High Throughput Native Glycan Profiling by MALDI-MS and Chip-based LC ESI-MS

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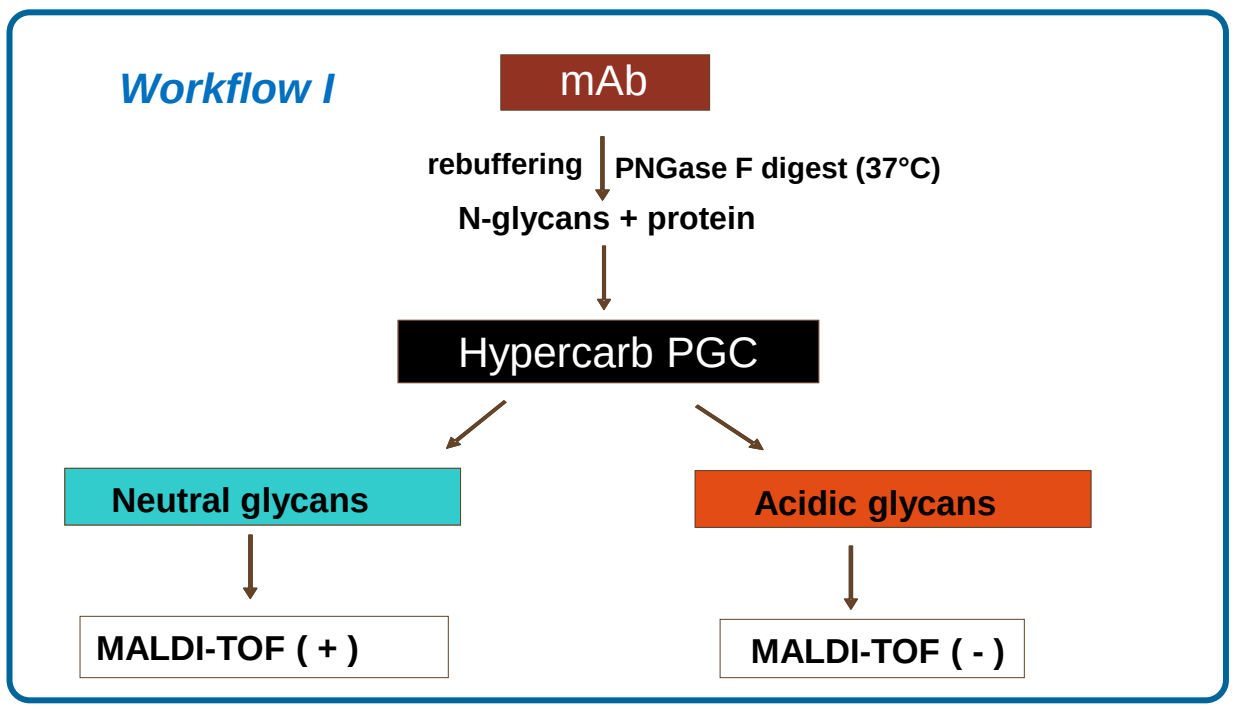
Introduction

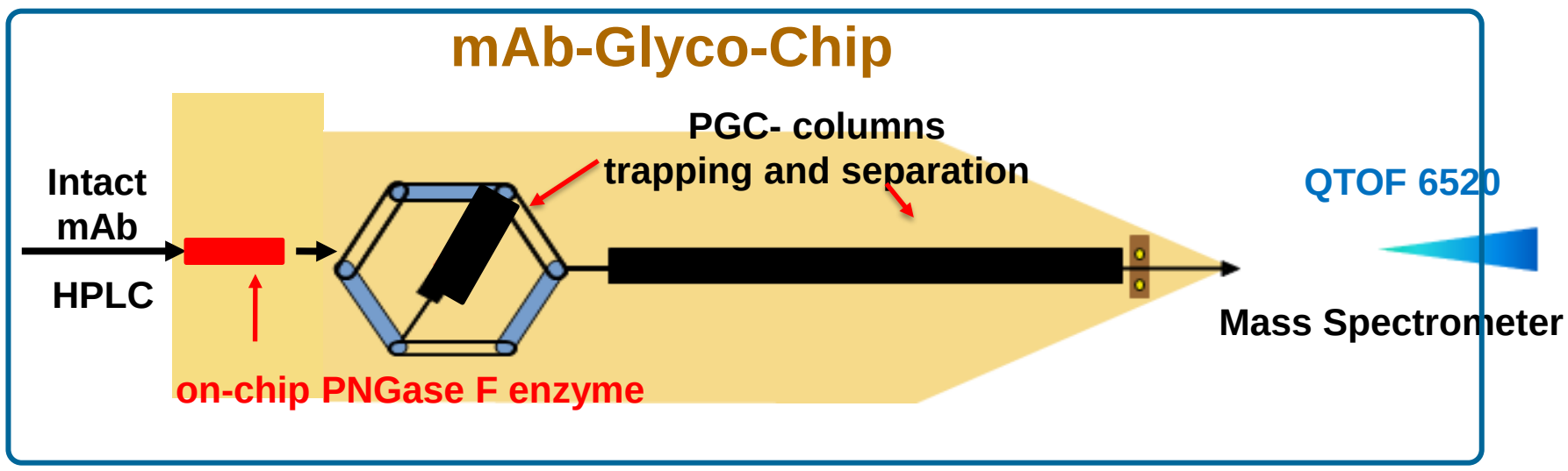
Glycosylation can modulate the structure and function of recombinant antibodies. Since glycan composition can be affected by the type of cell line, clonal selection, and specific production condition, it is important to closely monitor and control glycosylation during antibody development and production. Glycosylation analytics often include the analyses of glycoforms, glyco-sites, released glycans, and monosaccharides. For enzymatically released glycan, standard methods include glycan labeling using fluorescent agents and subsequent analysis by LC-FLD or CE-LIF. While these methods provide important structural information for glycan composition, they are tedious, time consuming, and not amenable to large sample set. Here, we present two facile methods for the analysis of native glycans: MALDI-Ms and chip-based LC/ESI-MS. The results from these methods are compared with the LC-FLD data.

Methods

*For MALDI-MS analysis (see **workflow I** below), N-glycans are released using PNGase F. The glycans are purified using hypercarb PGC column, neutral glycans and acidic glycans are separated and analyzed in the positive and negative ion modes respectively. The identities of the individual glycans are established by comparing the measured MW with the theoretical values. The relative abundances of each glycan is determined by dividing the intensity of individual glycan by the summed intensities of all glycans detected.*

*For chip-based LC ESI-MS method, the intact monoclonal antibodies (~100 ng) were directly loaded on a Agilent mAb-Glyco-Chip (see **workflow II** below) consisting of immobilized PNGase F and a porous graphitized carbon (PGC) column. After 4 min incubation with the PNGase F, the released N-glycans were captured by the subsequent porous graphitized carbon (PGC) trapping column. After washing, the trap column is then switched online with the analytical column (also PGC), which was coupled to an Agilent QTOF 6520 mass spectrometer. A linear 5 min gradient with formic acid/acetonitrile was used for analysis.*





Results and Discussion

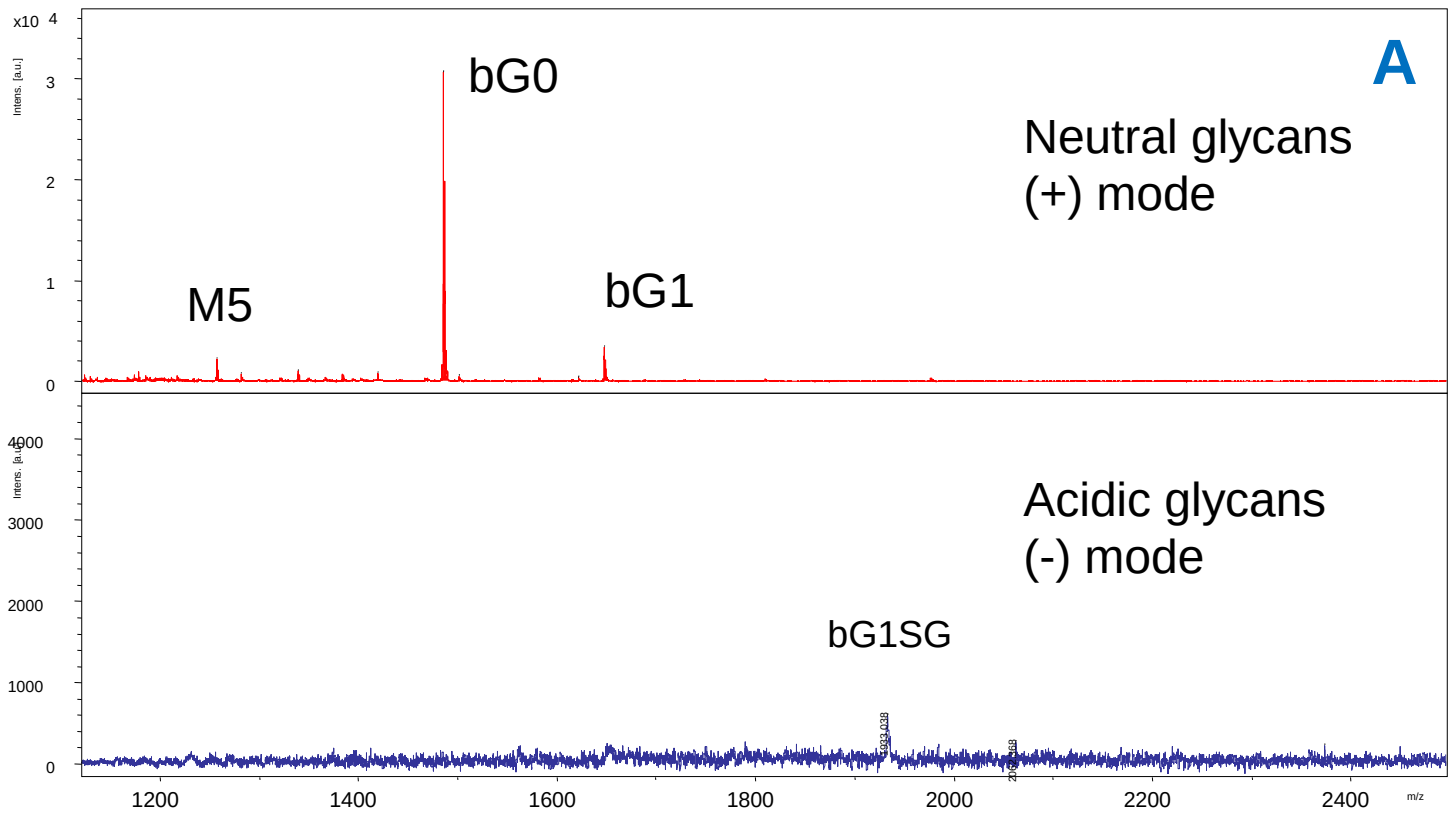


Figure A. MALDI mass spectra for unlabeled neutral and acidic glycans. For neural glycans, MALDI MS in the positive ion mode was used, and for acidic glycans, MALDI MS in the negative ion mode was used. (* unidentified ions)

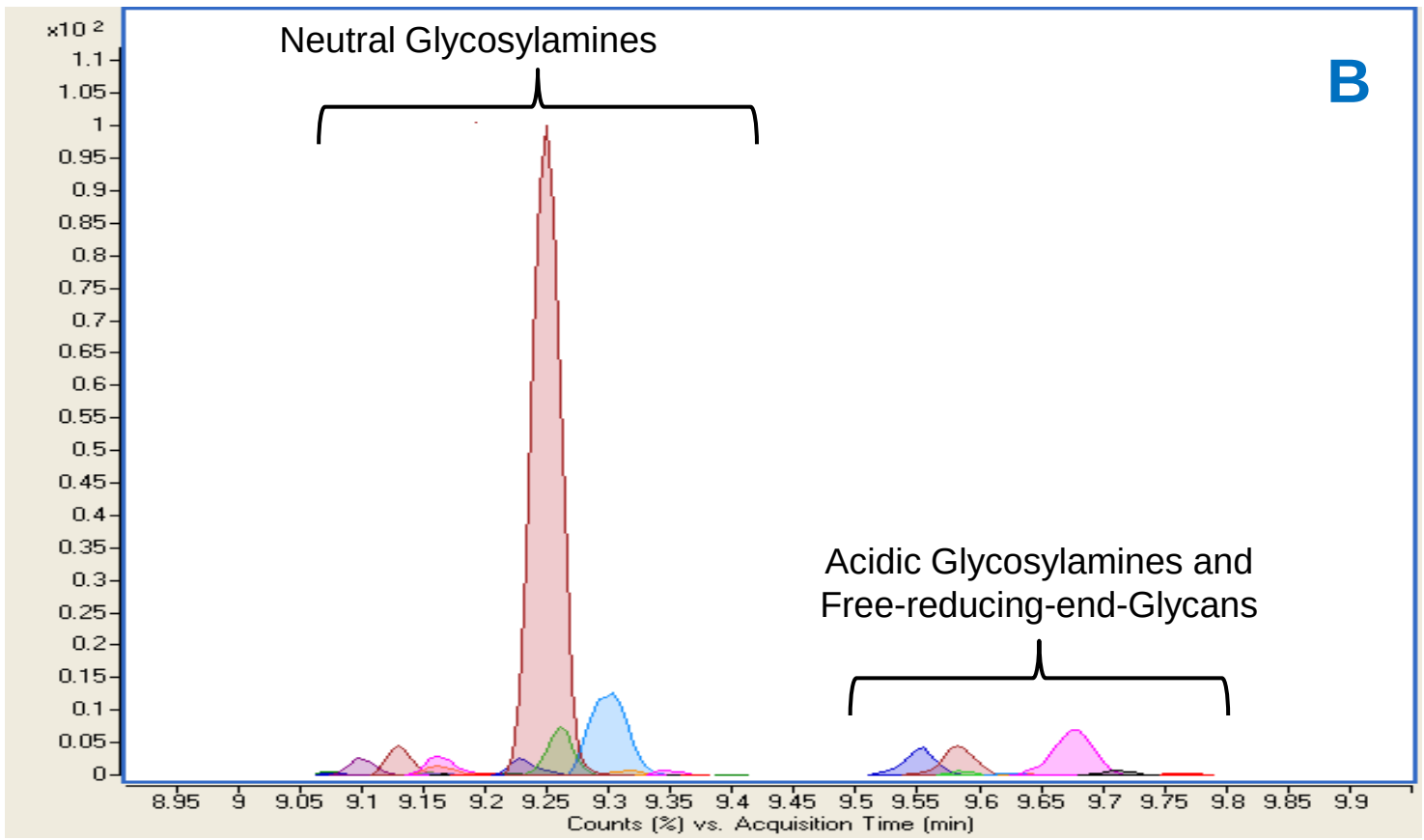


Figure B. mAb-Glyco-Chip Chromatogram for both neutral and acidic glycans. As the time between PNGase F enzymatic reaction and LC/MS analysis is less than 10 minutes, predominant amine form of N-glycans were present as compared to free-reducing end glycans.

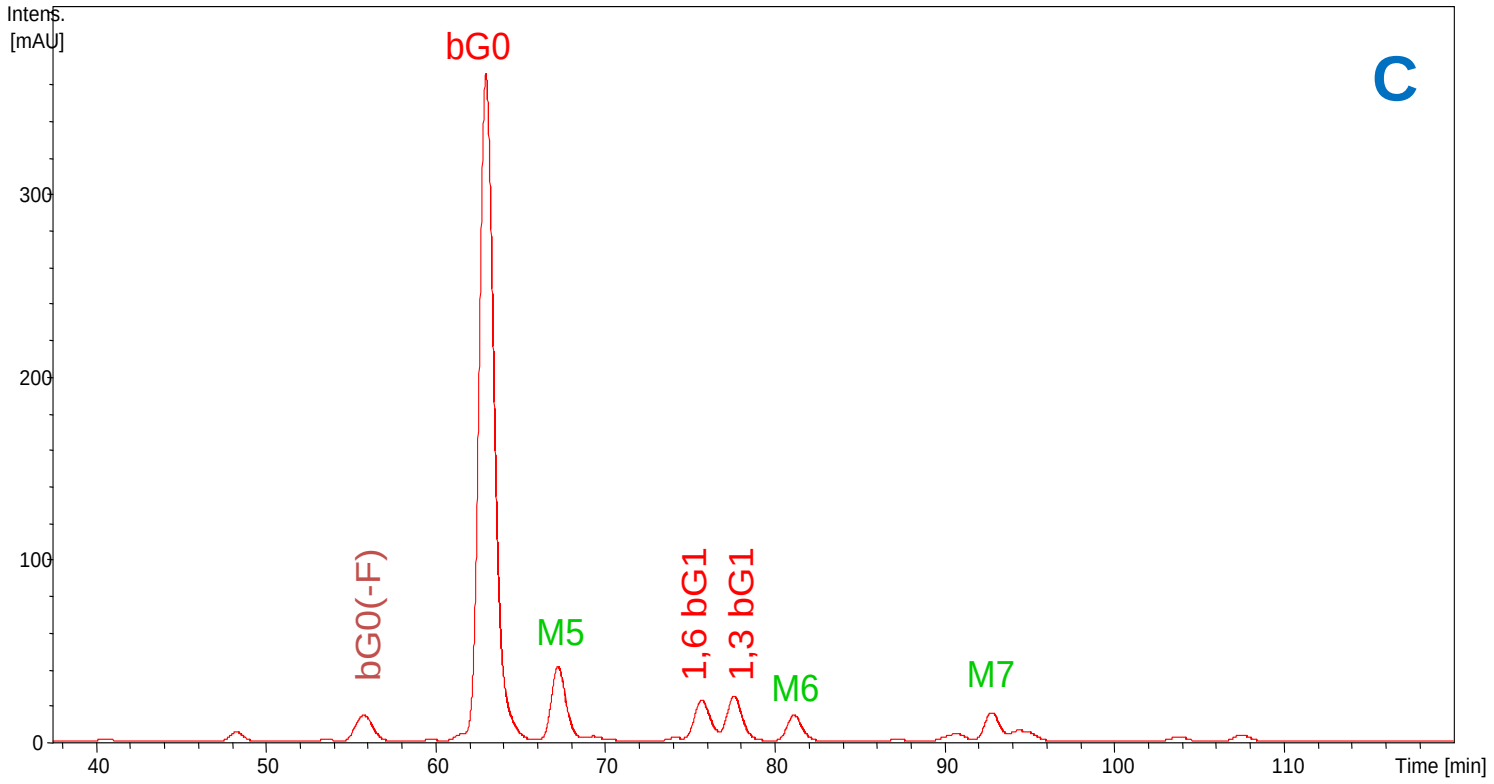


Figure C. FLD Chromatogram of 2-AB labeled glycans from NP-HPLC analysis (3 hour gradient)

Results and Discussion

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m/z	Glycan Identified	Alternative	mAb-Glyco-Chip/MS		MALDI-MS (+)				FLD 2-AB glycan
			Relative Ratio [%]	%RSD	m/z	Glycan Identified	Intensity %	RSD %	Area %
909.34	M3		0.1	3.14	933.31		0.2		
1071.397	Man4 N		0.2	8.13					
1071.397	M4 ?		0.1	8.57					
1112.42	bG0-N-F		1.5	5.50	1136.37	bG0-N-F	0.8	2.0	0.4
1233.45	M5		4.7	2.80	1257.40	M5	4.8	8.7	4.0
1258.48	bG0-N		1.5	2.86	1282.43	bG0-N	1.6	9.7	2.2
1315.50	bG0-F		2.4	2.55	1339.44	bG0-F	2.0	14.5	
1274.48	bG1-N-F		0.3	2.20	1298.41	bG1-N-F	0.2		3.1
1557.56	M7		1.0	4.62	1581.50	M7	0.9	8.8	
1395.50	M6		2.0	1.39	1419.43	M6	1.4	10.0	1.6
1461.56	bG0		71.1	0.66	1485.51	bG0	76.7	2.6	72.3
1477.56	bG1-F		0.2	6.31	1501.49	bG1-F	0.8	39.8	
1623.61	bG1		10.4	6.61	1647.55	bG1	9.0	4.2	8.7
1664.64	bNG0		0.4	3.54	1688.55	bNG0	0.4	6.9	0.3
1719.61	M8		0.4	9.10	1743.51	M8	0.4	7.9	0.3
1727.62	bG1SG-N		0.1	13.95					
1785.67	bG2		0.5	7.23	1809.58	bG2	0.4	14.6	
1826.69	bNG1		0.1	19.62	1850.52	bNG1	0.2		
1930.70	bG1SG	1,6 or 1,3 bG2SA-F	2.9	3.65	1930.7	bG1SG			
2092.76	bG2SG	1,6 or 1,3 bG3SA-F	0.3	0.82	2092.76	bG2SG			
					1460.45	hG0M5	0.2		0.1

Table D. A comparison of N-glycan data obtained from three completely independent methods, chip/MS, MALDI-MS, and FLD detection of labeled 2AB glycans. Though detection methods are drastically different from one to the other, strikingly similar relative ratio were obtained from all three methods. The chip/MS methods further validated MALDI and FLD methods while required a fraction of time per sample for sample prep and analysis. Ion/molecular species detected here are amino-glycans for mAb-Glyco-Chip, glycan-sodium adducts for MALDI-MS in positive mode, and 2-AB labeled glycans for LC-FLD.

High Throughput Parameters for mAb Glycan Analysis		MALDI-MS	LC-Chip/MS
Quantification	Linear range	10 fmol~1 pmol 100 X (+) 200 fmol~20 pmol 100 X (-)	5 fmol ~ 500 fmol
	Minimal sample	0.6 mg mAb	50 ng mAb
Reproducibility	Intra day RSD (>1% relative amt)	1~5%	1~5%
	Inter day RSD (>1% relative amt)	1~5%	1~5%
Identification	# glycan identified	Similar as LC-FLD	More glycans identified
	Specificity	Isobaric bG1 isomers, ionic adducts	Isomer can be separated
	Glycan characterization	yes	yes
Other High Throughput Considerations	Sample prep time	3 hr/100 samples	6 min/sample
	Instrument Run time	60 min/100 samples	6 min/sample
	Data analysis	60 min/100 samples	10min/sample

Table E. High throughput mode comparison between mAb-Glyco-Chip/MS and MALDI-MS. Comparing to the "gold standard" LC/CE-FLD method that takes days for sample preparation and analysis, both mAb-Glyco-Chip/MS and MALDI-MS methods provide a solution for high throughput glycan analysis.

Conclusions

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- Neither MALDI-MS nor LC-Chip/MS method requires any chemical labeling of the glycans; relative quantitation results provided by both MALDI-MS and LC-Chip/MS methods are highly comparable to those obtained by the conventional LC-FLD method while requiring a fraction of time.
- LC-Chip/MS method is more sensitive and provides more glycan identification, especially for acidic glycans; LC-Chip/MS is much faster for single or smaller batch of sample(s).
- MALDI-MS is more amenable to high throughput analysis of large sets of samples during process development and manufacture monitoring.