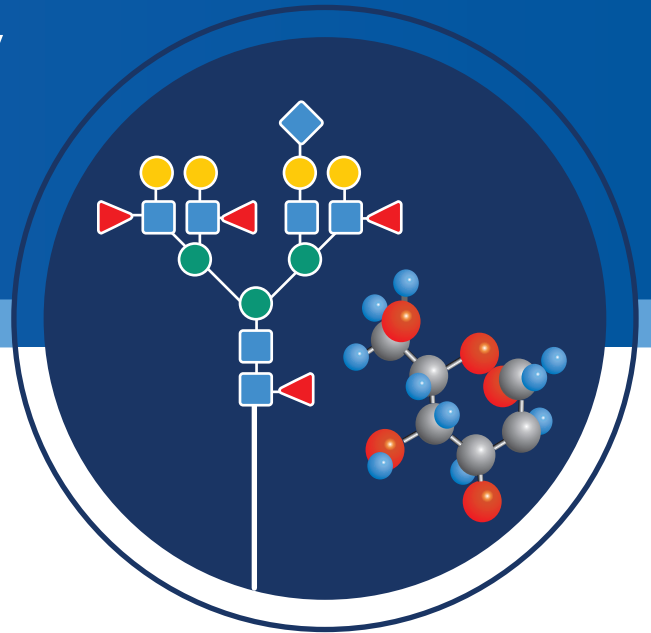


SimGlycan[®]

A high-throughput glycan and glycopeptide data analysis tool for LC-, MALDI-, ESI- Mass Spectrometry workflows

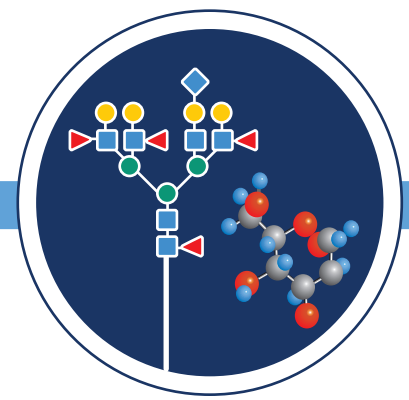


SimGlycan software processes and interprets the MS/MS and higher multi-stage/sequential (MSⁿ) data generated by LC-, MALDI-, ESI- Mass Spectrometry based glycomics and glycopeptide workflows. The software automatically matches experimental mass spectra against a comprehensive database and generates a scored list of candidate structures.

In addition to the built-in master database of over 9000 unique glycan structures, you can also create custom databases storing curated glycans from different biological sources. The glycan structures can be stored along with retention times and other chromatographic details. The retention times, biological source, fragment information etc. can later be used as search predicates for glycan identification.**

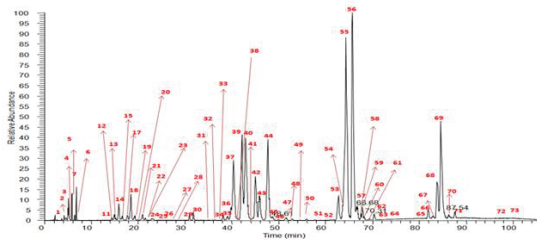
The program accepts raw data in:

- **Vendor-specific native data file formats** – *.raw (Thermo Scientific™), *.raw (Waters Corporation), *.wiff and *.t2d (SCIEX), *.fid, *.baf, and *.yep (Bruker Daltonics) and *.cef (Agilent Technologies)
- **Standard data file formats namely** – text, MS Excel, mzXML, mzML and mzData

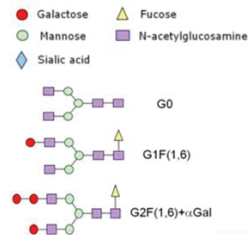


Database Creation

(A) Standard LC-MS and MS/MS Experiment for Samples



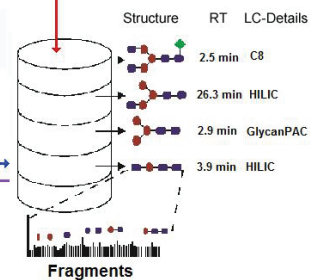
(B) Identified Glycans with Measured LC-Times



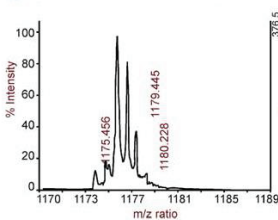
(C) SimGlycan Enterprise Server

Store Glycans along with Retention Time, LC Details, and MS/MS Data

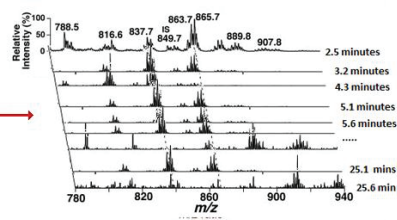
Glycan ID	Glycan Structure	Glycan Composition	Database Name	LC-MS Info	Retention Time	Chromatographic Details
Man5		(GlcNAc)2 (Man)5	IgG	<input checked="" type="checkbox"/>	1.62	View/Edit



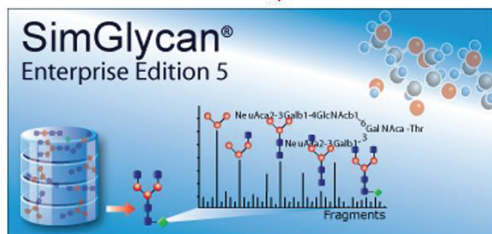
1 Exp. LC-MS Chromatogram



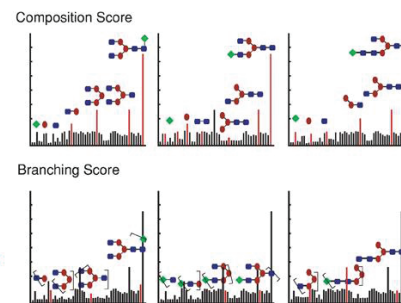
2 MS/MS Spectra



3 SimGlycan Client Tool



4 Match Experimental Data with Theoretical Data and Calculate Proximity Score



5 Predicted Glycans

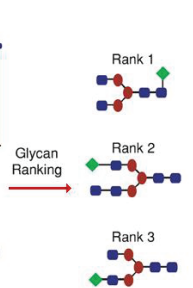
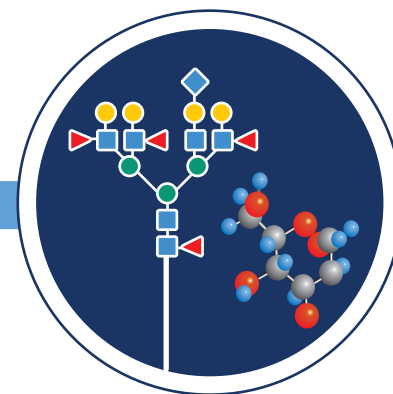


Figure 1: Schematic representation of glycan and glycopeptide data analysis workflow using SimGlycan

SALIENT FEATURES

1. LC-MS Data Processing: A fast and efficient algorithm performs peak detection, feature finding, and accurate precursor m/z selection for MS/MS data, making LC-MS glycomic data analysis – from structural identification to relative quantitation - simple, quick, and efficient.



SimGlycan® Enterprise Client 5.43 - C:\Program Files\SimGlycan Enterprise Client 5.43\SGProjects\Udainath.sgp

File Edit View Analyze Online Tools Help

Project Navigator

- Udainath
 - Profile
 - Sequence
 - Draw Glycan
 - Glycopeptide
 - LC-MS and MS/MS Data
 - XXX_P1
 - XXX_P2
 - XXX_P3
 - Glycan Quantitation

XXX_P1

Peaklist Search Results Annotated Peaklist

Glycan Search | GpQA Search |

	CID	Average Retentio...	Observed Retentio...	m/z	Delta Mass	Glycan ID	Glycan Sequence	Proximity Score	Intensity	Area
1	13.5735	13.5735	611.759	0.0		PB00307	Man(b1-3)Man(b...	79.8754	1215579.4205	203456.5967
5	15.6026	15.6026	684.788	0.0		PB00306	Man(a1-3)Man(a...	74.8788	97585.0946	23086.9525
7	16.5557	16.5557	735.2806	0.5474		PB00309	GlcNAc(b1-6)Man...	24.1567	117033.2474	29254.8693
12	18.5823	18.5823	808.3096	0.5474		PB00310	GlcNAc(b1-2)Man...	46.7146	1324408.2876	369526.891
12	18.5823	18.5823	808.3096	0.5467		PB00310	GlcNAc(b1-2)Man...	34.7207	179703.0757	48658.7188
20	19.5219	19.5219	836.8203	0.5467		PB00311	GlcNAc(b1-4)Man...	51.0563	184475.4221	50174.6455
24	21.2879	21.2879	899.8361	0.4771		PB00316	Gal(b1-4)GlcNAc...	70.9191	684874.1938	347862.4845
37	23.8157	23.8157	889.336	0.547		PB00315	Gal(b1-4)GlcNAc...	53.7547	524250.0365	171884.6787
37	23.8246	23.8246	889.336	0.5466		PB00315	Gal(b1-4)GlcNAc...	36.8146	233874.3484	75725.4391
39	23.8246	23.8246	795.7938	0.5462		PB00200	Man(a1-2)Man(a...	45.7175	1207175.2805	417090.996

Glycan Information

Name

Glycan Sequence

Composition

Chemical Formula (Underivatized)

Glycan Mass

Carbohydrate Mass

Theoretical Precursor m/z (z) OR Std DB Precursor m/z (z)

Class

Reaction

Pathway

Enzyme

Other DB links

% Glycosidic Match

% Cross ring Match

Glycan Structure (PB00307)

Sequence:

Man (b1-3) [Man (b1-6)] Man (a1-4) GlcNAc (b1-4) GlcNAc

Size: Small Medium Large With Anomeric Linkage info

Total no. of hits: 42

Figure 2: Typical SimGlycan interface showing the identified glycan structure of an LC detected compound

2. Glycan & Glycopeptide MS/MS Data Analysis: The program is capable of determining glycan, and glycopeptide heterogeneity and their isomeric forms. Additional key features are:

- Processes MSn data to resolve glycan heterogeneity, branching patterns, & isobaric structures.
- Searches for glycans with various chemical derivatives used for reducing end modifications e.g., 2-AB, 2-AA, RapiFlour, etc., even if the derivative is not available in the database.
- Characterizes Na, Li, Mg, K, and formate adducts, & adduct combinations such as Na + H, etc.
- Identifies complex glycosaminoglycan structures, even when modified with substituents such as sulfate, phosphate, and ethanolamine.
- Determines cross-ring and double glycosidic cleavages, through a combination of permethyl derivatization and MSn data.

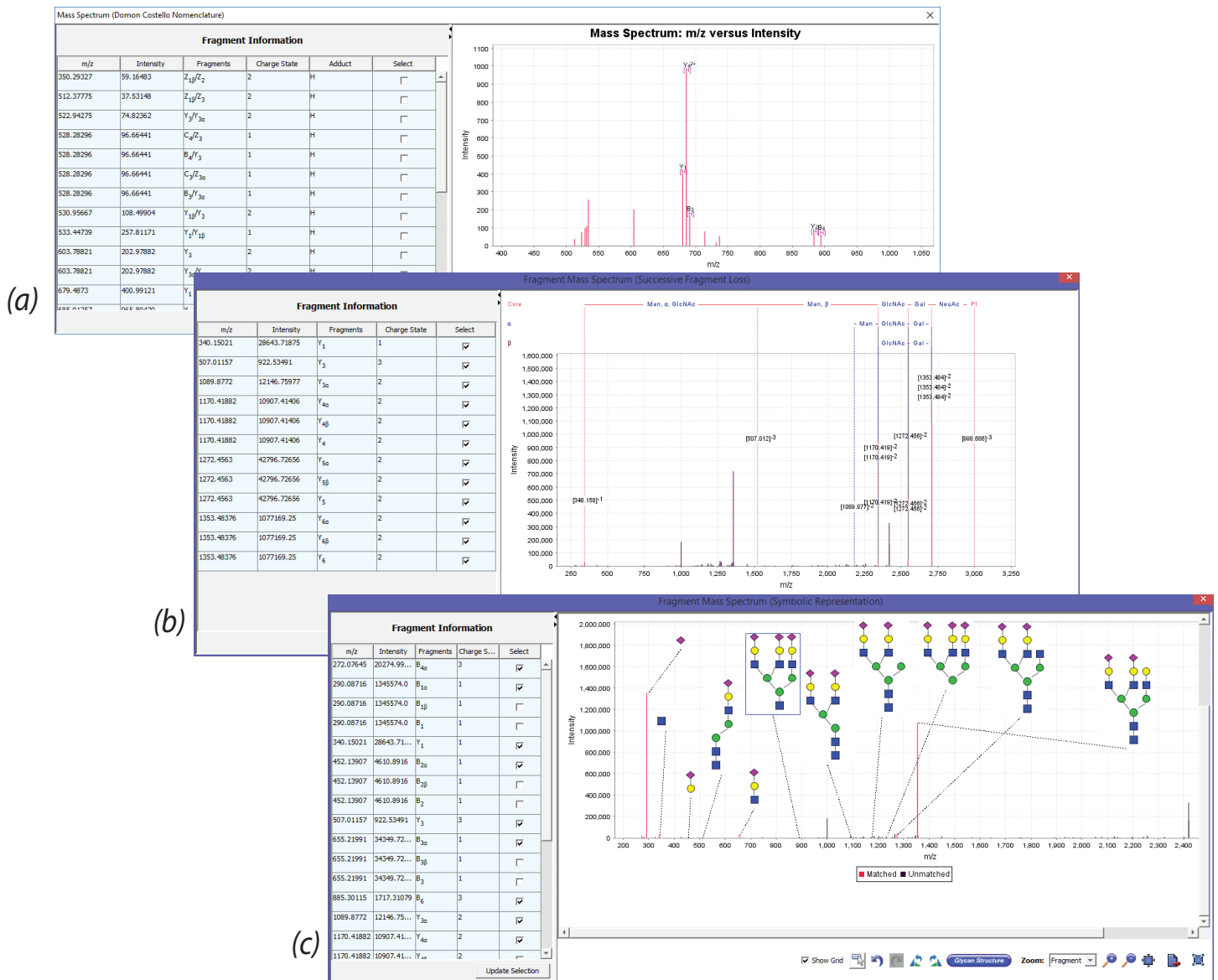
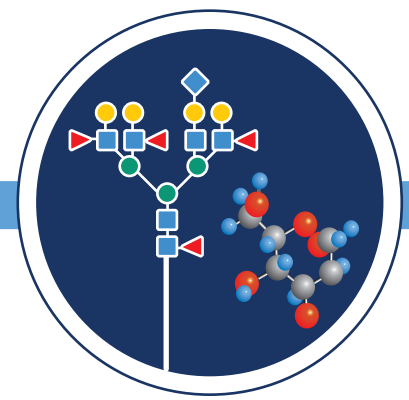


Figure 3: Typical SimGlycan software interface displaying mass spectrum annotated with (a) fragment names in Domon Costello Nomenclature, (b) successive fragment loss, and (c) symbolic representation of the fragments

3. Glycan Quantitation Using aminoxyTMT6 Reagent: Glycans labeled with aminoxyTandem Mass Tags (TMT) are quantified by measuring reporter ion peak intensities from MS/MS spectra. Various charts such as bar chart, dot-plot etc., enable visualization of the abundance of each glycan across biological samples.**

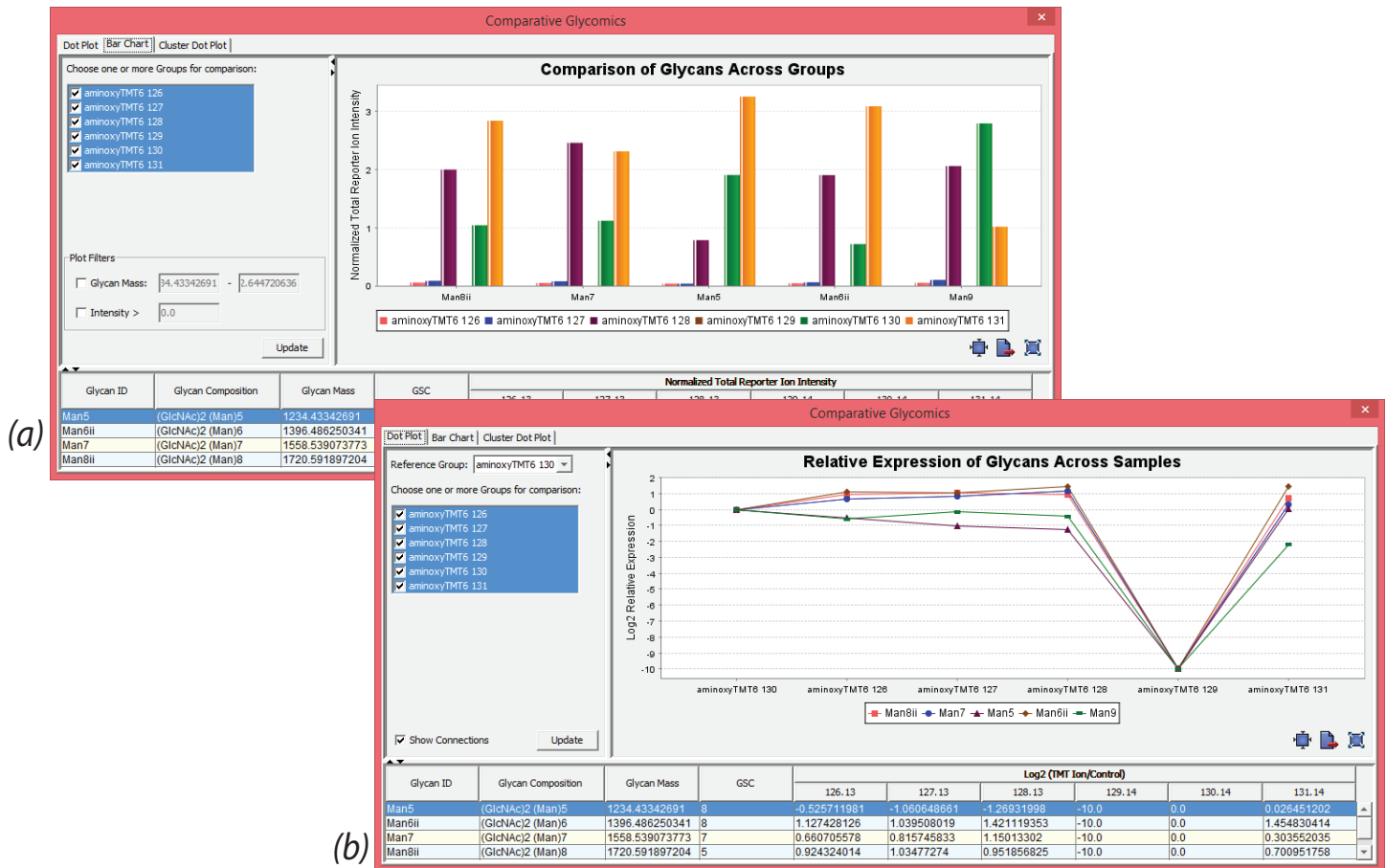
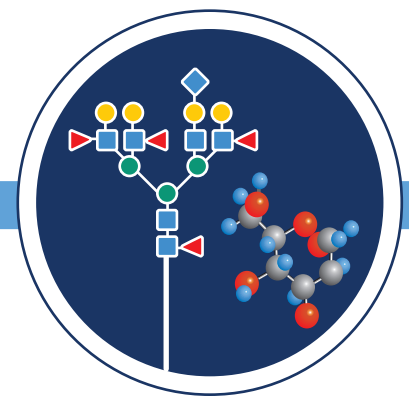


Figure 4: Typical SimGlycan software charts: (a) Bar Chart displaying the abundance glycans in different samples; (b) Dot plot depicting relative expression of glycans across samples**

** Available in SimGlycan Enterprise Edition only

4. Powerful Reporting: Export your analysis results in HTML and Microsoft Excel format for easy sharing of findings and publishing data.

- i. Creates reports that include glycan characteristics such as ID, name, composition, mass, precursor ion m/z, class, glycan image and theoretical fragments with corresponding mass and m/z values.
- ii. Exports images of glycan structures, fragments, and spectra as JPEG or PNG files for use in publications.