A novel method for high-throughput analysis of bovine milk oligosaccharides

Randall Robinson[1], Nina A. Poulsen[2], Lotte B. Larsen[2], and Daniela Barile[1,3]

[1]Department of Food Science and Technology, University of California, Davis, One Shields Ave., Davis, CA 95616

[2]Department of Food Science, Aarhus University, Blichers Allé 20, P.O. Box 50, DK-8830 Tjele, Denmark

[3]Foods for Health Institute, University of California, Davis, One Shields Ave., Davis, CA 95616

INTERNATIONAL MILK GENOMICS CONSORTIUM

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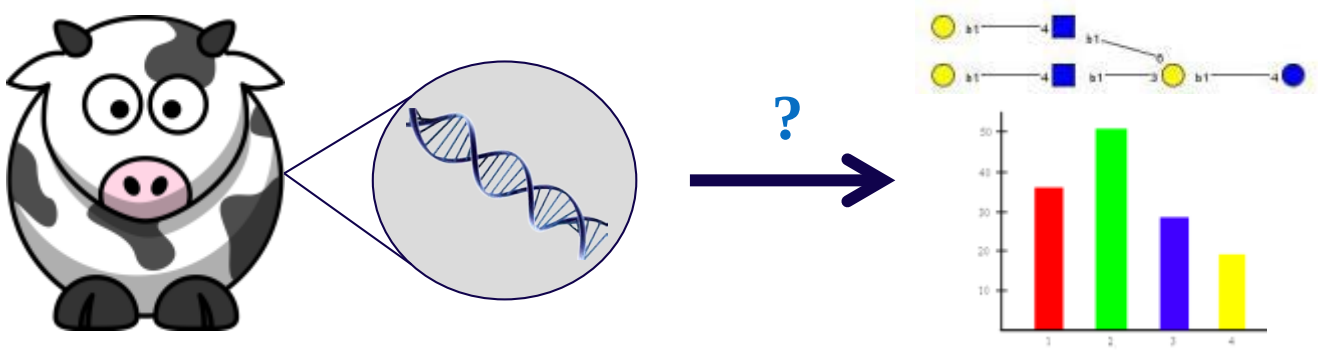
UCDAVIS FOOD SCIENCE AND TECHNOLOGY

Project background

- DNA does not directly code for OS production, but it likely affects milk OS content indirectly



- Identifying genes that influence OS production would allow for improved OS utilization in dairy products

Project background

- The Danish-Swedish Milk Genomics Initiative aims to correlate relevant attributes of milk with the bovine genome to improve milk quality
- OS profiling and relative quantification will be conducted for 336 Holstein and 300 Jersey samples
- Relative OS quantities will be correlated with the genomes of the cows in a genome-wide association study

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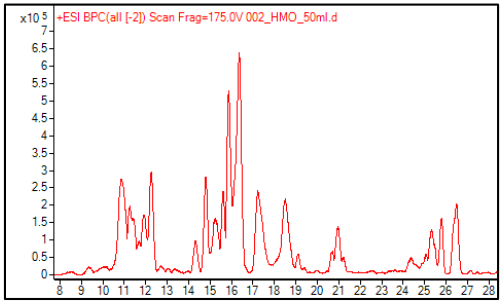
milkgenomics.dk

Instrumental platform

- nano-HPLC separation with a porous graphitized carbon column

- Agilent 6520 Quadrupole time-of-flight (Q-TOF) mass spectrometer

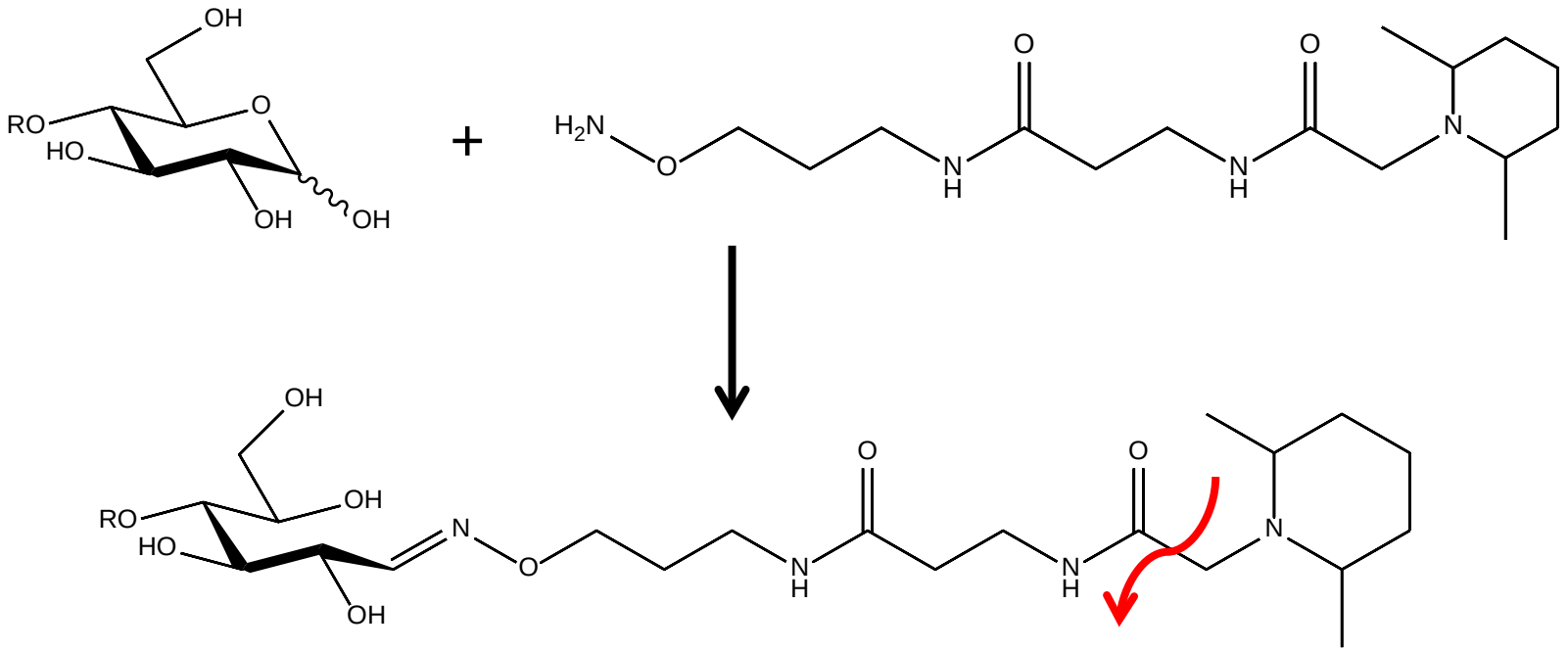
The throughput problem

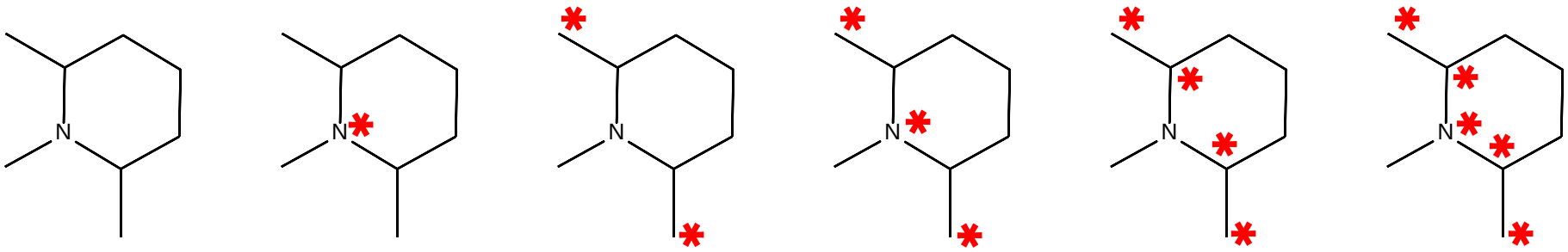


636 Samples X 70 min/sample = **31 days**

+ Blanks
+ Check standards
+ Tuning/calibration
+ Cleaning
+ Replicates

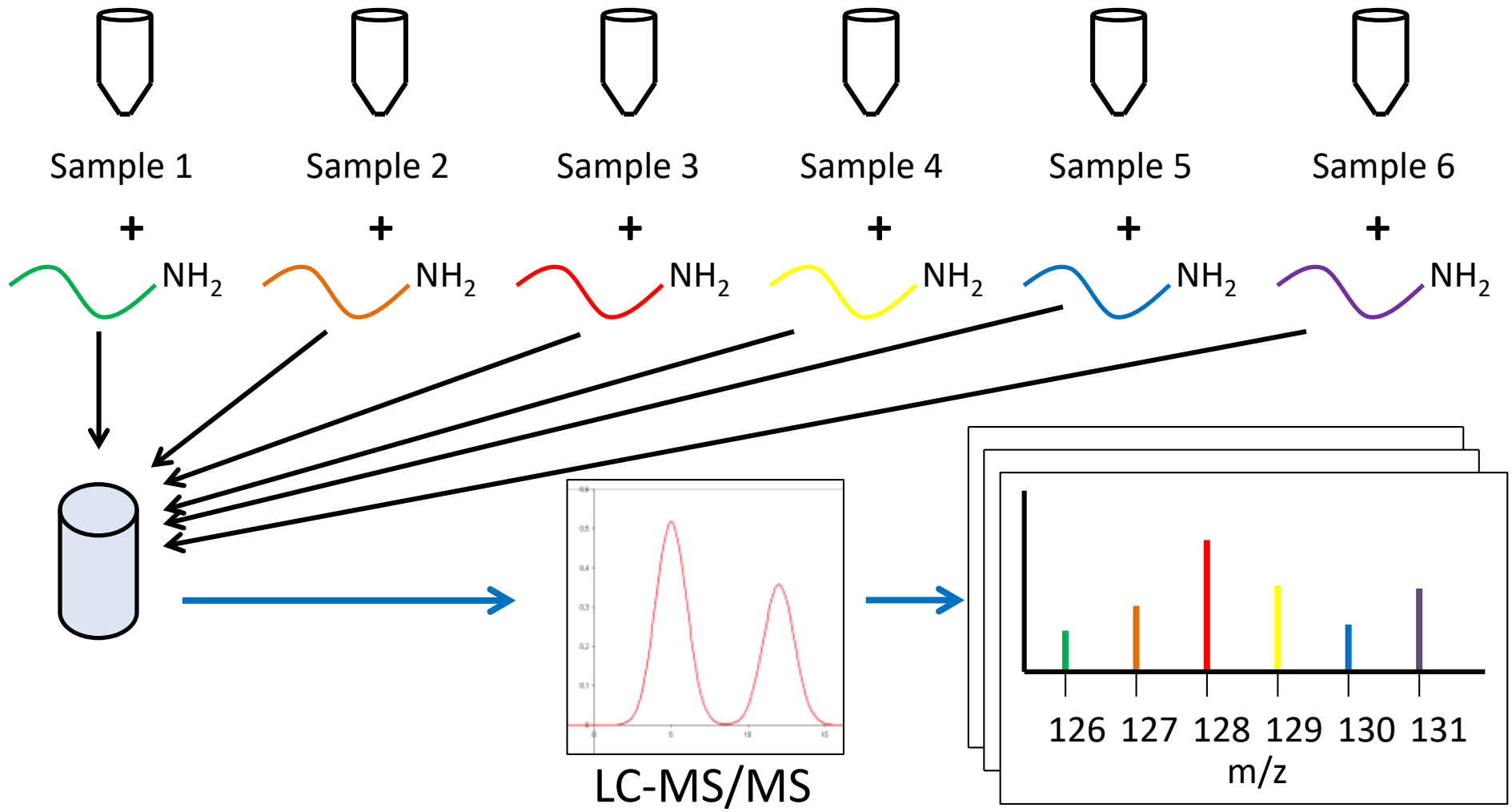
Tandem mass tags





126 127 128 129 130 131

Multiplexed analysis

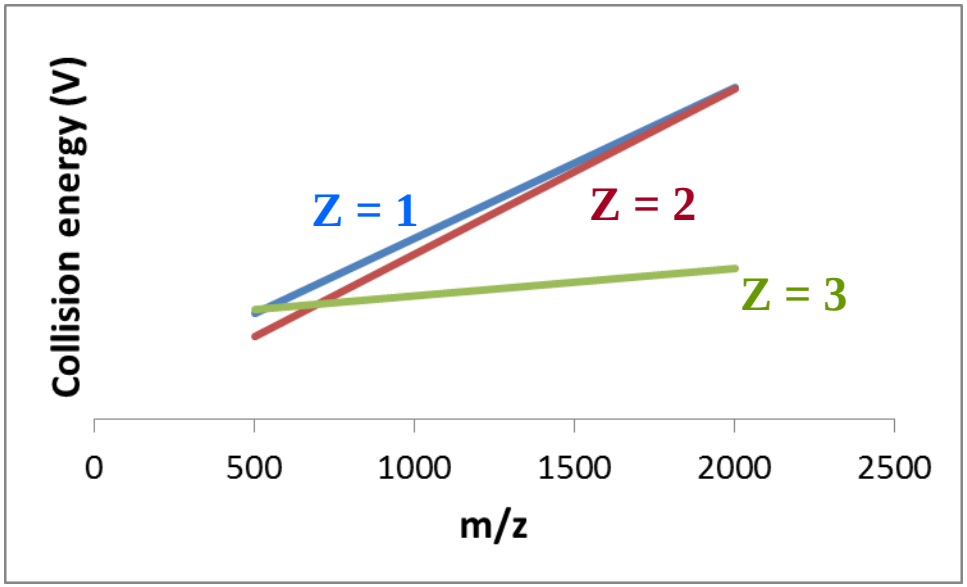


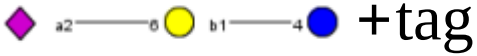
Advantages

- Reduction in instrumental runtime
- Less opportunity for drifts in sensitivity/performance
- Allows more samples to be run on a single column
- No manual peak integration
- Accurate relative quantification with optimized MS/MS method

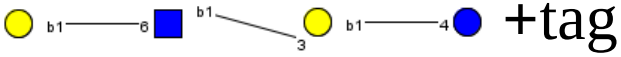
Collision energies

- Data quality is highly dependent on collision energies
- Charge state influences fragmentation

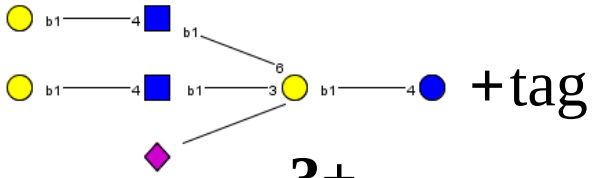




1+

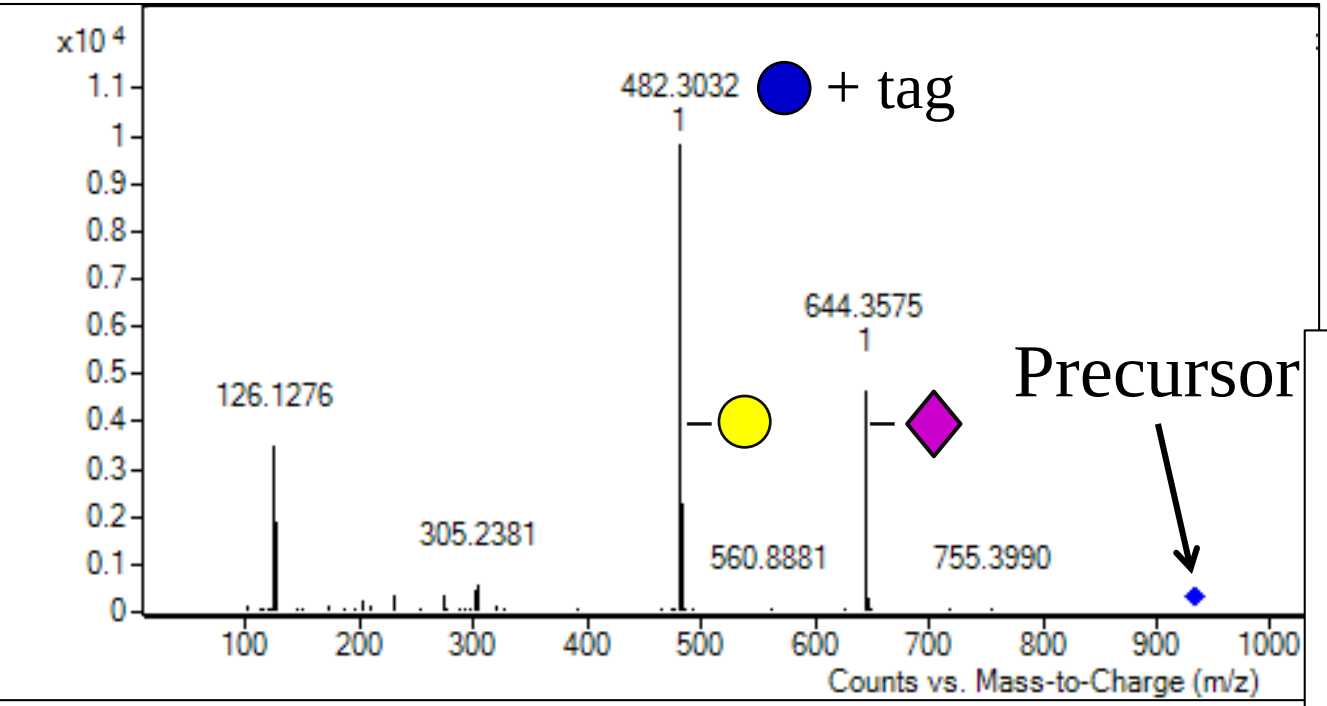


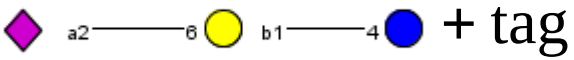
2+



3+

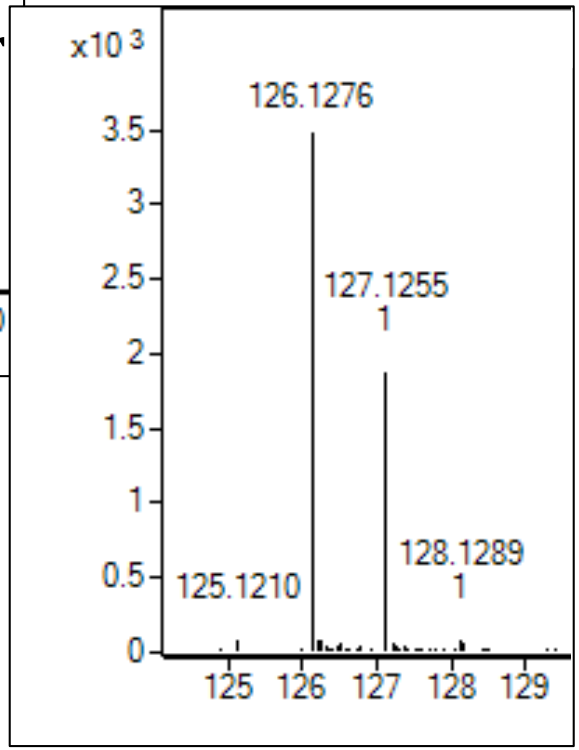
Rapid & automated glycan IDs



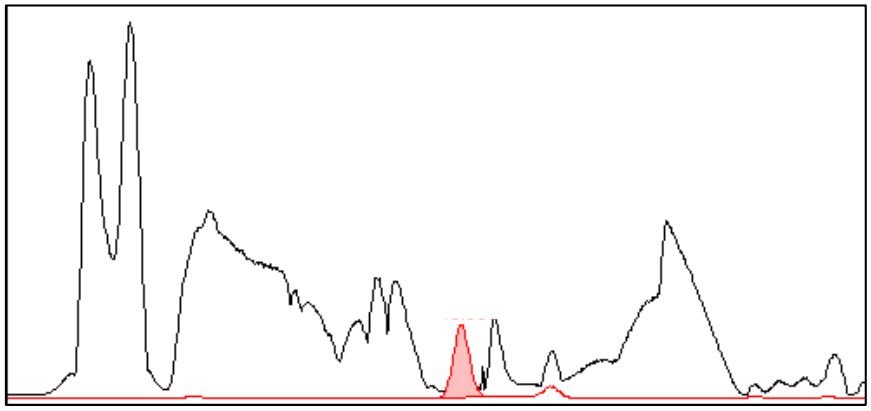


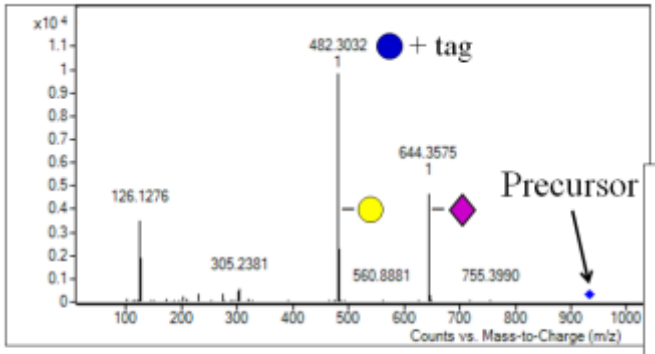
6’-sialyllactose

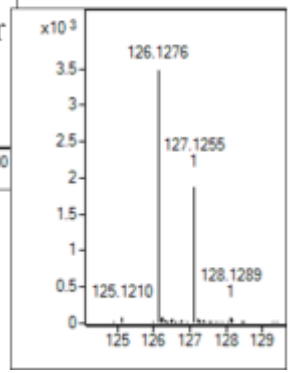
2 : 1

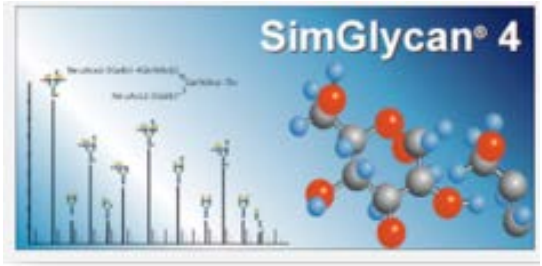


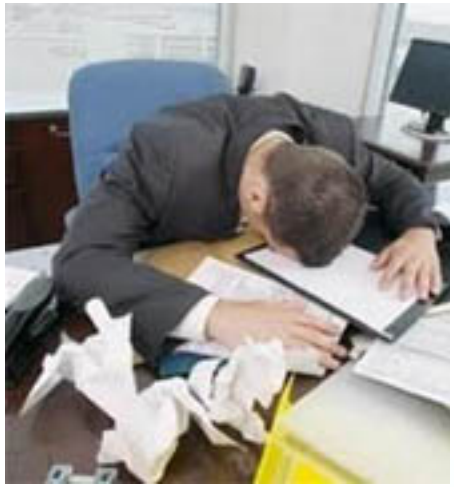
Data analysis







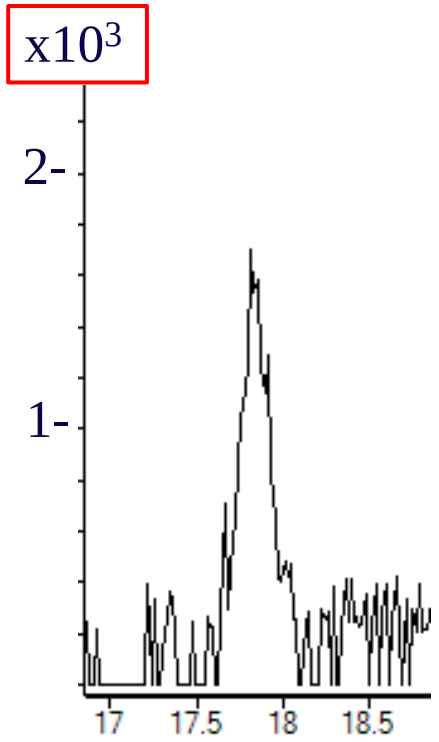




	A	B	C	D	E	F	G	H
1				Ratio (TMT Ion/Control)				
2	m/z	Composition	Mass	126.13	127.13	Glycan structure		
3	888.136093671	(Gal)1 (Glc)1 (NeuAc)1	633.211628077	1.0	0.671202247			
4	572.056922222	(Gal)1 (Glc)1 (NeuAc)1	633.211628077	1.0	0.479954764			
5	613.7788	(Hex)2 (NeuAc)2	924.307044605	1.0	1.225842485			
6	513.096678	(Hex)2 (HexNAc)1	545.195584082	1.0	0.377590916			
7	424.2222	(Gal)1 (Glc)1 (HexNAc)1	545.195584082	1.0	1.196550071			
8	549.2574	(Gal)2 (Glc)1 (NeuAc)1	795.264451509	1.0	0.642275801			
9	505.249	(Hex)3 (HexNAc)1	707.248407514	1.0	0.540409633			
10	606.7887	(Hex)3 (HexNAc)2	910.327780047	1.0	2.800464185			
11	708.3271	(Gal)3 (GlcNAc)1 (HexNAc)2	1113.40715258	1.0	0.095189109			
12	724.3227	(Fuc)1 (Hex)3 (HexNAc)6	1868.703178988	1.0	0.650984536			
13	586.276	(Gal)3 (Glc)1 (GlcNAc)1	869.301230945	1.0	2.015516586			

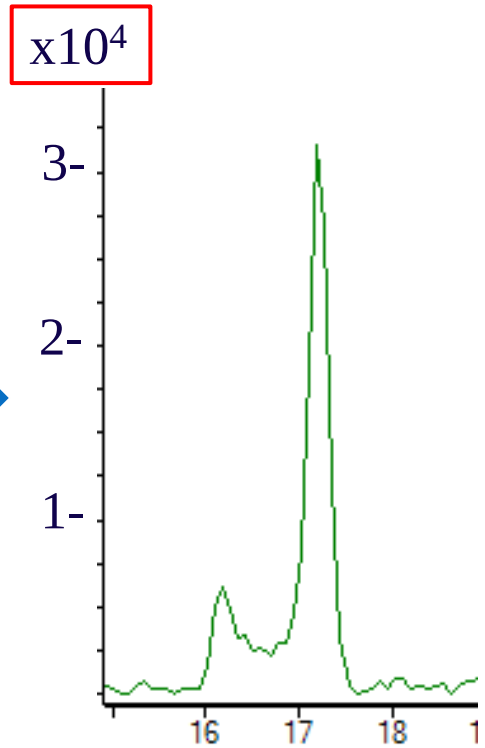
Improved sensitivity

5 Hex
4 HexNAc
1 Fuc



Underivatized

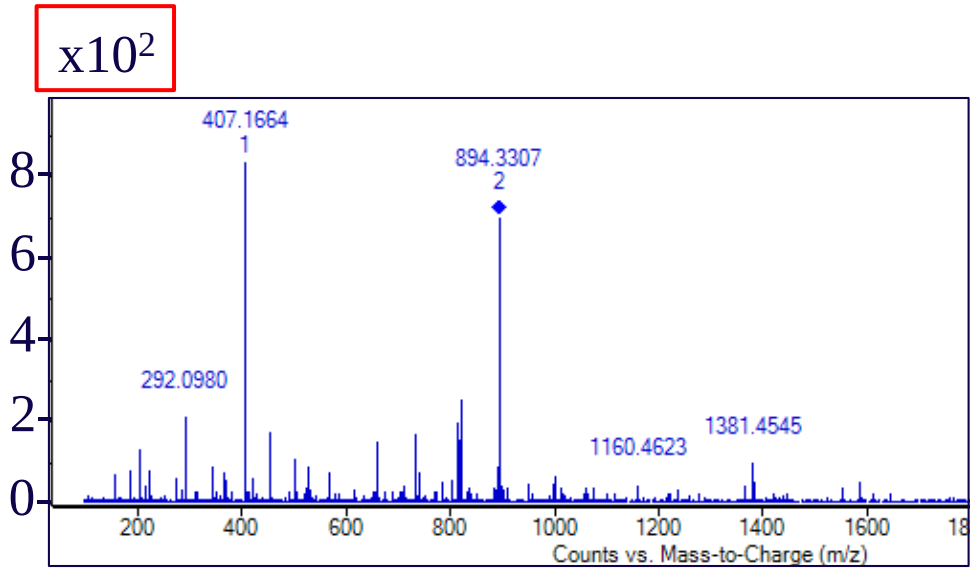
10X
Increase



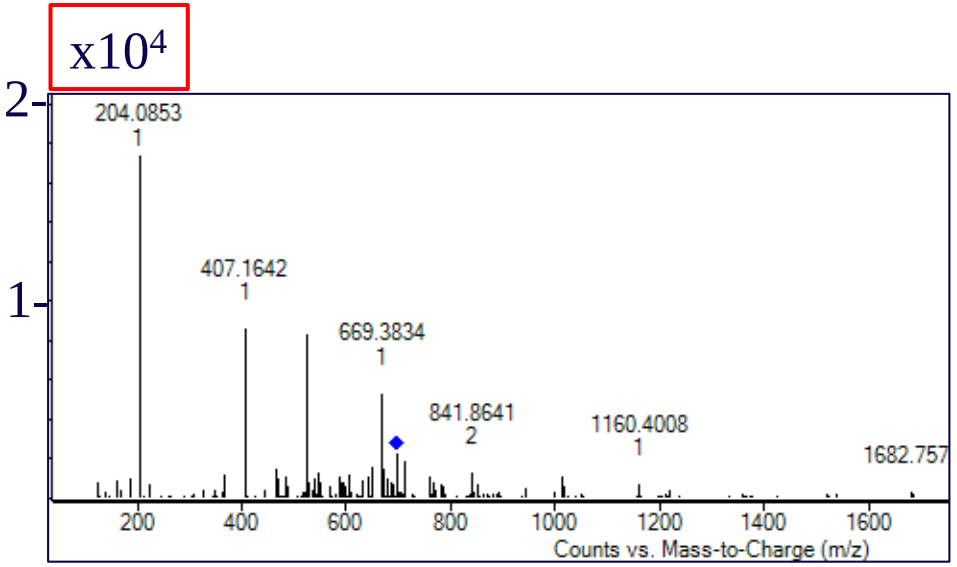
Aminoxy TMT-labeled sample

Improved sensitivity

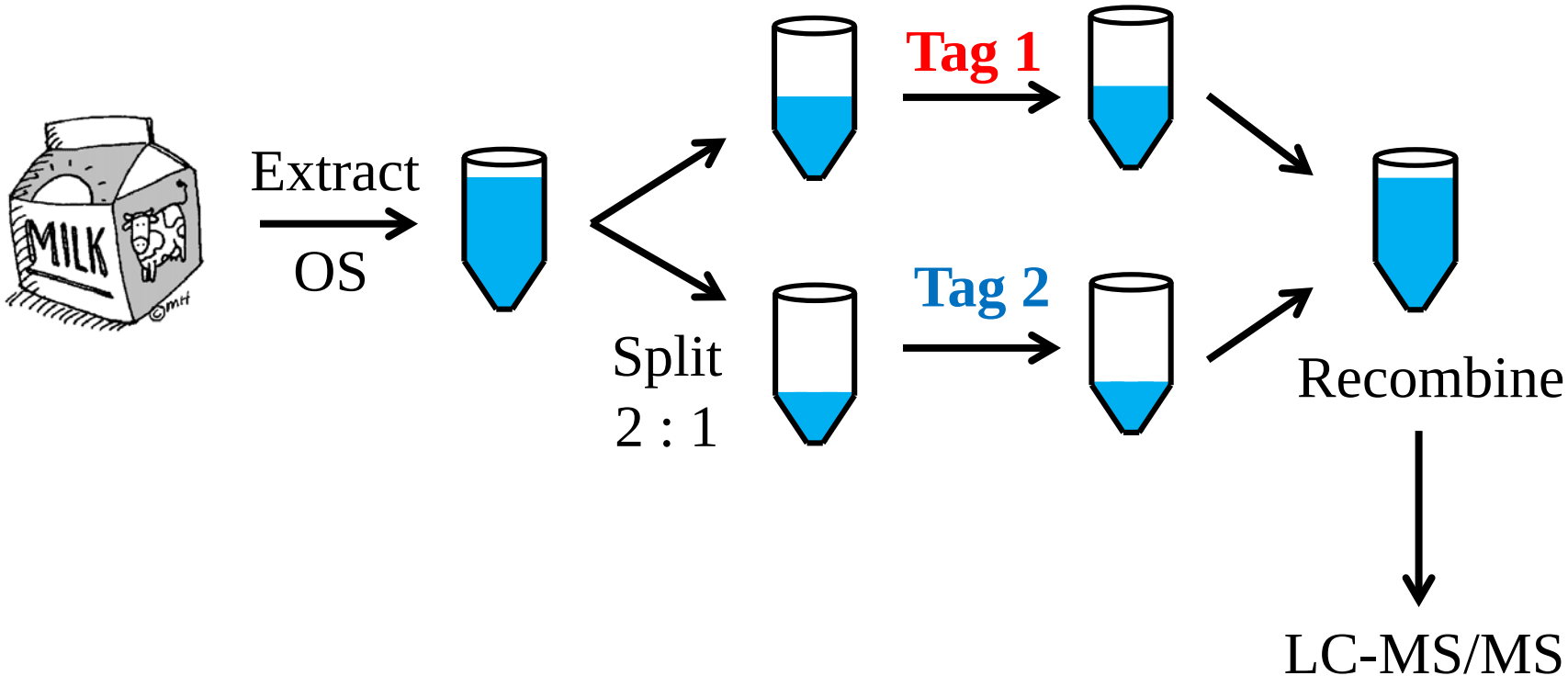
Underivatized



Aminoxy TMT-labeled sample



Test with milk



Acknowledgements

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