How to use software MS_Piano Version 1.0 (Mass Spectrum Peptide Annotation, for annotating peaks in CID tandem mass spectra of peptides and N-glycopeptides) and convert2msp

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I. MS_Piano.exe program (can be directly used without installation):

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…)

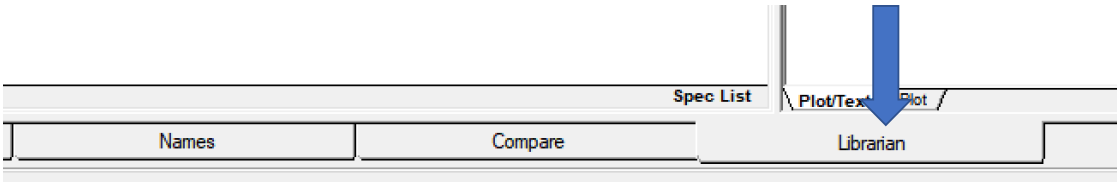
   You can also use add "-p n" for silent processing without any messages.
3. You can add a peptide modification with name and formula separated by tab in mod_added.txt.

- **MS_Piano .dll (for programmers use, peptide annotation only):**

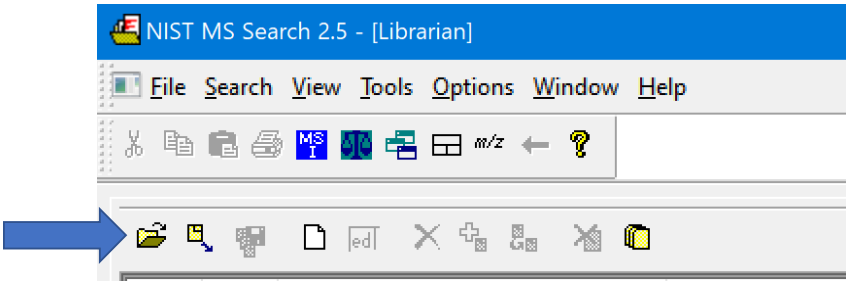
1. Download MS_Piano_dll and unzip the folder.
2. You can use MS_Piano_dll_app.cpp and MS_Piano_dll_app.h as an example to run MS_Piano_dll.dll.
3. You can add a peptide modification with name and formula separated by tab in mod_added.txt.

- **View annotated mass spectra with NISTms-gads:**

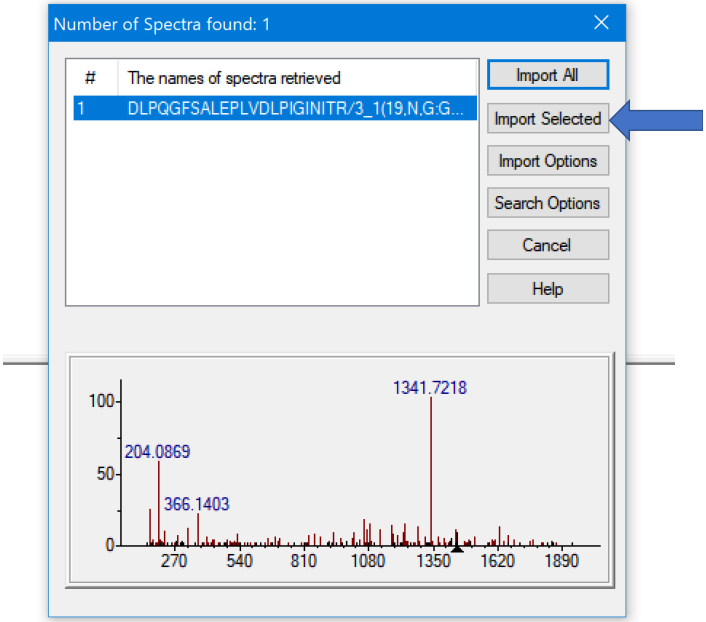
1. Go to https://chemdata.nist.gov/dokuwiki/doku.php?id=peptidew:nistmsgads and download NISTms-gads and unzip it.
2. Click on nistms-gads.exe in the NISTMS-GADS folder.
3. Once nistms-gads is open, click on Librarian tab at the bottom.



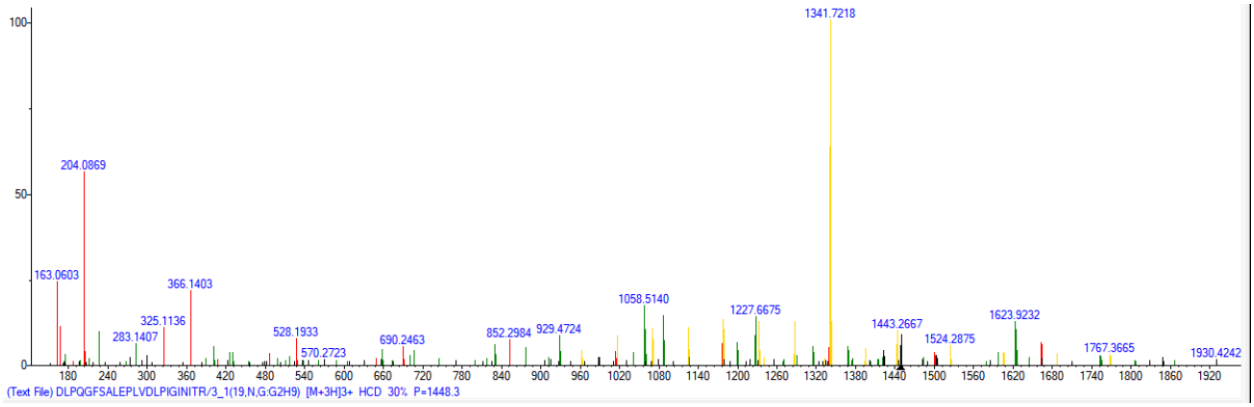
4. Click on file open button on the top, go to the MS_Piano_exe or another folder.



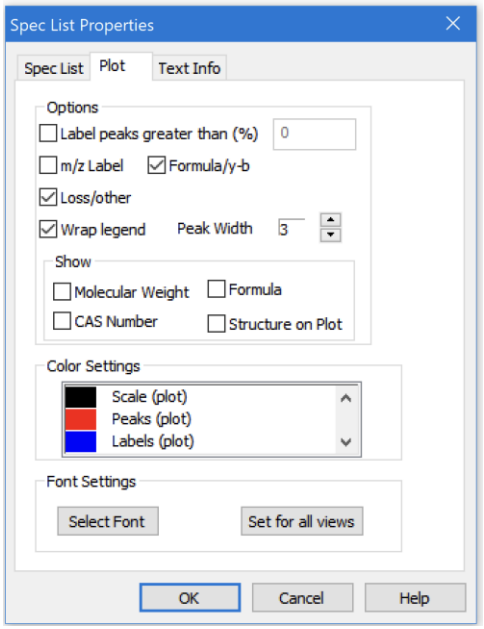
5. Select out.msp and select the spectra. Click on “Import Selected”.



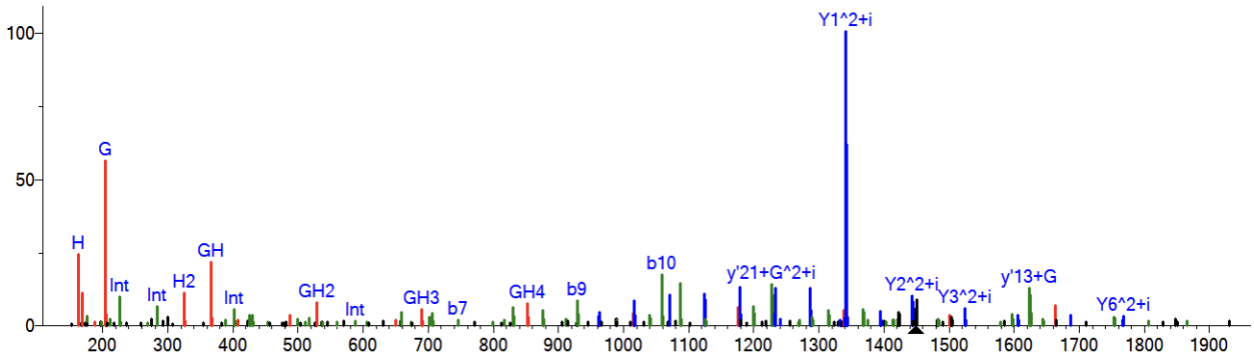
6. You will see the spectrum.



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.



II. 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