Getting Started with MASH Native

Version 1.1

Overview:

MASH Native, developed by the Ying Ge research group, is a comprehensive, universal, and user-friendly, free software environment for the analysis of top-down native and denatured proteomics data. MASH Native builds on previous work with MASH Suite, MASH Suite Pro, and MASH Explorer.

The tutorial below provides a brief introduction to the basic functions of MASH Native in both **Discovery Mode** (MS/MS datasets) and **Targeted Mode** (single or targeted protein datasets) for top-down proteomics native and denatured analysis.

- Tutorial 1: Analysis of native MS and MS/MS data for an unknown protein using Discovery Mode.
- Tutorial 2: Analysis of denatured MS/MS data for an unknown protein using Discovery Mode.
- Tutorial 3: Analysis of native data for the characterization of hemoglobin (*Bos Taurus)* using Targeted Mode.
- Tutorial 4: Analysis of denatured data for the characterization of NADH dehydrogenase [ubiquinone] iron-sulfur protein 5-like (*Sus scrofa)* using Targeted Mode.
- Tutorial 5: Post-Translational Modification Analysis using UniDec within MASH Native

Download the sample data in each section and follow the instructions below to begin using MASH Native. Video tutorials are also available on YouTube™. For a detailed description of the functions of the MASH Native software, please view the User Manual.

Privacy/Usage Reporting:

The MASH Native application may report data about your usage of the software to the MASH team. Each time you start a new version of the software, you will be asked to review your privacy/usage reporting settings. The GUI for this should be quite intuitive, but if you want more details, please see section 3 of the *User Manual*. Please see the MASH Privacy Notice (https://labs.wisc.edu/gelab/MASH_Explorer/doc/MASH%20Native%20Privacy_Notice.pdf) for information about what data may be reported and what is done with that data.

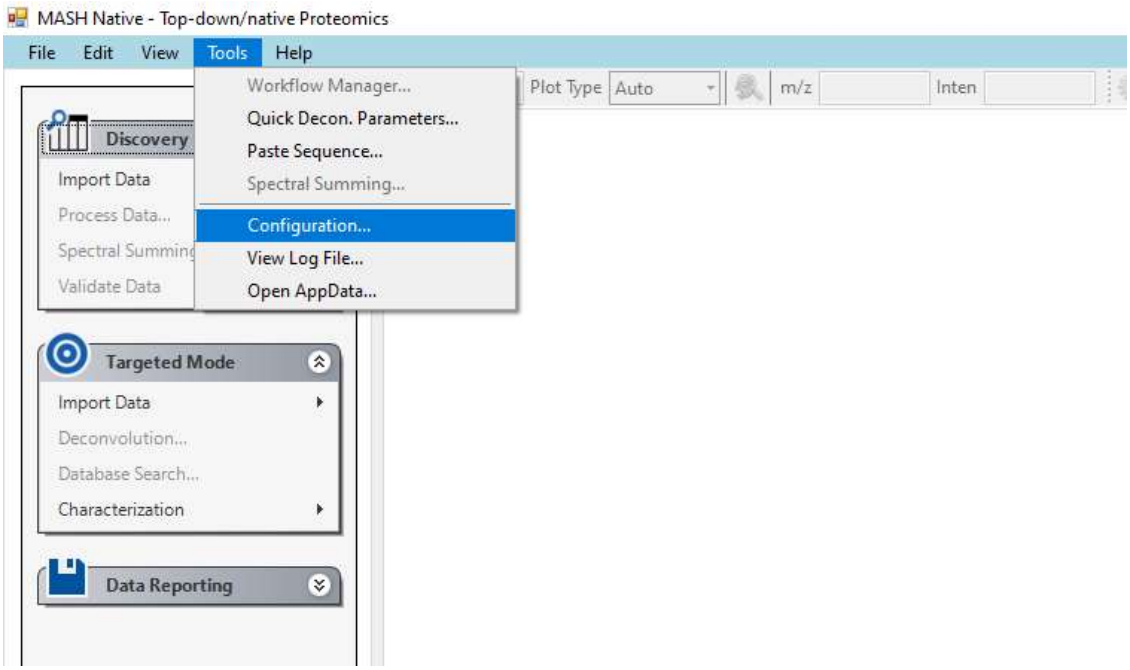
Installation and Configuration

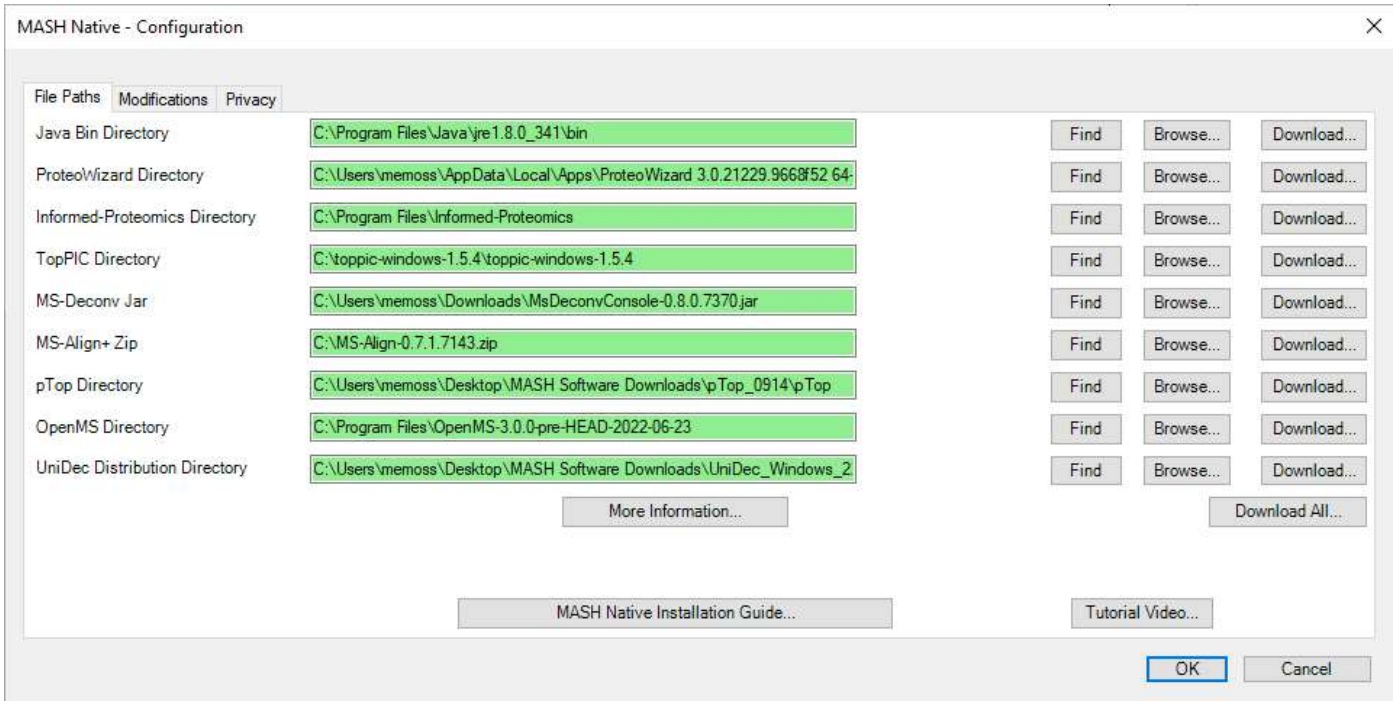
To begin, please install MASH Native by referring to the Installation Guide, available on our webpage and in the software download folder.

Changes needed:

- Show configuration dialog with only the required software green. --> Insert screenshot when configuration dialog is finalized **(I would insert this here and tell users this is the essential software needed to perform these basic familiarizations)**
- **Will add in splash screen to explain that the software gets downloaded then.**

Make sure that all associated software needed (for example, deconvolution and search tools) is installed and properly configured in the Configuration dialog (accessed via the Tools menu).

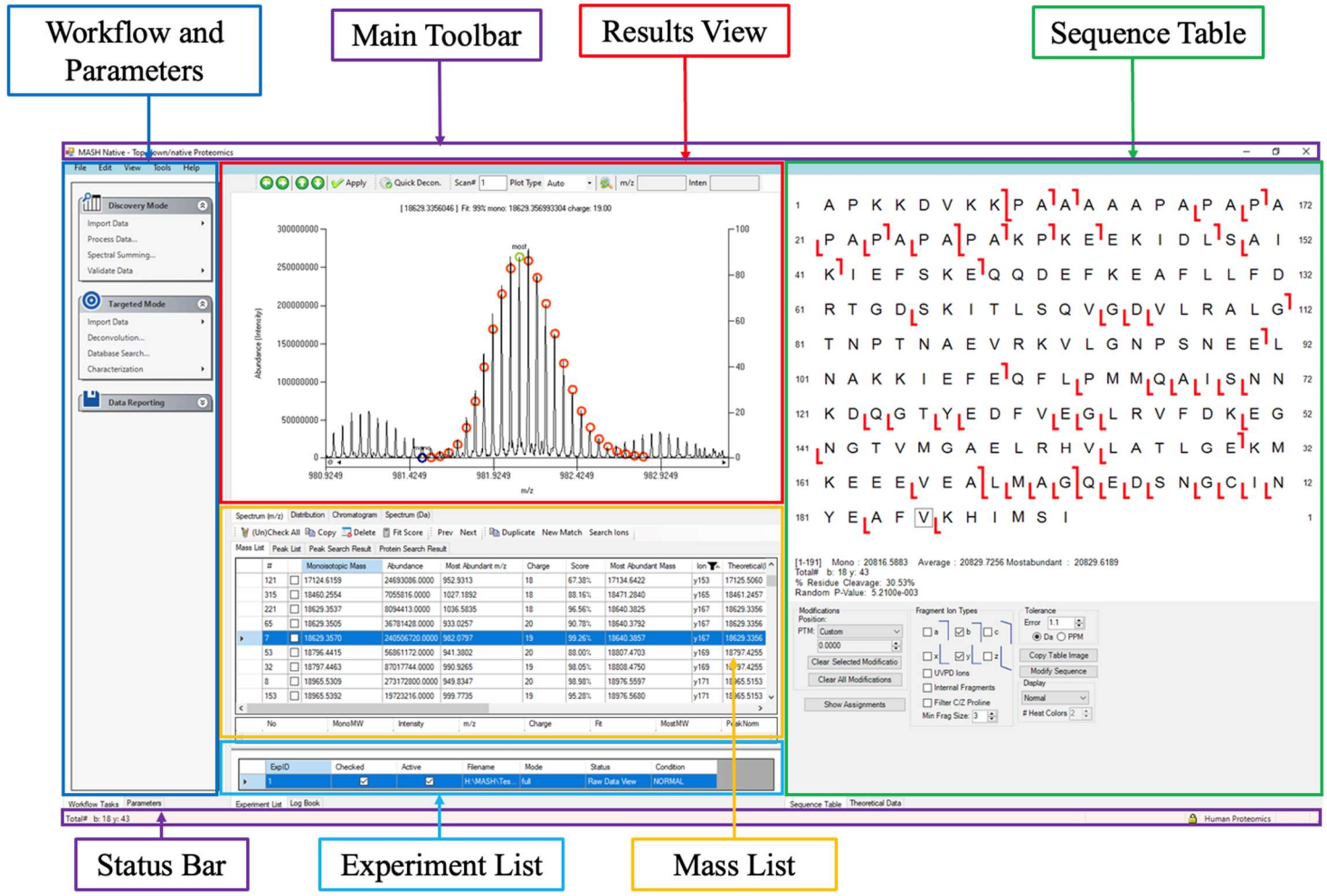




This tutorial uses TopPIC Suite and UniDec; the other search and deconvolution tools shown above are not required for the tutorial, but you may want to install them. You also need to install ProteoWizard.

Please note that this tutorial uses TopPIC Suite version 1.5.4 and UniDec 5.1.1.

Interface Layout



The components of the MASH Native main window are described below.

Workflow and Parameters: Users can follow the steps for LC-MS/MS and MS/MS workflow for data processing.

Results View: The mass spectra and chromatogram are shown in this panel

Mass List: The fragment ion information is presented in this panel.

Experiment List: This panel shows a list of the experiments that are currently loaded into MASH Native, and allows you to switch which experiment is active by double-clicking on the desired experiment. (The active experiment is highlighted in blue.)

Log Book: This panel displays processing and error messages. (Note that the Experiment List and Log Book are tabs in the same panel of the MASH Native App window.)

Status Bar: The progress of data processing is shown in this panel.

Sequence Table: This panel presents the protein sequence and top-down fragment ion information.

Discovery Mode Tutorials:

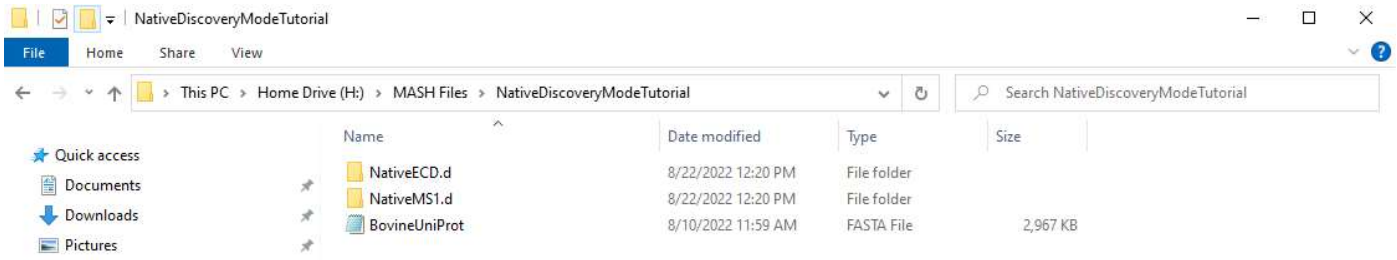
Discovery Mode is intended for MS/MS data analysis. These sample data show MASH Native's capability for analysis of both native and denatured MS/MS mass spectrometry data and includes spectral deconvolution and database search. Please follow the instructions in User Manual *Section 4 Discovery Mode: Protein Identification and Characterization from LC-MS/MS Native Mass Spectrometry Data,* if you require further assistance processing the data.

Tutorial 1: Analysis of MS/MS native top-down mass spectrometry data:

For the analysis of native mass spectrometry data, download the folder containing the native tutorial files and save them to a new local folder as seen below.

Download the folder below on the Google Drive for the native MS/MS tutorial

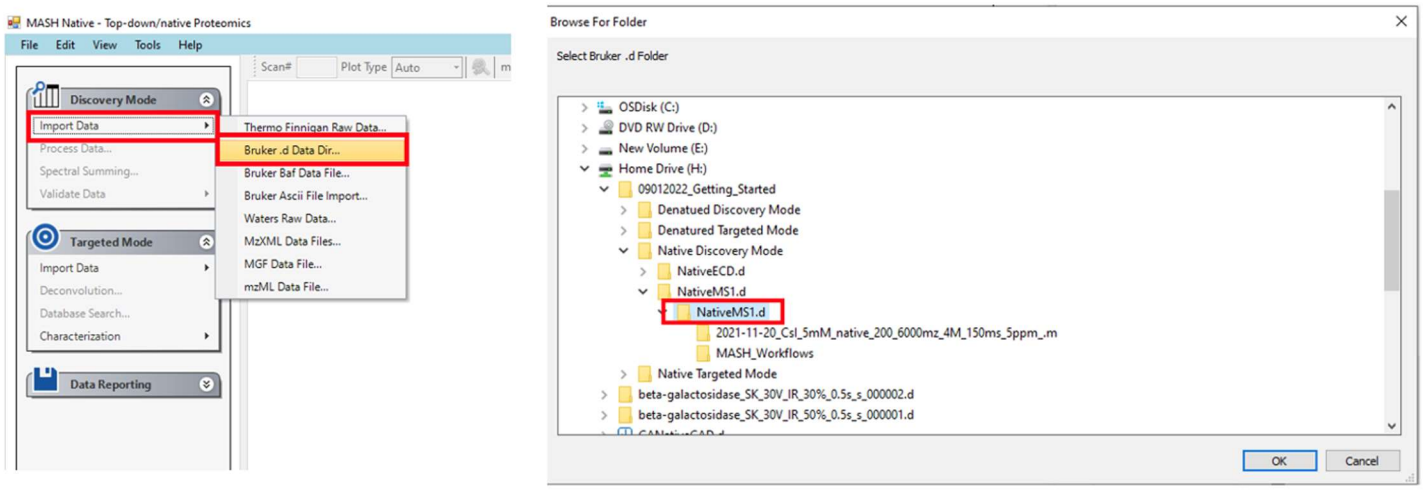
- Native Discovery Mode Tutorial folder which contains the Bruker NativeMS1 and NativeECD data files and BovineUniProt.fasta file.



Proceed with the following steps for analysis of native MS/MS mass spectrometry data to identify an unknown protein from a bovine extract.

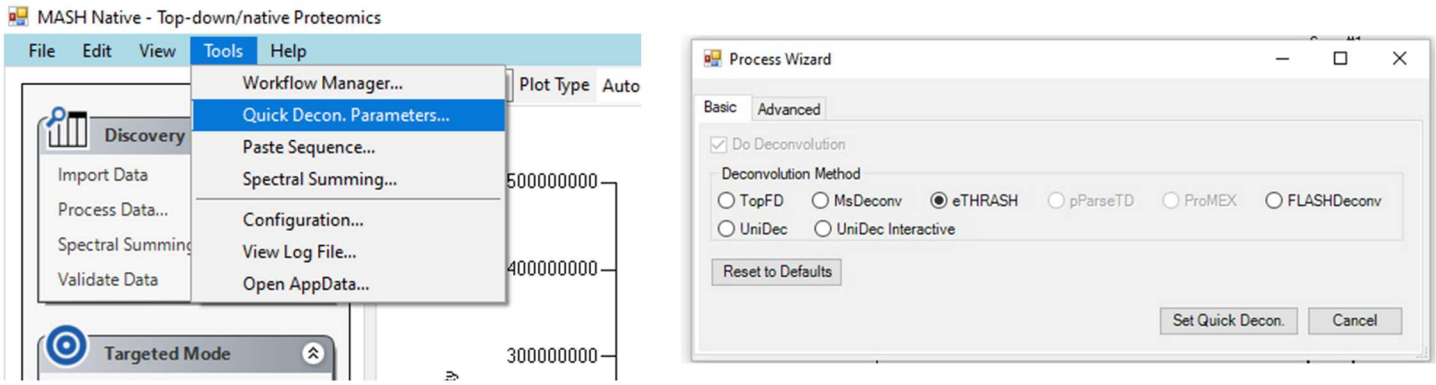
Step 1: Import MS1 native data

Use the Import Data function in the Discovery Mode to import the sample Bruker .d 'Native MS1' folder.

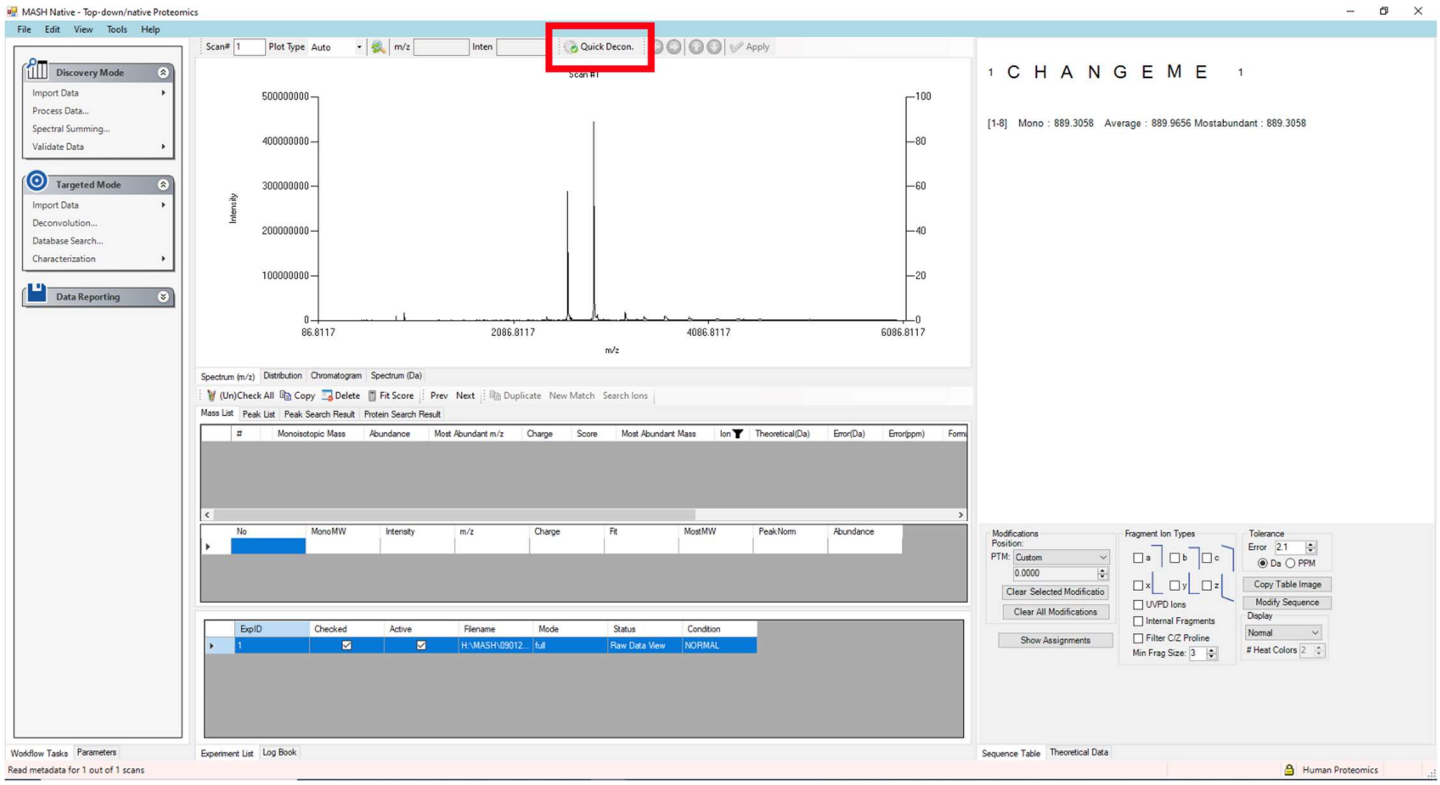


Step 2: Spectral deconvolution of the MS1 spectra and database search

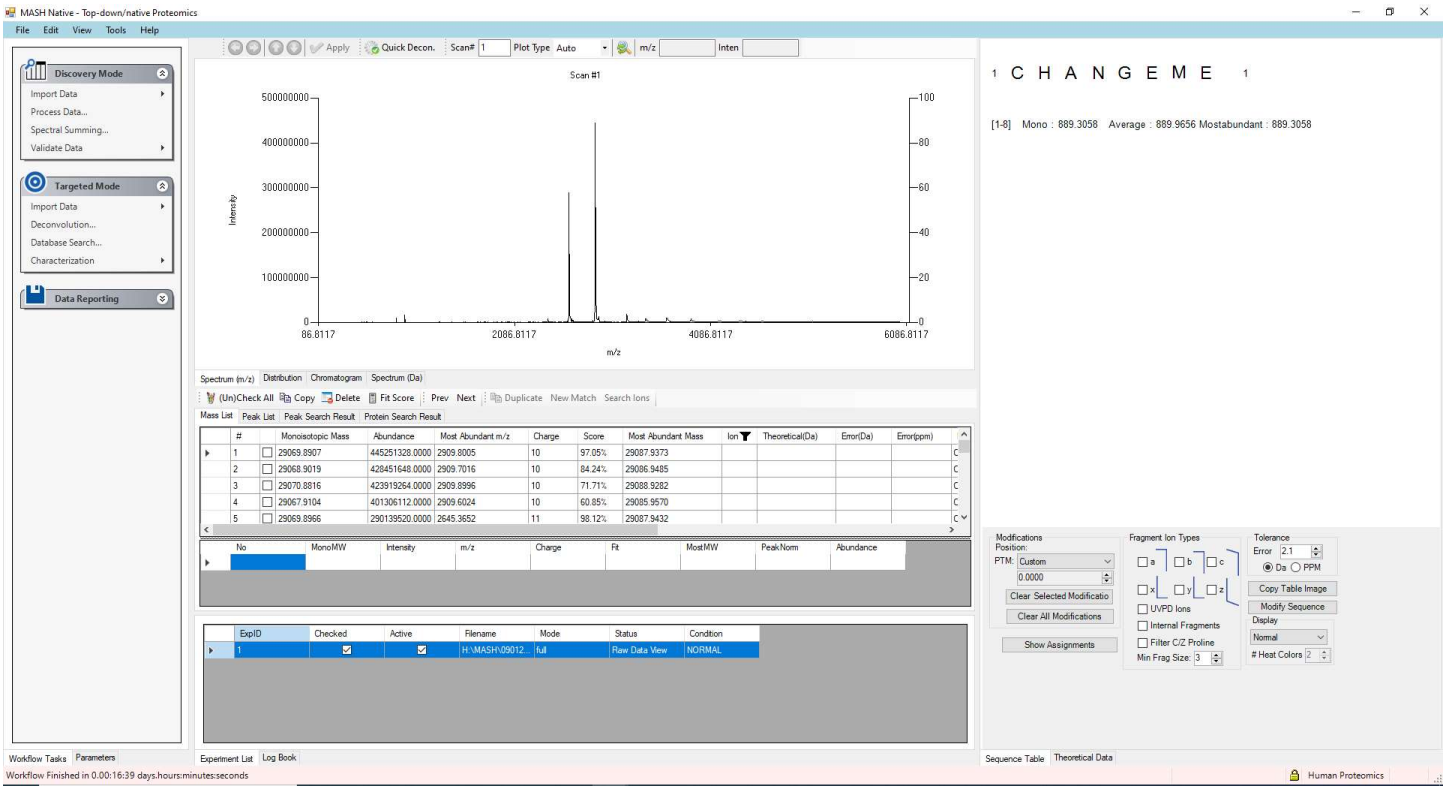
For this workflow, we will be using Quick Deconvolution with the eTHRASH algorithm. To ensure Quick Decon. will use the desired algorithm, under Tools, select "Quick Decon. Parameters." This will open the Process Wizard dialog and the user can select their deconvolution method. Select eTHRASH for this tutorial and click "Set Quick Decon."



Select the QuickDecon. button to deconvolute the native MS1 spectrum. This will open the Workflow Manager window and the terminal window. The terminal window is where the deconvolution and search are running. You can use the Workflow Manager to check on the status of your run, but note that closing this window will stop your workflow. When your run is completed, you will see the workflow in the Completed Workflows tab of the Workflow Manager and the line "Workflow Finished" in the Log Book.

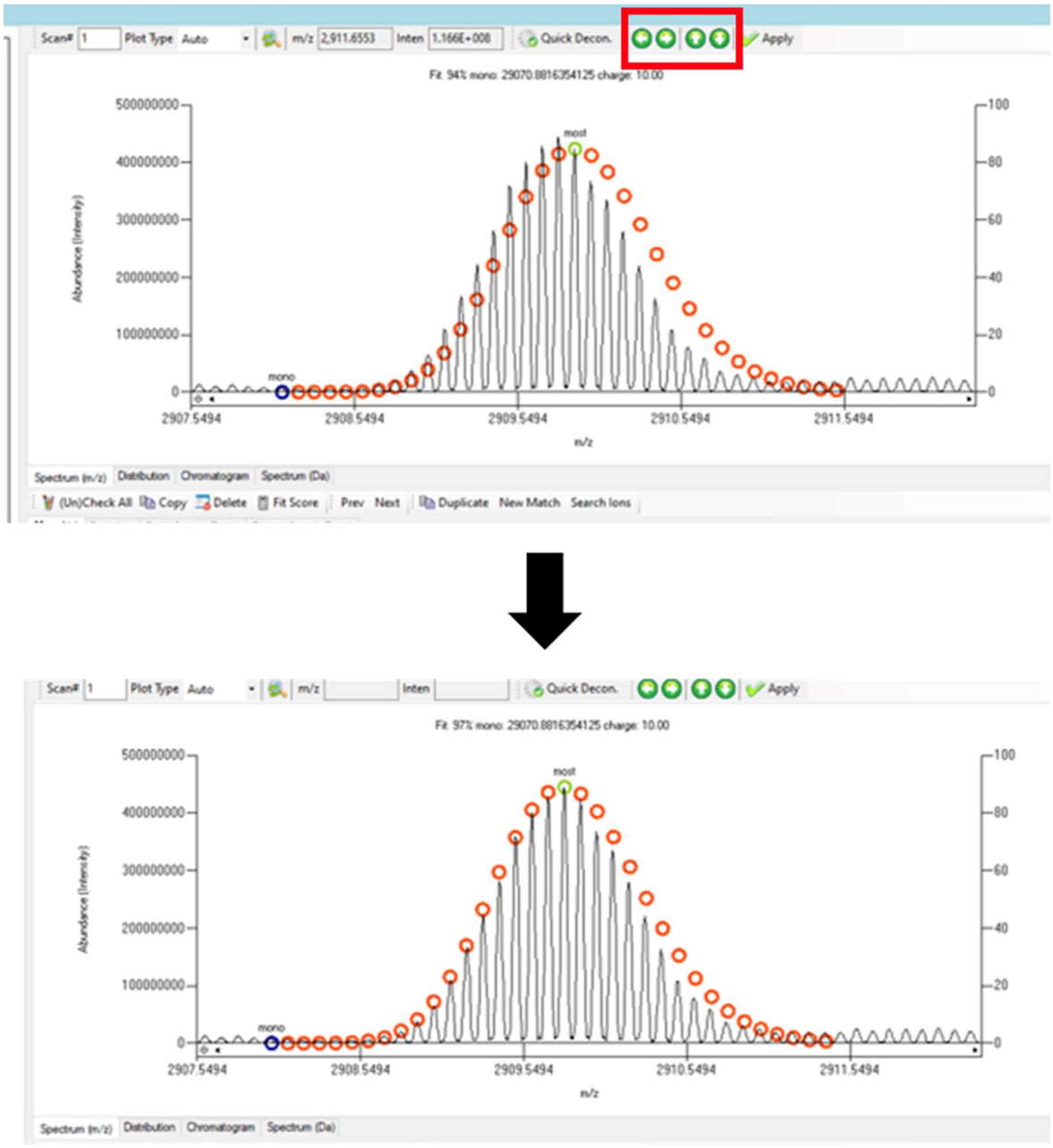


Once the deconvolution is finished, the mass list will populate, as seen below.



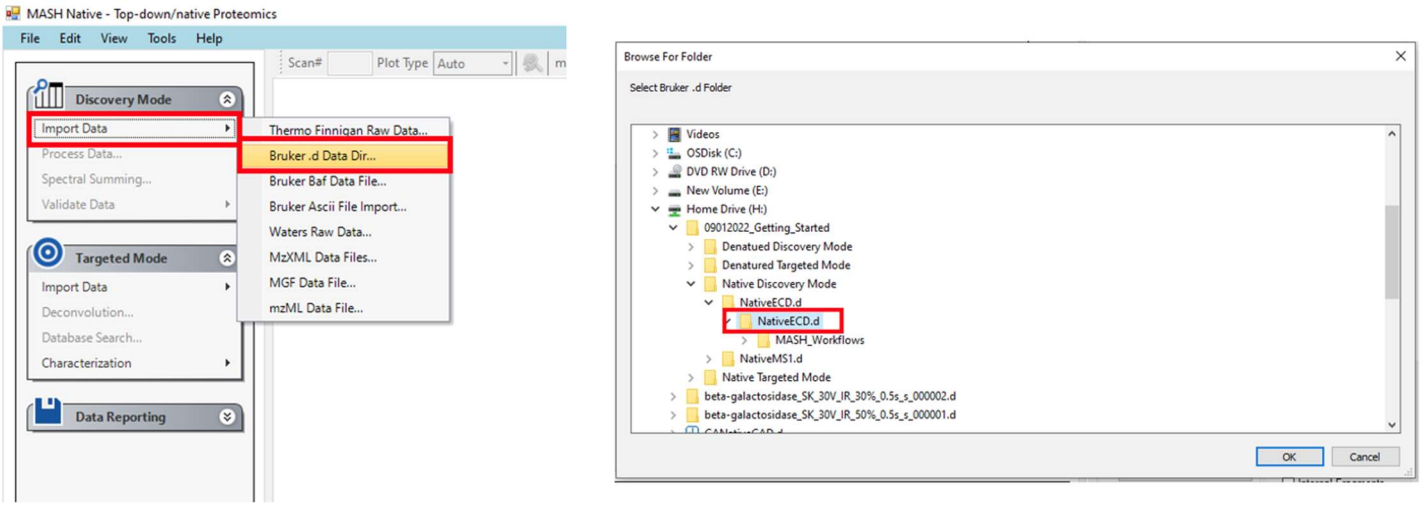
CHECKPOINT: Make sure your results match the photo above.

in the *Results View* to alter the theoretical fit and click the "Apply" button to save your adjustments, like the example below.



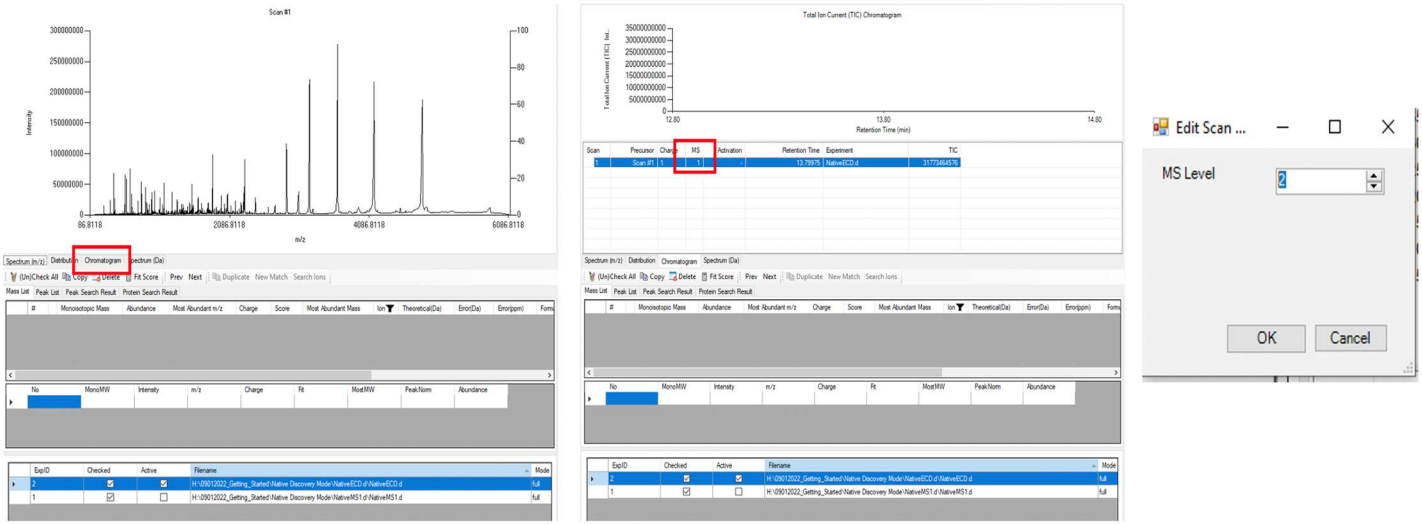
Step 3: Import MS/MS data

Use the Import Data function in the Discovery Mode to import the sample Bruker .d 'Native ECD' folder.



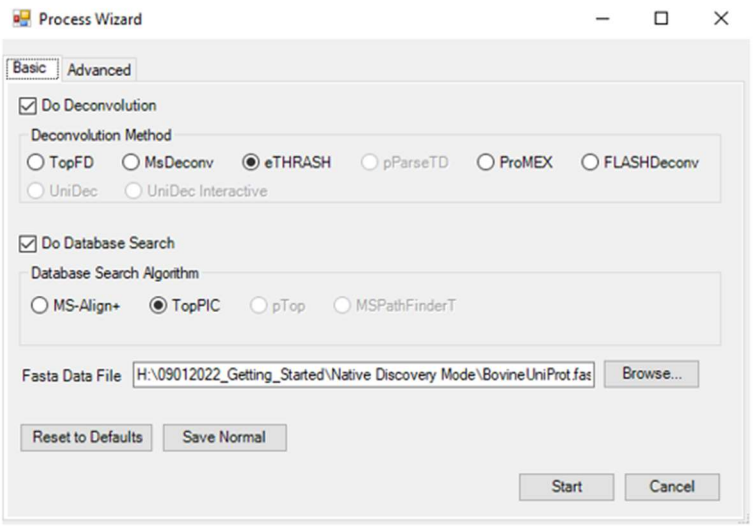
Step 4: Spectral deconvolution of the MS/MS spectra and database search

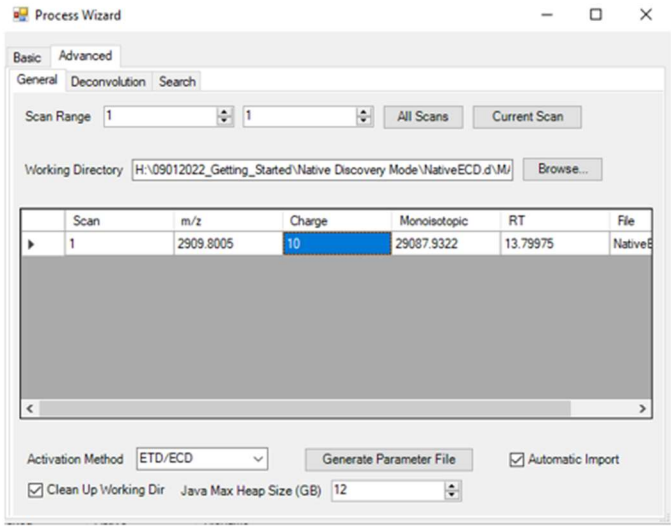
To complete the database search, the user must label the ECD data as an MS2 file. To do so, click the "Chromatogram" button, then double click where it says "MS" and in the popup window, change the MS level to 2 and press "OK."

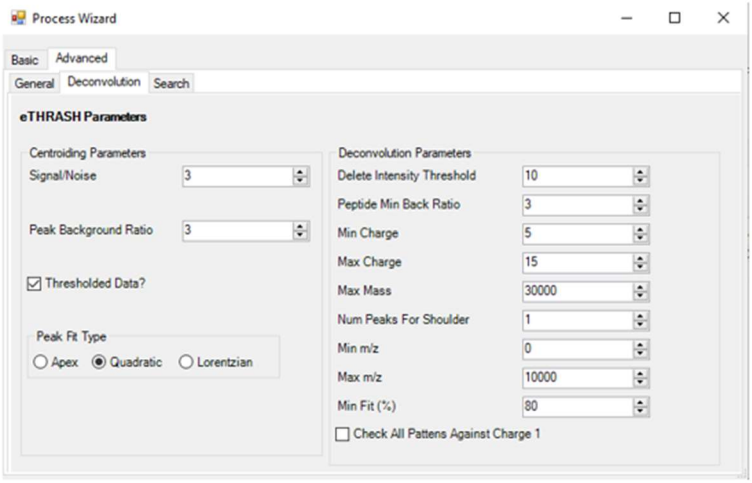


Now, click the Process Data menu item in the Discovery Mode section to open the Process Wizard dialog. The Process Wizard will setup the series of tasks for selecting, configuring, and running the deconvolution and database search algorithms. Select the eTHRASH deconvolution and TopPIC database search algorithms. Next, click on the Browse button and select the sample FASTA file to load into MASH Native. To set advanced parameters, open the Advanced tab and enter the precursor and charge state under the General tab as seen below.

Next, enter deconvolution parameters under the Deconvolution tab as seen below. Click on the Start button to begin the analysis.

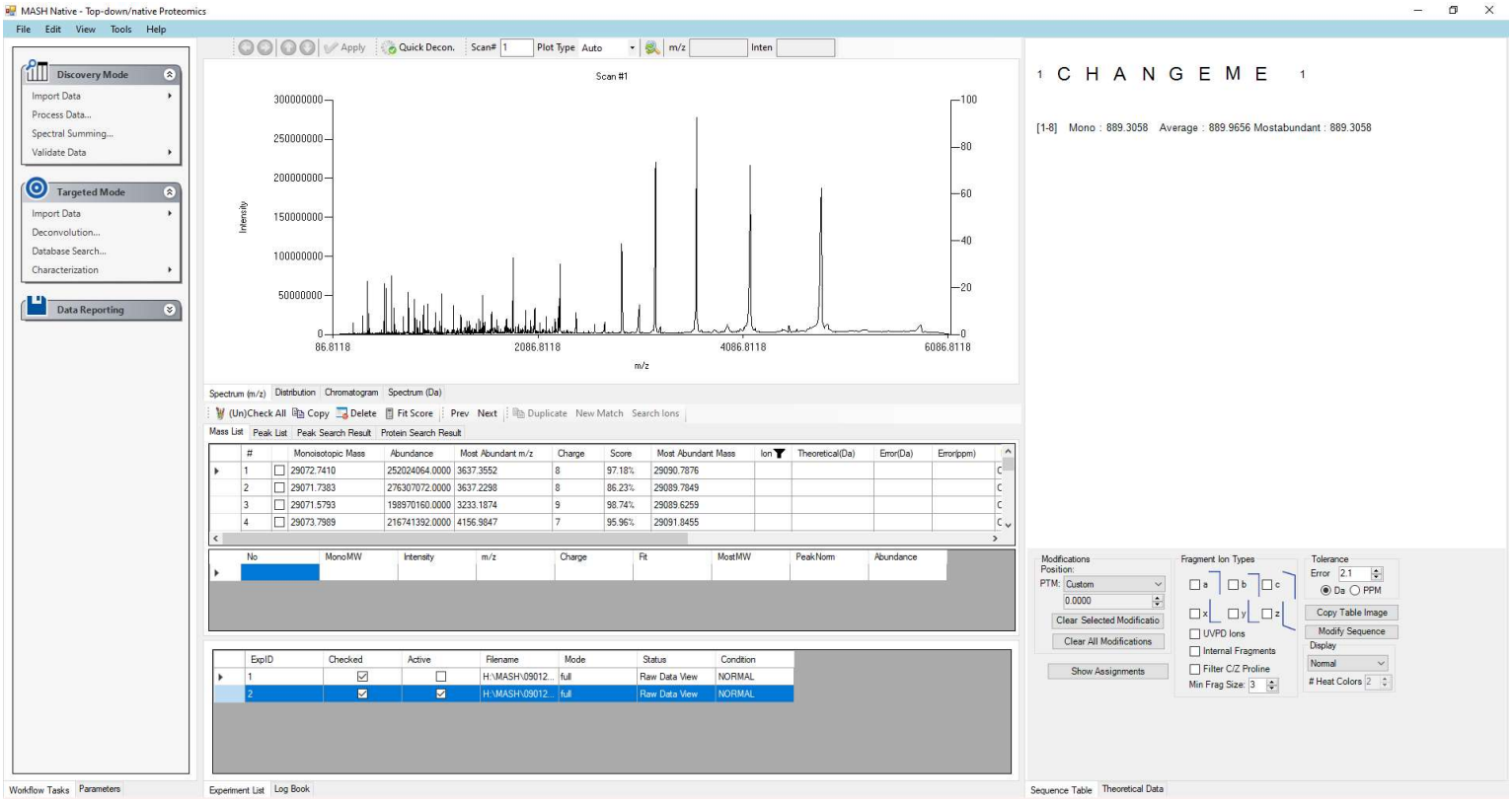






Step 5: Review results

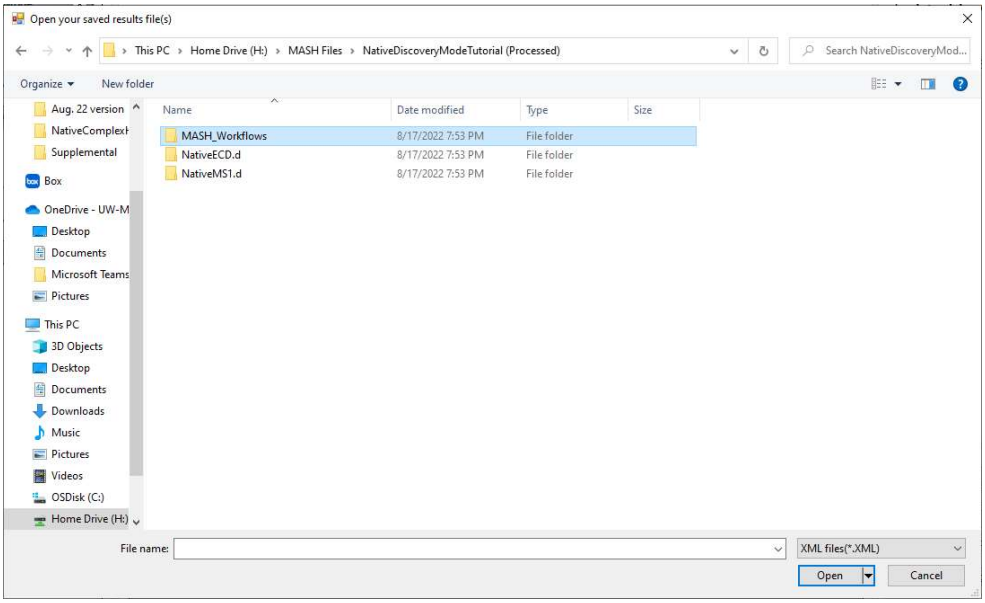
Your deconvolution and search results will be automatically imported into MASH Native if the run was successful.



You can find your deconvolution results in the Mass List and your search results in the Protein Search Results List.

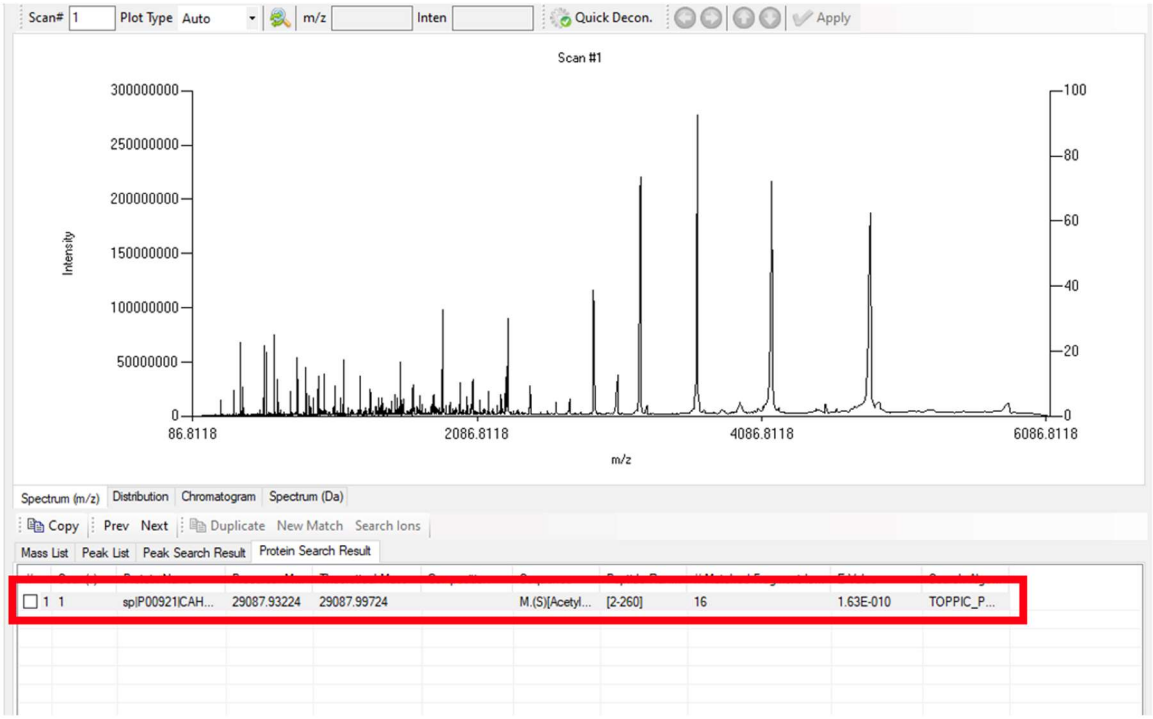
CHECKPOINT: Make sure your results match the photo above.

Your saved deconvolution results will be in your working directory under MASH_Workflows. You may wish to select “Overwrite” to avoid viewing duplicated results.

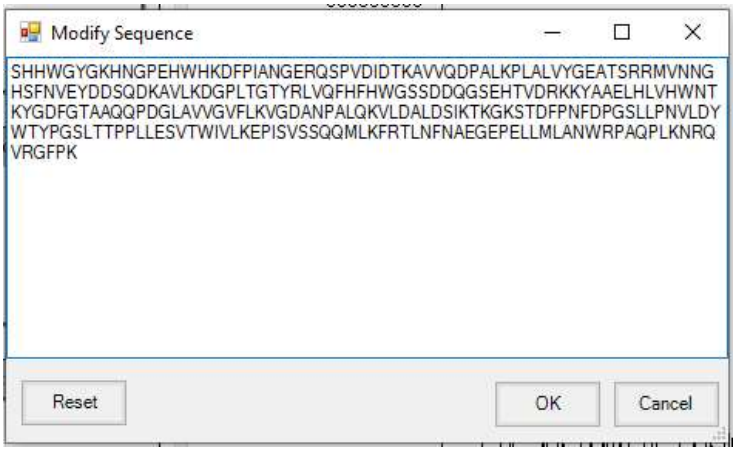


Step 6: Annotate results

MASH Native allows you to view and annotate your imported results with the Mass List and Protein Search Results List, the Results View, and the Sequence Table. For more details on these functions, refer to the *User Manual*. In the Mass List, click on some of the loaded masses to view the fragment ions of a selected scan in the dataset. Use the arrows in the results view to alter the theoretical fit and click the "Apply" button to save your adjustments. You can click on the entry in the Protein Search Results List.



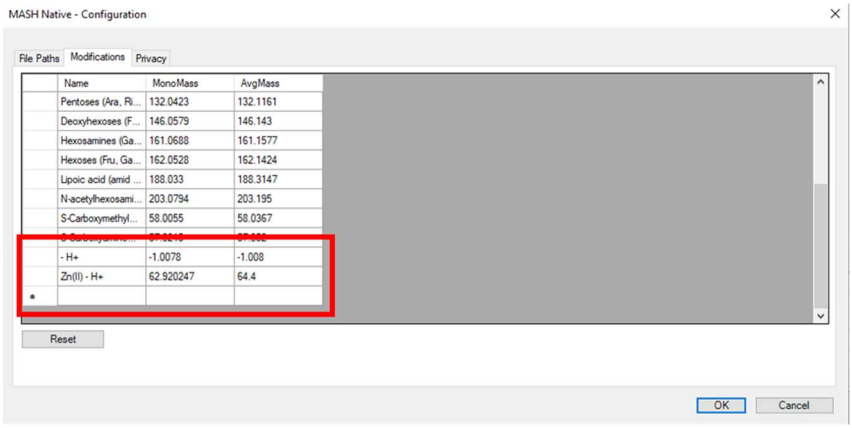
Known modifications for the protein CAH2 can be found at https://www.uniprot.org/uniprotkb/P00921/entry. With this, we can modify the protein sequence and modifications. In the Modify Sequence Dialog in the bottom right corner, remove the following characters so that the sequence matches the image below: “M.”, “(“, “)”, “[ACETYL]”, “(“, “)” “[81.3146]”, and finally “.”. Click OK to display these notation changes in the Sequence Table.



You can also manually type in (or copy/paste) the following sequence:

SHHWGYGKHNGPEHWHKDFPIANGERQSPVDIDTKAVVQDPALKPLALVYGEATSRRMVNNGHSFN
VEYDDSQDKAVLKDGPLTGTYRLVQFHFHWGSSDDQGSEHTVDRKKYAAELHLVHWNTKYGDFGT
AAQQPDGLAVVGVFLKVGDANPALQKVLDALDSIKTKGKSTDFPNFDPGSLLPNVLDYWTYPGSLTT
PPLLESVTWIVLKEPISVSSQQMLKFRTLNFNAEGEPELLMLANWRPAQPLKNRQVRGFPK

Next, add the following modifications in the Configuration Panel under the Tools menu. Add -H+ and Zn(II)-H+ in the table with their respective masses shown in the figure below. Then, using the PTM drop down box under the sequence table, add an acetylation to the N-terminal serine, -H+ to H93, and Zn(II)-H+ to H95. Check the *c* and *z*-ions and click the “Show Assignments” button. Additionally, users can restrict the mass tolerance and use the internal fragment options to improve sequence coverage.

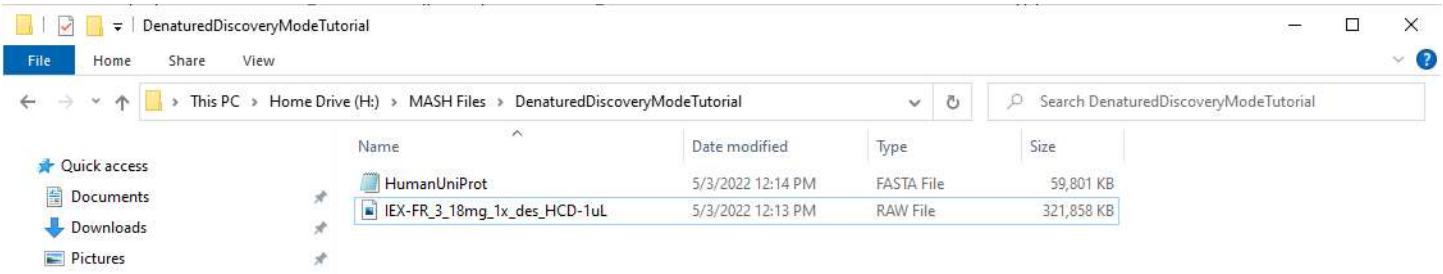


Analysis of MS/MS denatured top-down mass spectrometry data:

For the analysis of denatured mass spectrometry data, download the folder containing the denatured tutorial folder and save them to a new local folder as seen below.

Download the folder below on the Google Drive for the denatured MS/MS tutorial

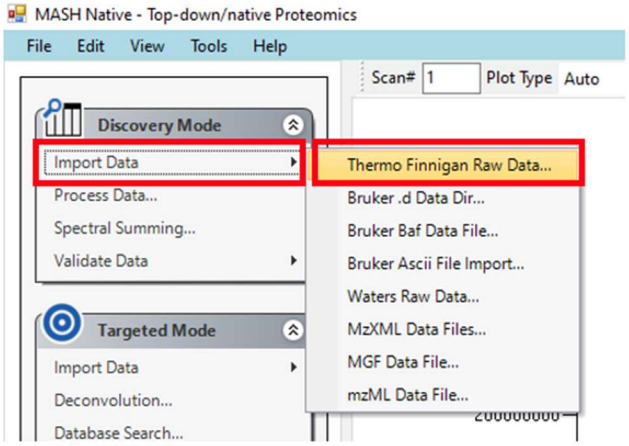
- Denatured Discovery Mode Tutorial folder which contains the IEX-FR_3_18mg_1X-des-HCD-1uL raw file and HumanUniProt.fasta file.

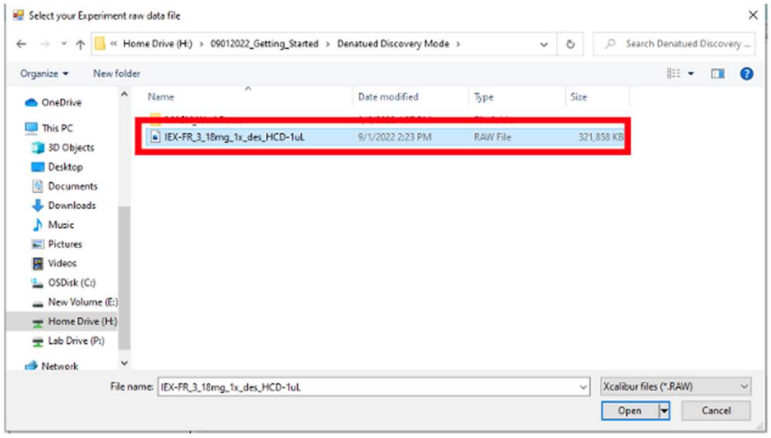


Proceed with the following steps for analysis of denatured MS/MS mass spectrometry data to identify an unknown protein from a human extract.

Step 1: Import the MS/MS denatured data

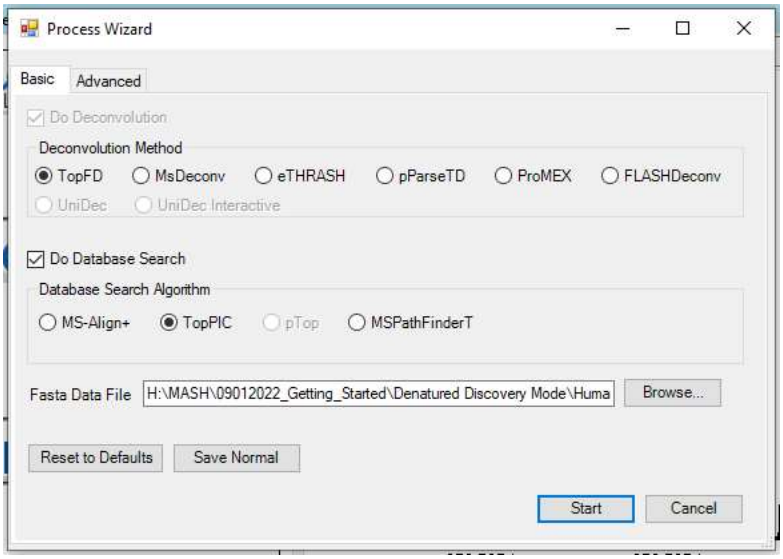
Use the Import Data function in the Discovery Mode to import the sample Thermo raw data file.

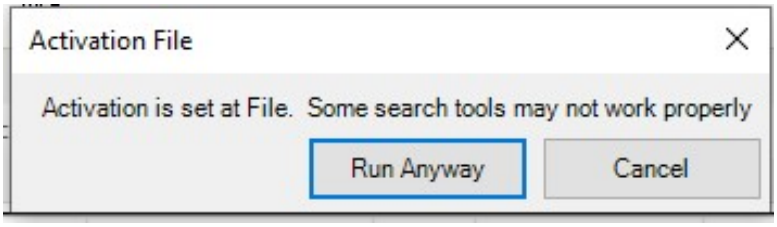




Step 2: Spectral deconvolution and database search

Click the Process Data menu item in the Discovery Mode section to open the Process Wizard dialog. The Process Wizard will setup the series of tasks for selecting, configuring, and running the deconvolution and database search algorithms. Select the TopFD deconvolution method and the TopPIC database search algorithm. Next, click on the Browse button and select the sample FASTA file to load into MASH. To set advanced parameters, open the Advanced tab; however, it is not necessary to set these parameters to begin your workflow. Click the "Start" button and an "Activation File" popup window will appear, please click "Run Anyway" to begin the search.





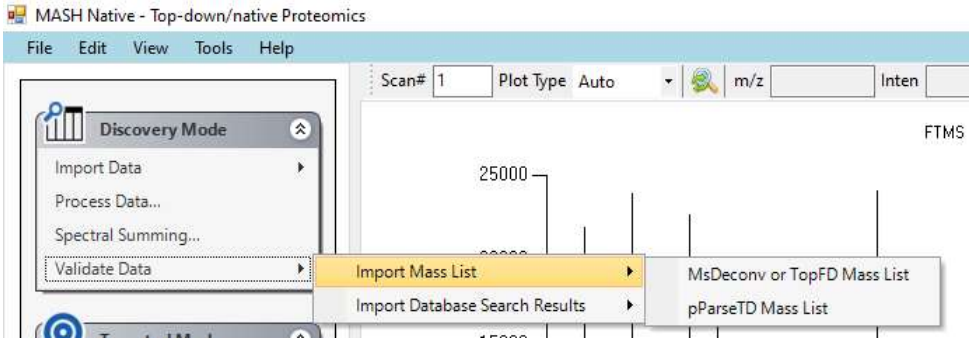
Starting your search will open the Workflow Manager window and the terminal window. The terminal window is where TopFD and TopPIC are running. You can minimize this window but closing this window will stop your workflow. You can close and open the Workflow Manager window as you wish, and you can use the Workflow Manager to check on the status of your run. When your run is completed, you will see the workflow in the Completed Workflows tab of the Workflow Manager and the line "Workflow Finished" in the Log Book.

Step 3: Import results

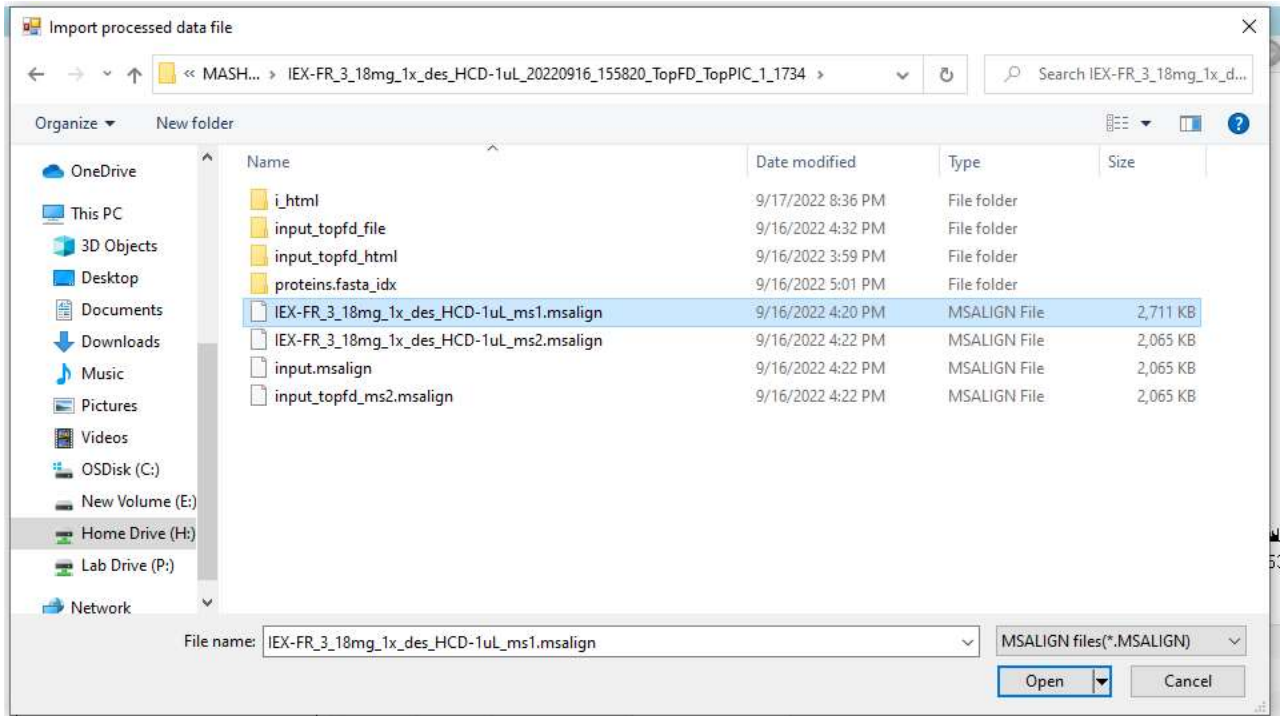
Your deconvolution and search results will be automatically imported into MASH Native if the run was successful. You can find your deconvolution results in the Mass List and your search results in the Protein Search Results List.

CHECKPOINT: Make sure your results match the photo above.

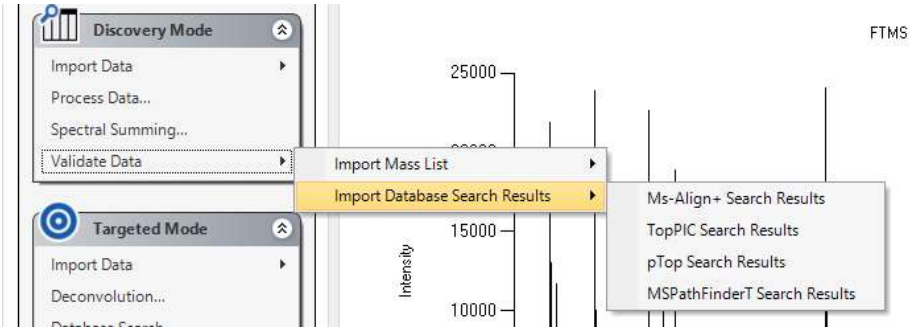
To import the deconvolution mass list for detailed analysis of the data set, select the Validate Data function in the Discover Mode panel, and click on Import Mass List.



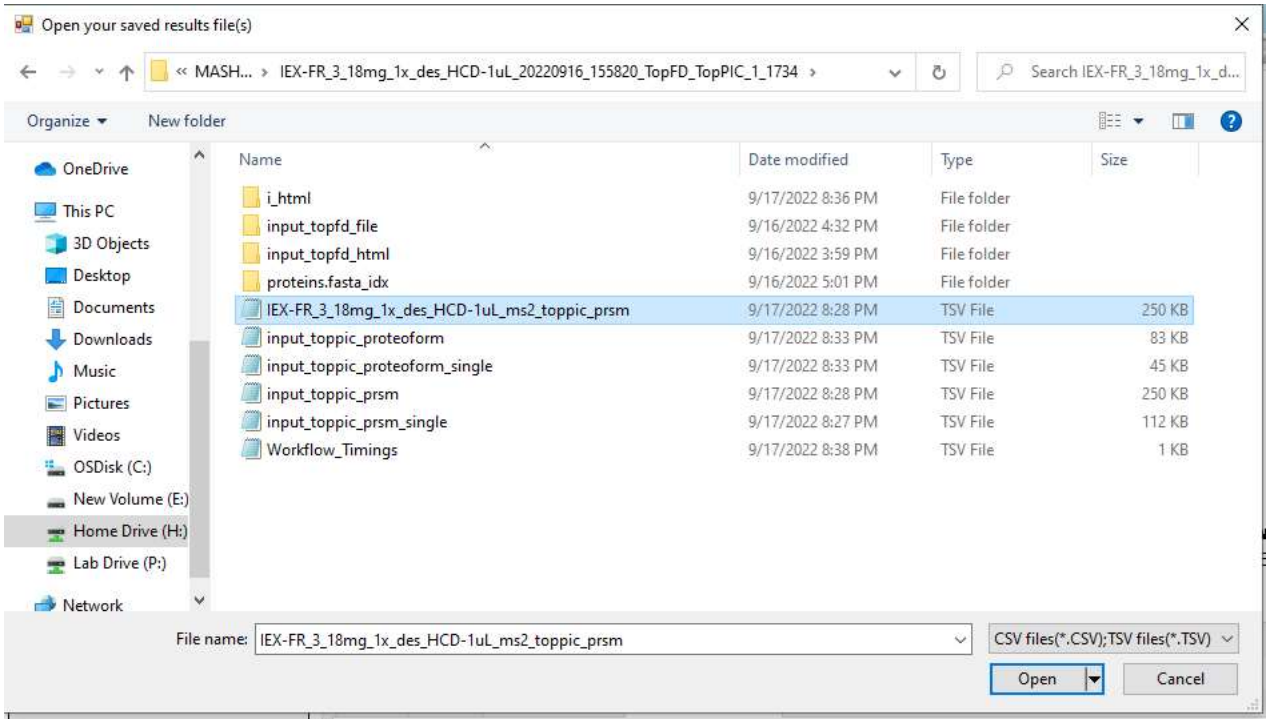
Your saved deconvolution results will be in your working directory under MASH_Workflows. Double click on the folder and select the file IEX-FR_3_18mg_1x_des_HCD-1uL_ms1.msalign as seen below. The loaded results will populate the Mass List. You may wish to select "Overwrite" to avoid viewing duplicated results.



To import the database search results for detailed analysis of the data set, select the Validate Data function in the Discovery Mode panel, and click on Import Database Search Results, and select TopPIC Search Results.



Navigate to your working directory and select the following file to import: IEX-FR_3_18mg_1x_des_HCD-1uL_ms2_toppic_prsm.tsv. You may wish to select "Overwrite" to avoid viewing duplicated results. Search results will be loaded into the Protein Search Results Table.



Step 4: Annotate results

MASH Native allows you to view and annotate your imported results with the Mass List and Protein Search Results List, the Results View, and the Sequence Table. For more details on these functions, refer to the *User Manual*. In the Mass List, click on some of the loaded masses to view the fragment ions of a selected scan in the dataset. Change the scan number in the Chromatogram or at the top of the Results View to view the mass fragments of a different scan. Use the arrows in the results view to alter the theoretical fit and click the "Apply" button to save your adjustments.

Targeted Mode:

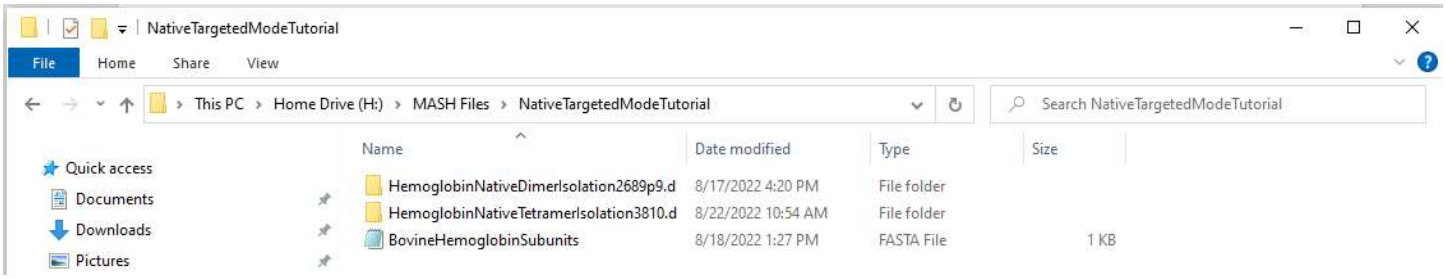
Targeted Mode is intended for targeted mass spectrometry data analysis. Detailed instructions on using this mode can be found in the User Manual at *Section 4 Targeted Mode: Protein Identification and Characterization from MS/MS Data*.

Analysis of native top-down mass spectrometry data:

For the analysis of native mass spectrometry data, download the folder containing the native tutorial files and save them to a new local folder as seen below.

Download the folder below on the Google Drive for the native targeted tutorial:

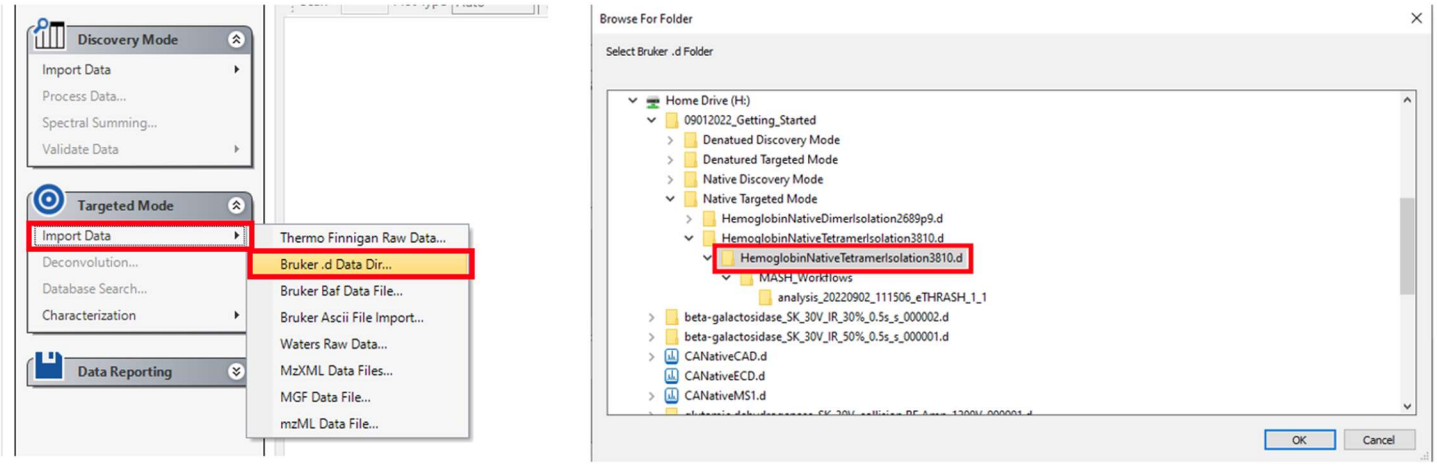
- Native Targeted Mode Tutorial folder which contains the Bruker HemoglobinNativeDimerIsolation2689p9.d and HemoglobinNativeTetramerIsolation3810.d data files as well as the BovineHemoglobineSubunits.fasta file.



Proceed with the following steps for analysis of native MS/MS mass spectrometry data to characterize hemoglobin from a bovine extract.

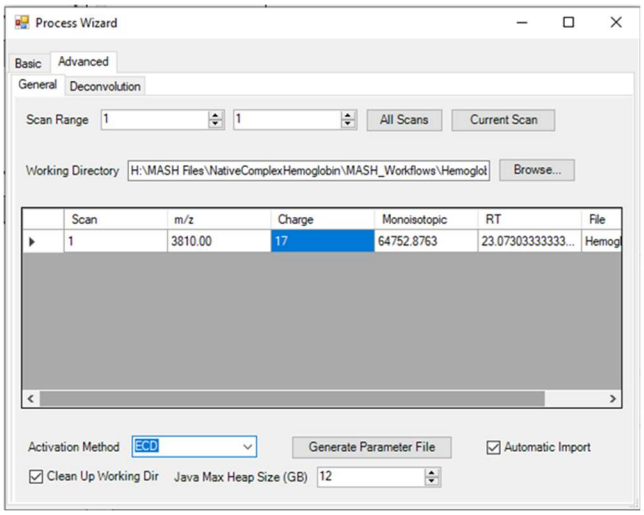
Step 1: Import data for analysis of hemoglobin tetramer

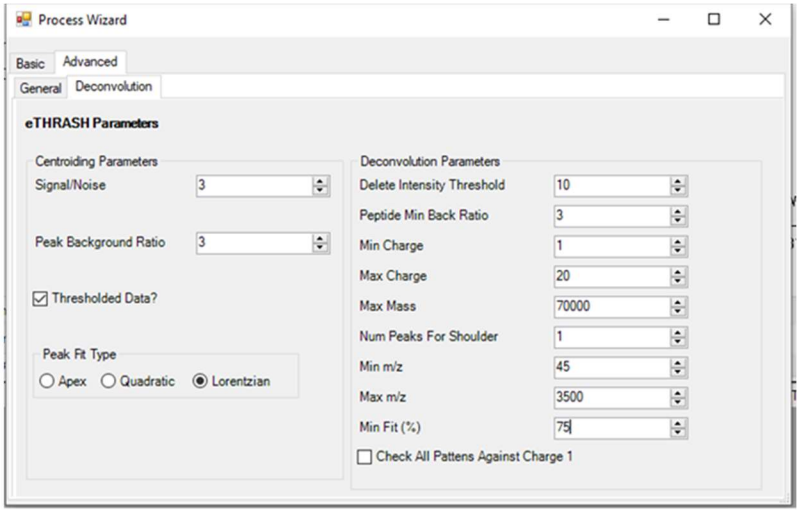
Use the Import Data function in Targeted Mode to import the HemoglobinNativeTetramerIsolation3810.d data file.



Step 2: Deconvolution

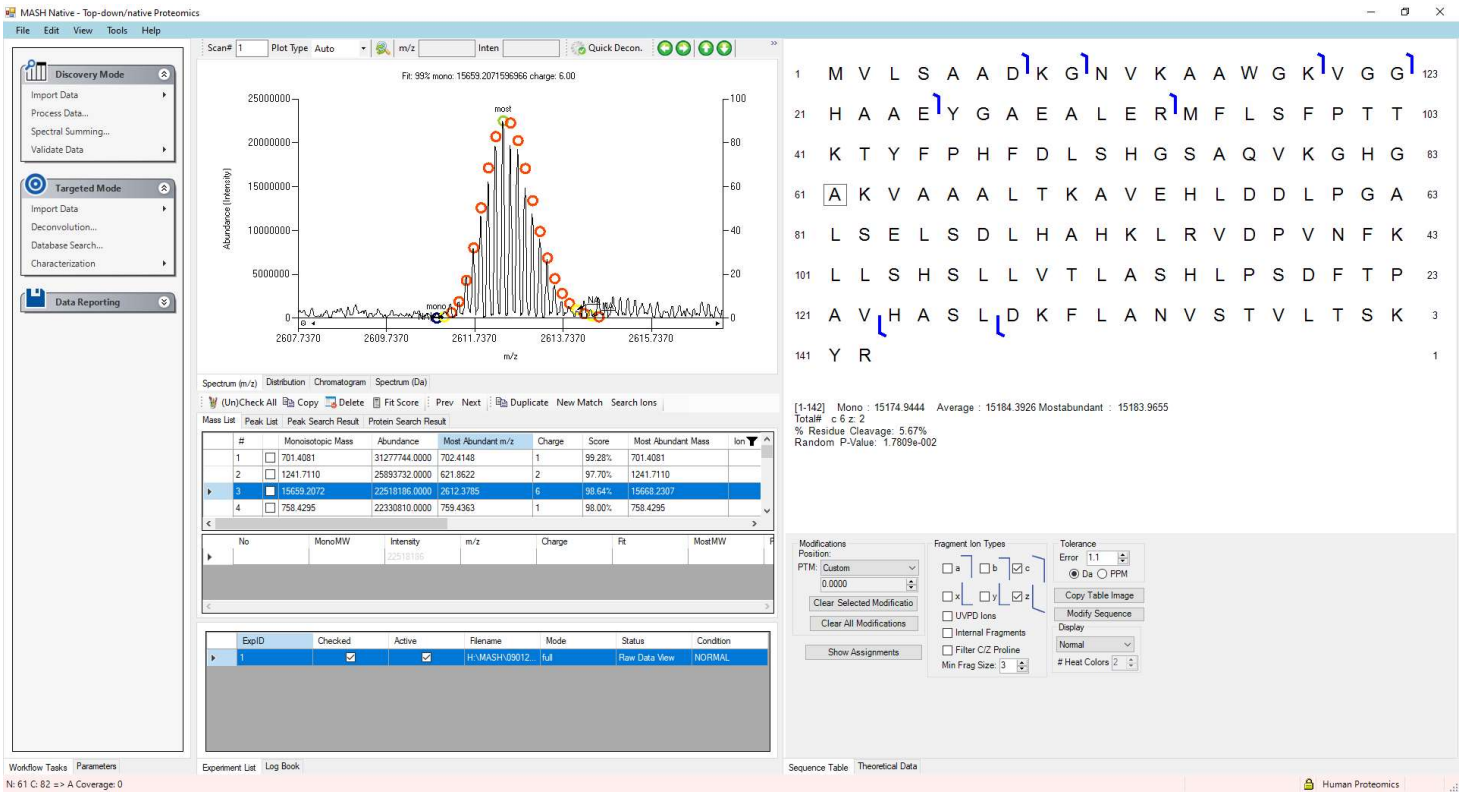
This data shown here is generated from the isolation of the 17+ charge state for the hemoglobin tetramer and ECD fragmentation applied. You can deconvolute the fragments and identify them in targeted mode. Under Targeted Mode, Process Wizard will guide users through the deconvolution step. Select the eTHRASH deconvolution method and input the parameters below in the and click the Start button. Enter the parameters below for the General and Advanced parameters in the Deconvolution settings. The Workflow Manager and terminal window will open. You can close the Workflow Manager if you wish, but do NOT close the terminal because this is where your computer is running the deconvolution. When the deconvolution is finished, the Mass List will automatically populate for fragment ion validation.



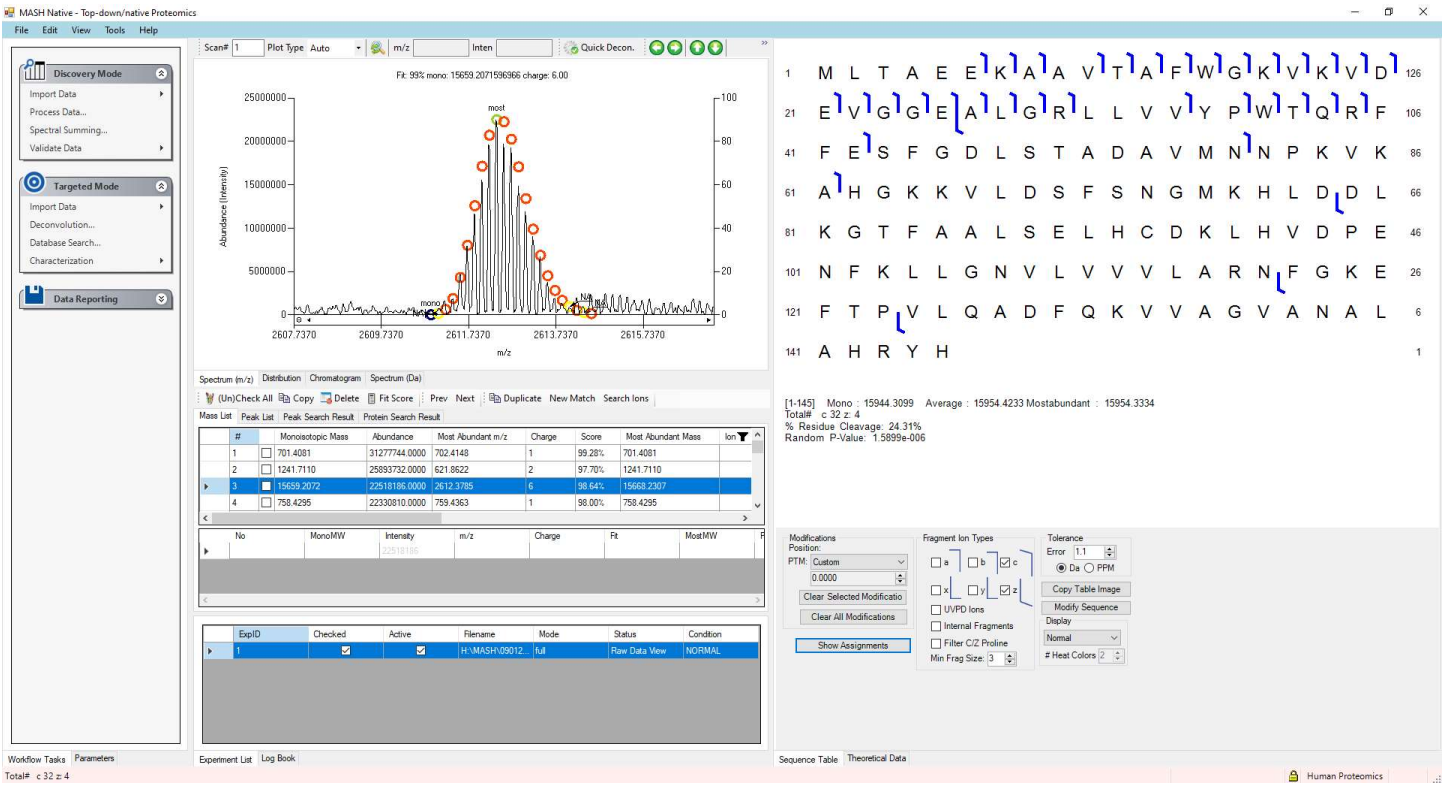


Step 3: Annotate results

When the fragment ions are verified, users can use the protein sequences from the alpha and beta hemoglobin subunits to identify fragments. Open the BovineHemoglobinSubunits FASTA file and copy the sequence for the alpha chain and paste into the modify sequence box in the bottom right corner. Users can then select *c* and *z*-ions and "Show Assignments."



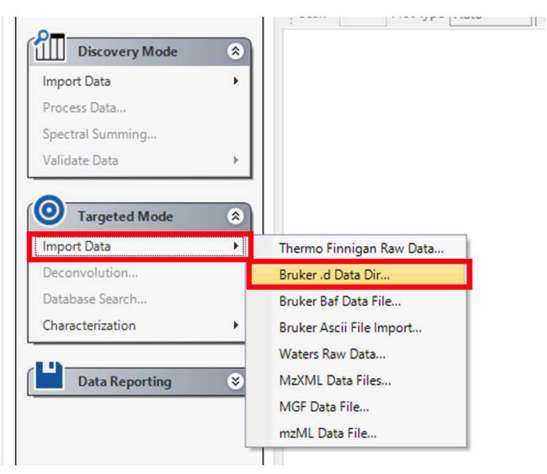
Similarly, users can identify fragments from the beta chain. Additionally, users can improve sequenece coverage by adding non-covalently bound iron and other known modifications and include internal fragment analysis into their data analysis. The internal fragments will populate in the mass list.

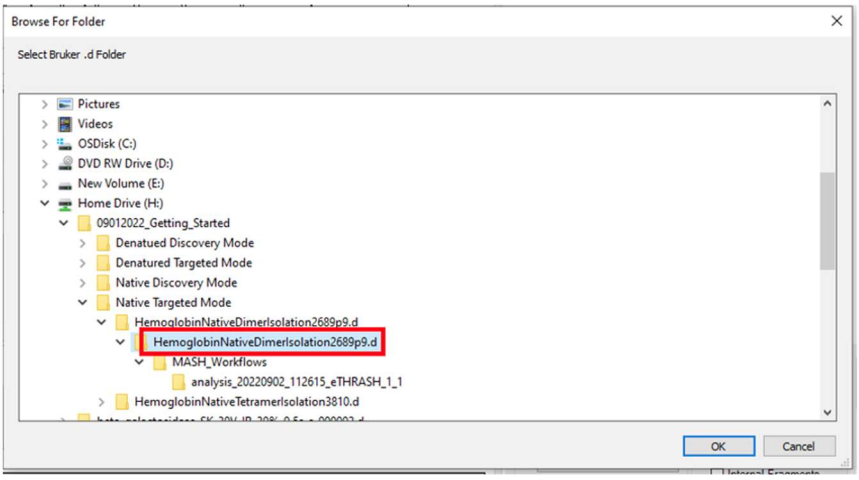


Save your results and close this experiment.

4. Import data for dissociation of hemoglobin subunits

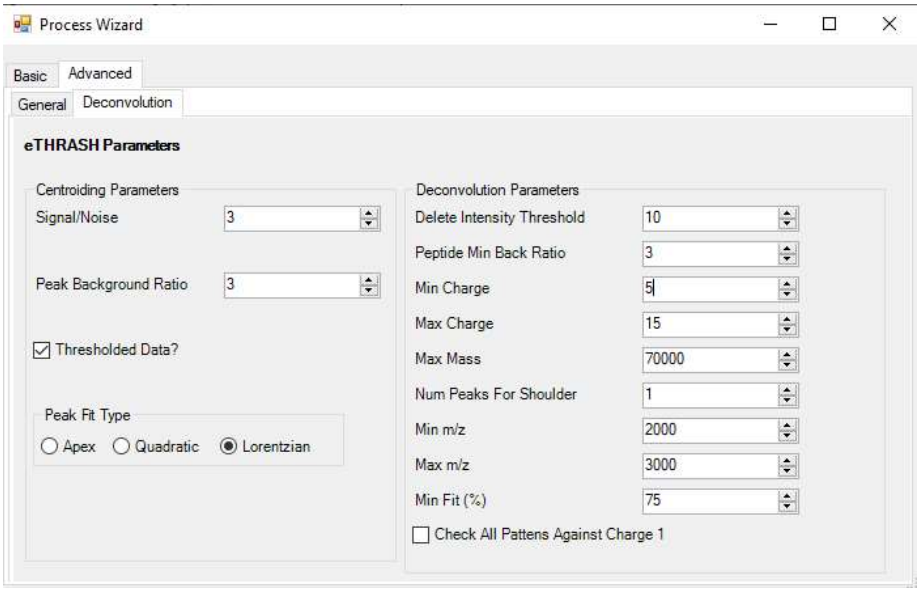
In a similar manner, import the HemoglobinNativeDimerIsolation2689p9.d data file for the isloation and the hemoglobin dimers and monomer dissociation experiment.





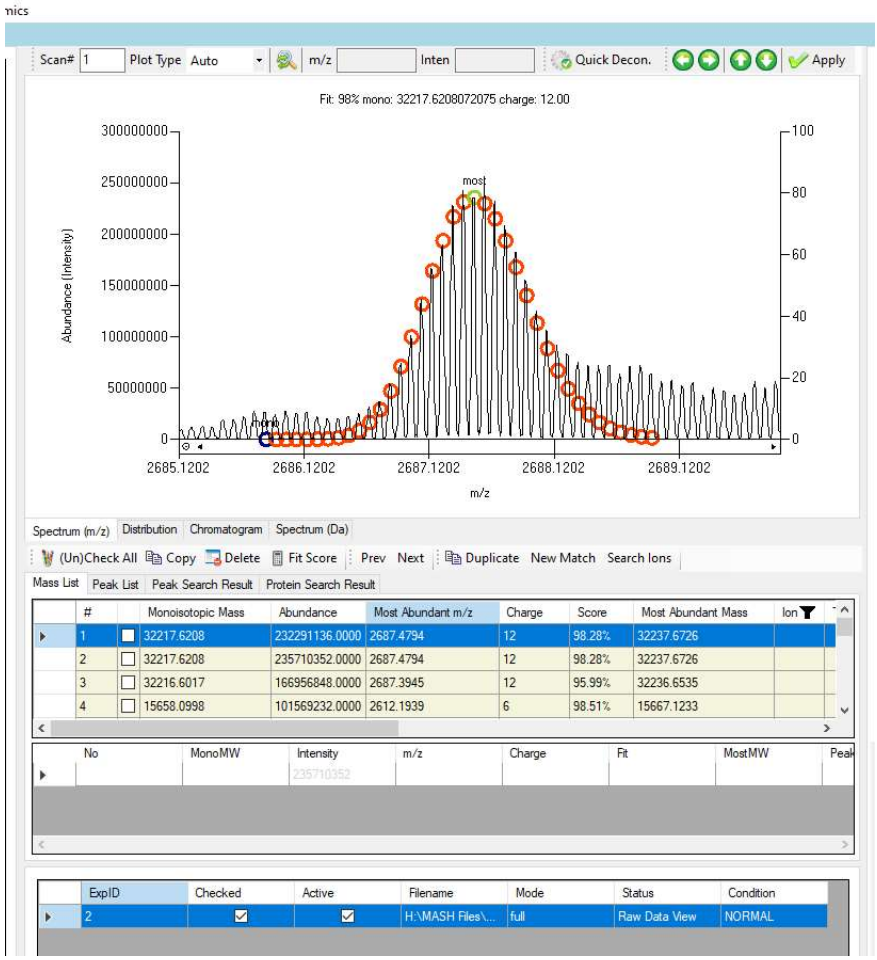
5. Deconvolution

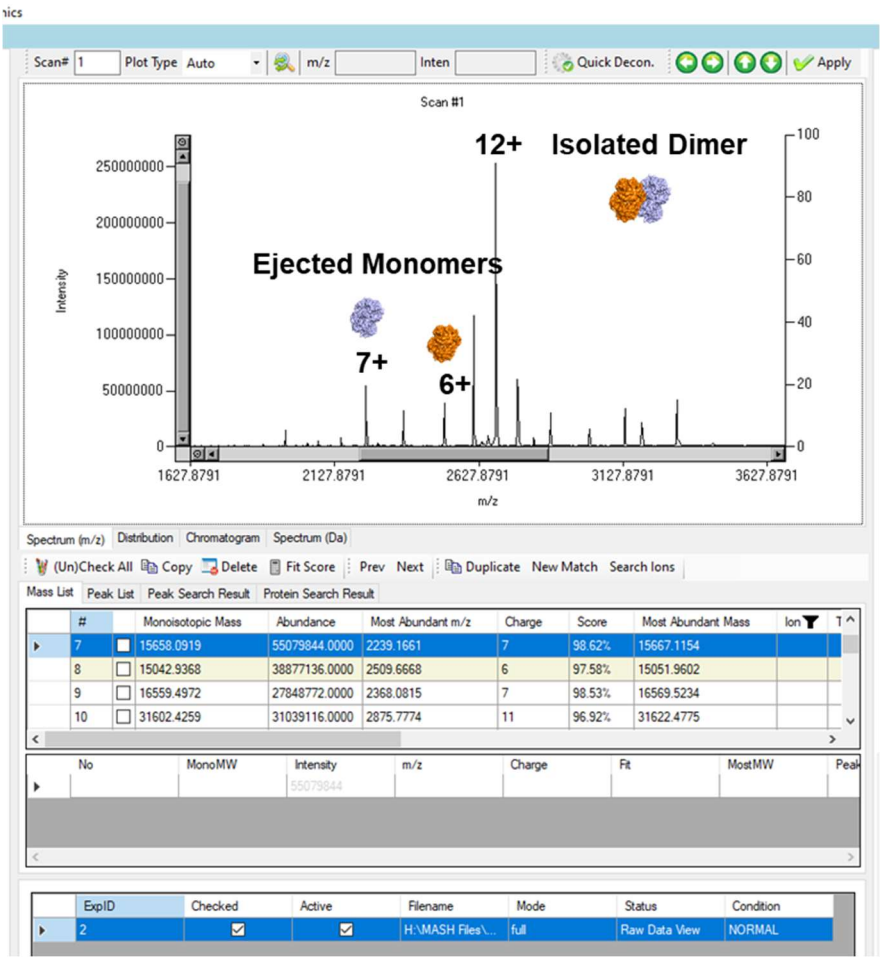
Deconvolute the spectra using the eTHRASH deconvolution algorithm and the advanced parameters shown below.



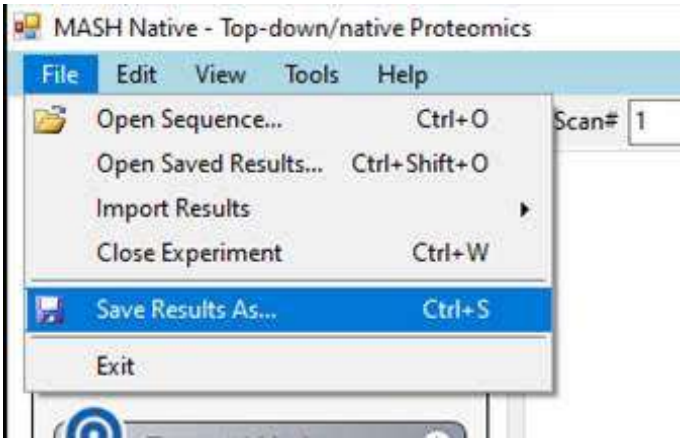
6. Review results

When the deconvolution finishes, results are automatically imported into the Mass List where they can be manually reviewed, and isotopic peak fit evaluated in a similar manner as above.





From this data, users can identify and annotate the ejected monomers. Users can click and drag on the spectrum to zoom in on a desired area of the spectrum, as seen in the photo above. Be sure to save your results as MASH will not prompt you to do so.

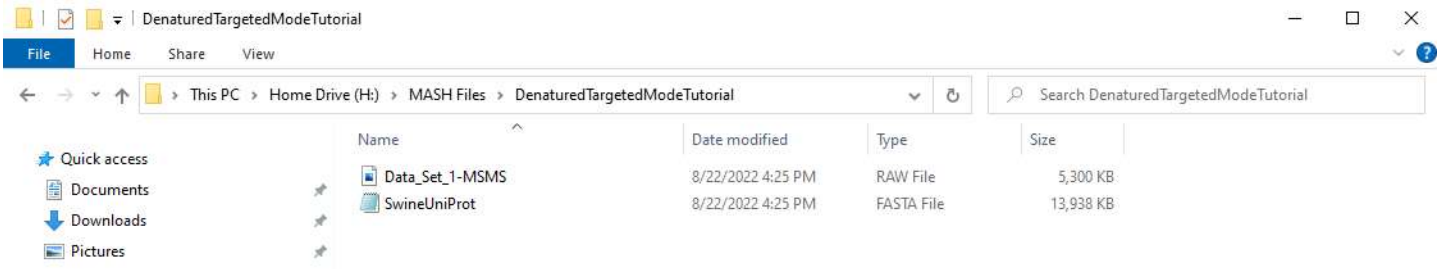


Analysis of denatured top-down mass spectrometry data:

For the analysis of denatured mass spectrometry data, download the folder containing the native tutorial files and save them to a new local folder as seen below.

Download the folder below on the Google Drive for the native targeted tutorial:

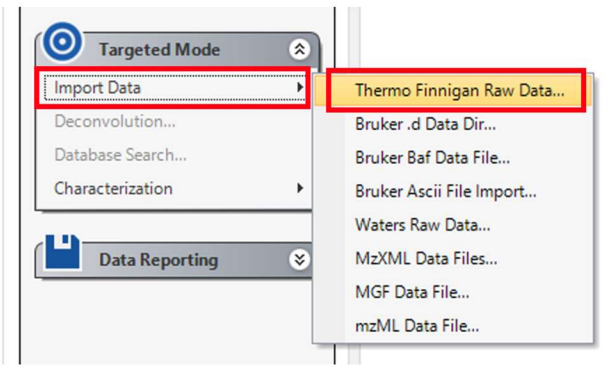
- Denatured Targeted Mode Tutorial folder which contains the Thermo Data_Set_1-MSMS.raw file and the SwineUniprot.fasta file.

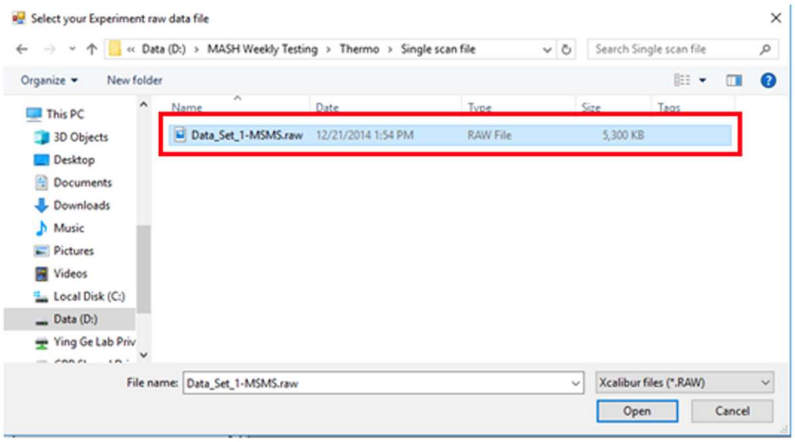


Proceed with the following steps for analysis of denatured MS/MS mass spectrometry data for protein characterization.

Step 1: Import data

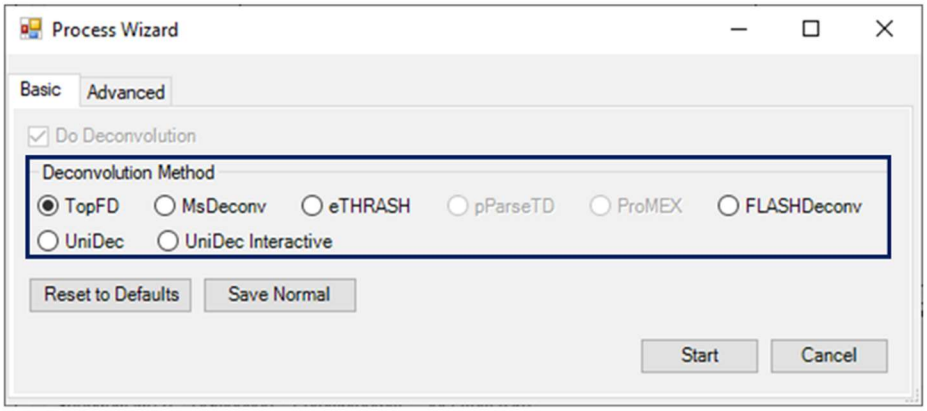
Use the Import Data function in Targeted Mode to import the sample Thermo data file.

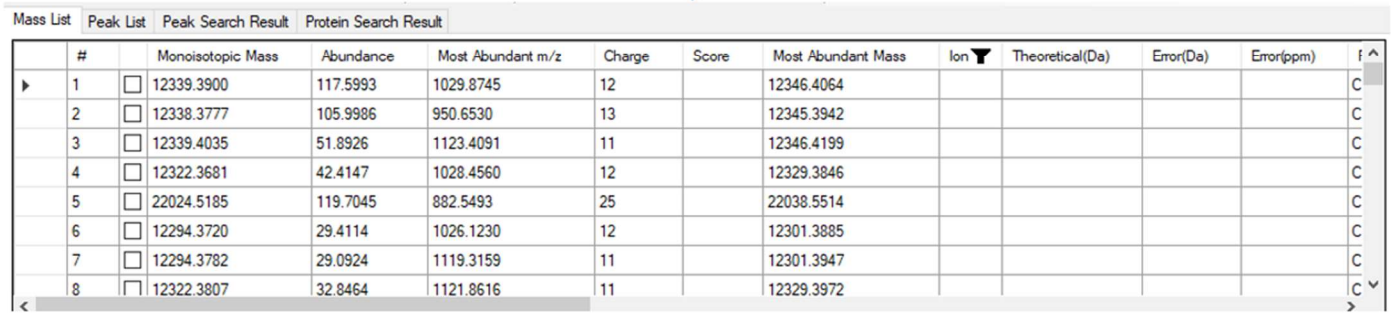




Step 2: Deconvolution

Under the Targeted Mode, Process Wizard will guide users through the deconvolution step. Select the TopFD deconvolution method and click the Start button. You have the option to set more advanced parameters in the Advanced tab, but this is not necessary to start your run. The Workflow Manager and terminal window will open. You can close the Workflow Manager if you wish, but do NOT close the terminal because this is where your computer is running TopFD. When the deconvolution is finished, the Mass List will automatically populate shown in the image below.



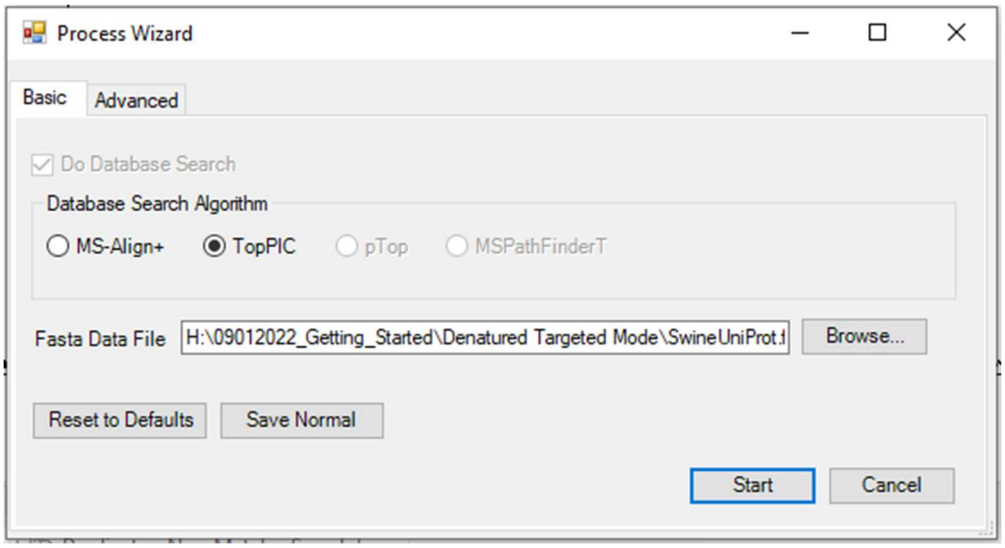


Mass List | Peak List | Peak Search Result | Protein Search Result

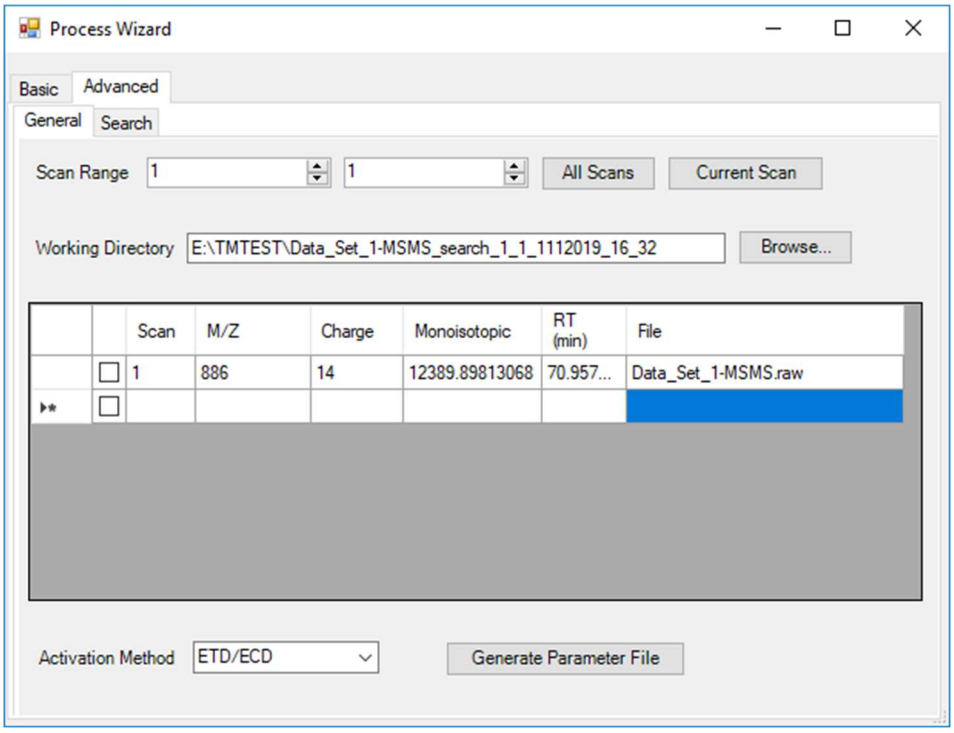
#		Monoisotopic Mass	Abundance	Most Abundant m/z	Charge	Score	Most Abundant Mass	Ion	Theoretical(Da)	Error(Da)	Error(ppm)	
1	☐	12339.3900	117.5993	1029.8745	12		12346.4064					C
2	☐	12338.3777	105.9986	950.6530	13		12345.3942					C
3	☐	12339.4035	51.8926	1123.4091	11		12346.4199					C
4	☐	12322.3681	42.4147	1028.4560	12		12329.3846					C
5	☐	22024.5185	119.7045	882.5493	25		22038.5514					C
6	☐	12294.3720	29.4114	1026.1230	12		12301.3885					C
7	☐	12294.3782	29.0924	1119.3159	11		12301.3947					C
8	☐	12322.3807	32.8464	1121.8616	11		12329.3972					C

Step 3: Database search

Following deconvolution, users can use the selected ion list for database search via the Process Wizard. In the Targeted Mode panel, select the Database Search function, which will open the Process Wizard. Select the TopPIC database search to run on the selected ion list shown in the Mass List. Click on the Browse button to load the SwineUniProt.fasta file.



This file requires Advanced Parameters to be set. Please click on the Advanced Parameters tab. Change the charge from 0 to 14 and hit Enter, so that the parameters in the list are exactly as shown below. The monoisotopic mass will automatically be calculated. Ensure that the Activation is set at ETD/ECD. Navigate back to the basic tab and click on the start button to begin your search.

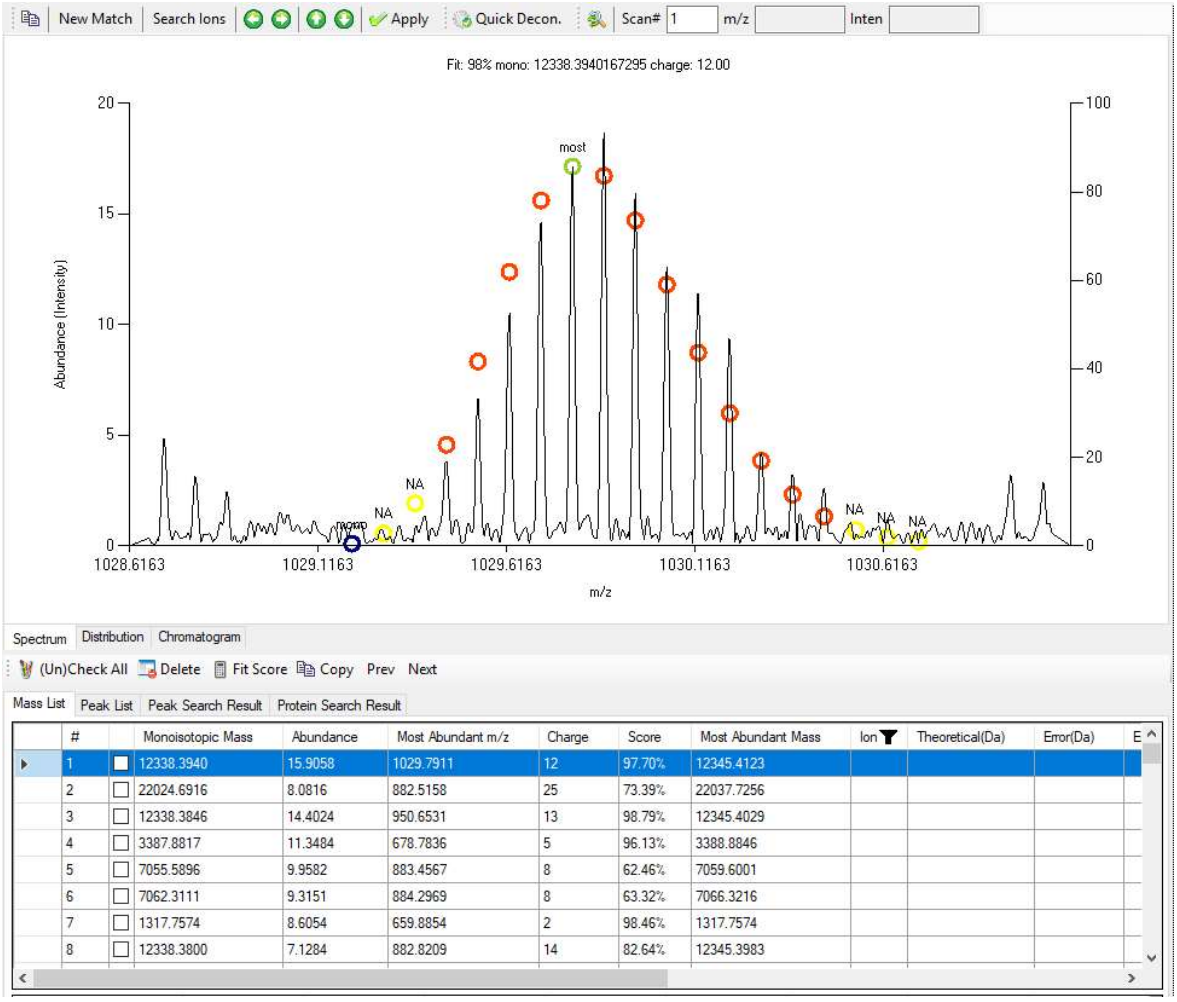


As before, the Workflow Manager and the terminal window will open. You can close the Workflow Manager if you wish, but do NOT close the terminal because this is where your computer is running TopPIC. When the search is finished, the terminal window will automatically close, the Log Book will show the line “Workflow Finished” and the Workflow Manager will move the workflow into the “Completed Workflows” Tab.

Your search results will be automatically imported into MASH Native. Look under the Protein Search Results list to view your results. If you would like to manually import your search results, please read Step 3: Import Results from the Discovery Mode and import your search results file with the Targeted Mode Characterization button.

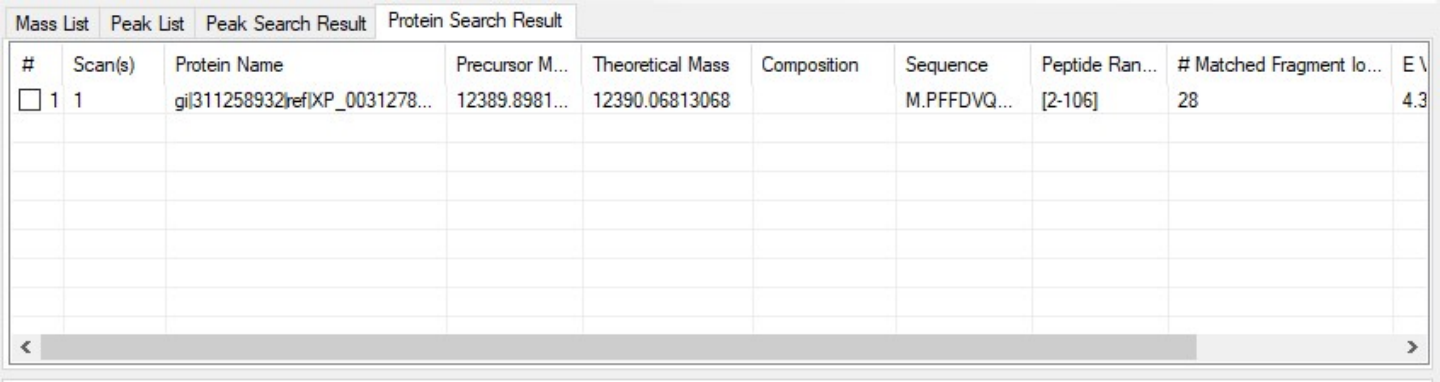
Step 4: Annotate results

MASH Native allows you to view and annotate your imported results with the Mass List and Protein Search Results List, the Results View, and the Sequence Table. For more details on these functions, refer to the *User Manual*.

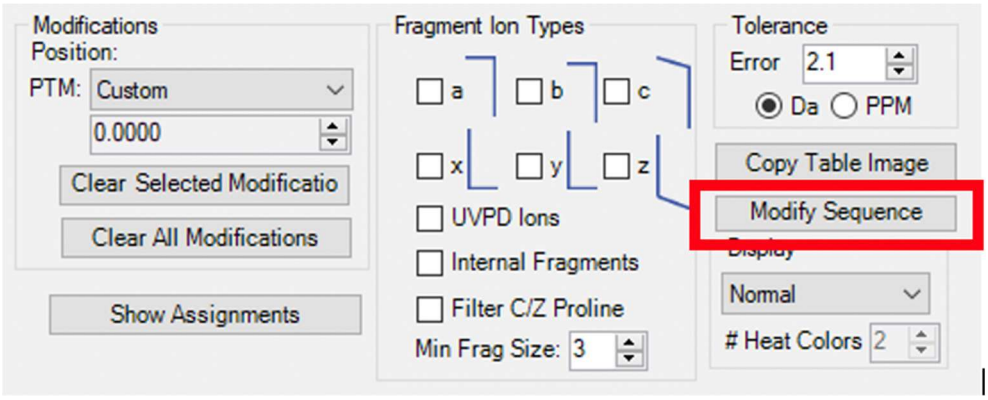


#		Monoisotopic Mass	Abundance	Most Abundant m/z	Charge	Score	Most Abundant Mass	Ion	Theoretical(Da)	Error(Da)	E
1	☐	12338.3940	15.9058	1029.7911	12	97.70%	12345.4123				
2	☐	22024.6916	8.0816	882.5158	25	73.39%	22037.7256				
3	☐	12338.3846	14.4024	950.6531	13	98.79%	12345.4029				
4	☐	3387.8817	11.3484	678.7836	5	96.13%	3388.8846				
5	☐	7055.5896	9.9582	883.4567	8	62.46%	7059.6001				
6	☐	7062.3111	9.3151	884.2969	8	63.32%	7066.3216				
7	☐	1317.7574	8.6054	659.8854	2	98.46%	1317.7574				
8	☐	12338.3800	7.1284	882.8209	14	82.64%	12345.3983				

Your TopPIC search results contain the protein sequence of the identified protein, which will be automatically imported. Go to the Protein Search Result list and click on the first result.

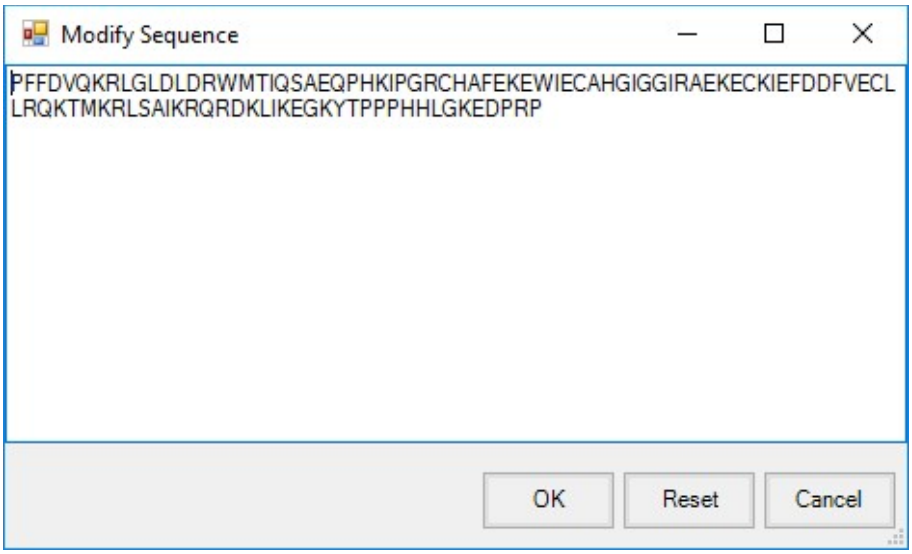


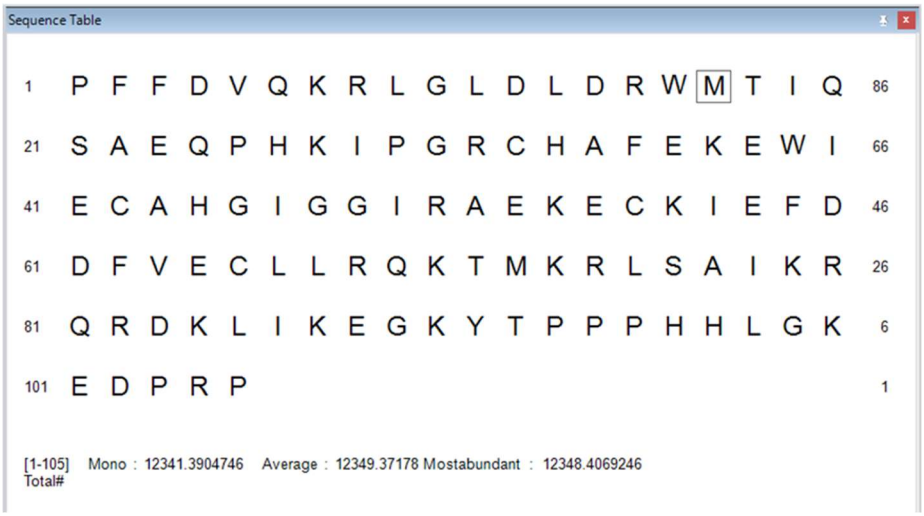
The Sequence Table will display this sequence. The protein sequence may need to be modified to remove some of TopPIC's notation. To do this, please click on the Modify Sequence button under the Sequence Table.



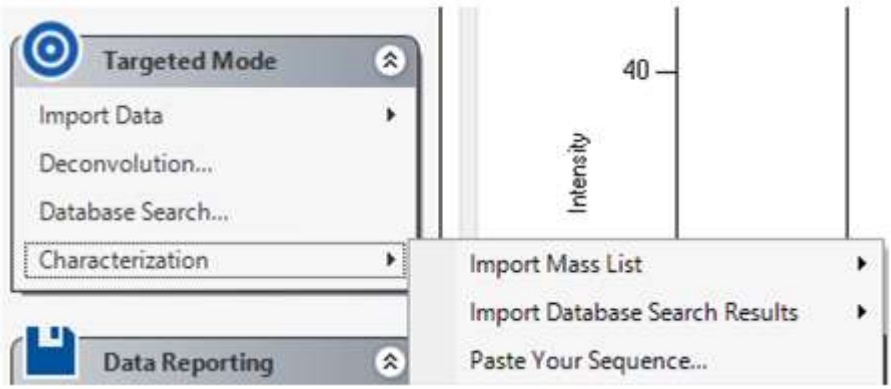
In the Modify Sequence Dialog, remove the following characters so that the sequence matches the image below: "M.", "(", ")" "[48.68]", and finally ".". Click OK to display these notation changes in the Sequence Table.

You can also manually type in (or copy/paste) the following sequence:
PFFDVQKRLGLDLDRWMTIQSAEQPHKIPGRCHAFEKEWIECAHGIGGIRAEKECKIEFDDFVECLLRQ
KTMKRLSAIKRQRDKLIKEGKYTPPPHHLGKEDPRP

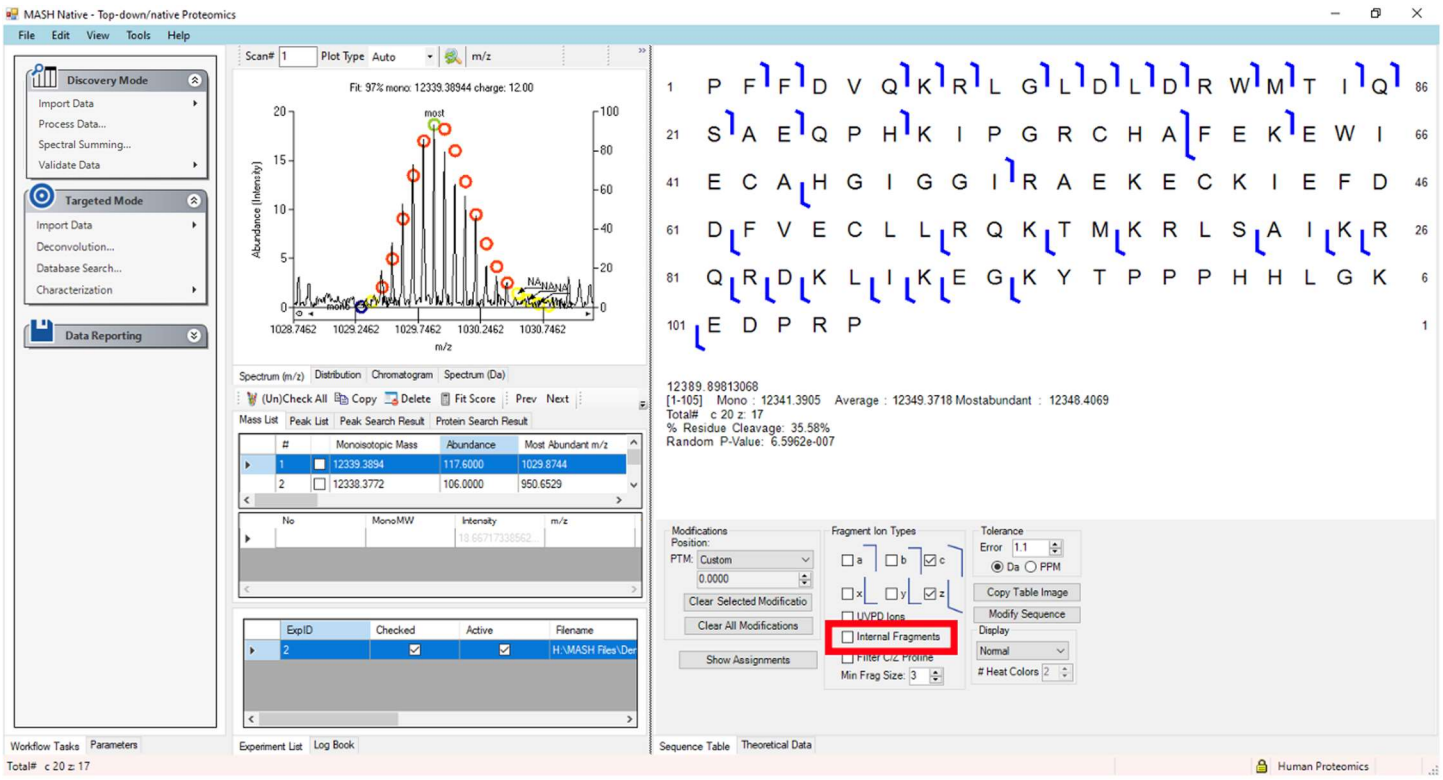




For manually pasting protein sequence, click on Paste Your Sequence and the Paste Sequence Information window will be opened. Paste the target sequence and click OK and the pasted sequence will be displayed in Sequence Table.



In the Sequence Table, check the c and z ion types and click on the Show Assignments button to view the fragmentation for that peptide sequence. If users want to view internal fragmentation, select the Internal Fragments checkbox before clicking the Show Assignments button, which matches monoisotopic masses with internal fragment ion types (e.g., cz). To search for internal ions such as *c* and *z*, the *c* and *z* ions should be checked on the interface. With internal fragmentation enabled, expect a longer wait time before the GUI becomes responsive. Users can click on each entry in the Mass List to see the profile data.

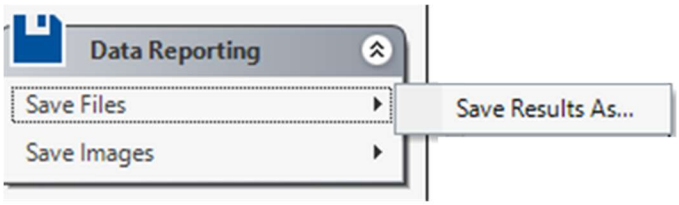


Step 5: Heat map

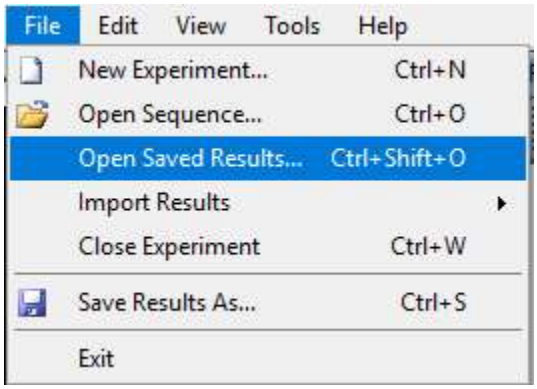
To view the heat map, select Heat Map in the menu under Display in the bottom right corner. This gives a visual representation of the relative number of ions in the mass list in which each amino acid is present. You can change the number of heat colors for the map, we have shown the map with 5 heat colors below.

Step 6: Save results

Saving annotated results is only available for Targeted Mode. In the Data Reporting panel click Save Files and then Save Results As. Your Mass List and Protein Search Results list will be saved as an XML file. Save this XML file in the same directory as your raw data file.



To load a saved XML file into MASH Native, in the top left-hand corner go to File and click Open Saved Results.



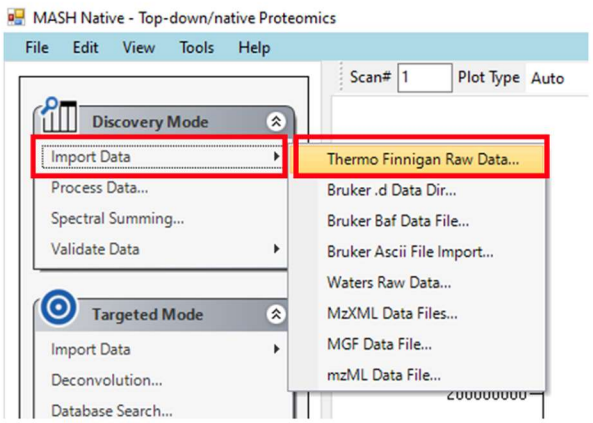
Spectral Summing:

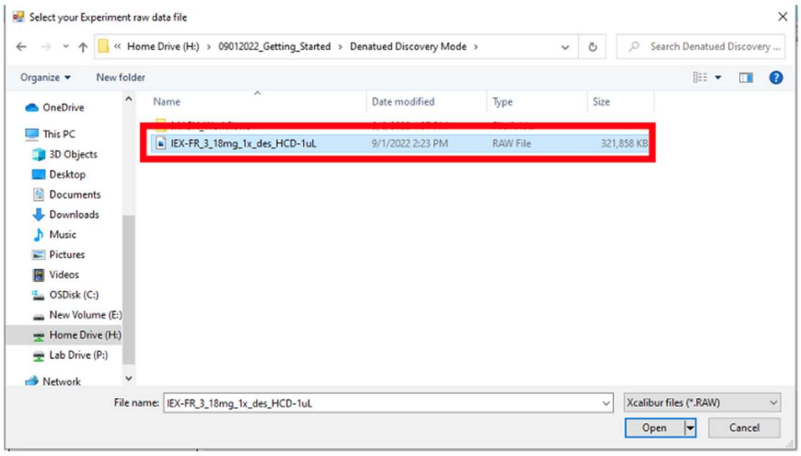
Sample Files: Use either of the files for discovery mode above.

- Sample Thermo raw data: IEX-FR_3_18mg_1x_des_HCD-1uL.raw.
- Sample 32-bit mzXML file: IEX-FR_3_18mg_1x_des_HCD-1uL.mzXML

Step 1: Import data

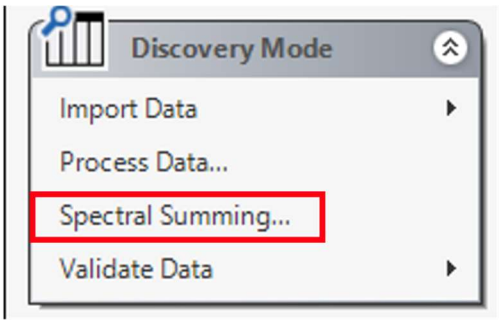
Use the Import Data function in the Discovery Mode to import the sample Thermo raw data file from the Denatured Discovery Mode Tutorial.



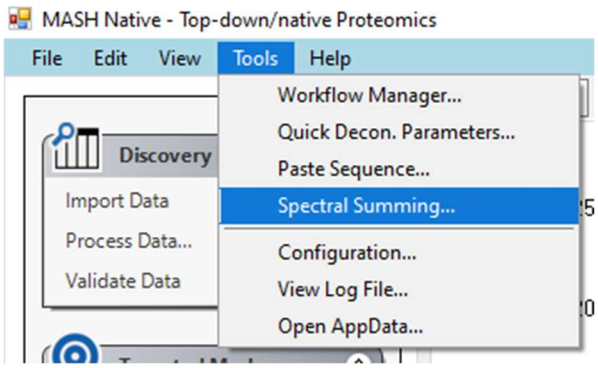


Step 2: Open spectral summing tool

Under the Discovery Mode panel, select Spectral Summing”



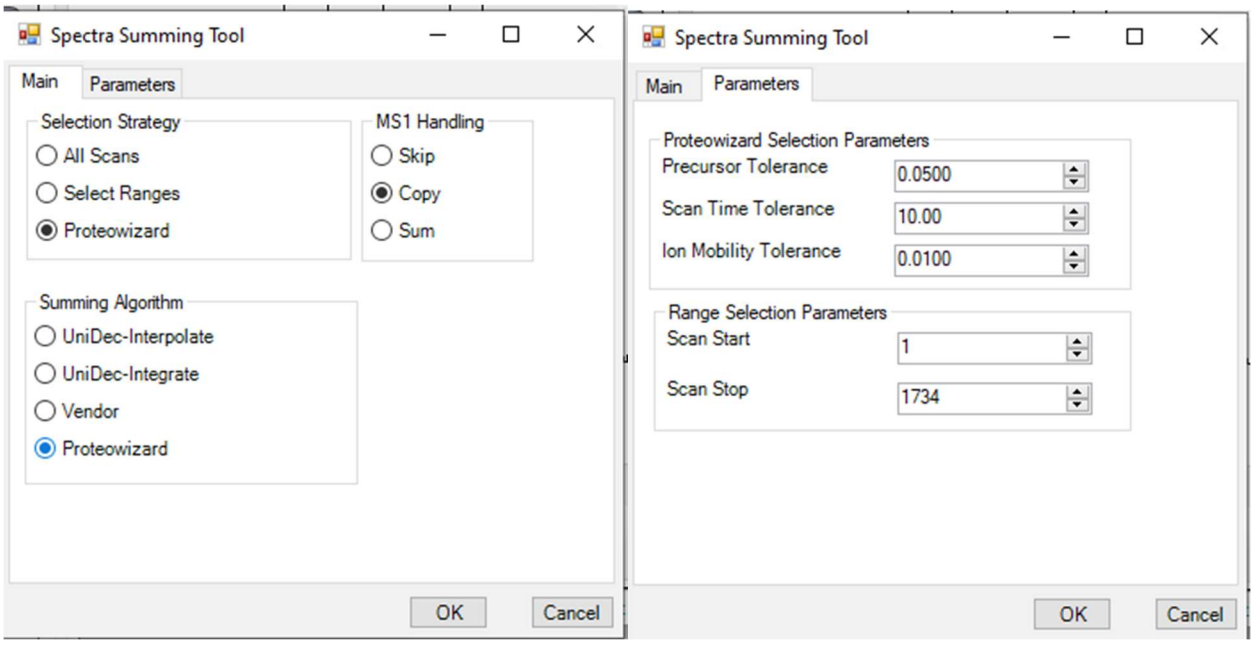
The user can also open the spectral summing tool from the “Tools” Tab.



Step 3: Set parameters

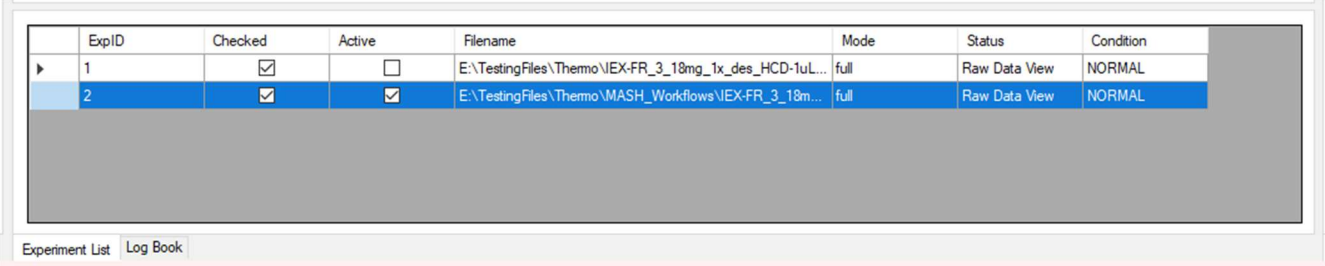
In the Spectra Summing Tool dialog, select "Proteowizard" for both the Selection Strategy and Summing Algorithm choices. Observe the parameters for running the Proteowizard selection in the parameters tab.

Click OK and the summing process should be started as a workflow and can be accessed using the workflow manager. Should take approximately 2-3 minutes to complete.



Step 4: Observe/process new experiment

Once the summing algorithm completes, a new .mzML has been generated and automatically loaded into MASH Native. You can observe the summed and original version of the experiment under the "Experiment List" in the bottom center of the MASH Native application. You can then process this experiment as you would in the Discovery Mode step mentioned above.



The mzML file of the summed scans is placed in a workflow folder, which you can find the location through the workflow manager. See User Manual for more details on using the workflow system and for more details on the other summing routes that MASH Native provides.

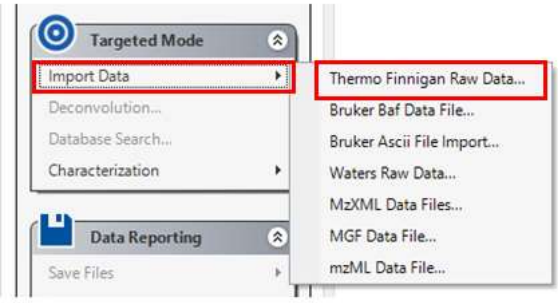
Spectral Deconvolution using UniDec:

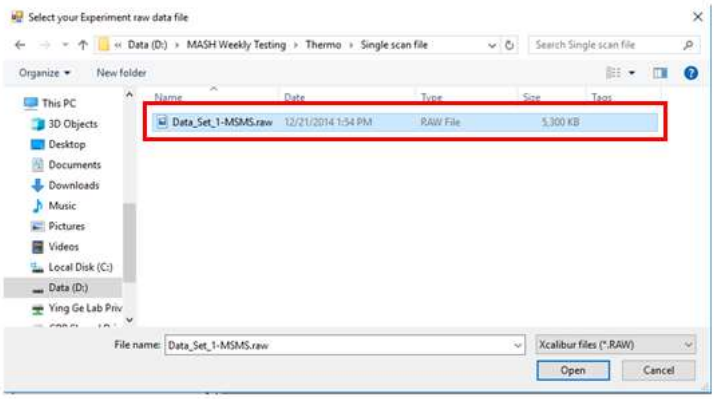
Sample Files: Use either of the targeted mode files above:

- Sample Thermo raw data: Data_Set_1-MSMS.raw.
- Sample 32-bit mzXML file: Data_Set_1-MSMS.mzXML.

Step 1: Import data

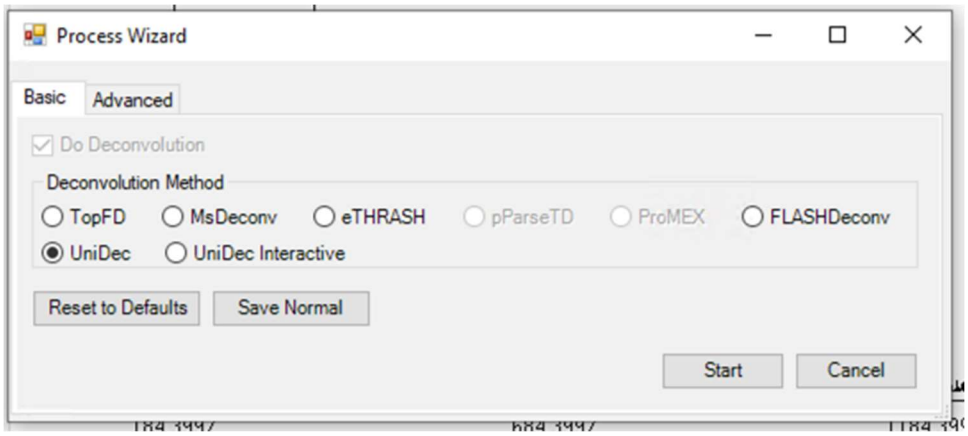
Use the Import Data function in Targeted Mode to import the sample Thermo data file from the Denatured Targeted Mode Tutorial.





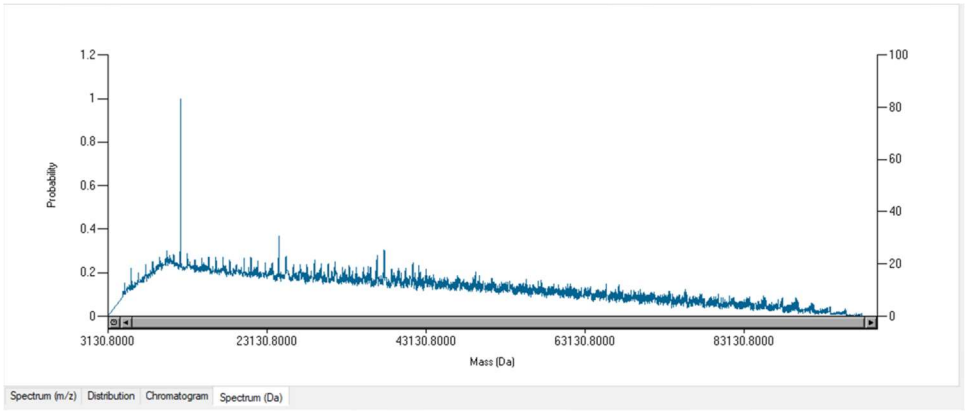
Step 2: Run UniDec deconvolution

Under the Targeted Mode, Process Wizard will guide users through the deconvolution step. Select the UniDec deconvolution method and click the Start button.



Step 3: Observe Mass List and deconvoluted spectrum results

Upon completion the Mass List will be populated using UniDec's results as previously described in the targeted mode spectrum above. The extracted deconvoluted spectrum can be observed by selecting the "Spectrum (Da)" tab in the spectrum view panel.



The zoom tools should work on the spectrum. Please see the "Best Practices for UniDec Deconvolution in MASH" file in the documentation for running UniDec on your particular type of targeted mode data.

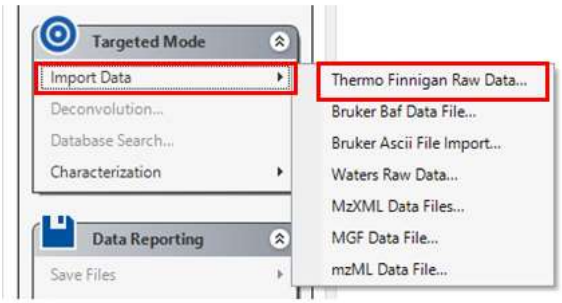
PTM Analysis using UniDec:

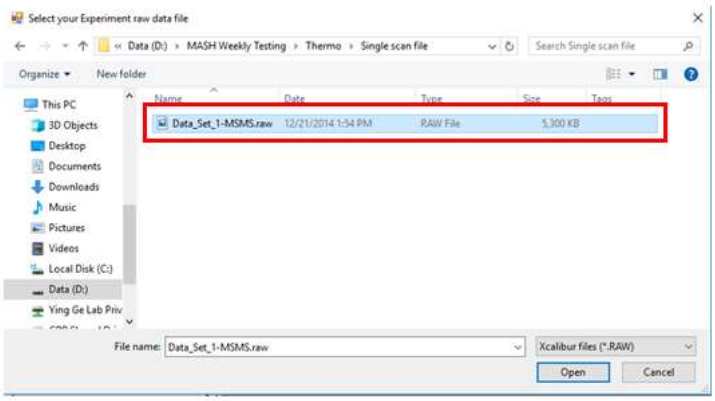
Sample Files: Use either of the targeted mode files below:

- Sample Thermo raw data: Data_Set_1-MSMS.raw.
- Sample 32-bit mzXML file: Data_Set_1-MSMS.mzXML.

Step 1: Import data

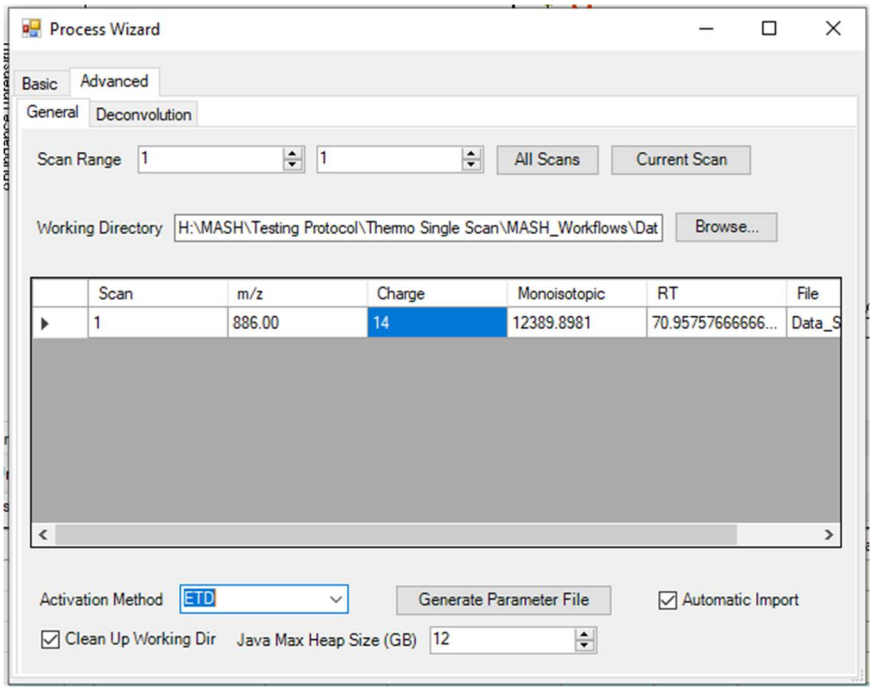
Use the Import Data function in Targeted Mode to import the sample Thermo data file.



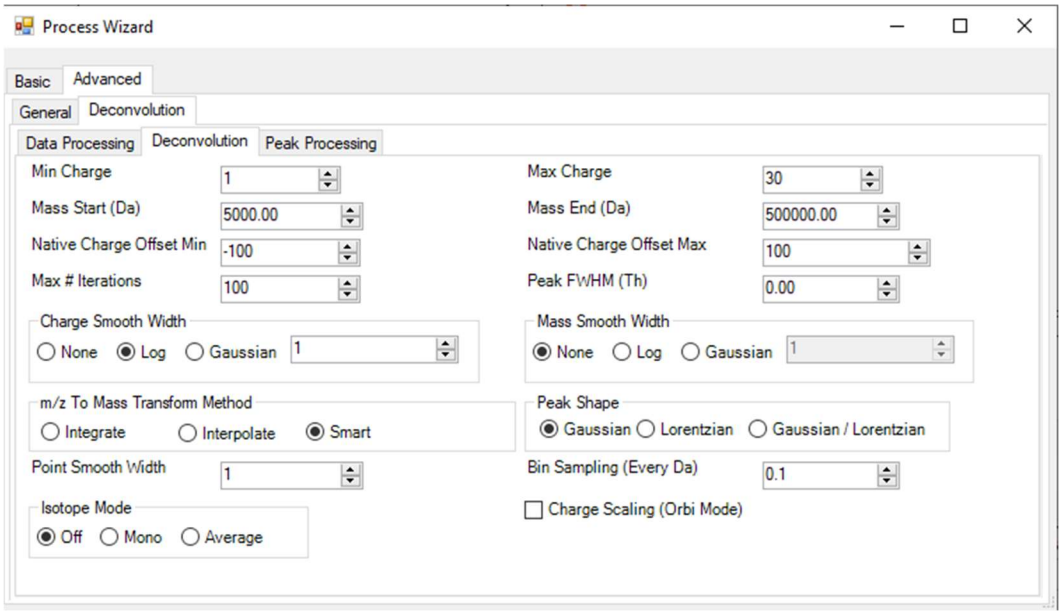


Step 2: Run UniDec deconvolution

Under the Targeted Mode, Process Wizard will guide users through the deconvolution step. Select the UniDec deconvolution method, under advanced, change the charge to 14, and change the activation method to ETD.



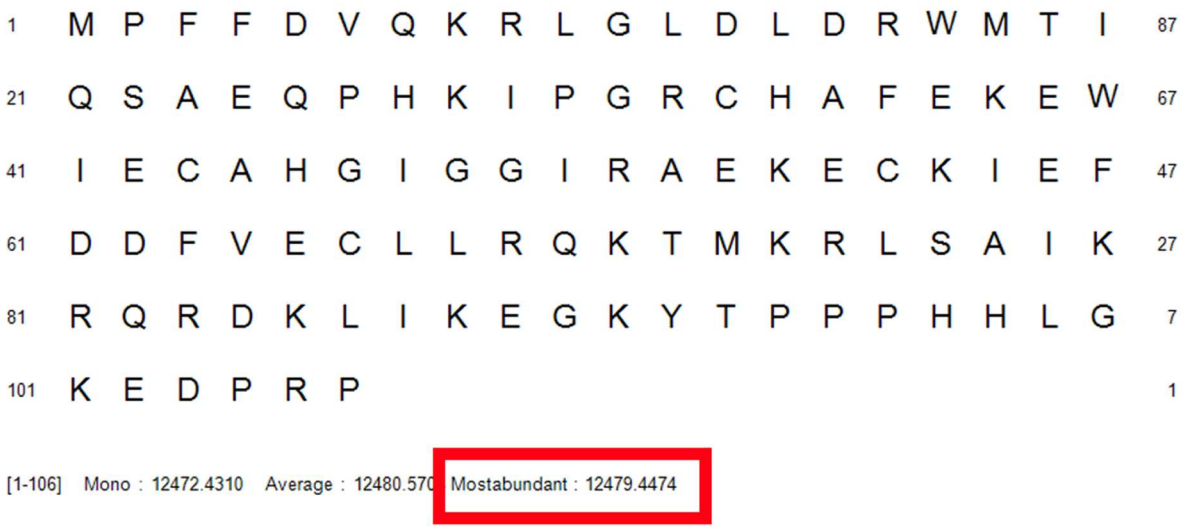
Then under Deconvolution → Deconvolution, change the max charge to 30 and bin sampling to 0.1, seen in the figure below, then go back to the Basic Tab and press “Start”.



Step 3: Import Sequence

- Sequence: NDUFS5 Sequence.

Copy the NDUFS5 protein sequence from the sequence .txt file. In MASH, click on "Modify Sequence" in the bottom left corner and the "Paste Sequence Information" window will open. Paste the sequence and click "OK" and the sequence will be displayed in the Sequence Table. In the table, it will calculate the most abundant mass of the sequence.



Step 4: Review mass list

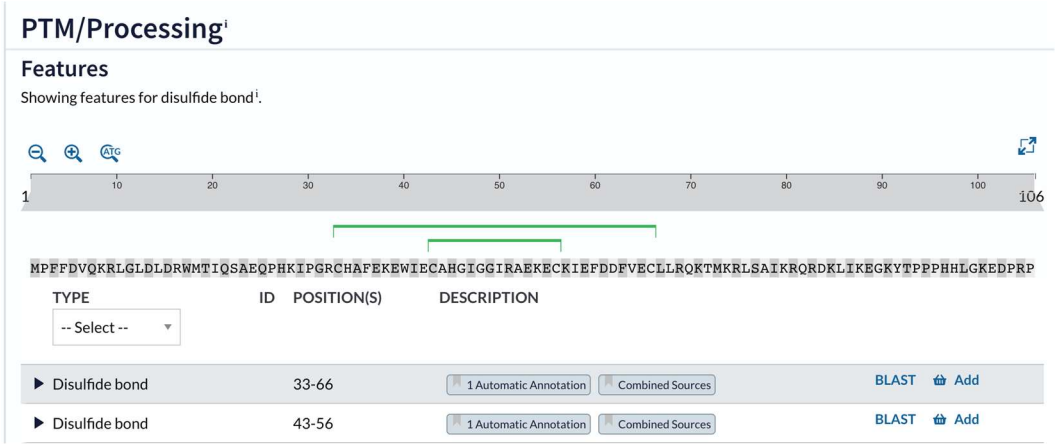
Upon completion the mass list will be populated using UniDec's results. Now, manually review the deconvolution and isotopic fit mass list to identify a high score charge assignment of most abundant masses that is closest to the Most Abundant Mass of the sequence.

#		Monoisotopic Mass	Abundance	Most Abundant m/z	Charge	Score	Most Abundant Mass
56	☐	0.0000	0.0930	1123.4073	11	94.88%	12346.4004
57	☐	0.0000	0.2607	1029.8740	12	97.48%	12346.4004
58	☐	0.0000	0.2002	950.7304	13	87.20%	12346.4004

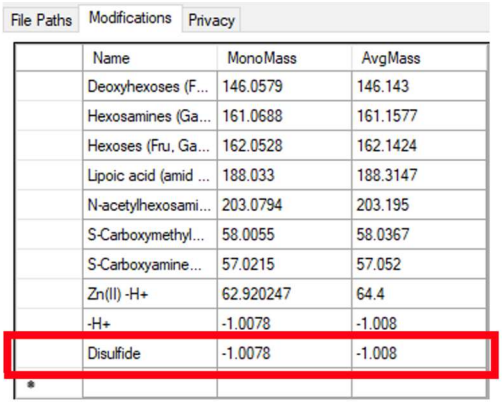
Step 5: Post-Translational Modifications

- NDUFS5 UniProt: https://www.uniprot.org/uniprotkb/F1SV23/entry

Using the UniProt link, identify the post-translational modifications (PTMs) for the NDUFS5 protein. From here, the user will see that there are two disulfide bonds in the sequence.

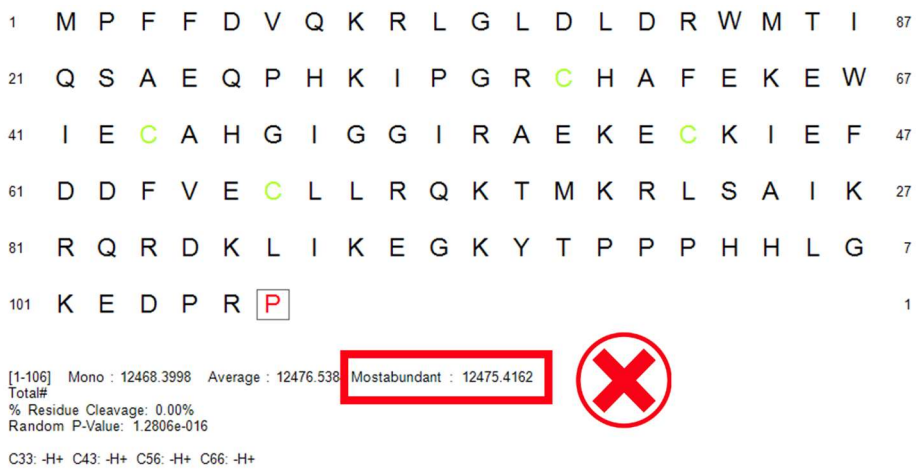


Under Tools → Configuration → Modifications, add a disulfide bond into the table with the respective masses shown in the figure below.



	Name	MonoMass	AvgMass
	Deoxyhexoses (F...	146.0579	146.143
	Hexosamines (Ga...	161.0688	161.1577
	Hexoses (Fru, Ga...	162.0528	162.1424
	Lipoic acid (amid ...	188.033	188.3147
	N-acetylhexosami...	203.0794	203.195
	S-Carboxymethyl...	58.0055	58.0367
	S-Carboxyamine...	57.0215	57.052
	Zn(II) -H+	62.920247	64.4
	-H+	-1.0078	-1.008
	Disulfide	-1.0078	-1.008
*			

Then using the PTM drop down box under the sequence table, add a disulfide to C33, C43, C56, and C66, then select “Show Assignments.” This will change the most abundant mass in the sequence table. If this mass matches the most abundant mass in the mass list, then these are the correct PTMs on your sample protein. If the masses do not match, then the combination of PTMs is not correct for your sample.



Because the masses do not match, the user must identify the PTMs based on the difference in masses. The difference between the mass in the mass list, and the raw sequence mass is 133.047 Da. From this, we can identify PTMs as the removal of the initiator methionine and one disulfide bond.

Under “Modify Sequence” delete M1, then in the PTM drop down box, add a disulfide bond to C43 and C56, and select “Show Assignments.” Now the most abundant mass in the sequence table is extremely close to the mass in the mass list and can be assumed as the modified sequence.