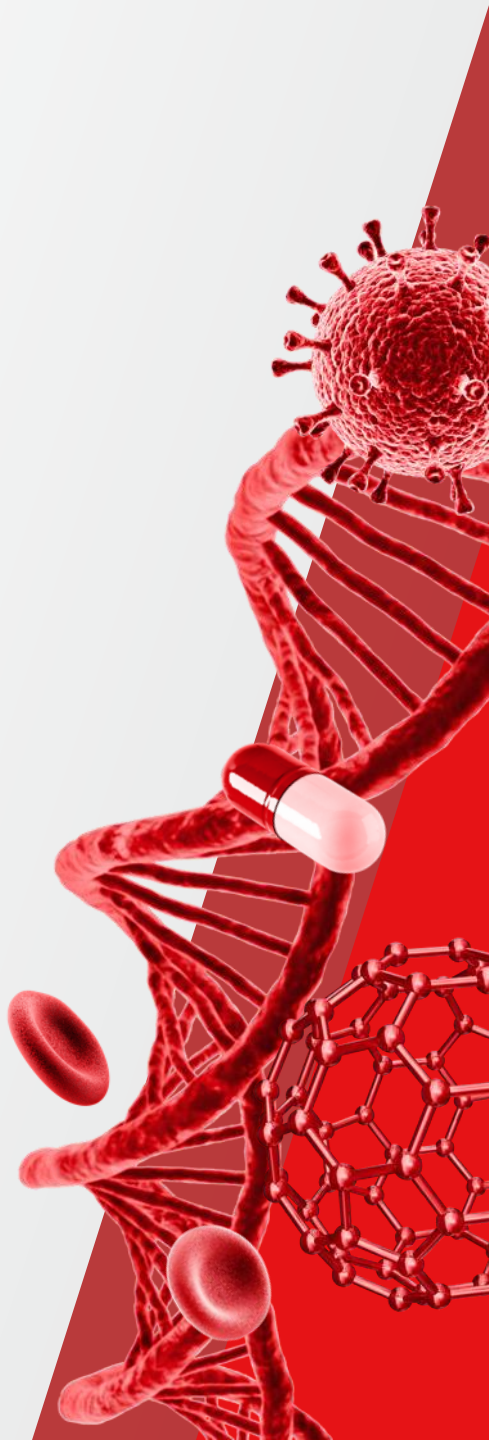
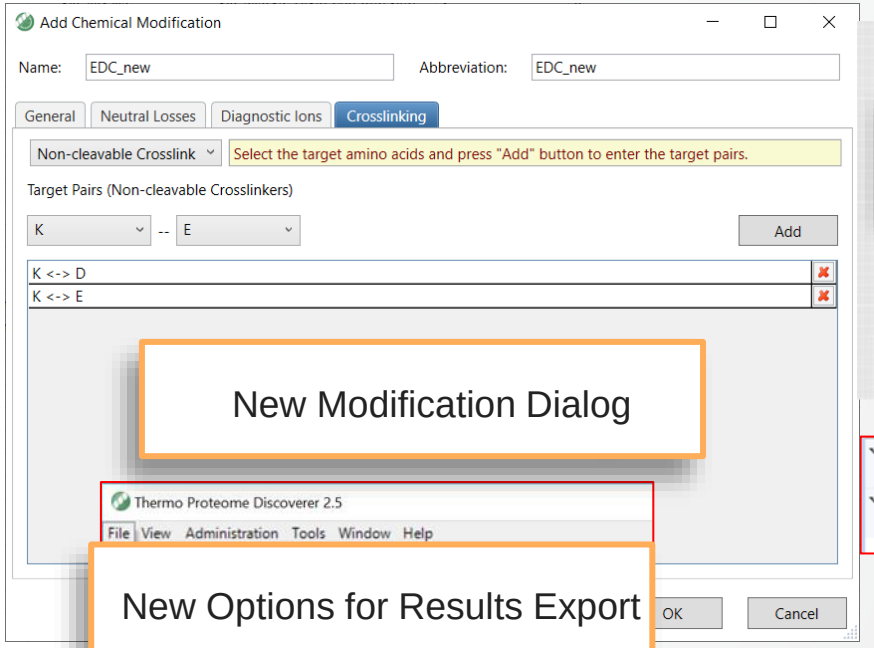
Getting Started on XlinkX 2.5 Node for Proteome Discoverer 2.5 Software

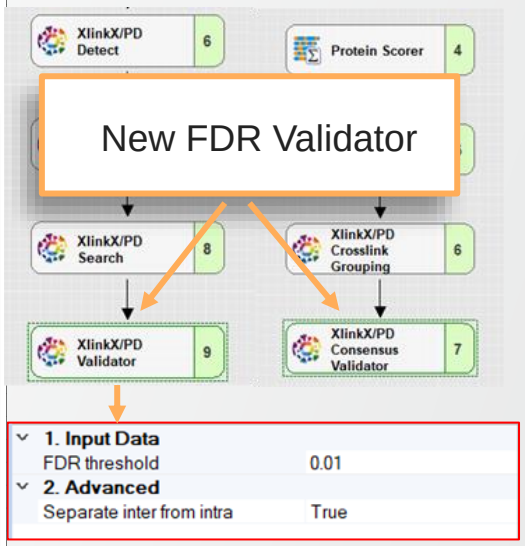


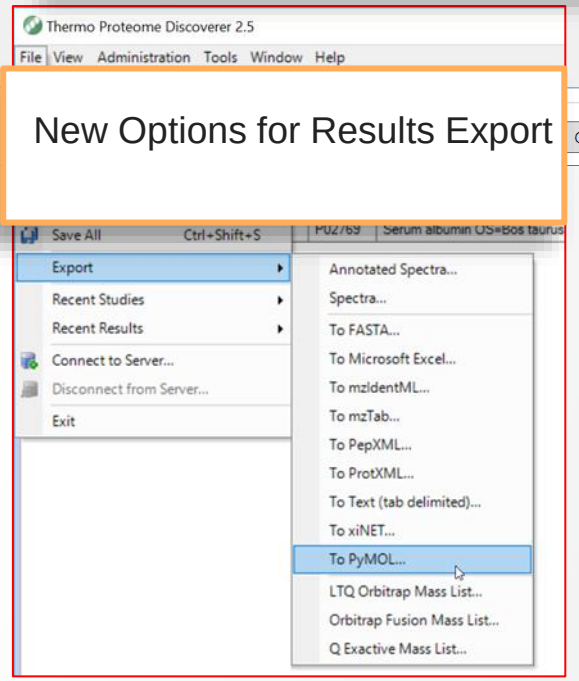
The world leader in serving science

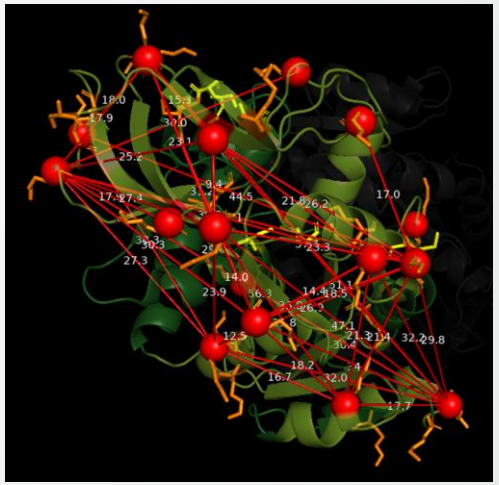
XlinkX 2.5 Node - New Features for Proteome Discoverer 2.5 Software

- New modifications dialog
  - Heterobifunctional crosslinkers support
  - Direct import of Unimod crosslinkers
- New FDR calculations including intra and inter validation
- New options for **results export :**
  - PyMol
  - xiView
  - Download: https://thermo.flexnetoperations.com/control/thmo/download?element=12199007



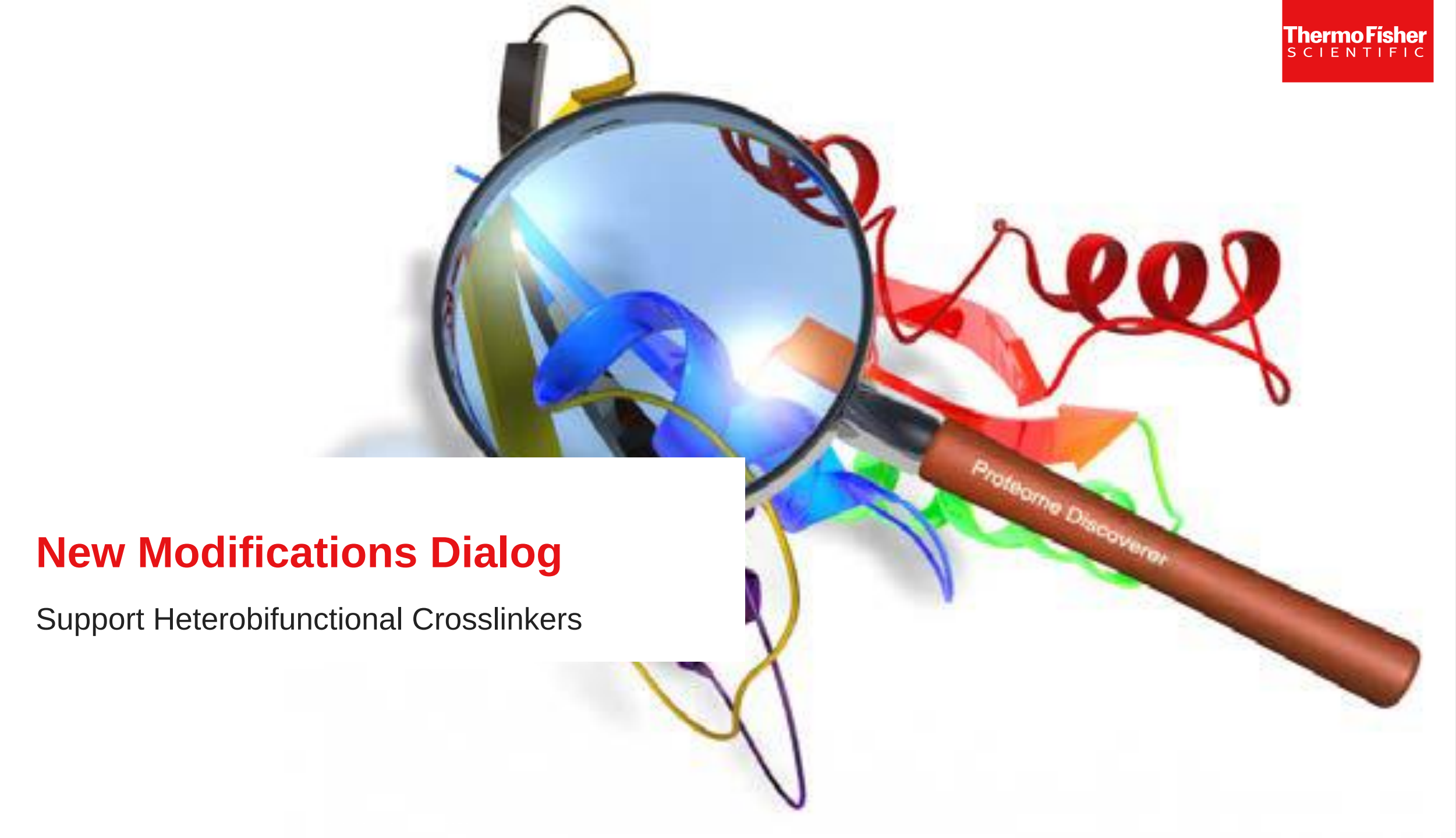






Outline

- New modifications dialog
  - Adding Crosslinkers
  - Adding Cleavable Crosslinkers
  - Adding Non-Cleavable Crosslinkers
  - Adding Monolinks Modification
- New FDR calculations
- Crosslinking Analysis Template
- Example of EDC Crosslinking Analysis
- New options for results export
  - Exporting Crosslinks to Pymol
  - Exporting Crosslinks to xiVIEW

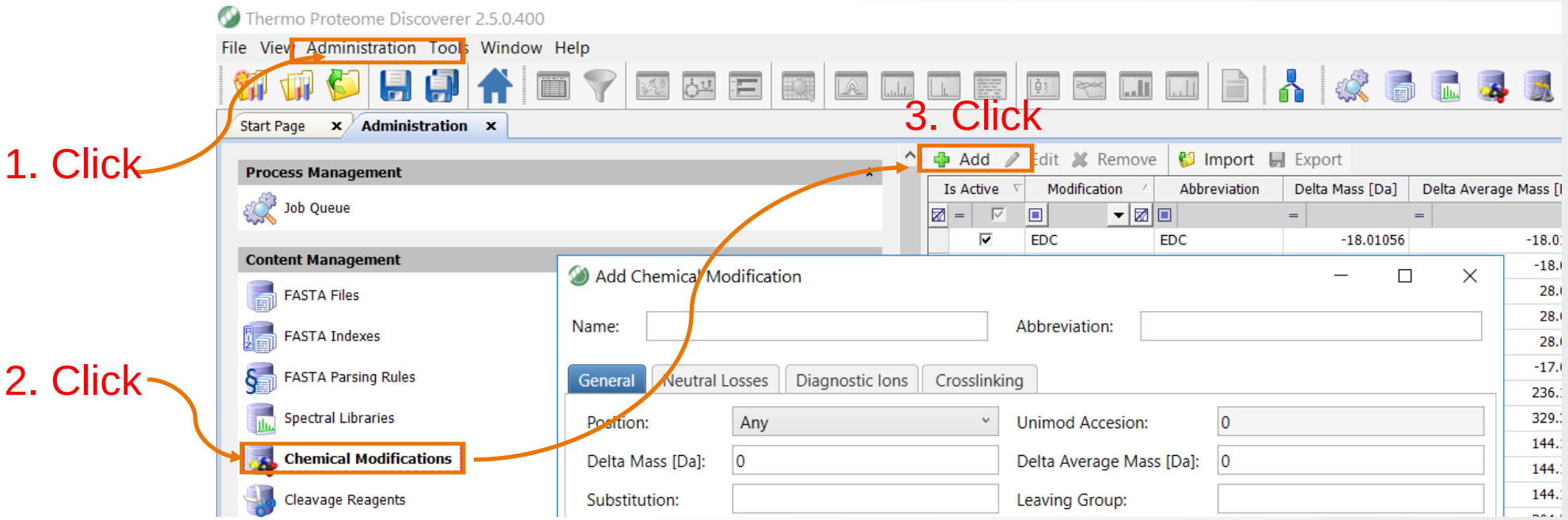


New Modifications Dialog

Support Heterobifunctional Crosslinkers

1. Adding Crosslinkers

- Common crosslinkers is available in the list of modifications in Thermo Scientific™ Proteome Discoverer Software.
- A new crosslinker can be added manually:
  - In the Modification Manager, Add a Modification for the crosslinker.



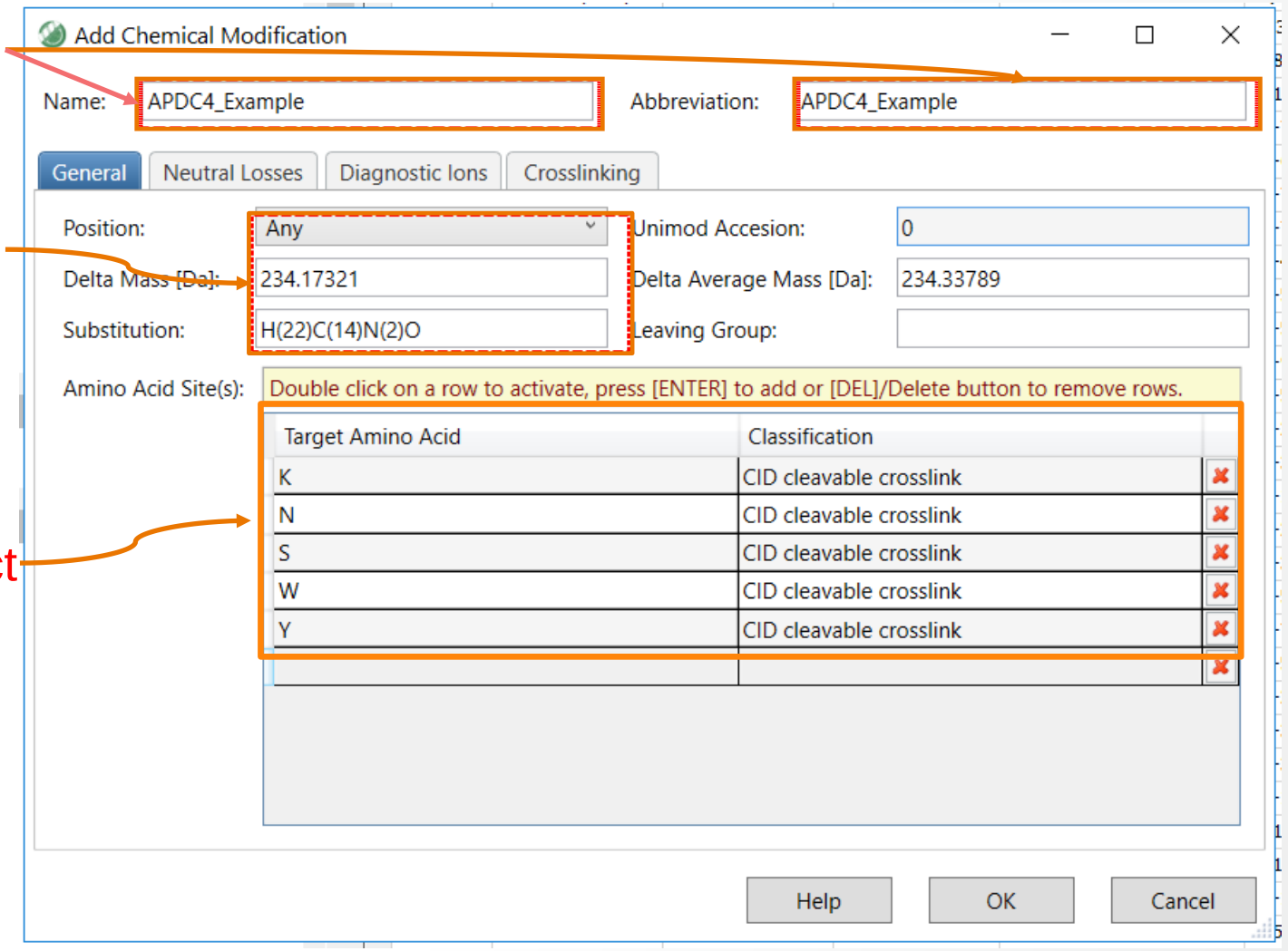
2. Adding Cleavable Crosslinkers

Step 1. Define a crosslink by using Name, Abbreviation, Substitution and Target Amino Acid Site(s) in General Tab.

1. Input

2. Input

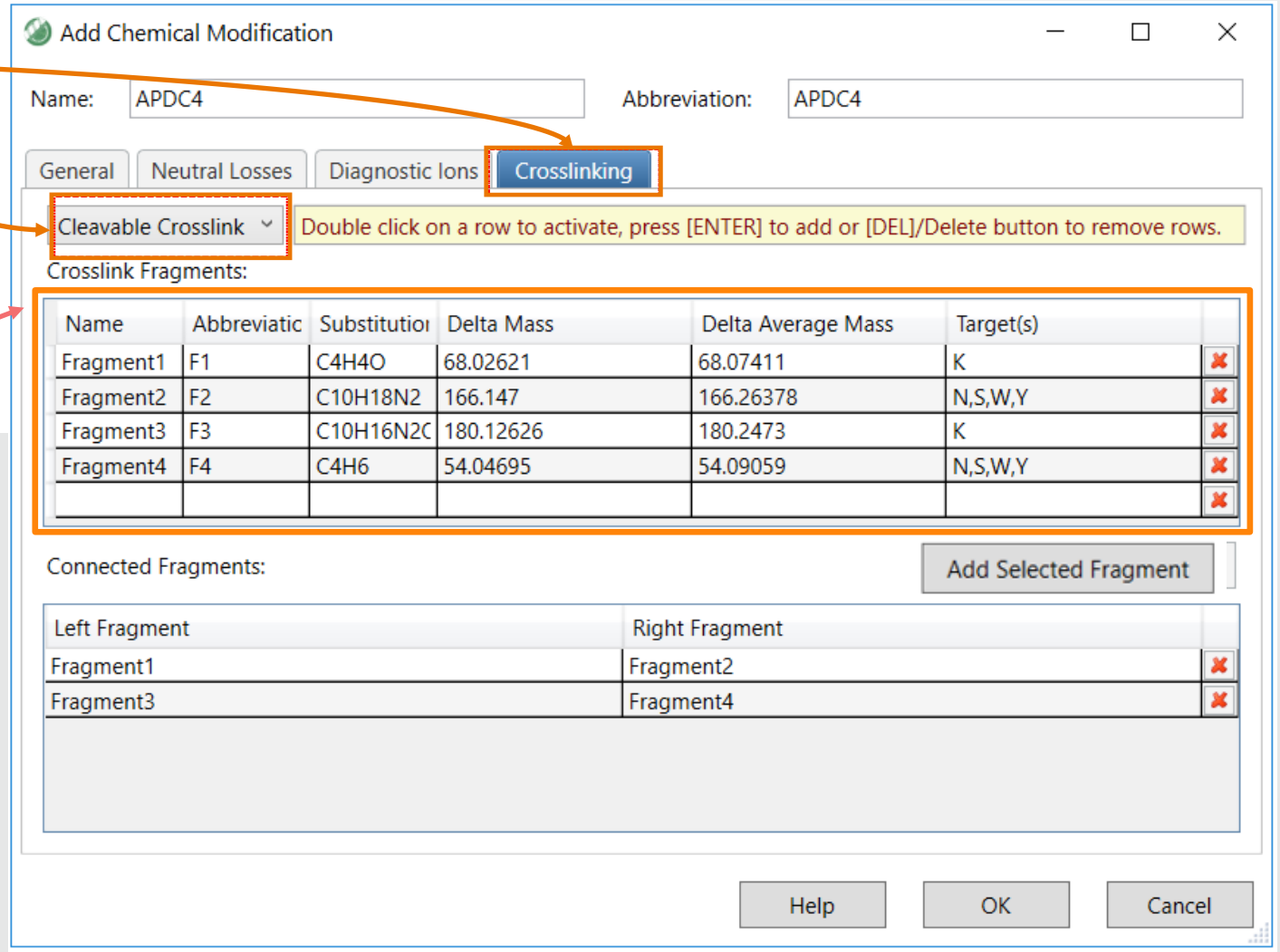
3. Click and Select



Adding Cleavable Crosslinkers

Step 2. Define a crosslink type, fragments in Crosslinking Tab.

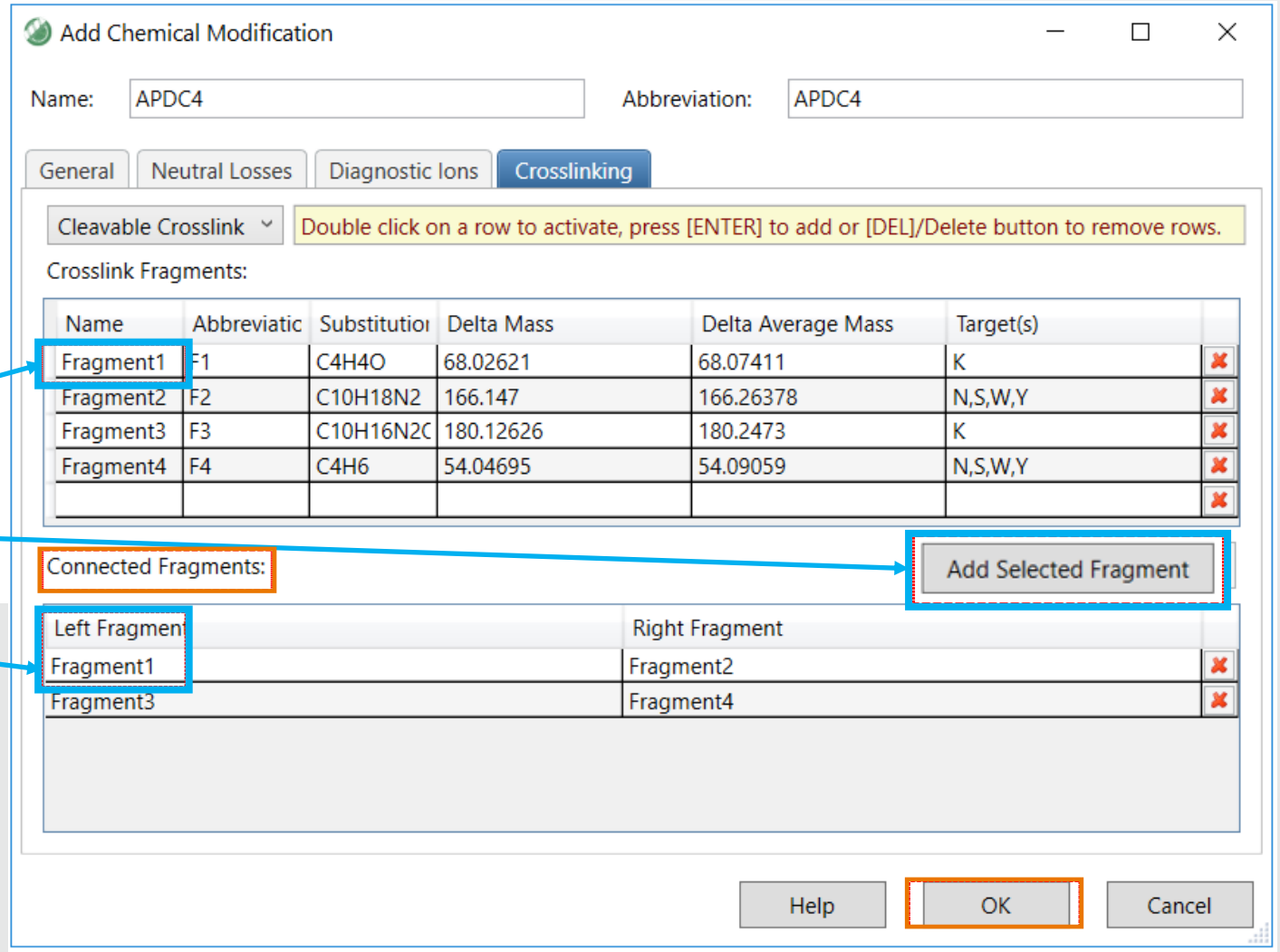
I. Click Crosslink to activate the crosslinking tab.
II. Click and define a Cleavable Crosslink type.
III. Click and Input crosslinking fragments Name, Abbreviate, Substitution, Delta Mass and Target(s) to the list.
IV. Select Left Fragments 1 from the list and click Add Selected Fragments to the Left Fragments.
V. Select Right Fragments 2 from the list and click Add Selected Fragments to the Right Fragments.
VI. Repeat step IV and V to connect all fragments.
VII. Click the OK button



Adding Cleavable Crosslinkers

Step 3. Add Left Fragments in Connected Fragments.

I. Click Crosslink to activate the crosslinking tab.
II. Click and define a Cleavable Crosslink type.
III. Click and Input crosslinking fragments Name, Abbreviate, Substitution, Delta Mass and Target(s) to the list.
IV. Select Left Fragments 1 from the list and click Add Selected Fragments to the Left Fragments.
V. Select Right Fragments 2 from the list and click Add Selected Fragments to the Right Fragments.
VI. Repeat step IV and V to connect all fragments.
VII. Click the OK button



Adding Cleavable Crosslinkers

Step 4. Add Right Fragments in Connected Fragments.

I. Click Crosslink to activate the crosslinking tab.

II. Click and define a Cleavable Crosslink type.

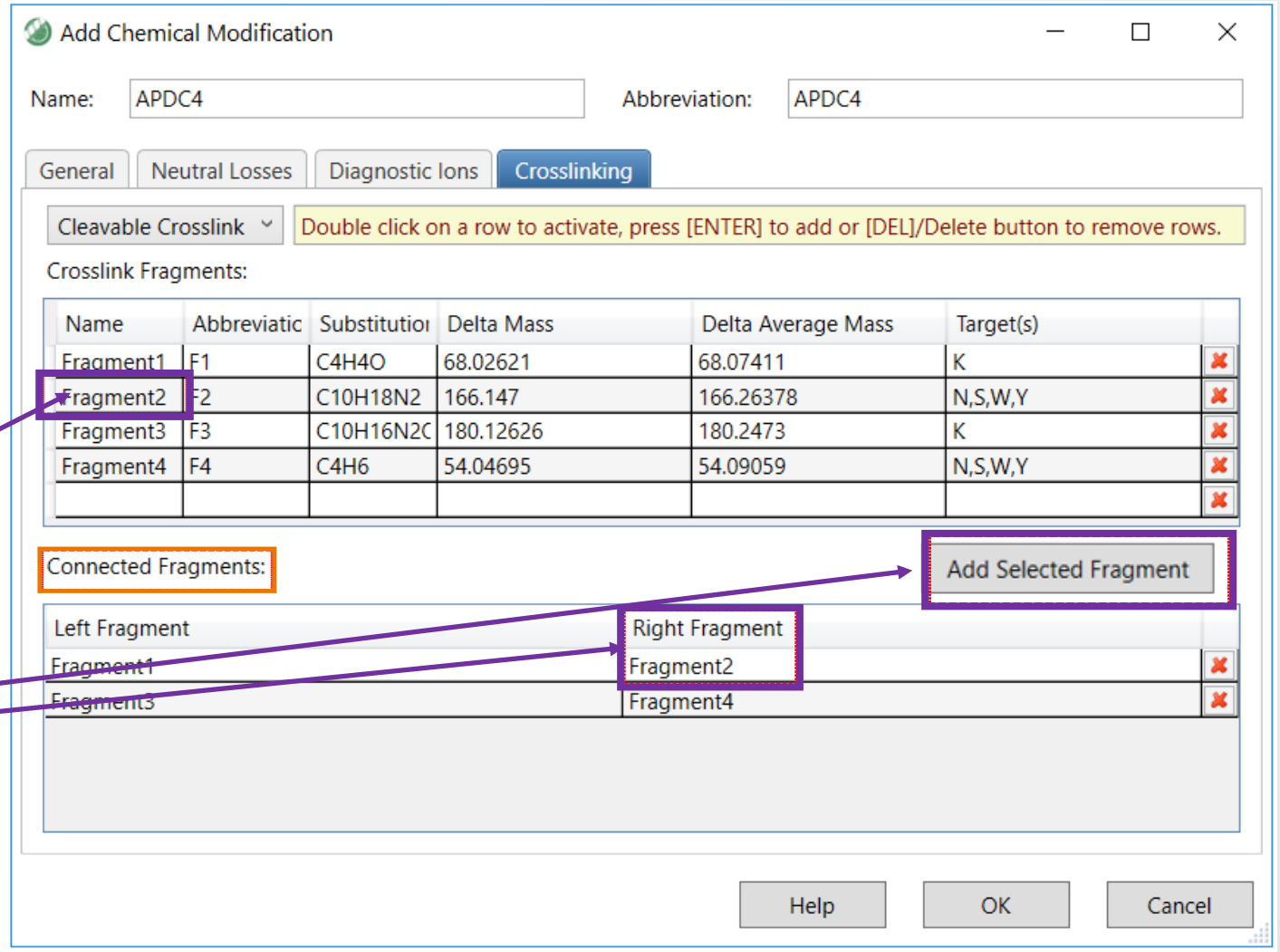
III. Click and Input crosslinking fragments Name, Abbreviate, Substitution, Delta Mass and Target(s) to the list.

IV. Select Left Fragments 1 from the list and click Add Selected Fragments to the Left Fragments.

V. Select Right Fragments 2 from the list and click Add Selected Fragments to the Right Fragments.

VI. Repeat step IV and V to connect all fragments.

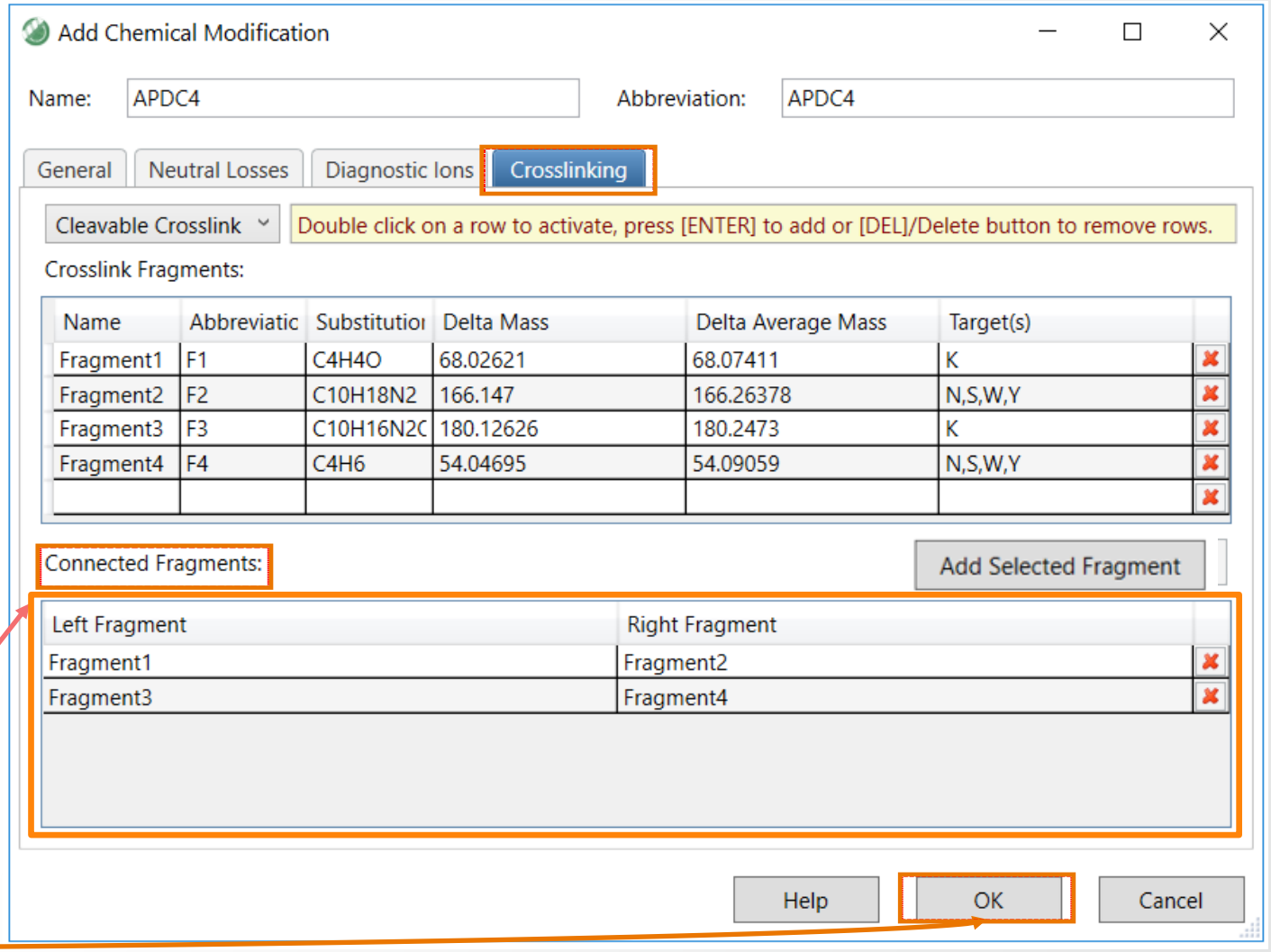
VII. Click the OK button



Adding Cleavable Crosslinkers

Step 5. Connect all fragments in Crosslinking Tab.

I. Click Crosslink to activate the crosslinking tab.
II. Click and define a Cleavable Crosslink type.
III. Click and Input crosslinking fragments Name, Abbreviate, Substitution, Delta Mass and Target(s) to the list.
IV. Select Left Fragments 1 from the list and click Add Selected Fragments to the Left Fragments.
V. Select Right Fragments 2 from the list and click Add Selected Fragments to the Right Fragments.
VI. Repeat step IV and V to connect all fragments one by one.
VII. Click the OK button



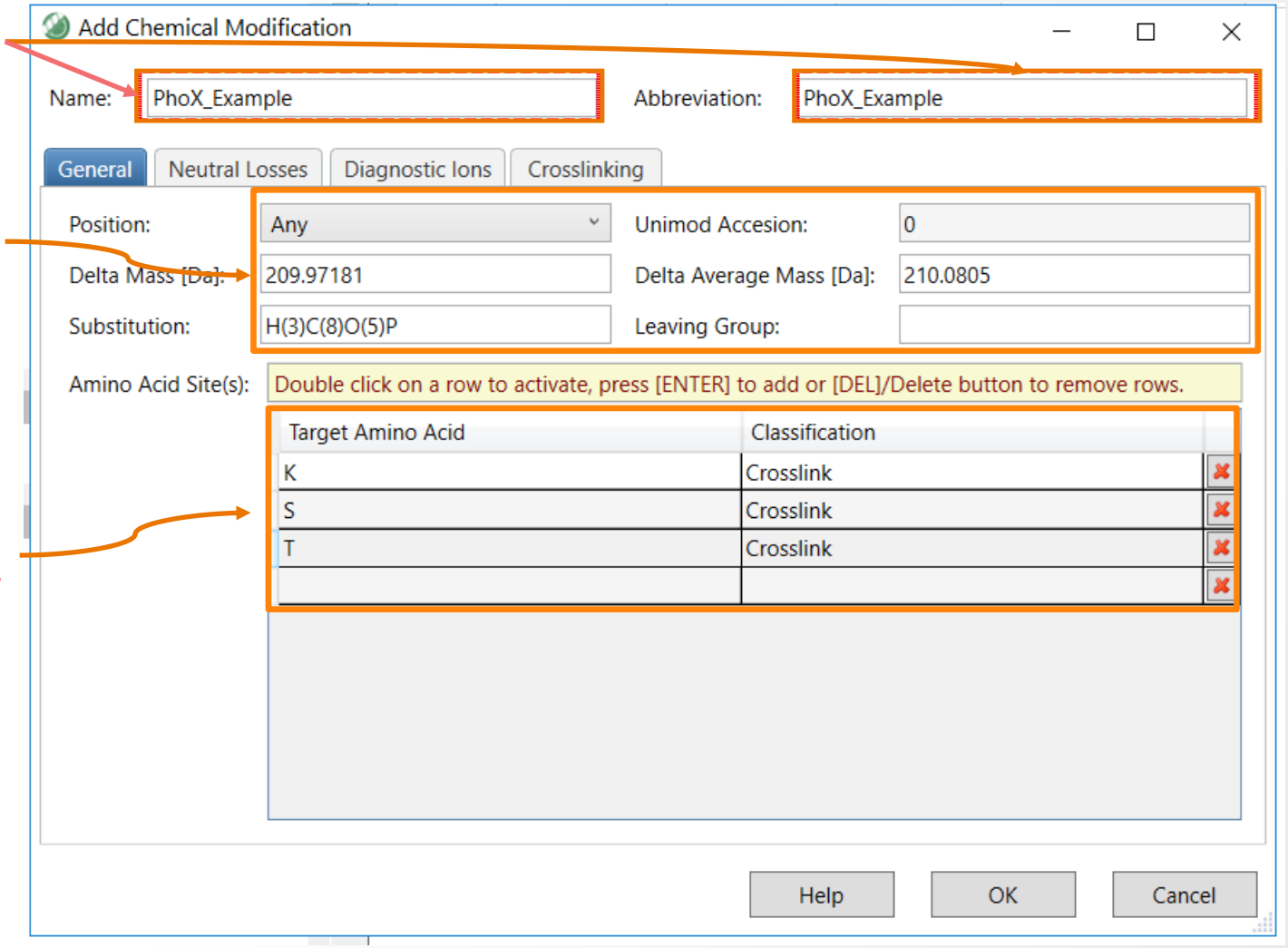
3. Adding Non-Cleavable Crosslinkers

Step 1. Define a crosslink by using Name, Abbreviation, Substitution and Target Amino Acid Site(s) in General Tab.

1. Click and add

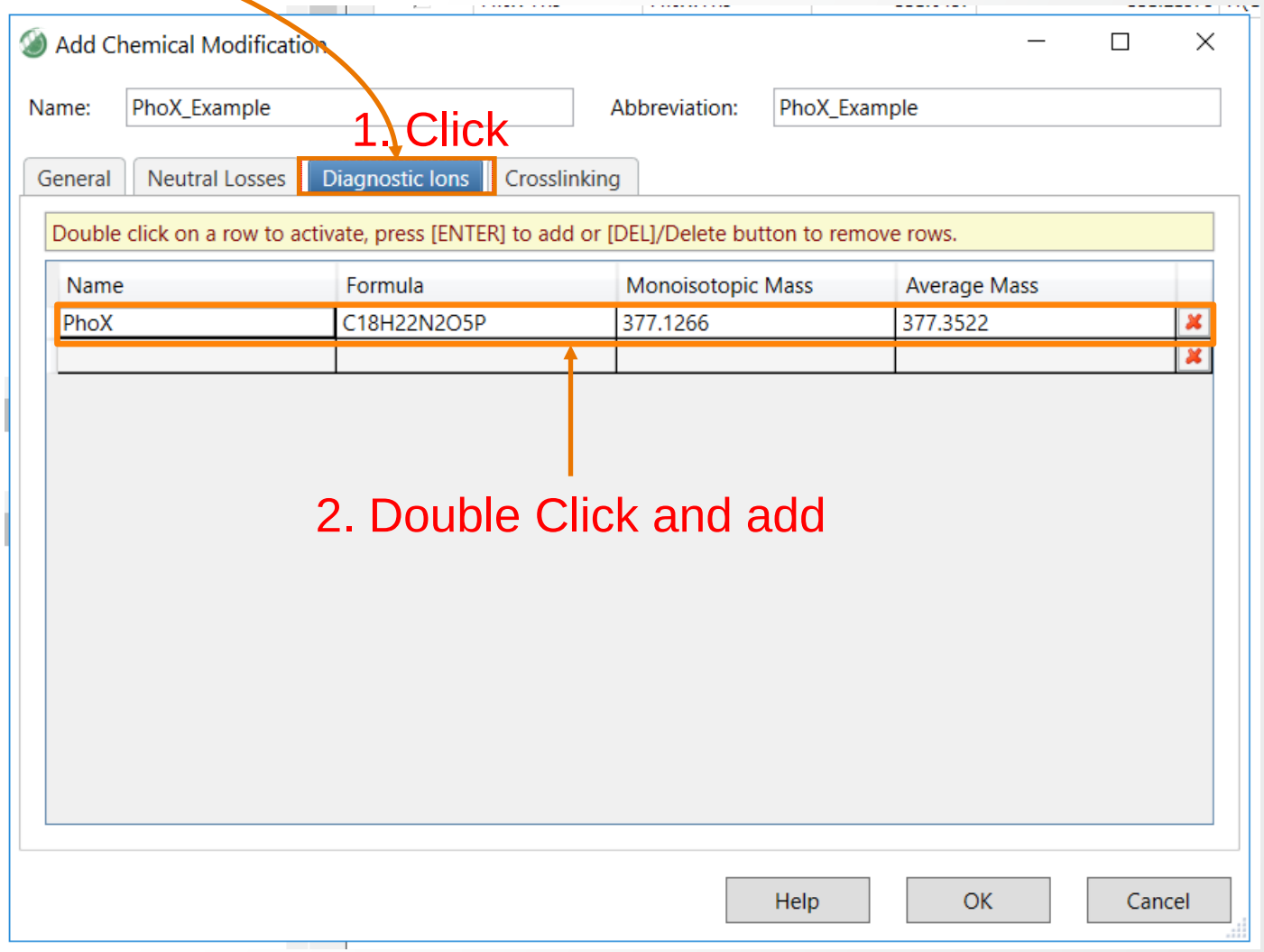
2. Click and add

3. Double click and select



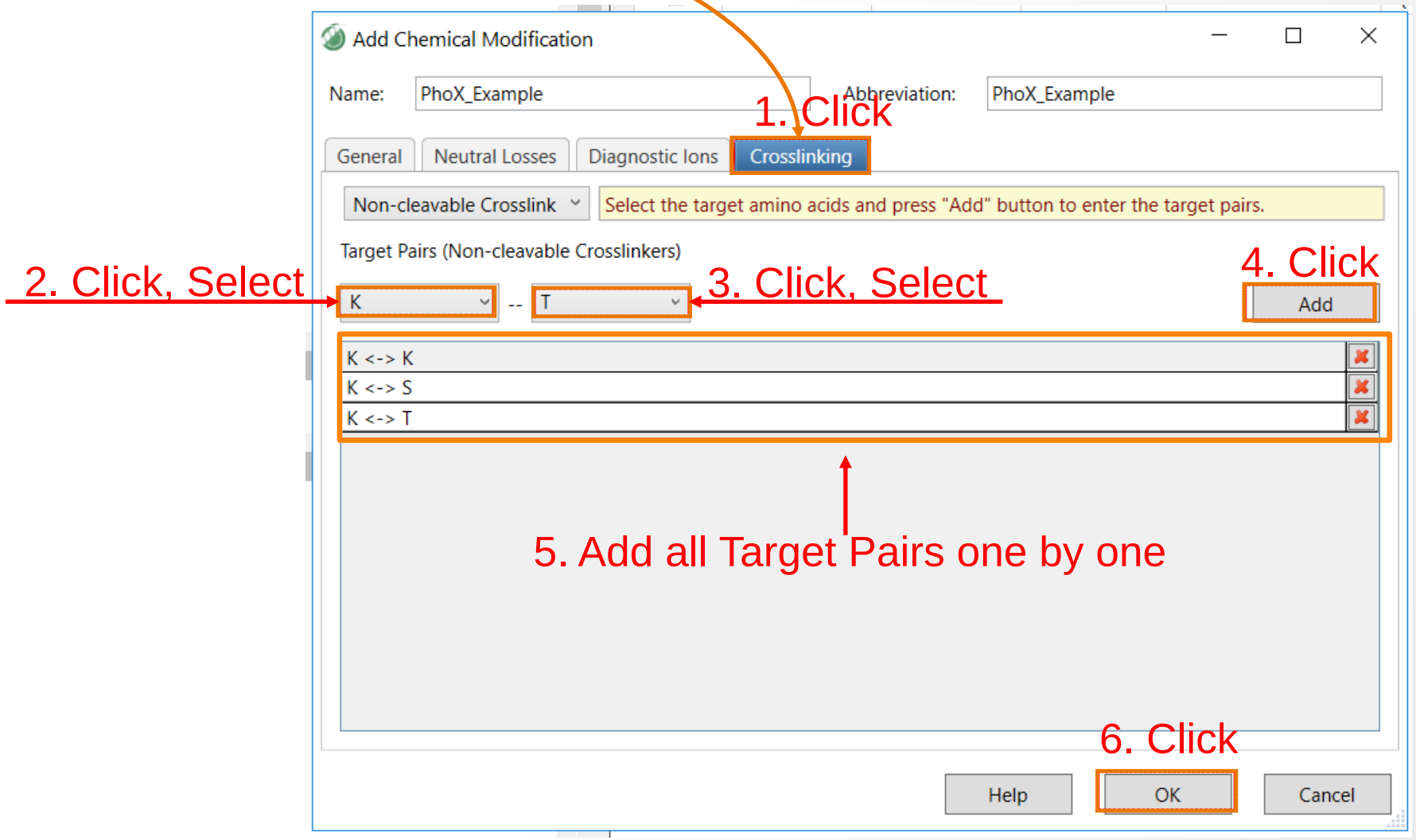
Adding Non-Cleavable Crosslinkers

Step 2. Define Diagnostic Ions by using Name and Formula. Monoisotopic Mass and Average Mass will automatic calculate based on Formula.



Adding Non-Cleavable Crosslinkers

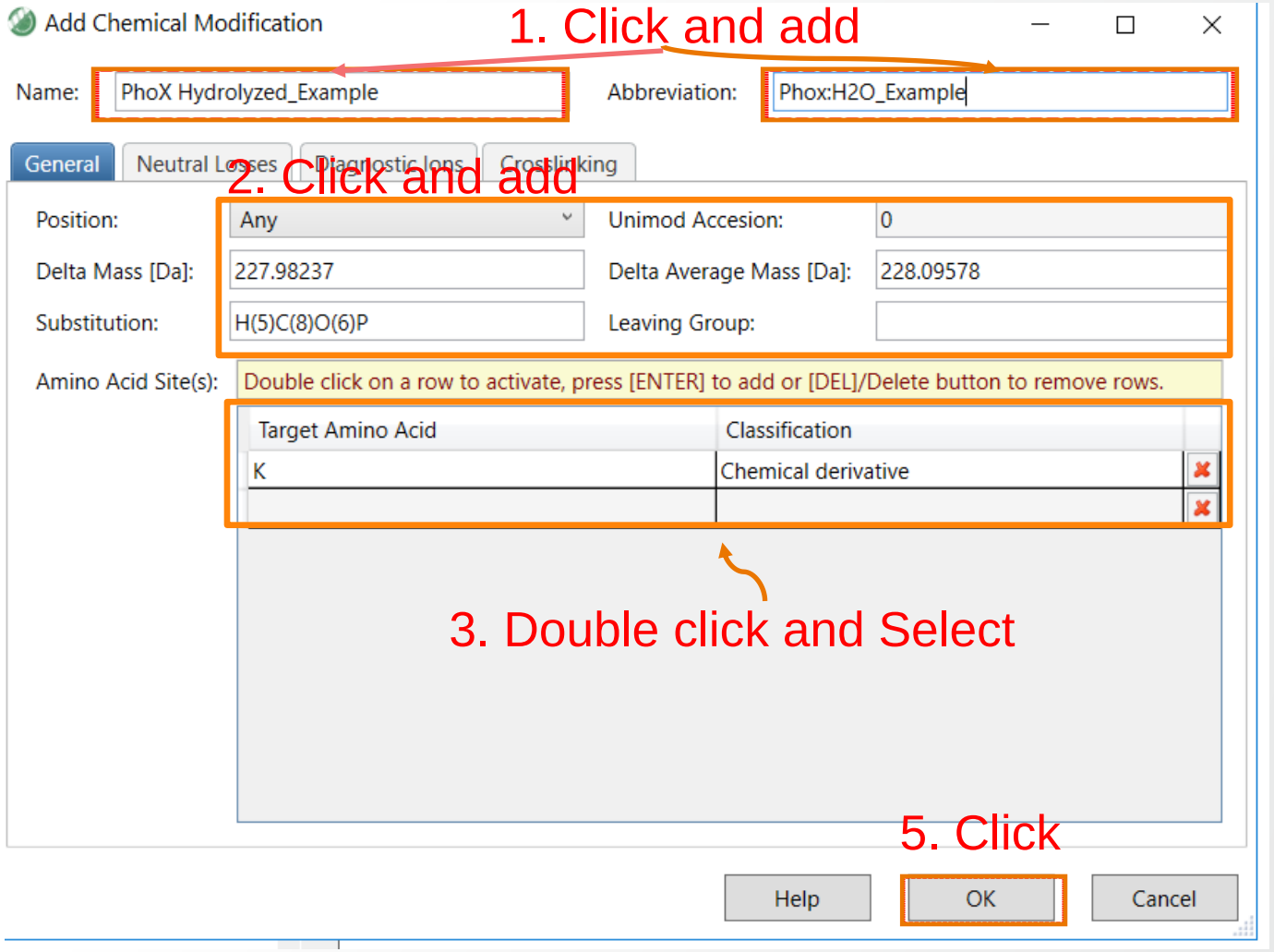
Step 3. Define Target Pairs in Crosslinking Tab.

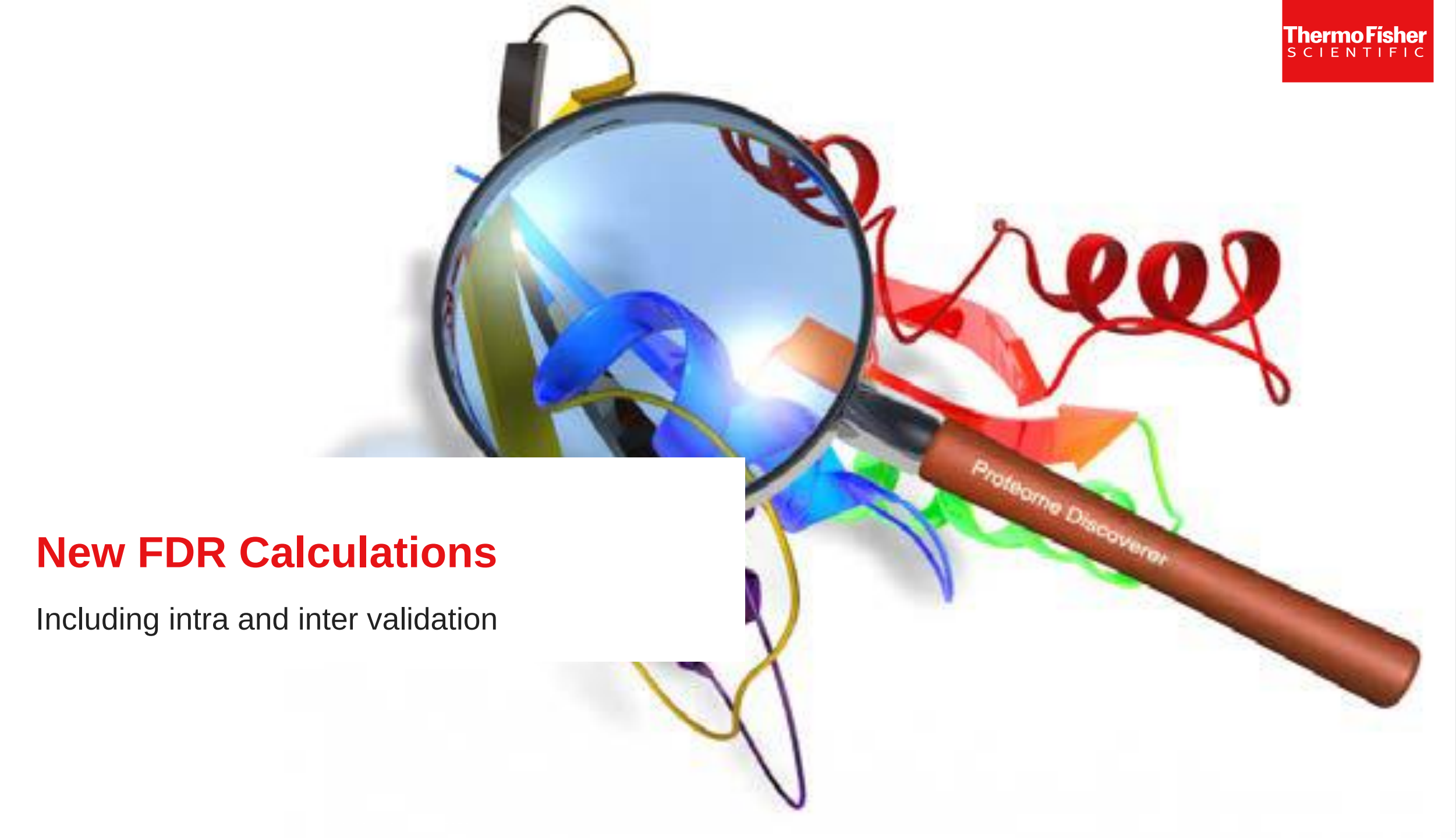


4. Adding Monolinks Modification

To set up the monolinks use the standard modification.

- Add a Chemical Modification of of PhoX dead ends by using Name, Abbreviation, Substitution and Target Amino Acid Site(s) in General Tab.





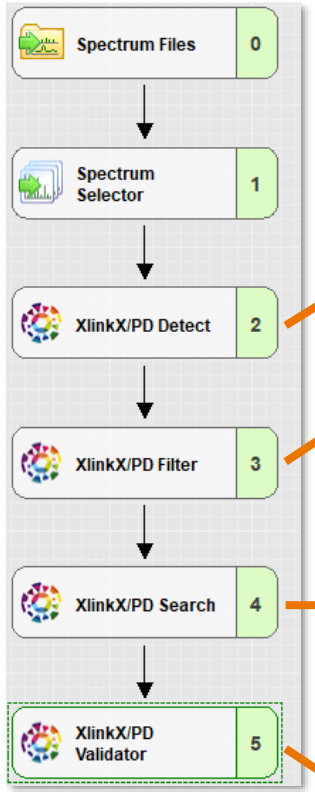
New FDR Calculations

Including intra and inter validation

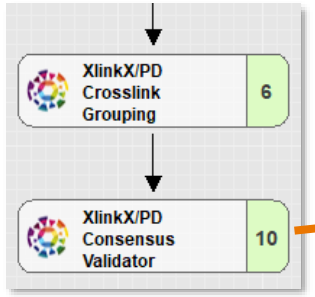
XlinkX 2.5 Node – Data Analysis Driven by XlinkX/PD

ThermoFisher SCIENTIFIC

Processing Workflow



Consensus Workflow (cutout)



1. Input Data

Acquisition strategy	MS2_MS2
Crosslink Modification	DSSO / +158.004 Da (K)
Minimum S/N	1.5
Enable protein N-terminus linkage	False

Select Crosslinker Reagent and acquisition strategy

1. Output Data

Select	Crosslinks

Filter for Spectra identified as being crosslinked

1. General Data	
Protein Database	pastrosepticum.fasta
Retain FASTA file indexes	True
Enzyme Name	Trypsin (Full)
Maximum Missed Cleavages	2
Minimum Peptide Length	5
2. Tolerances	
Precursor Mass Tolerance	10 ppm
FTMS Fragment Mass Tolerance	20 ppm
ITMS Fragment Mass Tolerance	0.5 Da
4. Static Modifications	
Static Modification	Carbamidomethyl / +57.021 Da (C)
Static Modification	None
Static Any N-term Modification	None
Static Any C-term Modification	None
Static Protein N-term Modification	None
Static Protein C-term Modification	None
5. Dynamic Modifications	
Dynamic Modification	Oxidation / +15.995 Da (M)
Dynamic Modification	None
Dynamic Modification	None
Dynamic Modification	None
Dynamic Modification	None
Dynamic Any N-term Modification	None
Dynamic Any N-term Modification	None
Dynamic Any N-term Modification	None
Dynamic Any C-term Modification	None
Dynamic Any C-term Modification	None
Dynamic Any C-term Modification	None
Dynamic Protein N-term Modification	Acetyl / +42.011 Da (N-Terminus)
Dynamic Protein C-term Modification	None

Select targeted modifications and protein database

1. Input Data

FDR threshold	0.01

Spectrum-Match-level (CSM) Validation (see slides below)

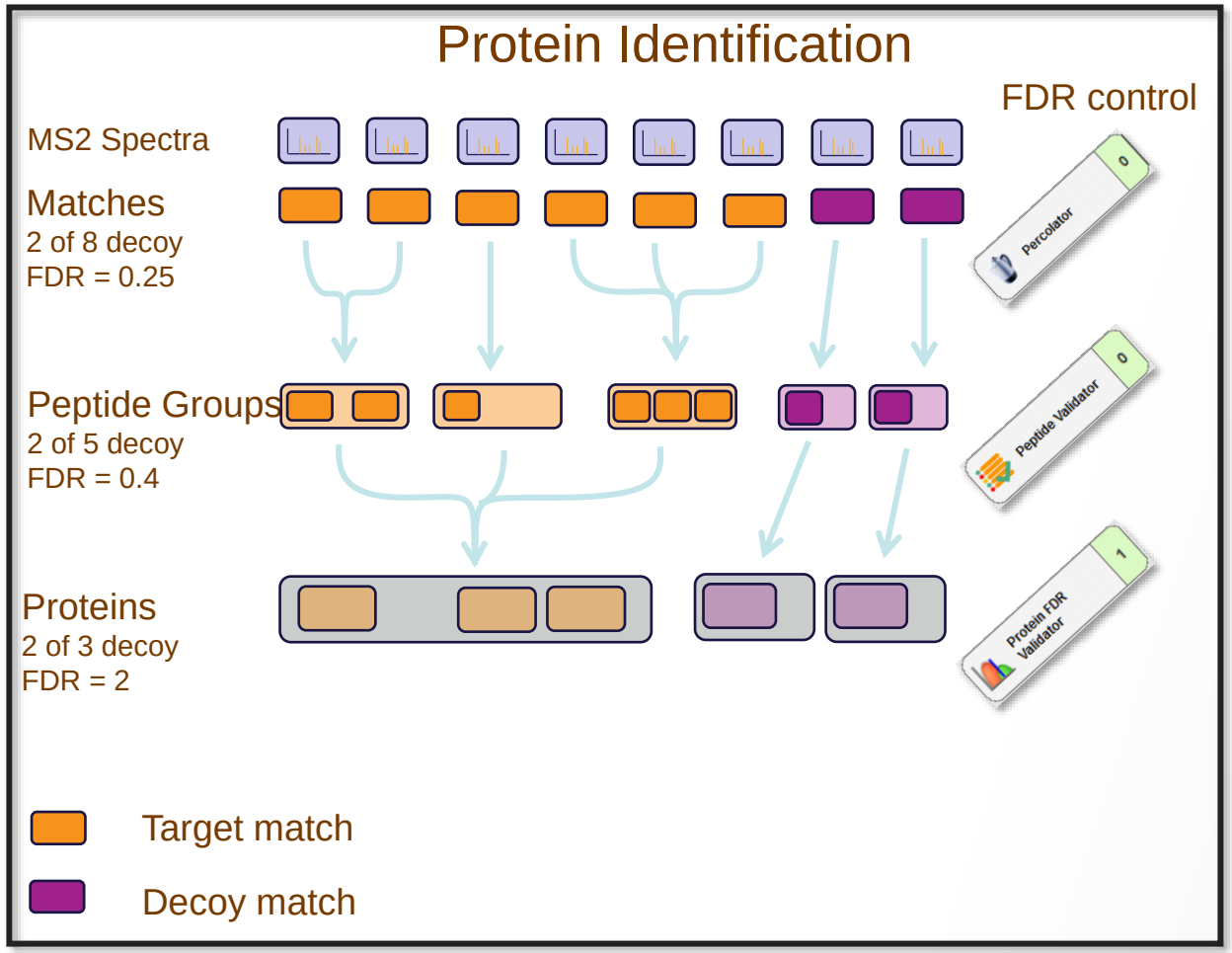
1. Input Data

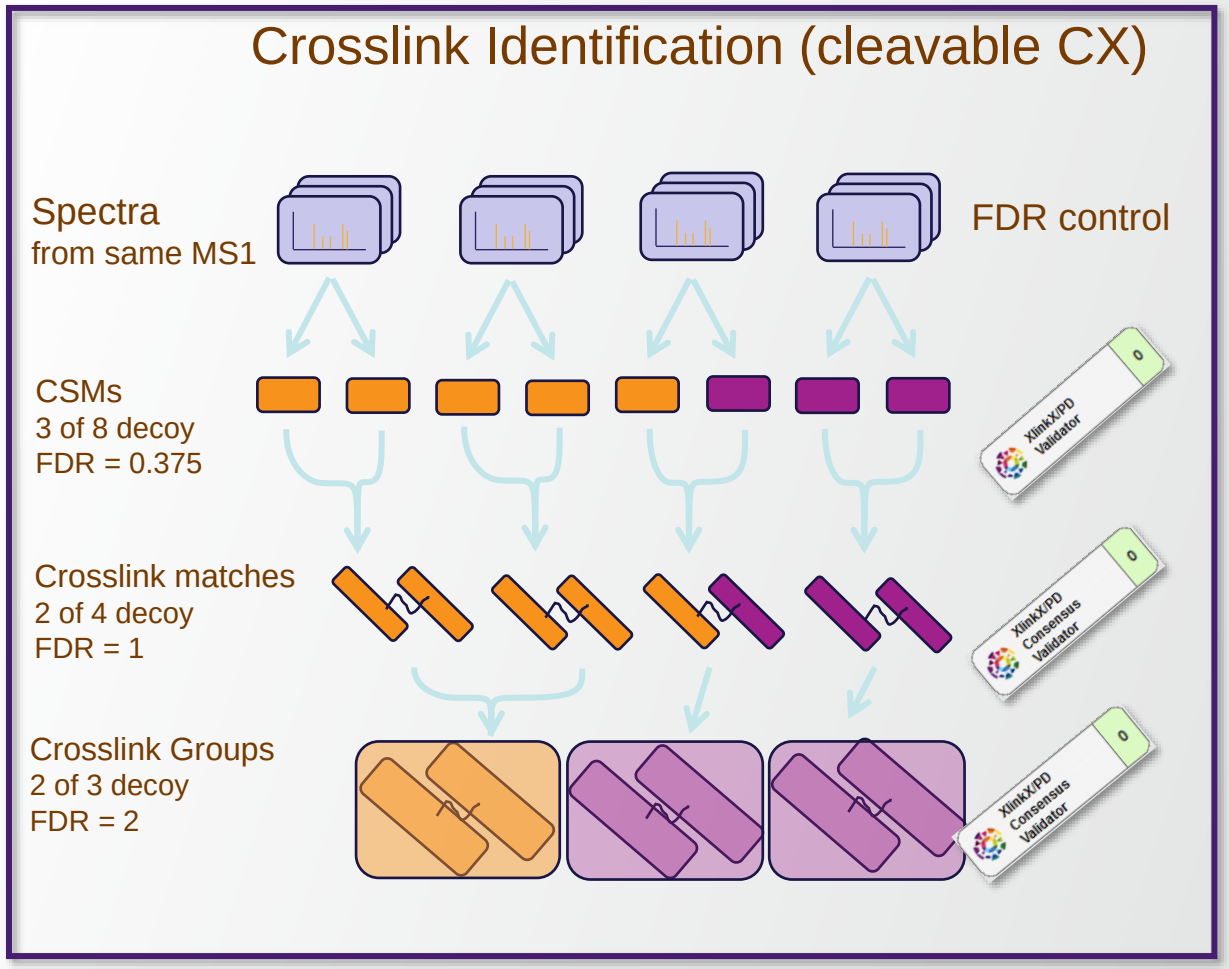
Cross-link FDR threshold	0.01
CSM FDR threshold	0.01

Crosslink-level Crosslink Validation (see slides below)

FDR Related Hierarchies Between Traditional and Crosslinked Analysis

- All levels of consolidation (Spectra, Matches, Peptide Groups, Proteins) need to be under FDR control
- Former versions of XlinkX node contained only FDR control on Matches – Level
- Higher level FDR was controlled by using Score Cutoffs
- In XlinkX 2.5 node FDR control an all Levels make Score Cutoffs obsolete (although these are still supported)

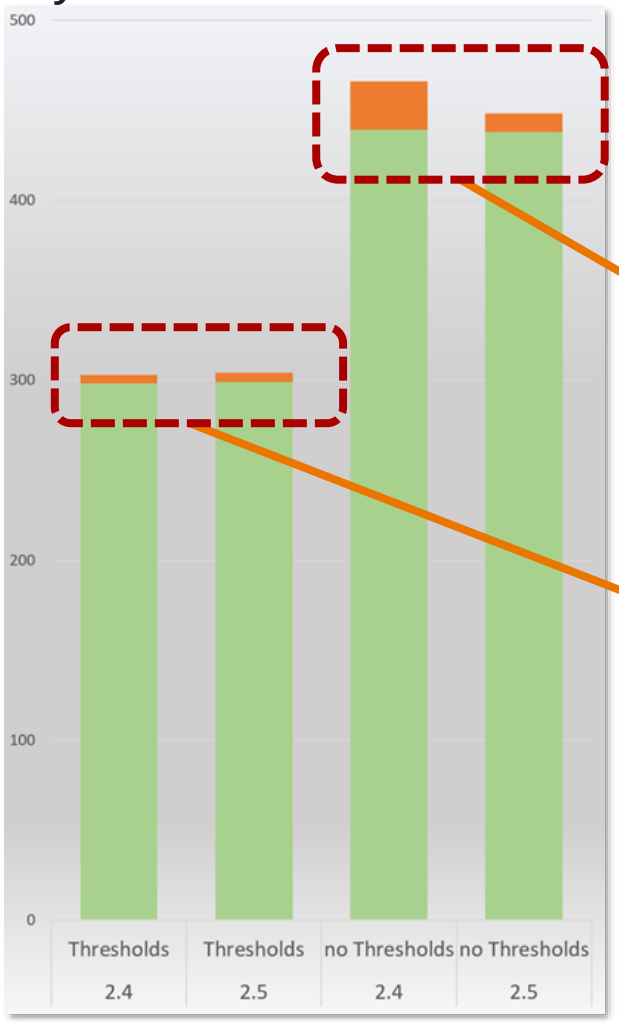




[1] S. Lenz et al bioRxiv. 2020

Case Studies – Improvements in Match Level

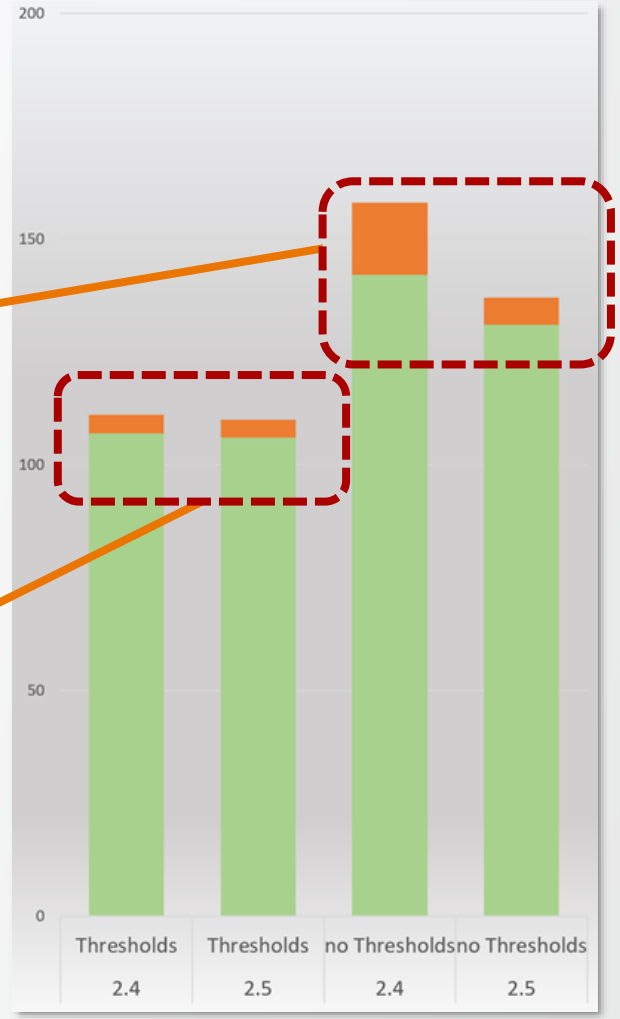
Synthetic Crosslinks from [2]



- Omitting Thresholds increases number of identified Crosslinks
- Omitting Thresholds drastically improves FDR in multi level approach
- Threshold based approach shows no difference between spectra-only FDR approach (PD 2.4) and hierarchical approach (PD 2.5)

CSM incorrect

CSM correct



Crosslink incorrect

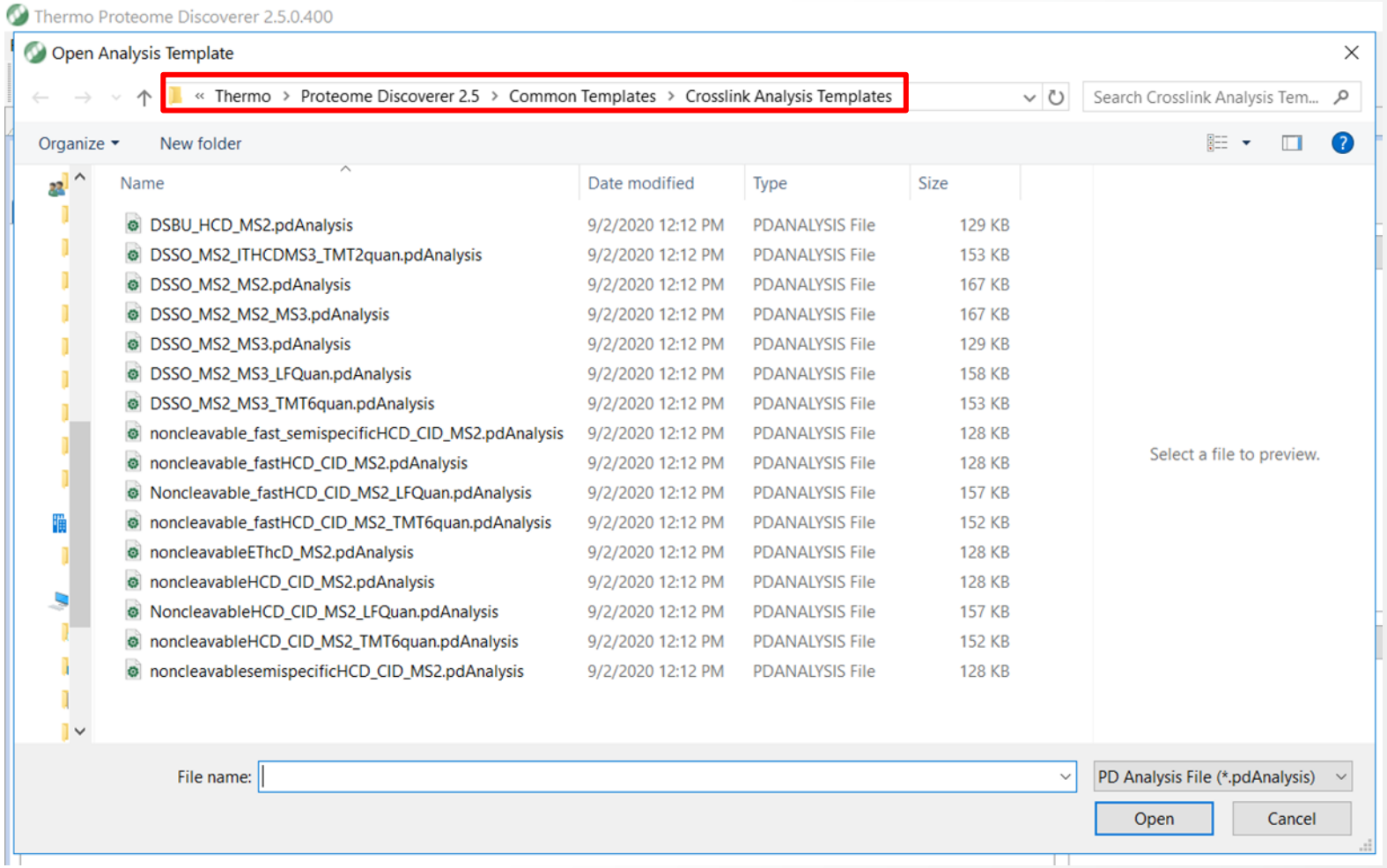
Crosslink correct

[2] R. Beveridge et al Nat Commun. 2020 Feb 6;11(1):742 PXD014337

Crosslinking Analysis Template

Preinstalled Crosslinking Analysis Templates

Crosslinking Analysis Templates located in C:/Users/Public/Public Documents/ Thermo/ Proteome Discoverer 2.5/ Common Templates/ Crosslink Analysis Templates

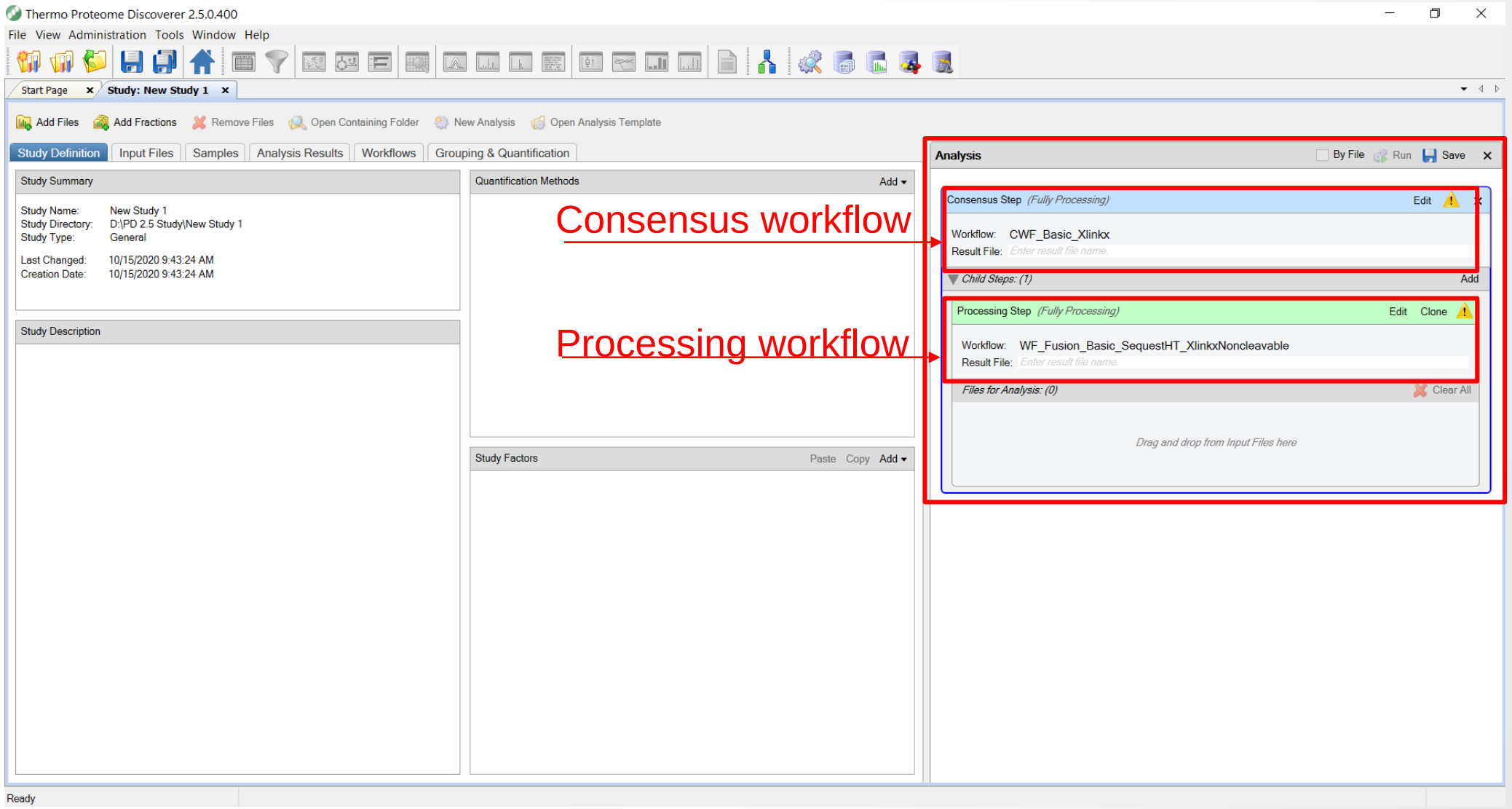


Xinkx 2.5 Node with Proteome Discoverer 2.5 Software Support:

- Non-Cleavable (n^2 database search)
- Non-Cleavable-fast (search distinct signature crosslinked fragments and sequence tags spectrum to reduce the search time)
- Cleavable-MS2-MS2 (e.g. CID for reporter detection-ETD for sequence readout)
- Cleavable-MS2 only (e.g. HCD for reporter detection and sequence readout)
- Cleavable-MS2-MS3 (e.g. MS2 CID for reporter detection and MS3 HCD for individual peptides)
- Cleavable MS2-MS2-MS3 (e.g. MS2 CID for reporter detection, MS3 HCD and MS2 EThcD for individual peptides)
- Cleavable semi-specific crosslinking data
- LFQ and TMT quantification for crosslinking data

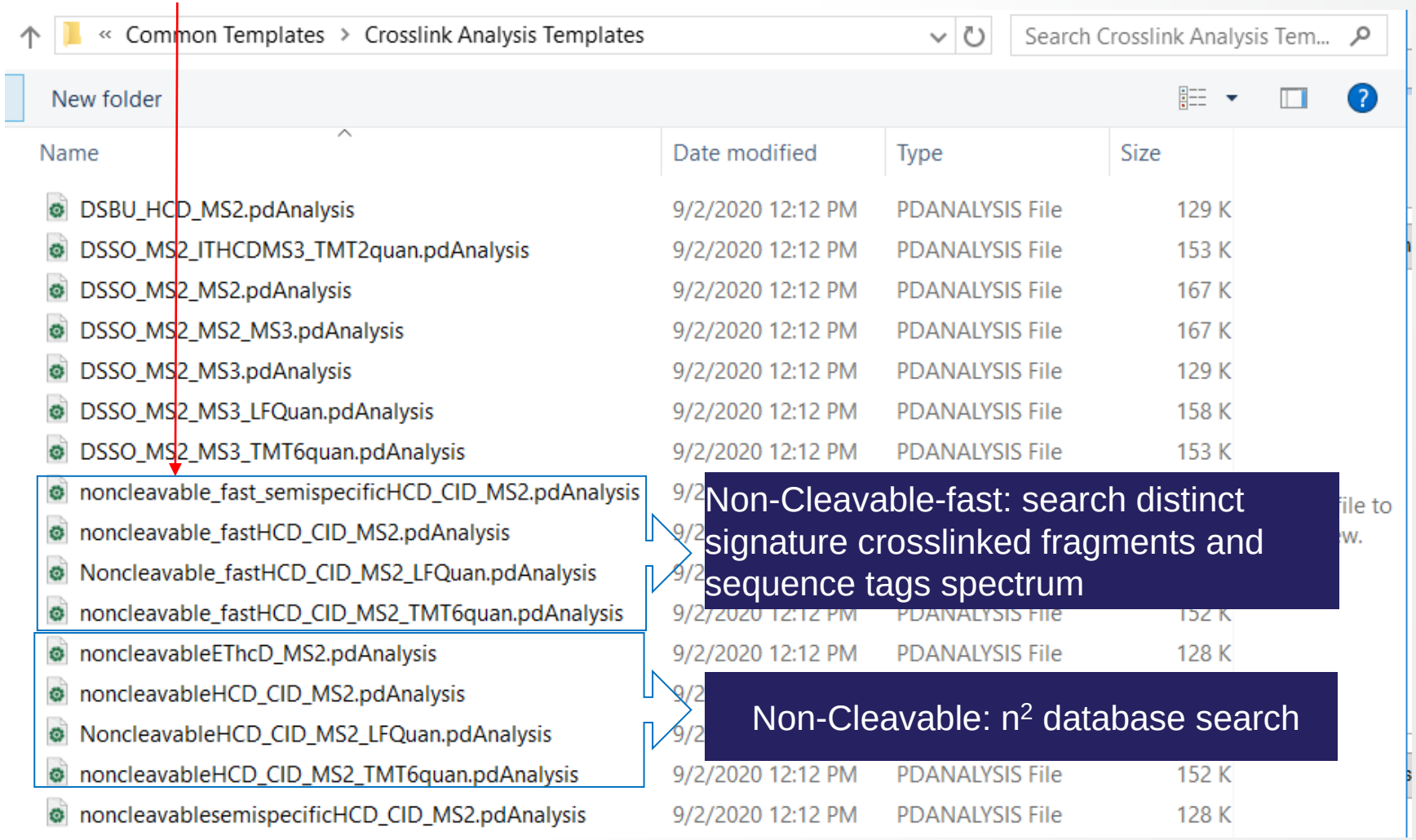
Analysis Template

Including Processing and Consensus workflow



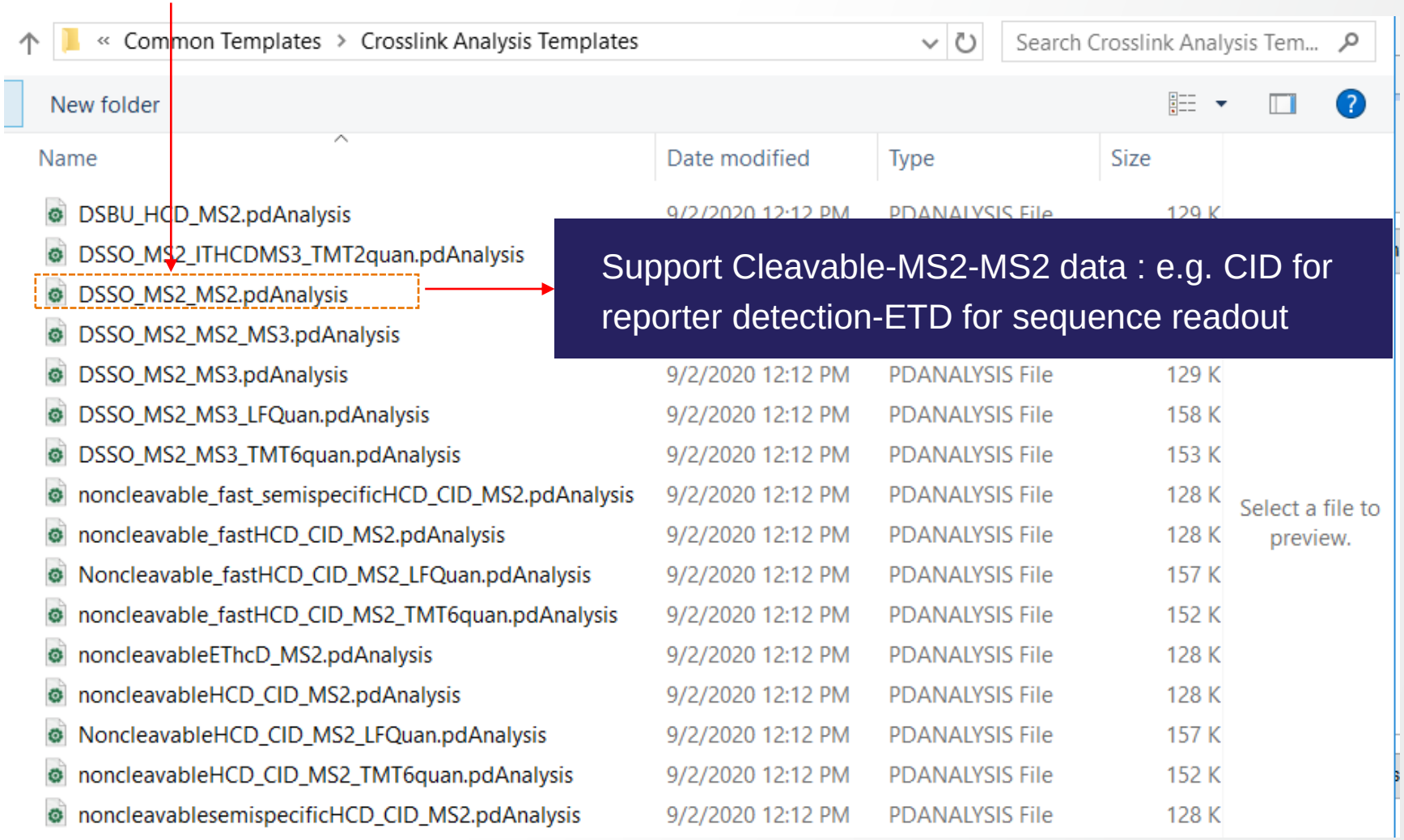
Analysis Template for Non-cleavable CID/HCD Data

Select and Open Analysis template



Analysis Template for DSSO Cleavable MS2-MS2 Data

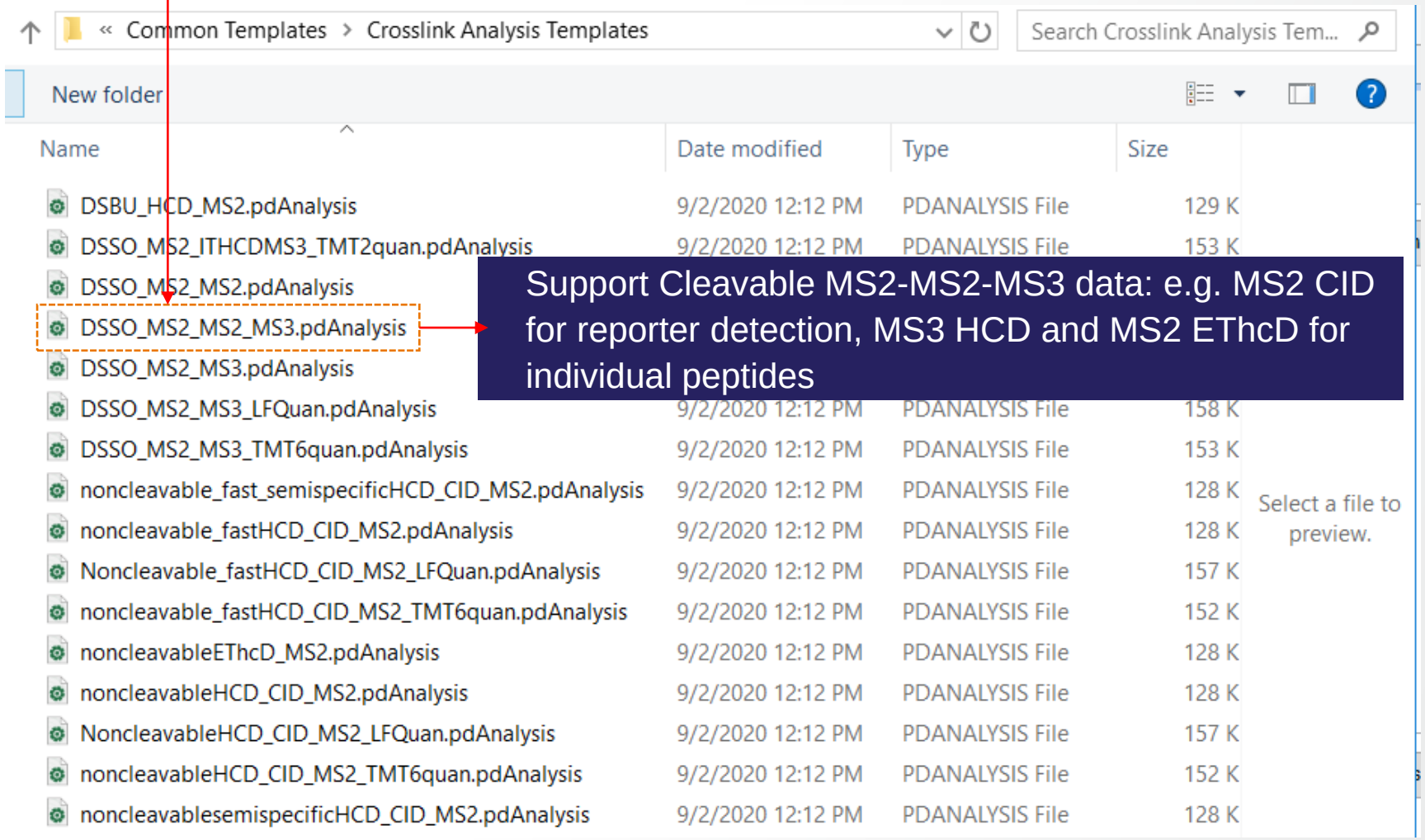
Select and open Analysis template



Support Cleavable-MS2-MS2 data : e.g. CID for reporter detection-ETD for sequence readout

Analysis Template for DSSO Cleavable MS2-MS2-MS3 Data

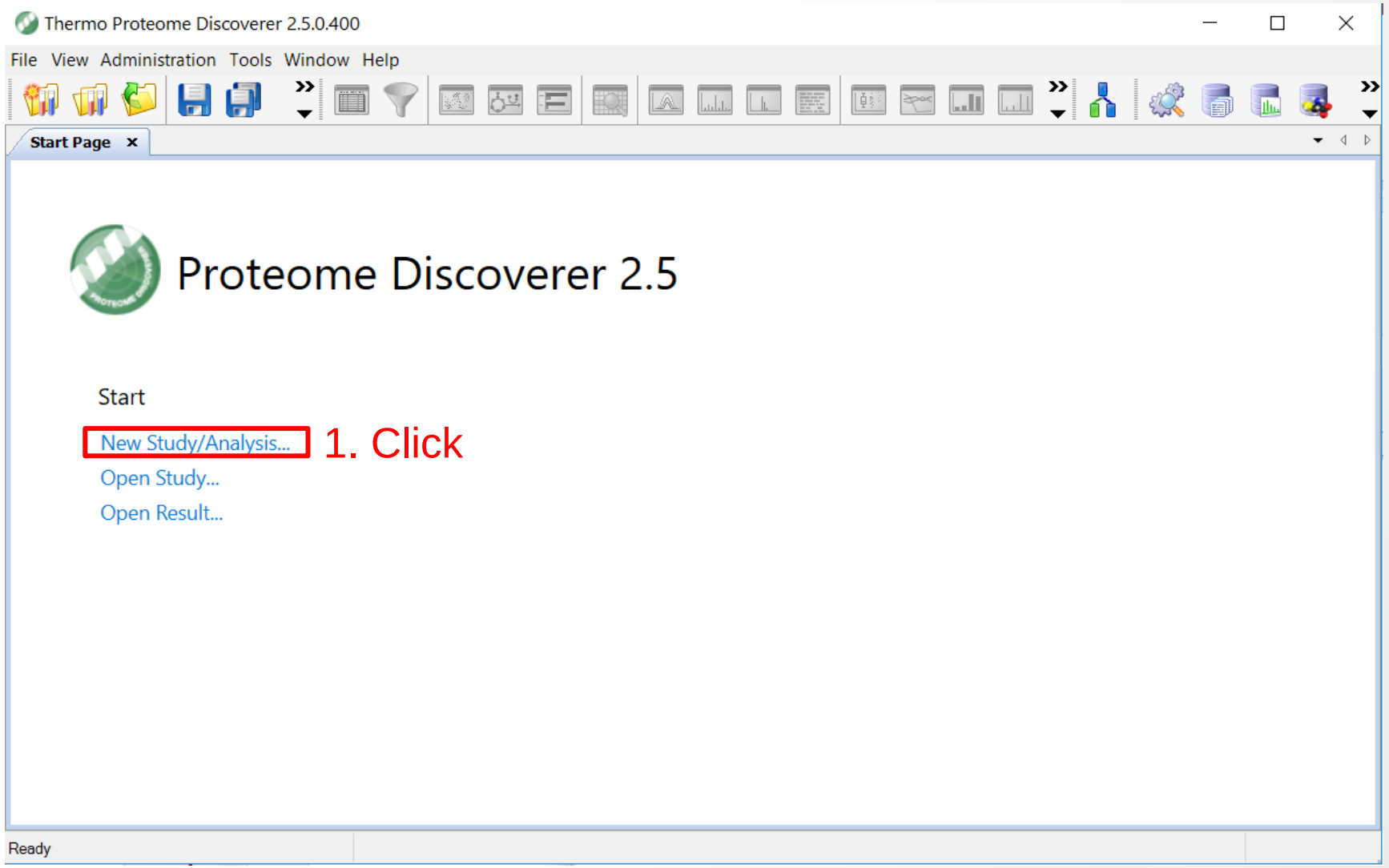
Select and Open Analysis template



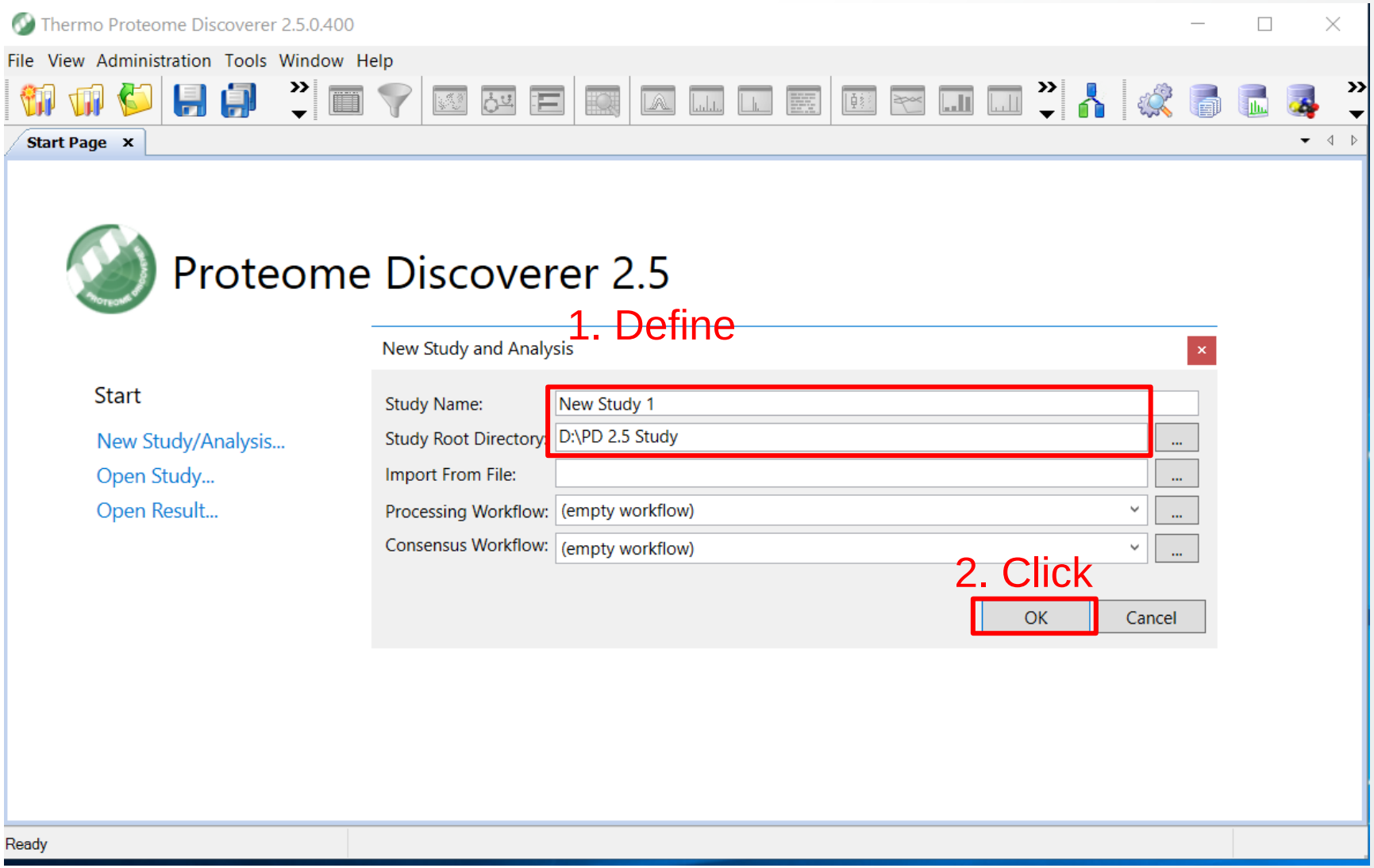
Support Cleavable MS2-MS2-MS3 data: e.g. MS2 CID for reporter detection, MS3 HCD and MS2 EThcD for individual peptides

Example of EDC Crosslinking Analysis

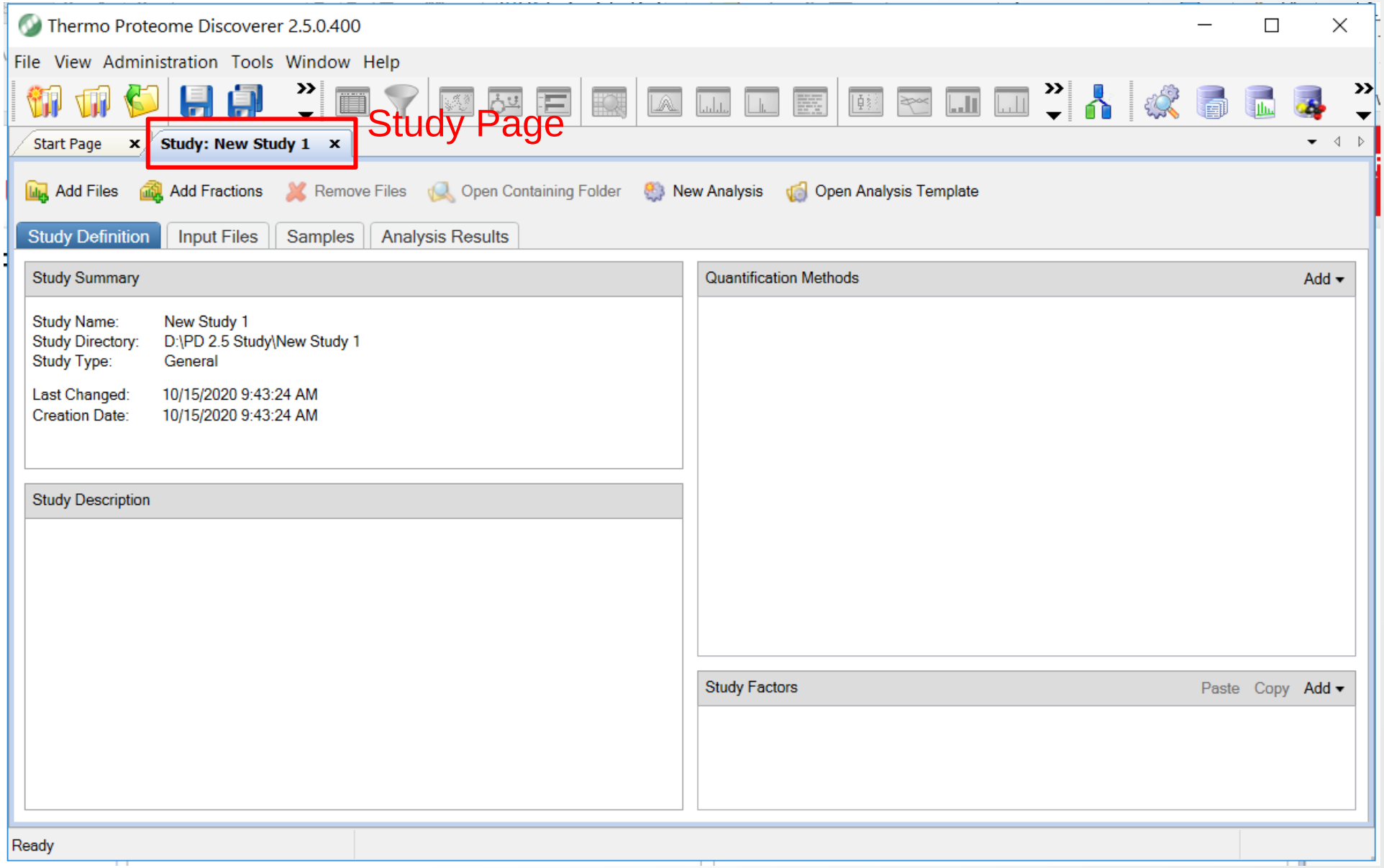
Step 1. Create and Setup a New Study



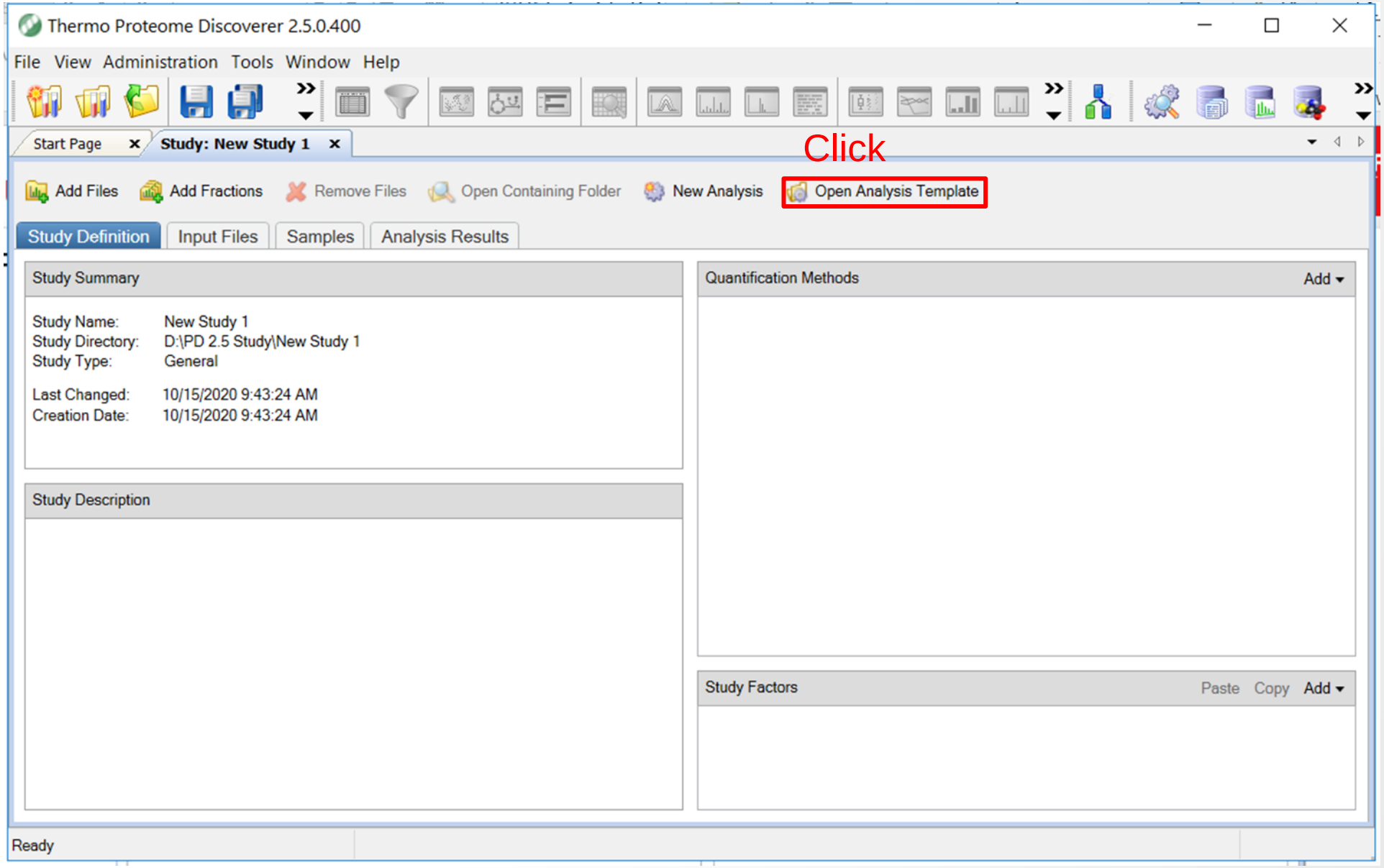
Step 2. Define the New Study



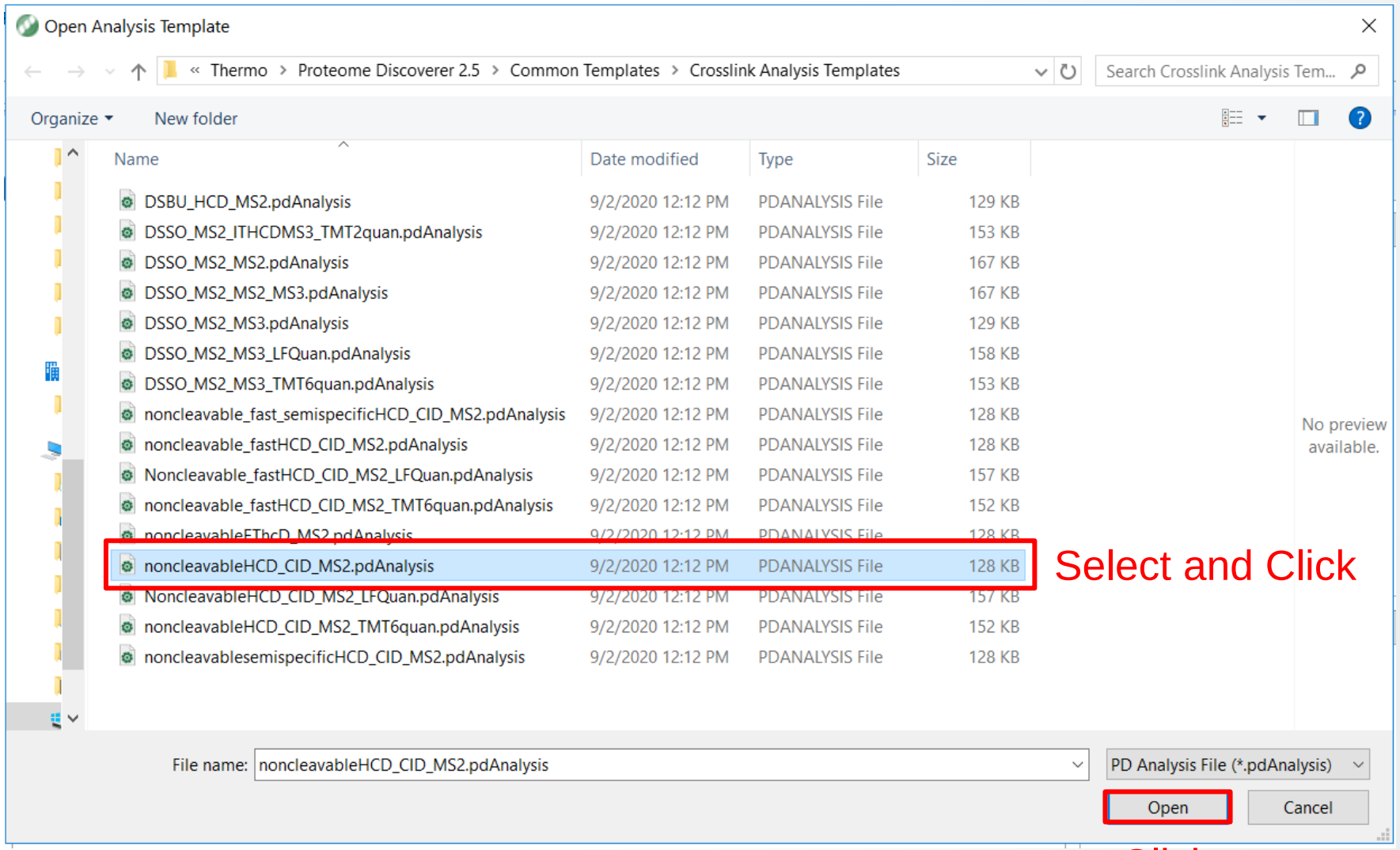
Study Page


Thermo Proteome Discoverer 2.5.0.400
File View Administration Tools Window Help
Study Page
Start Page
Study: New Study 1
Add Files
Add Fractions
Remove Files
Open Containing Folder
New Analysis
Open Analysis Template
Study Definition
Input Files
Samples
Analysis Results
Study Summary
Study Name: New Study 1
Study Directory: D:\PD 2.5 Study\New Study 1
Study Type: General
Last Changed: 10/15/2020 9:43:24 AM
Creation Date: 10/15/2020 9:43:24 AM
Study Description
Quantification Methods
Add
Study Factors
Paste
Copy
Add
Ready

Step 3. Select Analysis Template

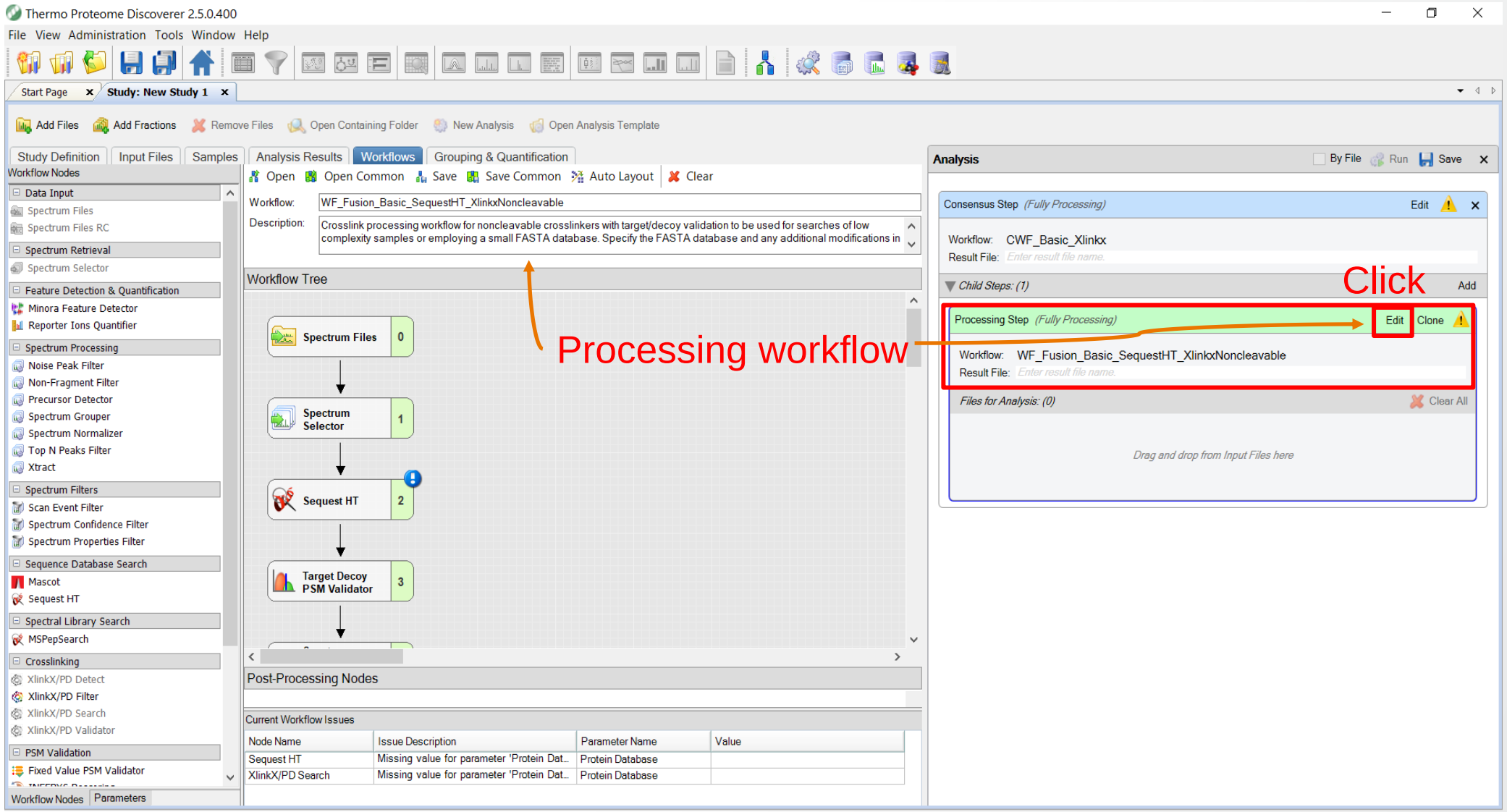


Step 4. Example of EDC Data Analysis



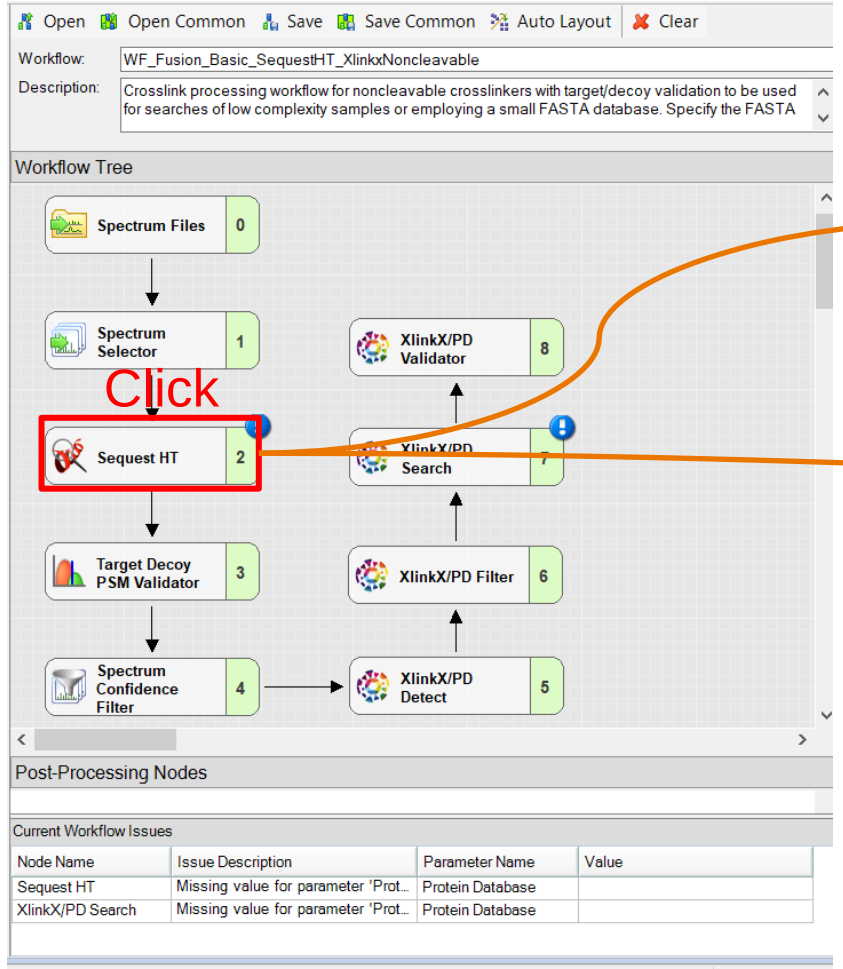
Step 5. Define Processing Workflow

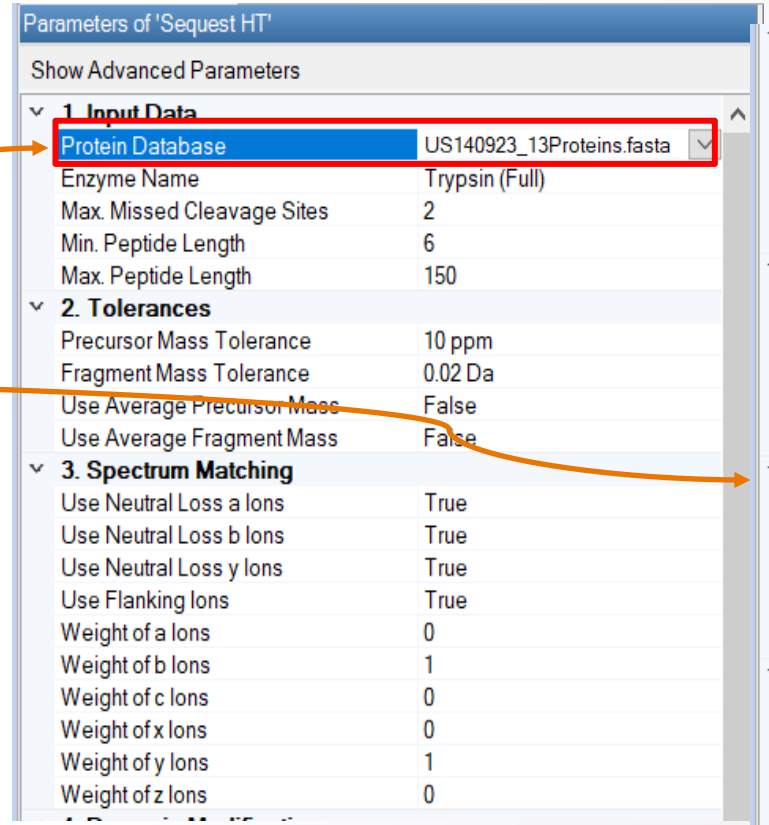
1) Active Processing workflow

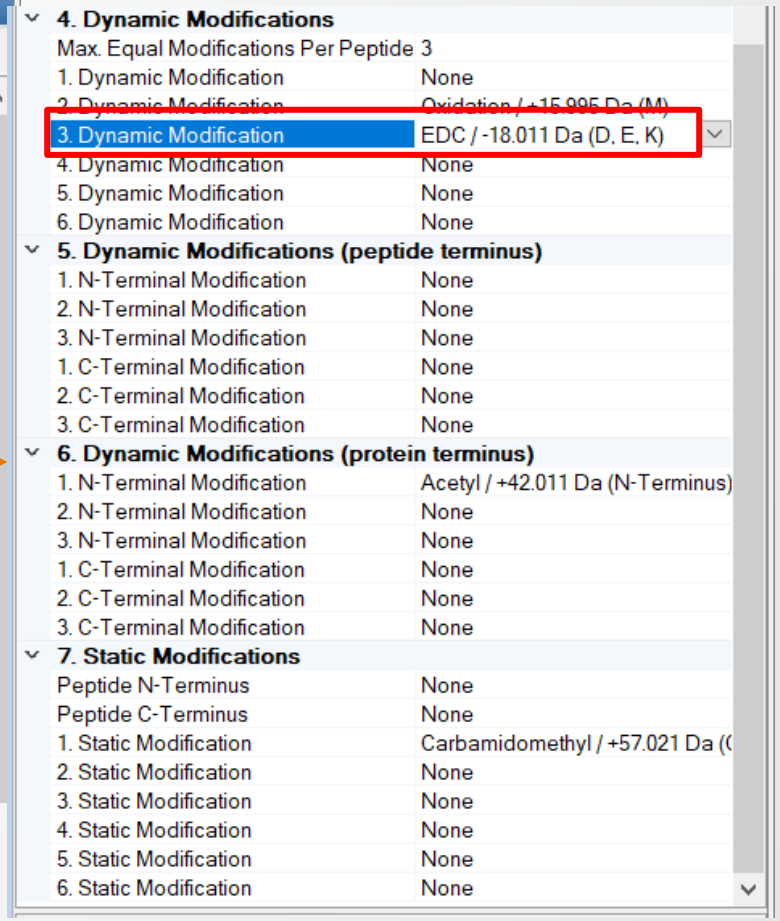


Step 5. Define Processing Workflow

2). Define Sequest HT node

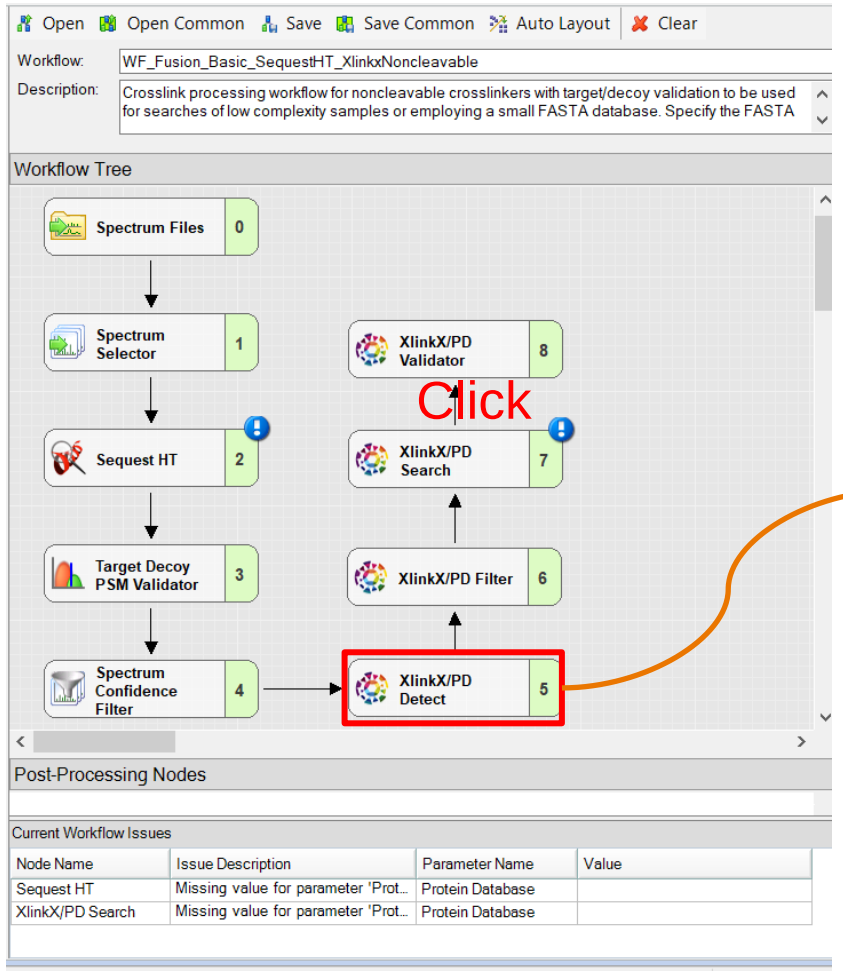


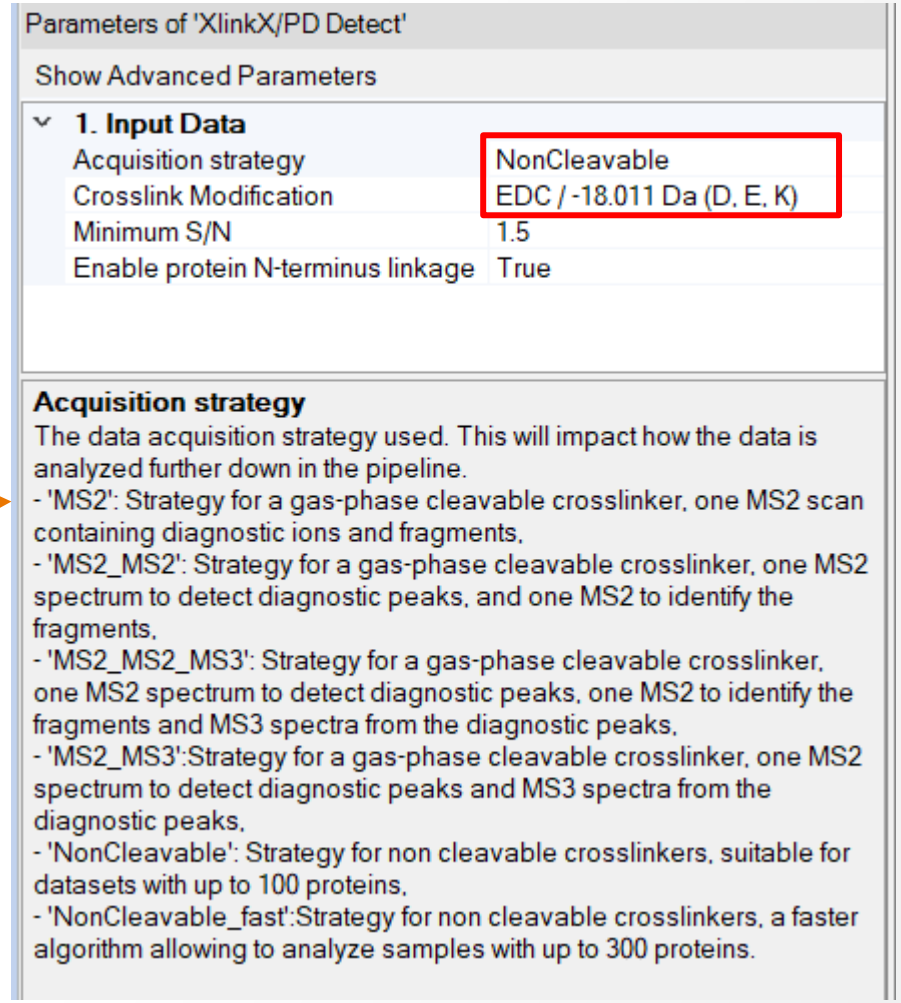




Step 5. Define Processing Workflow

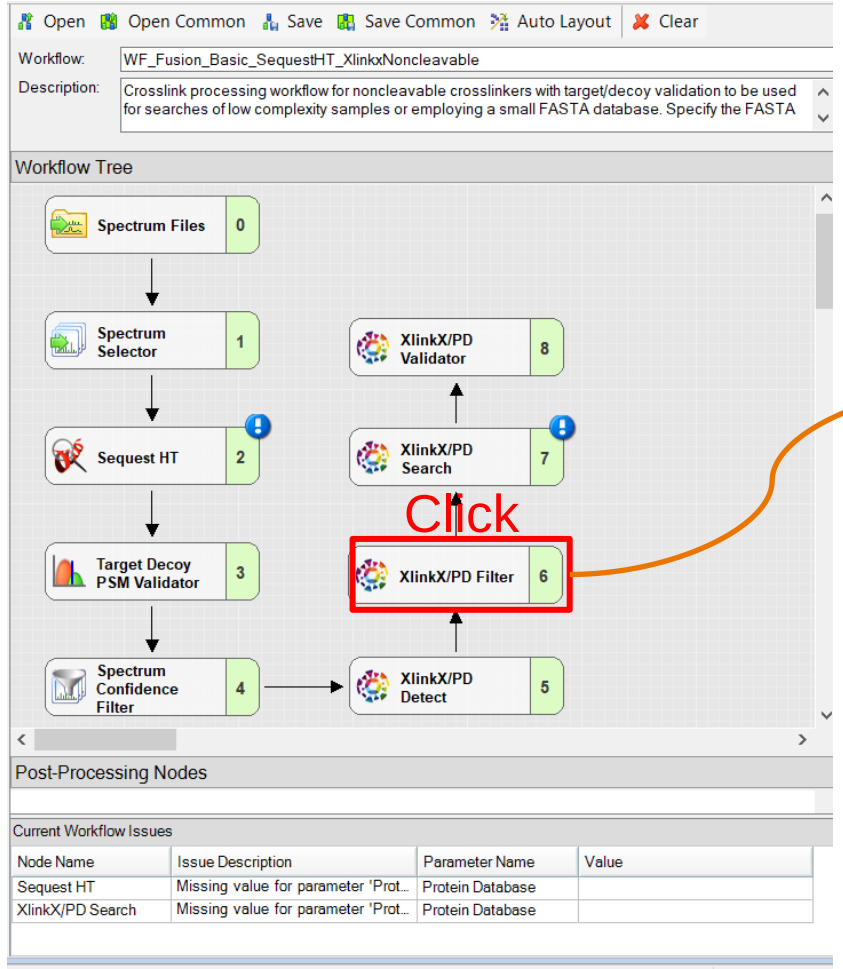
3). Define XlinkX PD Detect node

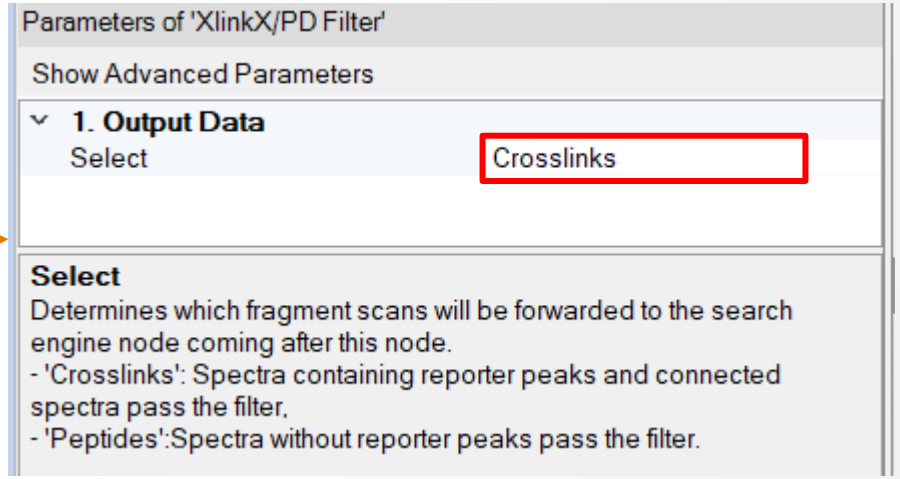




Step 5. Define Processing Workflow

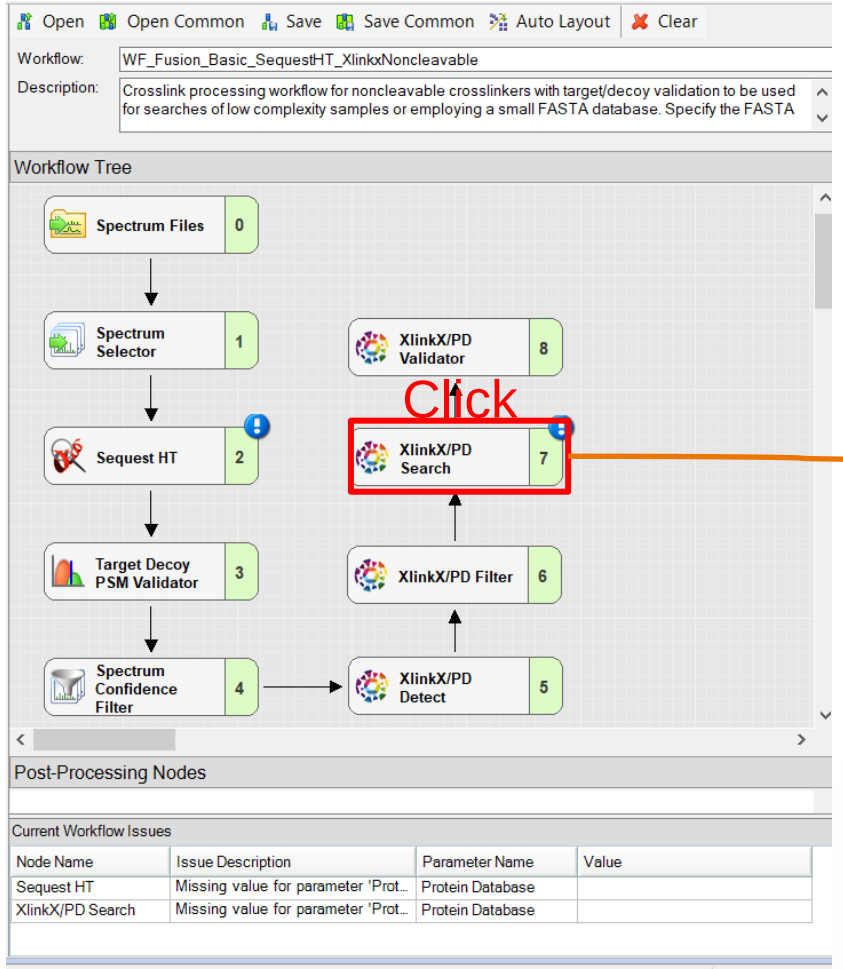
4). Define XlinkX/PD Detect node

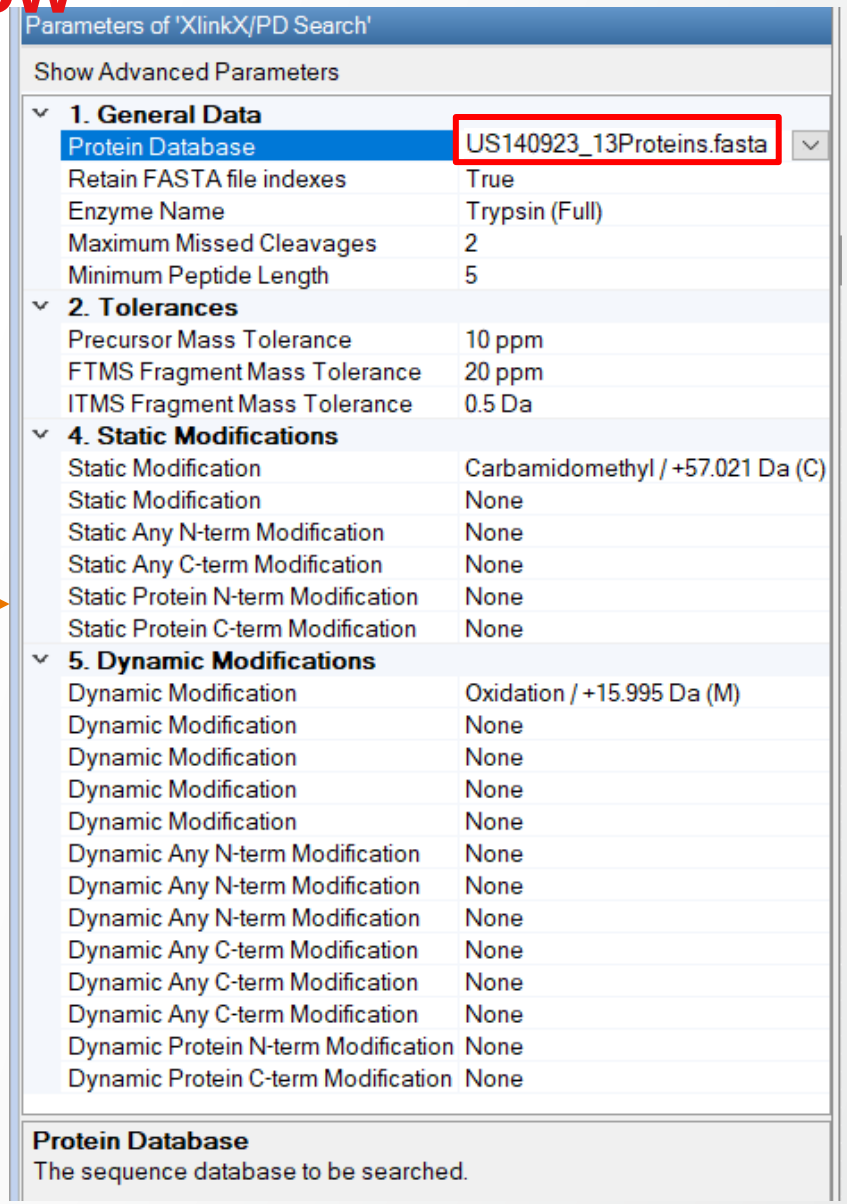




Step 5. Define Processing Workflow

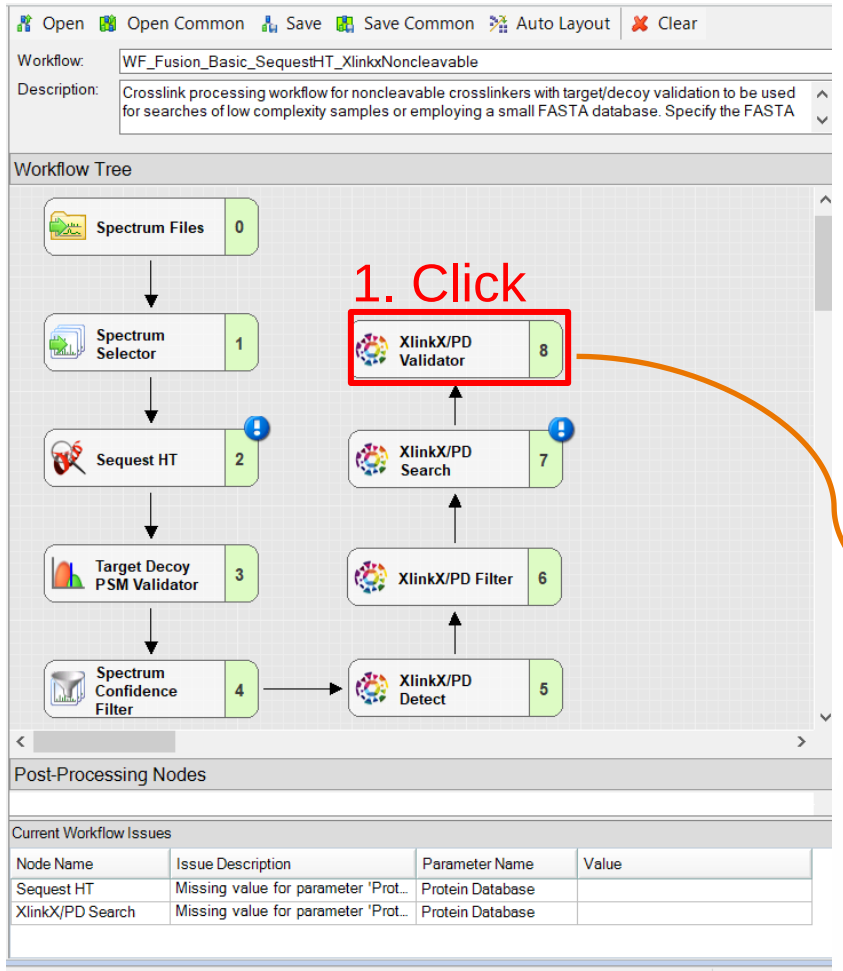
5). Define XlinkX/PD Search node

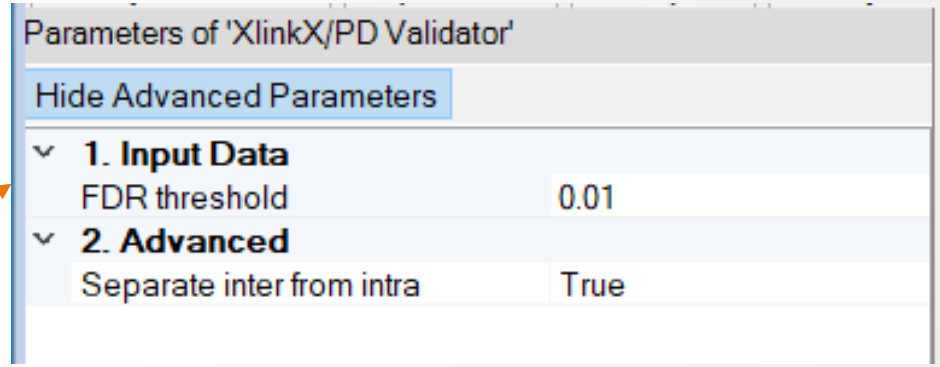


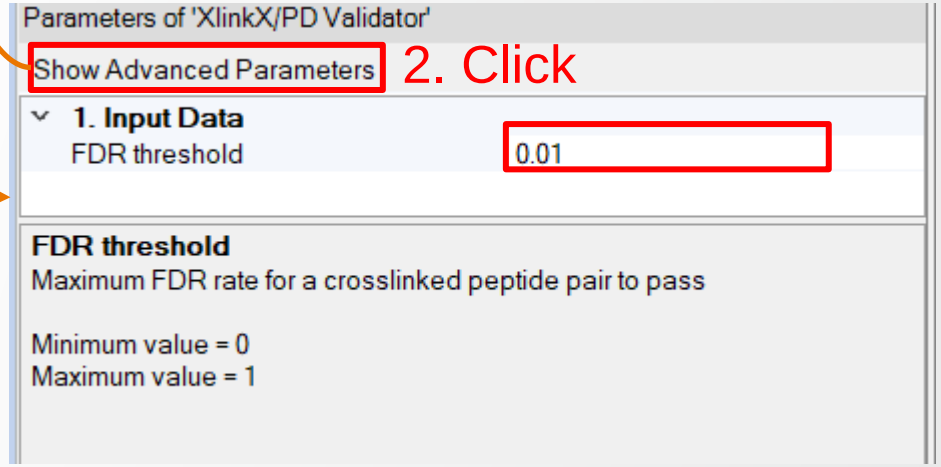


Step 5. Define Processing Workflow

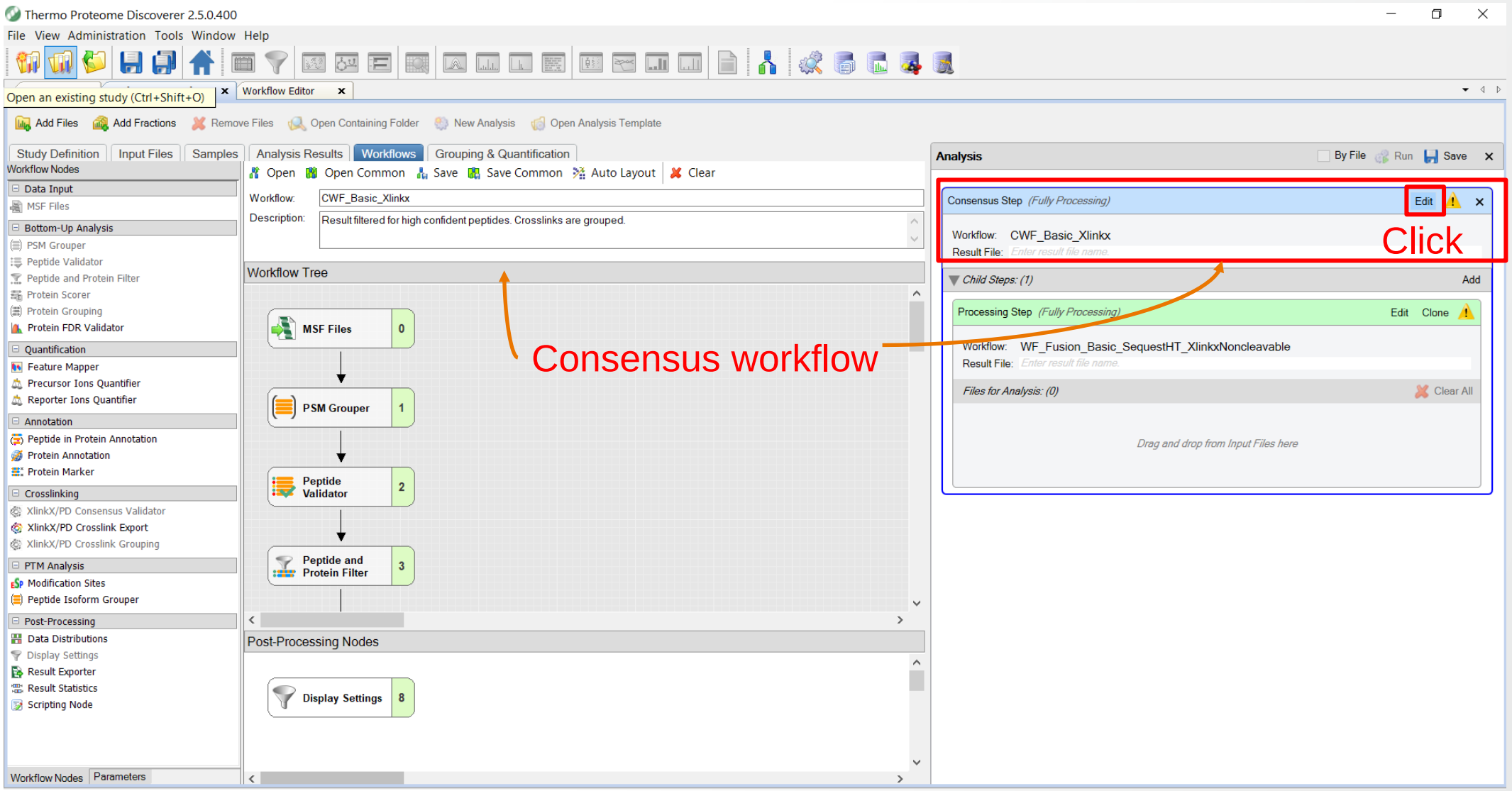
6). XlinkX/PD Detect node





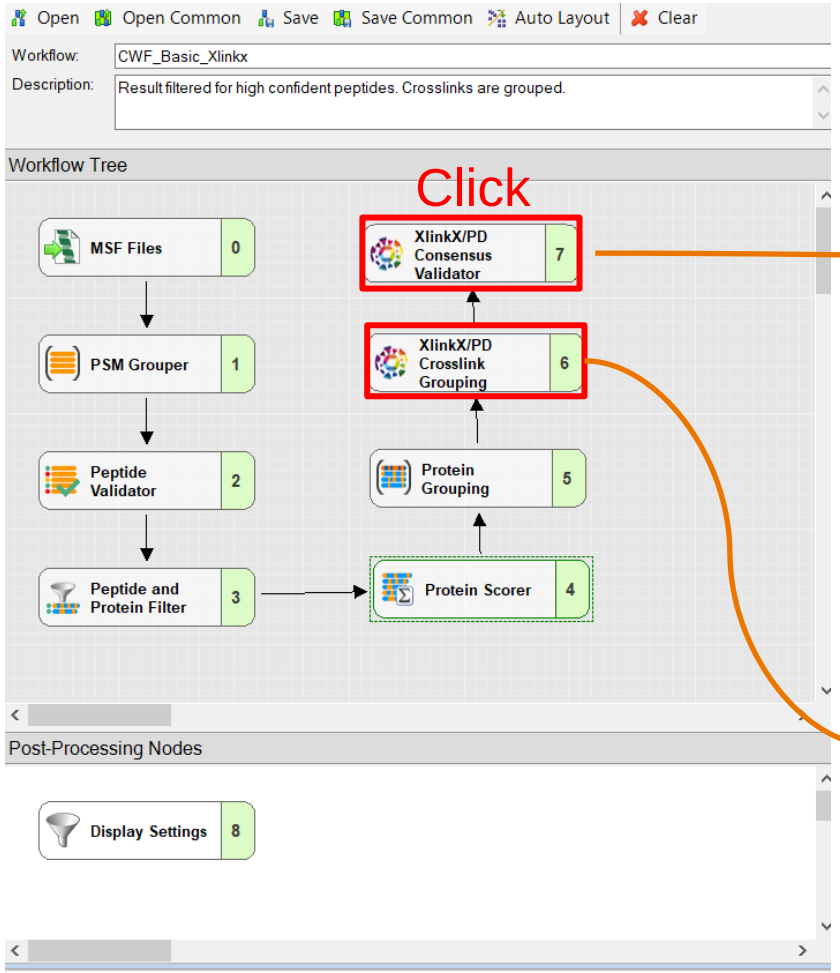


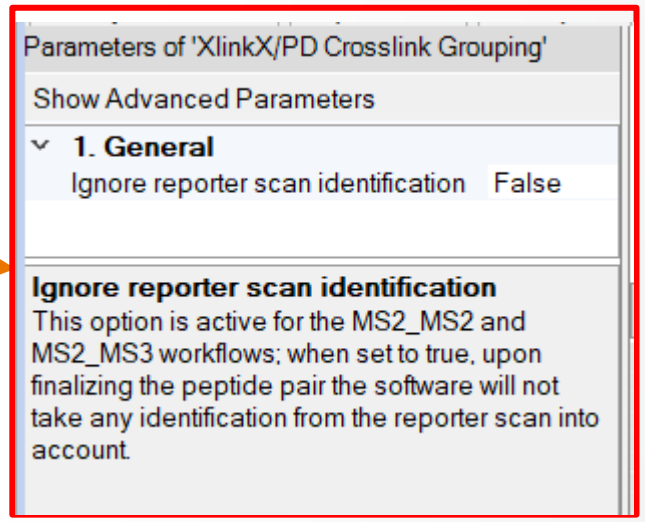
Step 6. Active Consensus Workflow

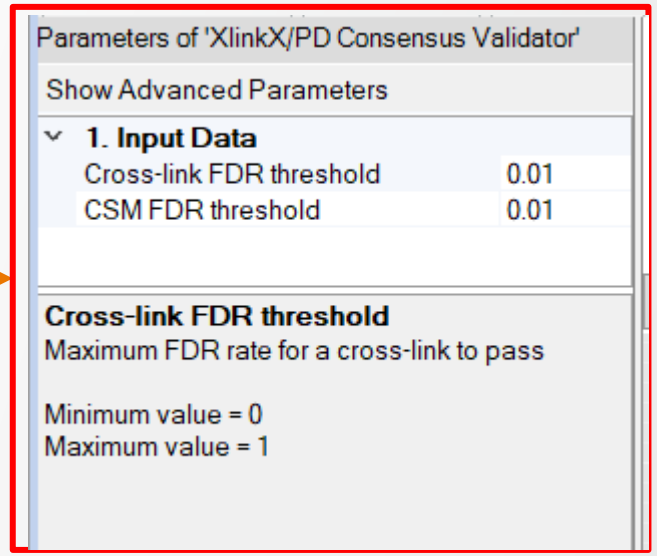


Step 7. Define Consensus Workflow

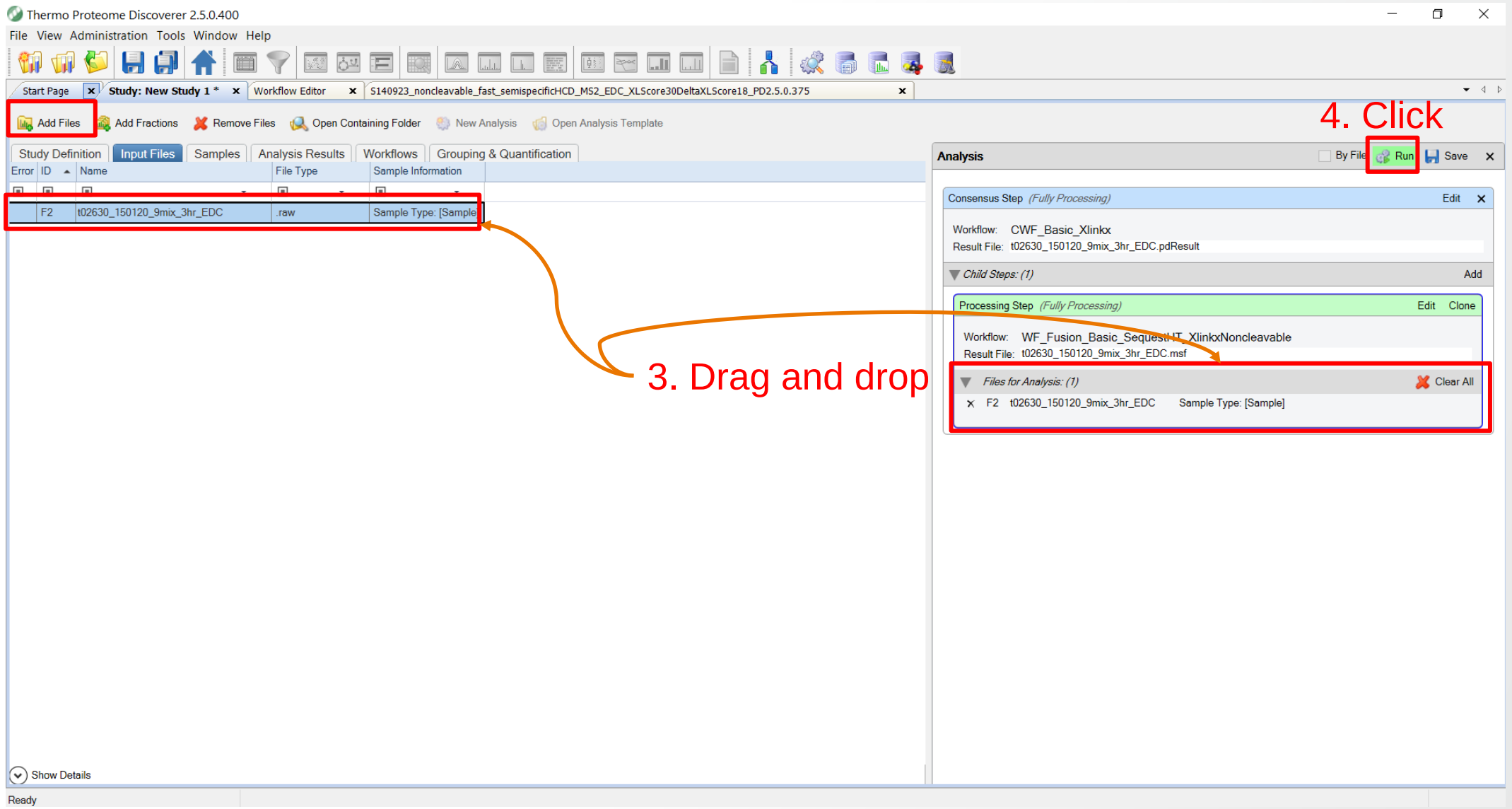
Define Xlinkx/PD Grouping and Validator node





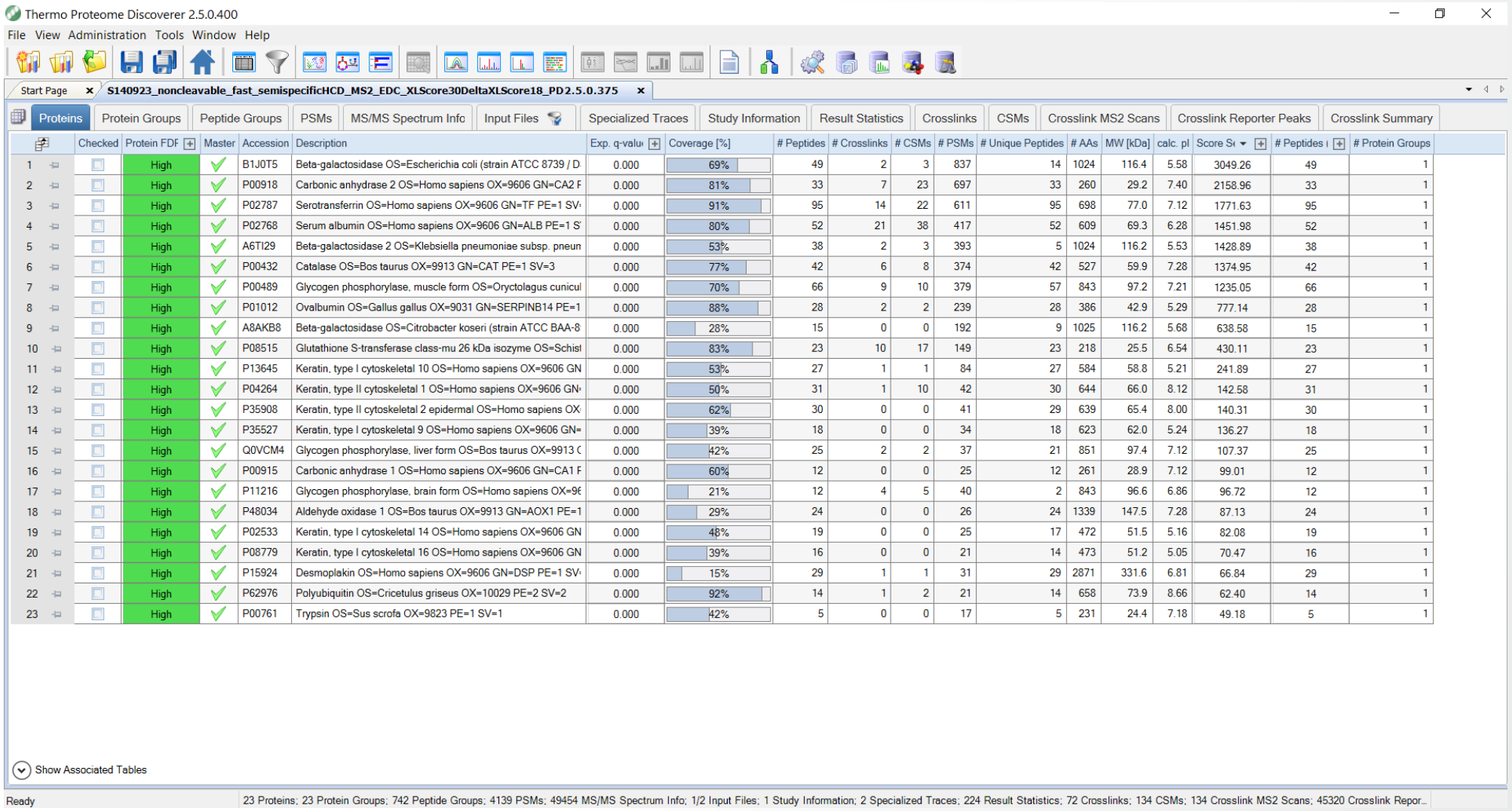


Step 8. Add Files and Run Analysis



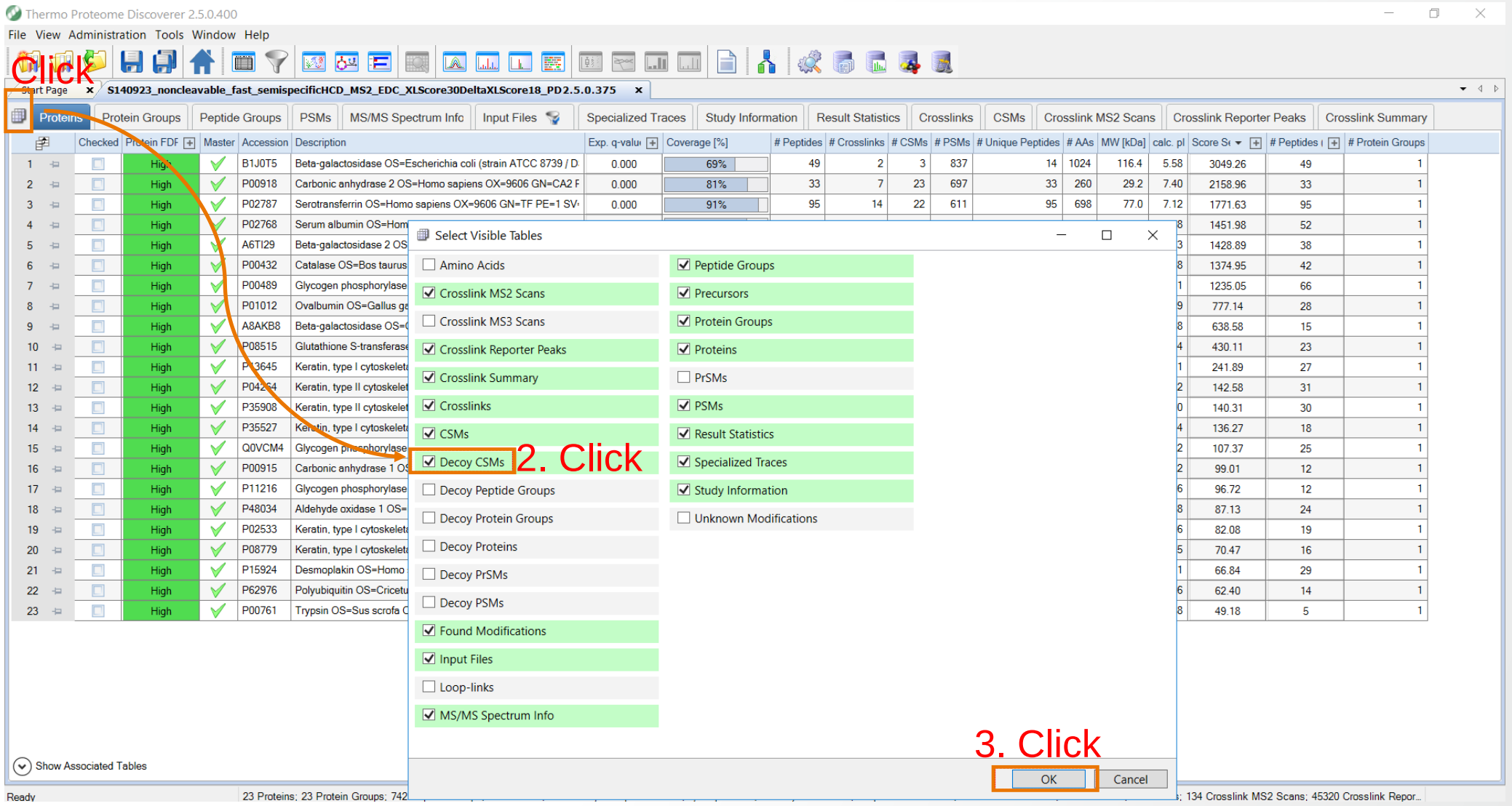
New Options for Results Export

Results Page

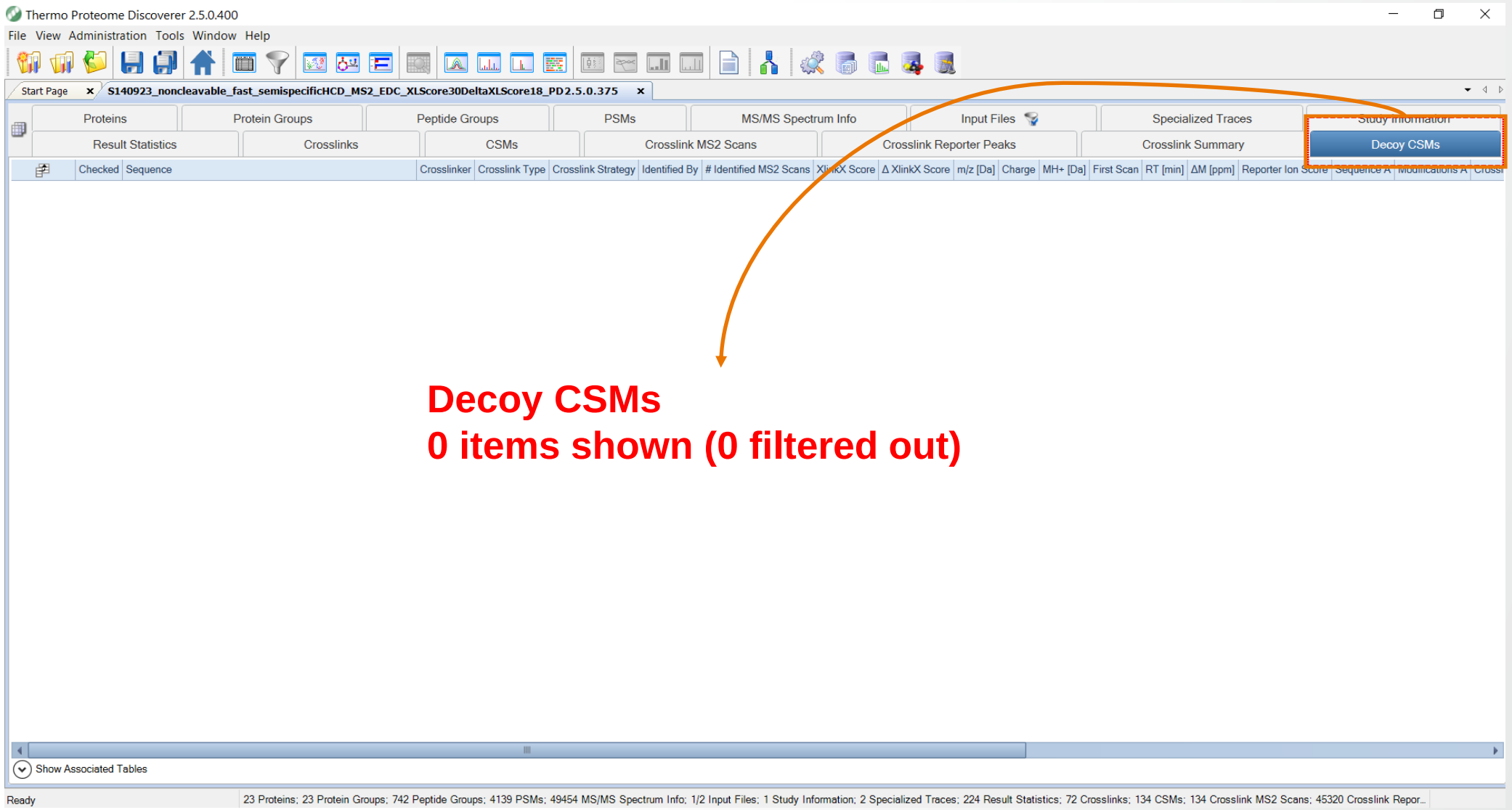


	Checked	Protein FDR	Master	Accession	Description	Exp. q-value	Coverage [%]	# Peptides	# Crosslinks	# CSMs	# PSMs	# Unique Peptides	# AAs	MW [kDa]	calc. pI	Score Se	# Peptides	# Protein Groups
1		High	✓	B1J0T5	Beta-galactosidase OS=Escherichia coli (strain ATCC 8739 / D	0.000	69%	49	2	3	837	14	1024	116.4	5.58	3049.26	49	1
2		High	✓	P00918	Carbonic anhydrase 2 OS=Homo sapiens OX=9606 GN=CA2 F	0.000	81%	33	7	23	697	33	260	29.2	7.40	2158.96	33	1
3		High	✓	P02787	Serotransferrin OS=Homo sapiens OX=9606 GN=TF PE=1 SV=	0.000	91%	95	14	22	611	95	698	77.0	7.12	1771.63	95	1
4		High	✓	P02768	Serum albumin OS=Homo sapiens OX=9606 GN=ALB PE=1 S	0.000	80%	52	21	38	417	52	609	69.3	6.28	1451.98	52	1
5		High	✓	A6TI29	Beta-galactosidase 2 OS=Klebsiella pneumoniae subsp. pneun	0.000	53%	38	2	3	393	5	1024	116.2	5.53	1428.89	38	1
6		High	✓	P00432	Catalase OS=Bos taurus OX=9913 GN=CAT PE=1 SV=3	0.000	77%	42	6	8	374	42	527	59.9	7.28	1374.95	42	1
7		High	✓	P00489	Glycogen phosphorylase, muscle form OS=Oryctolagus cunicul	0.000	70%	66	9	10	379	57	843	97.2	7.21	1235.05	66	1
8		High	✓	P01012	Ovalbumin OS=Gallus gallus OX=9031 GN=SERPINB14 PE=1	0.000	88%	28	2	2	239	28	386	42.9	5.29	777.14	28	1
9		High	✓	A8AKB8	Beta-galactosidase OS=Citrobacter koseri (strain ATCC BAA-8	0.000	28%	15	0	0	192	9	1025	116.2	5.68	638.58	15	1
10		High	✓	P08515	Glutathione S-transferase class-mu 26 kDa isozyme OS=Schist	0.000	83%	23	10	17	149	23	218	25.5	6.54	430.11	23	1
11		High	✓	P13645	Keratin, type I cytoskeletal 10 OS=Homo sapiens OX=9606 GN	0.000	53%	27	1	1	84	27	584	58.8	5.21	241.89	27	1
12		High	✓	P04264	Keratin, type II cytoskeletal 1 OS=Homo sapiens OX=9606 GN	0.000	50%	31	1	10	42	30	644	66.0	8.12	142.58	31	1
13		High	✓	P35908	Keratin, type II cytoskeletal 2 epidermal OS=Homo sapiens OX	0.000	62%	30	0	0	41	29	639	65.4	8.00	140.31	30	1
14		High	✓	P35527	Keratin, type I cytoskeletal 9 OS=Homo sapiens OX=9606 GN=	0.000	39%	18	0	0	34	18	623	62.0	5.24	136.27	18	1
15		High	✓	Q0VCM4	Glycogen phosphorylase, liver form OS=Bos taurus OX=9913 G	0.000	42%	25	2	2	37	21	851	97.4	7.12	107.37	25	1
16		High	✓	P00915	Carbonic anhydrase 1 OS=Homo sapiens OX=9606 GN=CA1 F	0.000	60%	12	0	0	25	12	261	28.9	7.12	99.01	12	1
17		High	✓	P11216	Glycogen phosphorylase, brain form OS=Homo sapiens OX=96	0.000	21%	12	4	5	40	2	843	96.6	6.86	96.72	12	1
18		High	✓	P48034	Aldehyde oxidase 1 OS=Bos taurus OX=9913 GN=AOX1 PE=1	0.000	29%	24	0	0	26	24	1339	147.5	7.28	87.13	24	1
19		High	✓	P02533	Keratin, type I cytoskeletal 14 OS=Homo sapiens OX=9606 GN	0.000	48%	19	0	0	25	17	472	51.5	5.16	82.08	19	1
20		High	✓	P08779	Keratin, type I cytoskeletal 16 OS=Homo sapiens OX=9606 GN	0.000	39%	16	0	0	21	14	473	51.2	5.05	70.47	16	1
21		High	✓	P15924	Desmoplakin OS=Homo sapiens OX=9606 GN=DSP PE=1 SV=	0.000	15%	29	1	1	31	29	2871	331.6	6.81	66.84	29	1
22		High	✓	P62976	Polyubiquitin OS=Cricetulus griseus OX=10029 PE=2 SV=2	0.000	92%	14	1	2	21	14	658	73.9	8.66	62.40	14	1
23		High	✓	P00761	Trypsin OS=Sus scrofa OX=9823 PE=1 SV=1	0.000	42%	5	0	0	17	5	231	24.4	7.18	49.18	5	1

Decoy CSMs

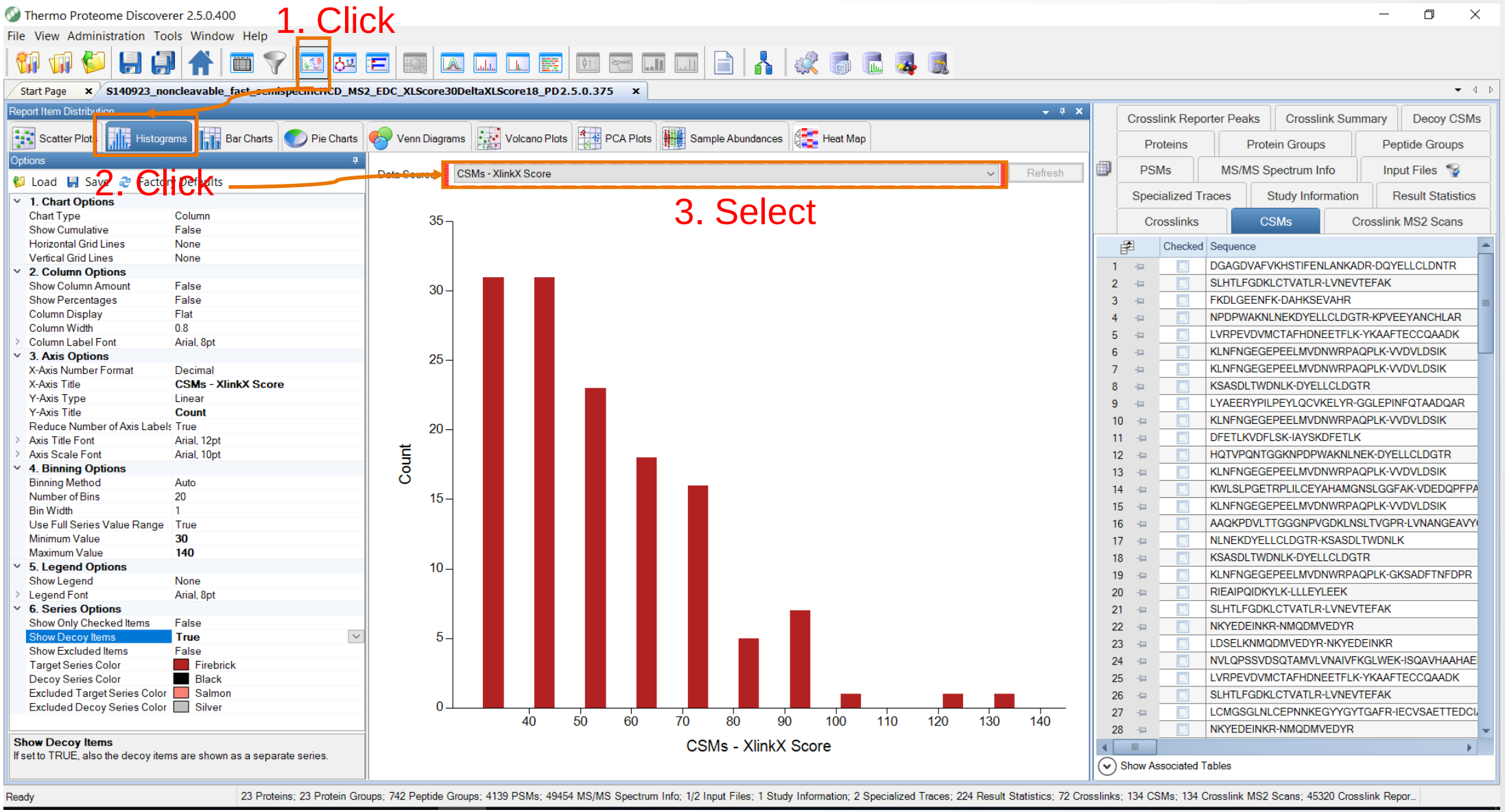

1. Click
2. Click
3. Click
Thermo Proteome Discoverer 2.5.0.400
File View Administration Tools Window Help
Start Page
S140923_noncleavable_fast_semispecificHCD_MS2_EDC_XLScore30DeltaXLScore18_PD2.5.0.375
Proteins
Protein Groups
Peptide Groups
PSMs
MS/MS Spectrum Info
Input Files
Specialized Traces
Study Information
Result Statistics
Crosslinks
CSMs
Crosslink MS2 Scans
Crosslink Reporter Peaks
Crosslink Summary
Select Visible Tables
Amino Acids
Crosslink MS2 Scans
Crosslink MS3 Scans
Crosslink Reporter Peaks
Crosslink Summary
Crosslinks
CSMs
Decoy CSMs
Decoy Peptide Groups
Decoy Protein Groups
Decoy Proteins
Decoy PrSMs
Decoy PSMs
Found Modifications
Input Files
Loop-links
MS/MS Spectrum Info
Peptide Groups
Precursors
Protein Groups
Proteins
PrSMs
PSMs
Result Statistics
Specialized Traces
Study Information
Unknown Modifications
OK
Cancel
Show Associated Tables
Ready

Decoy CSMs Page

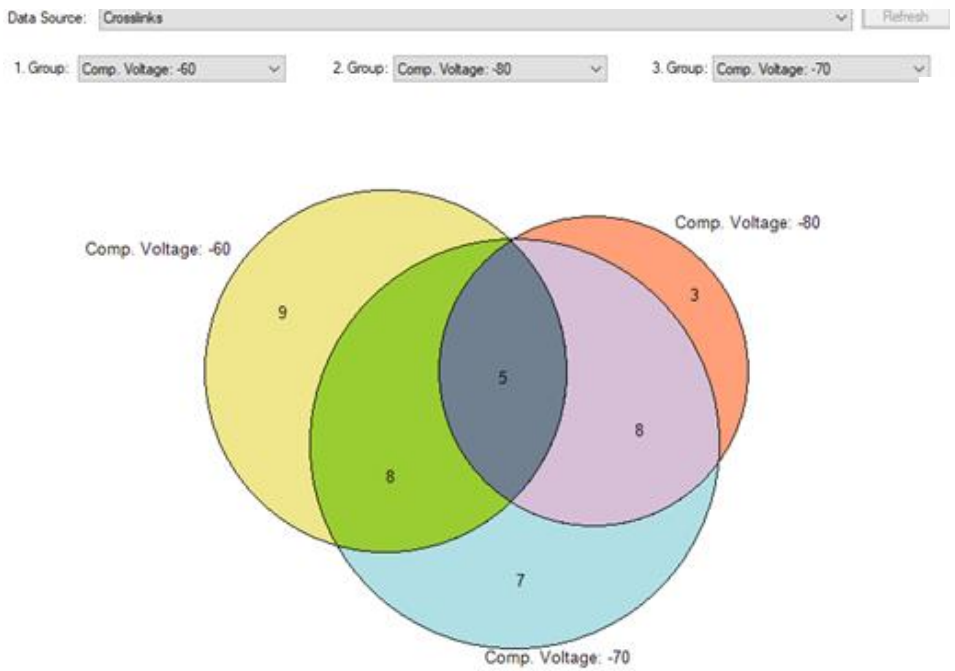


Decoy CSMs
0 items shown (0 filtered out)

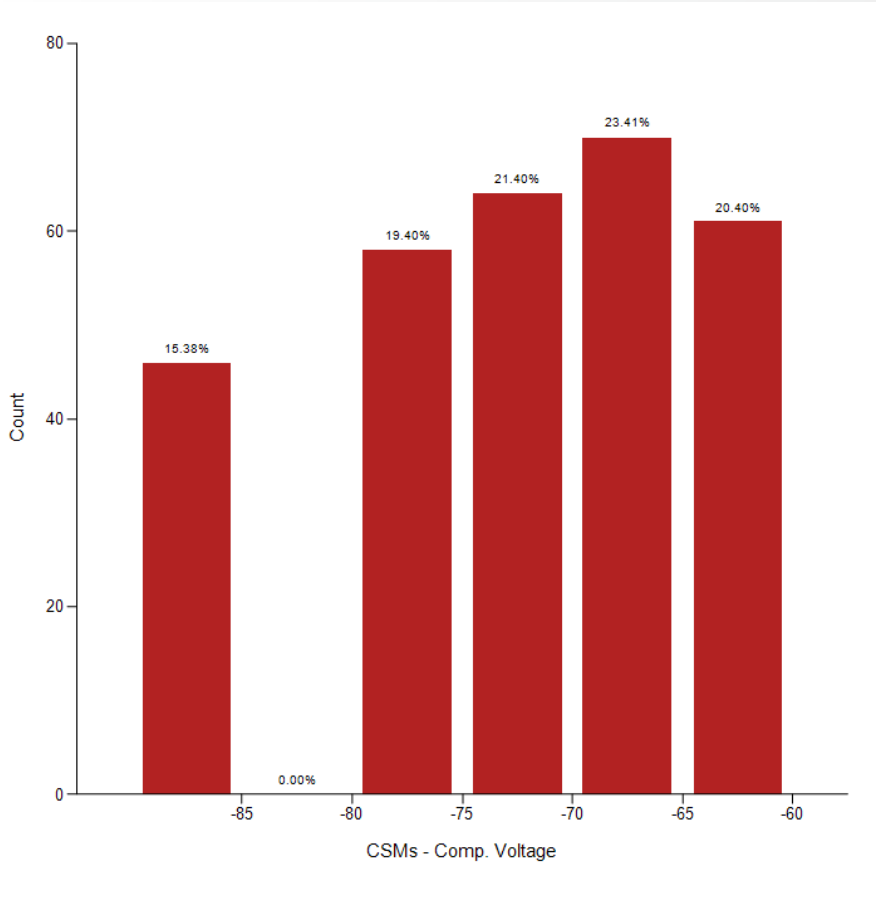
XlinkX Score Visualization



Compensation Voltage in CSM Table for FAIMS Data



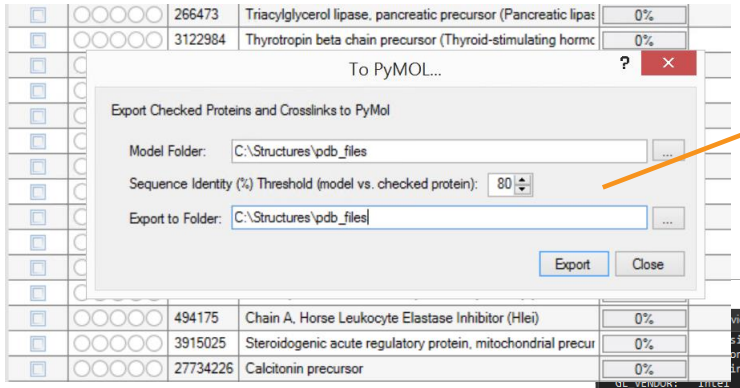
	Exclusive	Total	Label
A	9	22	Comp. Voltage: -60
B	3	16	Comp. Voltage: -80
C	7	28	Comp. Voltage: -70
B | C	8	13	Comp. Voltage: -80 | Comp. Voltage: -70
A | C	8	13	Comp. Voltage: -60 | Comp. Voltage: -70
A | B | C	5	5	Comp. Voltage: -60 | Comp. Voltage: -80 | Comp. Voltage: -70
Sum	40		



Exporting Crosslinks to Pymol

Automatic Export to Pymol (incl crosslink distances)

Export proteins containing crosslinkers in PD



Path to folder containing structure files for the exported protein, sequence similarities threshold between structure and PD sequence and destination folder

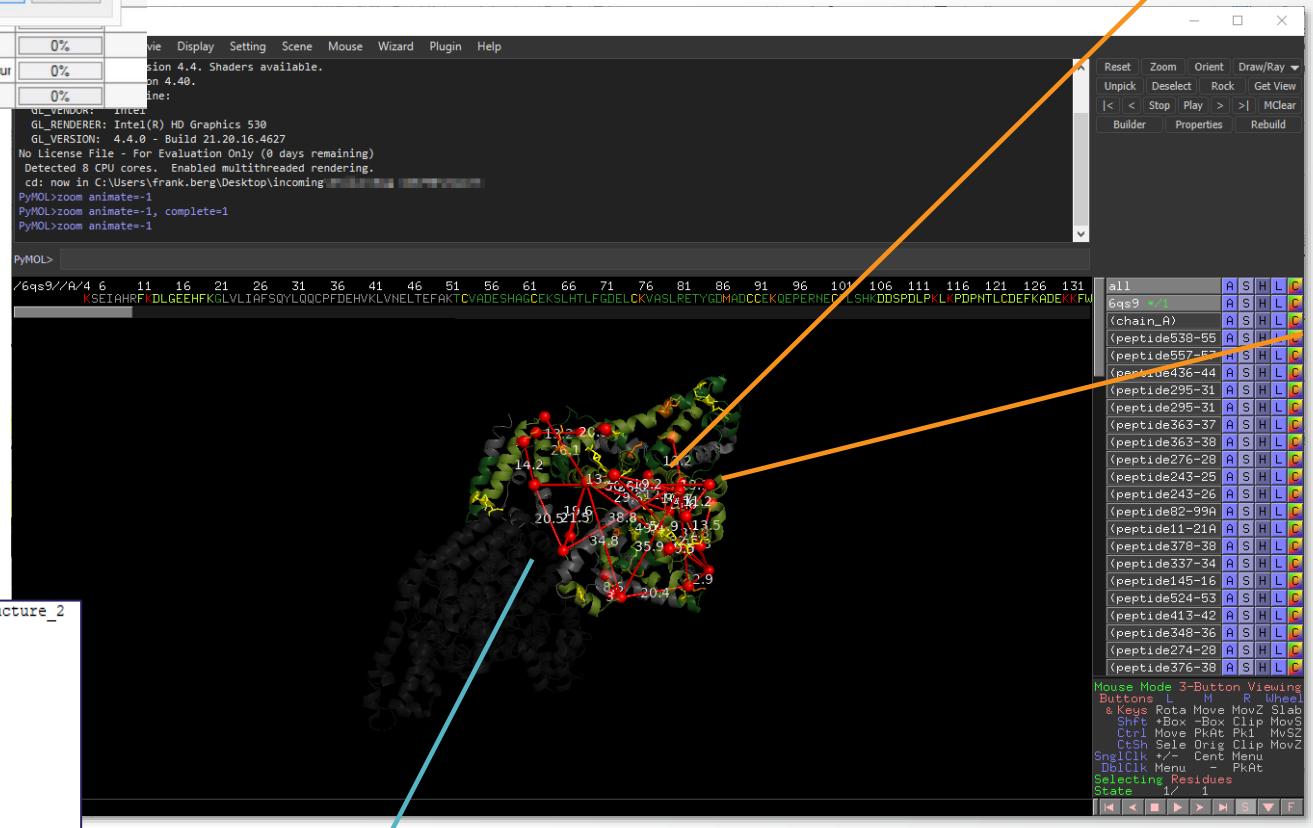
Color-coded display of protein sequence for easy highlighting of

- Links (red)
- Found Modifications (orange, yellow)
- Identified peptide chains (green)

… for pymol
Protein_chain.py

… for Xlink Analzyer
Protein_chain.txt

… add distances file
Protein_chain.Distances



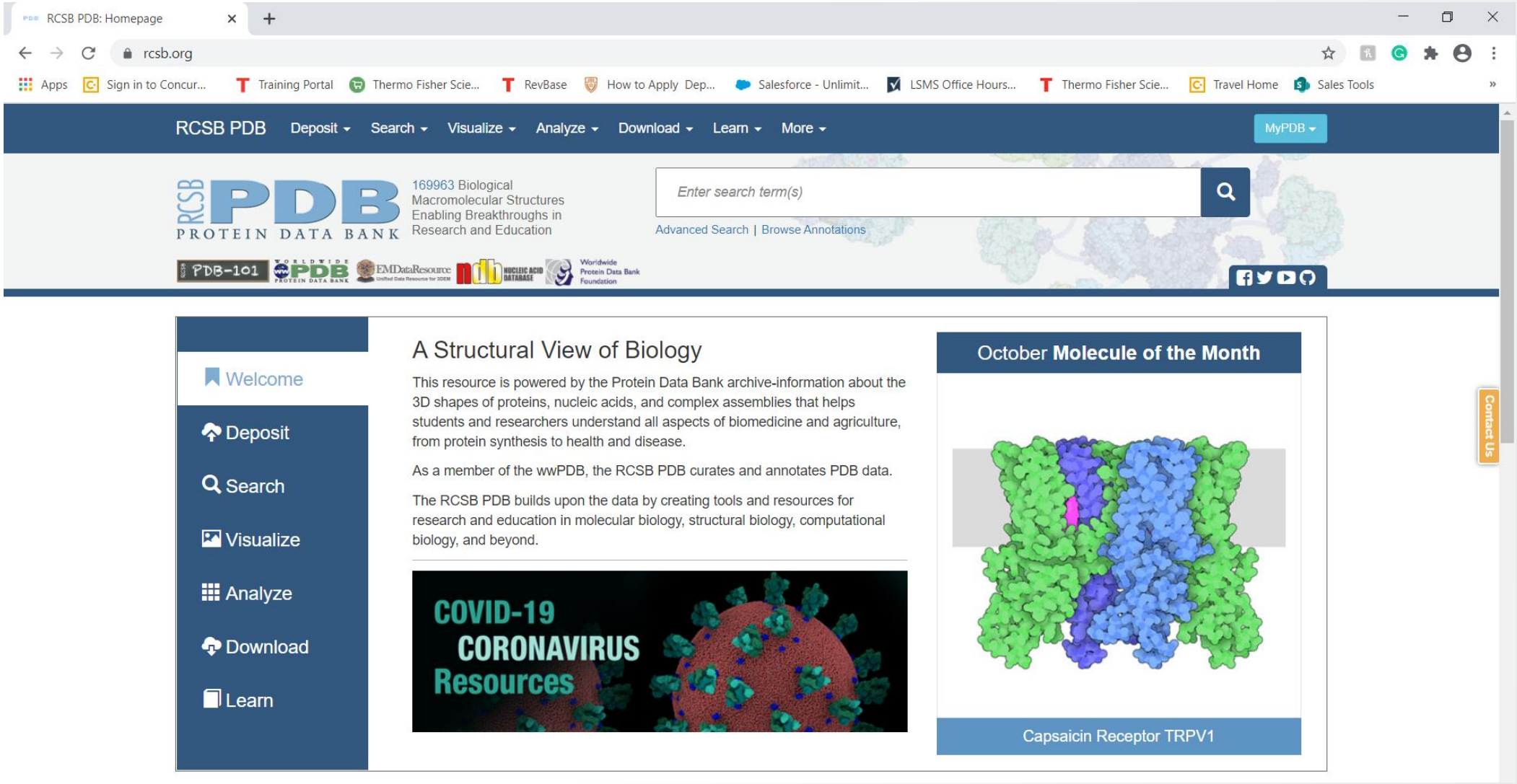
Graphical display of links including calculated crosslink distances

PDB_id	Chain_1	Position_in_structure_1	Chain_2	Position_in_structure_2	Position_in_PDresult_1	Position_in_PDresult_2	Type	Description	Distance (Angstroem)
6QS9	A	4	A	439	28	463	intra	intra_K4_K439	51.92
6QS9	A	431	A	131	455	155	intra	intra_K431_K131	34.84
6QS9	A	537	A	431	561	455	intra	intra_K537_K431	26.1
6QS9	A	4	A	431	28	455	intra	intra_K4_K431	49.87
6QS9	A	221	A	131	245	155	intra	intra_K221_K131	35.93
6QS9	A	439	A	431	463	455	intra	intra_K439_K431	13.21
6QS9	A	4	A	221	28	245	intra	intra_K4_K221	37.81
6QS9	A	132	A	131	156	155	intra	intra_K132_K131	3.81
6QS9	A	439	A	221	463	245	intra	intra_K439_K221	19.21
6QS9	A	4	A	239	28	263	intra	intra_K4_K239	20.81
6QS9	A	12	A	4	36	28	intra	intra_K12_K4	12.94
6QS9	A	12	A	131	36	155	intra	intra_K12_K131	20.37
6QS9	A	242	A	431	266	455	intra	intra_K242_K431	29.65
6QS9	A	221	A	431	245	455	intra	intra_K221_K431	25.97
6QS9	A	413	A	535	437	559	intra	intra_K413_K535	13.2

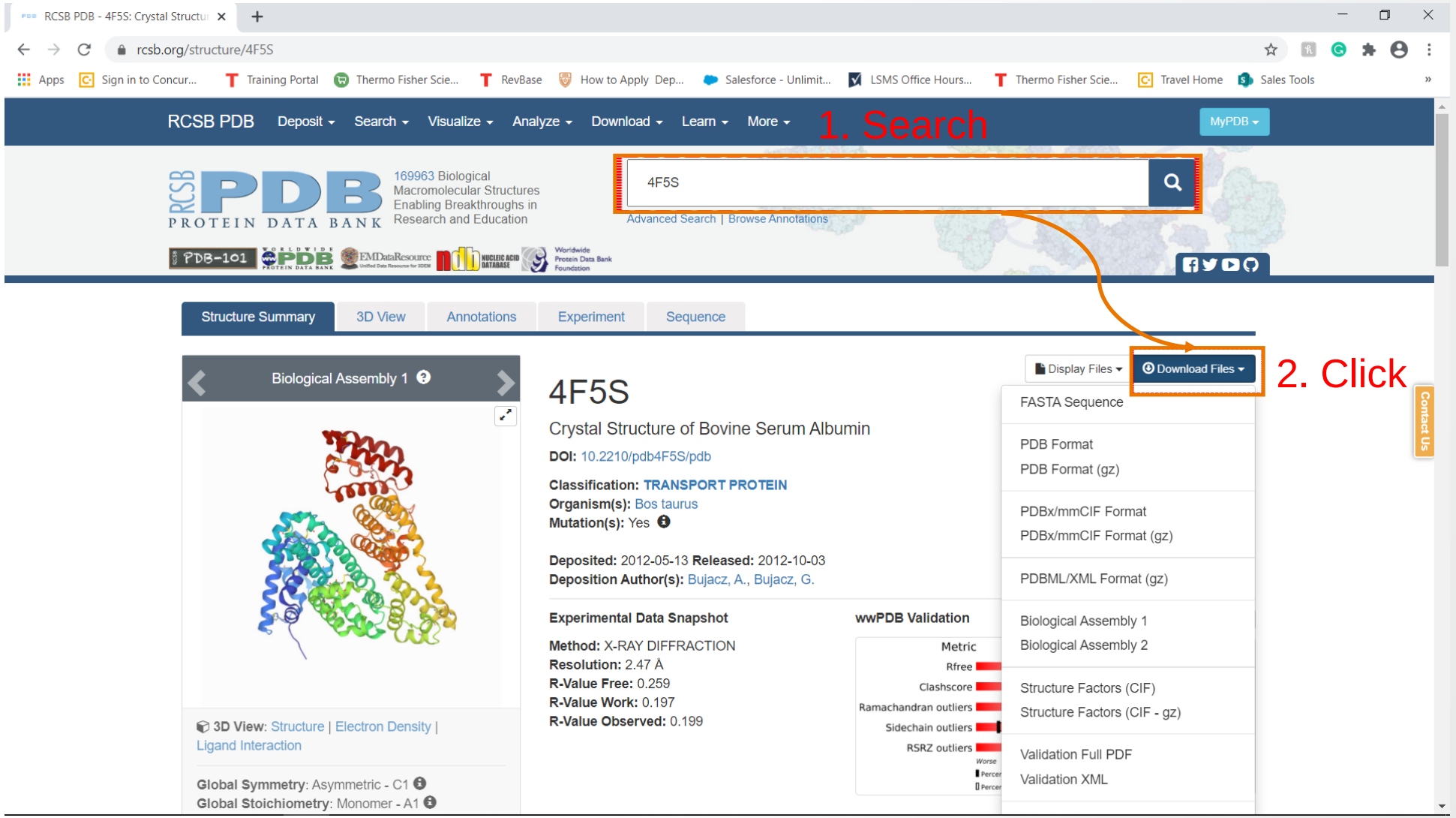
All positions aligned to PDB structure (done automatically)

Download a Protein Structure File

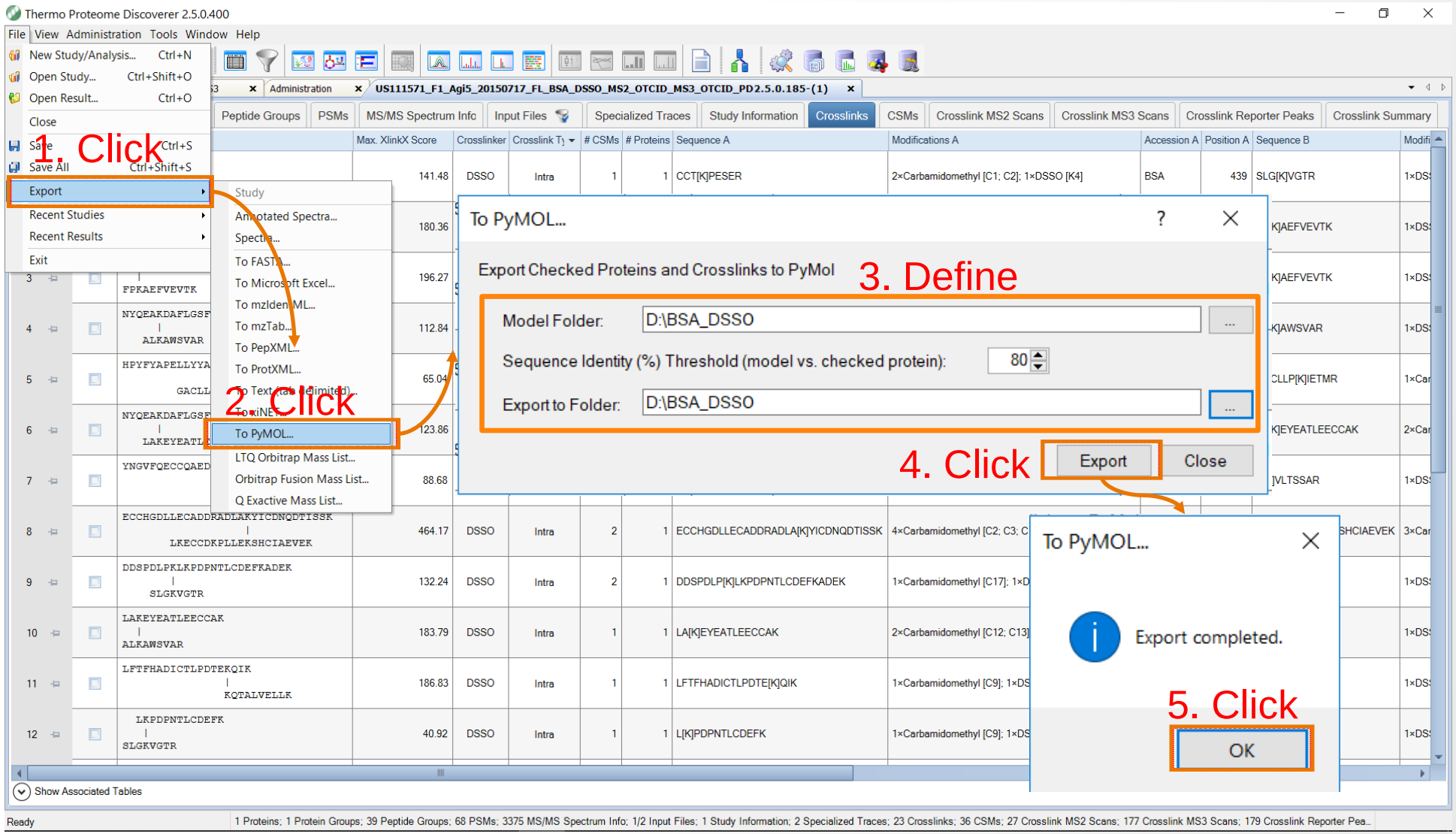
Access protein structure database https://www.rcsb.org/



Download a Protein Structure File in .pdb or .cif Format

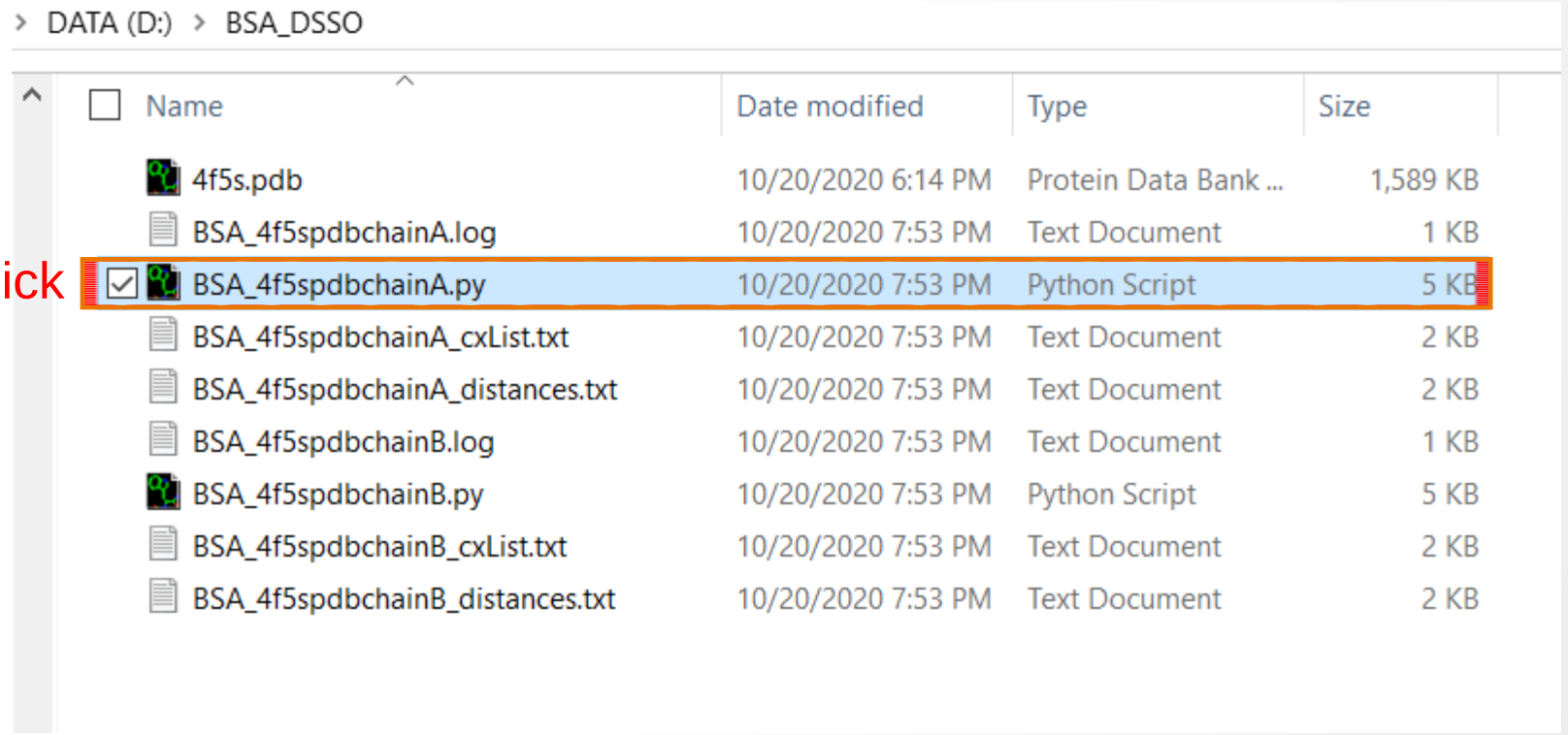


Export Crosslink Result to Pymol



Pymol Folder

Double Click



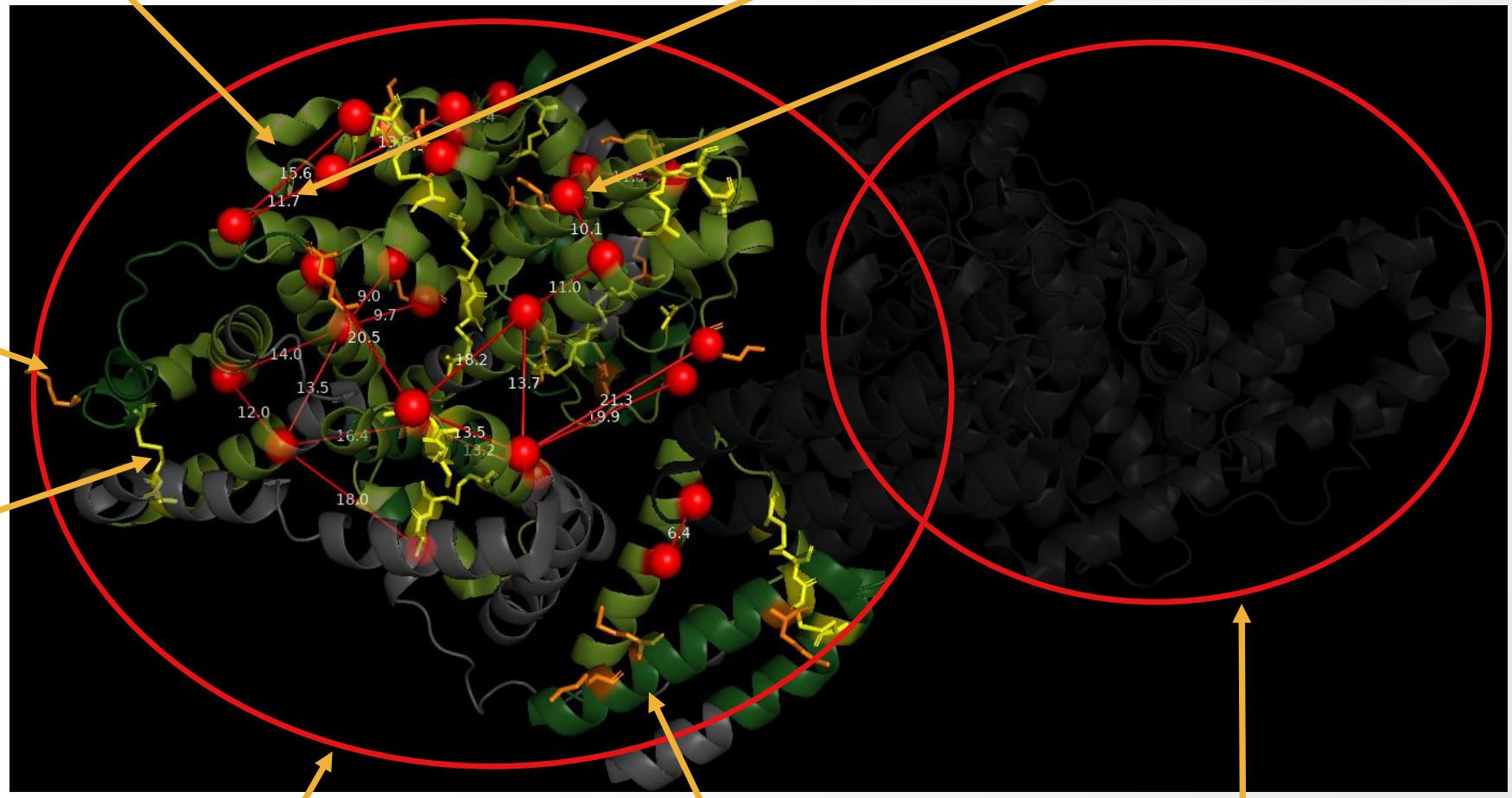
3D Display of Protein

Identified crosslink peptide (green)

Crosslink length measure

Crosslinked amino acid (Cα)

Modified sidechain
(inactive crosslinker, orange)



Modified cysteine (yellow)

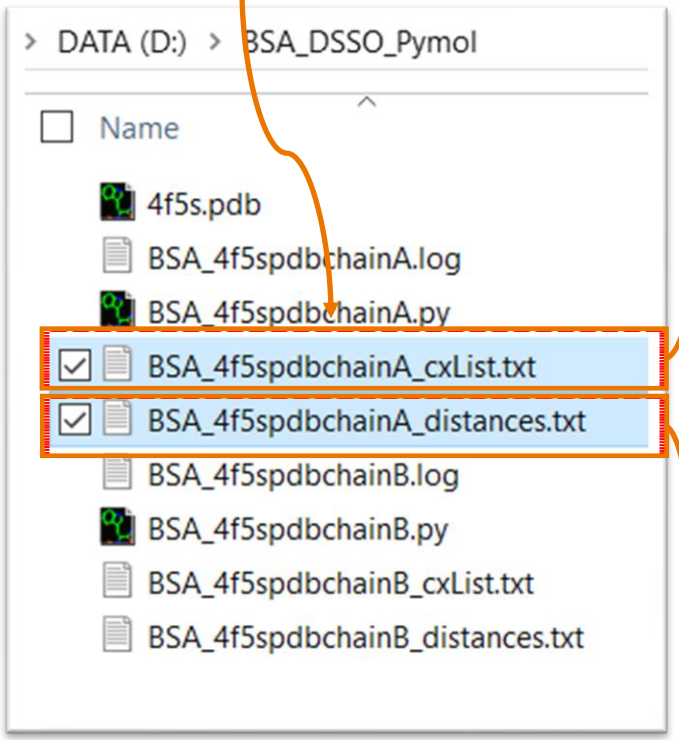
Protein chain used for export

Identified PSM (dark green)

Another protein chain in the structure file
(dark and transparent)

Extra Exporting

Select and double click



BSA_4f5spdbchainA_cxList.txt - Notepad

File Edit Format View Help

ID	Protein1	Protein2	AbsPos1	AbsPos2				
CCTKPESER-SLGKVGTR-a4K-b4K		4F5S	4F5S	436	428			
CCTKPESER-EKVLTSSAR-a4K-b2K		4F5S	4F5S	436	184			
CCTKPESER-LSQKFPK-a4K-b4K		4F5S	4F5S	436	218			
EKVLTSSAR-SLGKVGTR-a2K-b4K		4F5S	4F5S	184	428			
FKDLGEEHFK-DTHKSEIAHR-a2K-b4K		4F5S	4F5S	12	4			
LCVLHEKTPVSEK-CASIQKFGER-a7K-b6K		4F5S	4F5S	462	201			
LAKEYEATLEECCAK-VTKCCTESLVNR-a3K-b3K		4F5S	4F5S	347	471			
VHKECCHGDLLECADDRADLAK-ALKAWSVAR-a3K-b3K			4F5S	4F5S	239	208		
LAKEYEATLEECCAK-CASIQKFGER-a3K-b6K		4F5S	4F5S	347	201			
LVTDLTKVHK-ALKAWSVAR-a7K-b3K		4F5S	4F5S	236	208			
YICDNQDTISSKLK-ECCDKPLLEK-a12K-b5K		4F5S	4F5S	270	277			
LKPDPNTLCDEFK-SLGKVGTR-a2K-b4K	4F5S	4F5S	114	428				
LAKEYEATLEECCAK-ALKAWSVAR-a3K-b3K		4F5S	4F5S	347	208			
DDSPDLPKLKPDPNTLCDEFKADEK-SLGKVGTR-a8K-b4K		4F5S	4F5S	112	428			
ECCHGDLLECADDRADLAKYICDNQDTISSK-LKECCDKPLLEKSHCIAEVEK-a19K-b12K				4F5S	4F5S	258	282	
YNGVFQECCQAEDKGACLLPKIET-EKVLTSSAR-a21K-b2K		4F5S	4F5S	178	184			
NYQEAKDAFLGSFLYEYSR-LAKEYEATLEECCAK-a6K-b3K		4F5S	4F5S	319	347			
HPYFYAPELLYYANKYNGVFQECCQAEDK-GACLLPKIET-a15K-b7K			4F5S	4F5S	157	178		
NYQEAKDAFLGSFLYEYSR-ALKAWSVAR-a6K-b3K		4F5S	4F5S	319	208			
LKECCDKPLLEK-FPKAEFVEVTK-a2K-b3K		4F5S	4F5S	272	221			
YICDNQDTISSKLK-FPKAEFVEVTK-a12K-b3K		4F5S	4F5S	270	221			
LFTFHADICTLPDTEKQIK-KQTALVELLK-a16K-b1K		4F5S	4F5S	517	521			
DLGEEHFKGLVLIAFSQYLQQCPFDEHVK-KFWGK-a8K-b1K		4F5S	4F5S	20	130			

… for Xlink Analzyer
Protein_chain.txt

BSA_4f5spdbchainA_distances.txt - Notepad

File Edit Format View Help

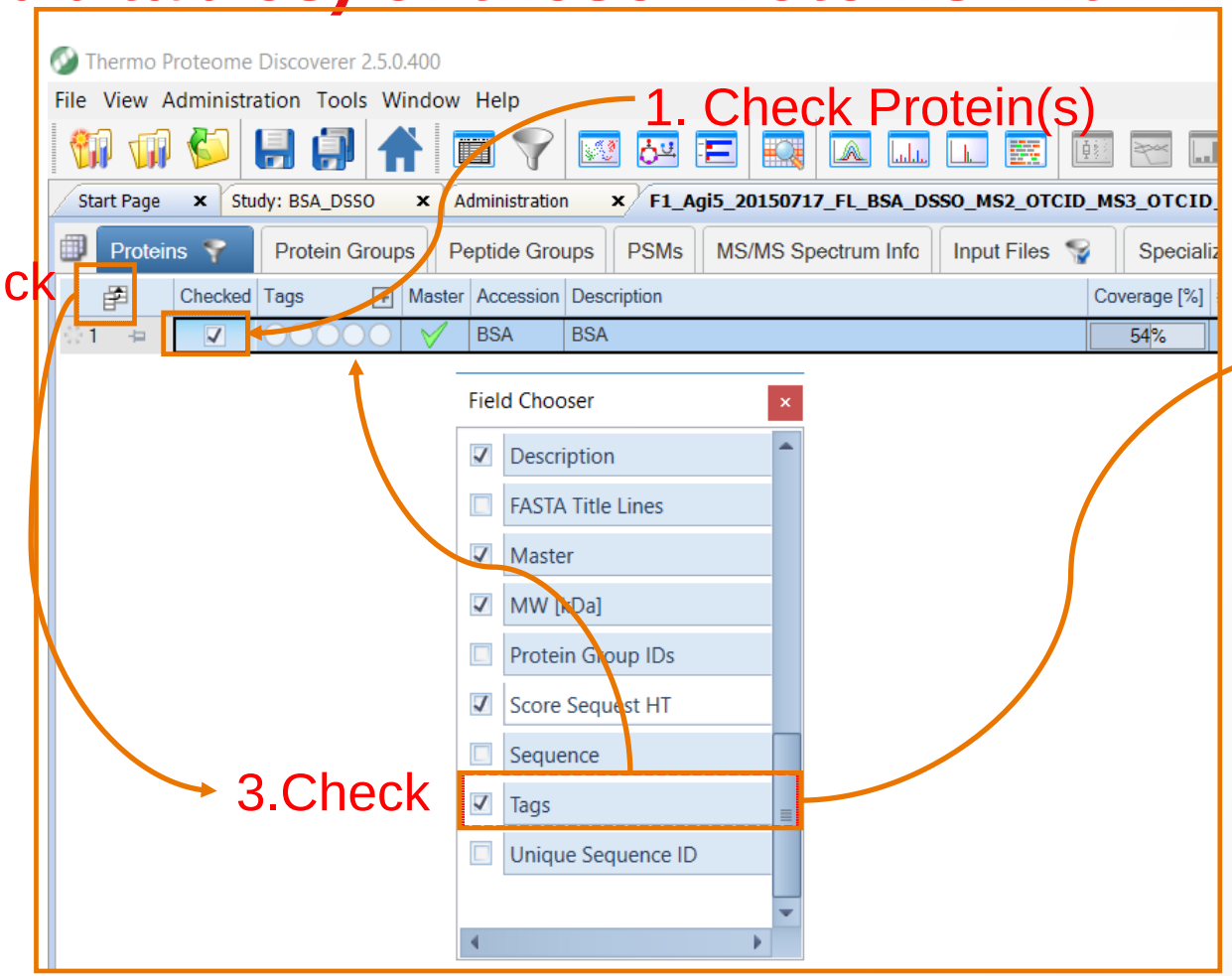
PDB_id	Chain_1	Position_in_structure_1	Chain_2	Position_in_structure_2	Position_in_PDresult_1	Position_in_PDresult_2	Type	Description	Distance(Angstroem)
4F5S	A	439	A	431	439	431	intra	intra_K439_K431	13.51
4F5S	A	439	A	187	439	187	intra	intra_K439_K187	18.22
4F5S	A	439	A	221	439	221	intra	intra_K439_K221	20.54
4F5S	A	187	A	431	187	431	intra	intra_K187_K431	13.69
4F5S	A	12	A	4	12	4	intra	intra_K12_K4	13.38
4F5S	A	465	A	204	465	204	intra	intra_K465_K204	13.24
4F5S	A	350	A	474	350	474	intra	intra_K350_K474	17.99
4F5S	A	242	A	211	242	211	intra	intra_K242_K211	9.73
4F5S	A	350	A	204	350	204	intra	intra_K350_K204	16.43
4F5S	A	239	A	211	239	211	intra	intra_K239_K211	8.96
4F5S	A	273	A	280	273	280	intra	intra_K273_K280	13.82
4F5S	A	116	A	431	116	431	intra	intra_K116_K431	21.27
4F5S	A	350	A	211	350	211	intra	intra_K350_K211	13.54
4F5S	A	114	A	431	114	431	intra	intra_K114_K431	19.9
4F5S	A	261	A	285	261	285	intra	intra_K261_K285	9.07
4F5S	A	180	A	187	180	187	intra	intra_K180_K187	10.97
4F5S	A	322	A	350	322	350	intra	intra_K322_K350	11.98
4F5S	A	159	A	180	159	180	intra	intra_K159_K180	10.07
4F5S	A	322	A	211	322	211	intra	intra_K322_K211	13.99
4F5S	A	275	A	224	275	224	intra	intra_K275_K224	15.59
4F5S	A	273	A	224	273	224	intra	intra_K273_K224	11.66
4F5S	A	520	A	524	520	524	intra	intra_K520_K524	6.42
4F5S	A	20	A	132	20	132	intra	intra_K20_K132	11.51

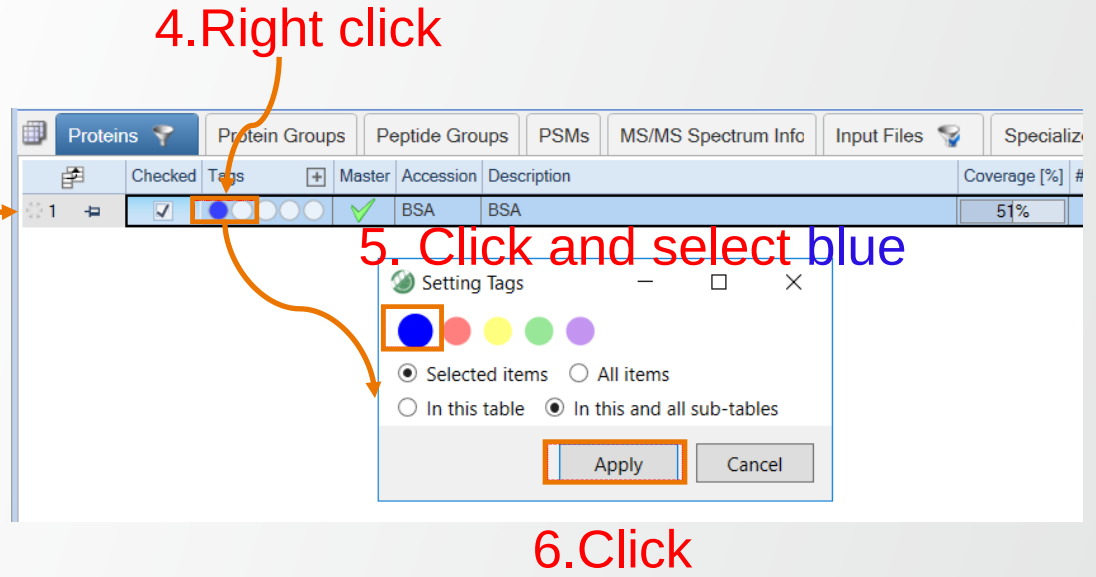
… add distances file
Protein_chain.Distance

Exporting Crosslinks to xiVIEW

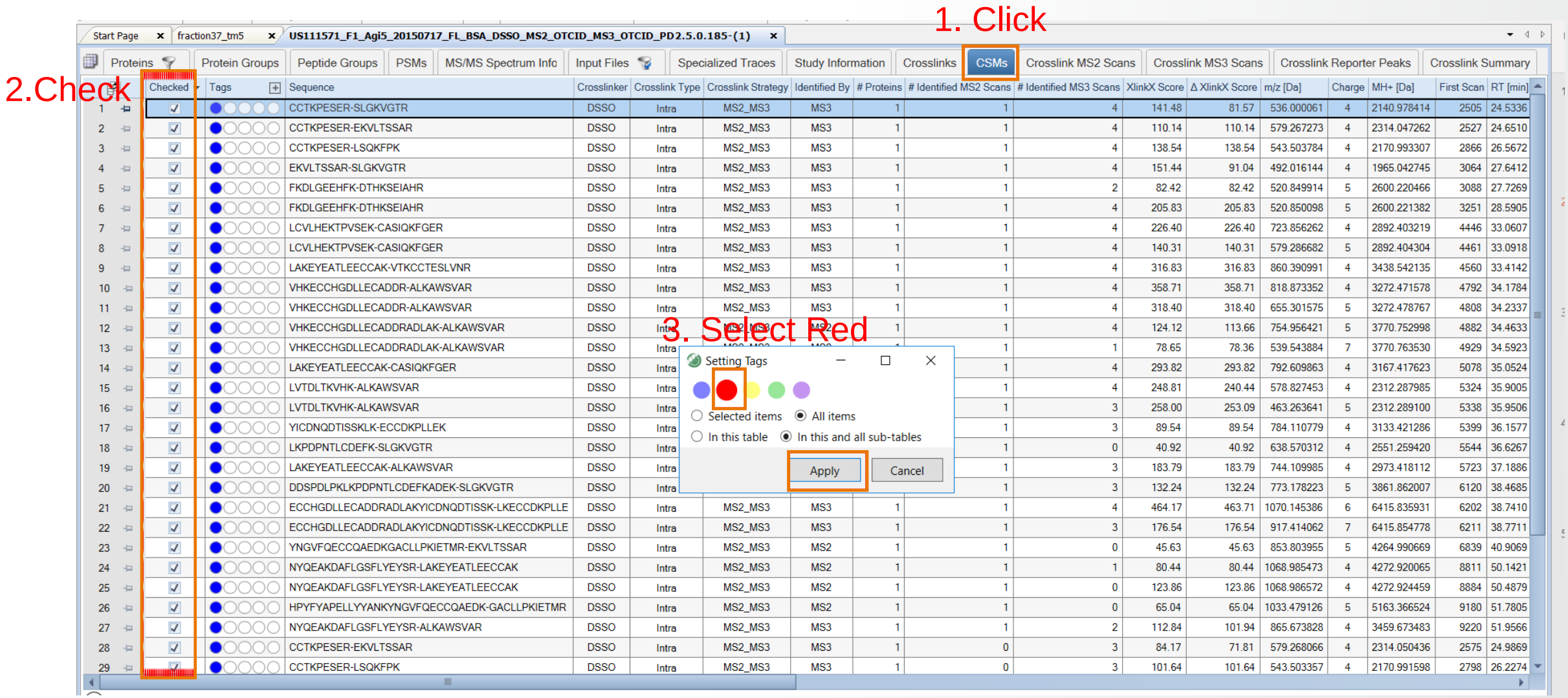
Selecting the correct set of spectra for proteins when exporting mzIdentML and mzML for uploading to XiView

Step 1: Check Proteins to be exported (only the Proteins), then select checked Proteins and tag all associated Items (sub tables) of these Proteins with A (Blue Tag)



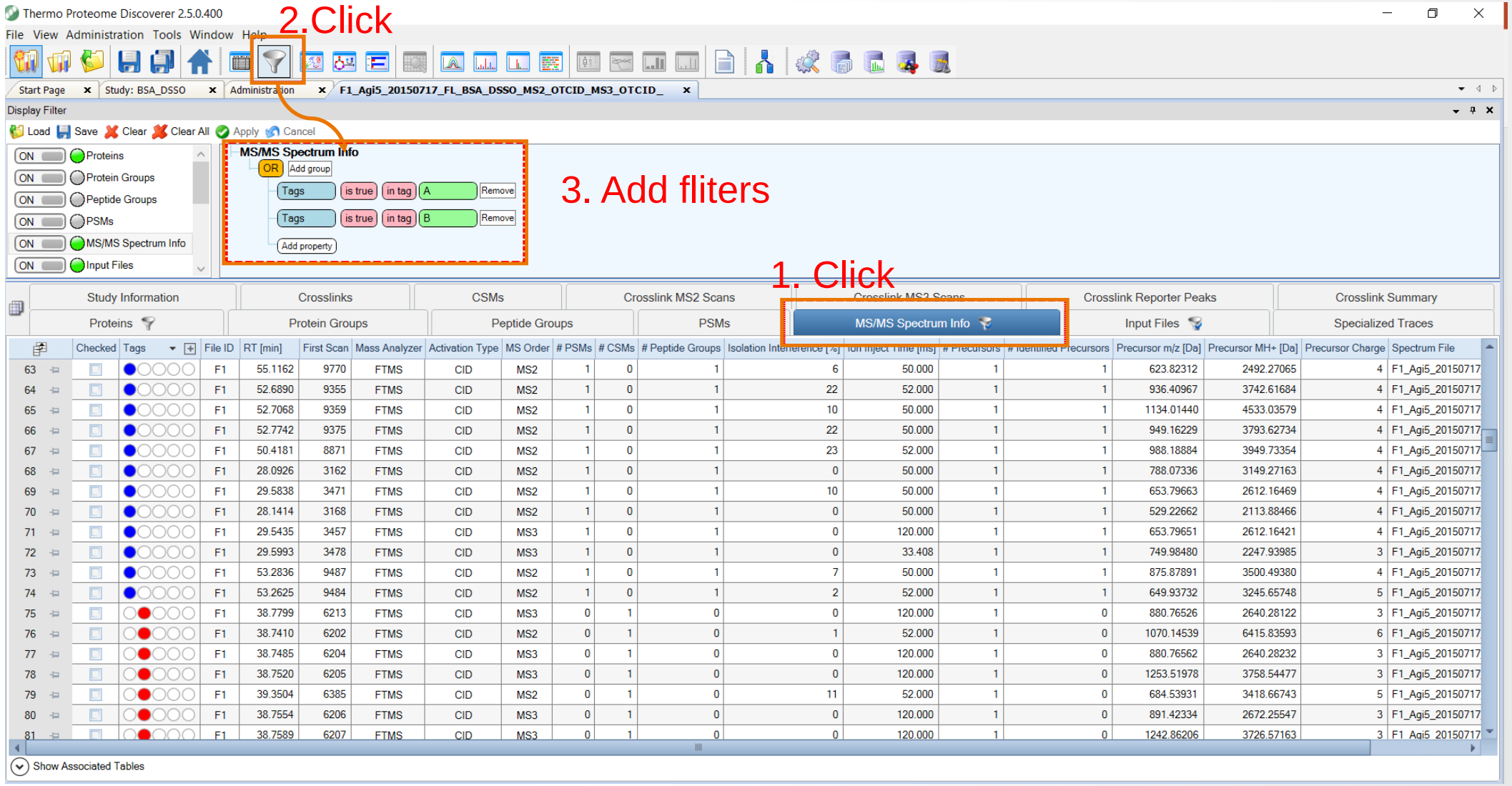


Step 2: Select all blue-tagged CSMs and tag all associated items with tag B (Red Tag)



	Checked	Tags	Sequence	Crosslinker	Crosslink Type	Crosslink Strategy	Identified By	# Proteins	# Identified MS2 Scans	# Identified MS3 Scans	XlinkX Score	Δ XlinkX Score	m/z [Da]	Charge	MH+ [Da]	First Scan	RT [min]
1	☑		CCTKPESER-SLGKVGTR	DSSO	Intra	MS2_MS3	MS3	1	1	4	141.48	81.57	536.000061	4	2140.978414	2505	24.5336
2	☑		CCTKPESER-EKVLTSSAR	DSSO	Intra	MS2_MS3	MS3	1	1	4	110.14	110.14	579.267273	4	2314.047262	2527	24.6510
3	☑		CCTKPESER-LSQKFPK	DSSO	Intra	MS2_MS3	MS3	1	1	4	138.54	138.54	543.503784	4	2170.993307	2866	26.5672
4	☑		EKVLTSSAR-SLGKVGTR	DSSO	Intra	MS2_MS3	MS3	1	1	4	151.44	91.04	492.016144	4	1965.042745	3064	27.6412
5	☑		FKDLGEEHFK-DTHKSEIAHR	DSSO	Intra	MS2_MS3	MS3	1	1	2	82.42	82.42	520.849914	5	2600.220466	3088	27.7269
6	☑		FKDLGEEHFK-DTHKSEIAHR	DSSO	Intra	MS2_MS3	MS3	1	1	4	205.83	205.83	520.850098	5	2600.221382	3251	28.5905
7	☑		LCVLHEKTPVSEK-CASIQKFGER	DSSO	Intra	MS2_MS3	MS3	1	1	4	226.40	226.40	723.856262	4	2892.403219	4446	33.0607
8	☑		LCVLHEKTPVSEK-CASIQKFGER	DSSO	Intra	MS2_MS3	MS3	1	1	4	140.31	140.31	579.286682	5	2892.404304	4461	33.0918
9	☑		LAKEYEATLEECCAK-VTKCCTESLVNR	DSSO	Intra	MS2_MS3	MS3	1	1	4	316.83	316.83	860.390991	4	3438.542135	4560	33.4142
10	☑		VHKECCHGDLLECADDR-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	4	358.71	358.71	818.873352	4	3272.471578	4792	34.1784
11	☑		VHKECCHGDLLECADDR-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	4	318.40	318.40	655.301575	5	3272.478767	4808	34.2337
12	☑		VHKECCHGDLLECADDRADLAK-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS2	1	1	4	124.12	113.66	754.956421	5	3770.752998	4882	34.4633
13	☑		VHKECCHGDLLECADDRADLAK-ALKAWSVAR	DSSO	Intra				1	1	78.65	78.36	539.543884	7	3770.763530	4929	34.5923
14	☑		LAKEYEATLEECCAK-CASIQKFGER	DSSO	Intra				1	4	293.82	293.82	792.609863	4	3167.417623	5078	35.0524
15	☑		LVTDLTKVHK-ALKAWSVAR	DSSO	Intra				1	4	248.81	240.44	578.827453	4	2312.287985	5324	35.9005
16	☑		LVTDLTKVHK-ALKAWSVAR	DSSO	Intra				1	3	258.00	253.09	463.263641	5	2312.289100	5338	35.9506
17	☑		YICDNQDTISSKLK-ECCDKPLLEK	DSSO	Intra				1	3	89.54	89.54	784.110779	4	3133.421286	5399	36.1577
18	☑		LKPDPNTLCDEFK-SLGKVGTR	DSSO	Intra				1	0	40.92	40.92	638.570312	4	2551.259420	5544	36.6267
19	☑		LAKEYEATLEECCAK-ALKAWSVAR	DSSO	Intra				1	3	183.79	183.79	744.109985	4	2973.418112	5723	37.1886
20	☑		DDSPDLPKLKPDPNTLCDEFKADEK-SLGKVGTR	DSSO	Intra				1	3	132.24	132.24	773.178223	5	3861.862007	6120	38.4685
21	☑		ECCHGDLLECADDRADLAKYICDNQDTISSK-LKECCDKPLLE	DSSO	Intra	MS2_MS3	MS3	1	1	4	464.17	463.71	1070.145386	6	6415.835931	6202	38.7410
22	☑		ECCHGDLLECADDRADLAKYICDNQDTISSK-LKECCDKPLLE	DSSO	Intra	MS2_MS3	MS3	1	1	3	176.54	176.54	917.414062	7	6415.854778	6211	38.7711
23	☑		YNGVFQECCQAEDKGACLLPKIETMR-EKVLTSSAR	DSSO	Intra	MS2_MS3	MS2	1	1	0	45.63	45.63	853.803955	5	4264.990669	6839	40.9069
24	☑		NYQEAKDAFLGSFLYEYSR-LAKEYEATLEECCAK	DSSO	Intra	MS2_MS3	MS2	1	1	1	80.44	80.44	1068.985473	4	4272.920065	8811	50.1421
25	☑		NYQEAKDAFLGSFLYEYSR-LAKEYEATLEECCAK	DSSO	Intra	MS2_MS3	MS2	1	1	0	123.86	123.86	1068.986572	4	4272.924459	8884	50.4879
26	☑		HPYFYAPELLYYANKYNGVFQECCQAEDK-GACLLPKIETMR	DSSO	Intra	MS2_MS3	MS2	1	1	0	65.04	65.04	1033.479126	5	5163.366524	9180	51.7805
27	☑		NYQEAKDAFLGSFLYEYSR-ALKAWSVAR	DSSO	Intra	MS2_MS3	MS3	1	1	2	112.84	101.94	865.673828	4	3459.673483	9220	51.9566
28	☑		CCTKPESER-EKVLTSSAR	DSSO	Intra	MS2_MS3	MS3	1	0	3	84.17	71.81	579.268066	4	2314.050436	2575	24.9869
29	☑		CCTKPESER-LSQKFPK	DSSO	Intra	MS2_MS3	MS3	1	0	3	101.64	101.64	543.503357	4	2170.991598	2798	26.2274

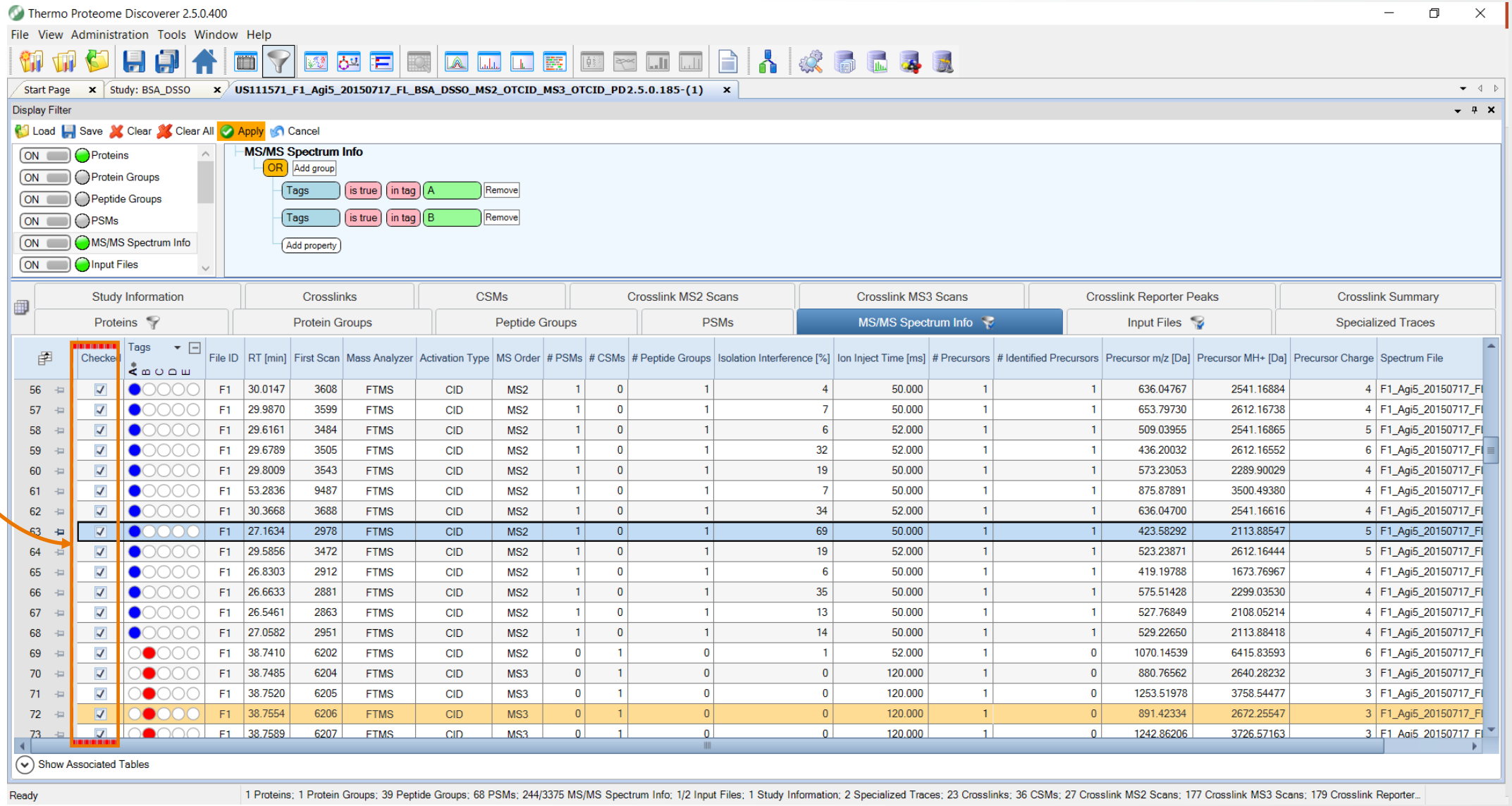
Step 3: Filter all MS/MS Spectrum Info items that carry tag A or B (Red or Blue)



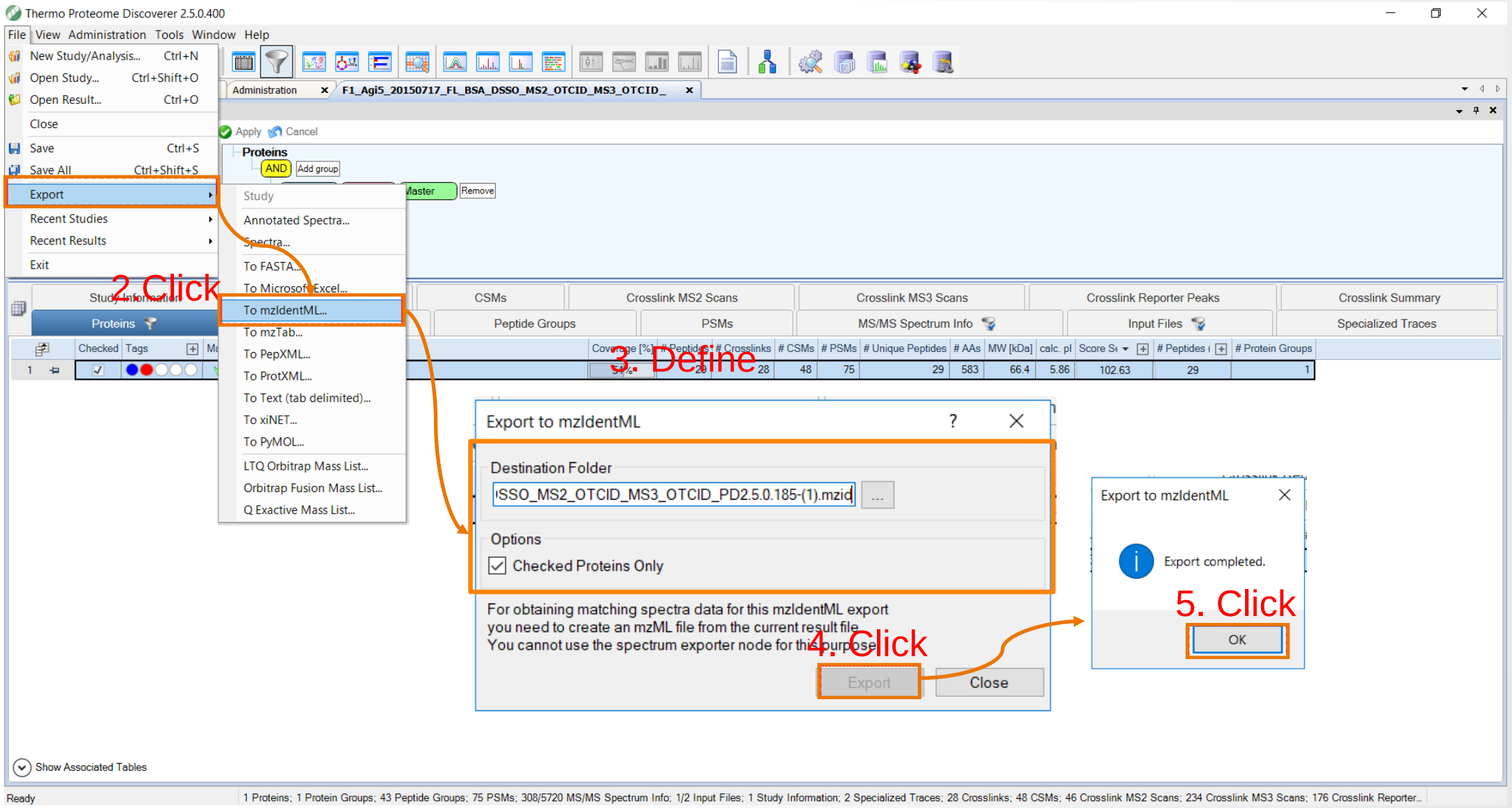
	Checked	Tags	File ID	RT [min]	First Scan	Mass Analyzer	Activation Type	MS Order	# PSMs	# CSMs	# Peptide Groups	Isolation Interference [%]	Ion Inject Time [ms]	# Precursors	# Identified Precursors	Precursor m/z [Da]	Precursor MH+ [Da]	Precursor Charge	Spectrum File
63			F1	55.1162	9770	FTMS	CID	MS2	1	0	1	6	50.000	1	1	623.82312	2492.27065	4	F1_Agi5_20150717
64			F1	52.6890	9355	FTMS	CID	MS2	1	0	1	22	52.000	1	1	936.40967	3742.61684	4	F1_Agi5_20150717
65			F1	52.7068	9359	FTMS	CID	MS2	1	0	1	10	50.000	1	1	1134.01440	4533.03579	4	F1_Agi5_20150717
66			F1	52.7742	9375	FTMS	CID	MS2	1	0	1	22	50.000	1	1	949.16229	3793.62734	4	F1_Agi5_20150717
67			F1	50.4181	8871	FTMS	CID	MS2	1	0	1	23	52.000	1	1	988.18884	3949.73354	4	F1_Agi5_20150717
68			F1	28.0926	3162	FTMS	CID	MS2	1	0	1	0	50.000	1	1	788.07336	3149.27163	4	F1_Agi5_20150717
69			F1	29.5838	3471	FTMS	CID	MS2	1	0	1	10	50.000	1	1	653.79663	2612.16469	4	F1_Agi5_20150717
70			F1	28.1414	3168	FTMS	CID	MS2	1	0	1	0	50.000	1	1	529.22662	2113.88466	4	F1_Agi5_20150717
71			F1	29.5435	3457	FTMS	CID	MS3	1	0	1	0	120.000	1	1	653.79651	2612.16421	4	F1_Agi5_20150717
72			F1	29.5993	3478	FTMS	CID	MS3	1	0	1	0	33.408	1	1	749.98480	2247.93985	3	F1_Agi5_20150717
73			F1	53.2836	9487	FTMS	CID	MS2	1	0	1	7	50.000	1	1	875.87891	3500.49380	4	F1_Agi5_20150717
74			F1	53.2625	9484	FTMS	CID	MS2	1	0	1	2	52.000	1	1	649.93732	3245.65748	5	F1_Agi5_20150717
75			F1	38.7799	6213	FTMS	CID	MS3	0	1	0	0	120.000	1	0	880.76526	2640.28122	3	F1_Agi5_20150717
76			F1	38.7410	6202	FTMS	CID	MS2	0	1	0	1	52.000	1	0	1070.14539	6415.83593	6	F1_Agi5_20150717
77			F1	38.7485	6204	FTMS	CID	MS3	0	1	0	0	120.000	1	0	880.76562	2640.28232	3	F1_Agi5_20150717
78			F1	38.7520	6205	FTMS	CID	MS3	0	1	0	0	120.000	1	0	1253.51978	3758.54477	3	F1_Agi5_20150717
79			F1	39.3504	6385	FTMS	CID	MS2	0	1	0	11	52.000	1	0	684.53931	3418.66743	5	F1_Agi5_20150717
80			F1	38.7554	6206	FTMS	CID	MS3	0	1	0	0	120.000	1	0	891.42334	2672.25547	3	F1_Agi5_20150717
81			F1	38.7589	6207	FTMS	CID	MS3	0	1	0	0	120.000	1	0	1242.86206	3726.57163	3	F1_Agi5_20150717

Step 4: Check all MS/MS Spectrum Info items that carry tag A or B (Red or Blue)

1.Check

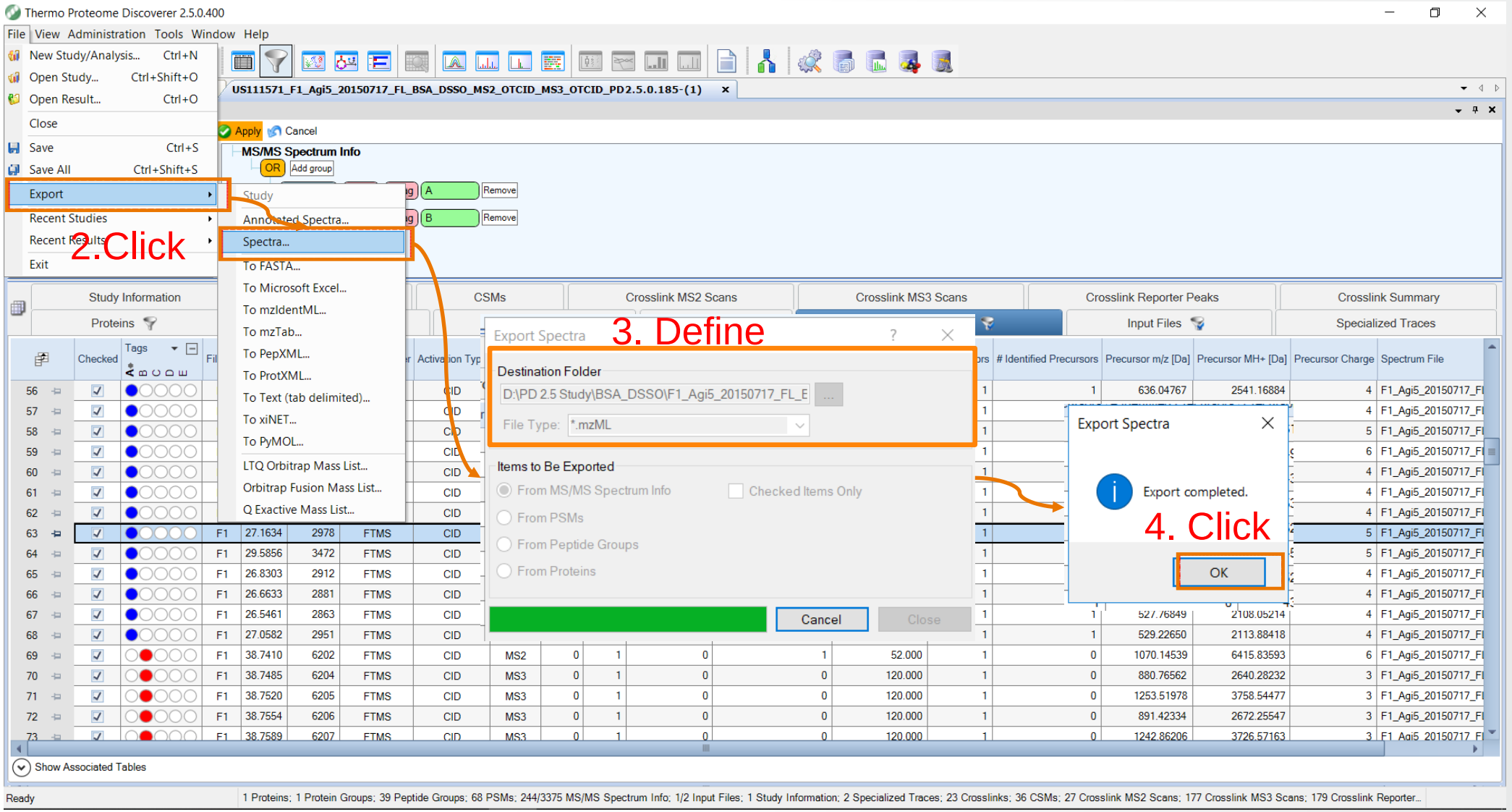


Step 5: Export checked Proteins to mzIdentML

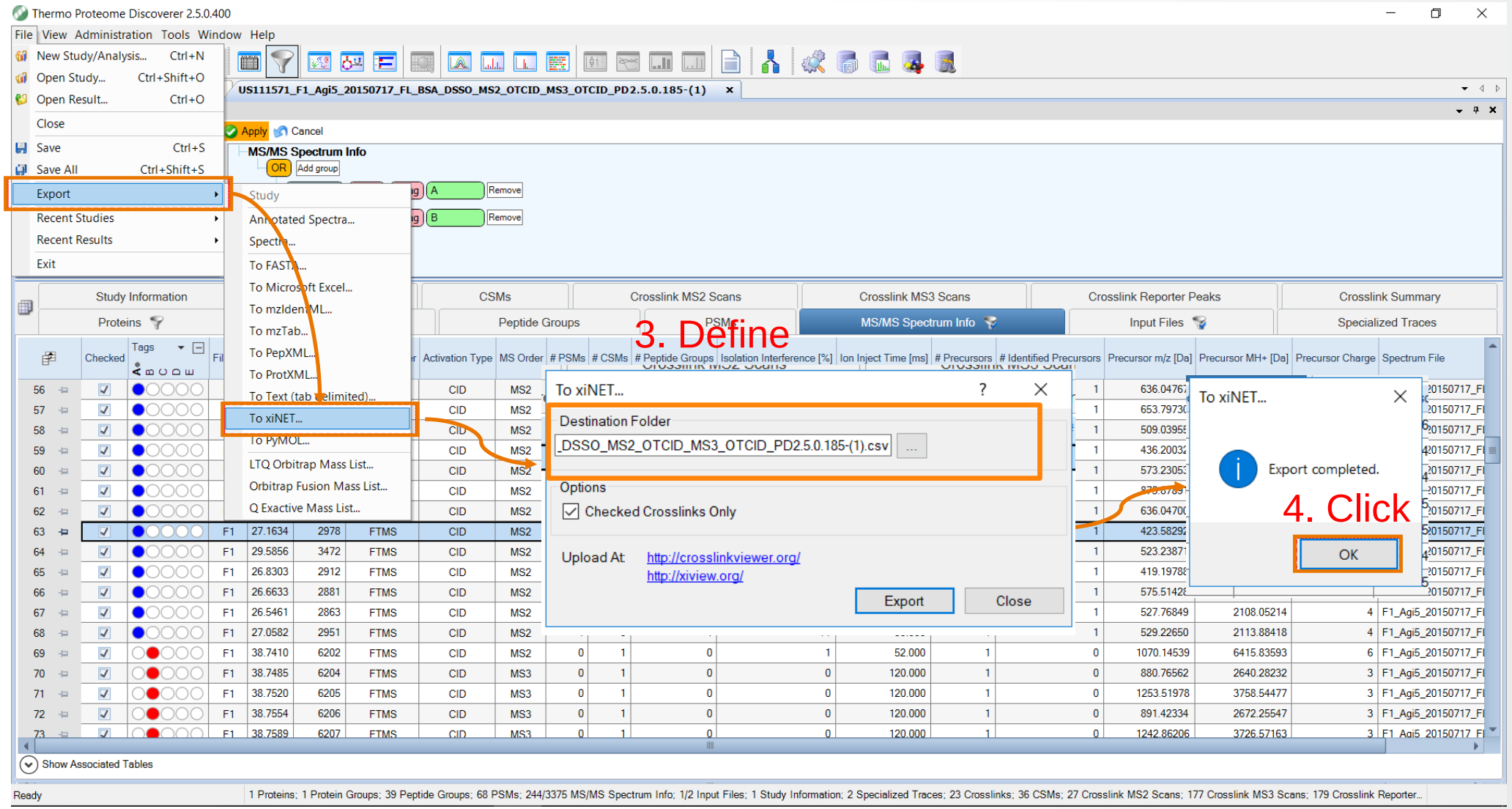


Step 6: Export checked MS/MS Spectrum Info to mzML file.

1. Click

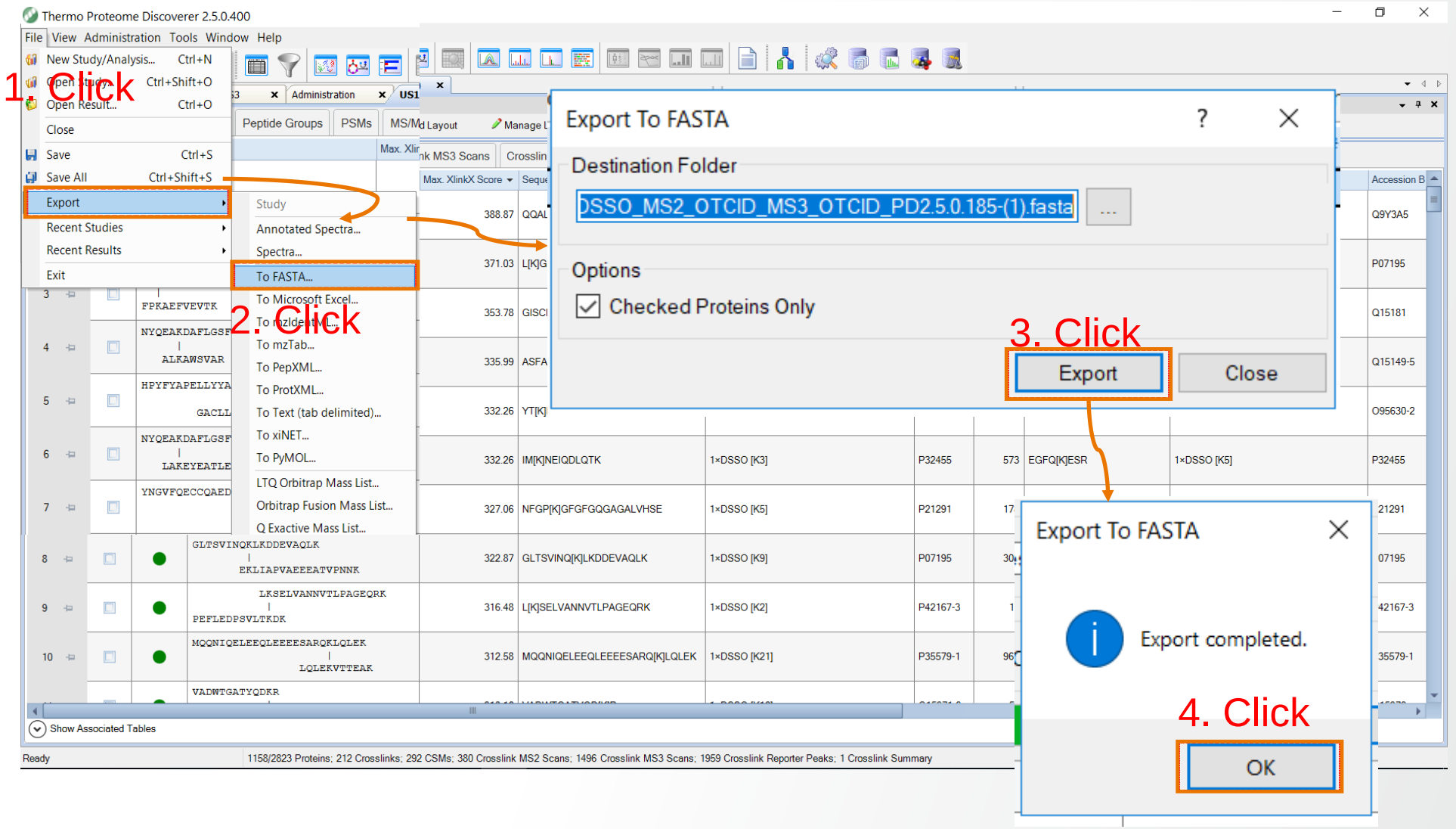


Step 7: Export crosslinks to xiNET CSV format



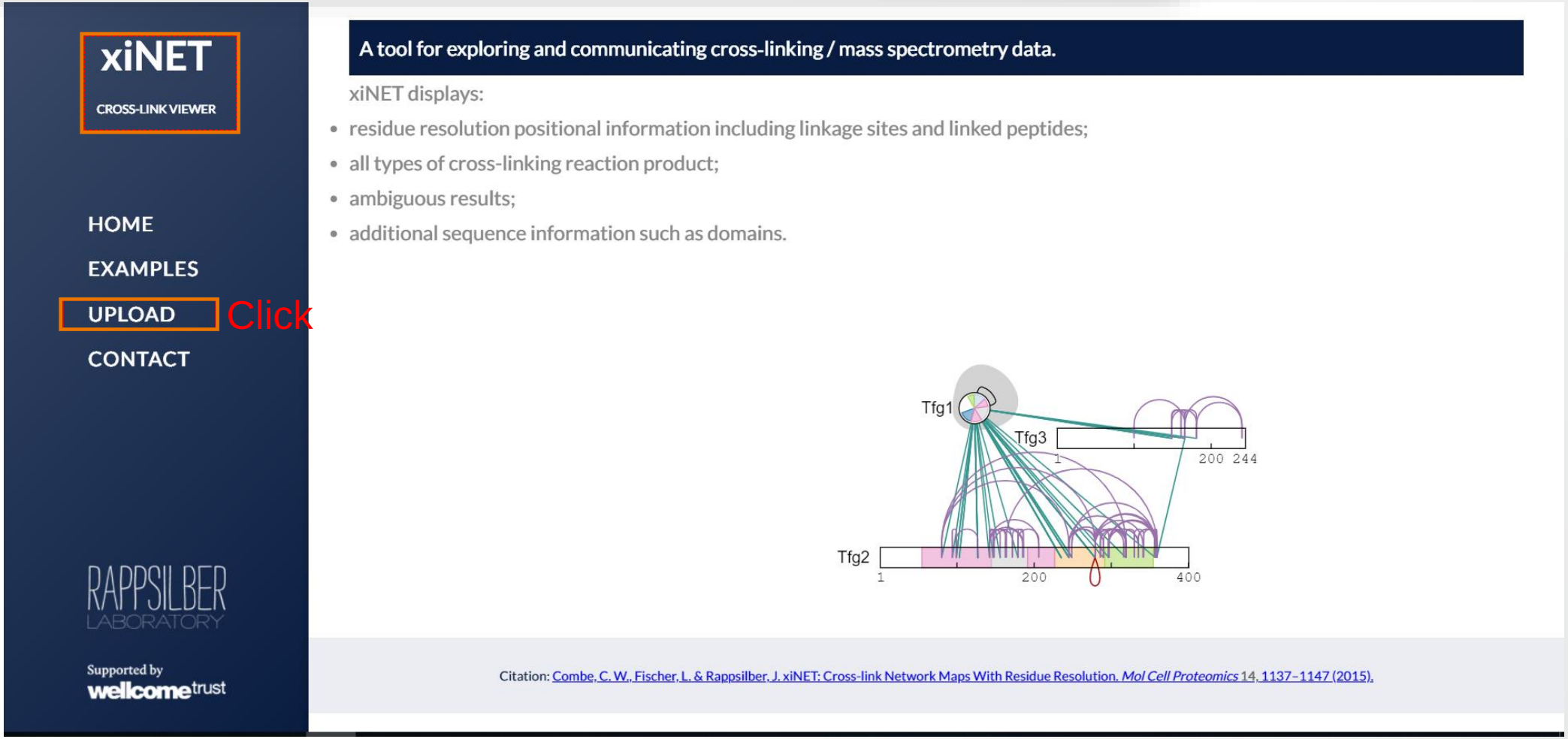
Note the xiVIEW URL on the ‘To xiNET…’ dialog (for later upload to website)

Step 8: Export result to *.fasta file

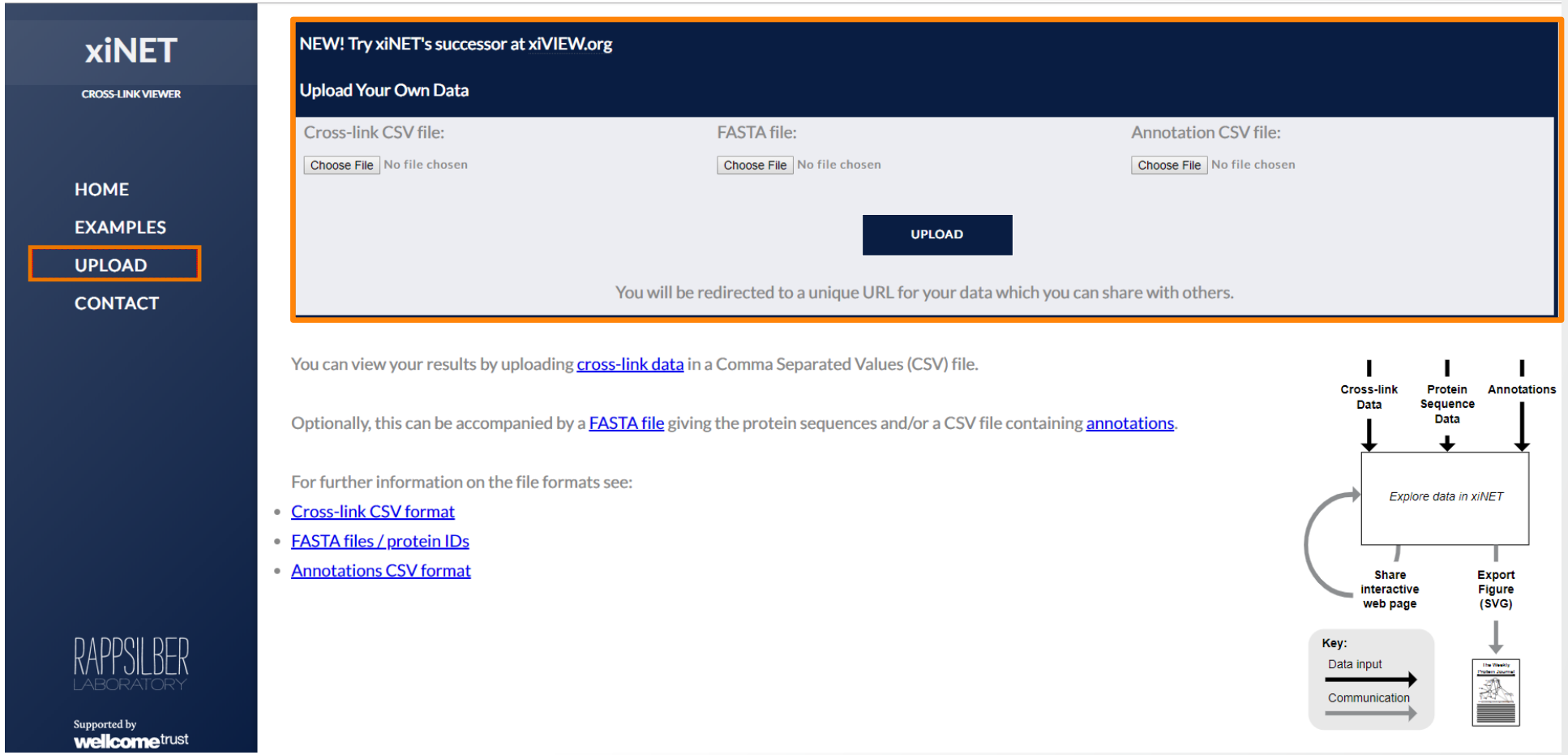


Step 9. Exproting crosslinks to xiNET

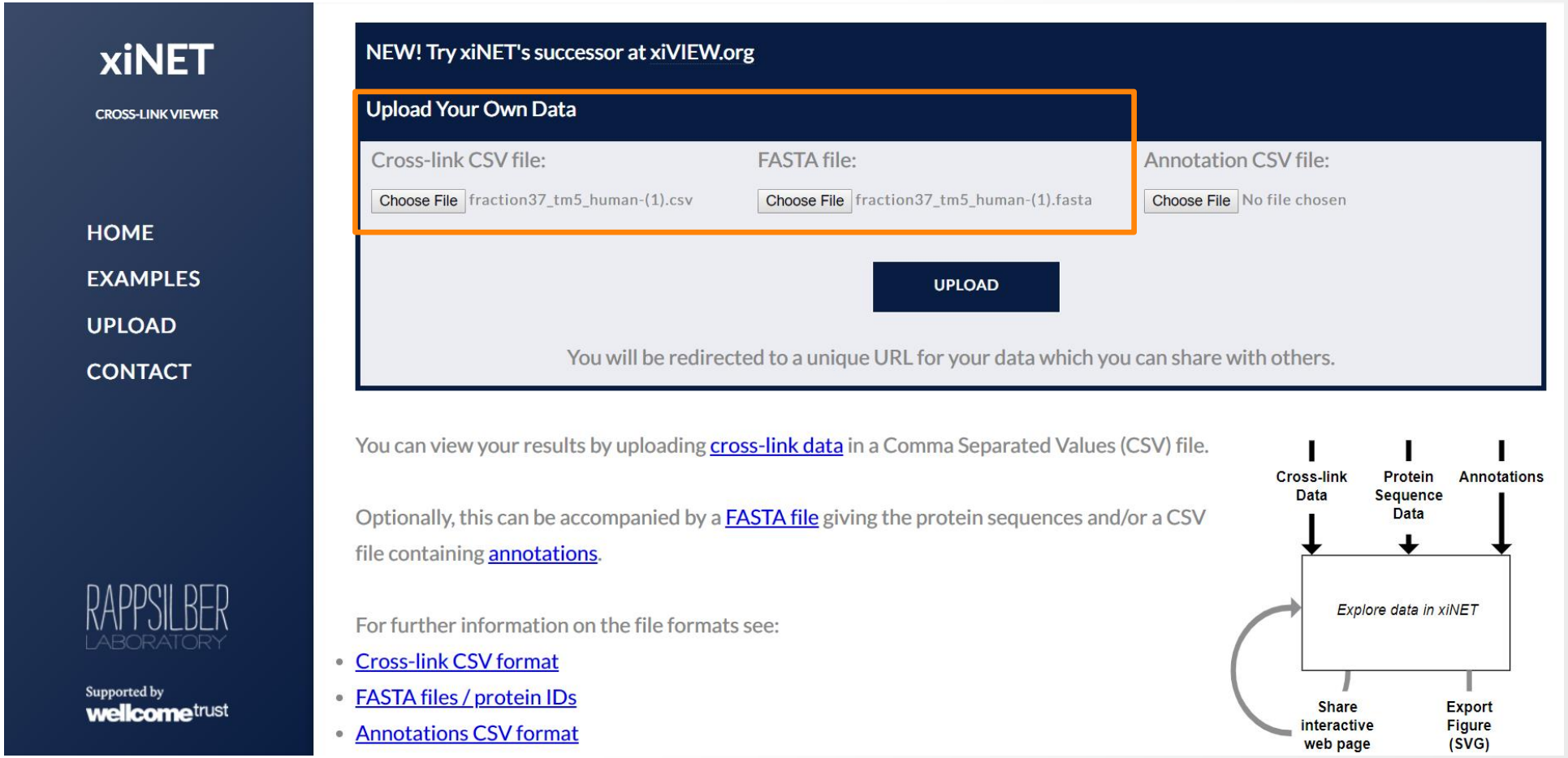
Access http://crosslinkviewer.org/ in Chrome



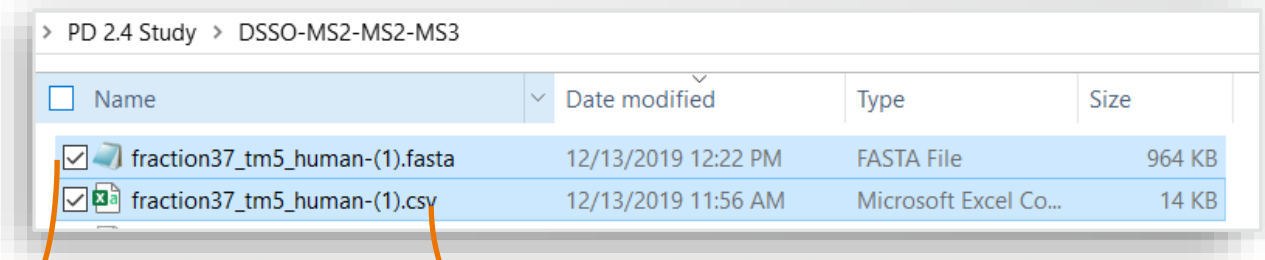
Click ‘UPLOAD’ then ‘Chose File’ to upload



Upload CSV and FASTA files



Upload CSV and FASTA files



xiNET

CROSS-LINK VIEWER

HOME

EXAMPLES

UPLOAD

CONTACT

RAPPSILBER LABORATORY

Supported by wellcome trust

NEW! Try xiNET's successor at xiVIEW.org

Upload Your Own Data

Cross-link CSV file: Choose File fraction37_tm5_human-(1).csv

FASTA file: Choose File fraction37_tm5_human-(1).fasta

Annotation CSV file: Choose File No file chosen

UPLOAD

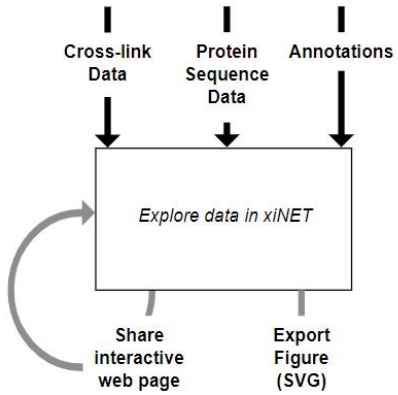
You will be redirected to a unique URL for your data which you can share with others.

You can view your results by uploading cross-link data in a Comma Separated Values (CSV) file.

Optionally, this can be accompanied by a FASTA file giving the protein sequences and/or a CSV file containing annotations.

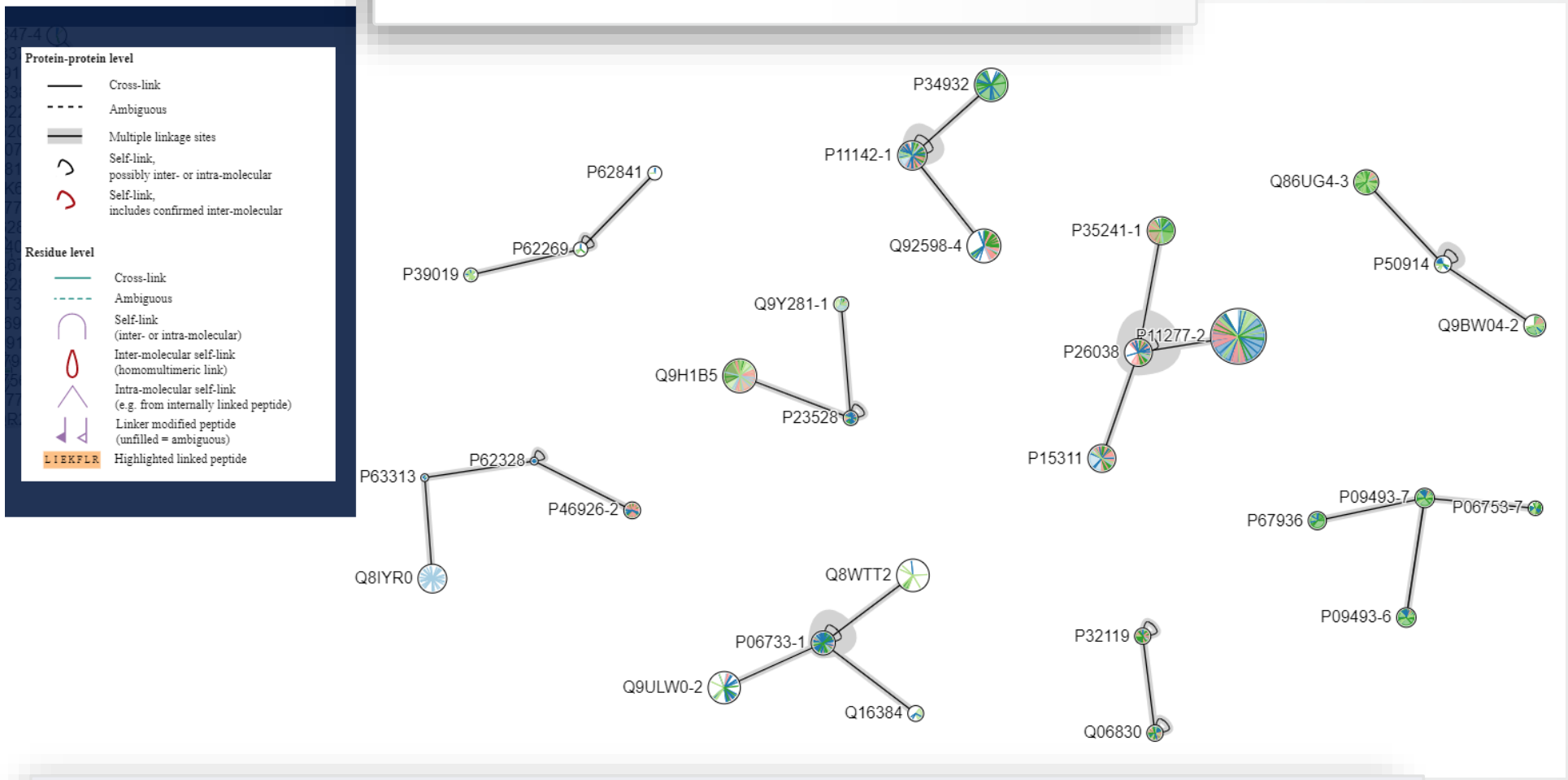
For further information on the file formats see:

- Cross-link CSV format
- FASTA files / protein IDs
- Annotations CSV format



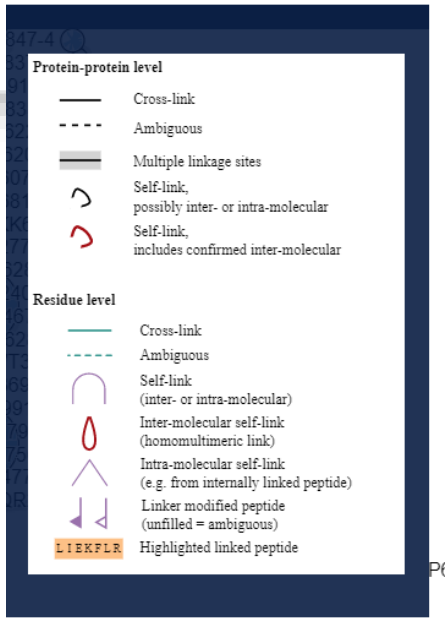
Visualization in xiNET

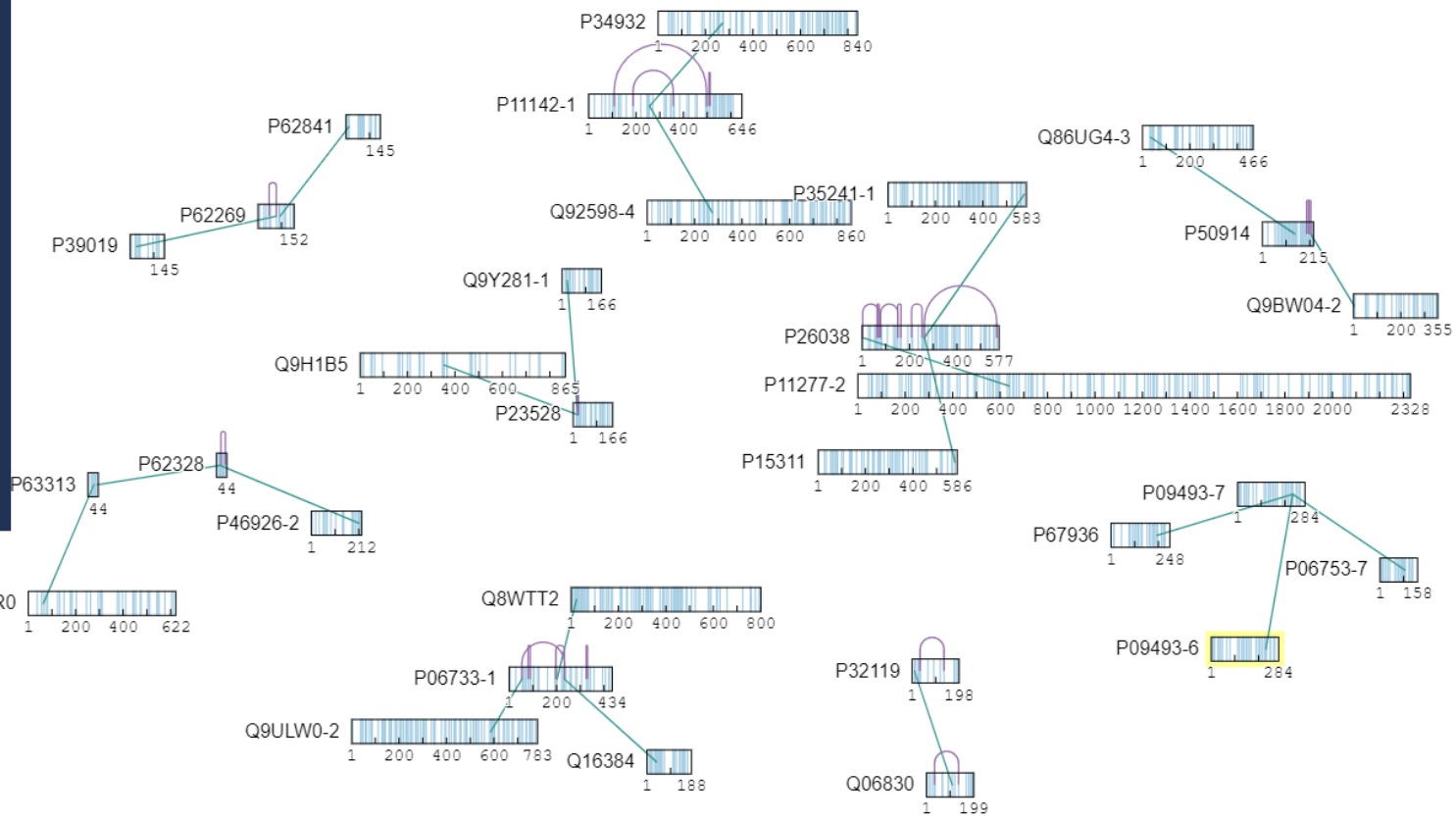
Displaying the crosslinks in xiNET

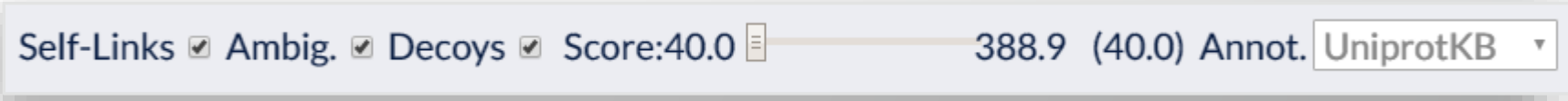


Visualization in xiNET

Expanding the proteins to show the linked positions (lysines) in the sequence.



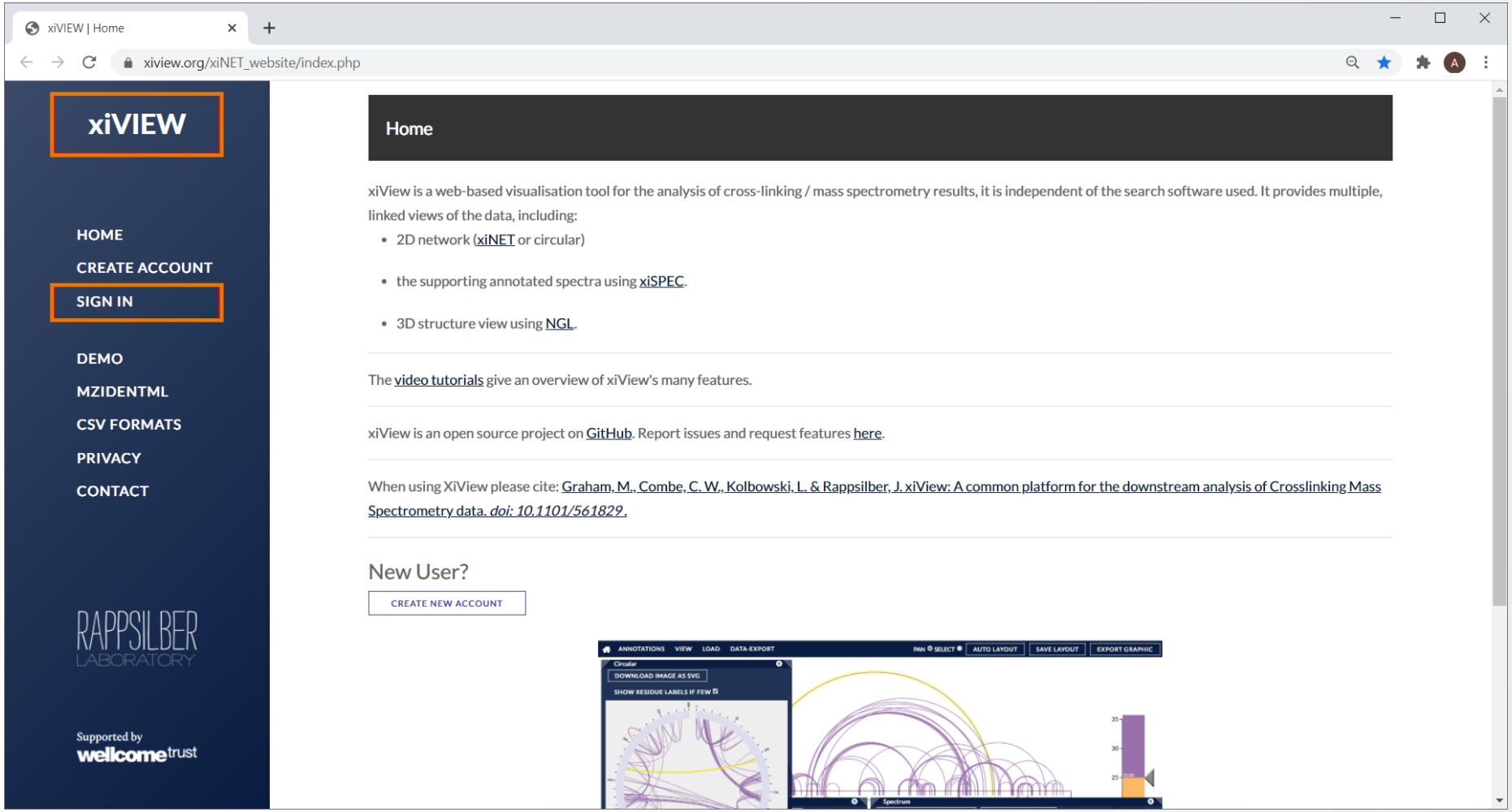




Step 10. Exporting crosslinks to xiVIEW

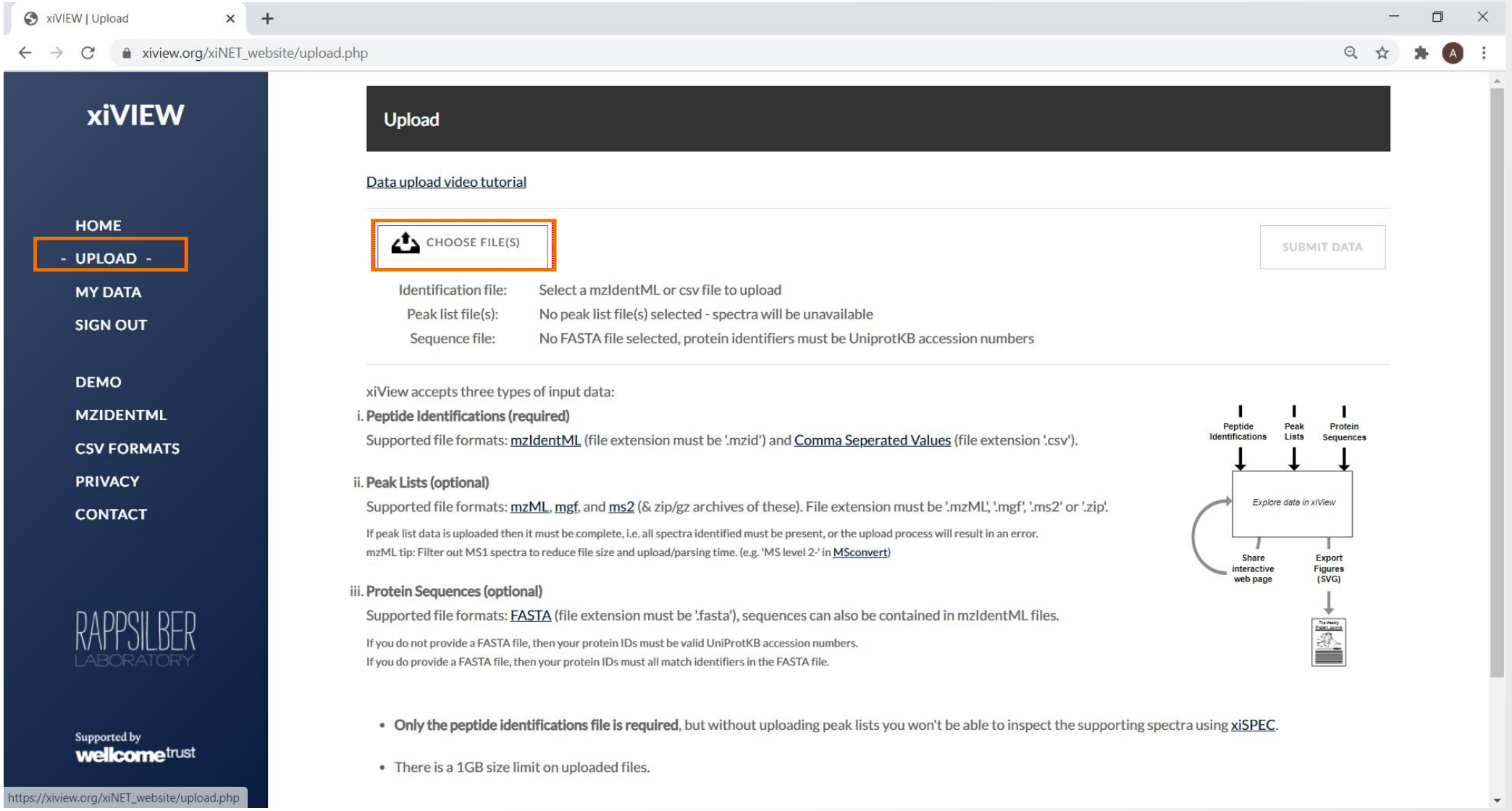
Access https://xiview.org/xiNET_website/index.php in Chrome

Sign into your account.

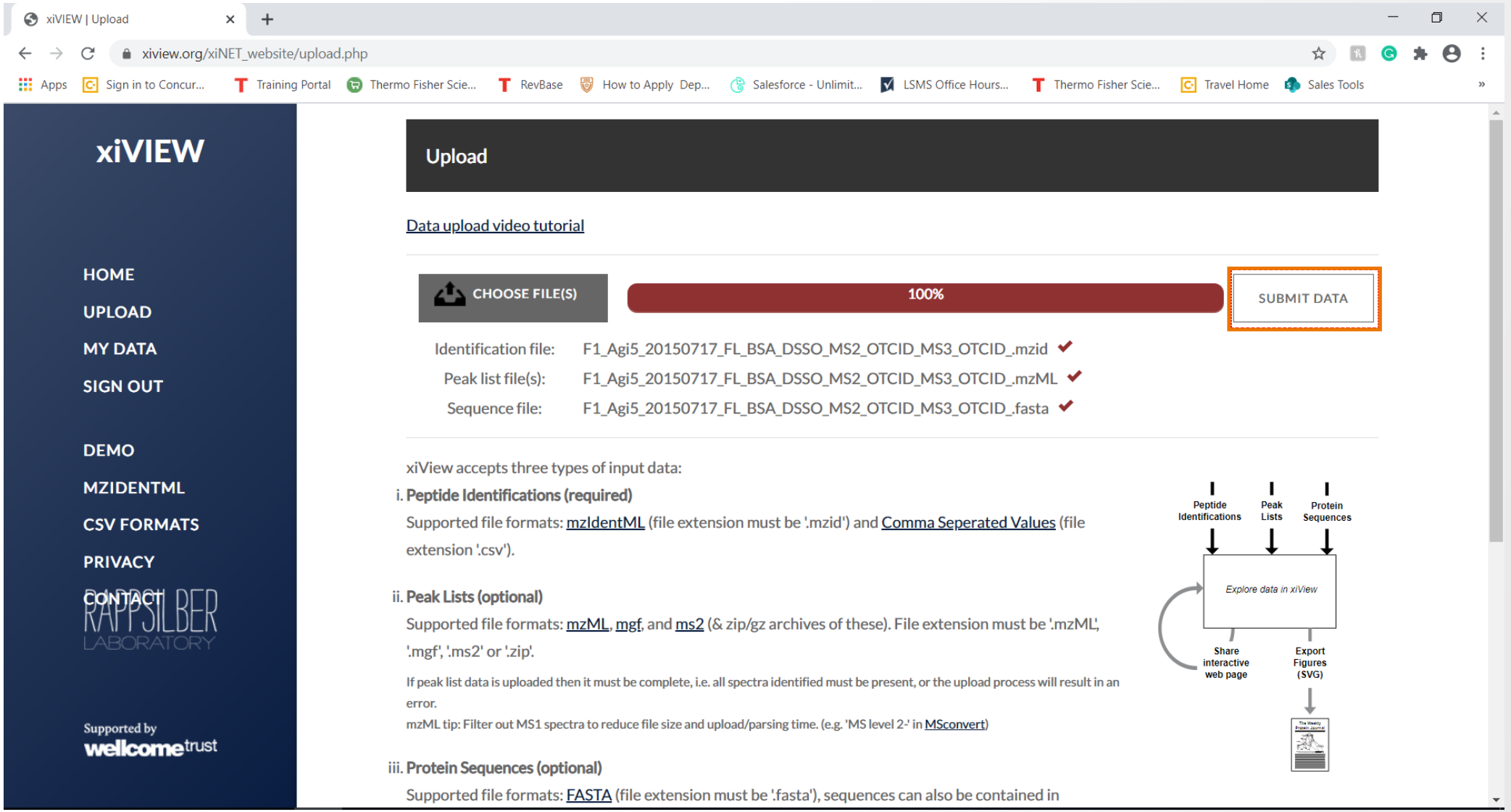


Navigate to the Upload page

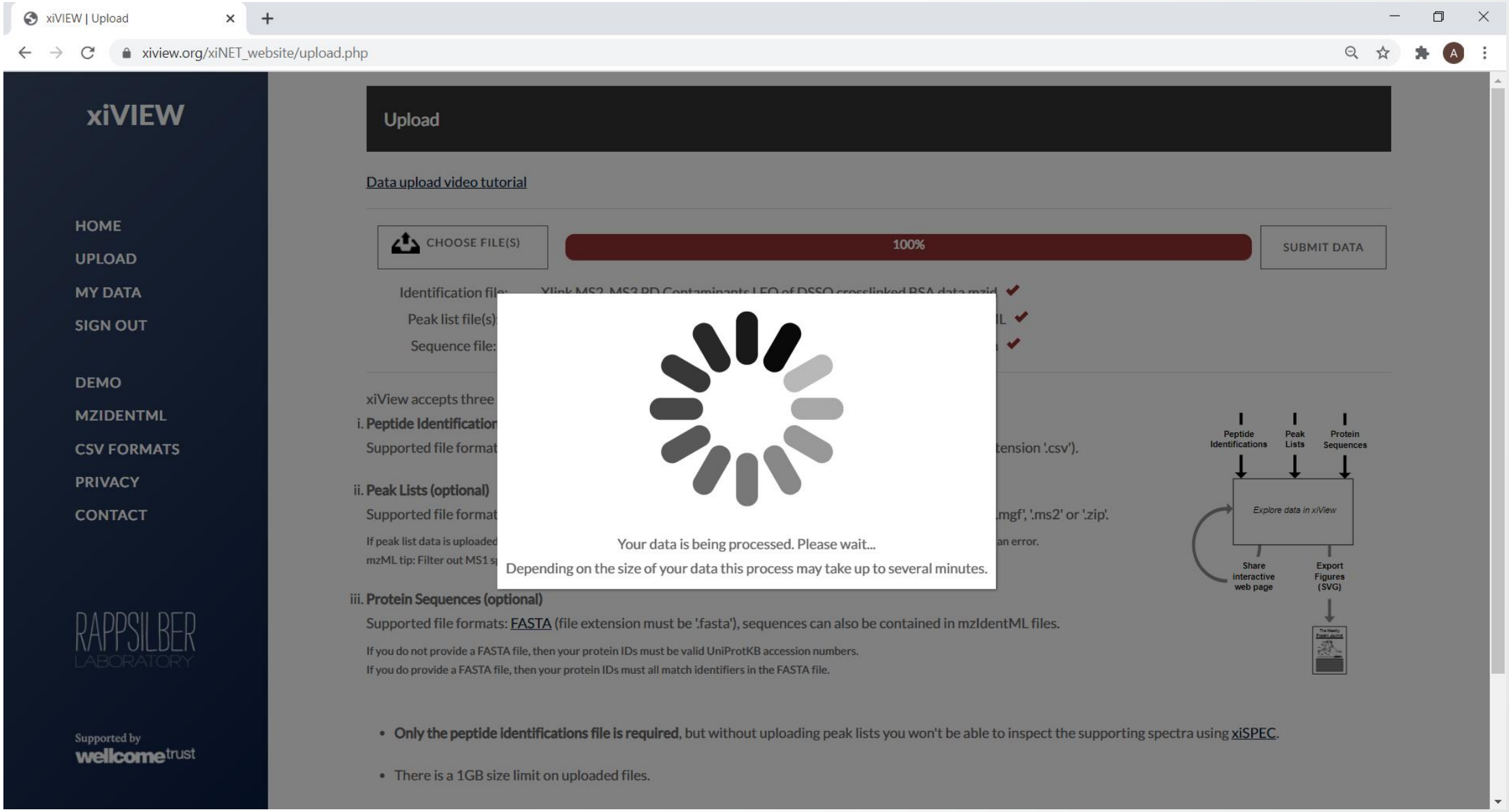
Choose files



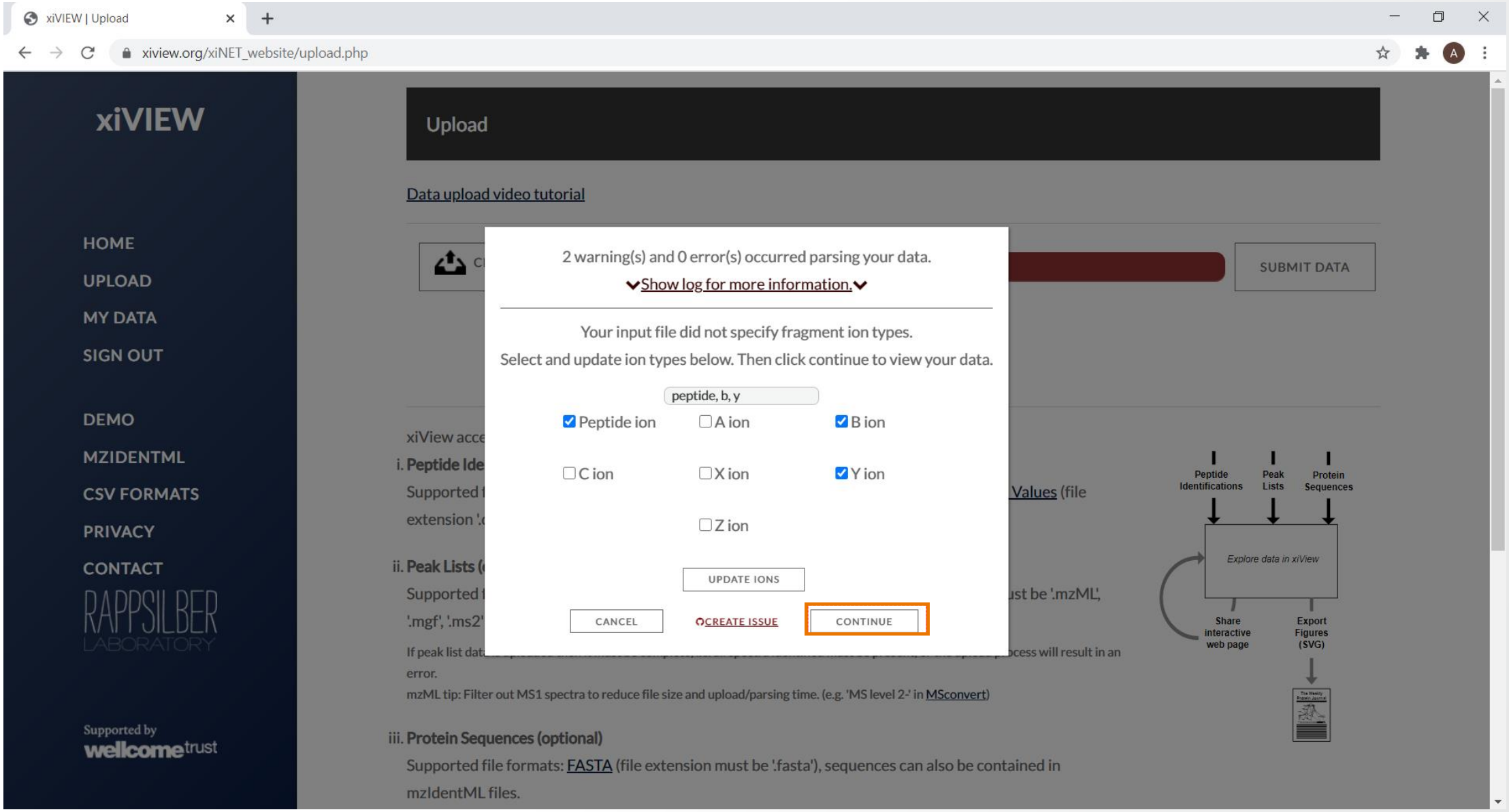
After the upload progress reaches 100%, submit the data



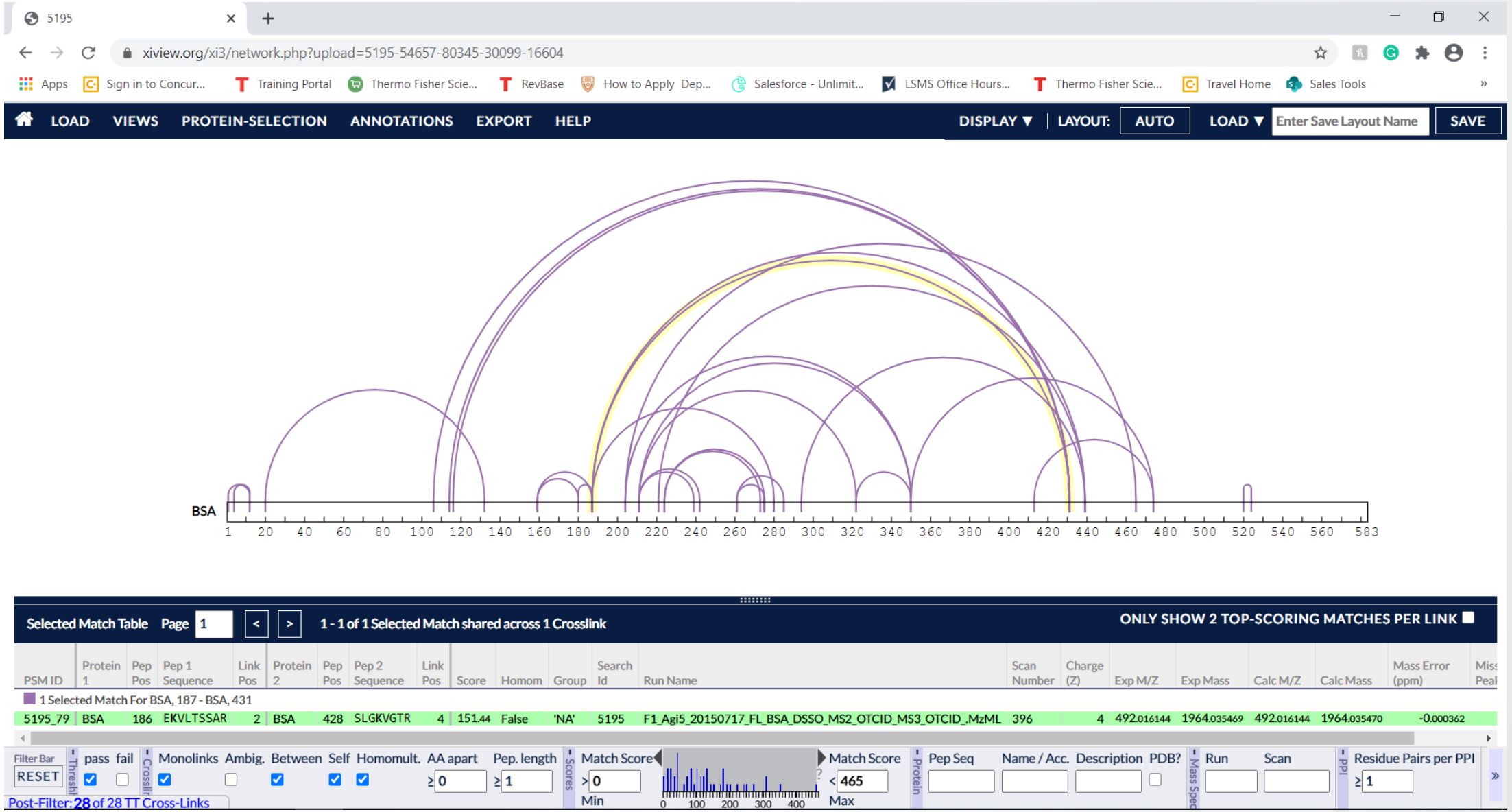
Depending upon file size, this process may take a little while…



If there are no errors, click ‘CONTINUE’

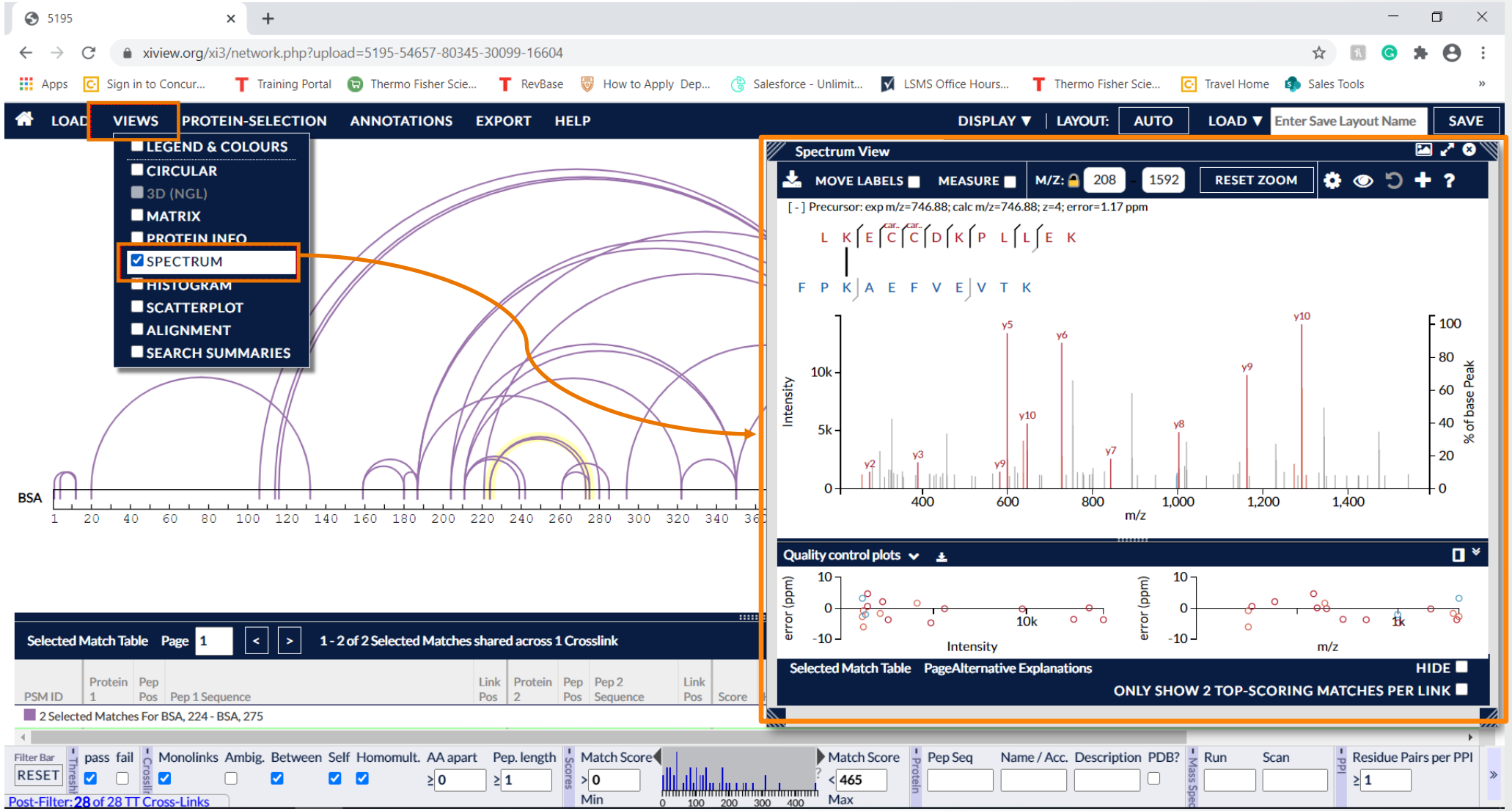


Viewing Your Data Online

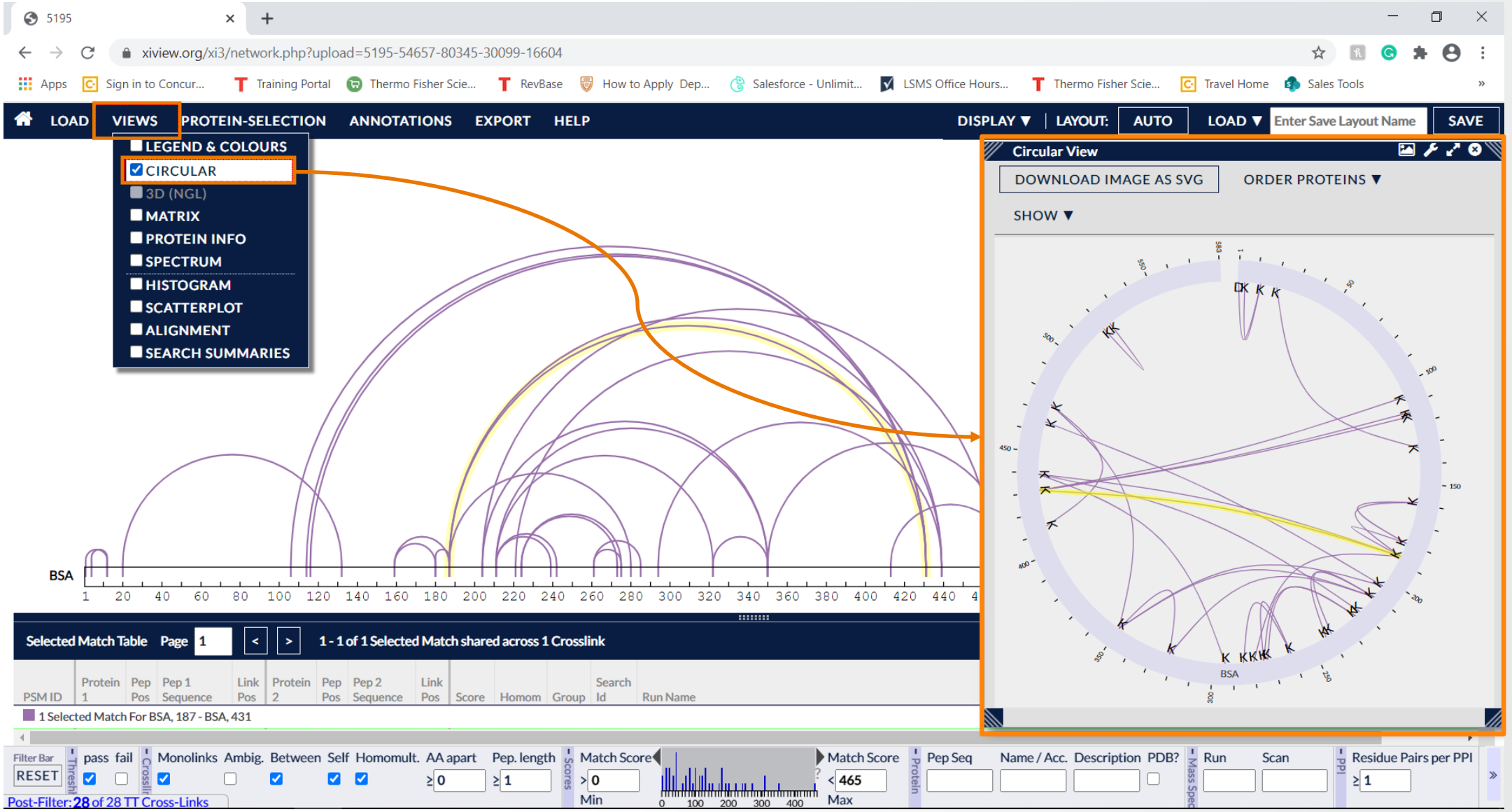


Re-arrange Proteins, Display Annotations and Views

Spectrum view



Circular View



The End