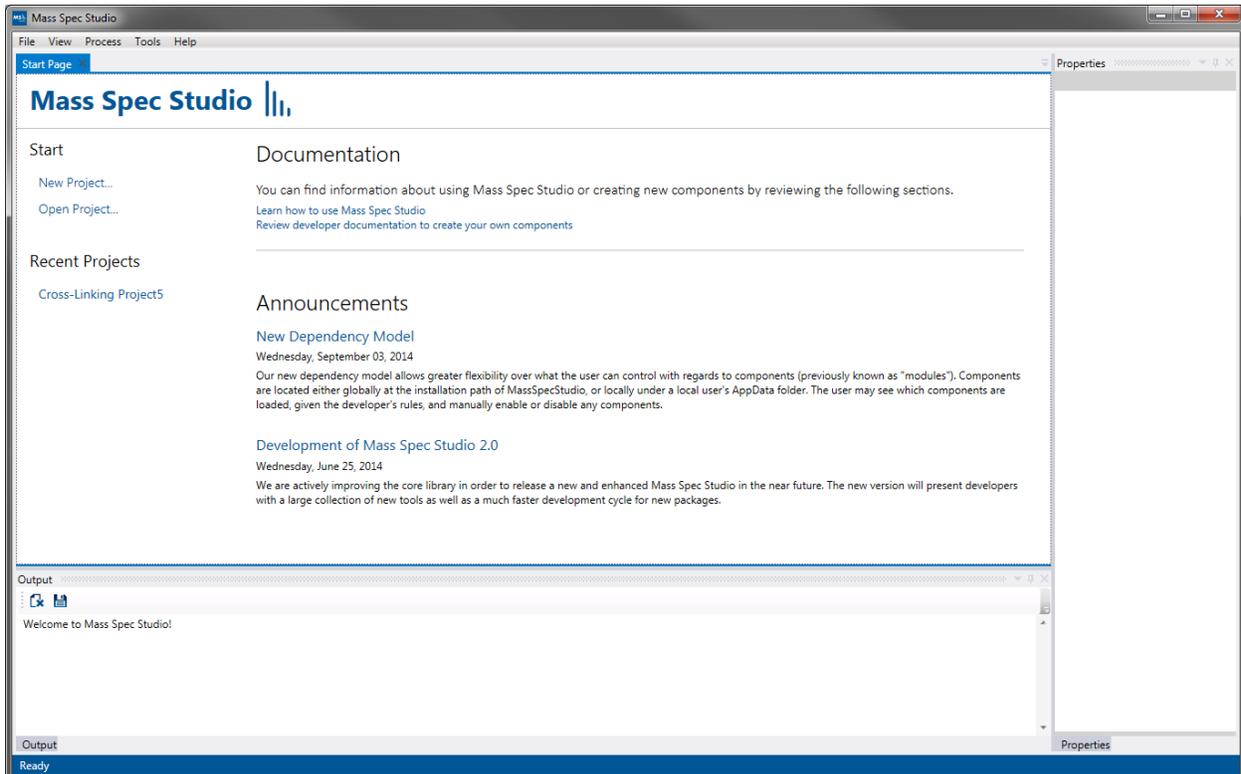


# Cross link analysis of LC-MS/MS data using Mass Spec Studio 2.3.0

*Contact: [dschriem@ucalgary.ca](mailto:dschriem@ucalgary.ca)*



## **Minimum requirements:**

1. Windows 7 or higher.
2. Dual Core processor.
3. 4 GB of RAM.
4. 10 GB of free disk space.

## **Download:**

Visit <http://www.msstudio.ca/downloads/crosslinking> to read the license agreement and download a copy of the software.

## **Pre-requisites:**

If you are analyzing original Thermo (.raw) data files, you need to install MSFileReader:  
<https://thermo.flexnetoperations.com/control/thmo/product?plneID=632401>.

If you don't have an account, you need to register here:  
<https://thermo.flexnetoperations.com/control/thmo/RegisterMemberToAccount>.

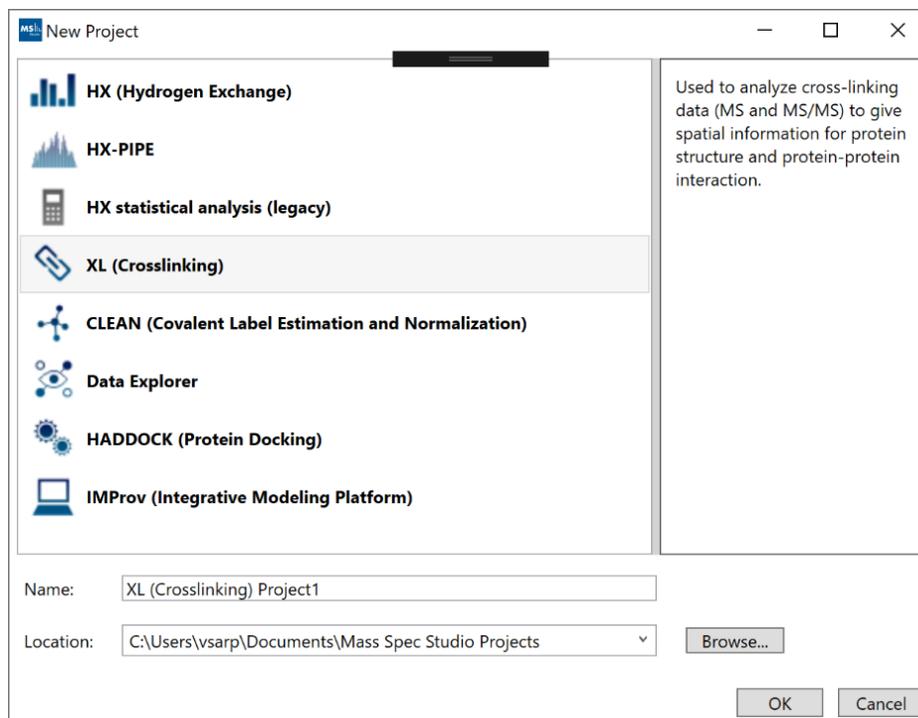
NOTE: If Mass Spec studio cannot find your Thermo .raw files, this means you do not have the 64-bit version of MSFileReader installed.

## **Installation:**

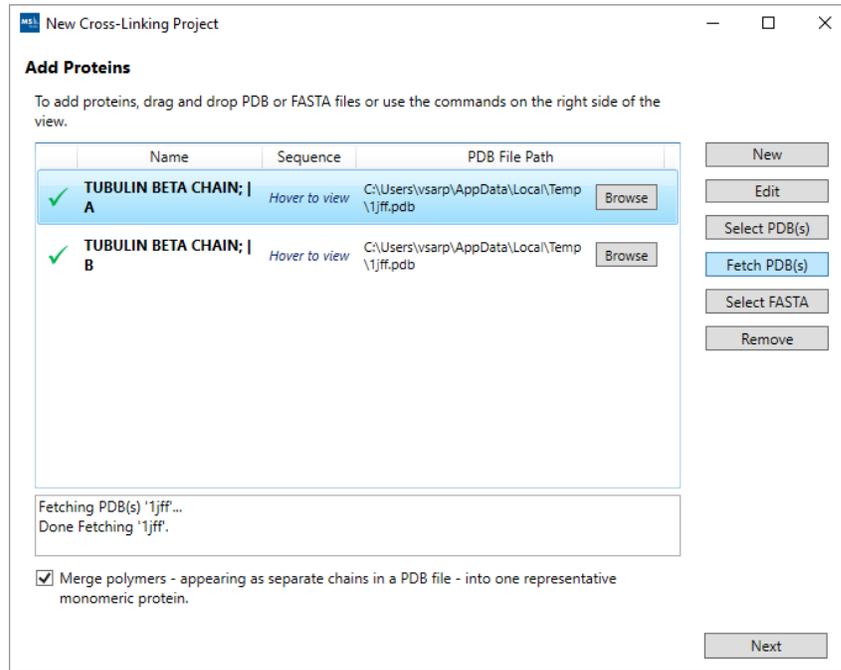
Run 'setup.exe' and follow the instructions to install the software.

## **Demo:**

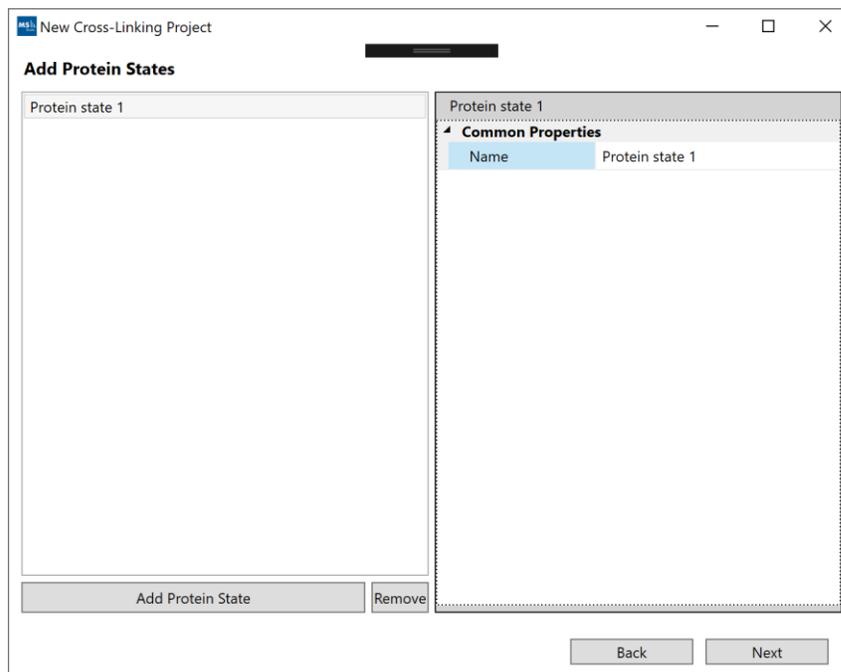
1. Download the demo data from <https://mssdownloads.s3-us-west-2.amazonaws.com/data/dss-demo.zip>.
2. Run Mass Spec Studio 2.3.0 and click on "New Project".
3. Select "Cross-Linking", give it a name and click "OK".



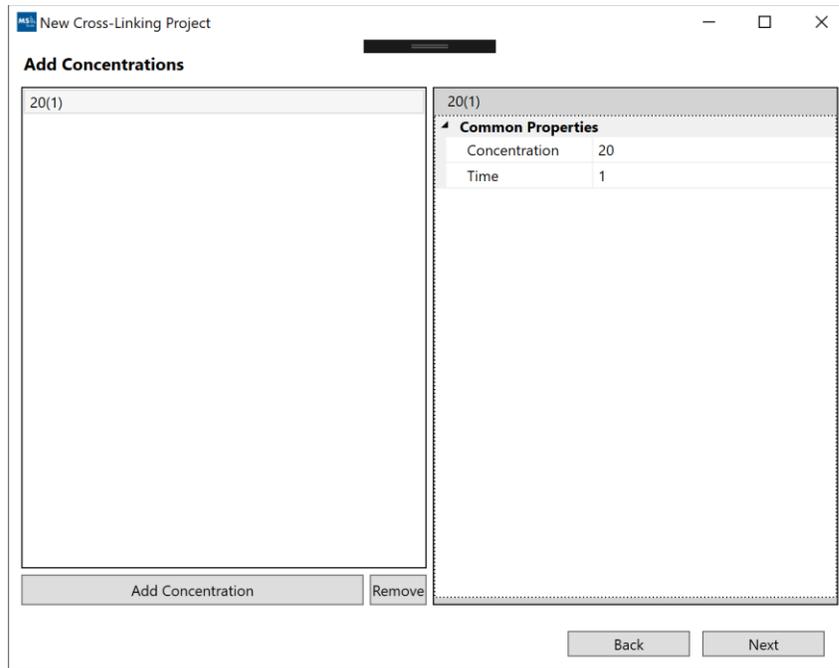
4. Add the protein system. For this demo, click "Fetch PDB(s)" and enter the code: **1jff**. Wait for the download to finish and click "Next".



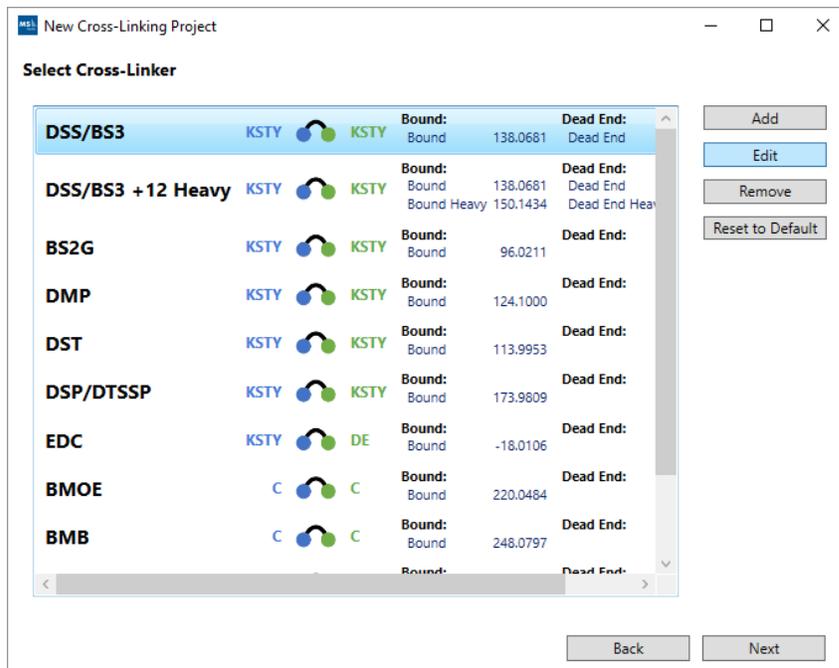
5. Add a protein state and name it anything. Protein states are used for separating searches/results for different experimental conditions. Our demo only uses a single run/condition.



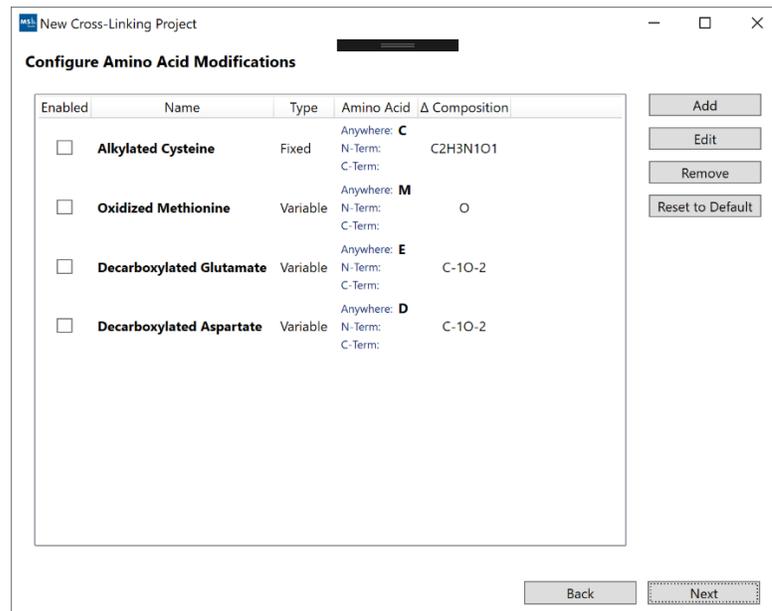
6. Add a concentration. Similar to protein states, concentrations are used to group runs for visualization purposes, but does not affect searching or export.



7. Select your cross-linker. For the demo, select **DSS/BS3** and click "Next":

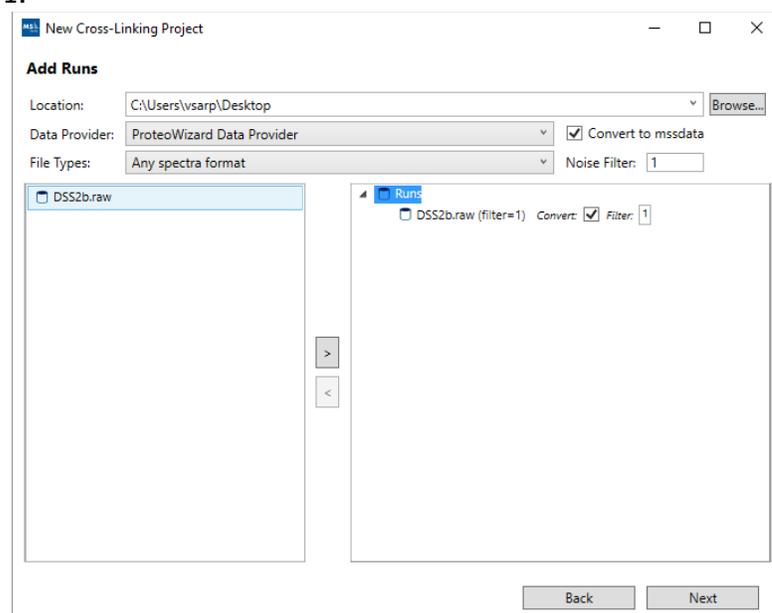


8. Select any post-translational modifications. For the demo, you can skip this step.

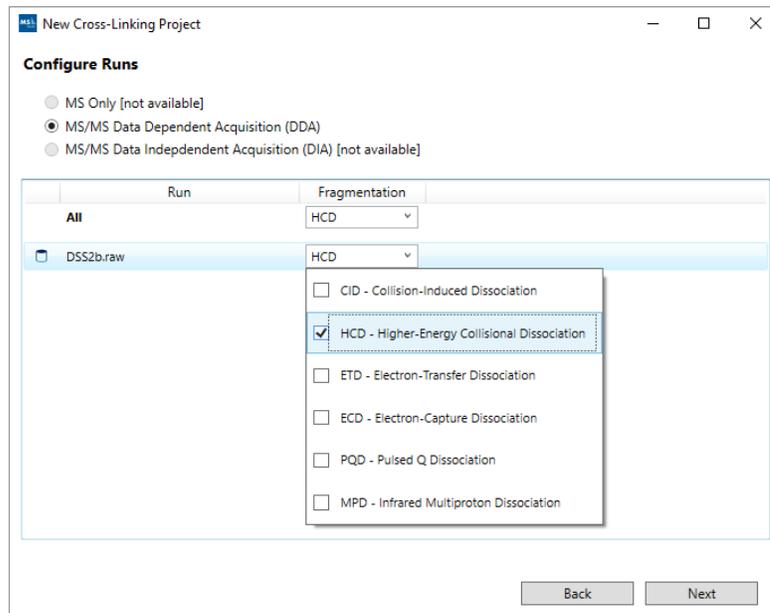


9. Add the data. For the demo, we will use **DSS2b.raw**.

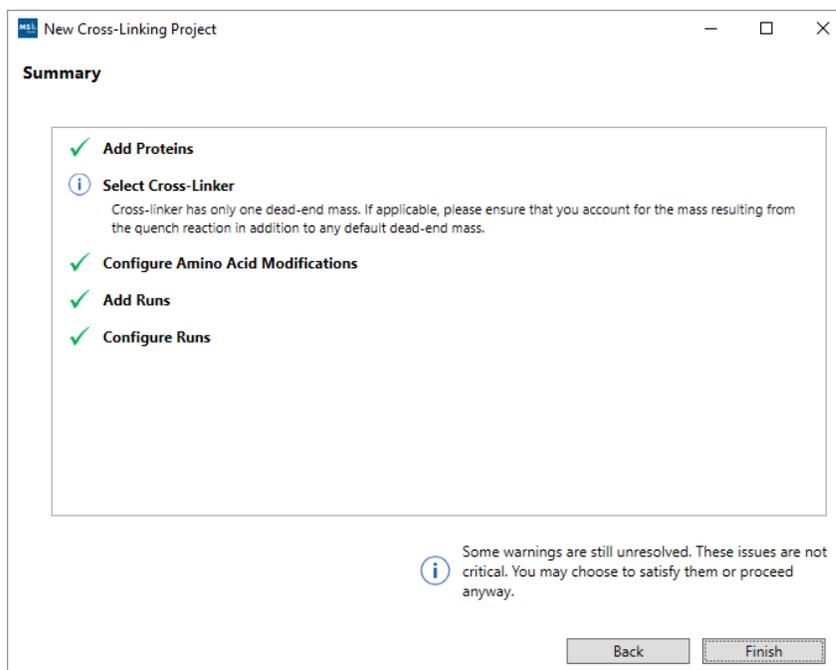
- Click "Browse" next to the Location field and find the folder which has the **DSS2b.raw** data file.
- Select "Thermo Data Provider" or "Proteowizard Data Provider" from the Data Provider drop down menu.
- Check ON the "Convert to mssdata" checkbox for faster processing.
- Select the **DSS2b.raw** file from the left view and click on the ">" button to add it under "Runs". Check to see that the convert option is enabled and the filter is set to 1.



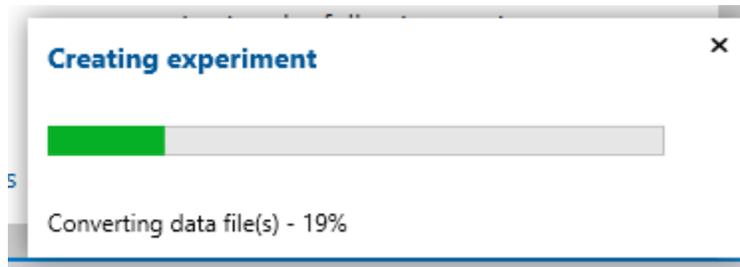
10. From fragmentation dropdown menu, check **ON** "HCD", check **OFF** "CID" and click "Next".



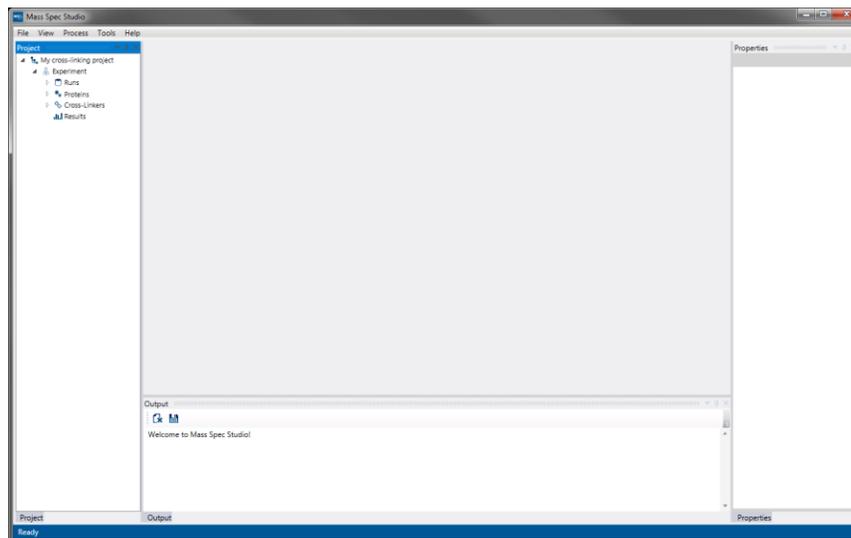
11. Verify that there are no errors and click "Finish" to create your project. The information for "Select Cross-Linker" is OK. Sometimes when using specific quenching reagents, one can have dead-end masses in addition to the default ones. Ignore this for now:



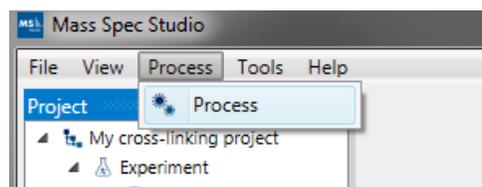
12. Wait for Mass Spec Studio to finish converting the data files and set up the experiment. This could take a few minutes for large files:



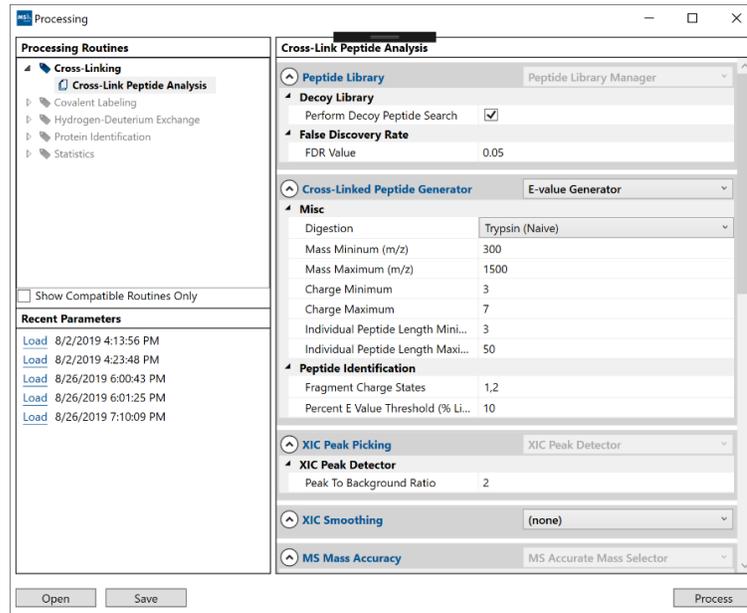
13. Your project will be saved under "**C:\Users\<you>\My Documents\Mass Spec Studio Projects**". Raw data files are copied and archived inside the project file structure for sharing and future retrieval.
14. After project creation is complete, you will see an empty project template. From here, you can navigate the left hand side "Project" view to explore your project setup:



15. Process the data. From the main menu -> Process, click "Process":

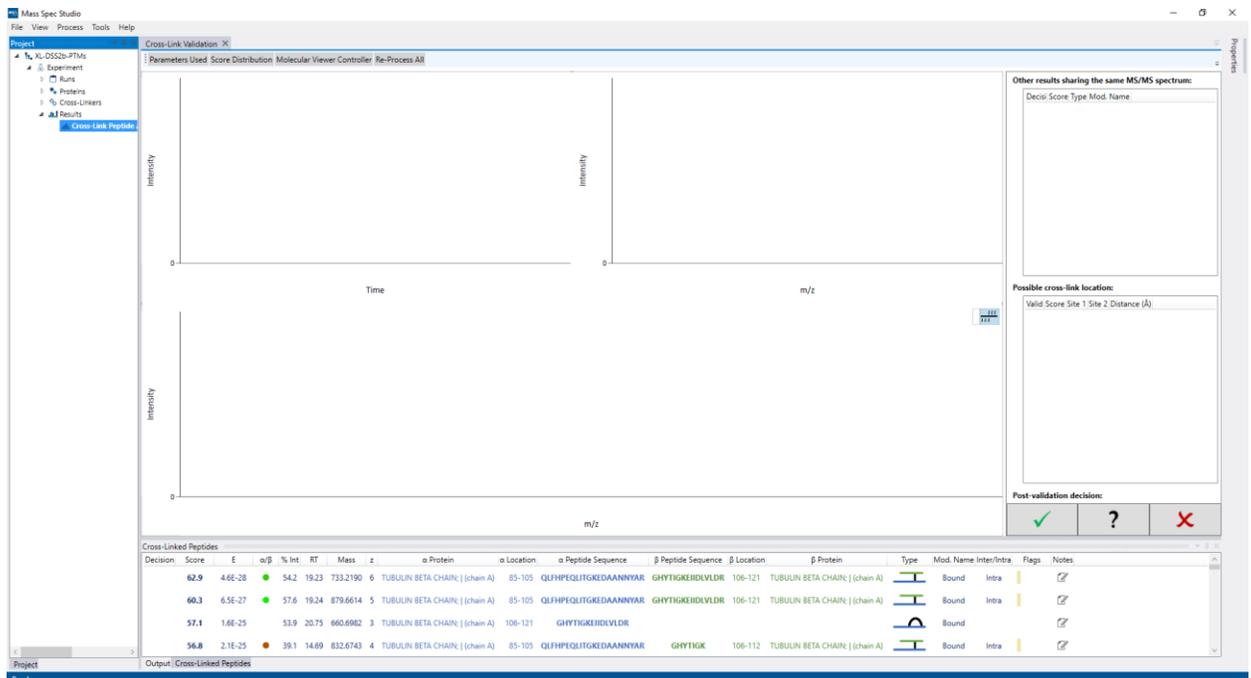


16. Select “**Cross-Link Peptide Analysis**”. Adjust your processing parameters. For the demo, leave everything default and click “Process”:

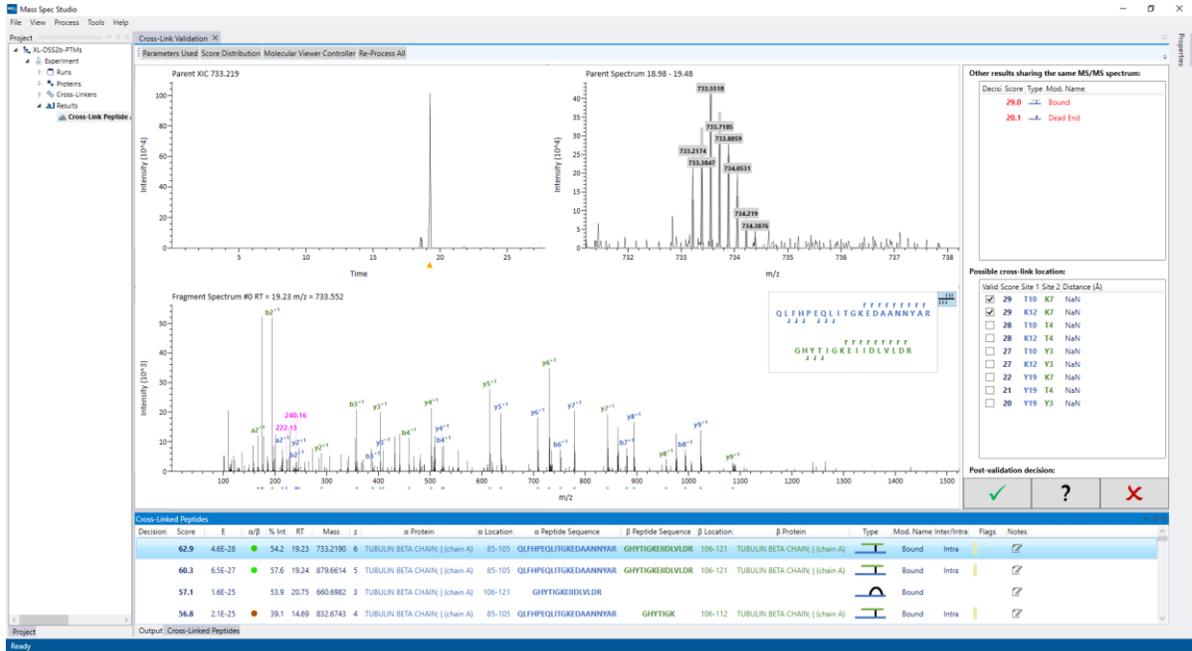


**Note:** Since version 2.2.0, the mass tolerance value represents single-side variance relative to a peak m/z. For example, 20PPM means plus or minus 20PPM on each side of a peak.

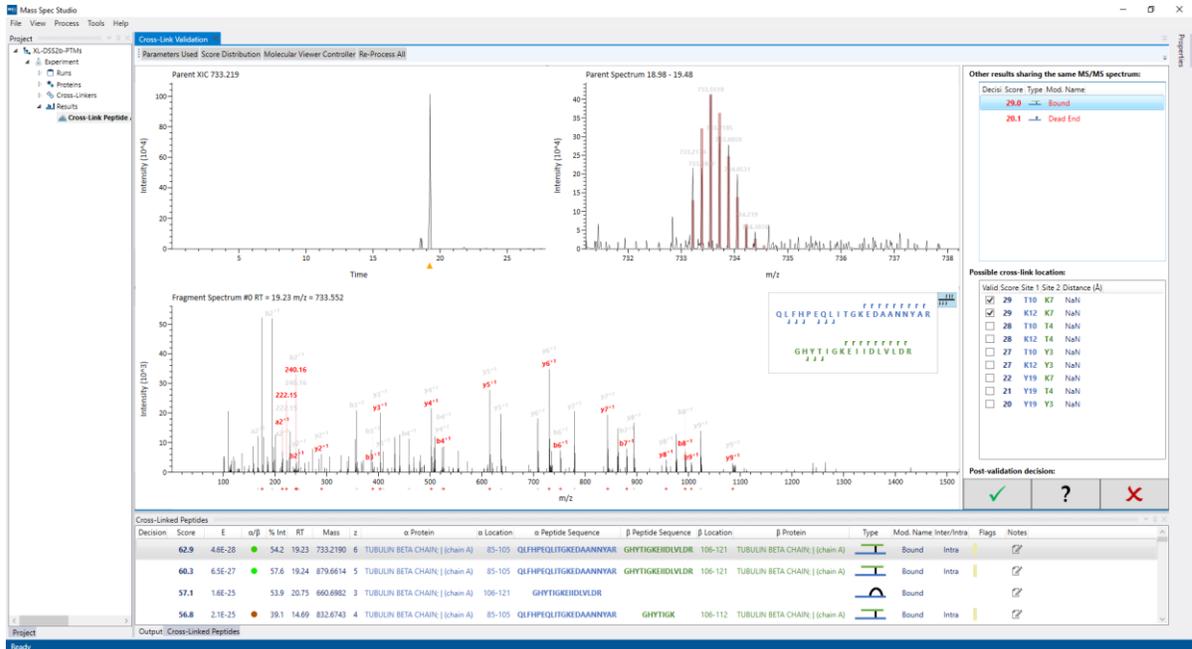
17. Once the processing is complete, you will see the validation viewer automatically opened for the result. The results from each processing run can be found under the “Results” tree section on the left hand side “Project” view:



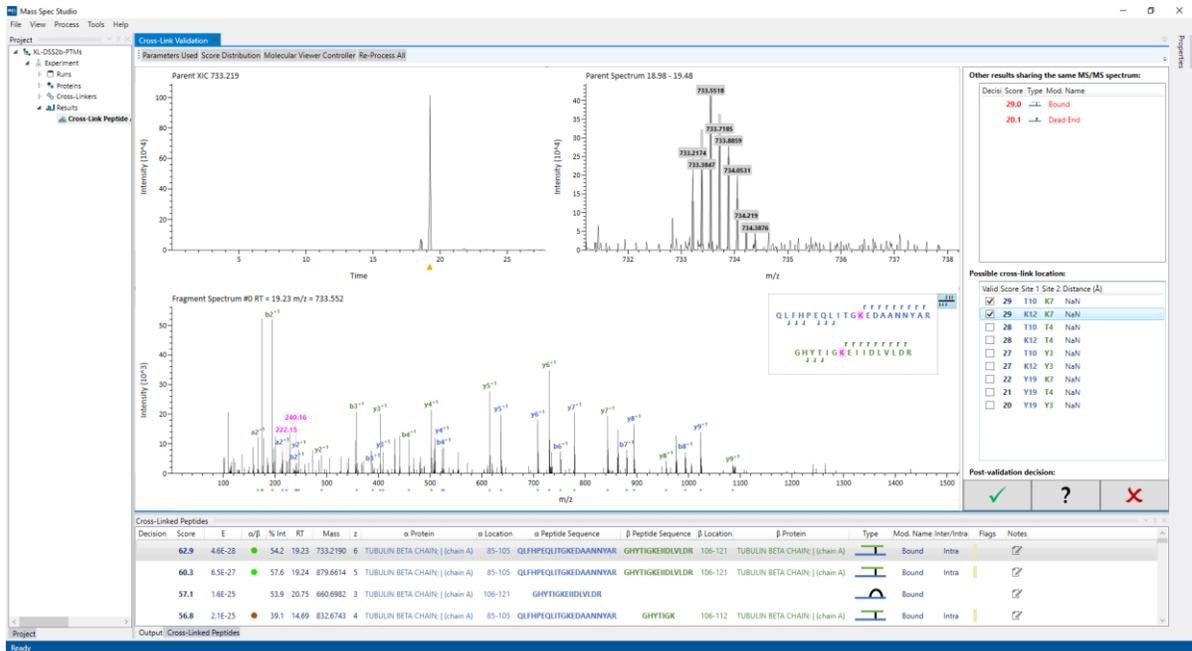
18. Select candidate peptides from the bottom "Cross-Linked Peptides" table to view and validate their site:



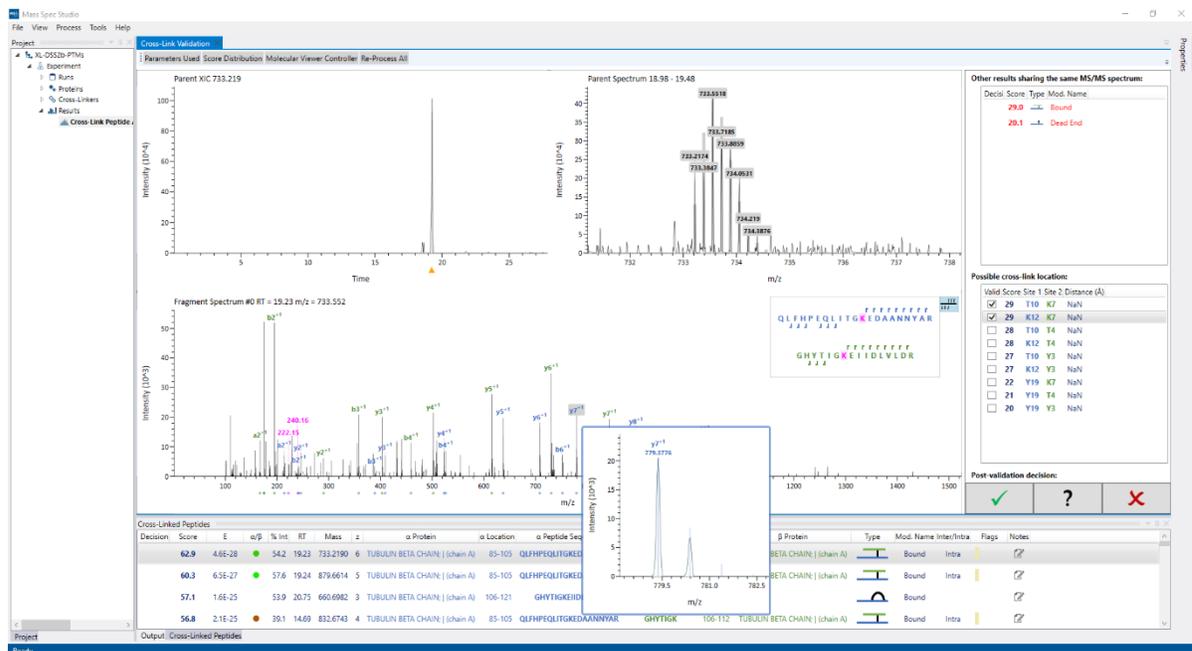
19. Click on overlapping results under the "Other results sharing the same MS/MS spectrum" view on the right hand side to overlay their data on your current view. Click away to dismiss it and view the original candidate again.



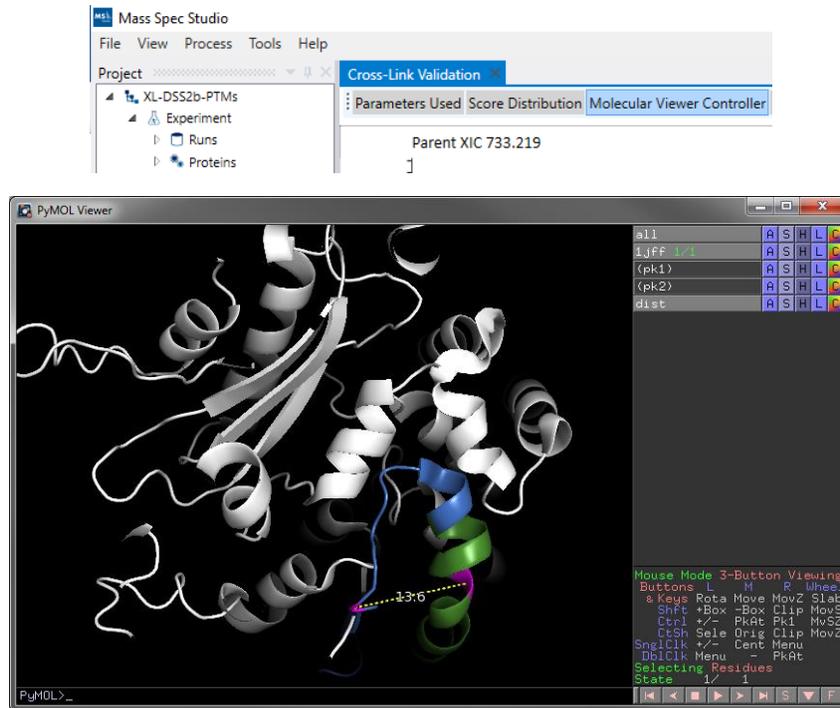
20. Click on a site under the "Possible cross-link location" view on the right side to highlight the site as well as the fragments for that selection. Fragments which disagree are shown in grey:



21. Hover over any fragment label to magnify its raw data and evaluate its fit with the expected profile.



22. If you have PyMOL installed, you can view the data live by clicking on the “Molecular Viewer Controller” button in the toolbar and click “OK” in the window popup. Selecting candidates or cross-link sites will update the molecular viewer live:



23. Accept or Reject a candidate and its sites via the buttons on the right side:

Parent XIC 733.219

Parent Spectrum 18.08 - 19.48

Fragment Spectrum #0 RT = 19.23 m/z = 733.552

Other results sharing the same MS/MS spectrum:

- 29.0 → Bound
- 20.1 → Dead End

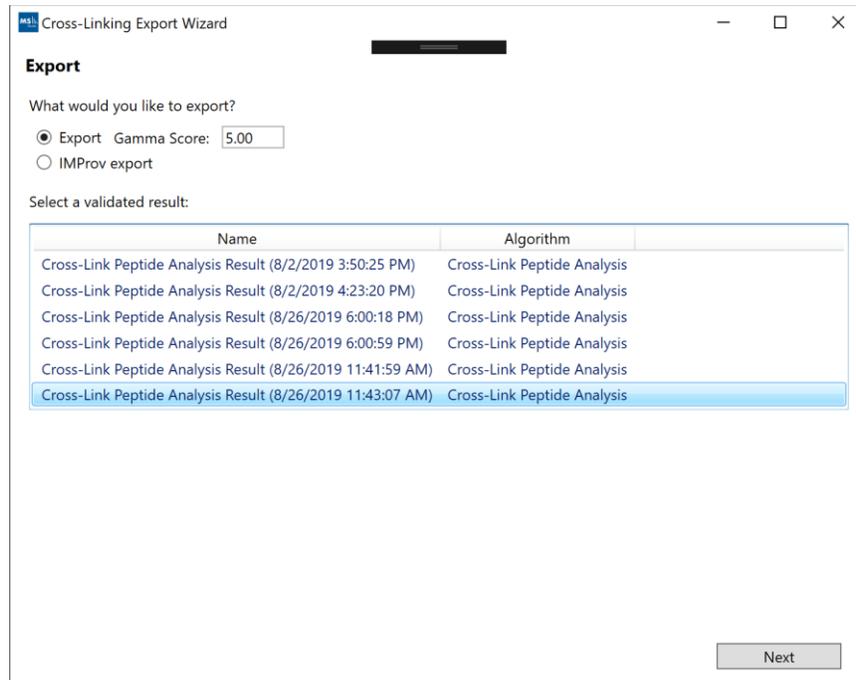
Possible cross-link location:

Valid Score	Site 1	Site 2	Distance (Å)
<input checked="" type="checkbox"/>	29	T10 K7	NaN
<input checked="" type="checkbox"/>	29	K12 K7	NaN
<input type="checkbox"/>	28	T10 T4	NaN
<input type="checkbox"/>	28	K12 T4	NaN
<input type="checkbox"/>	27	T10 Y3	NaN
<input type="checkbox"/>	27	K12 Y3	NaN
<input type="checkbox"/>	22	Y10 K7	NaN
<input type="checkbox"/>	21	Y10 T4	NaN
<input type="checkbox"/>	20	Y10 Y3	NaN

Decision	Score	E	ω/B	% Int	RT	Mass	z	α Protein	α Location	α Peptide Sequence	β Peptide Sequence	β Location	β Protein	Type	Mod. Name	Inter/Intra	Flags	Notes
✓	62.9	4.6E-28	54.2	19.23	733.2190	6		TUBULIN BETA CHAIN   (chain A)	85-105	QLFHPQLITGKDAANNYAR	GHYTYGKERIDVLDLR	106-121	TUBULIN BETA CHAIN   (chain A)	Bound	Intra			
✓	60.3	6.5E-27	57.6	19.24	879.6614	5		TUBULIN BETA CHAIN   (chain A)	85-105	QLFHPQLITGKDAANNYAR	GHYTYGKERIDVLDLR	106-121	TUBULIN BETA CHAIN   (chain A)	Bound	Intra			
?	57.1	1.6E-25	53.9	20.75	660.6962	3		TUBULIN BETA CHAIN   (chain A)	106-121	GHYTYGKERIDVLDLR								
✗	56.8	2.1E-25	39.1	14.69	832.6743	4		TUBULIN BETA CHAIN   (chain A)	85-105	QLFHPQLITGKDAANNYAR	GHYTYK	106-112	TUBULIN BETA CHAIN   (chain A)	Bound	Intra			

Post-validation decision: ✓ ? ✗

24. Export the cross-linked peptides or cross-link sites for by going to the menu -> File and clicking on "Export". Select the first "Export" option.



**Note:** Gamma score is a filtering mechanism for CrossLink sites where for each crosslinked peptide, only sites with scores  $> (\text{Max Score} - \text{Gamma})$  are exported, unless they are manually modified by the user. In the case of the latter, manual user decisions will override the Gamma score.