Creating a data analysis pipeline for producing high-quality glycopeptide mass spectral libraries

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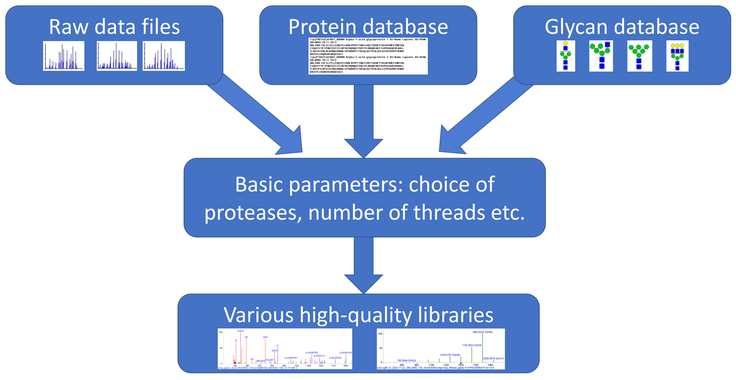
Introduction

- Previously[1], multiple tools developed at the NIST Mass Spectrometry Data Center were integrated into a single graphical user interface (GUI)
- This allowed creating series of sequentially operating programs to be configured and executed through a pre-defined 'pipeline'
- This poster describes extension of this pipeline for the specific task of comprehensive glycopeptide analysis and building high-quality glycopeptide mass spectral libraries from raw data
- The pipeline is implemented as a GUI with minimum required input parameters
- The generated libraries can be used with an updated version (not currently available for download) of the NIST Search Software[2]
- The NIST Search Software can be used for examining and comparing spectra in the resulting glycopeptide libraries

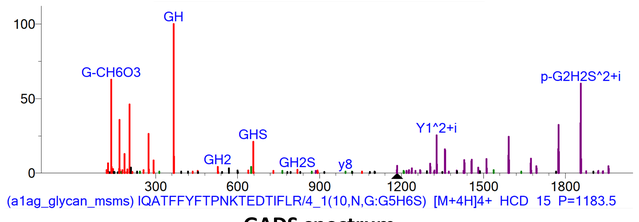
Input parameters



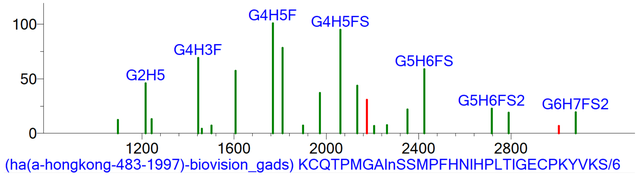
Types of generated libraries

(1) MS/MS glycopeptide libraries; (2) Glycopeptide abundance distribution spectra (GADS[3]) libraries; (3) Glycan libraries; (4) MS/MS peptide libraries.

Annotated glycopeptide spectrum



GADS spectrum



MSFragger[4,5] pipeline (for glycopeptide analysis)

ReAdw4Mascot2 (Dmitrii V. Tchekhovskoi): Input .RAW files are converted into the mzXML and MGF formats;
ProMS[6] (Xinjian Yan): analyzes search results (optional; run in parallel)

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FragPipe[4,5]: searching input RAW files, finding and validating glycopeptide matches.

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Format4gads (Guanghui Wang): combines and formats FragPipe output

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XICAnalyzer (Guanghui Wang): identifies peaks in an extracted ion chromatogram (XIC), calculates XIC theoretical isotope envelope, validates assignments of the search engine

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Validating spectra (Yuri A. Mirokhin): filtering spectra and creating libraries in the MSP text format

↓

MS_Piano[7] (Xiaoyu Yang): annotates peaks of peptide and glycopeptide spectra

↓

Make_GADS (Stephen E. Stein): builds GADS libraries and generates various summary statistics

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Building libraries in the NIST binary format (Dmitrii V. Tchekhovskoi): building libraries using the NIST tool Lib2NIST[2]

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Building Glycan libraries (Yuri A. Mirokhin)

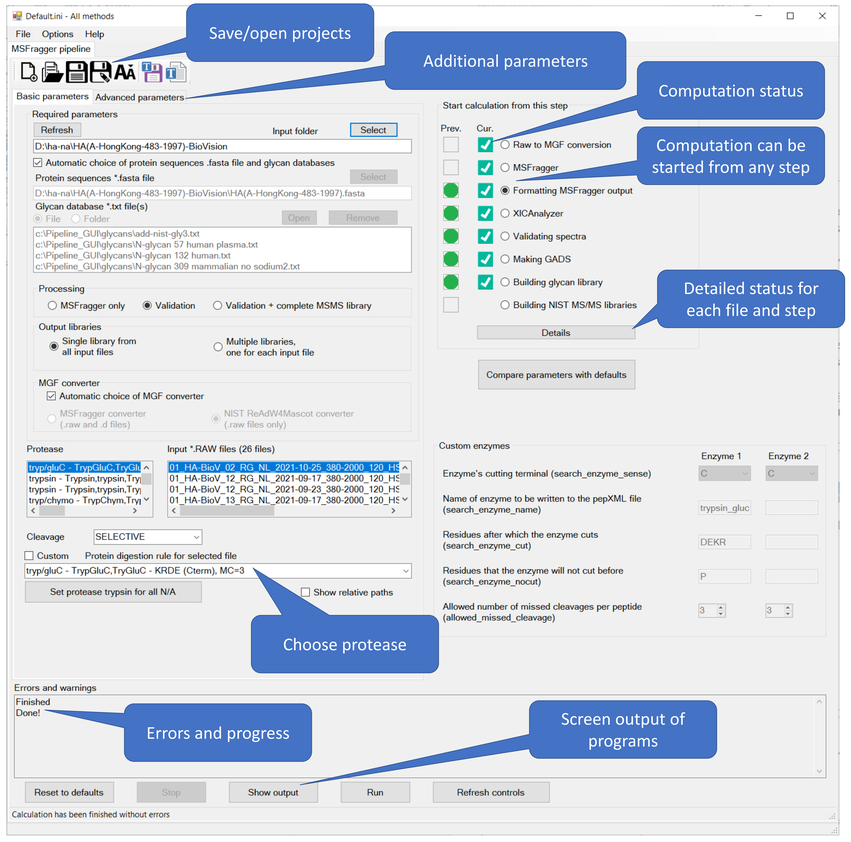
↓

MSPepSearch[2] (Dmitrii V. Tchekhovskoi): searches mass spectra in libraries using Hybrid Search[8] (optional)

- The GUI is implemented by Sergey Sheetlin
- FragPipe parameters are attuned by Guanghui Wang
- Extensive pipeline testing and conducting experiments are done by Stephen E. Stein, Yi Liu, Concepcion Remoroza, Zachary C. Goecker, Meghan C. Burke

Main features

- MSFragger and other components of FragPipe are used in the pipeline
- The computation is parallelized among computer cores
- There is an option for either N- or O-glycans
- A detailed description of the GUI is available through the menu "Help"
- Computation can be started from any step using the button "Run"
- The pipeline GUI analyzes the input settings and outputs warnings if some problems are detected during execution
- A batch software for processing multiple input data is available



Requirements

- The GUI is compatible with Windows 7, 10 and 11.
- **FragPipe** tools required licensing prior to use.

References

([1]) Sheetlin, Sergey L., at al, *Graphical User Interface for Integration of NIST Mass Spectrometry Data Center Library Building Tools.* Poster on ASMS 2021 Conference on Mass Spectrometry and Allied Topics.
([2]) NIST Search Software, NIST MSPepSearch, Lib2NIST: https://chemdata.nist.gov/dokuwiki/doku.php?id=chemdata:nist17
([3]) Remoroza, C.A., et al., *Representing and Comparing Site-Specific Glycan Abundance Distributions of Glycoproteins.* J. Proteome Res. 2021, 20, 9, 4475–4486.
([4]) Kong, A. T., et al., *MSFragger: ultrafast and comprehensive peptide identification in mass spectrometry–based proteomics.* Nature Methods 2017, 14, 5, 513-520.
([5]) FragPipe: https://fragpipe.nesvilab.org/
([6]) Rudnick, P.A., et al., *A Description of the Clinical Proteomic Tumor Analysis Consortium (CPTAC) Common Data Analysis Pipeline.* J Proteome Res, 2016. **15**(3): p. 1023-32.
([7]) Yang, X., et al., *MS_Piano: A Software Tool for Annotating Peaks in CID Tandem Mass Spectra of Peptides and N-Glycopeptides.* J. Proteome Res. 2021, 20, 9, 4603–4609.
([8]) Meghan C. Burke, et al., *The Hybrid Search: A Mass Spectral Library Search Method for Discovery of Modifications in Proteomics.* J. Proteome Res. 2017, 16, 5, 1924–1935.

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