

## How to use software MS\_Piano, NISTms-gads and convert2msp

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- I. **MS\_Piano 4.3 (Can be directly used without installation):** This software tool can be used for fast and reliable annotation of high- and low- resolution tandem mass spectra of CID and ETD for peptides, N- and O- linked glycopeptides in positive and negative modes.

1. Download MS\_Piano\_exe and unzip the folder. MS\_Piano is ready for use.
2. You can use in.msp file to test the program. Open the Command Prompt by searching "Command Prompt" on your computer.

If you copy the MS\_Piano\_exe to C: drive, type the following 3 lines:

```
cd \
```

```
cd MS_Piano_exe
```

```
MS_Piano in.msp out.msp
```

MS\_Piano will generate out.msp in the MS\_Piano\_exe folder. out.msp should be the same as out\_example.msp.

The default mass range is 20ppm. You can change it for high resolution spectra by using

```
MS_Piano in.msp out.msp -r Xppm      (X could be any integers, e.g. 1, 2, 5...)
```

or for low resolution spectra by using

```
MS_Piano in.msp out.msp -r XDa      (X could be any values, e.g. 0.4, 0.6...)
```

The followings are optional:

Check potential contaminants from NeuAc: default is off; use -c S

Annotating ETD spectra: default is off; ETD

Output loss from precursor: default is off; use -l loss

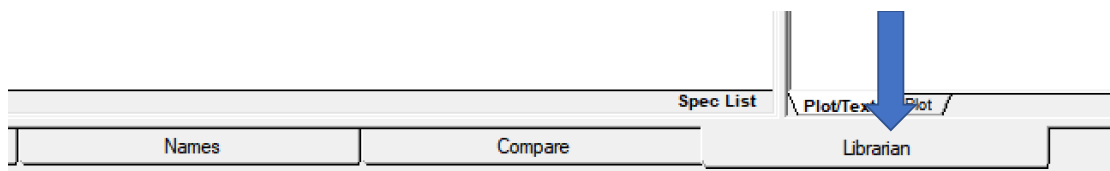
Print annotations sorted by mass difference for high res. spectra: default is off; use -p s

```
MS_Piano "C:\test\test.msp" "C:\test\test_annotated.msp" -l loss -c S -p s ETD
```

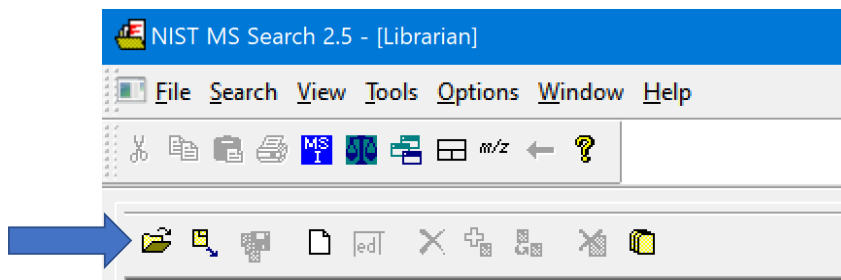
3. You can add a peptide modification with name and formula separated by tab in mod\_added.txt.

## II. View annotated mass spectra with NISTms-gads 1.0:

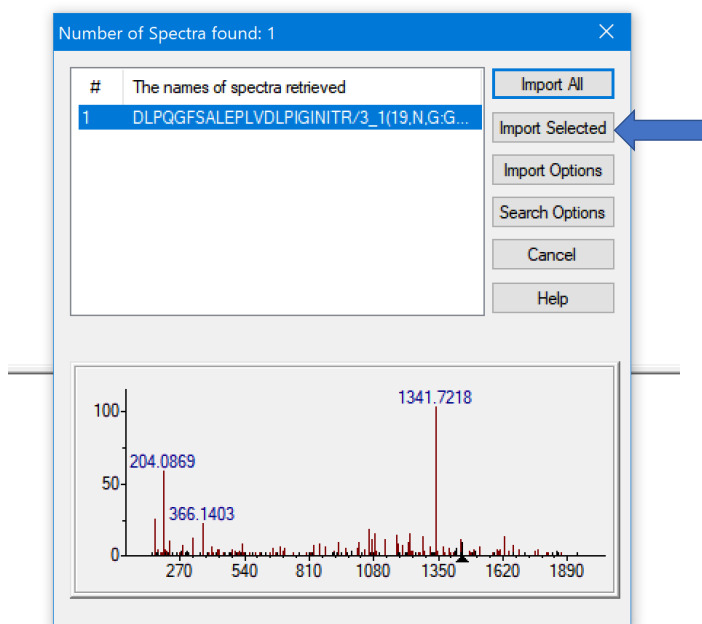
1. Go to <https://chemdata.nist.gov/dokuwiki/doku.php?id=peptidew:nistmsgads> and download NISTms-gads and unzip it.
2. Click on nistms-gads.exe in the NISTMS-GADS folder.
3. Once nistms-gads is open, click on Librarian tab at the bottom.



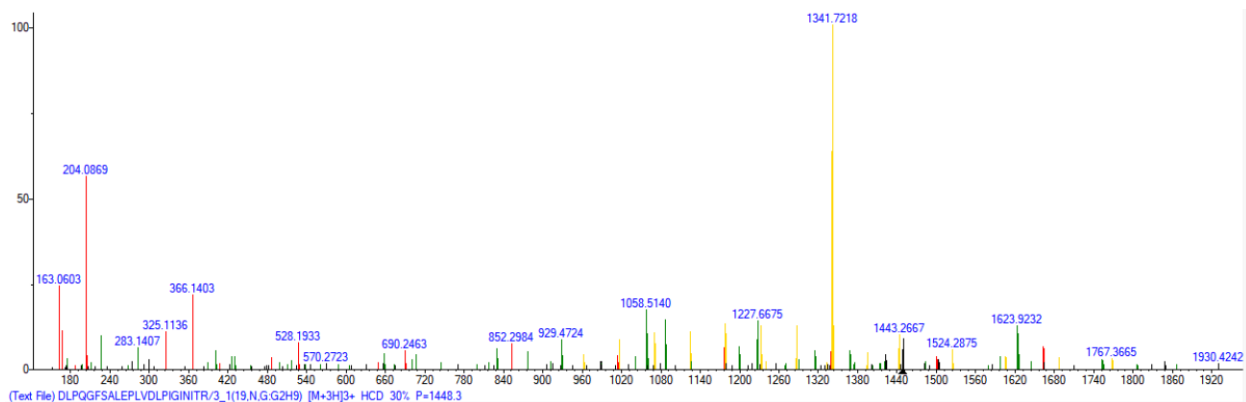
- Click on file open button on the top, go to the MS\_Piano\_exe or another folder.



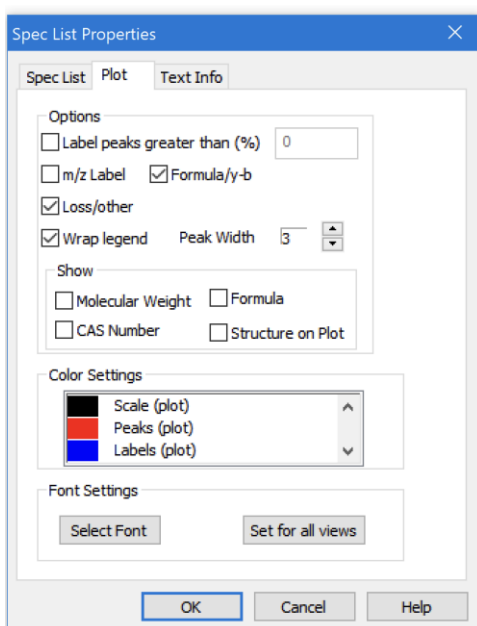
- Select out.msp and select the spectra. Click on "Import Selected".



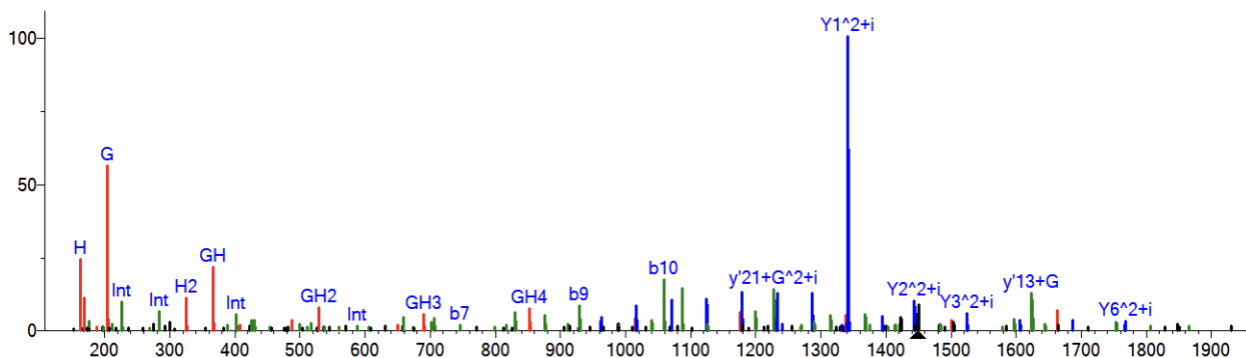
- You will see the spectrum.



7. Right click on the spectrum and select Property. You can change the color, fonts and ions, etc.



8. You will see peaks of different ions labeled with different colors.



### III. convert2msp 1.0 (can be directly used without installation):

This software tool converts the tsv and calibrated mgf files from MSFragger searching and the txt and mgf files from pGlyco searching to msp files respectively. It also annotates spectra in the converted msp files.

When you download MS\_Piano\_exe, convert2msp will in the same folder. You can use the files in MSFragger and pGlyco folder to test the program.

Open the Command Prompt by searching "Command Prompt" on your computer.

1. If you copy the MS\_Piano\_exe to C: drive, type the following 2 lines:

```
cd \
```

```
cd MS_Piano_exe
```

For MSFragger, type

```
convert2msp "C:\MS_Piano_exe\MSFragger\psm.msp MSFragger
```

convert2msp will generate psm.msp in C:\MS\_Piano\_exe\MSFragger folder. It should be the same as psm\_example.msp.

For pGlyco, type

```
convert2msp "C:\MS_Piano_exe\pGlyco\pGlycoDB-GP-FDR-Pro-Quant-Site.msp pGlyco
```

convert2msp will generate pGlycoDB-GP-FDR-Pro-Quant-Site.msp in C:\MS\_Piano\_exe\pGlyco folder. It should be the same as pGlycoDB-GP-FDR-Pro-Quant-Site\_example.msp.

2. To use Hyperscore in MSFragger or TotalScore in pGlyco as a threshold of any values:

e.g. convert2msp "C:\MS\_Piano\_exe\MSFragger\psm.msp MSFragger score 5

e.g. convert2msp "C:\pglyco\pGlycoDB-GP-FDR-Pro-Quant-Site.txt" pGlyco score 5

3. To annotate spectra in the converted msp file:

e.g. convert2msp "C:\MSFragger\psm.tsv" MSFragger annotation

e.g. convert2msp "C:\pglyco\pGlycoDB-GP-FDR-Pro-Quant-Site.txt" pGlyco annotation