Contents

1. Requirements

1) Computing system

pIon search requires a computer with recommended configuration as follows:

- ➢ Microsoft Windows 64-bit
- ➢ Intel Core i7/i9/Xeon Processor
- ➢ 32GB of RAM or more

Note: pIon v1.0 is NOT supported by non-Windows operating systems (incl. MacOS, Linux and so on).

2) MS Data

- ➢ Data dependent acquisition (DDA) with BOTH MS1 and MS/MS spectra recorded in the High-Resolution mode

Note: 1) For automatic performance assessment of chemoproteomic probes, it is recommended to acquire MS data from probe-labeled samples with DMP-tag.

2. Download

1) pIon can be freely downloaded from the following website:

http://pfind.org/software/pIon/index.html

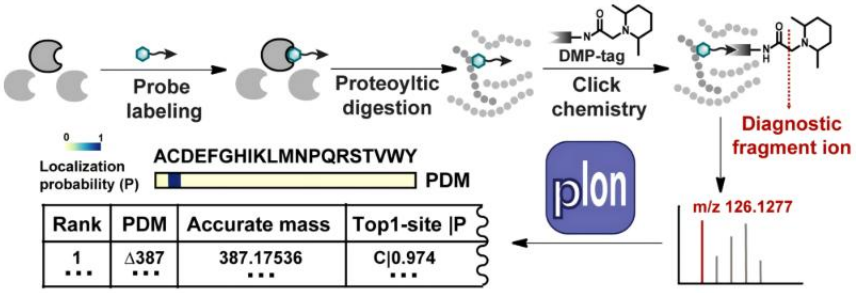
pFind Studio: a computational solution for mass spectrometry-based proteomics

pIon

Introduction - Cite us - Downloads

Introduction

The ever-growing field of covalent drug discovery and chemoproteomics has fueled a need for bioconjugation methods with high selectivity in a native context. Given that the sheer number of functional groups present, achieving residue-specificity in biological systems remains a challenge. In order to identify the best residue-specific bioconjugation method, numerous small molecule models and/or purified proteins have been employed for rigorous method validation in terms of selectivity, efficiency and stability. Such in vitro experiments therefore became increasingly time consuming, yet they were unable to enumerate all functional groups present in complex, native biological systems. pIon is a computational tool that enables a cost-efficient pipeline for high-throughput evaluation of residue-specific bioconjugation chemistries. It starts with a rapid experimental phase, in which the proteome conjugated by a reactive probe is chemically coded for generating diagnostic report ions in tandem mass spectrometry analysis. The resulting MS data can be directly imported into pIon, which automatically calculates the accurate modification masses derived from a tested probe as well as the corresponding residue preferences. Thus, pIon has the potential to become a valuable option for routine evaluation of bioconjugation chemistries, thereby driving the field of bioconjugation chemistry to unprecedented dimensions and interfacing the worlds of biological and synthetic chemistry.



Downloads

Notice: Nov. 27, 2024 - pIon is currently available for free use. Click to download.

For demo data, please refer to Click to download..

For source code, please refer to github.

For detailed usage, please refer to user guide.

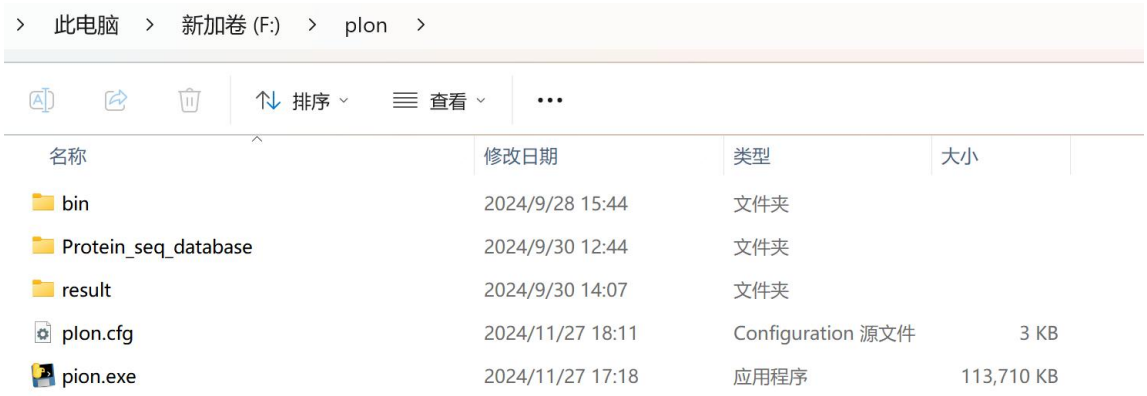
For other issues, please contact pengyaping21s@ict.ac.cn.

2) Click to download, download the ZIP compressed file.

3) Un-zip the “plon.zip” package into a specified file folder (e.g., Local disk F).

3. Configuration

1) Double click *plon* to open the main folder.



2) Open configuration file "*plon.cfg*" using a text editor, *e.g.*, Microsoft Notepad or Notepad++ (https://notepad-plus.en.softonic.com/).

3) Setting "plon.cfg".

```
################################################################################
# pIon general parameter settings
# Path to the output file
output_path=F:\pIon\result1

# Path to the protein sequence database
fasta_path=F:\pIon\Protein_seq_database\Homo_sapiens_uniprot_canonical_20395_entries_20210516.fasta

# Format of MS data, RAW or MZML or MGF
msmstype=MGF

# The number and path of MS data
msmsnum=1
msmspath1=H:\pIon\pIon1\demo_dataset\IPM_demo.mgf


# Type of MS dissociation method
activation_type=HCD-FTMS

# Usage of open search (True/ False), against Unimod, the common modification can be set if not
open_flag=False
common_modification_number=2
common_modification_list=Carbamidomethyl[C];Oxidation[M];

# Mass range of unknown modification (Da)
min_mass_modification=200
max_mass_modification=500

# Mass shifts with PSMs less than X% of that of overall PDMs were neglected
filter_frequency=0

# If consider the N-side or C-side for amino acid localization (True or False)
side_position=True

# P-value threshold enabling confident amino acid localization
p_value_threshold=0.001

# If report the statistical information (True or False)
report_statistics=True

################################################################################
# If isotope coding is adopted to facilitate the discovery of unknown modifications (True or False)
isotope_labeling=False


# Mass tolerance of the mass shift between light isotope and heavy isotope
mass_of_diff_diff=6.020132


# Isotopic mass difference within empirically defined tolerance(Da)
mass_diff_diff_range=0.005


################################################################################
# If ion labeling is adopted to facilitate the discovery of unknown modifications (True or False)
ion_labeling=True

# One charge mass of ion, it is recommended to keep at least three decimal places
ion_mass=126.128

# In the 0-1 range, the higher the score, the stricter the filtering, and the recommended value is 0.7
ion_filter_ratio=0.7
```

General Note 1:

For the first-time users, custom settings are required for ①-⑤, ⑧ default settings can be adopted for ⑥, ⑦, ⑨-⑭.

General Note 2:

All parameters (shown in red below) are case sensitive.

General Note 3:

The blank space should be avoided.

1. General Parameters Setting

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① # Path to the output file

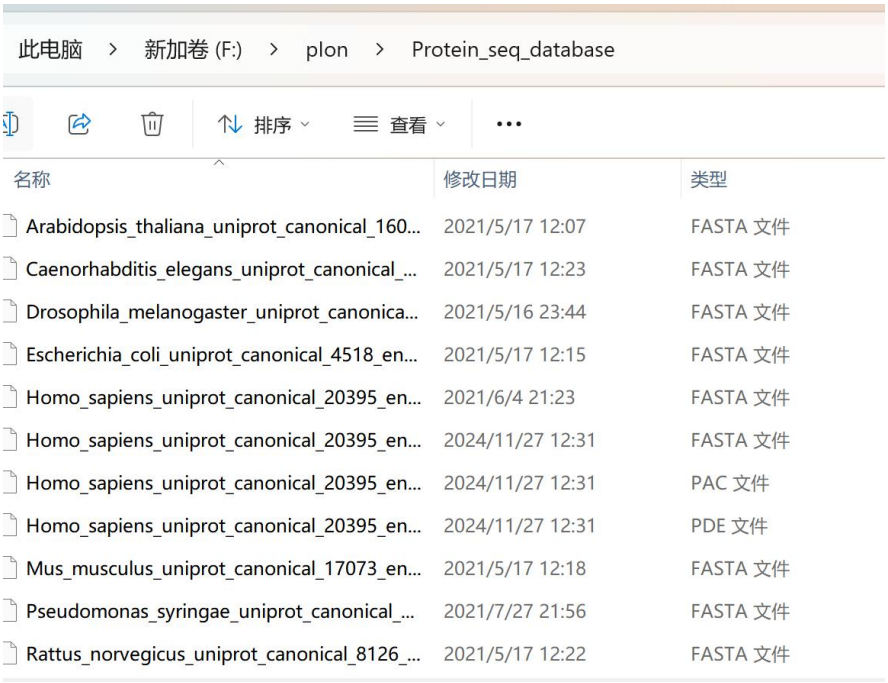
output_path=F:\pIon\result1

Note: If the output file folder does not exist, an error will be reported.

② # Path to the protein sequence database

fasta_path=F:\pIon\Protein_seq_database\Homo_sapiens_uniprot_canonical_20395_entries_20210516.fasta

Note: The protein *.fasta database databases of several commonly used species (*e.g.*, *home sapiens*) are included in the subfolder (named as Protein_seq_database) of pIon. Note that the databases of other species can be downloaded from Uniprot as described in **Supporting Protocol 1**.



③ # Format of MS data (RAW or MZML or MGF)

msmstype=RAW

Note: Non-Thermo MS data need to be converted into mzML files before pIon search. The users can refer to **Supporting Protocol 2**.

④ # The number and path of MS data

msmsnum=N
msmspath1=X:\XXX\XXX.raw
msmspath2=X:\XXX\XXX.raw
……
msmspathN=X:\XXX\XXX.raw

Note: The suffix of MS data files MUST be input.

Example:

msmsnum=1
msmspath1=G:\data\HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.raw

⑤ # Type of MS dissociation method

activation_type=HCD-FTMS
illustration: default
Note: 1) pIon and pChem v1.0 can NOT support MS data generated under electron-transfer dissociation ETD, electron-transfer/higher-energy collision dissociation EThcD, and the likes.

⑥ # Usage of open search (True/ False) against Unimod, the common modification can be set if not

open_flag=False
common_modification_number=2
common_modification_list=Carbamidomethyl[C];Oxidation[M];
illustration: default
Note: The names of common modifications should be the same as those appeared in Unimod database. Specifically, you can refer to the modification.ini file in the bin directory.

⑦ # Mass range of unknown modification (Da)

min_mass_modification=200
max_mass_modification=500

illustration: default
Note: The PDMs generated from the use of bioorthogonal cleavable linkers typically possess masses higher than 200 Da and less than 500 Da.

⑧ # Mass shifts with PSMs less than X% of that of overall PDMs were neglected

filter_frequency=5

illustration: default
Note: This parameter can be set as 0 if one wants to retrieve all PDMs including those with just a few PSMs.

⑨ # If consider the N- or C-termini for amino acid localization (True or False)
side_position=True
illustration: default

⑩ # P-value threshold enabling confident amino acid localization
p_value_ threshold=0.001
illustration: default

⑪ # if report the statistical information (True or False)
report_statistics=True
illustration: default

2. The parameter settings for pChem v1.0: if it is not in isotope mode, you can set *isotope_labeling* to False, and the remaining parameters are the same as in the previous version
No changes are needed for pIon in this section.(i.e., ⑫ -1 , ⑫ -2, ⑫ -3)
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3. The parameter settings for pIon: If ion labeling is adopted to facilitate the discovery of unknown modifications
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⑬-1 # If ion labeling is adopted to facilitate the discovery of unknown modifications (True or False)

ion_labeling=True

⑬-2 # One charge mass of ion, it is recommended to keep at least three decimal places

ion_mass=126.128

⑬-3 # In the 0-1 range, a higher score indicates stricter filtering, with a recommended value of 0.7.

ion_filter_ratio=0.7

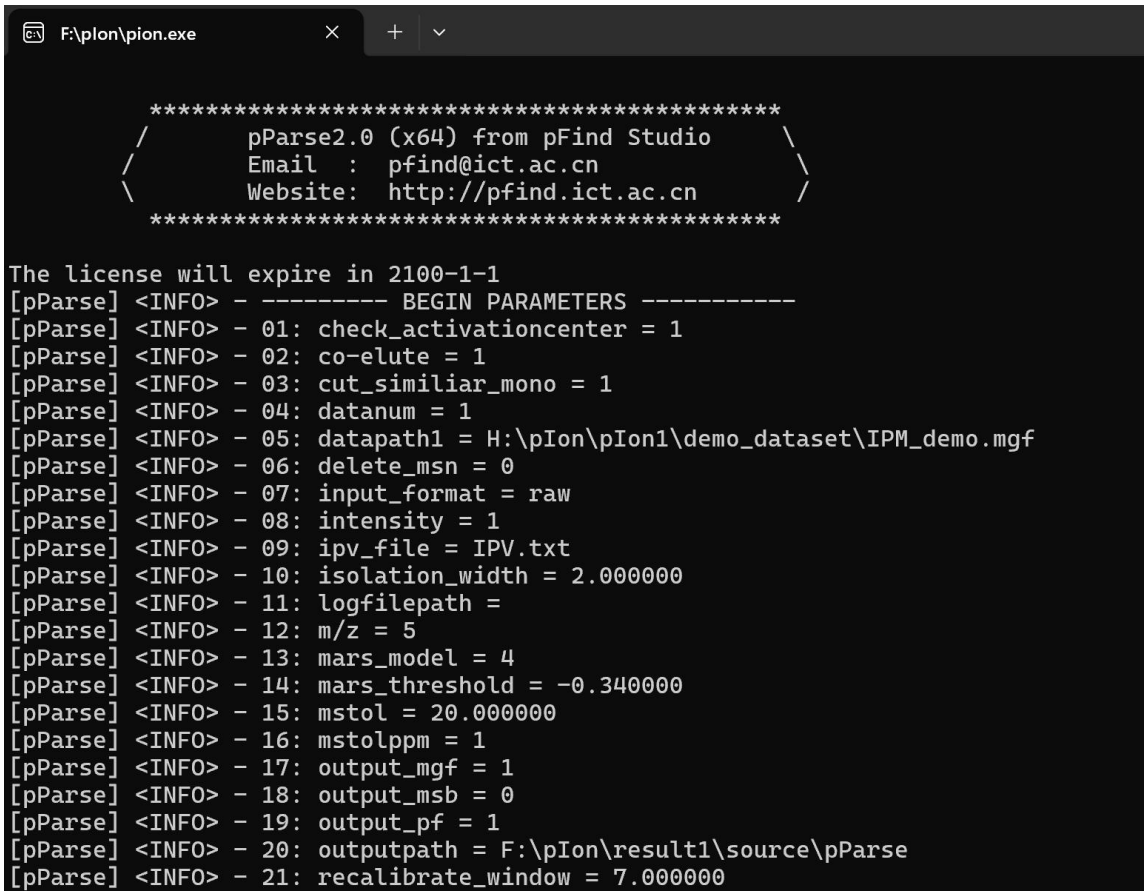
illustration: default

4. Run

Once all parameters have been set, double click “*pIon.exe*”

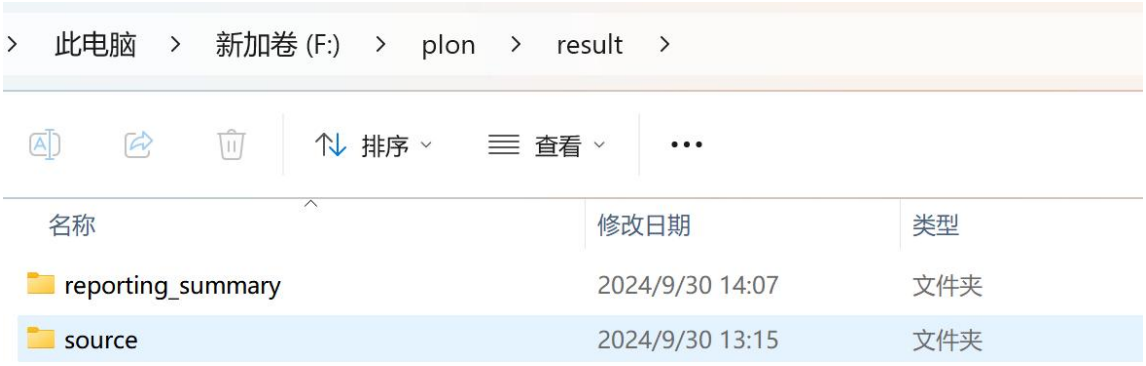
to execute the programming. The message “**Please press any key to continue**” means that program runs to completion.

Note: pIon search will generate several intermediate files in the main folder. do NOT open those files during program running.

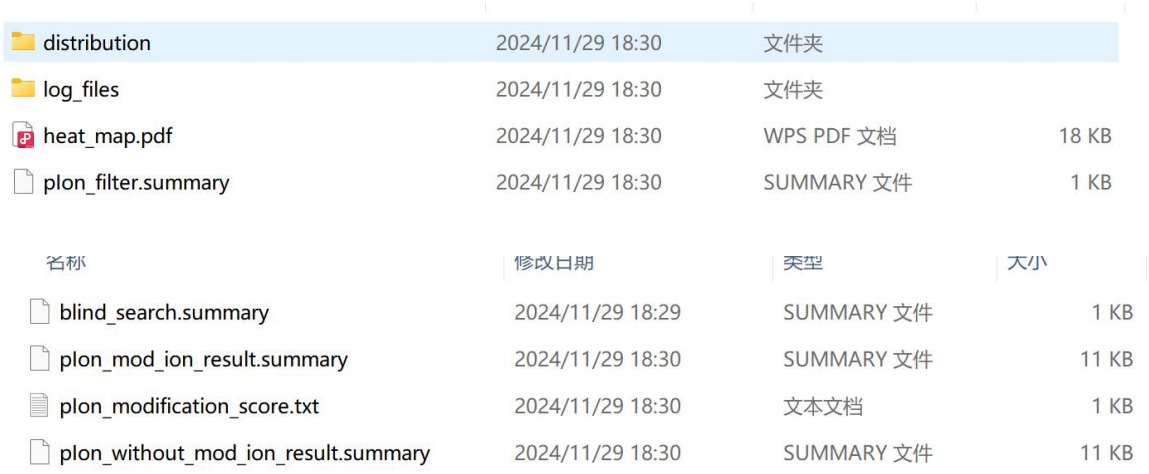


5. Output

1) Double click “*result*” file for searching results.
2) Double click “*reporting summary*”.



3) There are six major output documents. (2 files in the reporting_summary directory and 4 files in the reporting_summary/log_files directory.)



Note: Users are recommended to copy these output documents and paste into another file. Otherwise, they can be covered by those generated from the next search event.

① plon_filter.summary

plon_filter.*summary* is a tab-delimited text file contains the details of every PDM.

plon_filter.summary

Rank	Modification	Accurate Mass (std, r-squared)	Top1 Site | Probability | p-value	Others	#PSM
1	PFIND_DELTA_334	334.211930 (0.001866, 0.852884)	C|0.868|0.0000	N-SIDE(0.09, 0.0000);	4087

PDM: Probe-derived modifications

#PSM: The number of PSMs corresponding to modified peptides identified by search engine.

Top1 site | Top1 Probability: The amino acid most likely to be modified with the corresponding localization probability.

Others: Other amino acid sites that may also be labeled by probes and their corresponding localization probability values.

② blind_search.summary

blind_search.summary is a tab-delimited text file contains the details of every candidate mass shifts identified by blind search.

blind_search.summary

Rank	Modification	Accurate Mass (std, r-squared)	Top1 Site | Probability | p-value	Others	#PSM
1	PFIND_DELTA_334	334.211930 (0.001866, 0.852884)	C|0.868|0.0000	N-SIDE(0.09, 0.0000);	4087
2	PFIND_DELTA_348	348.227635 (0.001862, 0.856077)	C|0.732|0.0000	N-SIDE(0.132, 0.0000); M(0.07, 0.0000);	302
3	PFIND_DELTA_350	350.206857 (0.002106, 0.856508)	C|0.456|0.0000	M(0.263, 0.0000); N-SIDE(0.144, 0.0004);	160
4	PFIND_DELTA_429	429.249386 (0.002686, 0.870266)	C|0.803|0.0000	76	
5	PFIND_DELTA_332	332.196673 (0.003081, 0.852405)	N-SIDE|0.353|0.0000	34	
6	PFIND_DELTA_376	376.221967 (0.002086, 0.861753)	C|0.483|0.0000	N-SIDE(0.483, 0.0000);	29
7	PFIND_DELTA_366	366.203410 (0.003235, 0.859825)	C|0.643|0.0000	28	
8	PFIND_DELTA_335	335.214838 (0.002373, 0.853121)	C|0.667|0.0000	27	

#PSM: The number of PSMs corresponding to modified peptides identified by search engine.

Top1 site | Top1 Probability: The amino acid most likely to be modified with the corresponding localization probability.

Others: Other amino acid sites that may also be labeled by probes and their corresponding localization probability values.

③ plon_mod_ion_result.summary & plon_without_mod_ion_result.summary

Stores detailed information about all diagnostic ions in both modified and unmodified spectrum categories

plon_mod_ion_result.summary

Modification info for PFIND_DELTA_334:(PSM = 4087) (filtered PSM = 3620)(neighbor filtered PSM = 2754)
Top 300 characteristic ions in the PFIND_DELTA_334 spectra:

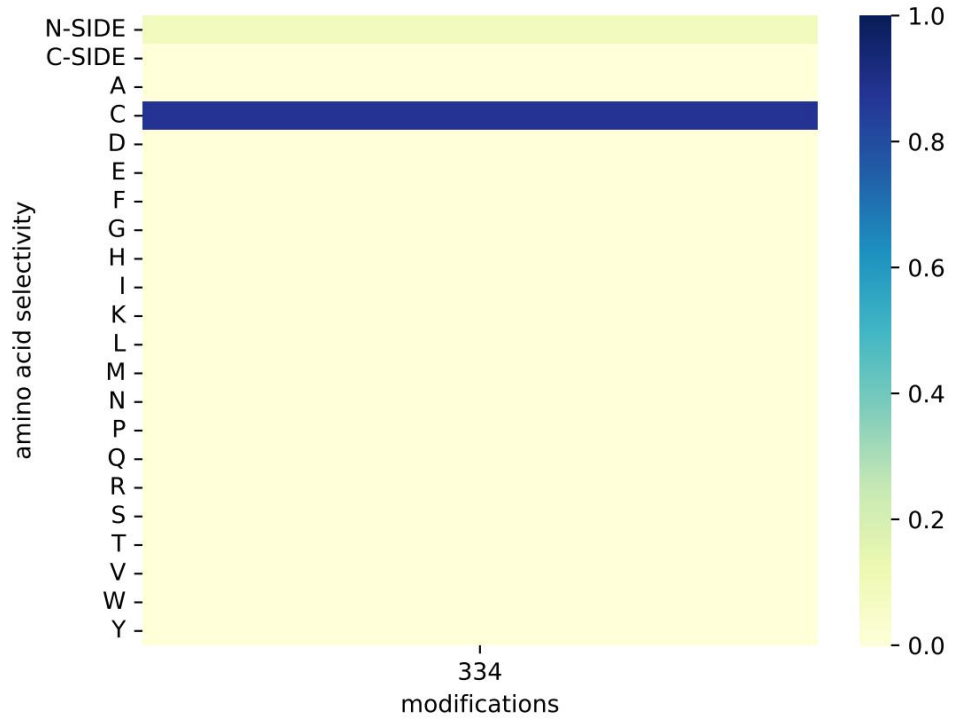
Rank	ion type	ion count	ion accuracy	ion relative peak
1	126.128	3618	126.128188	0.311837
2	120.082	3080	120.081296	0.226492
3	129.102	3451	129.102656	0.204547
4	369.208	2324	369.207957	0.158625
5	136.076	3349	136.076015	0.158356
6	110.072	3376	110.071808	0.107049
7	130.086	3143	130.086605	0.07344
8	201.124	2702	201.123842	0.068785
9	393.208	1334	393.20782	0.064449
10	197.166	2501	197.165394	0.056755
11	159.092	2124	159.091926	0.054985
12	175.12	1683	175.119505	0.053225
13	215.14	2039	215.139706	0.04411
14	199.18	1364	199.18054	0.04006
15	157.134	1871	157.133866	0.039547
16	143.118	2194	143.118127	0.039459
17	173.128	1613	173.128608	0.039152
18	185.166	1600	185.165479	0.037046
19	147.114	1095	147.113209	0.035794
20	187.144	1565	187.144382	0.033035
21	183.15	1935	183.149748	0.031068
22	169.134	1982	169.133864	0.031039

plon_without_mod_ion_result.summary

Top 300 characteristic ions in the unmodified spectra: (PSM: 77190)

Rank	ion type	ion count	ion accuracy	ion relative peak
1	120.082	70545	120.081292	0.315512
2	129.102	73529	129.10266	0.259933
3	136.076	72815	136.07603	0.200565
4	126.128	74350	126.128164	0.177321
5	110.072	72995	110.071825	0.138545
6	130.086	67284	130.08664	0.091378
7	201.124	60452	201.123831	0.086791
8	175.12	36254	175.119487	0.07954
9	159.092	51097	159.091952	0.062918
10	199.18	33825	199.1806	0.060641
11	185.166	40326	185.16547	0.060548
12	215.14	47651	215.139678	0.058763
13	173.128	37941	173.12864	0.054535
14	143.118	52825	143.118143	0.053201
15	147.114	26350	147.1132	0.052815
16	157.134	45958	157.133858	0.05074
17	183.15	48202	183.14972	0.049322
18	187.144	37803	187.144401	0.047983
19	169.134	49574	169.13387	0.044049
20	233.166	27961	233.165639	0.037421
21	244.166	36233	244.166071	0.037032
22	200.14	37016	200.139892	0.036031
23	197.128	35023	197.128566	0.035934
24	227.176	35568	227.176026	0.031801

④ **heat_map.pdf**



Horizontal coordinate: The ∆mass of each PDM

Longitudinal coordinate: The types of amino acids

Color gradient: The localization probability that the modification occurs at each potential site.

Note: Those amino acids with p-value higher than p_value_ threshold (0.001 by default) are considered mis-localized sites. As such, their localization probability values are defined to be null. 2) For data generated from non-isotope-labeled or non-ion-labeled samples, heatmap will NOT be provided.

⑤ PSM-level results

F:\plon\result\source\blind\pFind-Filtered.spectra

This file contains the PSM-level information regarding all possible modifications on peptides.

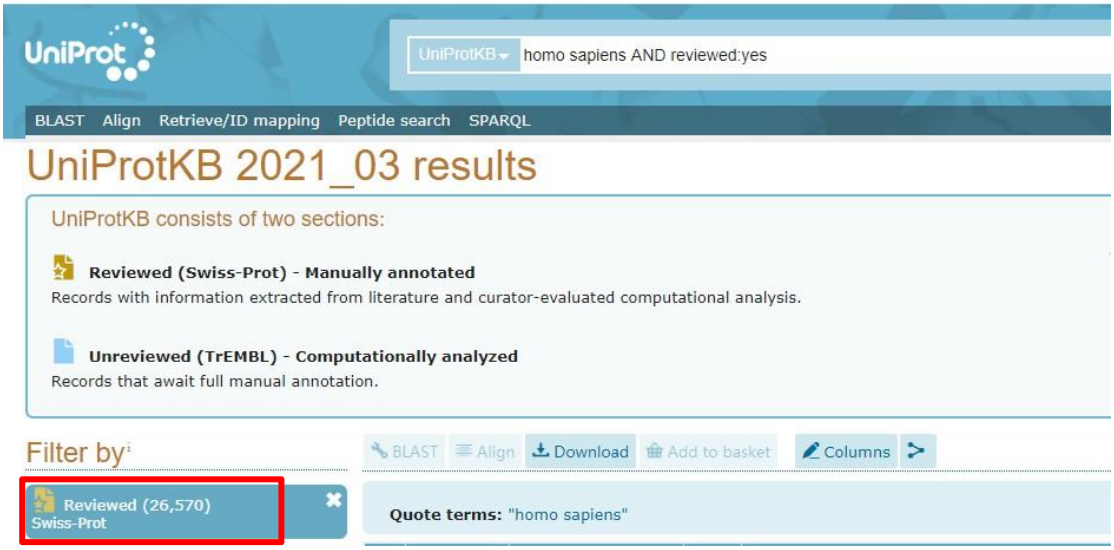
File_Name	Scan_No	Exp.MH+	Charge	Q-value	Sequence	Calc.MH+	Mass_Shift(Exp.-Calc.)	Raw_Score	Final_Score	Modification	Specificity	Proteins	Positions	Label	Target/Decoy	Miss.Clv.Sites	Avg.Frag.Mass.Shift	Others	Accurate modification mass
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.80136.80136.3.0.dta	80136	4051.128439	3	0	KLHEEEIQELQAQIQEQHVQIDVDVSKPDLTAALR	4051.109269	0.019170	50.413527	9.1228e-15		3	sp|P08670|VIME_HUMAN/	235,K,D/	1|	target	2	2.550422	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.81355.81355.2.0.dta	81355	2929.475516	2	0	TVSLGAGAKDELHIVEAEAMNYEGSPIK	2929.461027	0.014489	41.451767	1.10475e-12		3	sp|P06748|NPM_HUMAN/	45,R,V/	1|	target	1	3.400282	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.90651.90651.3.0.dta	90651	4204.073378	3	0	LASLMNLGMSSSLNQQGAHSALSSASTSSHNLQSIFNIGSR	4204.050778	0.022600	36.745504	1.59364e-12		3	sp|P43243|MATR3_HUMAN/	51,R,G/	1|	target	0	3.744516	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.86312.86312.3.0.dta	86312	3923.034311	3	0	LHEEEIQELQAQIQEQHVQIDVDVSKPDLTAALR	3923.014313	0.019998	40.635572	1.92653e-12		3	sp|P08670|VIME_HUMAN/	236,K,D/	1|	target	1	1.285594	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.79810.79810.3.0.dta	79810	3481.685716	3	0	KEEQEVQATLESEEVDLNAGLHGNWTLENAK	3481.671600	0.014116	39.679732	2.0799e-12		3	sp|Q08211|DHX9_HUMAN/	151,R,A/	1|	target	1	4.626725	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.31208.31208.3.0.dta	31208	4246.570279	3	0	LAADEDDDDDDEEDDDEDDDDDDFDDEEAEEKAPVKK	4246.550379	0.019900	38.226992	2.54421e-12		3	sp|P06748|NPM_HUMAN/	157,K,S/	1|	target	2	2.886990	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.95117.95117.2.0.dta	95117	2947.530735	2	0	ILAAALTQHNGDAAASLTVAEQYVSAFSK	2947.515838	0.014897	40.220500	4.23135e-12		3	sp|Q9UJZ1|STML2_HUMAN/	259,R,L/	1|	target	0	4.684390	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.79475.79475.3.0.dta	79475	3481.685527	3	0	KEEQEVQATLESEEVDLNAGLHGNWTLENAK	3481.671600	0.013927	38.666853	4.51191e-12		3	sp|Q08211|DHX9_HUMAN/	151,R,A/	1|	target	1	3.665085	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.79117.79117.3.0.dta	79117	3481.687216	3	0	KEEQEVQATLESEEVDLNAGLHGNWTLENAK	3481.671600	0.015616	37.241112	1.77755e-11		3	sp|Q08211|DHX9_HUMAN/	151,R,A/	1|	target	1	1.875068	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.92566.92566.3.0.dta	92566	4062.005986	3	0	QVFAENKDEIALVLFGTDGTDNPLSGGDQYQNITVHR	4061.983741	0.022245	36.337211	1.91044e-11		3	sp|P13010|XRCC5_HUMAN/	44,R,H/	1|	target	1	4.714821	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.92350.92350.3.0.dta	92350	3603.802556	3	0	SMTEAEQQQLIDDHFLFDKPVSPLLLASGMAR	3603.782014	0.020542	42.741930	1.91159e-11	30,Oxidation[M];	3	sp|P12277|KCRB_HUMAN/	177,K,D/	1|0|	target	1	3.088547	100	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.90143.90143.2.0.dta	90143	2611.366213	2	0	VIHDNFGIVEGLMTTVHAITATQK	2611.354726	0.011487	42.504578	2.10905e-11	13,Oxidation[M];	3	sp|P04406|G3P_HUMAN/	162,K,T/	1|0|	target	0	3.498282	100	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.96801.96801.2.0.dta	96801	2891.456104	2	0	VHTVEDYQAIVDAEWNILYDKLEK	2891.446035	0.010069	36.410365	3.02266e-11		3	sp|Q99832|TCPH_HUMAN/	256,R,I/	1|	target	1	2.946987	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.86828.86828.2.0.dta	86828	2915.466990	2	0	VSDSISAQYPVVDHEFDAVVVGAGGAGLR	2915.453242	0.013748	33.066752	3.30269e-11		3	sp|P31040|SDHA_HUMAN/	46,K,A/	1|	target	0	3.949114	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.90495.90495.2.0.dta	90495	2611.367774	2	0	VIHDNFGIVEGLMTTVHAITATQK	2611.354726	0.013048	42.248081	3.92606e-11	13,Oxidation[M];	3	sp|P04406|G3P_HUMAN/	162,K,T/	1|0|	target	0	1.911314	100	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.75094.75094.2.0.dta	75094	2730.285134	2	0	LPTGYYFGASAGTGDLSDNHDIISMK	2730.271450	0.013684	36.251030	4.40851e-11		3	sp|Q12907|LMAN2_HUMAN/	246,R,L/	1|	target	0	3.933429	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.92100.92100.3.0.dta	92100	3172.608822	3	0	GQYISPFHDIPIYADKDVFHMVVEVPR	3172.592310	0.016512	34.929387	5.25267e-11		3	sp|Q15181|IPYR_HUMAN/	25,K,W/	1|	target	1	4.740456	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.90153.90153.3.0.dta	90153	3620.859618	3	0	ANLEAFTVDKDITLTNDKPATAIGVIGNFTDAER	3620.844076	0.015542	36.543951	5.36682e-11		3	sp|P33176|KINH_HUMAN/	384,K,R/	1|	target	2	2.850358	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.96223.96223.2.0.dta	96223	2649.455494	2	0	SKDDQVTVIGAGVTLHEALAAAELLK	2649.445643	0.009851	35.824011	5.54023e-11		3	sp|P29401|TKT_HUMAN/	497,K,K/	1|	target	1	3.670571	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.57024.57024.3.0.dta	57024	3595.740903	3	0	LHSFESHKDEIFQVQWSPHNETILASSGTDR	3595.719886	0.021017	39.569068	5.97591e-11		3	sp|Q09028|RBBP4_HUMAN/	309,K,R/	1|	target	1	2.889461	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.31957.31957.3.0.dta	31957	4473.734086	3	0	VKLAADEDDDDDDEEDDDEDDDDDDFDDEEAEEKAPVKK	4473.713743	0.020343	35.970966	6.08141e-11		3	sp|P06748|NPM_HUMAN/	155,K,S/	1|	target	3	3.225640	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.85865.85865.2.0.dta	85865	2617.289652	2	0	FLAAGTHLGGTNLDFQMEQYIYK	2617.275418	0.014234	36.356151	6.16468e-11		3	sp|P08865|RSSA_HUMAN/	17,K,R/	1|	target	0	2.565410	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.98436.98436.2.0.dta	98436	2801.429629	2	0	DGAGFLINLIDSPGHVDFSSEVTAALR	2801.410319	0.019310	34.044486	6.2823e-11		3	sp|P13639|EF2_HUMAN/	93,K,V/	1|	target	0	3.118144	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.83308.83308.2.0.dta	83308	2802.395165	2	0	NTELAVFHDETEIQNQTDLLSLSGK	2802.379078	0.016087	34.375863	6.56227e-11		3	sp|P41252|SYIC_HUMAN/	1125,K,T/	1|	target	0	4.482609	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.17913.17913.4.1.dta	17913	4307.986307	4	0	KKGDGGGAGGGGGGGGSGGGGSGGGGGGGSSRPPAPQENTTSEAGLPQGEAR	4307.960257	0.026050	41.966421	6.94232e-11		3	sp|Q9UKM9|RALY_HUMAN/	222,K,T/	1|	target	3	1.233280	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.75831.75831.3.0.dta	75831	4036.912992	3	0	GILGYTEHQVVSSDFNSDTHSSTFDAGAGIALNDHFVK	4036.894597	0.018395	39.945993	7.14445e-11		3	sp|P04406|G3P_HUMAN/	271,K,L/	1|	target	0	4.682275	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.49633.49633.2.0.dta	49633	2514.256338	2	0	WNTEDKVSHVSTGGGASLELLEGK	2514.246955	0.009383	34.322203	7.62427e-11		3	sp|P00558|PGK1_HUMAN/sp|P07205|PGK2_HUMAN/	382,K,V/382,K,I/	1|	target	1	1.190555	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.18390.18390.2.0.dta	18390	2606.196693	2	0	SPPSTGSTYGSSQKEESAASGGAAYTK	2606.185141	0.011552	32.056759	1.0637e-10		3	sp|Q9Y2W1|TR150_HUMAN/	319,K,R/	1|	target	1	0.149424	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.81240.81240.3.0.dta	81240	3245.659613	3	0	TAVIDHHNYDISDLGQHTLIVADTENLLK	3245.643544	0.016069	40.150333	1.09434e-10		3	sp|P39656|OST48_HUMAN/	153,K,A/	1|	target	0	4.833907	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.72944.72944.3.0.dta	72944	3784.824176	3	0	YGPQYGHPPPPPPPPEYGPHADSPVLMVYGLDQSK	3784.810277	0.013899	36.245317	1.14265e-10		3	sp|P14866|HNRPL_HUMAN/	358,R,M/	1|	target	0	4.364400	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.95309.95309.3.0.dta	95309	3438.713031	3	0	TIFTGHTAVVEDVSWHLLHESLFGSVADDQK	3438.696304	0.016727	36.000088	1.17134e-10		3	sp|Q09028|RBBP4_HUMAN/	220,K,L/	1|	target	0	3.593828	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.82819.82819.2.0.dta	82819	2703.409741	2	0	KTFSHELSDFGLESTAGEIPVVAIR	2703.398696	0.011045	32.919731	1.2222e-10		3	sp|P30101|PDIA3_HUMAN/	304,R,T/	1|	target	1	4.142107	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.68413.68413.2.0.dta	68413	2884.478650	2	0	KIEPELDGSAQVTSHDASTNGLINFIK	2884.468556	0.010094	36.271620	1.3297e-10		3	sp|P06744|G6PI_HUMAN/	523,K,Q/	1|	target	1	2.989336	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.98454.98454.2.0.dta	98454	2801.429629	2	0	DGAGFLINLIDSPGHVDFSSEVTAALR	2801.410319	0.019310	32.613609	1.33089e-10		3	sp|P13639|EF2_HUMAN/	93,K,V/	1|	target	0	2.982055	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.88409.88409.2.0.dta	88409	3011.581753	2	0	HIADLAGNSEVILPVPAFNVINGGSHAGNK	3011.569601	0.012152	33.010505	1.37211e-10		3	sp|P06733|ENOA_HUMAN/	132,R,L/	1|	target	0	3.433382	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.80155.80155.3.0.dta	80155	3481.685252	3	0	KEEQEVQATLESEEVDLNAGLHGNWTLENAK	3481.671600	0.013652	36.300910	1.3884e-10		3	sp|Q08211|DHX9_HUMAN/	151,R,A/	1|	target	1	5.434585	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.62436.62436.2.0.dta	62436	2764.374866	2	0	LPGPTGSVVSTGTSFSSSSPGLASAGAAEGK	2764.363429	0.011437	34.233419	1.40924e-10		3	sp|Q7Z434|MAVS_HUMAN/	239,R,Q/	1|	target	0	2.691226	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.70332.70332.2.0.dta	70332	2623.339190	2	0	LHGGTPANFLDVGGGATVHQVTEAFK	2623.326204	0.012986	35.974552	1.44607e-10		3	sp|Q9P2R7|SUCB1_HUMAN/	336,K,L/	1|	target	0	0.473340	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.75470.75470.3.0.dta	75470	4036.913733	3	0	GILGYTEHQVVSSDFNSDTHSSTFDAGAGIALNDHFVK	4036.894597	0.019136	39.360870	1.56119e-10		3	sp|P04406|G3P_HUMAN/	271,K,L/	1|	target	0	1.922608	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.72605.72605.3.0.dta	72605	3784.826234	3	0	YGPQYGHPPPPPPPPEYGPHADSPVLMVYGLDQSK	3784.810277	0.015957	35.709469	1.58701e-10		3	sp|P14866|HNRPL_HUMAN/	358,R,M/	1|	target	0	3.717392	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.91136.91136.3.0.dta	91136	3589.801605	3	0	AALANLCIGDVITAIDGENTSNMTHLEAQNR	3589.793096	0.008509	36.799667	1.6293e-10	7,PFIND_DELTA_334.22;	3	sp|O00151|PDLI1_HUMAN/	38,K,I/	1|PFIND_DELTA_334.22|	target	0	2.792105	36	334.2132
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.73249.73249.3.0.dta	73249	3116.438791	3	0	FWEVISDEHGIDPTGTYHGDSDLQLER	3116.423061	0.015730	31.162655	1.6875e-10		3	sp|P04350|TBB4A_HUMAN/sp|P68371|TBB4B_HUMAN/	19,K,I/19,K,I/	1|	target	0	2.125186	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.73425.73425.3.0.dta	73425	3102.422121	3	0	FWEVISDEHGIDPTGTYHGDSDLQLDR	3102.407411	0.014710	30.973769	1.7562e-10		3	sp|P07437|TBB5_HUMAN/	19,K,I/	1|	target	0	3.485326	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.42181.42181.2.0.dta	42181	2479.090776	2	0	VHNDAQSFDYDHDAFLGAEEAK	2479.079565	0.011211	33.891734	1.90666e-10		3	sp|O43852|CALU_HUMAN/	37,K,T/	1|	target	0	2.829188	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.46161.46161.2.0.dta	46161	2683.332161	2	0	IHVSDQELQSANASVDDSRLEELK	2683.316816	0.015345	33.535942	2.16052e-10		3	sp|P22314|UBA1_HUMAN/	806,K,A/	1|	target	1	2.846873	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.83169.83169.2.0.dta	83169	2472.278205	2	0	TVEEVLGHFGVNESTGLSLEQVK	2472.261544	0.016661	34.622542	2.17766e-10		3	sp|P16615|AT2A2_HUMAN/	7,K,K/	1|	target	0	2.773707	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.72172.72172.3.0.dta	72172	3154.534082	3	0	PAPVAVAAAATAAAYGGYPTAHTATDYGYTQR	3154.522711	0.011371	29.402265	2.19801e-10		3	sp|Q96KR1|ZFR_HUMAN/	91,R,Q/	1|	target	0	4.093649	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.61224.61224.3.0.dta	61224	3933.737431	3	0	DTHEDHDTSTENTDESNHDPQFEPIVSLPEQEIK	3933.716758	0.020673	33.950893	2.20417e-10		3	sp|P43487|RANG_HUMAN/	5,K,T/	1|	target	0	3.707479	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.96027.96027.3.0.dta	96027	3951.992407	3	0	DLINEACWDISSSGVNLQSMDSSHVSLVQLTLR	3951.977252	0.015155	36.559683	2.22121e-10	7,PFIND_DELTA_334.22;	3	sp|P12004|PCNA_HUMAN/	20,K,S/	1|PFIND_DELTA_334.22|	target	0	1.389466	100	334.2183
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.97263.97263.2.0.dta	97263	2714.380649	2	0	KLDGETTDLQDQIAELQAQIDELK	2714.372932	0.007717	35.579958	2.22699e-10		3	sp|P35580|MYH10_HUMAN/	1058,R,L/	1|	target	1	2.345676	4	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.92320.92320.2.0.dta	92320	2502.247539	2	0	ENTEGEYSGIEHVIVDGVVQSIK	2502.235723	0.011816	32.558977	2.44991e-10		3	sp|P50213|IDH3A_HUMAN/	146,R,L/	1|	target	0	4.715387	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.89003.89003.3.0.dta	89003	3898.831242	3	0	AHESAVTGLSLHATGDYLLSSSDDQYWAFSDIQTGR	3898.815290	0.015952	33.810457	3.17768e-10		3	sp|Q9UMS4|PRP19_HUMAN/	303,R,V/	1|	target	0	4.509088	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.59107.59107.2.0.dta	59107	2693.390965	2	0	TAHLDEEVNKGDILVVATGQPEMVK	2693.381329	0.009636	33.932617	3.50767e-10		3	sp|P11586|C1TC_HUMAN/	198,K,G/	1|	target	1	2.609834	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.65458.65458.2.0.dta	65458	2635.351867	2	0	KYGGPPPDSVYSGVQPGIGTEVFVGK	2635.340124	0.011743	31.960907	3.60258e-10		3	sp|O43390|HNRPR_HUMAN/	145,R,I/	1|	target	1	1.810302	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.56298.56298.2.0.dta	56298	2673.348118	2	0	PAHLLQDDISSSYTTTTTITAPPSR	2673.336490	0.011628	30.870622	3.78199e-10		3	sp|O00767|SCD_HUMAN/	1,M,V/	1|	target	0	3.589815	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.21903.21903.2.0.dta	21903	2982.406701	2	0	EELQANGSAPAADKEEPAAAGSGAASPSAAEK	2982.392154	0.014547	35.116592	3.8665e-10		3	sp|P29966|MARCS_HUMAN/	55,K,G/	1|	target	1	5.709081	68	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.98773.98773.2.0.dta	98773	2933.552289	2	0	VGAGAPVYMAAVLEYLTAEILELAGNAAR	2933.543967	0.008322	31.255114	4.02792e-10		3	sp|Q16777|H2A2C_HUMAN/sp|Q6FI13|H2A2A_HUMAN/	43,R,D/43,R,D/	1|	target	0	3.379331	4	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.73419.73419.3.0.dta	73419	3116.438225	3	0	FWEVISDEHGIDPTGTYHGDSDLQLER	3116.423061	0.015164	29.647997	4.14354e-10		3	sp|P04350|TBB4A_HUMAN/sp|P68371|TBB4B_HUMAN/	19,K,I/19,K,I/	1|	target	0	3.331819	64	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.97443.97443.2.0.dta	97443	2874.416453	2	0	LFENQLVGPESIAHIGDVMFTGTADGR	2874.408936	0.007517	33.370379	4.17808e-10		3	sp|Q9HDC9|APMAP_HUMAN/	93,R,V/	1|	target	0	3.576504	4	
HFX_YangJing_HeJiXiang_IPM_20230701_F1_R1.73068.73068.2.0.dta	73068	2577.347472	2	0	AITVESPDGHLFQVEYAQEAVKK	2577.334647	0.012825	32.545159	5.20516e-10		3	sp|Q8TAA3|PSMA8_HUMAN/sp|O14818|PSA7_HUMAN/	7,R,G/5,R,G/	1|	target	1	2.564896	64	

6. Supporting protocol 1: Protein sequence database

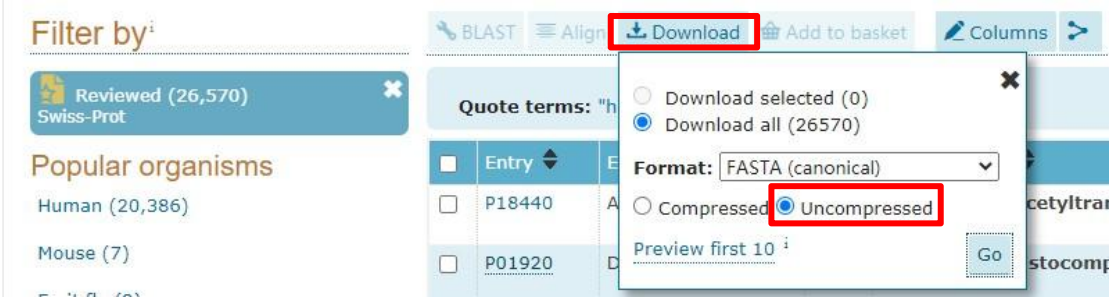
This protocol is used to download protein *.fasta files for database search.

1) Open https://www.uniprot.org/, enter the Latin name of the species (e.g., *home sapiens*), then click search.

2) Click "*Reviewed*" (Swiss-Prot).



3) Select "*Uncompressed*", then Click "*Download*" and "*Go*".

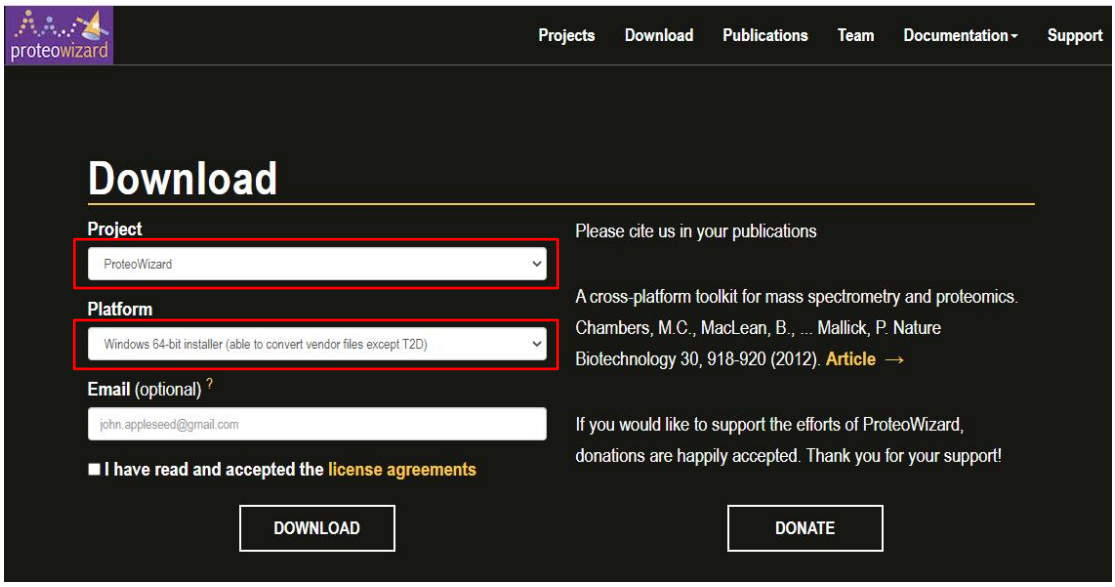


4) Get the *.fasta file.

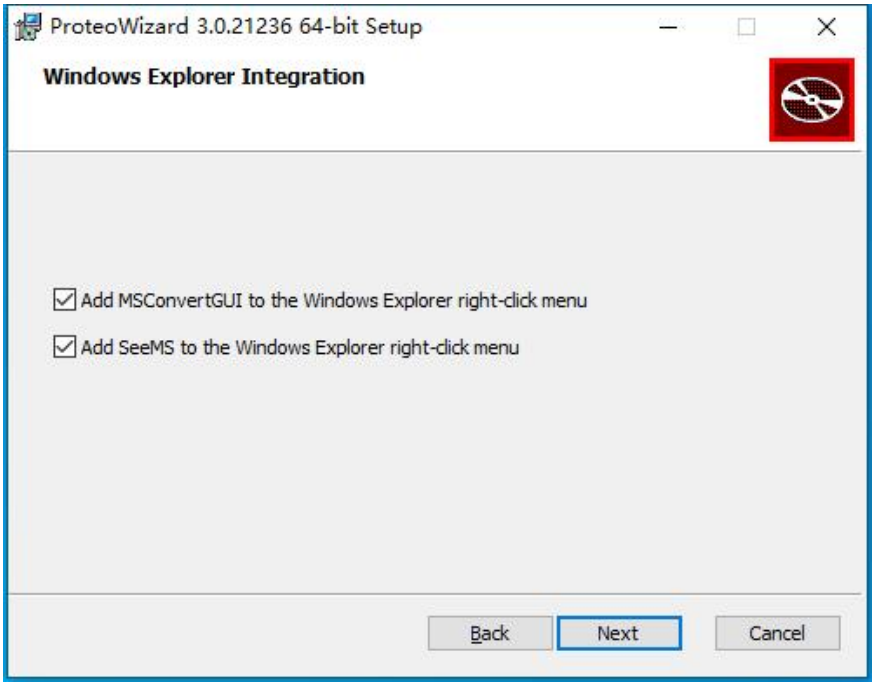
7. Supporting protocol 2: MSconvert

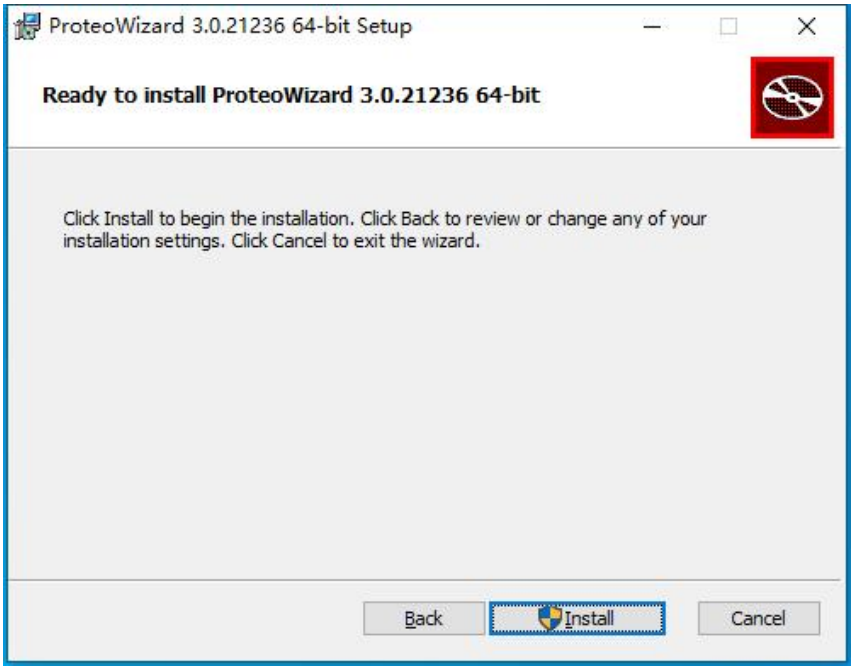
This protocol is used to convert non-Thermo MS data into mzML format files for pIon search.

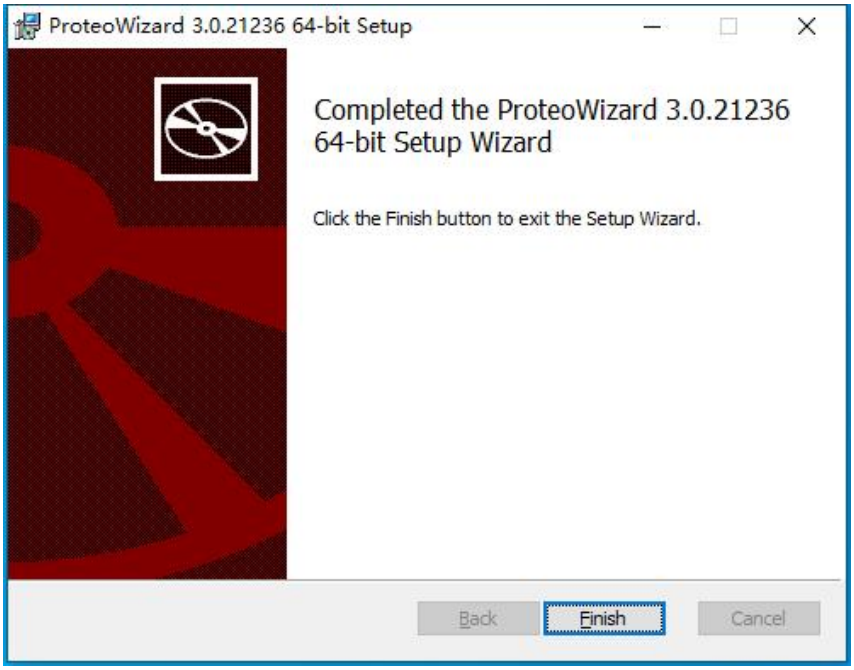
1) Download MSconvertGUI that is embedded in the ProteoWizard platform from: https://proteowizard.sourceforge.io/download.html .



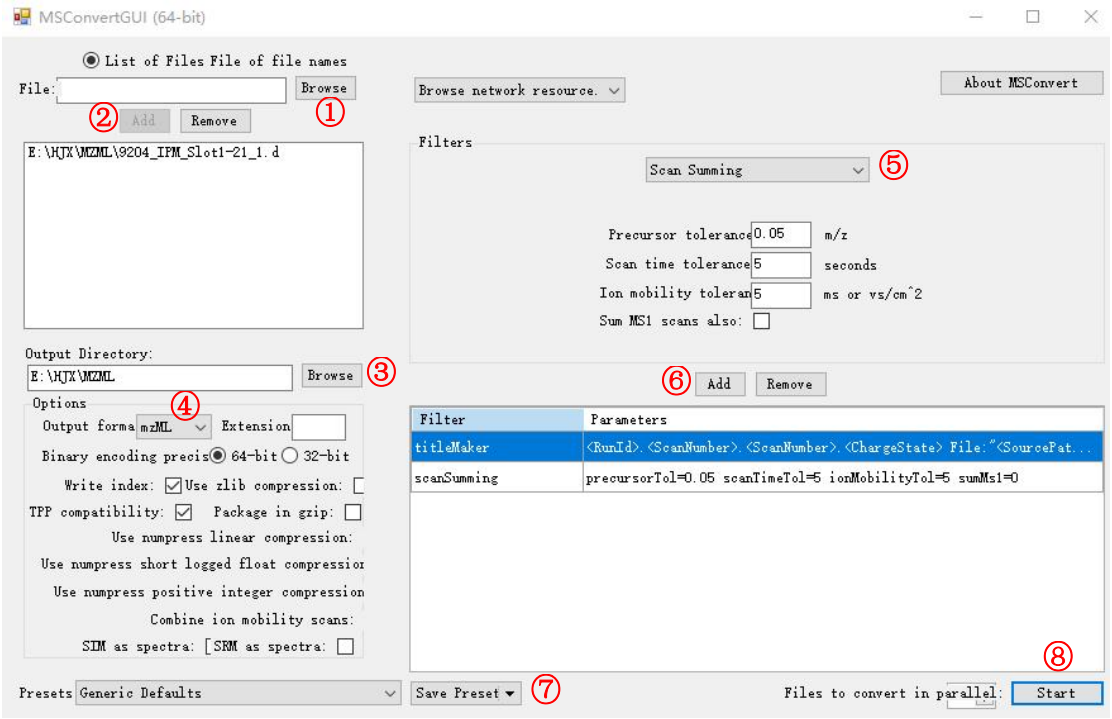
2) Install ProteoWizard according to the following instruction.




ProteoWizard 3.0.21236 64-bit Setup
Ready to install ProteoWizard 3.0.21236 64-bit
Click Install to begin the installation. Click Back to review or change any of your installation settings. Click Cancel to exit the wizard.
Back
Install
Cancel


ProteoWizard 3.0.21236 64-bit Setup
Completed the ProteoWizard 3.0.21236 64-bit Setup Wizard
Click the Finish button to exit the Setup Wizard.
Back
Finish
Cancel

3) Open MSconvertGUI



①-②Browse and add MS data (e.g., *.d, *.WIFF files)

③ Define output route

④ Choose *.mzML as the output data format

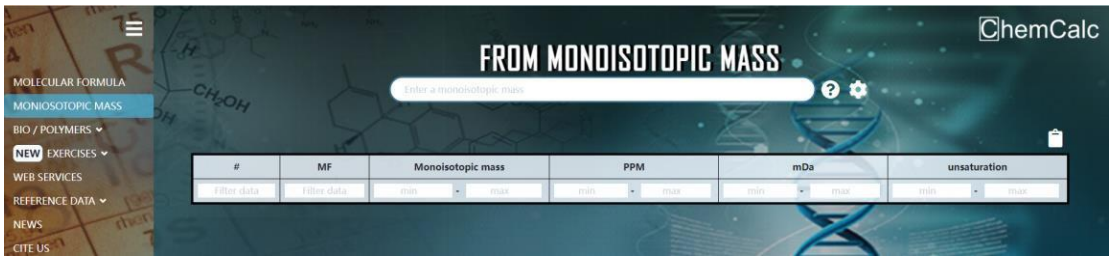
⑤ -⑥ Define parameters for Scan Summing

⑥ -⑧ Save and run

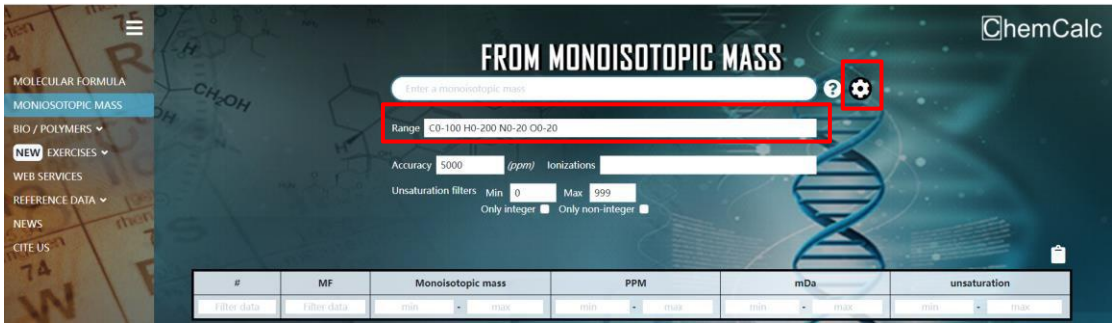
8. Supporting protocol 3: ChemCalc

This protocol is used to estimate candidate molecular formulas from the pChem/pIon-determined accurate masses.

1) Open **https://www.chemcalc.org/mf-finder** .



2) Click  , check the element composition.



3) Input the monoisotopic mass of each PDM shown in *pChem.summary* or *pChem_ion_filter.summary* file. The candidate molecular formulas will immediately appear below.

Rank	PDM	Accurate Mass	Top1 Site|Probability	Others	#PSM	#PSM L|H	DFLs
1	PFIND_DELTA_252	252.122339	C|0.988		13876	7368|6508