

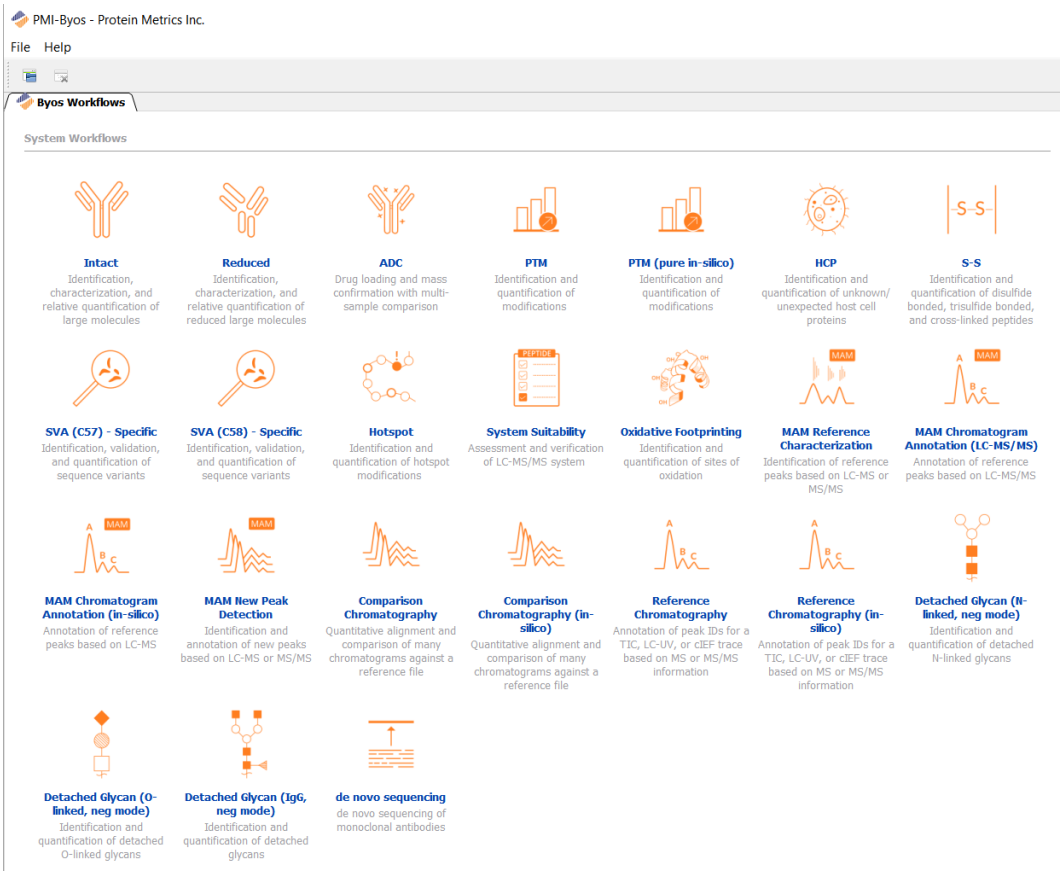
PMI Byos®

Quick Start Guide

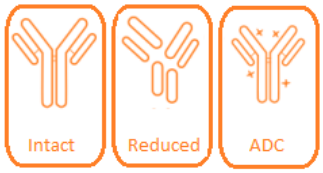
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3.9

Protein Metrics Inc., Cupertino, California, USA

Byos Quick Start Guide



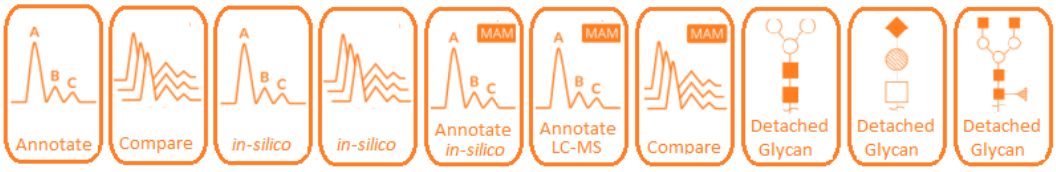
Intact Mass Analysis



Peptide Analysis



Chromatogram Analysis



Byos® workflows are based on the legacy products MS/MS Analysis (Byonic™), Peptide Analysis (Byologic®), Chromatogram Analysis (Byomap™), Intact Analysis (Intact Mass™), and de novo Sequencing (Supernovo™)

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Launching a Byos Workflow to Generate an Analysis and Report

The user may select any default workflow from the System Workflows section or customized workflow from the My Workflows section. Default parameters for each workflow, including a report template, have been set in the **Sequences and masses** (Intact, Reduced, and ADC workflows) and Processing Nodes (all workflows) tabs.

The user just needs to drag and drop their sample data file(s) and a FASTA file and click Create Project. A completed analysis and optimized report will then be available.

This Quick Start Guide has five sections:

1. Intact Analysis: Intact, Reduced, and ADC workflows
2. Peptide Analysis: HCP, HotSpot, PTM, PTM (in-silico), SVA (C57)[1], SVA (C58)[1], S-S, MAM Reference Characterization, System Suitability, and Oxidative Footprinting workflows
3. Chromatogram Analysis: Reference Chromatography, Characterization Chromatography, Reference Chromatography (in-silico) and Characterization Chromatography (in-silico), MAM Chromatogram Annotation (LC-MS), MAM Chromatogram Annotation (in-silico), and MAM New Peak Detection workflows
4. Detached Glycan Analysis: Detached Glycan (N-linked), Detached Glycan (O-linked), and Detached Glycan (IgG) workflows
5. De novo Sequencing Analysis

[1] Specific to the alkylant (iodoacetamide or iodoacetic acid)

Intact Analysis

Intact, Reduced, and ADC

These Intact Analysis workflows all have four separate tabs: **Samples**, **Sequences and masses**, **Sample-protein input**, and **Processing nodes**.

a. Click to launch any of the mentioned workflows. In Figure 1, the Intact workflow is selected.

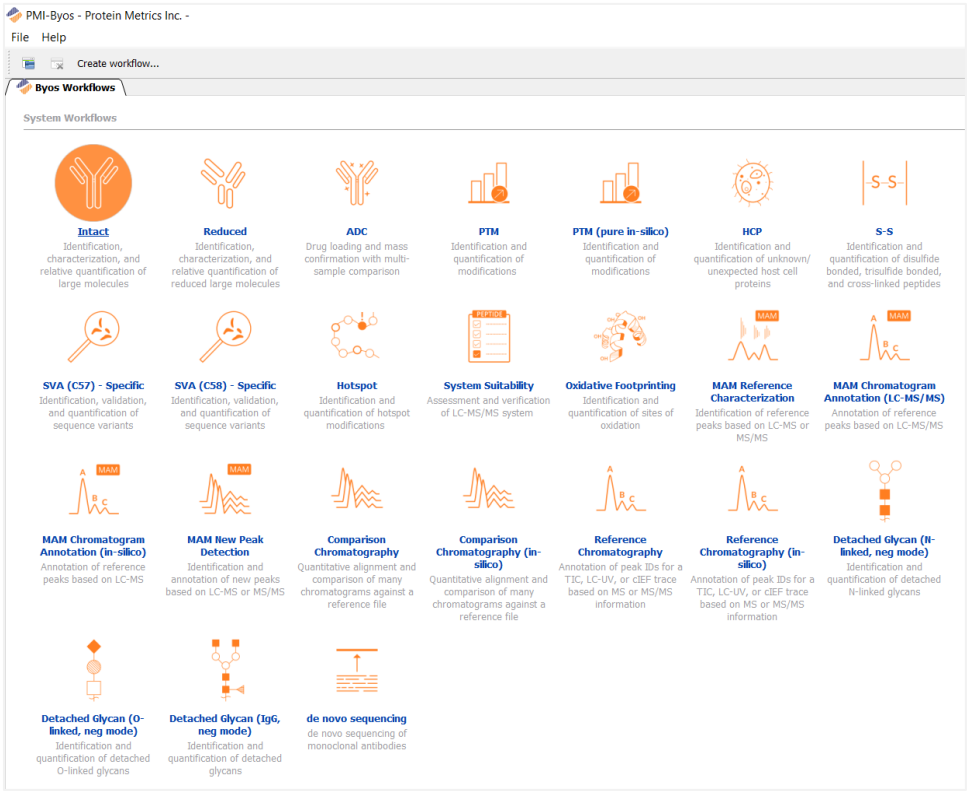


Figure 1: Byos System Workflows. These are the default workflows included with installation.

b. Drag and drop the sample data file(s) to be processed into the **Samples** tab, as shown in Figure 2. These can be from any vendor.

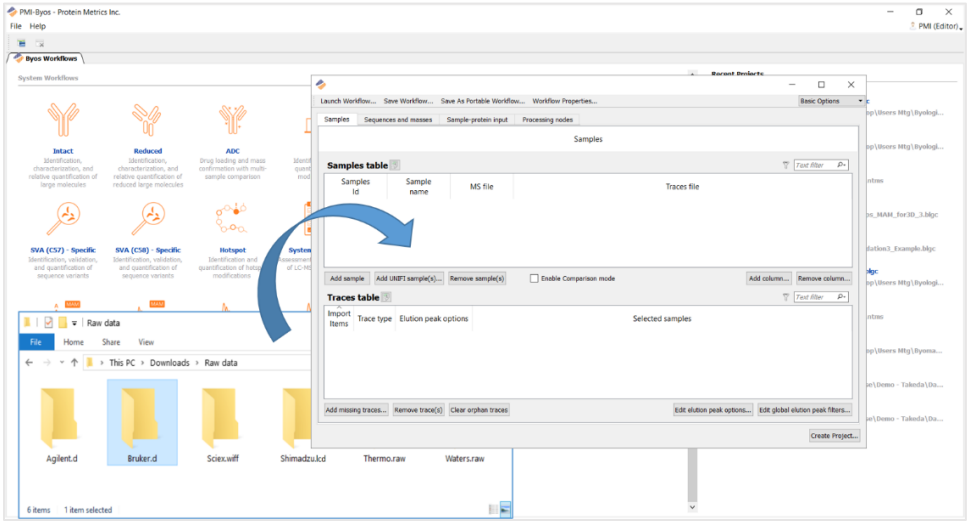


Figure 2: Drag and drop sample data file(s) to be processed into the **Samples** tab.

c. Drag and drop the FASTA file to be processed into the **Sequences and masses** tab.

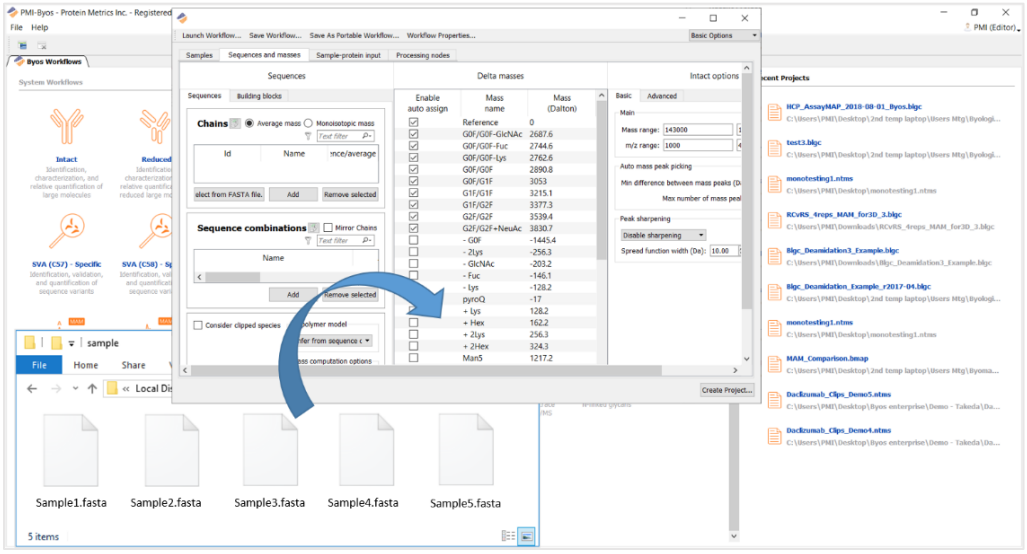


Figure 3: Drag and drop FASTA file to be processed into the **Sequences and masses** tab.

d. Click **Create Project** to start processing and to generate the report.

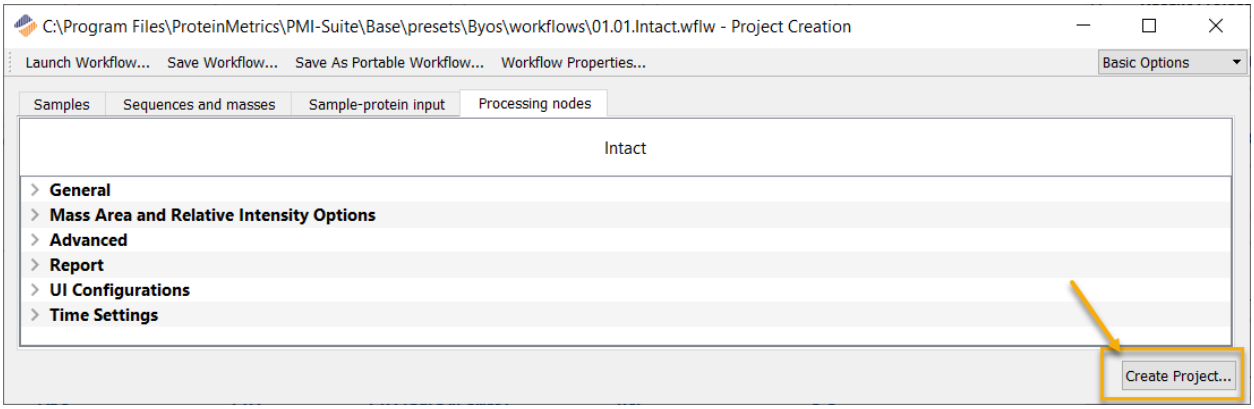


Figure 4: Click **Create Project**.

e. The user will be prompted to name and save the project file. Please note Byos will save this type of project as a *.ntms file.

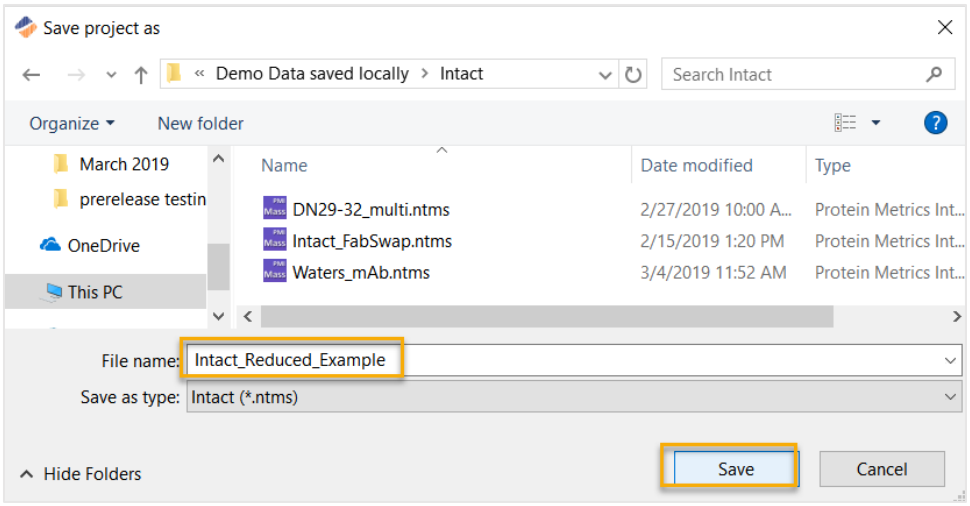


Figure 5: Name the project and save in a convenient location.

f. Processing will begin. Once completed, the project and associated report will appear as additional tabs after the **Byos Workflows** tab, as shown in Figure 6.

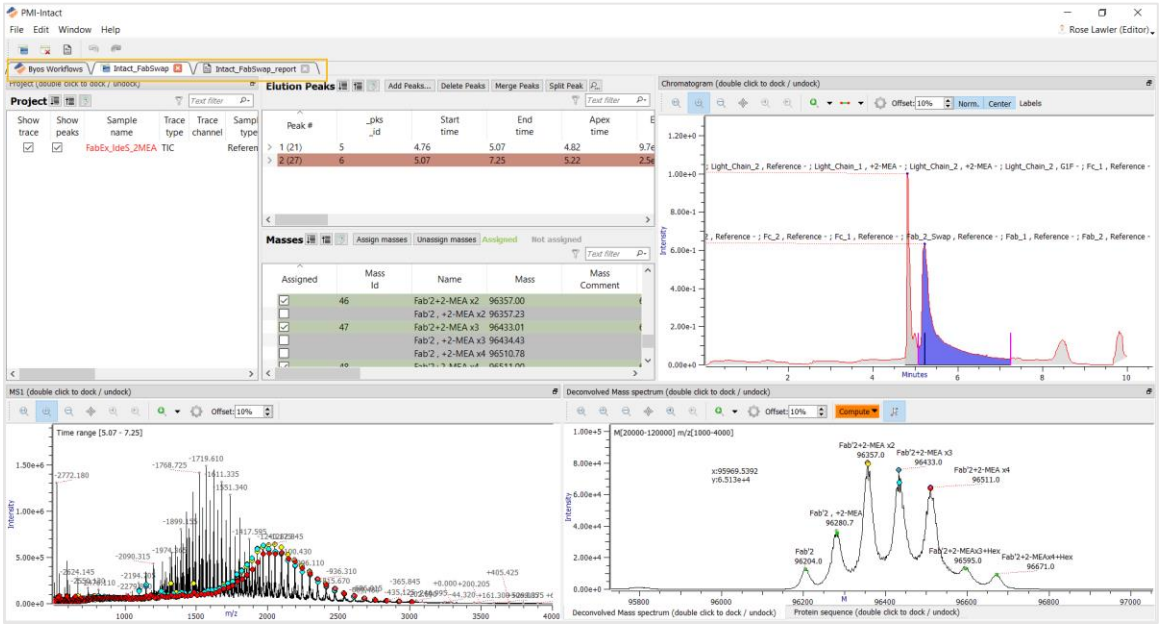


Figure 6: **Byos Workflows** tab, completed project, and associated report are available as separate tabs.

Peptide Analysis

HCP, HotSpot, PTM, SVA (C57) – Specific, SVA (C58) – Specific, S-S, MAM Reference Characterization, System Suitability, Oxidative Footprinting, and PTM (in-silico)

These Peptide Analysis workflows all have three separate tabs: **Samples**, **Sequences**, and **Processing nodes**.

NOTE: Considerations specific to the HCP and System Suitability workflows in the **Sequences** tab are called out.

a. Click to launch any of the mentioned workflows. In Figure 7, the Post-translational Modifications (PTM) workflow is selected.

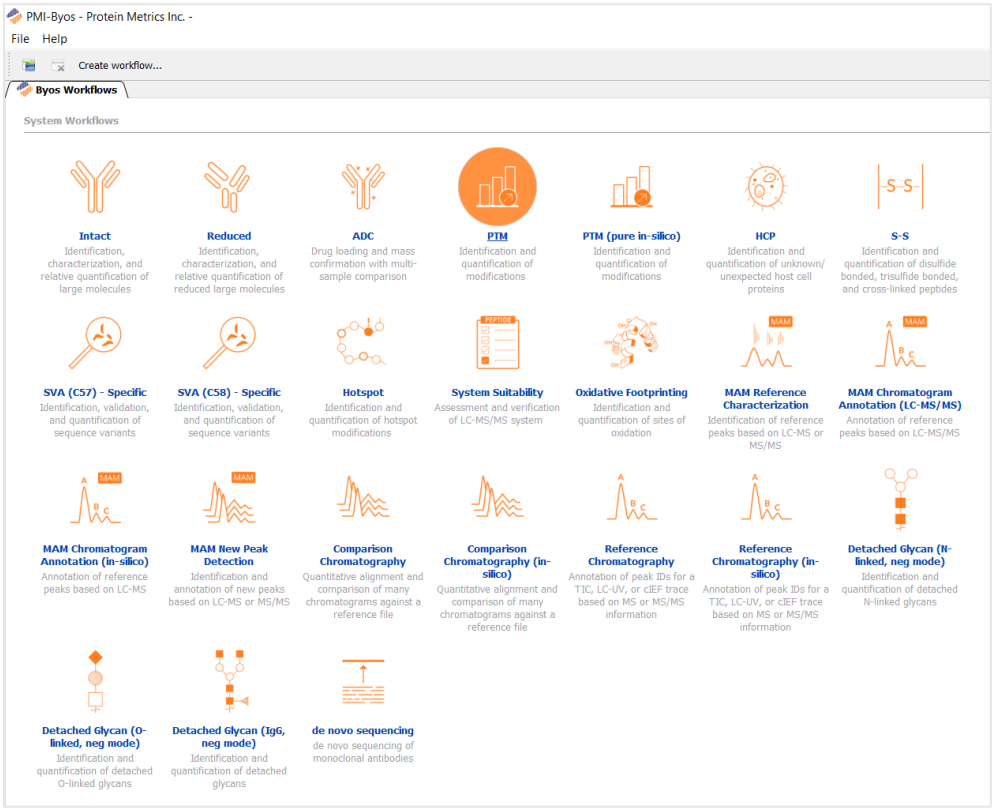


Figure 7: Byos System Workflows with PTM selected.

b. Drag and drop the sample data file(s) to be processed into the **Samples** tab, as shown in Figure 8. These can be from any vendor.

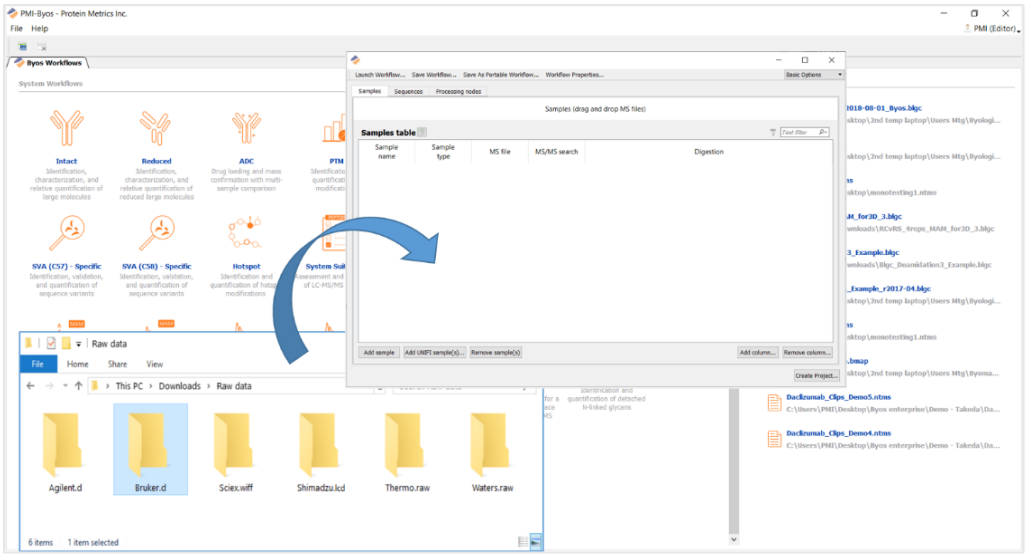


Figure 8: Drag and drop sample data file(s) to be processed into the **Samples** tab.

c. Drag and drop the FASTA file to be processed into the **Sequences** tab.

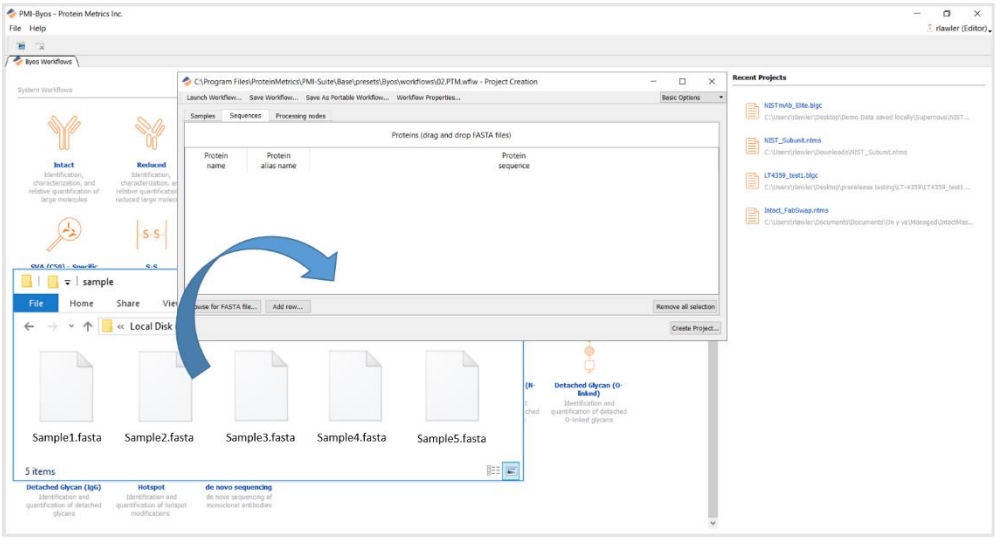


Figure 9: Drag and drop FASTA file to be processed into the **Sequences** tab.

NOTE: In the HCP workflow, the user must instead point to a protein database. The user can either drag and drop as shown in Figure 10 or direct Byos to a database file. This is done by clicking in the space to activate the light blue "…" box. The user is then prompted to select a file as shown in Figure 10.

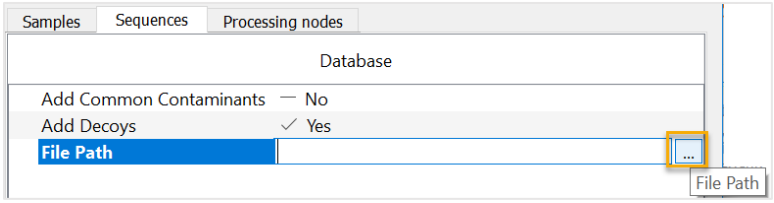


Figure 10: Click to activate the file path prompt.

NOTE: In the System Suitability workflow, the **Sequences** tab is populated by default to include the Pierce RT standard. The user can replace this with another standard if desired. The user can

change this by clicking to highlight the line and clicking **Remove all selection** (shown in Figure 11). Another protein could be brought in using drag and drop (shown in Figure 9).

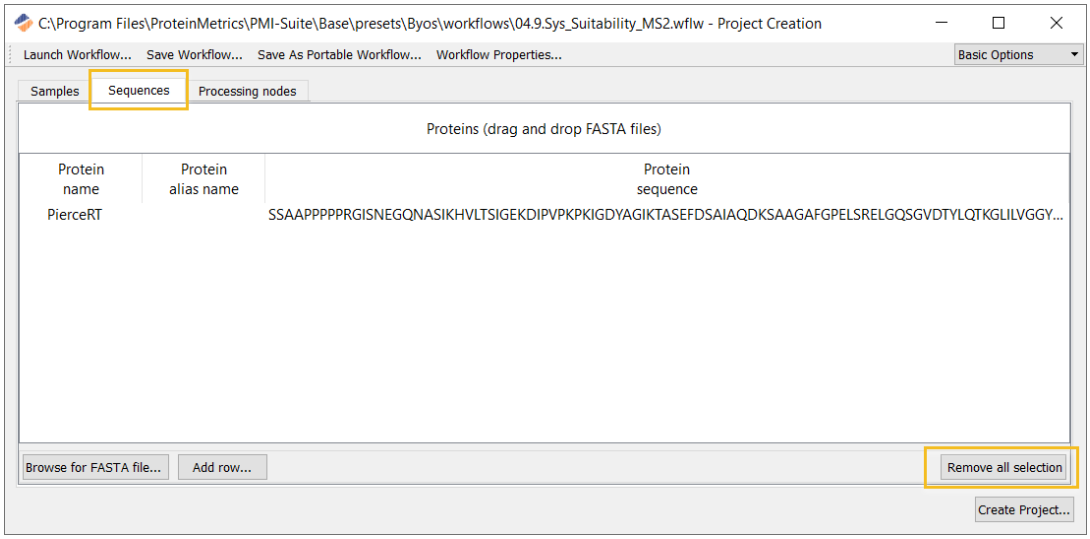


Figure 11: The Pierce RT standard is included by default in the System Suitability workflow.

d. Click **Create Project** to start processing and to generate the report.

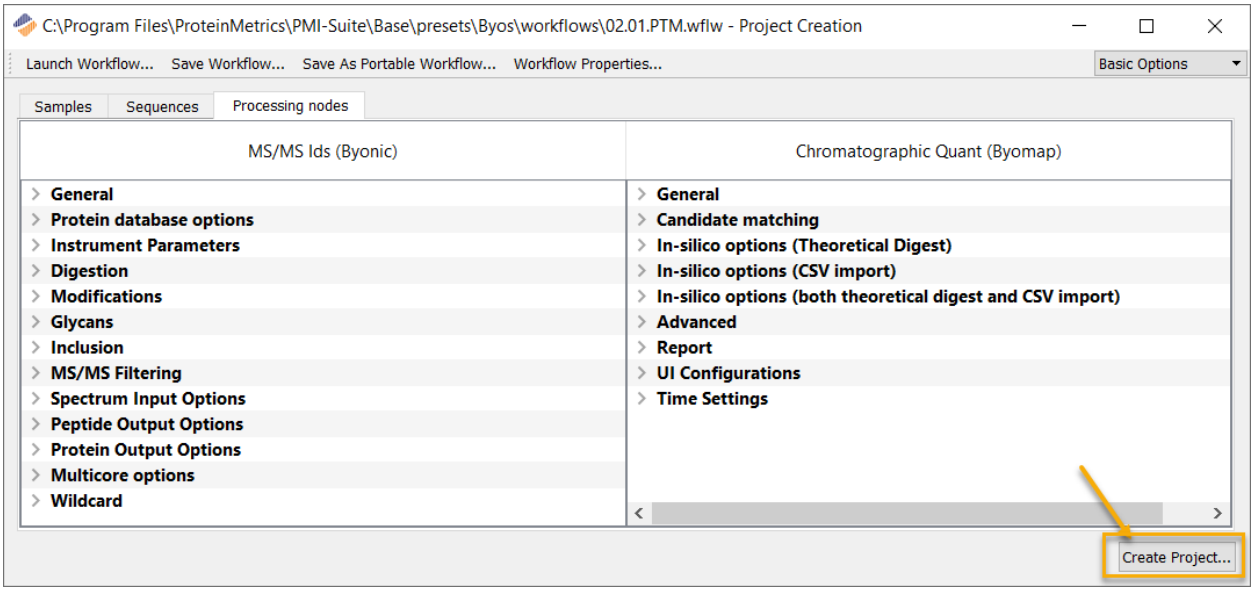


Figure 12: Click **Create Project**.

e. The user will be prompted to name and save the project file. Please note Byos will save this type of project as a *.blgc file.

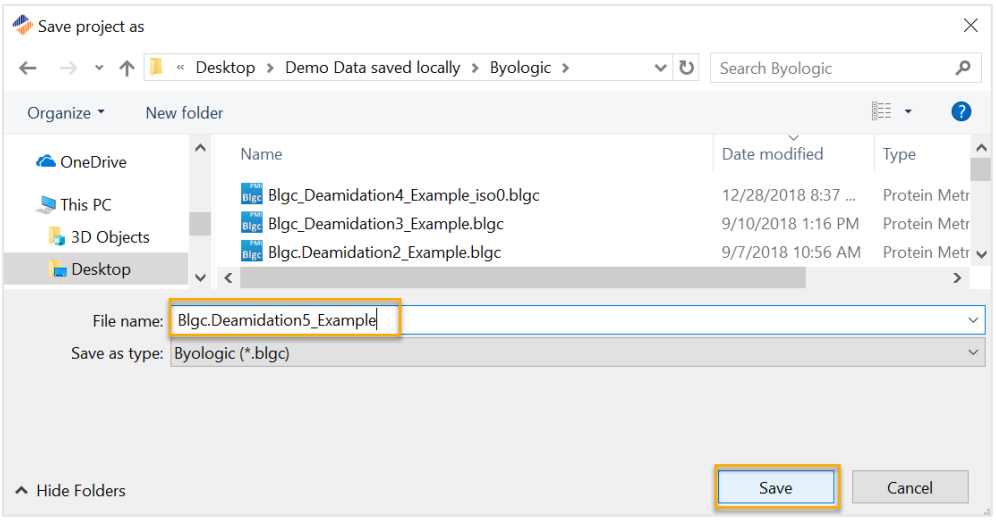


Figure 13: Name the project and save in a convenient location.

f. Processing will begin. Once complete, the project and associated report will appear as additional tabs after the **Byos Workflows** tab, as shown in Figure 14.

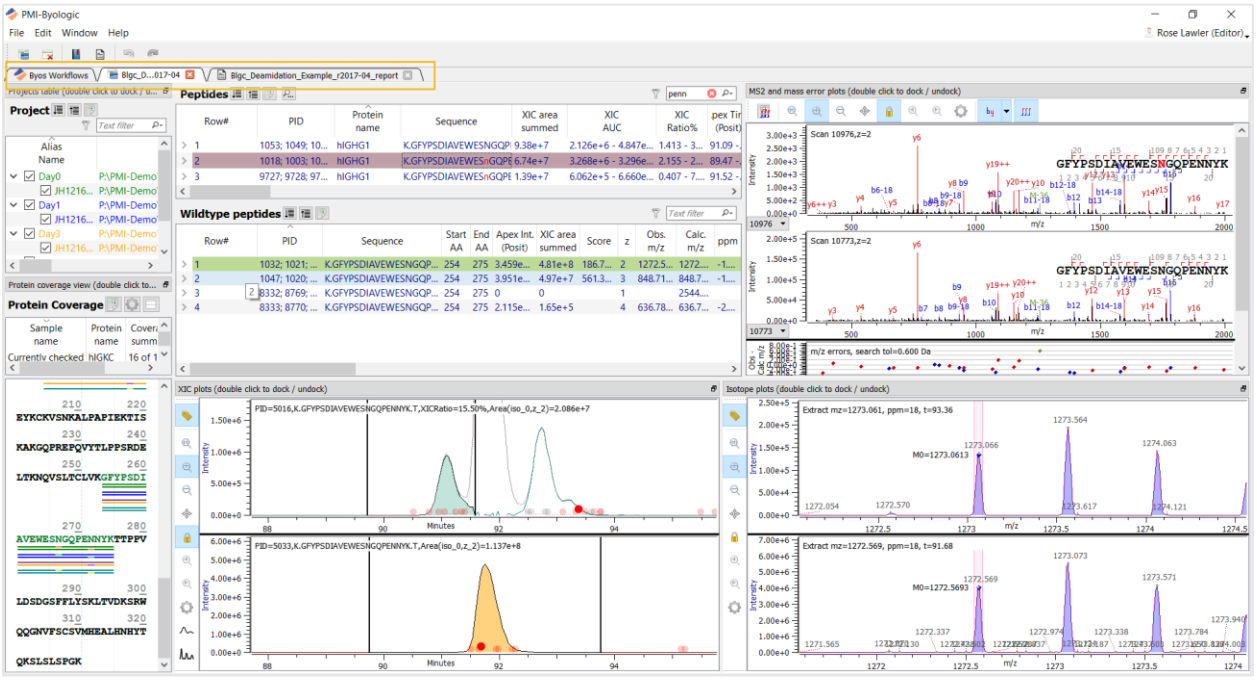


Figure 14: **Byos Workflows** tab, completed project, and associated report are available as separate tabs.

Chromatogram Analysis

Reference Chromatogrpahy, Characterization Chromatography, MAM Chromatogram Annotation (LC-MS), Reference Chromatogrpahy (in-silico), Characterization Chromatography (in-silico), MAM Chromatogram Annotation (in-silico), and MAM New Peak Detection

These Chromatogram Analysis workflows all have three separate tabs: **Samples**, **Sequences**, and **Processing nodes**.

a. Click to launch any of the above mentioned workflows. In Figure 15, the Reference Chromatogrpahy workflow is selected.

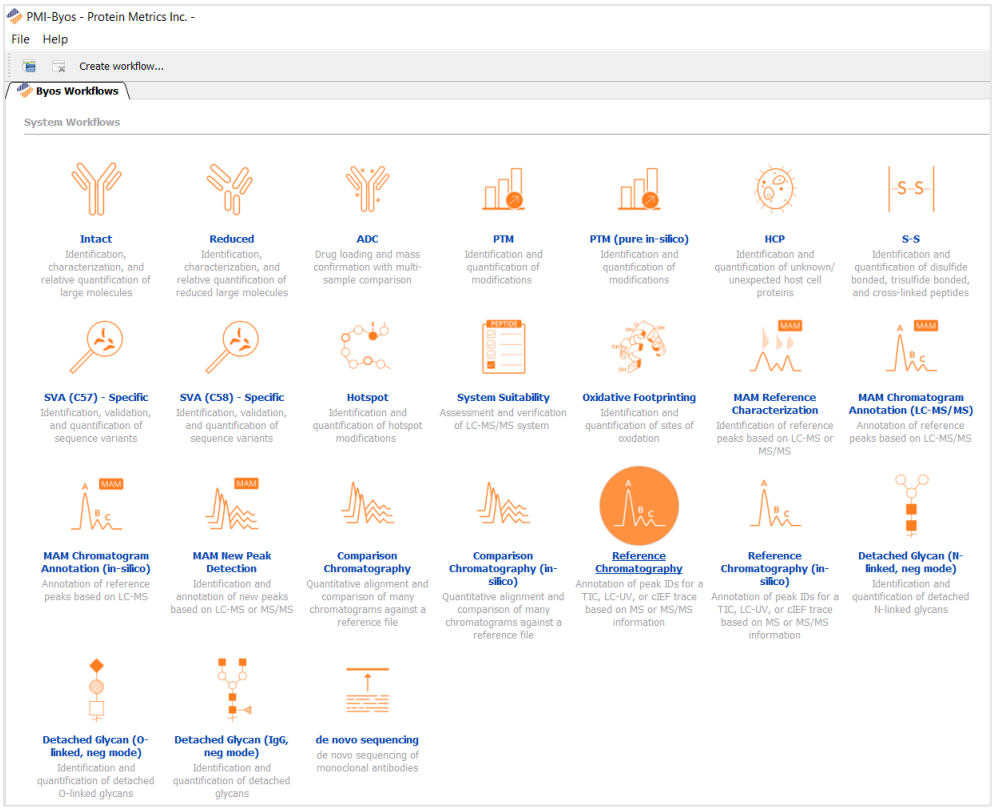


Figure 15: Byos System Workflows with Reference Chromatography selected.

b. Drag and drop the sample file(s) to be processed into the **Samples** tab, as shown in Figure 16. These can be from any vendor.

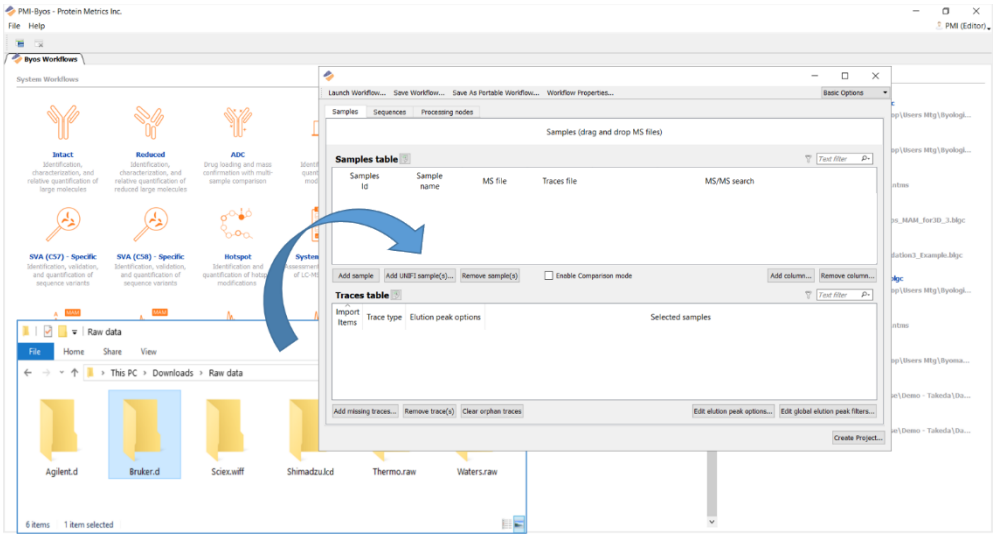


Figure 16: Drag and drop sample data files of interest into the **Samples** tab.

c. The user can load a Traces file and an MS/MS search file. To load these files, click within the box to activate the light blue "…" box, as shown in Figure 17.

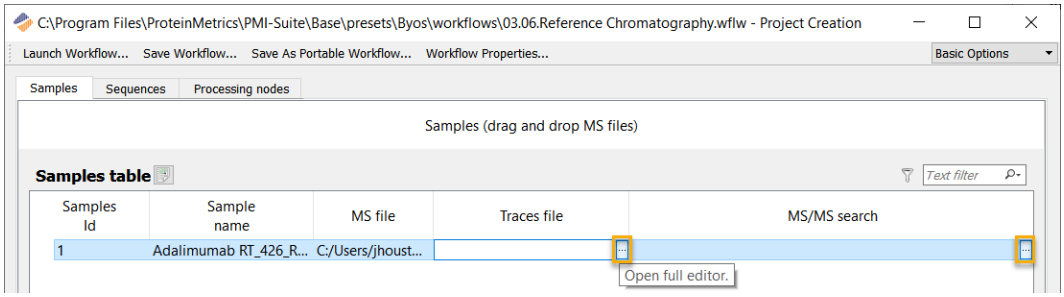


Figure 17: Click to open file editors to load a trace file or an MS/MS search file (*.byrslt).

d. The user can associate traces with samples. To associate traces, click within the box to activate the light blue "…" box, as shown in Figure 18.

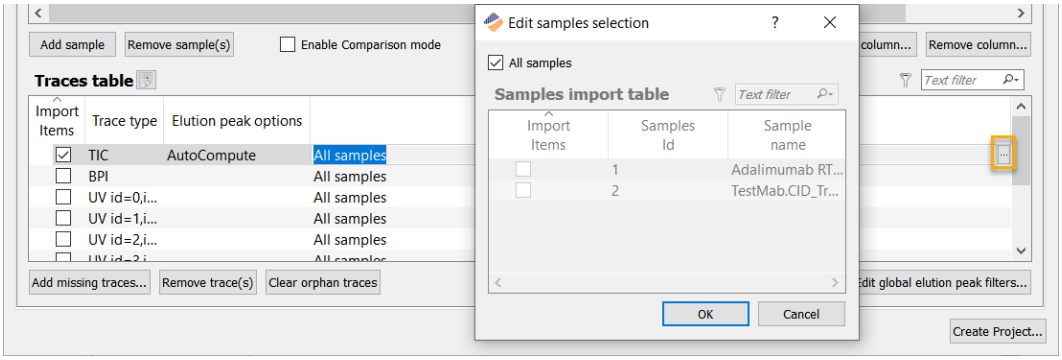


Figure 18: Associate traces with samples. Traces are assigned to all samples by default.

e. Drag and drop the FASTA file to be processed into the **Sequences** tab.

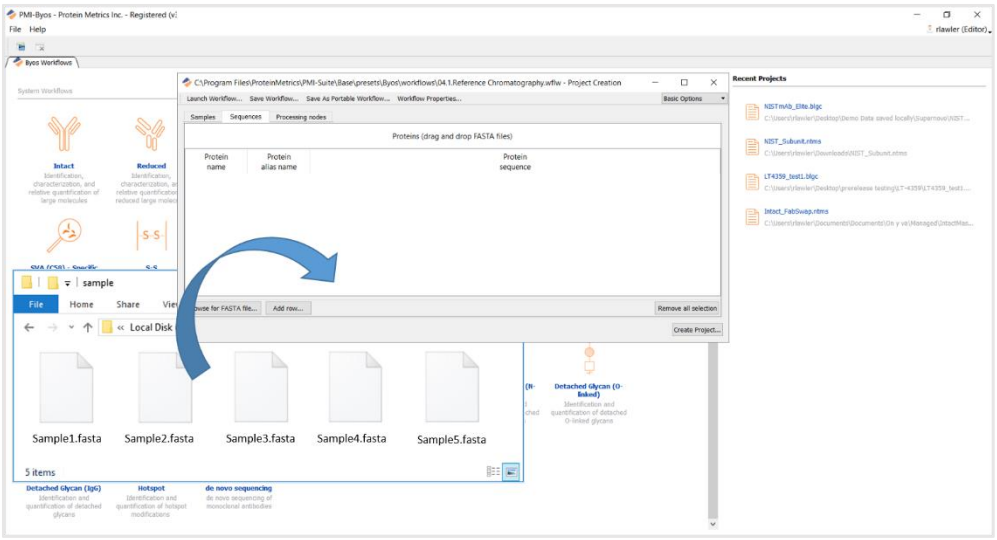


Figure 19: Drag and drop FASTA file to be processed into the **Sequences** tab.

f. Click **Create Project** to start analysis and report generation. Please note Byos will save this type of project as a *.bmap file.

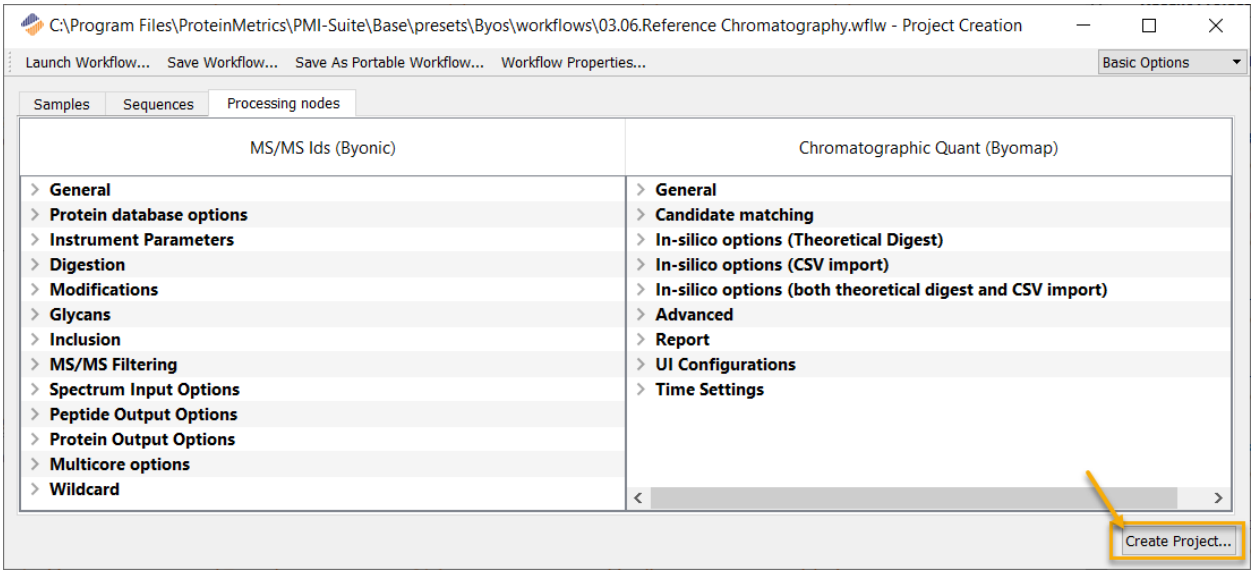


Figure 20: Click **Create Project**.

g. The user will be prompted to name and save the analysis file.

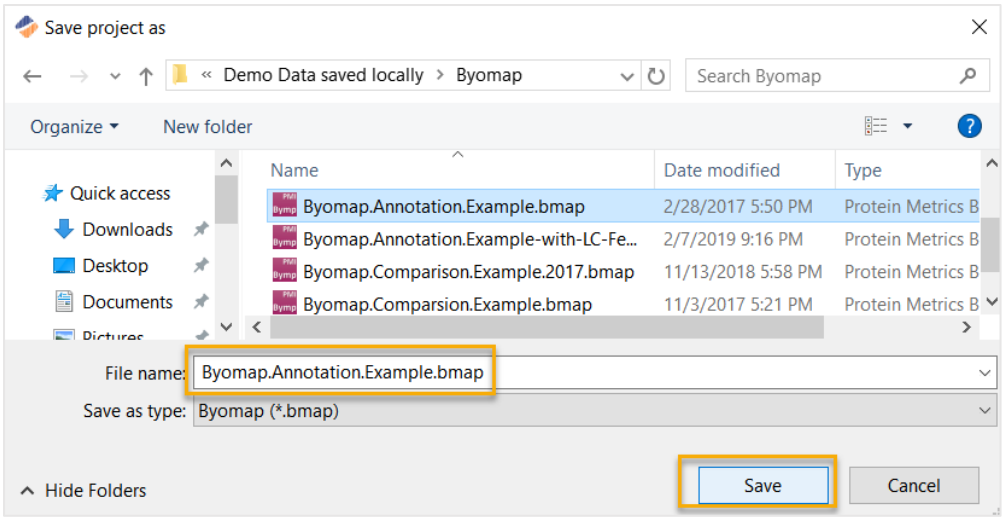


Figure 21: Name the project and save in a convenient location.

h. Processing will begin. Once complete, the project and associated report will appear as additional tabs after the **Byos Workflows** tab, as shown in Figure 22.

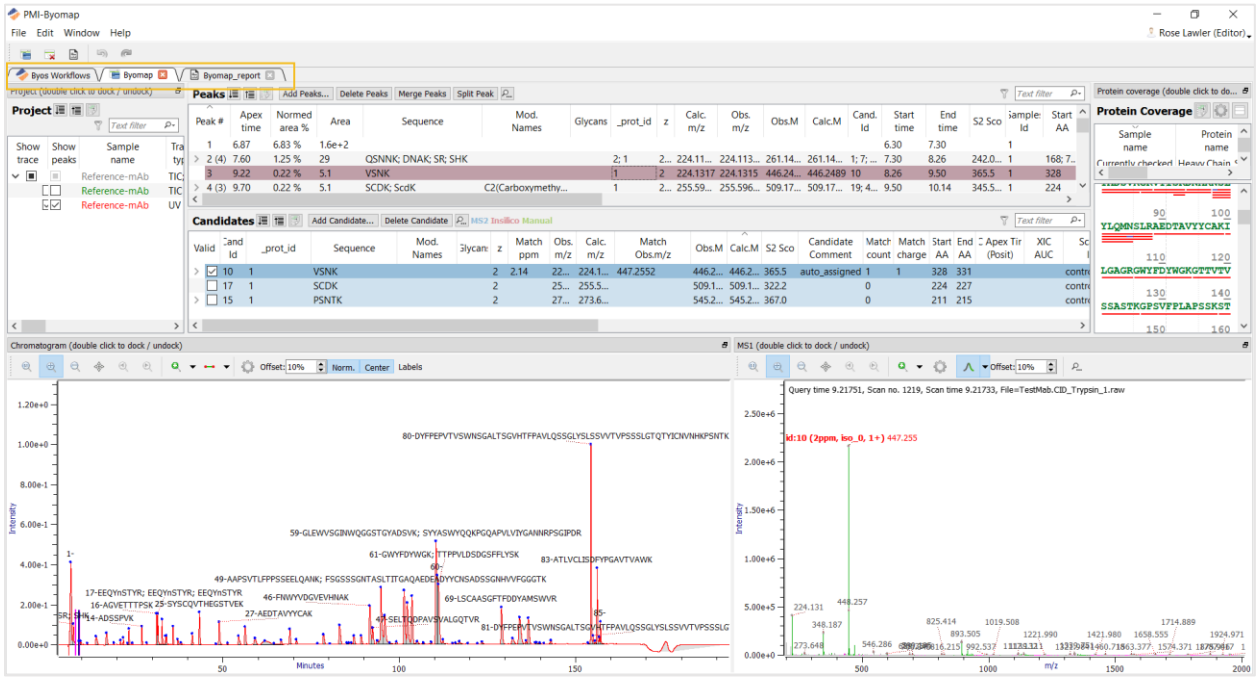


Figure 22: **Byos Workflows** tab, completed project, and associated report are available as separate tabs.

Detached Glycan Analysis

Detached Glycan (N-linked), Detached Glycan (O-linked), and Detached Glycan (IgG)

These Detached Glycan workflows all have four separate tabs: **Samples**, **Glycan table**, **Glycan options**, and **Processing nodes**. Please note the default setting for these analyses are set to process in **negative mode**.

a. Click to launch any of the above mentioned workflows. In Figure 23, the Detached Glycan (N-linked, neg mode) workflow is selected.

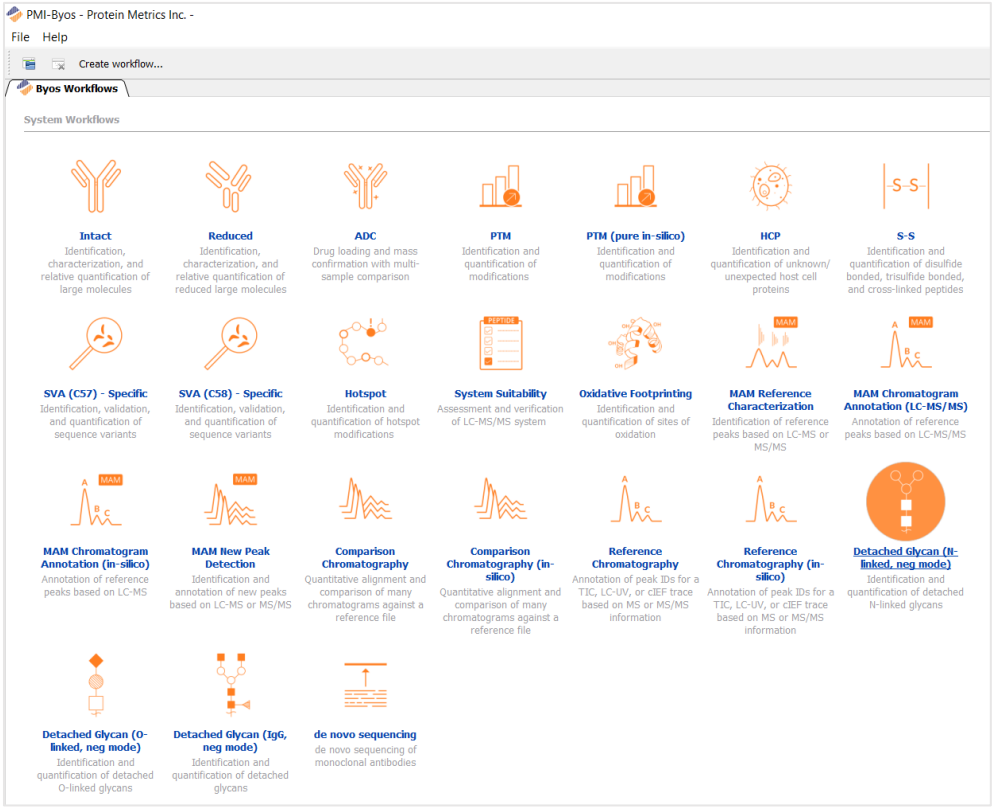


Figure 23: Byos System Workflows with Detached Glycan (N-linked, neg mode) selected.

b. Drag and drop the sample file(s) to be processed into the **Samples** tab, as shown in Figure 24. These can be from any vendor.

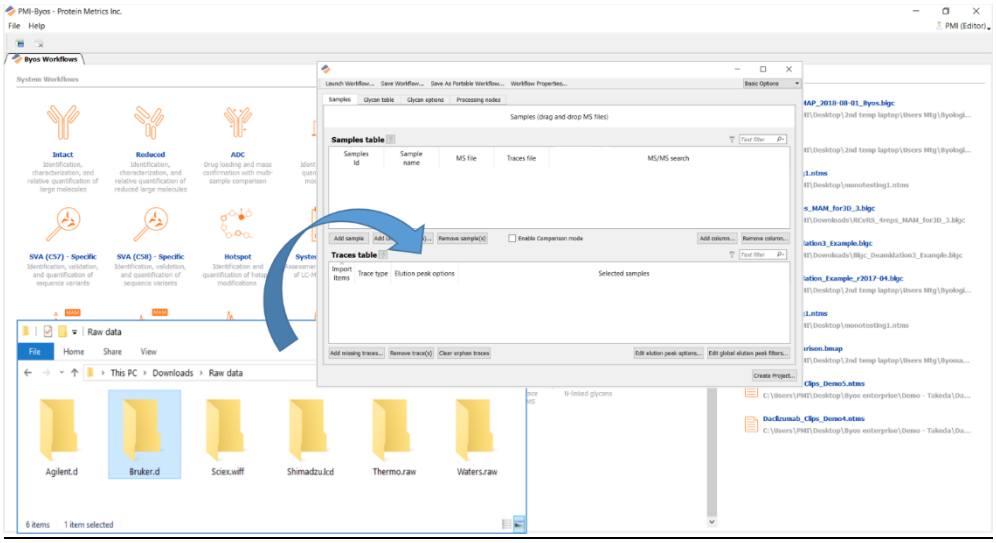


Figure 24: Drag and drop sample data files to be processed into the **Samples** tab.

c. The user can load a Traces file and an MS/MS search file. To load these files, click within the box to activate the light blue "…" box, as shown in Figure 25.

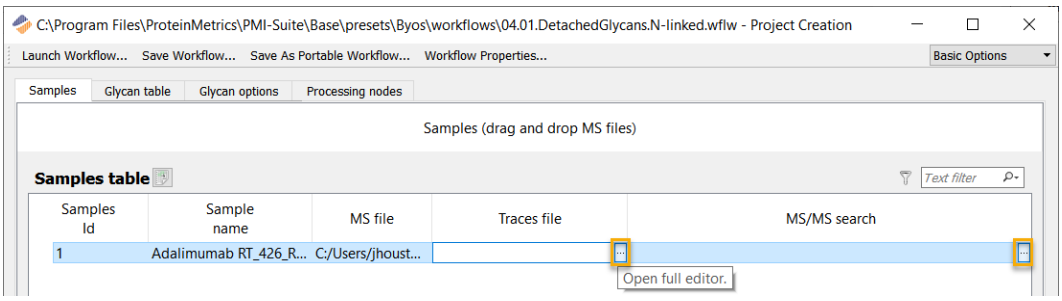


Figure 25: Click to activate the traces editor in TracesCsv or TracesMs.

d. The user can associate traces with samples. To associate traces, click within the box to activate the light blue "…" box, as shown in Figure 26.

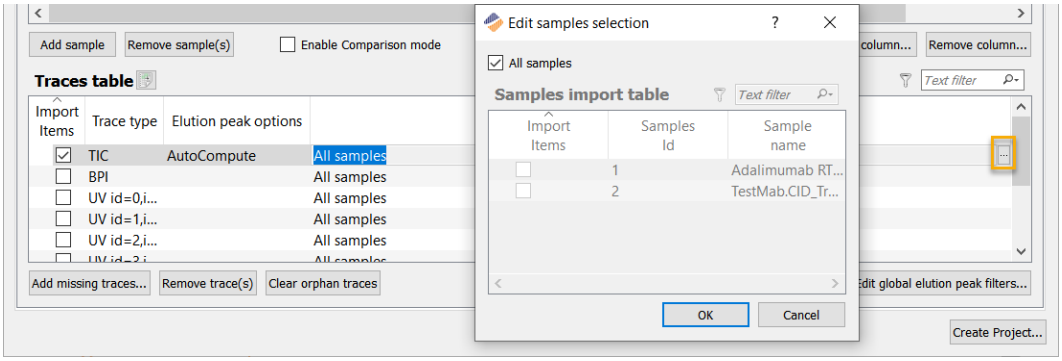


Figure 26: Associate traces with samples. Traces are assigned to all samples by default.

e. Each default workflow includes a table of common glycans. The user can choose to use the default list, add to it, or replace it entirely with a customized list with the **Add from CSV file** button.

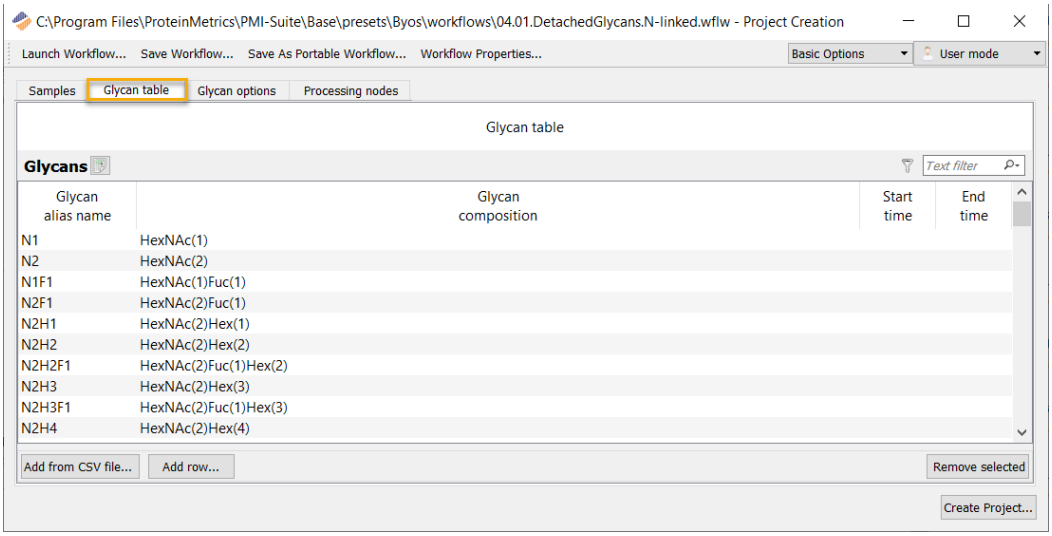


Figure 27: **Glycan table** tab..

f. If using a fluorescent tag, the user can set this in the **Glycan options** tab. Several commonly used tags are included by default; if an alternative mass is preferred, please use the **Custom** radio button. The user can also add adduct masses to be considered in calculations using the **Add Adduct** button.

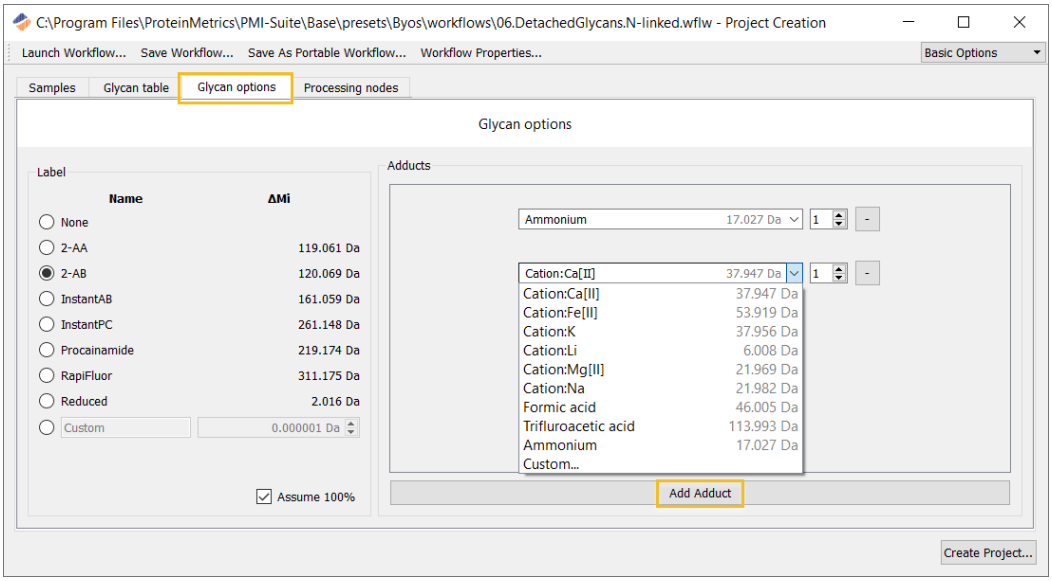


Figure 28: **Glycan options** tab. Set the label, include adducts using the **Add Adduct** button.

g. Click **Create Project** to start analysis and report generation. Please note Byos will save this type of project as a *.bmap file.

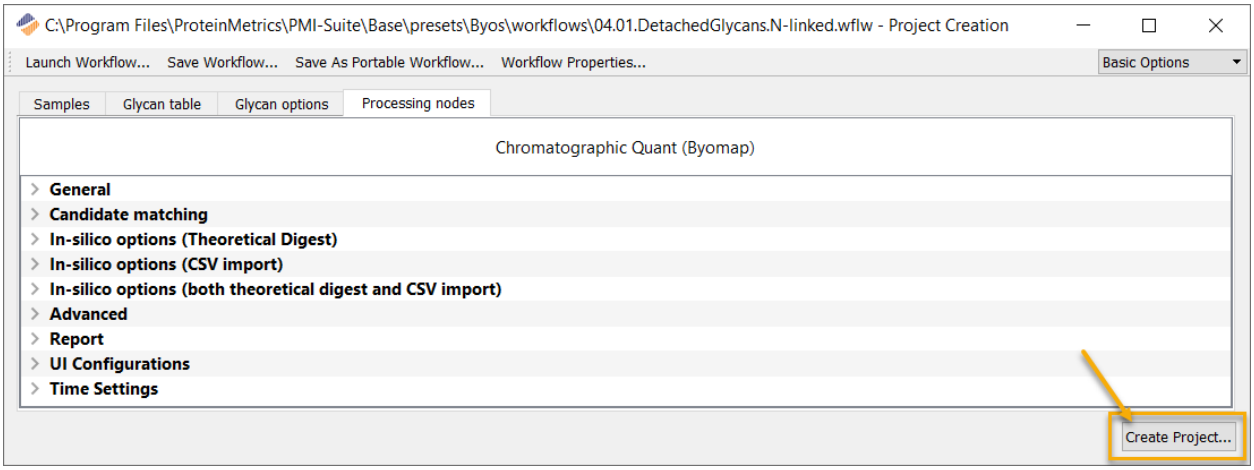


Figure 29: Click **Create Project**.

h. The user will be prompted to name and save the analysis file.

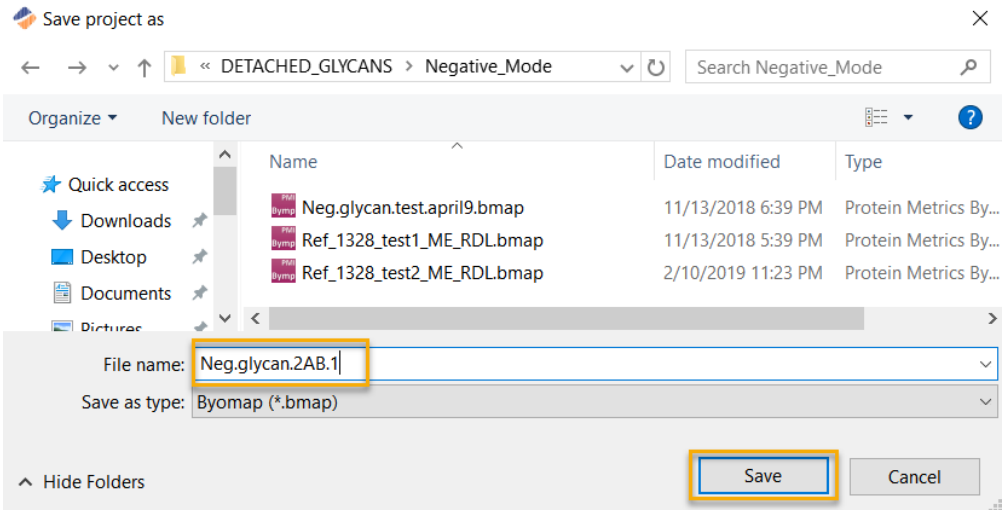


Figure 30: Name the project and save in a convenient location.

i. Processing will begin. Once complete, the project and associated report will appear as additional tabs after the **Byos Workflows** tab, as shown in Figure 31.

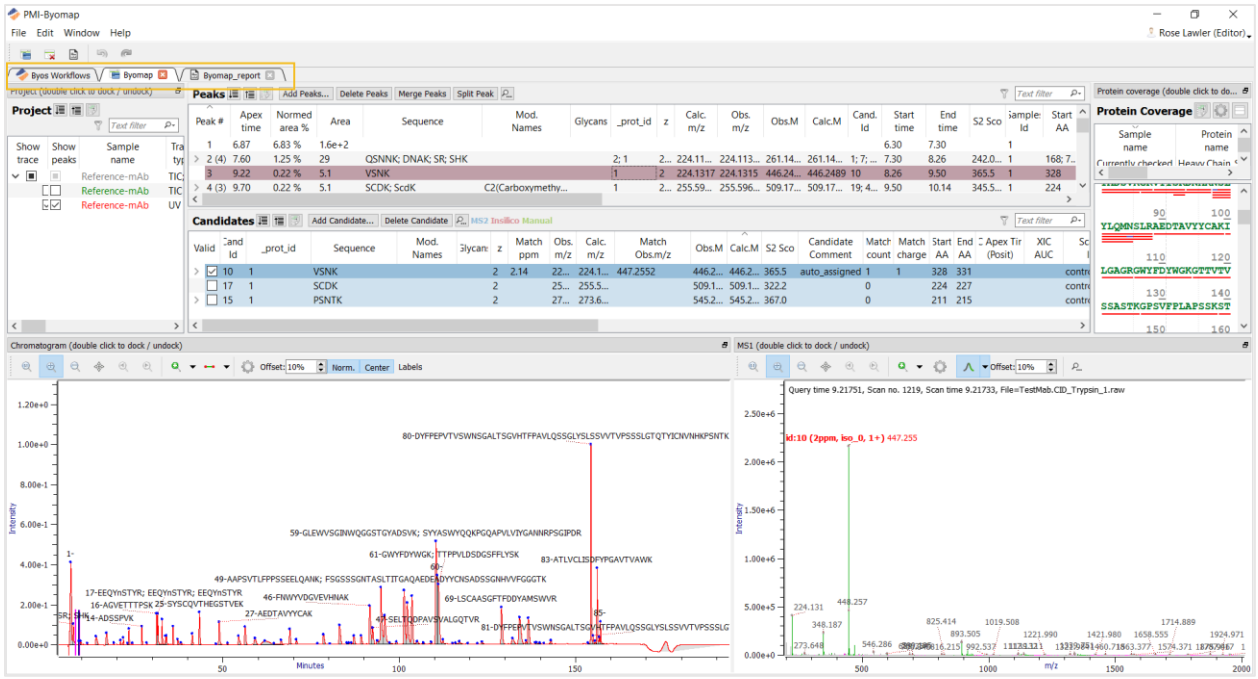


Figure 31: **Byos Workflow** tab, completed project, and associated report are available as separate tabs.

De novo Sequencing Analysis

The de novo sequencing workflow has the **Samples** and **Processing nodes** tabs.

a. Click to launch the de novo sequencing workflow.

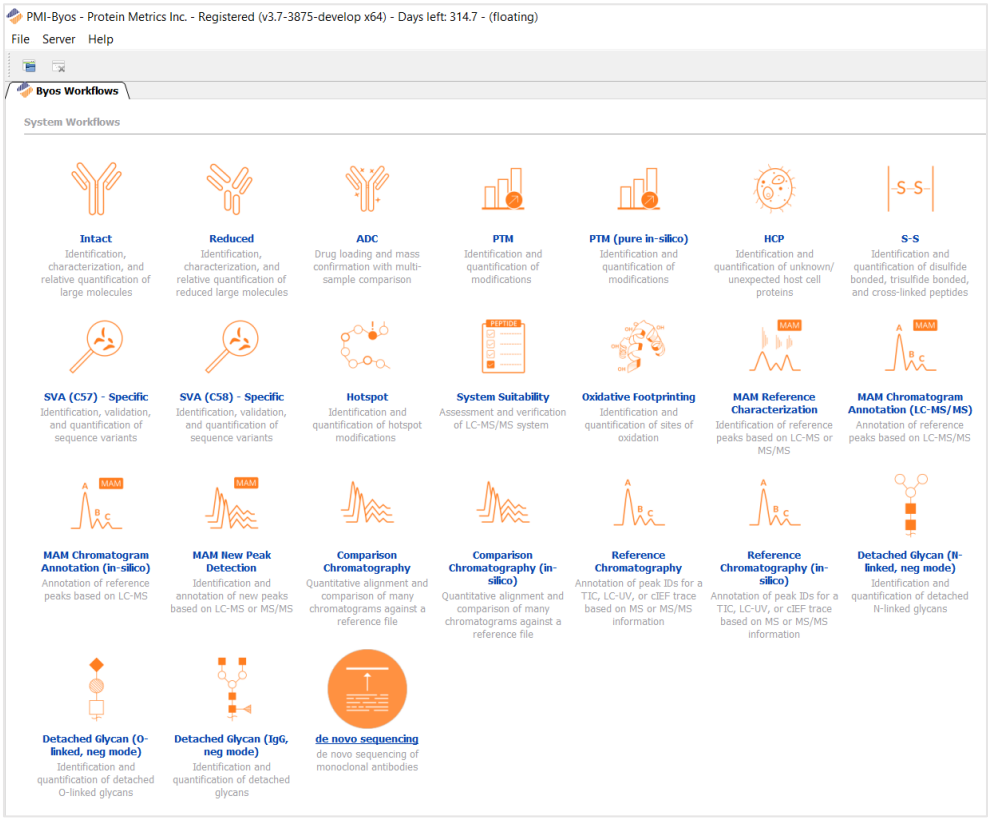


Figure 32: Byos System Workflows with de novo sequencing selected.

b. Drag and drop the sample data file(s) to be processed into the **Samples** tab, as shown in Figure 33. These can be from any vendor.

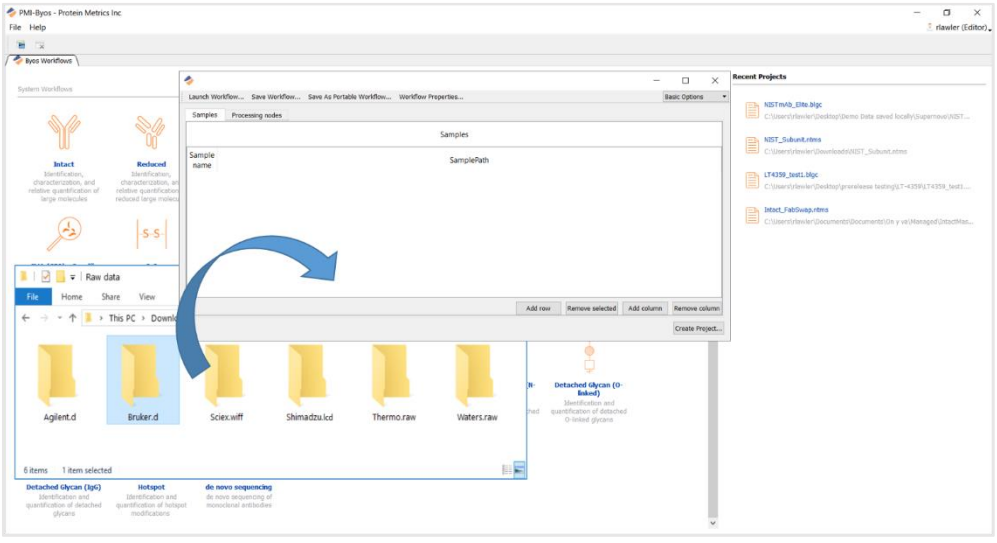


Figure 33: Drag and drop sample data files of interest into the **Samples** tab.

c. [OPTIONAL] Specify an alternative Byonic parameter file (*.byparms) by clicking within the box to activate the light blue "…" box, as detailed in Figure 34.

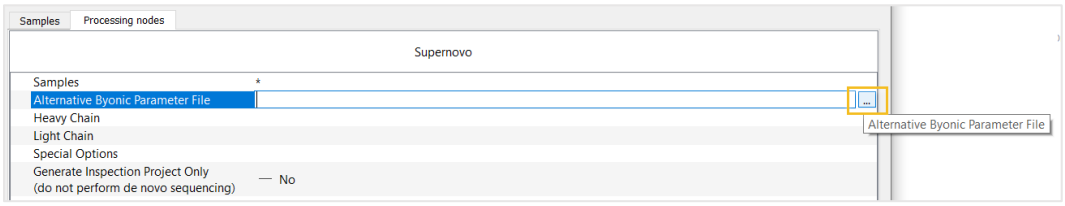


Figure 34: Click to activate the Alternative Byonic Parameter File and select the *.byparms file to be applied during processing.

d. [OPTIONAL] If a starting sequence template is to be used, paste the **Heavy Chain** and **Light Chain** sequences.

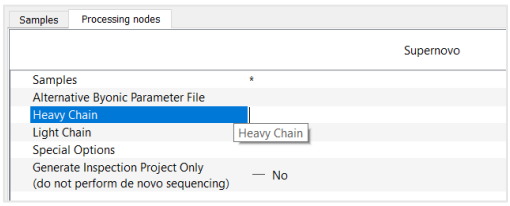


Figure 35: The sequences for the **Heavy Chain** and **Light Chain** can be pasted in as text.

e. Click **Create Project** to start analysis and report generation. Please note Byos will save this type of project as a *.blgc file.

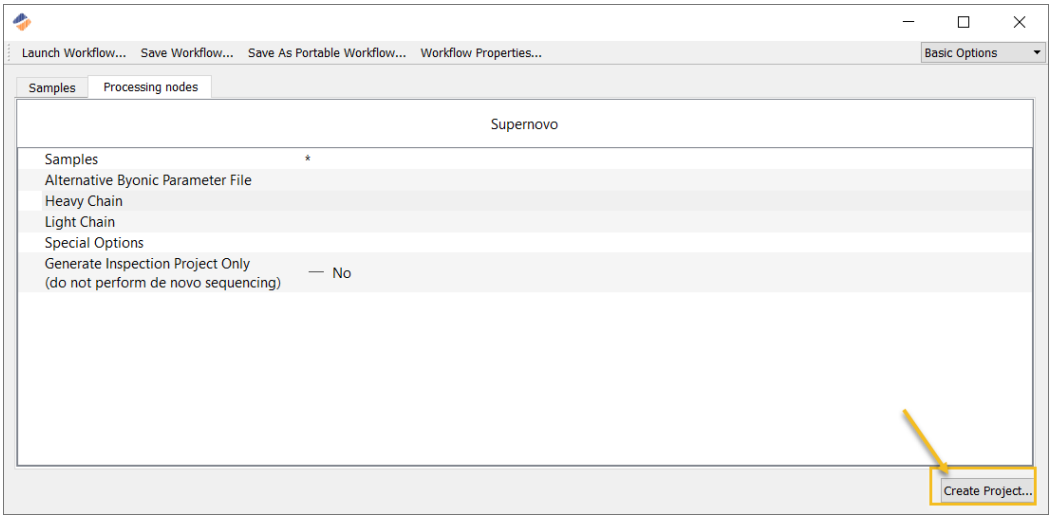


Figure 36: Click **Create Project**.

f. Name the project and save in a convenient location.

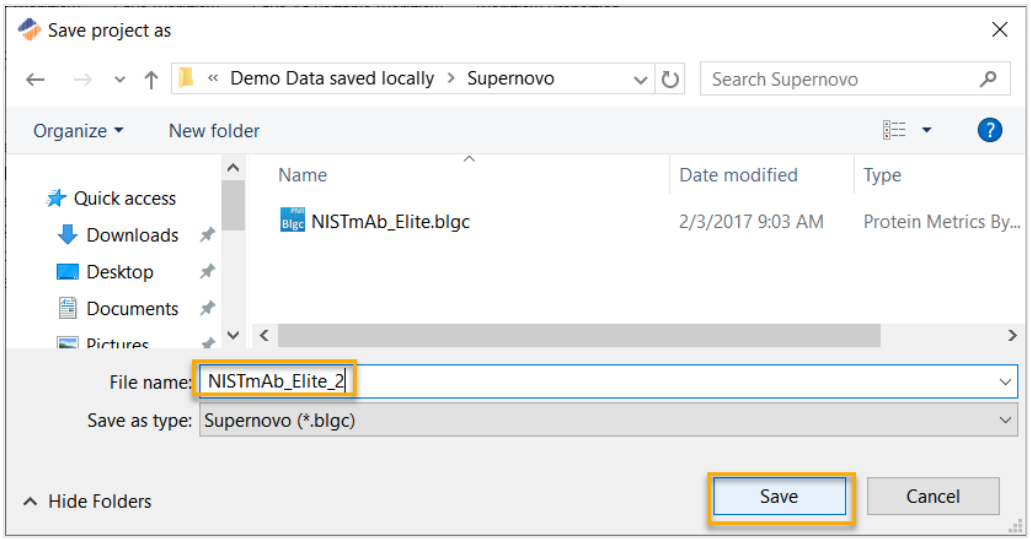


Figure 37: Name the project and save in a convenient location.

g. Processing will begin. Once complete, the project and associated report will appear as additional tabs after the **Byos Workflows** tab, as shown in Figure 38.

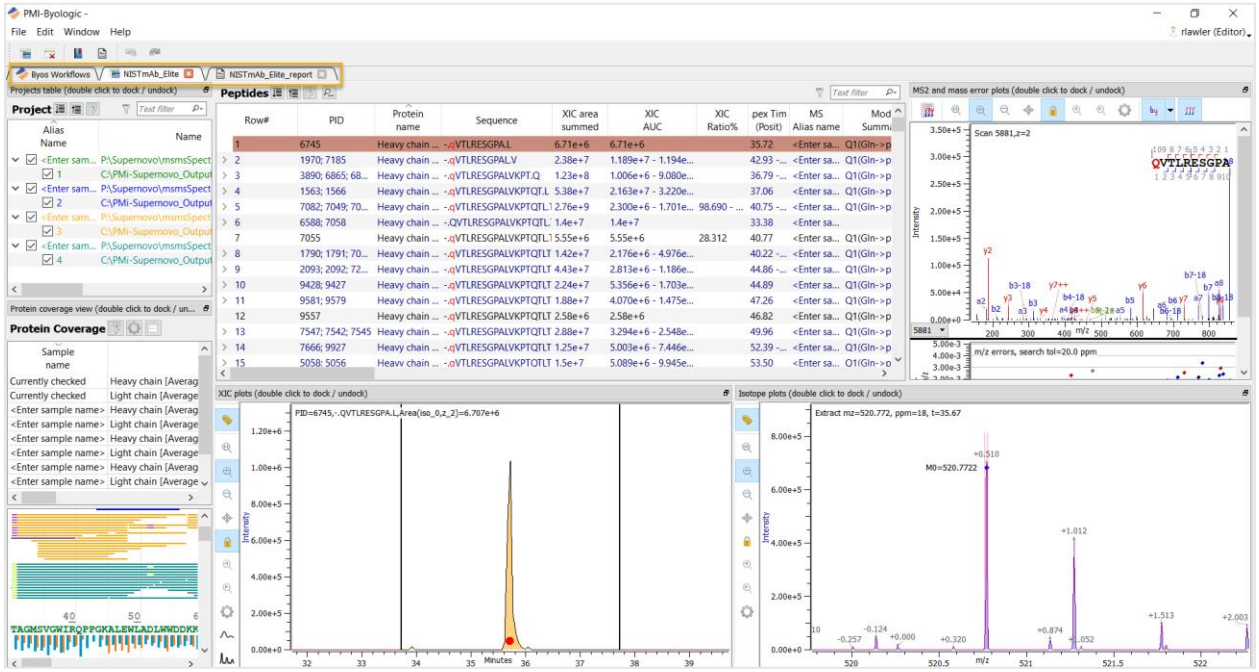


Figure 38: **Byos Workflow** tab, completed project, and associated report are available as separate tabs.