

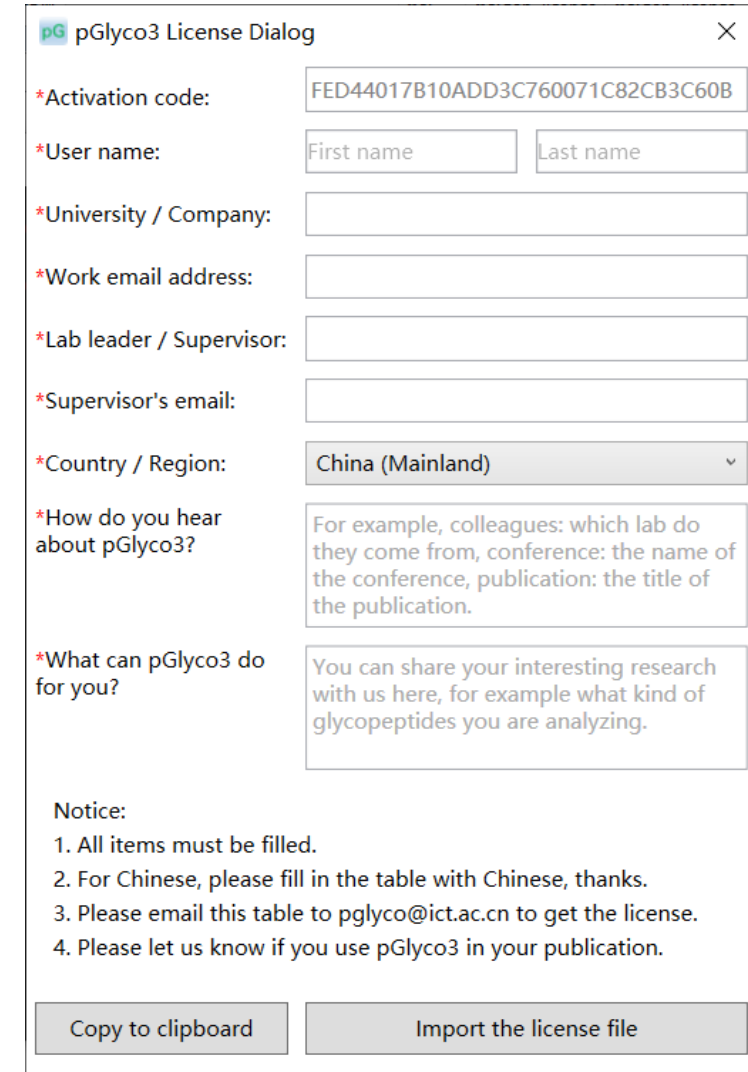
pGlyco3 User Guide

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2021.04.01

pGlyco3

- Download the latest version from:
<https://github.com/pFindStudio/pGlyco3/releases>
- Complete the form, click “Copy to clipboard”
- Paste the form in the main text of an email, and send it to pglyco@ict.ac.cn
- After you receive the pGlyco3.license file, click “Import the license file”
- Restart pGlyco3



The image shows a 'pGlyco3 License Dialog' window. It contains several input fields for user information, a dropdown for country/region, and two text areas for additional comments. At the bottom, there are two buttons: 'Copy to clipboard' and 'Import the license file'. A 'Notice' section at the bottom provides instructions for filling out the form.

pGlyco3 License Dialog

*Activation code: FED44017B10ADD3C760071C82CB3C60B

*User name: First name Last name

*University / Company:

*Work email address:

*Lab leader / Supervisor:

*Supervisor's email:

*Country / Region: China (Mainland)

*How do you hear about pGlyco3?
For example, colleagues: which lab do they come from, conference: the name of the conference, publication: the title of the publication.

*What can pGlyco3 do for you?
You can share your interesting research with us here, for example what kind of glycopeptides you are analyzing.






Notice:

1. All items must be filled.
2. For Chinese, please fill in the table with Chinese, thanks.
3. Please email this table to pglyco@ict.ac.cn to get the license.
4. Please let us know if you use pGlyco3 in your publication.

Copy to clipboard Import the license file






Runners

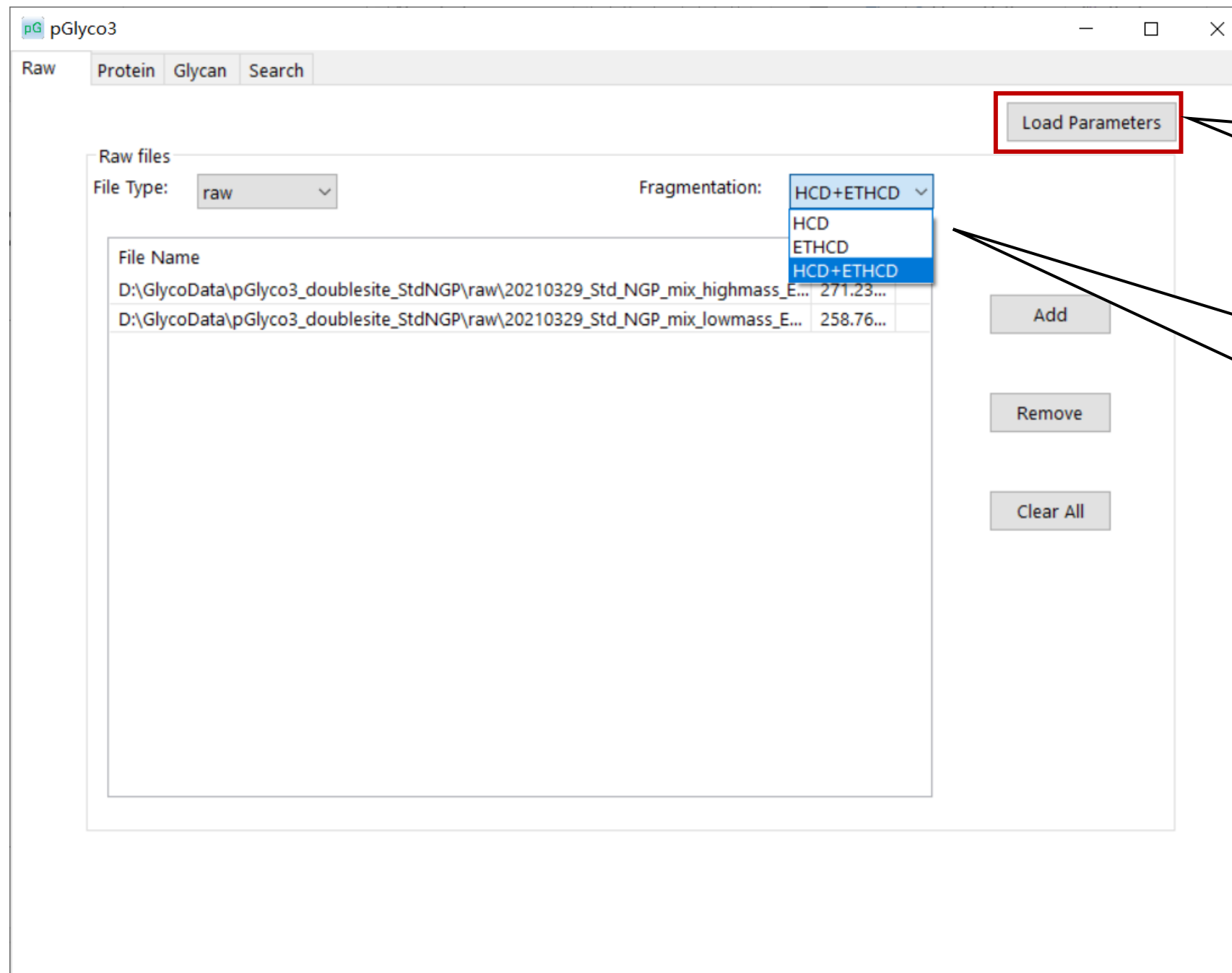
- run_pGlycoGUI.bat
 - pGlyco search engine
- run_gLabel.bat
 - Spectrum annotation
- edit_glycoini.bat
 - Add/edit monosaccharides

 bin	2021/3/30 11:10
 edit_glycoini.bat	2020/12/27 16:20
 readme.txt	2020/10/13 9:26
 run_gLabel.bat	2020/11/17 11:14
 run_pGlycoGUI.bat	2020/5/16 16:27

Runners

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Load Parameters

Loading existing parameters (pGlyco.cfg)

Add

Remove

Clear All

HCD+ETHCD could be: HCD-pd-ETxxD, HCD-couple-ETxxD, or even HCD-pd-CID-ETxxD, etc.

pGlyco3

Raw Protein Glycan Search

Fasta:

Enzyme

Name: Digest N-Term: ☐ Digest C-Term: Max Miss Cleavage:

Digestion:

Fixed Protein Modifications

<< >>

Variable Protein Modifications

<< >>

Modification List

- 2-dimethylsuccinyl[C]
- 2-monomethylsuccinyl[C]
- 2-nitrobenzyl[Y]
- 2-succinyl[C]
- 2HPG[R]
- 3-deoxyglucosone[R]
- 3-phosphoglyceryl[K]
- 3sulfo[AnyN-term]
- 4-ONE+Delta_H(-2)O(-1)[C]
- 4-ONE+Delta_H(-2)O(-1)[H]
- 4-ONE+Delta_H(-2)O(-1)[K]
- 4-ONE[C]
- 4-ONE[H]
- 4-ONE[K]
- 4AcAllylGal[C]
- ADP-Ribosyl[C]
- ADP-Ribosyl[D]
- ADP-Ribosyl[E]
- ADP-Ribosyl[K]

Filter:

Max Var Mod on Peptide: Peptide Length: from to Peptide Mass: from to

non-specific (only
for small fasta)

Digest C-Term:
ABCDEFGHIJKLMN
OPQRSTUVWXYZ
Max Miss Cleave: 25

Supporting multi-
enzyme

It does not include
glycosylation

For O-Glycan,
Multi-Site-O-
Glycan.gdb is
recommended.

Multi-site N-
glycans could also
be localized by
ETxxD using xxx-
multi.gdb

pGlyco3

Raw Protein Glycan Search

Glycan DB: pGlyco-N-Human-multi.gdb Glycan Type: N-Glycan Convert GlycoWorkbench

Multi-Site-O-Glycan.gdb
pGlyco-N-HighMannose.gdb
pGlyco-N-Human-multi.gdb
pGlyco-N-Human.gdb
pGlyco-N-Mouse.gdb
pGlyco-N-Plant-multi.gdb
pGlyco-N-Plant.gdb
pGlyco-O-Glycan.gdb
pGlyco-O-HexOnly.gdb
pGlyco-O-Man.gdb

<< Glyco: Modified as:

>>

Variable Glycan Modifications

<< Glyco: H Modified as: pH

>>

Max Var Mod on Glycan: 1 Max Number of (Modified) Glycans to Search: 100000

Converting Glycoworkbench .gwp file
to gdb. Users can draw their own
glycan database using
GlycoWorkbench. If you have a
better O-glycan database, it will be
great to share it with others😊

Max allowed monosaccharide-modification (e.g. phoHex) for each glycan. If this number is too
large, the glycan database will “explode” while searching. The total size of glycan database can
be controlled by “Max Number of Glycans to Search”

pGlyco3

Raw Protein Glycan Search

Precursor Tolerance: \pm 10 ppm Fragment Tolerance: \pm 20 ppm

Number of Processes: 5 Glycopeptide FDR: 0.01

☐ Percolator ☐ FMM for Peptide FDR

☒ pGlycoSite: Localized Glycans Must Be in GDB

Output Folder: D:\GlycoData\pGlyco3_doublesite_StdNGP\ETXXD Browse

Run Save Stop

pGlycoNovo

☐ Run pGlycoNovo

Glyco: H Max:

Glyco: N Max: 7

Glyco: F Max: 5

Glyco: A Max: 4

Glyco: Max:

Glyco: Max:

Glyco: Max:

Glyco: Max:

Allow Max Glyco Gap: 3

CPU threads

Keep these as default

Only for professional users. Searching glycopeptides without glycan databases. Note that it sometimes generates false positives, like peptide de novo sequencing.


```
C:\pGlyco_Release\pGlyco3\bin\pGlyco3GUI.exe
.pGlyco3GUI.exe
Process ID=21372: [XIC] Smoothing window = 21
Process ID=21372: [XIC] Smoothing method = savgol_filter
Process ID=21372: [XIC] Loading pGlyco results ...
Process ID=21372: [XIC] RT window is [-120.0, +120.0] seconds
Process ID=21372: [XIC] Indexing d:\glycodata\pglyco3_doublesite_stdngp\ETXXD\proc
Process ID=21372: c:\pGlyco_Release\pGlyco3\bin>pGlycoSite.exe "D:\GlycoData\pGlyco3_doublesite_stdngp\ETXXD\proc
lyco.cfg"
Process ID=91408: Reading pGlyco results ...
Process ID=91408: Inferring proteins ...
Process ID=91408: End inference
Process ID=91408: D:\GlycoData\pGlyco3_doublesite_stdngp\ETXXD\proc
Process ID=91408: c:\pGlyco_Release\pGlyco3\bin>XIC.exe -p "D:\GlycoData\pGlyco3_doublesite_stdngp\ETXXD\proc
lyco.cfg"
Process ID=21372: Already registered!
Process ID=21372: [pGlycoSite] Glycosylation site localization finished!
Process ID=91408: [XIC] Smoothing window = 21
Process ID=91408: [XIC] Smoothing method = savgol_filter
Process ID=91408: [XIC] Loading pGlyco results ...
Process ID=91408: [XIC] RT window is [-120.0, +120.0] seconds
Process ID=91408: [XIC] Indexing d:\glycodata\pglyco3_doublesite_stdngp\ETXXD\proc
Process ID=91408: c:\pGlyco_Release\pGlyco3\bin>pGlycoSite.exe "D:\GlycoData\pGlyco3_doublesite_stdngp\ETXXD\proc
lyco.cfg"
Process ID=91408: Already registered!
Process ID=91408: [pGlycoSite] Glycosylation site localization finished!
[pGlyco] All results are merged!
[pGlyco] Running time = 3.3 minutes.
[pGlyco] Task completed!
```

pGlyco3

Raw Protein Glycan Search

Precursor Tolerance: \pm 10 ppm Fragment Tolerance: \pm 20 ppm

Number of Processes: 5 Glycopeptide FDR: 0.01

☐ Percolator ☐ FMM for Peptide FDR

☒ pGlycoSite: Localized Glycans Must Be in GDB

Task does not start

A task is still running, please wait or click 'Stop'

OK

pGlycoNovo

☐ Run pGlycoNovo

Glyco: H Max: 20

Glyco: N Max: 7

Glyco: F Max: 5

Glyco: A Max: 4

Glyco: Max:

Glyco: Max:

Glyco: Max:

Glyco: Max:

Allow Max Glyco Gap: 3





Output Folder: D:\GlycoData\pGlyco3_doublesite_stdngp\ETXXD Browse

Run Save Stop

Bug: if we click "Run" for the second time, it always shows "Task does not start" even after the previous task is completed. You can click "Stop" any time when the command line shows "Task completed!" (shown in left).

Runners

- run_pGlycoGUI.bat
 - pGlyco search engine
- run_gLabel.bat
 - Spectrum annotation
- edit_glycoini.bat
 - Add/edit monosaccharides

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gLabel for glycopeptide

Load gLabel config

Tolerance: 20.0 ppm plot glycan and peptide

MGF: D:\GlycoData\pGlyco3_doublesite_StdNGP\raw\20; browse

pGlycoRes: ETXXD-PRM\pGlycoDB-GP-FDR-Pro-Quant-Site.txt browse

MaxPlotMZ: 4100.0 ETD HCD ETD ETHCD ☐ show mass ☒ pGlycoSite

Spectrum: 0329_Std_NGP_n RM.4231.4231.4.1.dta show

self defined glycopeptide

Glycan: H N|8 2|5 2;6 2;7 2;8 2

Peptide: ACMJESGK|4|2,Carbamidomethyl[C];3,Oxidation[M] show this

Out Folder: D:\GlycoData\pGlyco3_doublesite_StdNGP\ETXXD- Batch Plot

20210329_Std_NGP_mix_ETXXD_PRM_ETHCDFT.mgf **ETHcd**

20210329 Std NGP mix ETXXD PRM HCDFT.mgf **HCD**

20210329_Std_NGP_mix_ETXXD_PRM_HCDpdETXXDFT.mgf **Do not use it**

pGlycoDB-GP-FDR.txt

pGlycoDB-GP-FDR-Pro.txt

pGlycoDB-GP-FDR-Pro-Quant.txt

pGlycoDB-GP-FDR-Pro-Quant-Site.txt

If not sure, choose the longest one

gLabel for glycopeptide

Load gLabel config

Tolerance: 20.0 ppm

MGF: D:\GlycoData\pGlyco3_doublesite_StdNGP\raw\20; browse

pGlycoRes: ETXXD-PRM\pGlycoDB-GP-FDR-Pro-Quant-Site.txt browse

MaxPlotMZ: 4100.0

Spectrum: 0329_Std_NGP_n RM.4231.4231.4.1.dta show

self defined glycopeptide

Glycan: H N|8 2|5 2;6 2;7 2;8 2

Peptide: ACMJESGK|4|2,Carbamidomethyl[C];3,Oxidation[M] show this

Out Folder: D:\GlycoData\pGlyco3_doublesite_StdNGP\ETXXD- Batch Plot

plot glycan and peptide

plot glycan: Y ions (with peptide attached)
plot peptide: b/y/c/z ions
plot glycan and peptide: both
B ions are always plotted

ETD
HCD
ETD
ETHCD

Plot glycan-localized c/z ions, only works for ETD mgf

HCD: b/y ions
ETD: c/z ions
ETHCD: b/y/c/z ions

gLabel for glycopeptide

Load gLabel config

Tolerance: 20.0 ppm

plot glycan and peptide

MGF: D:\GlycoData\pGlyco3_doublesite_StdNGP\raw\20% browse

pGlycoRes: ETXXD-PRM\pGlycoDB-GP-FDR-Pro-Quant-Site.txt browse

MaxPlotMZ: 4100.0 ETD ☐ show mass ☒ pGlycoSite

Spectrum: 0329_Std_NGP_mix_ETXXD_PRM.4231.4231.4.1.dta show

self defined glycopeptide

Glycan: H N|8 2|5 2;6 2;7 2;8 2

Peptide: ACMJESGK|4|2,Carbamidomethyl[C];3,Oxidation[M] show this

Out Folder: D:\GlycoData\pGlyco3_doublesite_StdNGP\ETXXD- Batch Plot

A

GlySpec

20210329_Std_NGP_mix_ETXXD_PRM.3738.3738.4.8.dta
20210329_Std_NGP_mix_ETXXD_PRM.3792.3792.4.7.dta
20210329_Std_NGP_mix_ETXXD_PRM.3837.3837.4.1.dta
20210329_Std_NGP_mix_ETXXD_PRM.3972.3972.4.3.dta
20210329_Std_NGP_mix_ETXXD_PRM.3990.3990.4.1.dta
20210329_Std_NGP_mix_ETXXD_PRM.4035.4035.4.3.dta
20210329_Std_NGP_mix_ETXXD_PRM.4043.4043.3.2.dta
20210329_Std_NGP_mix_ETXXD_PRM.4044.4044.4.1.dta
20210329_Std_NGP_mix_ETXXD_PRM.4052.4052.3.4.dta

First column in the pGlycoRes file, it will automatically switch between HCD scan and ETD scan.

Ignore this section!!

Save all annotated plots into a folder, for lazy people like me.

Result Interpretation

- MonoArea:
 - Monoisotopic peak intensity
- IsotopeArea:
 - Summed intensity of all isotopes (including mono)
- ETDScore: ETDScore for site localization
- LocalizedSiteGroups:
 - format: site1,site2,glycan composition,probability;
s1,s2,glycan,prob;...
 - if site1≠site2, it means ETD spectrum is not able to distinguish sites between site1 and site2, site1-to-site2 is regarded as a site group

AN	AO	AP	AQ	AR	AS	AT
MonoArea	IsotopeArea	ETDScore	LocalizedSiteGroups	LocalizedS	LocalizedI	PreLocalizedI
87948.9	596593	3742	J9,J9,(5 4 0 0),0.57	3	0.083333	3
591603	4.34E+06	3796	J3,J9,(5 4 0 0),0.91	14	0.388889	14
5.45E+06	5.04E+07	3841	J9,J9,(5 4 0 0),0.85	17	0.472222	15
965899	6.38E+06	3976	J3,J3,(5 4 0 0),0.94	20	0.555556	20
8.22E+06	1.32E+08	3994	J3,J3,(5 4 0 0),0.97	24	0.666667	24
562654	5.09E+07	4039	J3,J9,(5 4 0 0),0.81	7	0.194444	7
700256	1.50E+07	4047	J3,J9,(5 4 0 0),0.81	3	0.083333	3
562654	5.09E+07	4048	J9,J9,(5 4 0 0),0.88	15	0.416667	15
700256	1.50E+07	4056	J3,J3,(5 4 0 0),0.80	6	0.166667	6
562654	5.09E+07	4057	J9,J9,(5 4 0 0),0.90	21	0.583333	21
700256	1.50E+07	4065	J3,J9,(5 4 0 0),0.60	2	0.055556	2
562654	5.09E+07	4066	J3,J3,(5 4 0 0),0.89	11	0.305556	11
562654	5.09E+07	4075	J3,J9,(5 4 0 0),0.73	5	0.138889	5

Python Codes for Post Analysis

- https://github.com/pFindStudio/pGlyco3/blob/main/protein_site_analysis.py
 - Generate protein level information (localized sites, intensities)
- Usage: `python protein_site_analysis.py C:/xx/output_dir/pGlycoDB-GP-FDR-Pro-Quant-Site.txt`

Python Codes for Post Analysis

- https://github.com/pFindStudio/pGlyco3/blob/main/Y_ion_extractor.py
 - Extract Y ions, e.g. Y-N(1)F(1) and Y-N(2)F(1) to check core fucosylation
 - Y-N(1)F(1) and Y-N(2)F(1) could be modified in the codes
- Usage: `python Y_ion_extractor.py C:/xx/output_dir/pGlyco3.cfg`

We will upload more Python codes

- See <https://github.com/pFindStudio/pGlyco3>

Advanced usages: to be continued