

# An integrated solution for automated analysis of glycoproteins

Analysis of glycans—the oligosaccharide portion of glycoproteins—presents analytical challenges. Glycoproteins are usually available only in small amounts. The glycans themselves are generally complex in structure. Even glycans attached to a single site of a glycoprotein are mixtures of structures. Standards to compare with unknown glycans generally don't exist. The analytical sensitivity, accuracy, and expertise needed for glycoproteomics has, until now, rested in a small number of specialized laboratories.

Proteome Systems and Agilent
Technologies have combined their
expertise to develop a system for
automated analysis of glycans
released from glycoproteins. The
combination of technologies can be
used for identification of glyco-markers
in biological events such as cancer
and autoimmune diseases, or for
analysis of recombinant glycoprotein
products such as erythropoietin,
interferon beta, and antibodies.



# Integrated solution for automated analysis of glycoproteins

The GlycomIQ solution from Proteome Systems includes protocols, checkout sample, and columns for the LC-MS/MS analysis of glycoprotein samples. It also includes the powerful GlycoSuite software tools and database. When the GlycomIQ solution is combined with an Agilent Technologies liquid chromatograph and ion trap mass spectrometer, glycan structural determination can be carried out on amounts of glycoproteins typically detected in Coomassie-stained 2D gels, or carried out on glycoproteins in solution.

# Sample preparation

The GlycomIQ solution helps you prepare samples for capillary or nanoflow

LC-ESI-MS/MS analysis of oligosaccharide mixtures derived from both N- and O-linked glycans released from glycoproteins. Proper sample preparation leads to better, more consistent results. Included with the solution are:

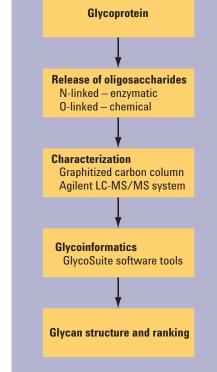
- Application protocols for sample preparation
- Checkout sample
- Porous graphitized-carbon capillary column

# Easy, accurate LC-MS/MS analysis

Agilent's 1100 Series liquid chromatographs and LC/MSD XCT series ion trap mass spectrometers are ideal for the LC-MS/MS analysis of the glycans released from glycoproteins separated

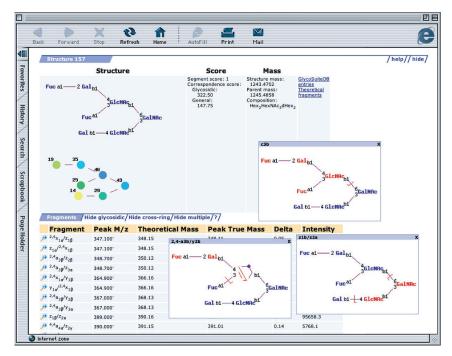
LC system produces stable flows that improve MS sensitivity and repeatability, increasing confidence in your results. The LC/MSD XCT ion traps are easy to use, yet provide the outstanding sensitivity required to find even small amounts of glycans. The resulting MS/MS information is easily transferred to the GlycomlQ server for further data analysis.

via 2D gels. The 1100 Series Capillary



GlycomIQ workflow





Glycosid1Q

# GlycoSuite tools turn raw data into answers

After MS/MS data are acquired and the meaningful spectra are located, Proteome Systems's suite of bioinformatic tools enables the interpretation of mass spectrometric data from glycans.

#### **GlycosidIQ**

A glycofragment mass fingerprinting (GMF) tool that takes the researcher's MS/MS fragmentation spectra and matches them with the in silico fragmentation of the GlycoSuite database of glycan structures. The output is ranked according to proprietary algorithms that take into account the structural and fragmentation properties of branched glycans.

#### **GlycoComp**

A calculator of glycan compositions corresponding to parent mass data of released glycans or glycopeptides, with the results ranked according to biological probability.

#### **GlycoFrag**

An *in silico* MS/MS fragmentation tool of user-entered glycan structures.

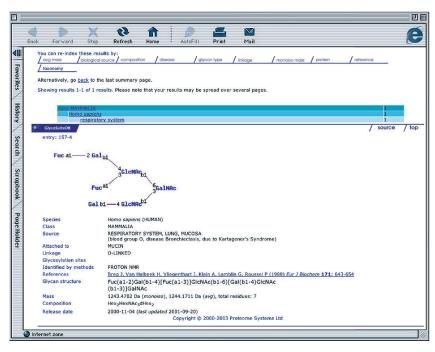
#### **GlycoBuild**

A tool to draw 2D glycan structures.

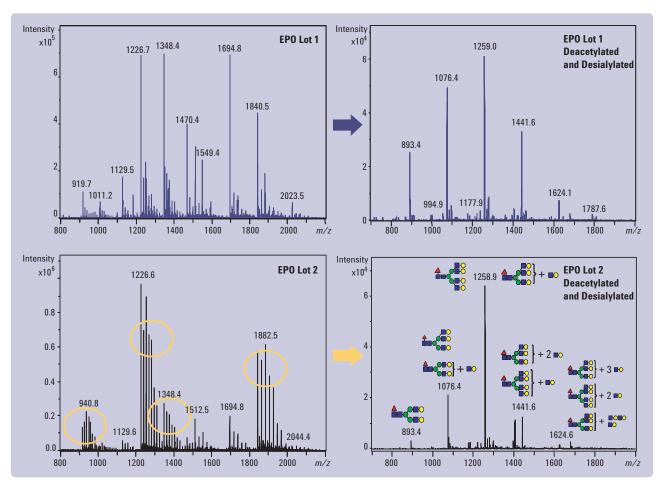
#### **GlycoSuiteDB**

A database of most N- and O-linked characterized glycan structures published in the literature:

- N- and O-linked linear and branched glycan structures
- Epitope structure (internal and terminal)
- Linkage, anomeric configuration, mass and composition
- Biological source information
- · Native and recombinant sources
- Tissue/cell type, cell line, strain and disease
- Protein name (SWISS-PROT/TrEMBL)
- · Site of attachment
- · Methods used
- Literature references (PubMed)



**GlycoSuiteDB** 



Analysis of two recombinant EPO lots reveals similar core oligosaccharide structures.

## Glycan analysis example

The cleaved glycans from two recombinant erythropoietin (EPO) lots were analyzed. Major differences between them are apparent (left two spectra), with the mass differences in the MS spectrum of one sample consistent with extensive multiple acetylation of that sample. Treatment of the samples to remove acetyl and sialic acid groups, followed by MS analysis, produced very similar spectra (right), indicating that the core oligosaccharide structures were similar.

#### For more information

For more information about the integrated glycomics solution, you can go to:

www.agilent.com/chem/glycomics

You can also call:

## 1-800-227-9770 (U.S. and Canada)

In other countries, please call your local Agilent Technologies analytical sales office or authorized Agilent Technologies distributor.

This information is subject to change without notice.

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