GRITS Toolbox – A freely available software suite for the interpretation of glycomics high-throughput MS/MS data

René Ranzinger, D. Brent Weatherly, Shahnawaz Khan, Sena Arpinar,
Khalifeh AlJadda, Mindy Porterfield, Michael Tiemeyer and William S. York

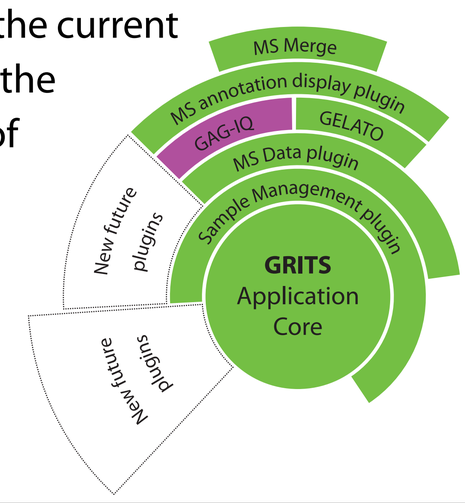
Complex Carbohydrate Research Center, University of Georgia, Athens, GA, USA

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.**

http://www.grits-toolbox.org

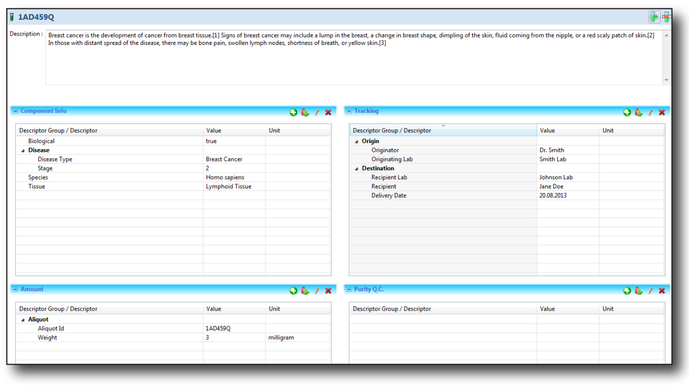
Extendable Software Platform

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 functionality while reusing existing program components.



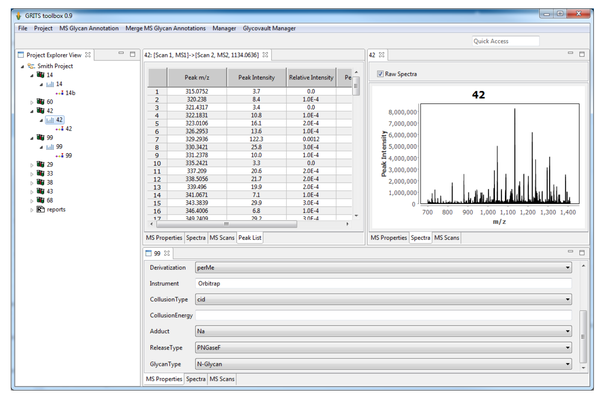
Sample Information

To allow a comprehensive overview of the processed data when exchanging GRITS projects with colleagues, the software allows **storing of meta-data about the analyzed sample**. This can happen as a free text description or a set of defined terms for origin, species, disease, data about sample amount and quality control. Providing this optional information and using the defined terms simplifies data exchange, and data submission to repositories.



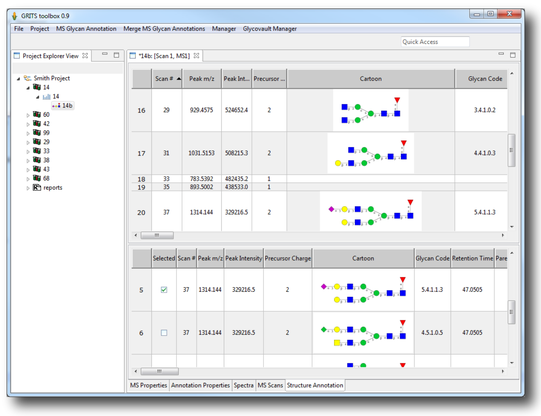
Mass Spectrometry Data

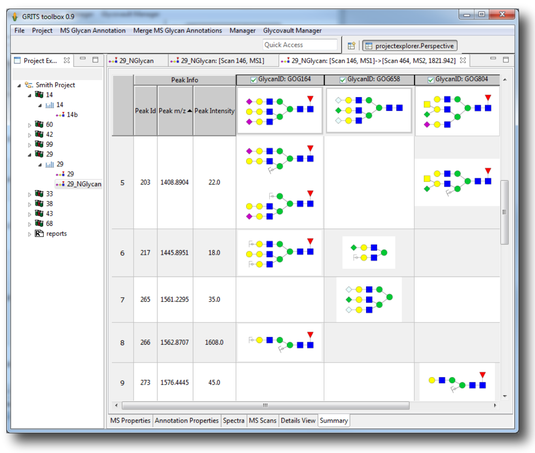
MS data in **mzXML format** can be loaded into GRITS toolbox. Thermo RAW file format is supported as well but auto generation of corresponding mzXML files using a public web service is restricted to files smaller than 10 MB. Uploaded files are copied and become part of the GRITS project to facilitate full access for colleagues 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.



MS Annotation

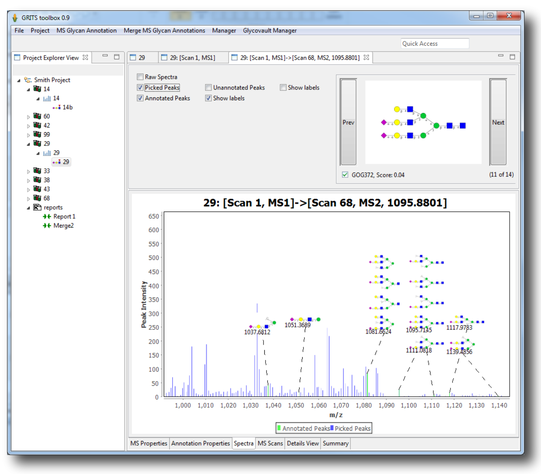
After automatic annotation of the MS/MS data the annotation results can be **manually inspected using diverse display options**. The MS^1 annotation overview shows the MS^2 precursor annotation of each sub spectrum in the upper part of the application and the different candidate structures for each peak in the lower part.





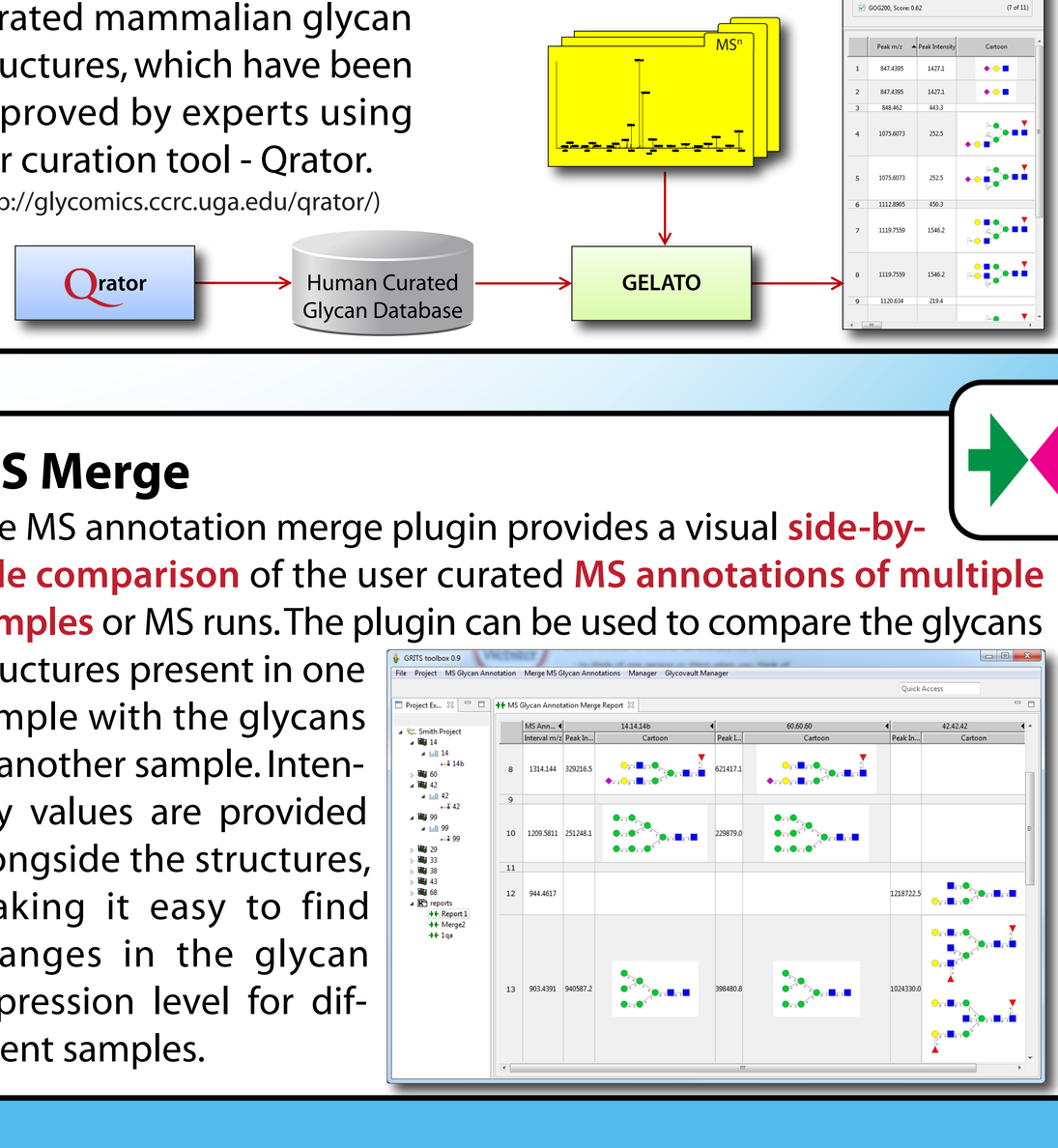
Double clicking the MS^2 precursor annotation, opens the fragment summary view to show all **precursor candidate structures side by side** with the MS^2 fragment ions supporting each structure. The same procedure can be used to study MS^n annotations.

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 **MS^1 spectra with intact structures** and annotation of **MS^n spectra with fragment ions**.



GELATO

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 structures, which have been approved by experts using our curation tool - Qrator.
(http://glycomics.ccrc.uga.edu/qrator/)



MS Merge

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

The University of Georgia

Complex Carbohydrate Research Center

NATIONAL INSTITUTES OF HEALTH