Converting Raw LC-HRMS/MS Files into mzML files

B. Place

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In order to use the data analysis tools, all files must be converted into *.mzML files. In order to convert proprietary vendor files, all users *must* download ProteoWizard MSConvert tool, which can be acquired by going to https://proteowizard.sourceforge.io/ and downloading the most recent version of ProteoWizard.

Once downloaded and install, follow the next steps to convert the raw file(s) to *.mzML format.

1) Run ProteoWizard MSConvert program

Select the program from the start Menu > Proteowizard > MSConvert

🖳 MSConvertGUI (64-bit)		– 🗆 X
List of Files File of file names File: Browse	Browse network resource	~ About MSConvert
Outrue Directory:	Filters CWT (1	Peak Picking Algorithm: continuous wavelet transform; works for any profile data) IS Levels: Min SNR: Min peak spacing: - 0.1 0.1
Browse		Add Remove
Output format: mzMI V Extension:	Filter	arameters
Binary encoding precision:	titleMaker <	RunId>. <scannumber>.<scannumber>.<chargestate> File:"<sourcepath>", Nati</sourcepath></chargestate></scannumber></scannumber>
Write index:	threshold al	solute 1 most-intense
TPP compatibility: Package in gzip:	peakPicking c	vt snr=0.1 peakSpace=0.1 msLevel=1-
Use numpress linear compression:		
Use numpress short logged float compression:		
Use numpress positive integer compression:		
Combine ion mobility scans:		
SIM as spectra: SRM as spectra:		
Presets: Generic Defaults	✓ Save Preset ▼	Files to convert in parallel: 10 🖨 Start

Figure 1: Initial screen when running MSConvert

2) Select the files to be converted

The MSConvert software can convert the following vendors:

Thermo (*.raw), Waters (*.raw), SCIEX (*.wiff2), Agilent (*.D), Shimadzu (*.LCD,) Bruker (*.D)

• Select the files using the **Browse** button.

Note: If selecting only one file at a time, you must press the Add button to include the file in the list.

• Select the *Output Directory* Folder

Note: It will default to the same directory as the original file.

After loading the file, the program should look like the below image.

🖳 MSConvertGUI (64-bit)			– 🗆 ×
List of Files File of file names File: Add Remove S_PFAC30PAR_Spectra\PFAC30PAR_PFCA2.raw <	Browse network resource Filters MS lev Scan numb Scan time (second Scan eve	Subset Subset Subset Subset Charge states: er: - Number of data points: s): - Collision energy: ent: - Activation type:	About MSConvert
Output Directory:	Scan polar	Add Remove	viy ~
Output format: mzML V Extension:	Filter	Parameters	
Binary encoding precision: 64-bit 32-bit	titleMaker	<runid>.<scannumber>.<scannumber>.<chargestate< td=""><td>> File:"<sourcepath>", Nati</sourcepath></td></chargestate<></scannumber></scannumber></runid>	> File:" <sourcepath>", Nati</sourcepath>
Write index: 🔽 Use zlib compression: 🔽	threshold	absolute 1 most-intense	
TPP compatibility: Package in gzip:	peakPicking	vendor msLevel=1-2	
Use numpress linear compression:			
Use numpress short logged float compression:			
Use numpress positive integer compression:			
Combine ion mobility scans:			
SIM as spectra: SRM as spectra:			
Presets: Generic Defaults	Save Preset	Files to convert in para	llel: 10 🚖 Start

Figure 2: MSConvert with data file loaded

3) Adjust the mzML file parameters

- Select mzML from the *Output format* input and make sure the *Extension* input is blank
- Select 64-bit under Binary encoding precision
- Check the box next to *Write Index*, *TPP Compatibility*, and *Use zlib compression* and leave all other boxes unchecked.

See the image above for the proper selection

4) Add the conversion filters

To use the data analysis tool the following filters must be used, after selecting the proper parameters click the **Add** button:

Select the *Peak Picking* filter and include the following parameters:

• Algorithm: Vendor or CWT

Note: Vendor filter does not work for Waters files

• MS Levels: 1 -

If using CWT:

- Min. SNR: 0.1
- Min. peak spacing: 0.1

Parameters should look as follows

Filters	Peak Picking ~
	Algorithm: Vendor (does not work for UNIFI, and it MUST be the first filter!) ~
	MS Levels: Min SNR: Min peak spacing: 1 - 0.1 0.1
	Add Remove

Figure 3: Peak picking filter parameters

Press the \mathbf{Add} Button

Select the *Threshold Peak Filter* and include the following parameters:

- Threshold type: Absolute intensity
- Orientation: Most intense
- Value: 1

Parameters should look as follows

Filters	Threshold F	⁹ eak Filter	~	
	Threshold type: Orientation: Value:	Absolute intensity Most intense 1	~	
	Ac	dd Remove		

Figure 4: Threshold peak filter parameters

Press the \mathbf{Add} Button

Note: The TitleMaker filter shows up upon opening MSConvert every time, this can be included and will not affect the data analysis.

5) Press Start Button

This will convert all added files to *.mzML format and put them in the assigned Output directory.

Filter	Parameters
peakPicking	vendor msLevel=1-
threshold	absolute 1 most-intense
Save Preset	Files to convert in parallel: 10 🚖 Start

Figure 5: MSConvert Start button

6) Save the settings as a preset using the *Save Preset* dropdown menu (optional)

Save Preset	
Save Preset As	
Save as defaults for Thermo RAW data	

Figure 6: MSConvert presets menu