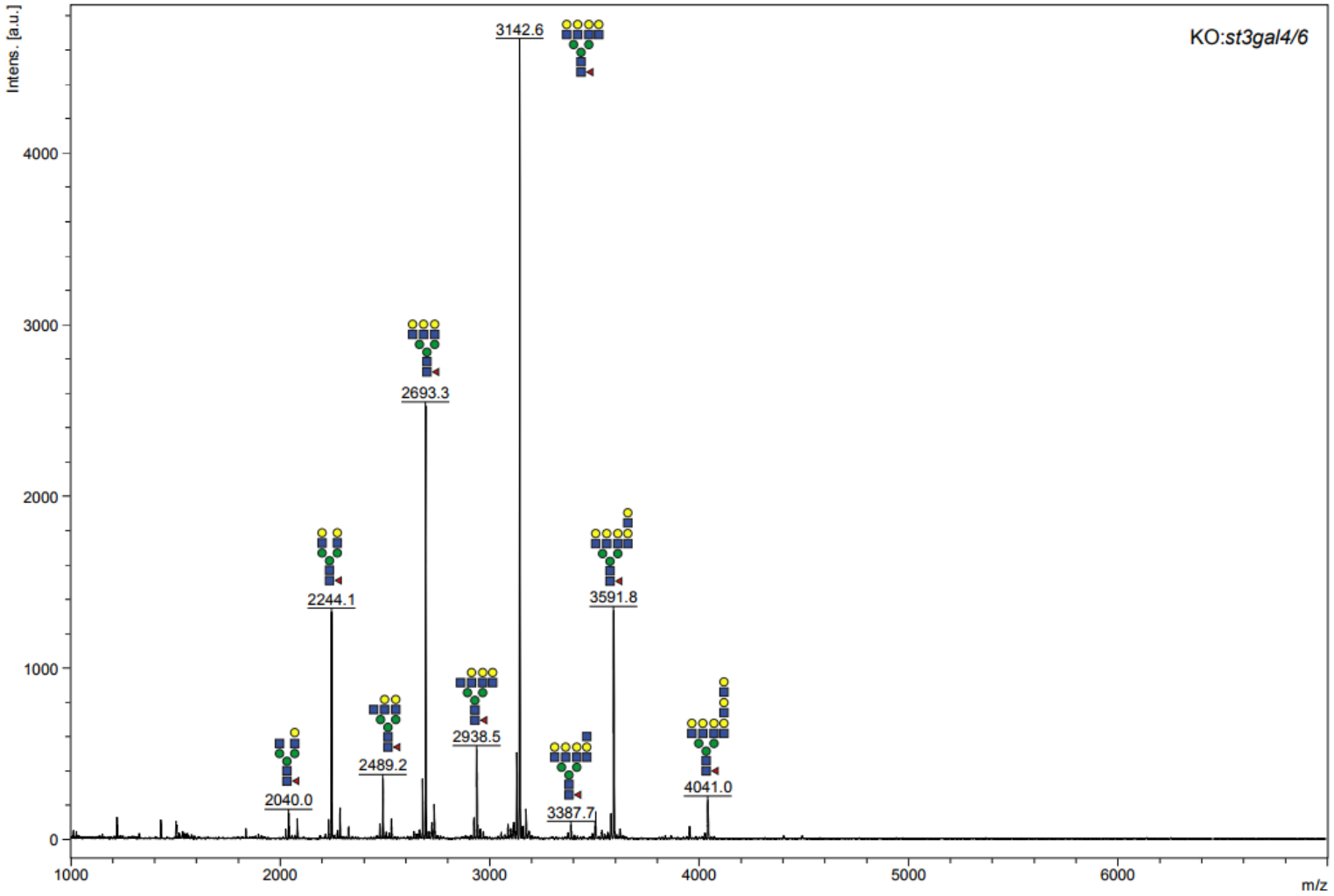


They should result in the same glycoforms (compositions should be identical)

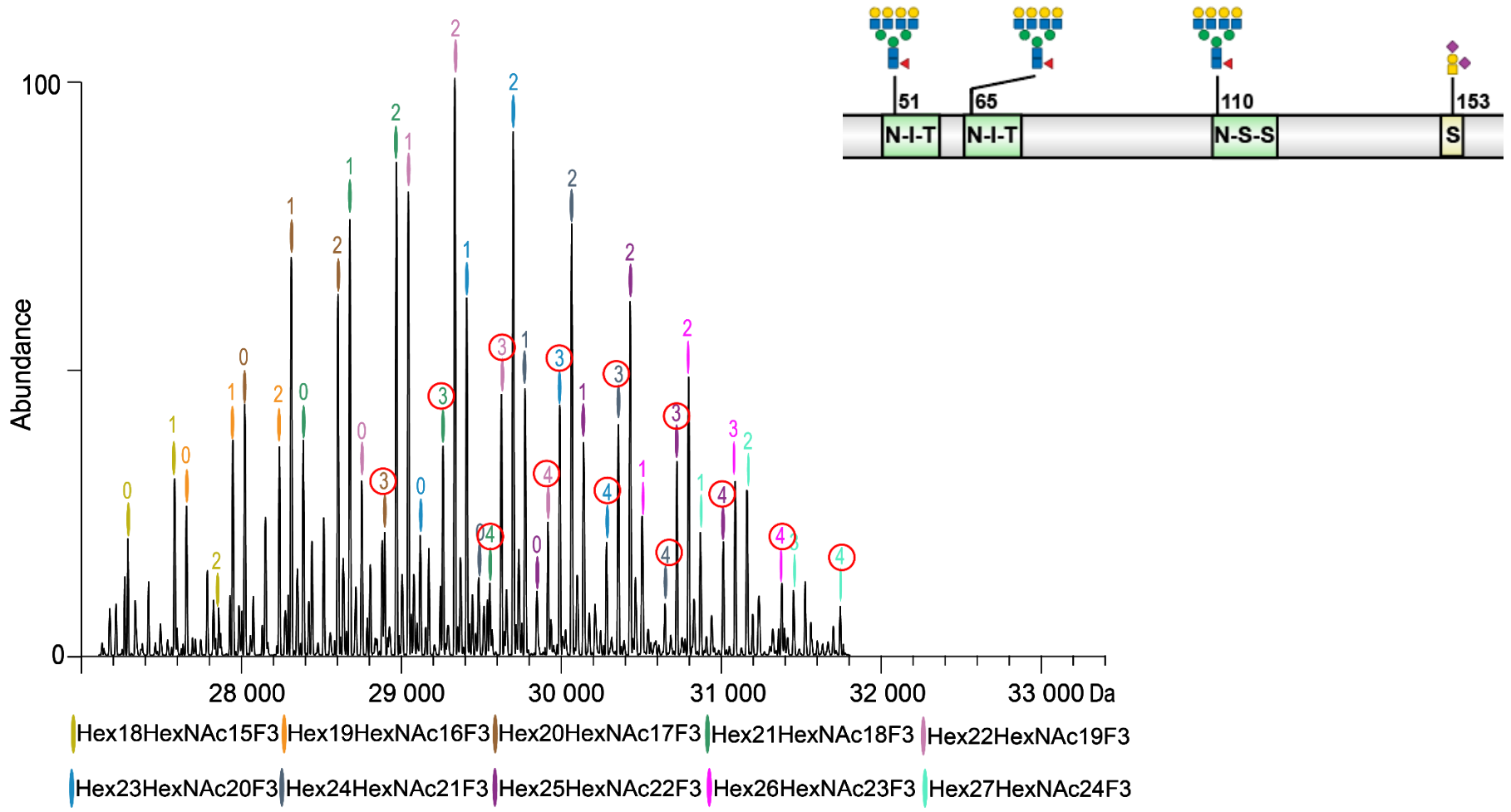
Custom-engineered EPO: Mgat5 versus Mgat 4a/4b example



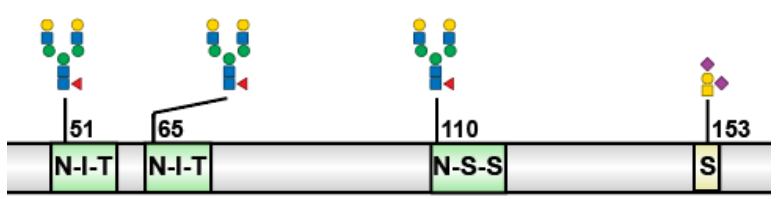
Sialylation knock out (MALDI)

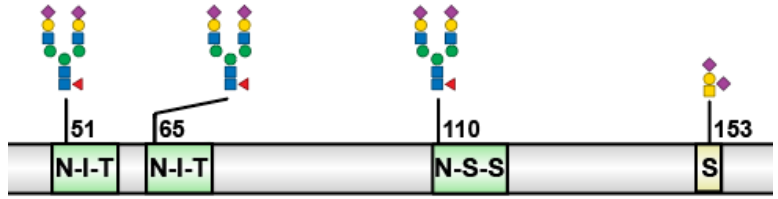


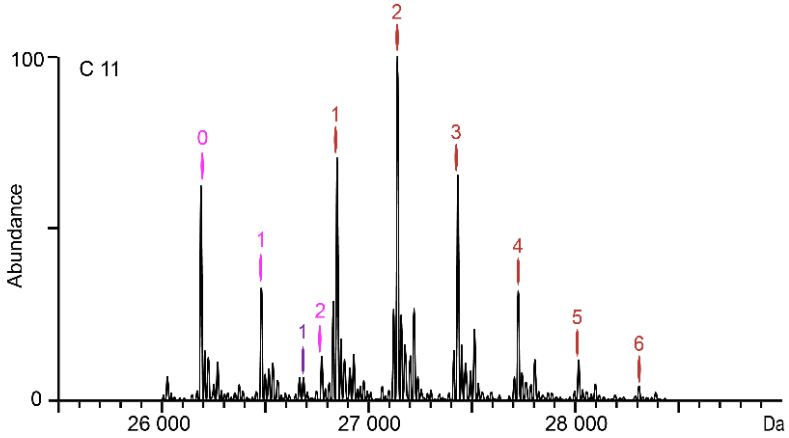
Sialylation knock out (EMR)

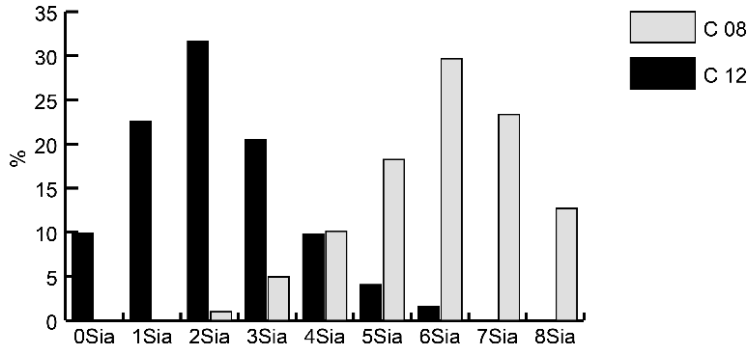


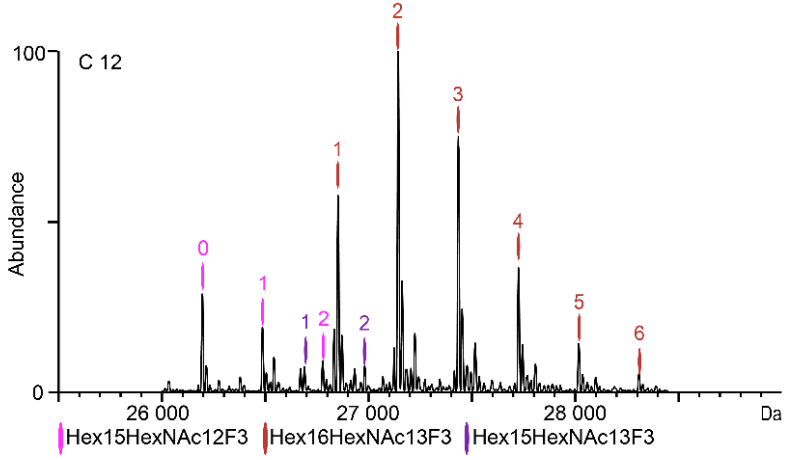
Sialylation knock out (EMR)

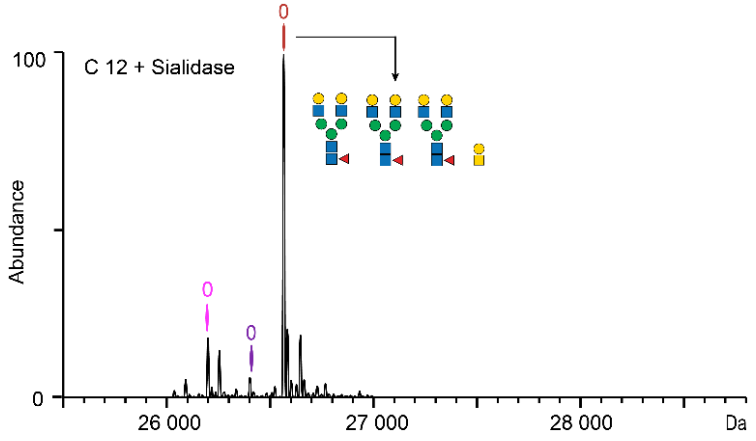




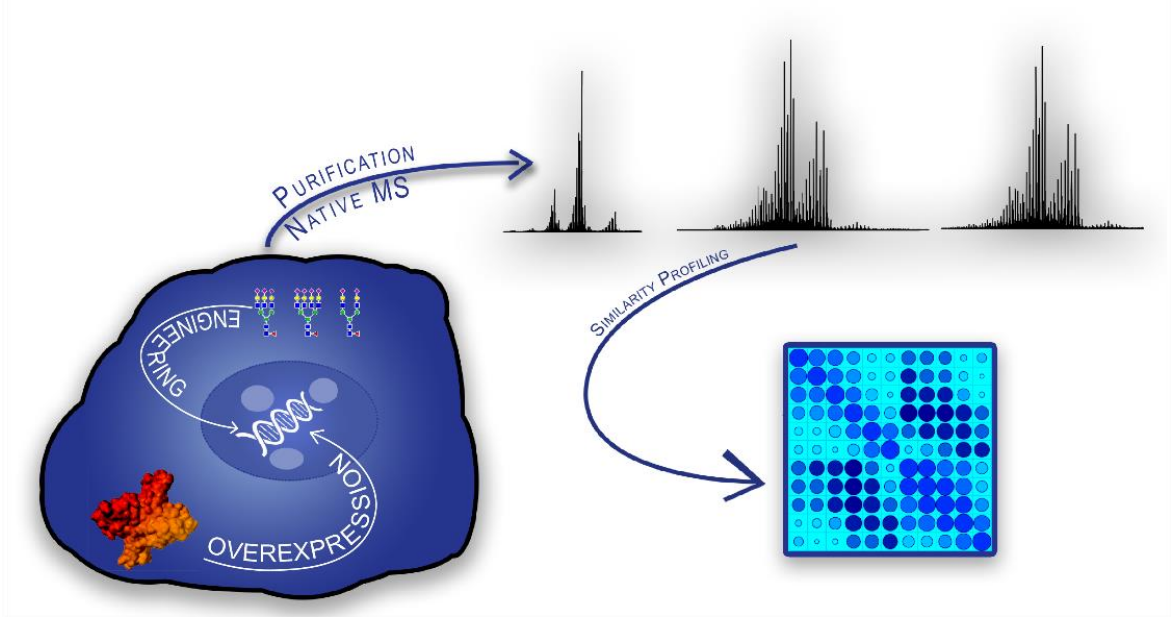


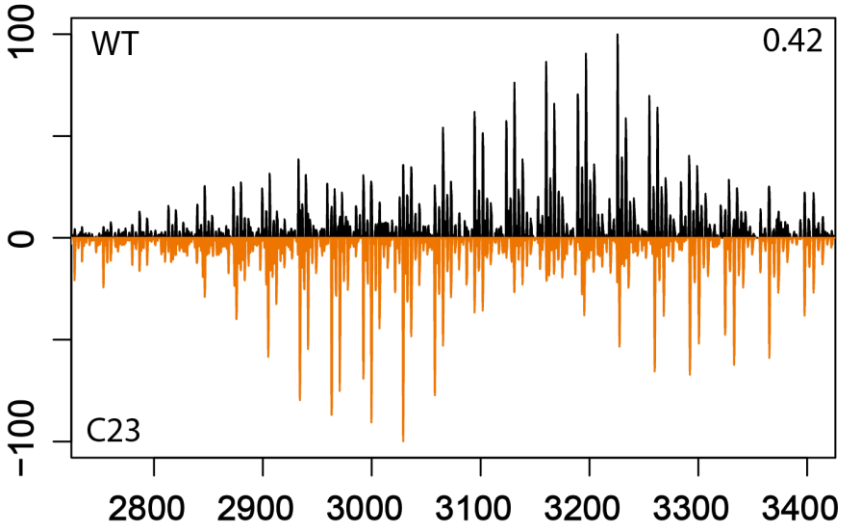


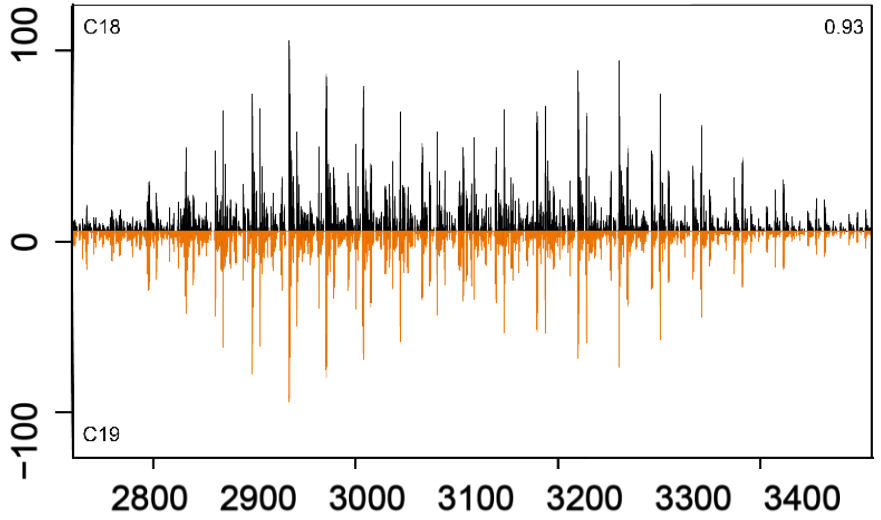




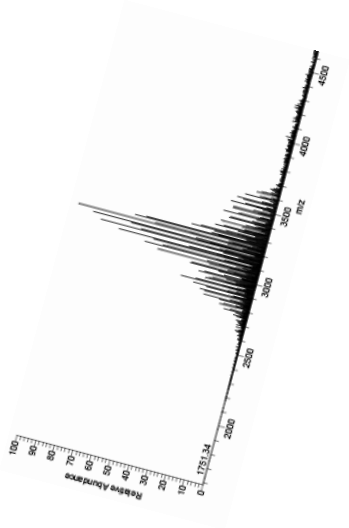
Can we profile quicker?

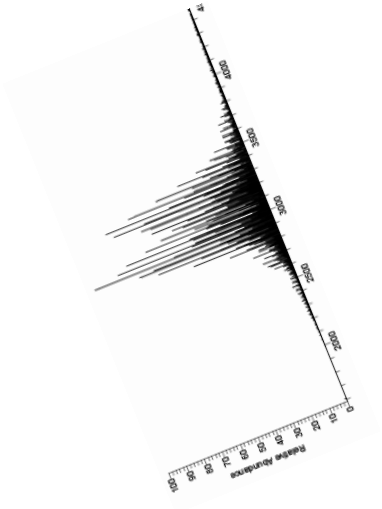


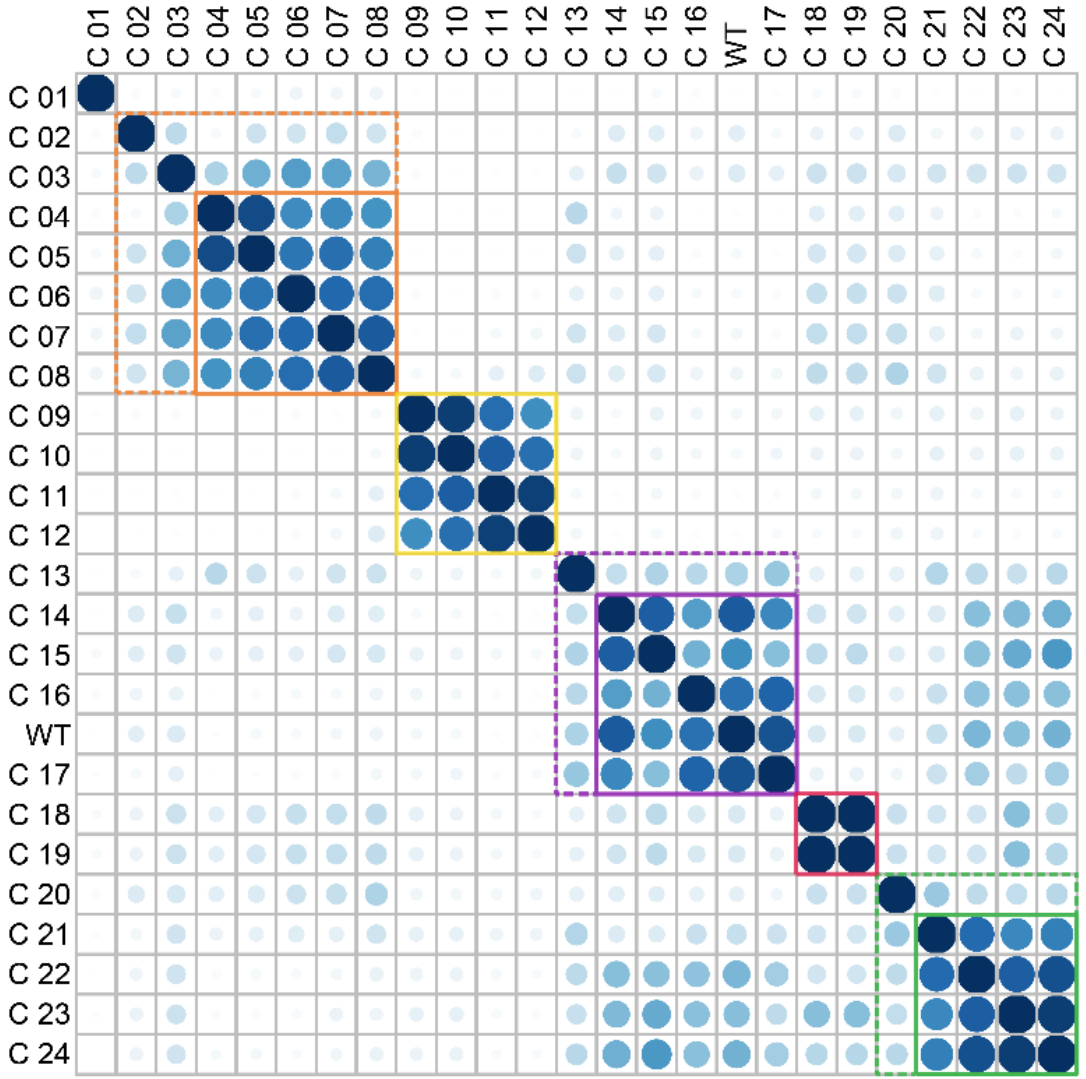


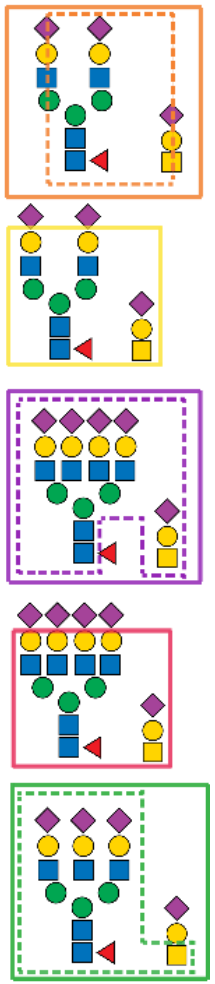


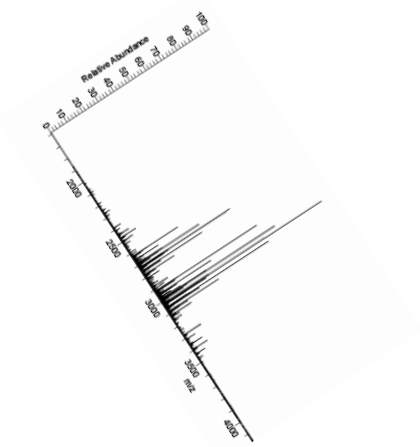
Similarity based EPO profiling

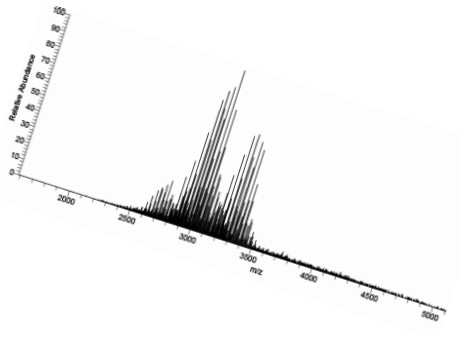


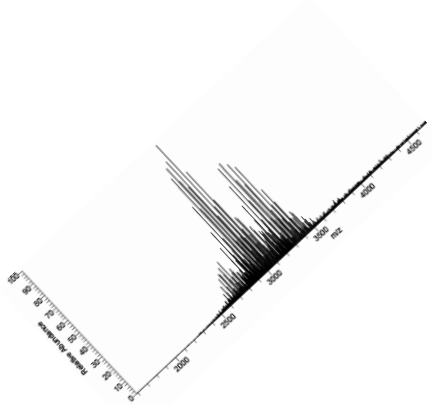




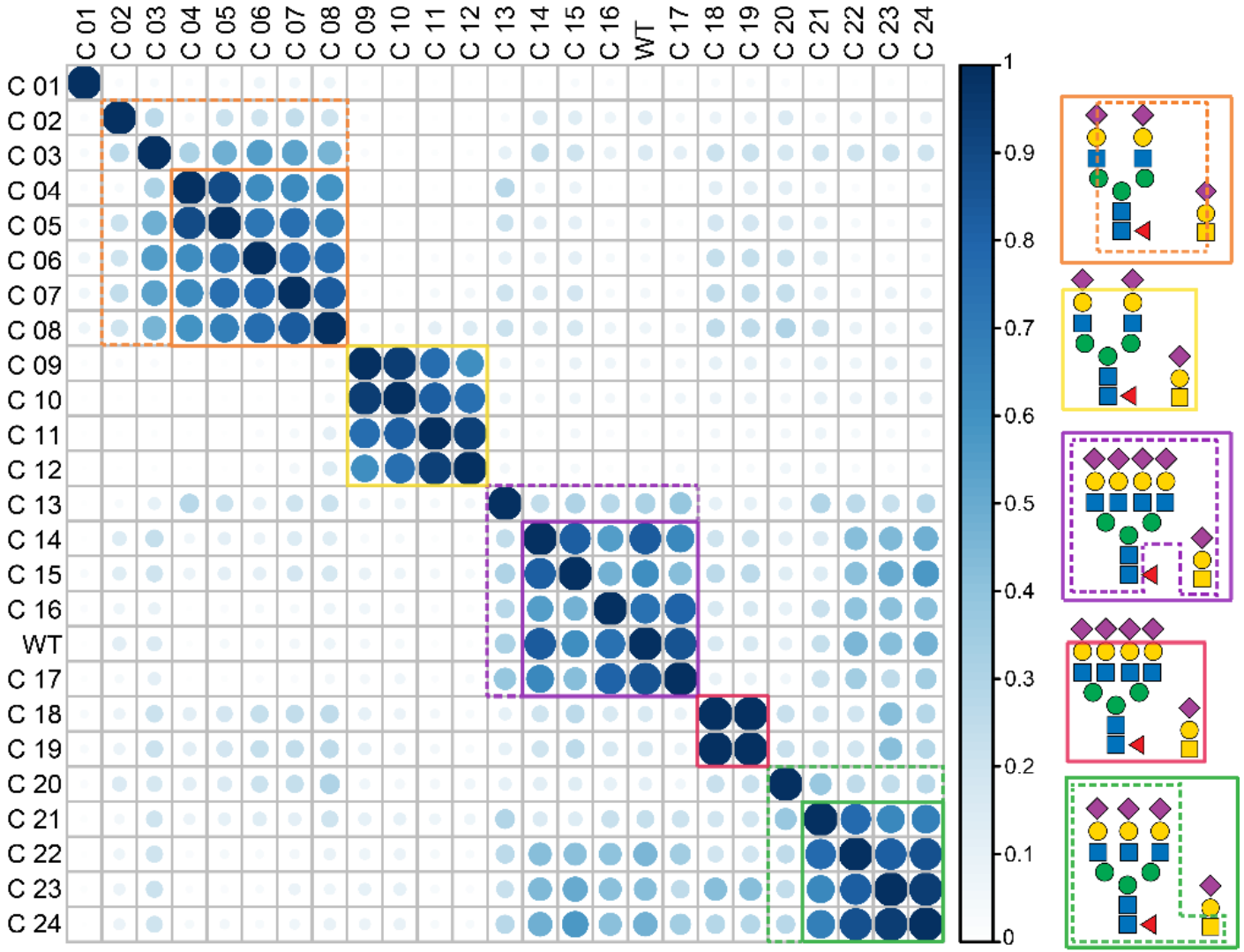


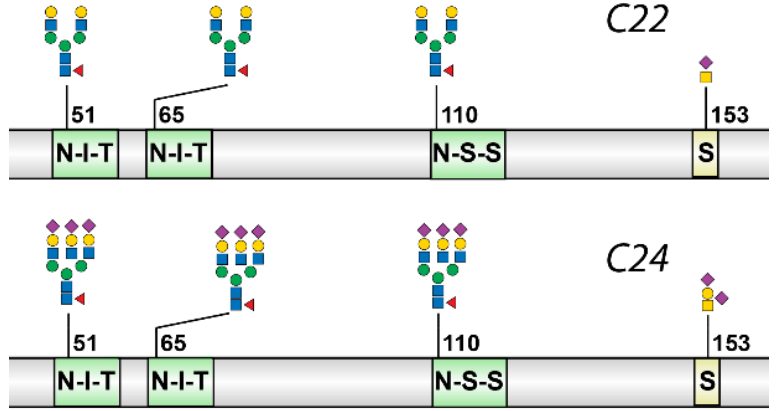


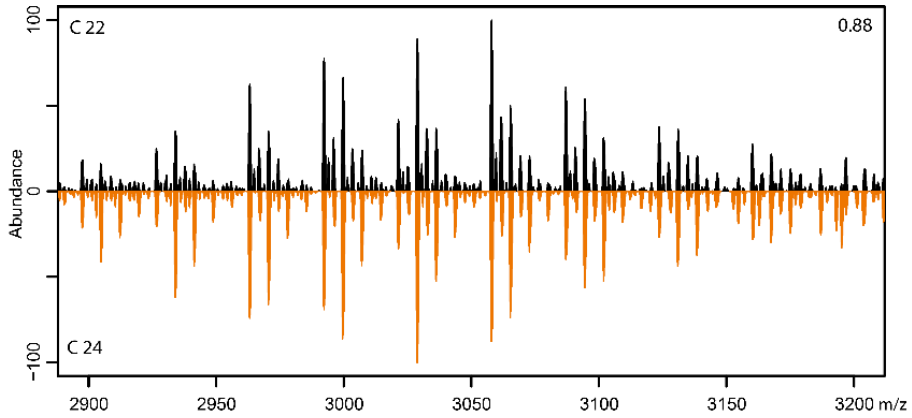




Similarity based EPO profiling (II)







C 22 mgat4A/4B/5/st3gal4/6 cosmc KO

C 24 mgat4B KO

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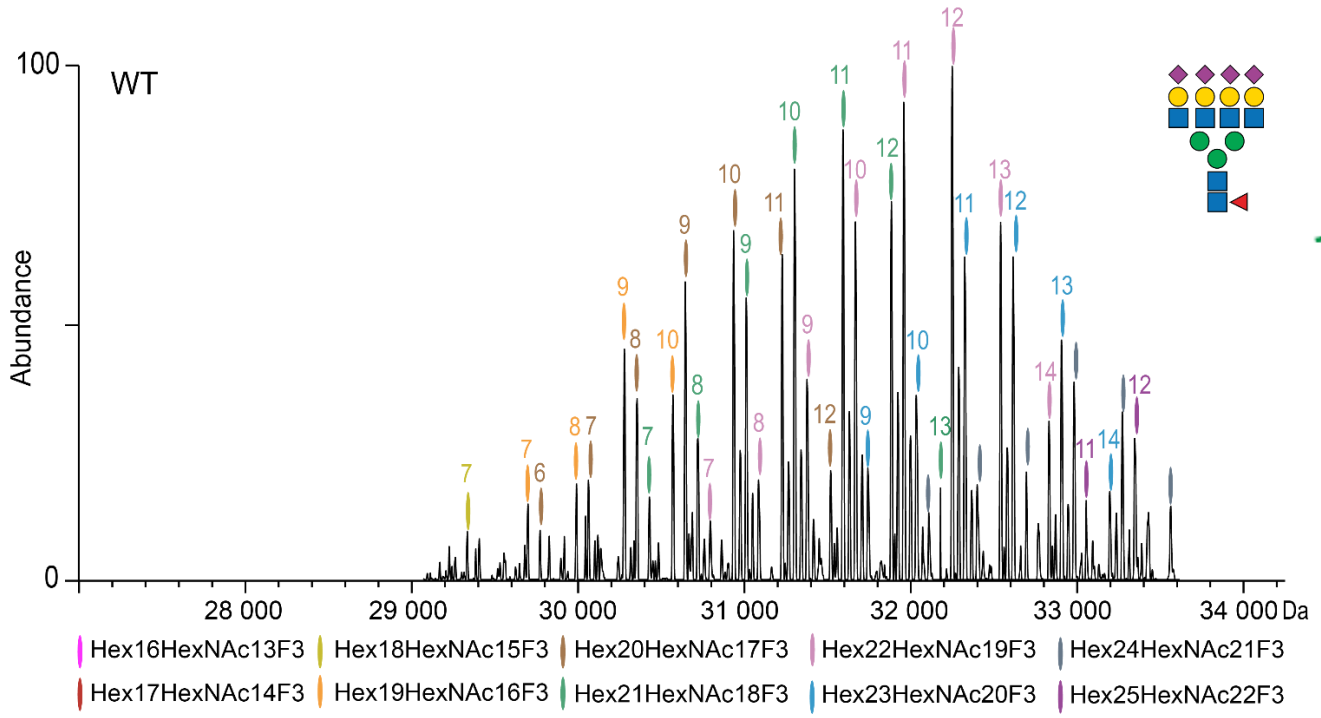
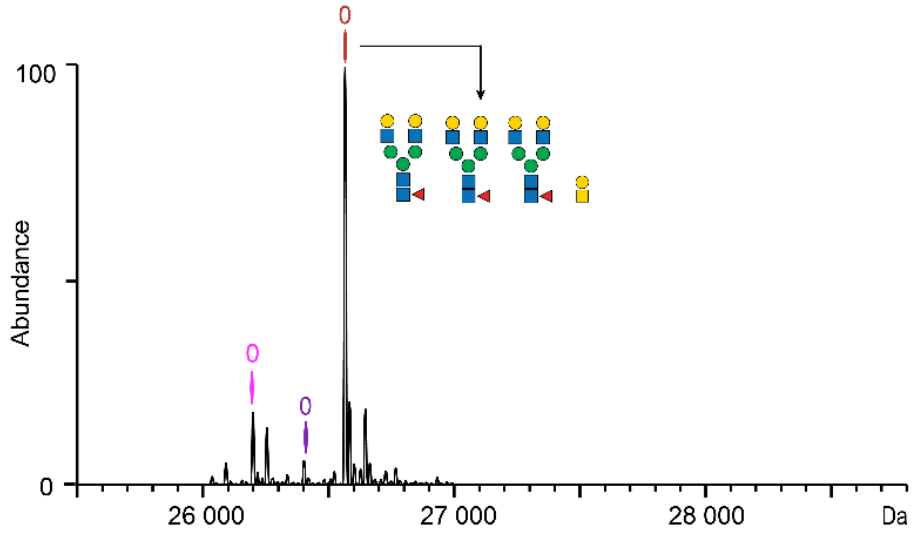
Conclusions (1/3)

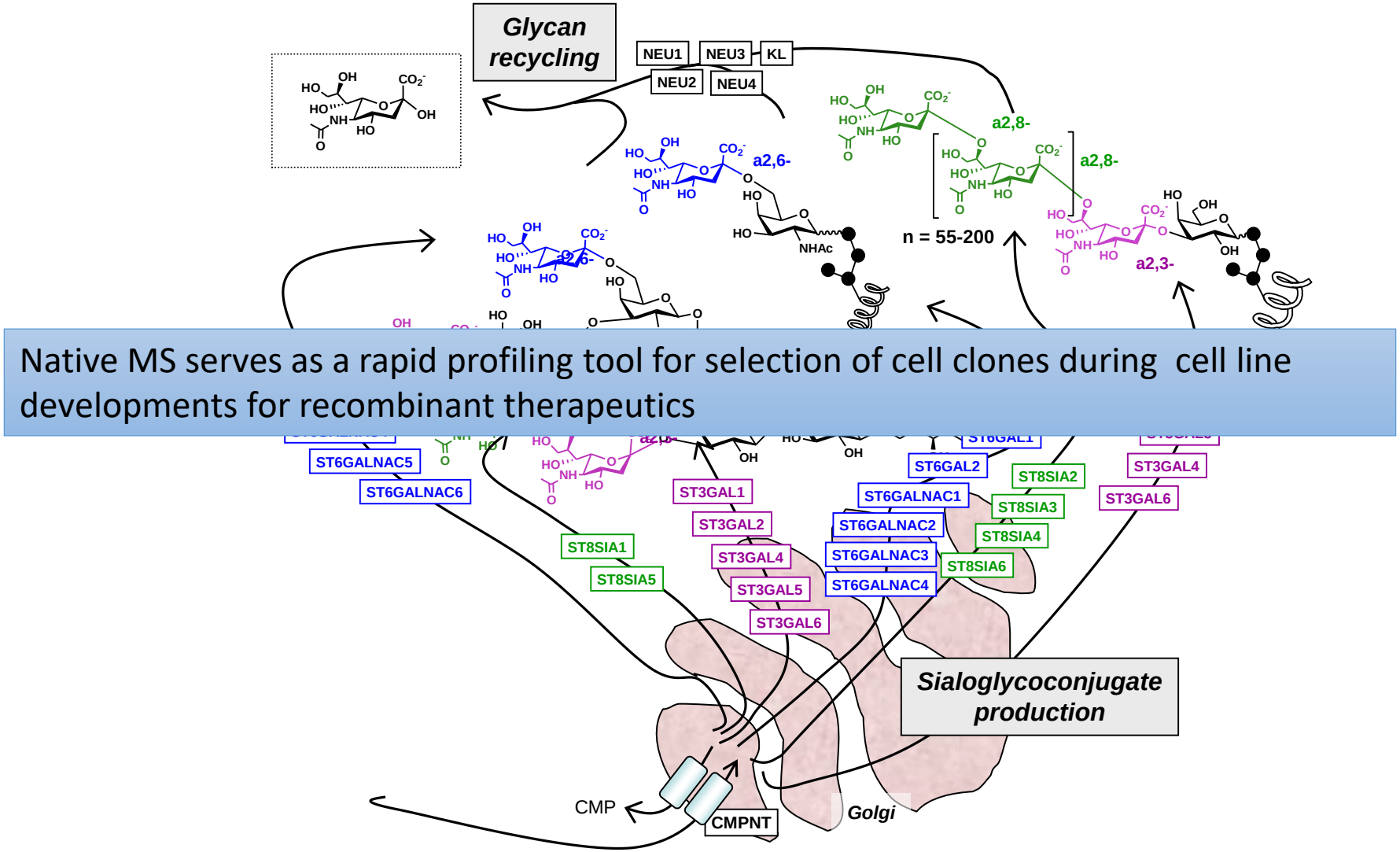


Glycoengineering



Glycoengineering enables the study of structure to function relationship of glycans

Conclusions (2/3)



Native MS serves as a rapid profiling tool for selection of cell clones during cell line developments for recombinant therapeutics

Conclusions (3/3)

Glucose limitation reduces site occupancy

Galactose feeding increases galactosylation

Increase in glutamine/ammonia reduces galactosylation and sialylation

Native MS:

1. enables screening of consistency in glycoprotein products

2. especially suited for studying glycosylated biotherapteuics
   - differences between biosimilars and originator
   - divergence of a biologic through its product lifecycle

pH

Carbon dioxide

Temperature

Dissolved oxygen

Mode of culture

DMSO

Shear Stress

Glycerol

Manganese

Cell growth speed

Cell density

Expression rate

Sodium-butyrate

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