

Ongoing technical advancements have made mass spectrometry (MS) the dominant experimental technique used for the analysis and identification of glycan structures, whether as purified glycans or as complex mixtures of glycans extracted from biological samples. Currently, most interpretation and annotation of the high throughput MS/MS data generated in this context is done manually, due to lack of software support. The freely available tools (e.g. GlycoWorkbench, GlycoPeakfinder, GlycoMOD) and commercial systems (e.g. SimGlycan®) that have been developed over the last decades are not suited for analysis of datasets that include hundreds or thousands of MSⁿ spectra. Thus, the development of new software tools capable of processing such high throughput datasets and assisting in their interpretation and annotation is crucial to keep up with the rapid pace of technological development and data generation.

We have, therefore, developed the GRITS Toolbox, a modular software suite for functionality while reusing existing the processing, interpretation and storage of glycomics data with a focus on MS data. After loading MS data into the software (as RAW and/or mzXML files), program components. users can invoke various integrated data processing modules, including the Sample Information 3 Glycomics Elucidation and Annotation Tool (GELATO), which associates spectra features in the data sets with structures supplied by customizable databases. To allow a comprehensive overview of the processed data Alternatively, annotations generated by SimGlycan® can be imported into when exchanging GRITS projects with colleagues, the software GRITS. An extensive graphical user interface allows the annotated data to be browsed, visualized, manually modified and exported to Excel for further allows storing of meta-data about the analyzed sample. This can processing. The results of several different experiments can also be merged and happen as a free text description or a set of defined terms for origin, displayed side by side to identify differences in the glycosylation patterns of species, disease, data about Labers by the sease of the development of cancer from breast tissue [2] Signs of breast cancer may include a lump in the breast, a change in breast shape, In these with diseast greast of the diseast, there may be bere pair, swellen hymph nodes, shortness of breast, any about press of the diseast, there may be bere pair, swellen hymph nodes, shortness of breast, any about press of the diseast of the diseas analyzed samples.

The validity of the data annotation depends critically on the quality of the databases that provide structures that are associated with spectral features. Therefore, GRITS provides several of its own N-glycan, O-glycan and glycolipid databases that have been generated using knowledge from a highly curated glycan structure ontology. We have populated this ontology using a web-based curation system, called Qrator (http://glycomics.ccrc.uga.edu/qrator/). The current version of the software suite is <u>freely available</u> via the project website: http://www.grits-toolbox.org.

In addition, software has been developed that utilizes Consecutive Reaction MS data in mzXML format can be loaded into GRITS toolbox. Monitoring MS² data (iCRM) that allows the identification and quantification of O-Thermo RAW file format is supported as well but auto generation of linked glycans from a complex mixture released from glycoproteins or cells. The next steps are to automate the collection and processing of these data and corresponding mzXML files using a public web service is restricted incorporate them into the GRITS software suite, as well as to expand coverage to files smaller than 10 MB. Uploaded files are copied and become to N-linked glycans and glycolipids using shotgun proteomics. This technology part of the GRITS project to fahas been applied to identify potential diagnostic and prognostic cancer markers cilitate full access for colleagues in sera

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Ongoing technical advancements have made tandam mass spectrometry (MS/MS) the dominant experimental technique used to identify structures of purified glycans as well as complex mixtures of glycans extracted from biological samples. Currently, most interpretation of the generated high throughput MS/MS data are done manually, due to the lack of software support. The freely available tools and commercial systems that have been developed over the last decades are not suited for analysis of large datasets that include

hundreds or thousands of MS/MS spectra. **GRITS** toolbox is a freely available software suite for the processing of these datasets.





Development of a software suite for the interpretation of glycomics high-throughput MS/MS and CRM-based data

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Extendable Software Platform 2

- GRITS toolbox is a platform independent software based on the Eclipse software framework (https://www.eclipse.org) and the Java programming language. Although the current
- version of GRITS is primarily focused on the processing, interpretation and storage of glycomics MS data, its modular architecture based on plugins can reduce development time by allowing **easy**
- extension of the software with new

sample amount and quality control. Providing this optional information and using the defined terms simplifies data exchange, and data submission to repositories.



Mass Spectrometry Data

when sharing projects. MS data can be **displayed** as peak lists or in a spectral viewer. Meta data about sample preparation and instrument setup can be stored as well.

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GELATO 5

The Glycomics Elucidation and Annotation Tool (GELATO) is the **integrated MS/MS annotation module** within GRITS, which associates spectral features in the MS/MS data sets with structures supplied by customizable databases. The default ::::-databases used by GRITS consist of sets of human curated mammalian glycan GOG200, Scores 0.62 Peak m/z 🔺 Peak Intensity Cartoon structures, which have been 847,4895 1477.1 +-----847/4395 1427.1 🔶 🗧 approved by experts using ज़क़ॶॖॾॾऀॖ॒ॖॗॖॿॶॖॣख़ॻॖॖऀॿ our curation tool - Qrator. •••**••**•• 1118.2599 1546.2 •**•••**•• 1119.7559 1545.2 Qrator GELATO 🔭 Human Curated 🛛 🗕 🛶 🍋

Glycan Database



After automatic annotation of the MS/MS data the annotation results can be manually inspected using diverse display options. The MS¹ annotation overview shows the MS^2 precursor annotation of each sub spectrum in the upper part of the application and the different candidate structures for each peak in the lower part.



The annotation **spectrum viewer** shows the raw spectrum and highlights peaks that are annotated or are not annotated. In addition the viewer shows both the annotation of MS¹ spectra with intact structures and annotation of MSⁿ spectra with fragment ions.

structures present in one sample with the glycans in another sample. Intensity values are provided alongside the structures, making it easy to find changes in the glycan expression level for different samples.



MS Annotation 6

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Double clicking the MS² precursor annotation, opens the fragment summary view to show all precursor candidate structures side by side with the MS² fragment ions supporting each structure. The same procedure can be used to study MSⁿ annotations.



MS Merge

The MS annotation merge plugin provides a visual side-byside comparison of the user curated MS annotations of multiple samples or MS runs. The plugin can be used to compare the glycans

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12 🛉 iCRM 🐐

- Targeted approach to glycan assignment and quantification
- Front-end can be automated
- Separation of Isobaric Glycans for Quantification
- Quantification is in MSn (IceCream Software)
- Automated data extraction for Assignment
- Automated Normalized Quantification
- Automated Comparative Glycomics



inearity of response from serial dilution of O-Man tetrasaccharide structure. O-Glycans were prepared from mouse brain and analyzed via CRM for generation of diagnostic fragment at varying concentrations (1, .33, I). Theoretically the line should be y=1x+0. We observed y=1.03x-0.03 with an R²=.996. Data was normalized to a spiked in control and the intensity of the most concentrated sample was set to 1.

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