Getting started with GlyCombo

Thank you for your interest in our software, GlyCombo, an open-source, freely available software tool designed to rapidly assign monosaccharide combinations to observed and fragmented precursors in an MS experiment.

Please find below a User Guide to help you understand our software and how to use it to accelerate your glycoscience research.

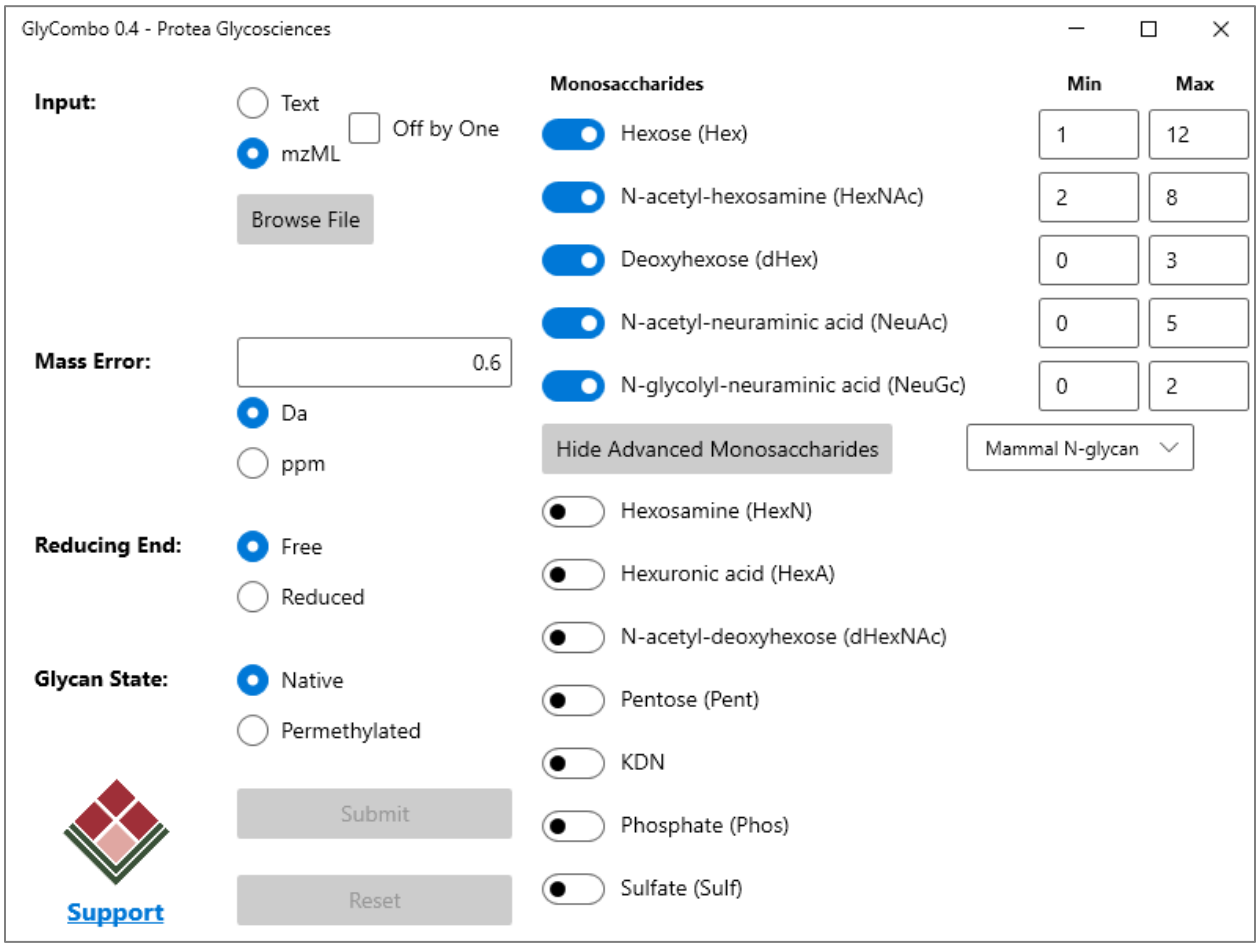
Prerequisites

- Raw files acquired from Thermo, Bruker, or Sciex mass spectrometers, that feature MS2 scans
    - This is an important aspect; we only assign compositions to MS2 scans.
- MSConvert to convert your raw files to mzML format
    - MSConvert is part of the Proteowizard software suite, please follow this link to install the software: https://proteowizard.sourceforge.io/download.html
- GlyCombo is downloaded and installed. It can be downloaded directly from the following link: https://github.com/Protea-Glycosciences/GlyCombo/releases/latest/download/GlyCombo_setup.exe
    - This installation self-updates when new updates are released.
    - If your institution's IT security policies prevent the installation of the software, we also provide a non-updating version at the following link: https://github.com/Protea-Glycosciences/GlyCombo/releases/latest/download/GlyCombo_Portable.exe

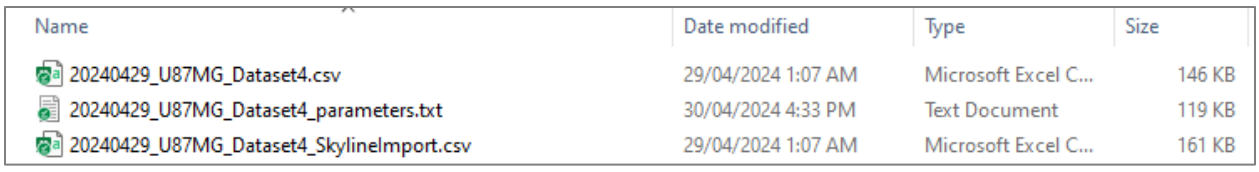
Procedure

1. Open GlyCombo
2. Choose your input and add it accordingly (Browse file if using mzML files, or add your neutral precursor masses to the text box for text)
    a. A summary of the mzML file/s should appear briefly after selecting them
3. Provide the run-specific options:
    a. Mass error (ppm if using Orbitrap or Da if using any other mass analyser)
    b. Reducing end format
    c. Glycan state
4. Define your monosaccharide search space
    a. We recommend starting with a preset and modifying according to your preferences

b. Use the toggle to enable the monosaccharide, and define the range with the Min and Max text boxes
c. Click the “Show Advanced Monosaccharides” button if your monosaccharide of interest is missing



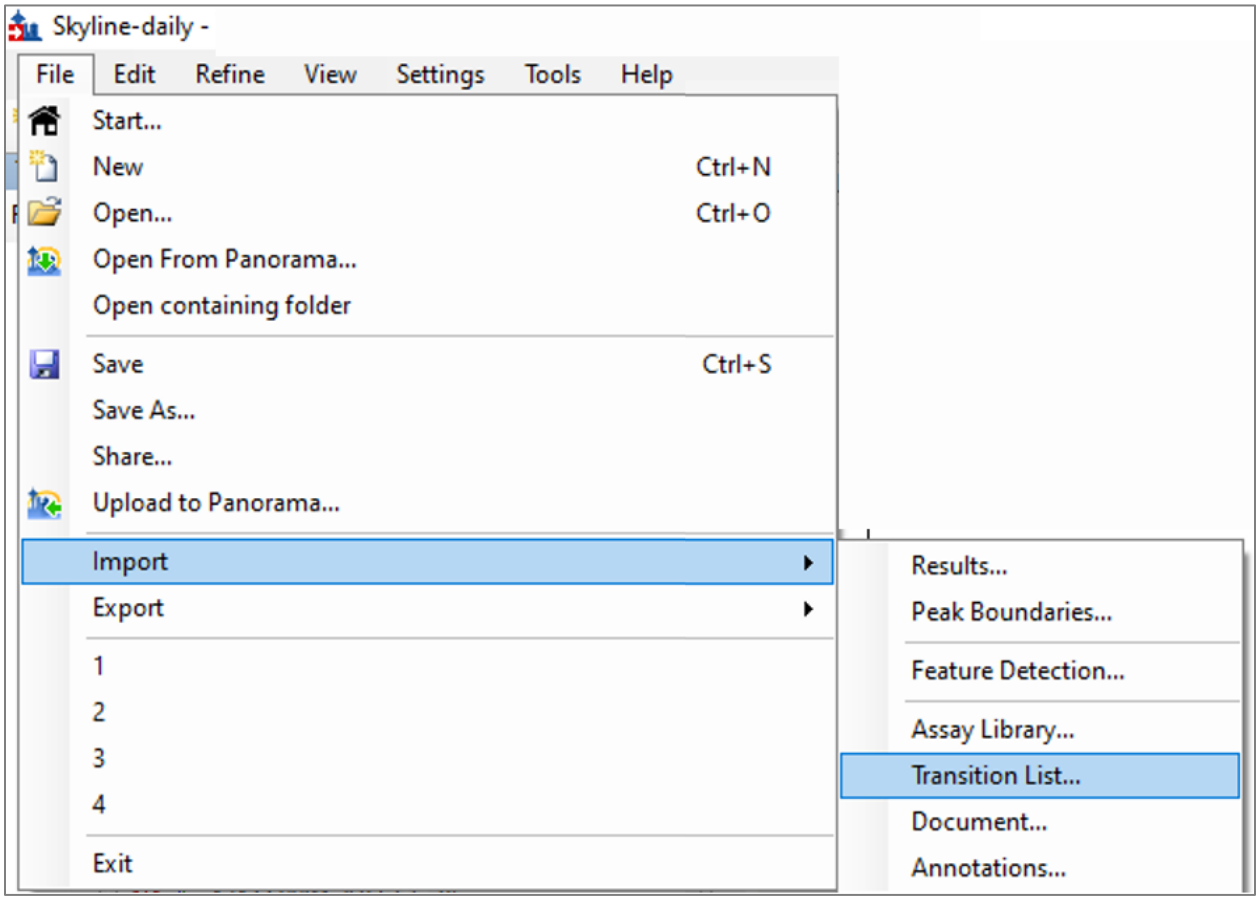
5. Click the Submit button
    a. This may take several seconds to process
    b. Once complete, pop-ups should appear for each mzML file providing information regarding number of combinations identified
6. Review the output
    a. Three files should be generated if using mzML files



b. The first output .csv should look like this, with one row per composition

Composition	Observed mass	Theoretical mass	Molecular Formula	Mass error	Scan number	Precursor Charge	Retention Time	TIC
(dHex)2 (Hex)2 (NeuG	2362.845556	2362.877962	C91H150N8O63	0.032406	164.11	-2	1.5705	371915.684
(Hex)3 (NeuAc)3 (Neu	2090.711588	2090.704357	C78H126N6O59	-0.007231	177.9	-2	2.229416667	61464.6534
(dHex)1 (Hex)2 (NeuAc	2090.711588	2090.704357	C78H126N6O59	-0.007231	177.9	-2	2.229416667	61464.6534

c. The file ending with the _SkylineImport.csv suffix can be directly imported into Skyline to define transitions as shown below. After this step, import your results and Skyline will automatically extract chromatograms for each composition identified by GlyCombo



7. Continue exploring the data in your preferred downstream applications
   a. GlyCombo identifies matching glycan compositions however it is up to the user to identify which is correct, and if desired, annotate MS2 spectra to identify the corresponding glycan structure
   b. Skyline serves as a great second filter for the glycans identified due to its ability to extract out the full expected isotopic envelope for the precursor and give it a corresponding score (idotp).