Automated Identification and Quantitation of Stable Isotope Labeled Released N-glycans by LC-MS with SimGlycan® Software

PREMIER Biosoft
Accelerating Research in Life Sciences

Ningombam Sanjib Meitei[1,2], Sohini Saha[1], Rupanjan Goswami[1], Arun Apte[2], Haiying Li[3], Richard S. Lee[3,4]
[1]PREMIER Biosoft, Indore, India; [2]PREMIER Biosoft, CA, USA; [3]Department of Urology Boston Children's Hospital; [4]Harvard Medical School, MA, USA.
Corresponding author e-mail: sanjib@premierbiosoft.com

1

INTRODUCTION

Glycans play a critical quality attribute for the therapeutic monoclonal antibodies, where terminal sugars of the Fc glycans are responsible for determining their efficacy and safety. One of the emerging trends of glycomics research include quantitative and qualitative analysis of glycans labeled with tandem mass tags or isotope labels. The majorly reported methods for stable isotope labelling include:

[1] Mass shift based methods e.g., isotopic labeling using ^{12}[C6]-2-AA)/^{13}[C6]-2-AA or the Dual Reactions for Analytical Glycomics (DRAG)

[2] Isobaric tags based methods e.g., aminoxyTMT6, and

[3] QUANTITY: Quantification relies on reporter ions generated in MS2 or MS3.

However among all these methods, stable isotope labeling monitors specific proteomes and hence is accurate in quantification of the glycans using LC-MS workflows. However, one of the main challenges in MS-based glycomics analysis is lack of adequate software tools to exploit the recently developed glycomics methods. We have developed SimGlycan 5.90 software modules to streamline the analysis of data generated by these workflows.

METHODS

Sample Preparation: N-glycans were released from a mixture of five glycoprotein standards by PNGase F. The released N-glycans underwent dual-reaction based on a previously published method. Briefly, two equivalent N-glycan aliquots were respectively labeled with a pair of ''light'' and ''heavy'' 2-AA reagents, which were then mixed together and purified by cellulose beads. The purified 2-AA labeled glycans were further methylamidated followed by another cellulose purification (Figure 1).

LC-MS Methods: The purified N-glycans derived from 1.5µg protein mixture were injected into an Ekspert nanoLC 415 coupled to a Thermo Q-Exactive mass spectrometer.

Data Analysis: SimGlycan 5.90 software (PREMIER Biosoft, USA) was used for data analysis.

Database Curation: To facilitate rapid and accurate annotation of glycans, a custom database containing 68 curated compositional structures probable structural isomers from five standard glycoprotein mixtures was created in SimGlycan server database (Table 1). This custom database was used as the MS/MS database search template.

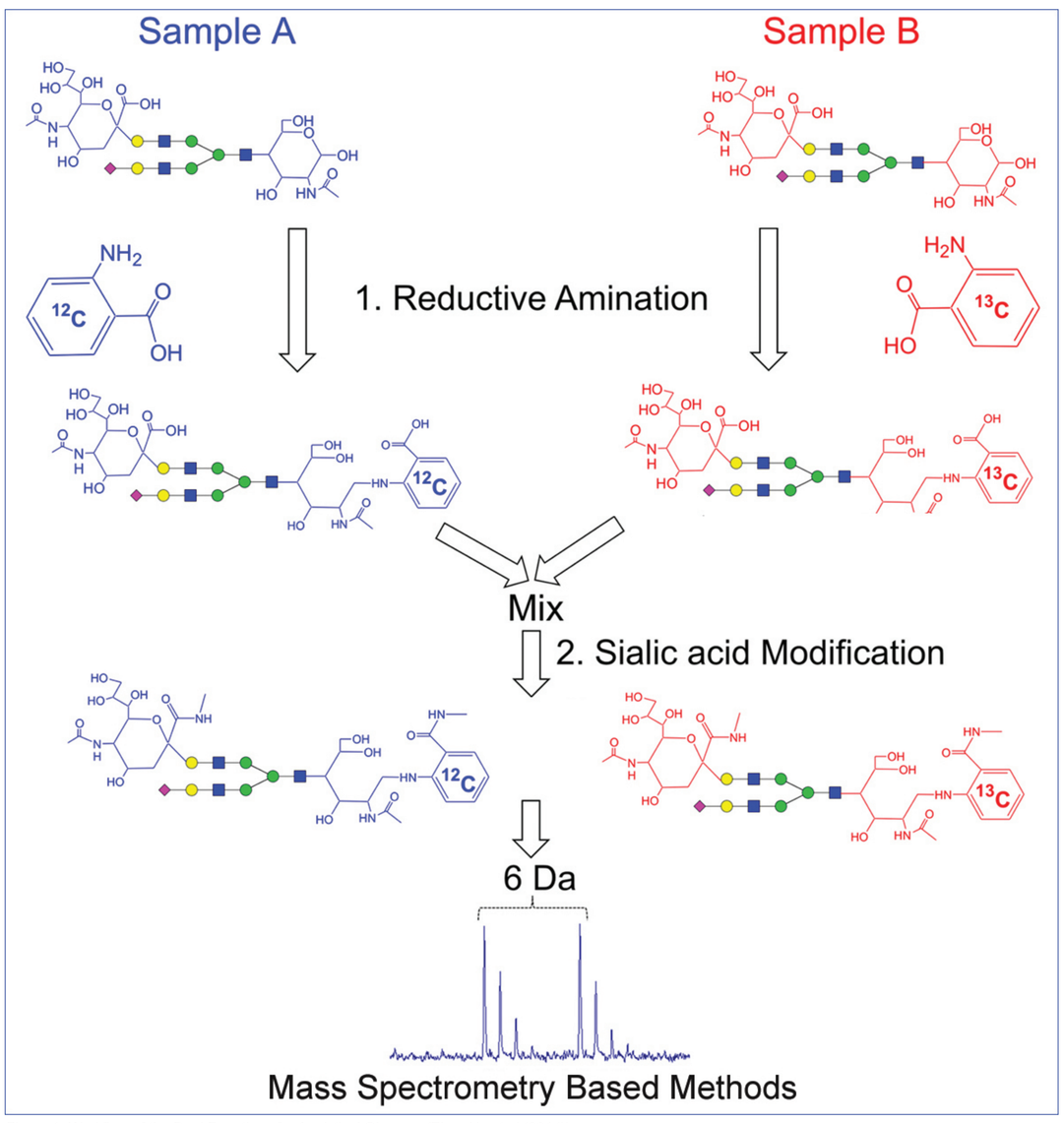

Figure 1: Workflow of the Dual Reactions for Analytical Glycome (Zhou H., et al (2014)).

1	HexNAc2	18	Hex5HexNAc4	35	Hex3HexNAc5Fuc1	52	Hex6HexNAc5NeuAc2
2	Hex1HexNAc2	19	Hex5HexNAc4Fuc1	36	Hex3HexNAc5Fuc2NeuAc1	53	Hex6HexNAc5NeuAc3
3	Hex2HexNAc2	20	Hex3HexNAc4Fuc1	37	Hex4HexNAc5	54	Hex6HexNAc5NeuAc4
4	Hex3HexNAc2	21	Hex4HexNAc4	38	Hex4HexNAc5Fuc1	55	Hex4HexNAc6Fuc2NeuAc1
5	Hex4HexNAc2	22	Hex4HexNAc4Fuc1	39	Hex4HexNAc5Fuc1NeuAc1	56	Hex4HexNAc6Fuc2NeuAc2
6	Hex5HexNAc2	23	Hex4HexNAc4Fuc1NeuAc1	40	Hex5HexNAc5	57	Hex4HexNAc6Fuc2NeuAc3
7	Hex5HexNAc4NeuGc1	24	Hex5HexNAc4Fuc1NeuAc1	41	Hex5HexNAc5Fuc1	58	Hex4HexNAc6Fuc3NeuAc2
8	Hex6HexNAc2	25	Hex5HexNAc4Fuc1NeuAc2	42	Hex5HexNAc5Fuc1NeuAc1	59	Hex7HexNAc6Fuc1NeuAc4
9	Hex7HexNAc2	26	Hex5HexNAc4Fuc2NeuAc1	43	Hex5HexNAc5Fuc1NeuAc2	60	Hex7HexNAc6NeuAc1
10	Hex8HexNAc2	27	Hex5HexNAc4NeuAc1	44	Hex5HexNAc5Fuc1NeuAc3	61	Hex7HexNAc6NeuAc2
11	Hex9HexNAc2	28	Hex5HexNAc4NeuAc1NeuGc1	45	Hex5HexNAc5NeuAc1	62	Hex7HexNAc6NeuAc3
12	Hex6HexNAc3	29	Hex5HexNAc4NeuAc2	46	Hex5HexNAc5NeuAc2	63	Hex7HexNAc6NeuAc4
13	Hex5HexNAc3	30	Hex5HexNAc4NeuAc3	47	Hex6HexNAc5Fuc1NeuAc2	64	Hex7HexNAc6Fuc1NeuAc2
14	Hex4HexNAc3	31	Hex5HexNAc4NeuGc2	48	Hex6HexNAc5Fuc1NeuAc3	65	Hex8HexNAc7NeuAc4
15	Hex3HexNAc3Fuc1	32	Hex6HexNAc4Fuc1NeuAc3	49	Hex6HexNAc5Fuc2NeuAc2	66	Hex5HexNAc7Fuc2NeuAc3
16	Hex6HexNAc3Fuc1	33	Hex7HexNAc4Fuc2NeuAc2	50	Hex6HexNAc5Fuc2NeuAc3	67	Hex6HexNAc8Fuc2NeuAc1
17	Hex6HexNAc3Fuc2NeuAc1	34	Hex3HexNAc5	51	Hex6HexNAc5NeuAc1	68	Hex4HexNAc3Fuc1

Table 1: List of the 68 curated compositional glycan structures derived from five standard glycoprotein mixtures.

RESULTS AND DISCUSSION

The LC-MS data (Figure 2) was subjected into SimGlycan's LC-MS data processing module for peak detection, peak picking, molecular feature finding, and cluster MS/MS data to LC compounds (Figure 3). The molecular feature finding algorithm reported 5058 LC-compounds out of which 1982 has MS/MS data (Figure 4).

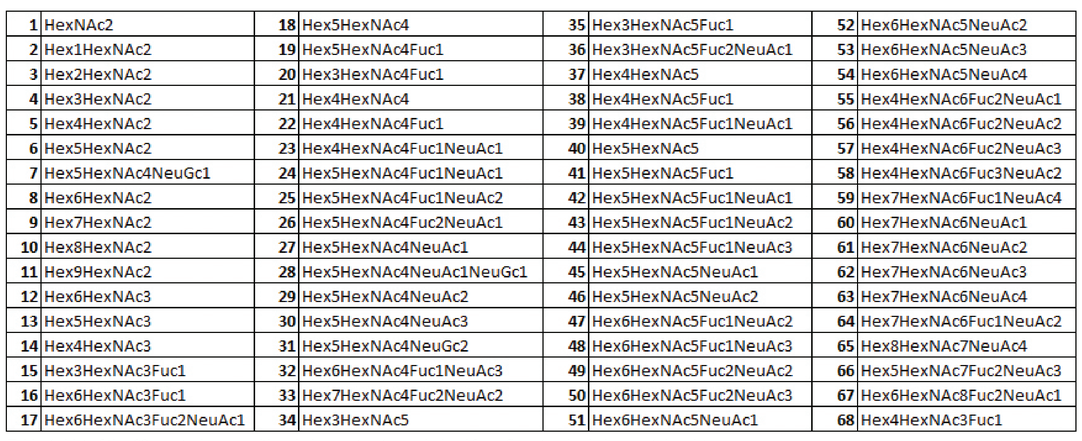

Figure 2: Typical Thermo Xcalibur Software graphical user interface showing Total Ion Chromatogram and mass spectrum.

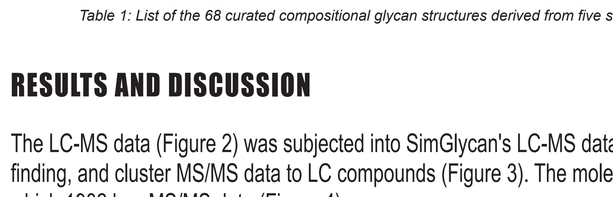

Figure 3(A): Typical GUI of SimGlycan software showing Peak Detection and Peak Picking parameters using the DRAG LC-MS template.

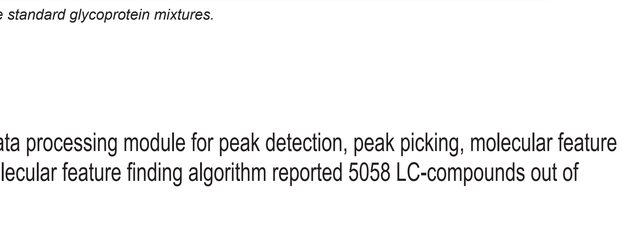

Figure 3(B): Typical GUI of SimGlycan software showing peak detection, and molecular feature finding parameters for the DRAG LC-MS template.

2

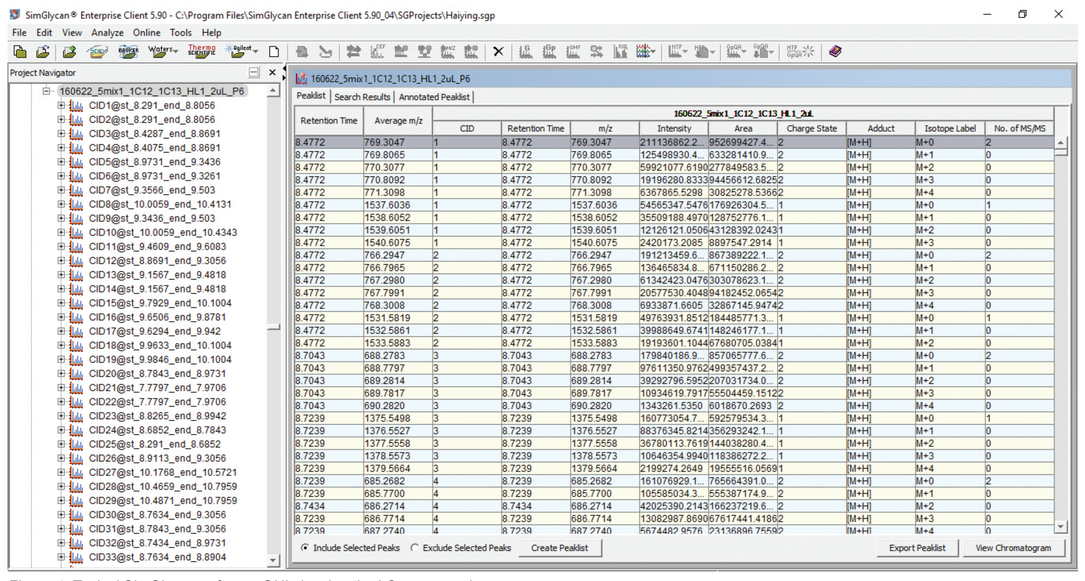

Figure 4: Typical SimGlycan software GUI showing the LC-compounds.

The MS/MS data was subjected to SimGlycan database search for glycan annotation using the parameter settings displayed in Figure 5. The search was performed on the custom database containing 68 curated compositional structures using 10 ppm tolerance for the precursor and product ions. Also, only the MS/MS scans with precursor m/z having only the H^+ adducts were considered for the database search.

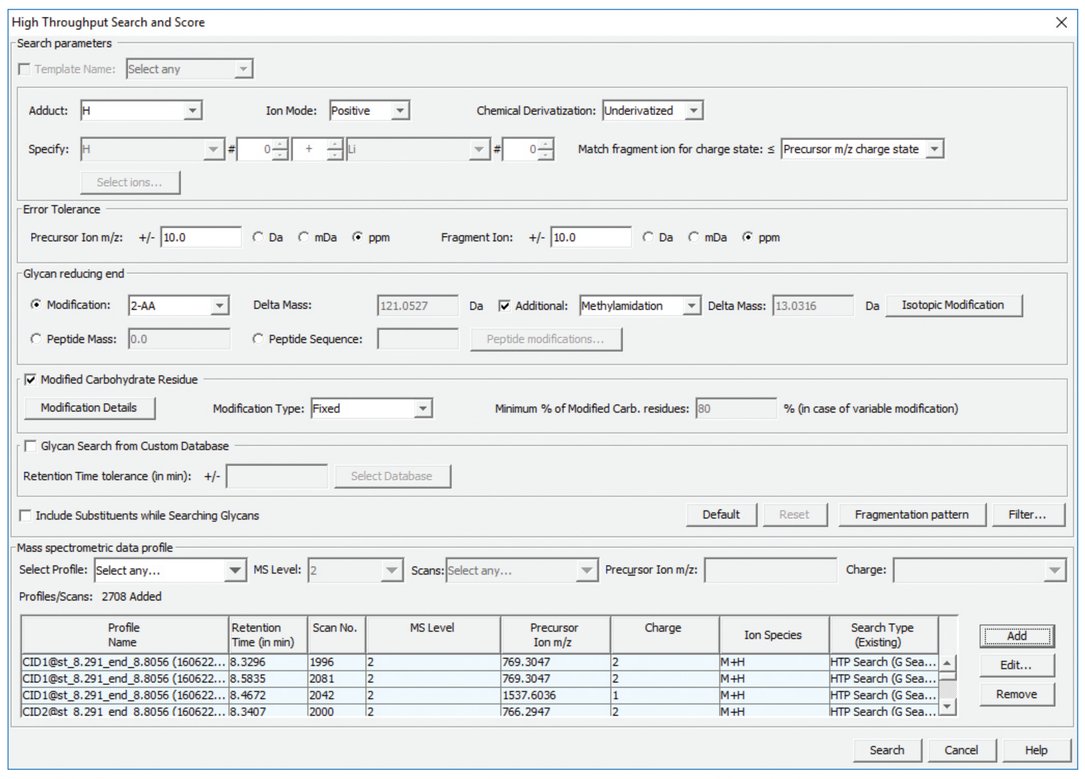

Figure 5(A): Typical SimGlycan software search window showing the database search parameters for the DRAG LC-MS workflow.

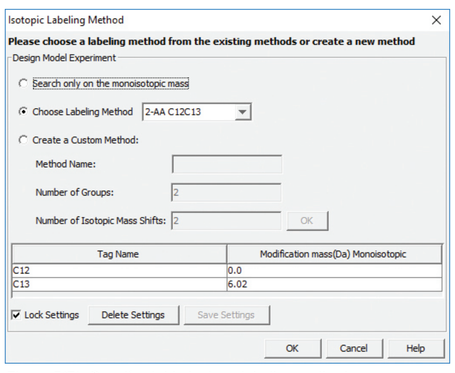

Figure 5(B): Specify stable isotope labeling method

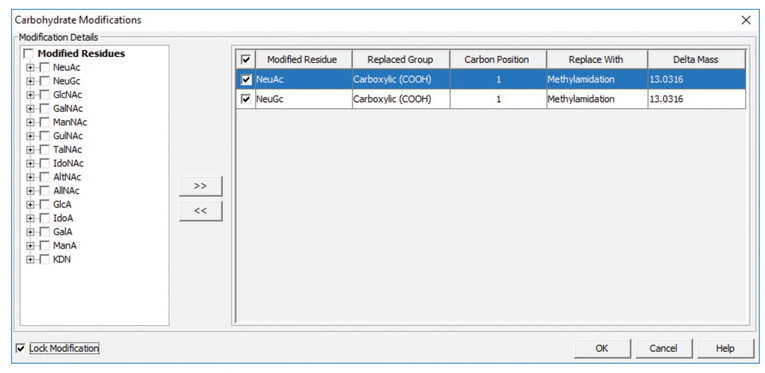

Figure 5(C): Carbohydrate modifications e.g., methylamidation of sialic acid residues

The database search annotated 143 LC-compounds with 65 compositional structures (Figure 6). The identified glycans are labeled with Tag Name and Mass Shift e.g., C12 as the Tag Name to represent the identified glycan is labeled with light 2-AA reagent, C13 as the Tag Name to indicate the glycan labeled with heavy 2-AA reagent. Area under curve of extracted ion chromatograms of the identified glycans were used as their measure of quantity in the samples. Finally, the results were exported into a Microsoft Excel file for further analysis (Figure 7).

Figure 6: Typical GUI of SimGlycan software showing identification of 2-AA[^{12}C] labeled (Fuc)2 (GlcNAc)5 (Man)3 (NeuAc)1 as the compositional structure.

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S
1	Scan No.	CID	RT	Delta Mass	Glycan ID	Polarity	Adduct	z	Mass Shifts	#Modified Residues	Proximity Score	Intensity	Composition	Glycan structure					
2	942	209	3.7035	6.5491	G25	Positive	H	3	0	1	53.3189	495910.92	(Fuc)2 (GlcNAc)5 (Man)3 (NeuAc)1						
3	888	209	3.7643	6.2963	G25	Positive	H	2	0	1	55.7343	136048.77	(Fuc)2 (GlcNAc)5 (Man)3 (NeuAc)1						
4	1098	229	4.5191	4.6849	G25	Positive	H	3	0	1	53.3798	177218.15	(Fuc)2 (GlcNAc)5 (Man)3 (NeuAc)1						
5	1148	2420	4.6474	6.8174	G8	Positive	H	2	6.02	0	71.5166	157013.28	(GlcNAc)2 (Man)6						
6	1152	206	4.6687	0.1056	G17	Positive	H	2	0	2	71.1835	112928.94	(Gal)2 (GlcNAc)4 (Man)3 (NeuAc)2						
7	1138	205	4.6792	0.7642	G17	Positive	H	3	6.02	2	71.1548	559524.27	(Gal)2 (GlcNAc)4 (Man)3 (NeuAc)2						

Figure 7: Typical SimGlycan software generated MS Excel format report file showing detailed information of the identified glycans.

The identified glycans can then be aligned between the biological groups – group A labeled with light 2-AA reagent, and group B labeled with heavy 2-AA reagent. Intensity or Area under curve of extracted ion chromatograms of the identified glycans may be used as their measure of quantity in the samples.

CONCLUSION

New software modules of SimGlycan v.5.90 provides the following key features:
1. High resolution accurate mass (HRAM) LC-MS data processing – accurate peak picking, and charge state identification.
2. Identification of glycans using data from MS based Isotopic labeling methods.
3. Identification of glycans using MS based DRAG workflow.

REFERENCES

1. Prien, J.M., et al. (2010), Anal. Chem. 82, 1498-1508.
2. Zhou H., et al, (2014), Anal Chem. 86(13): 6277–6284.
3. Yang S., et al (2015), Scientific Reports 5, Article number: 17585.