Tutorial for the XLink PRM Skyline Plugin

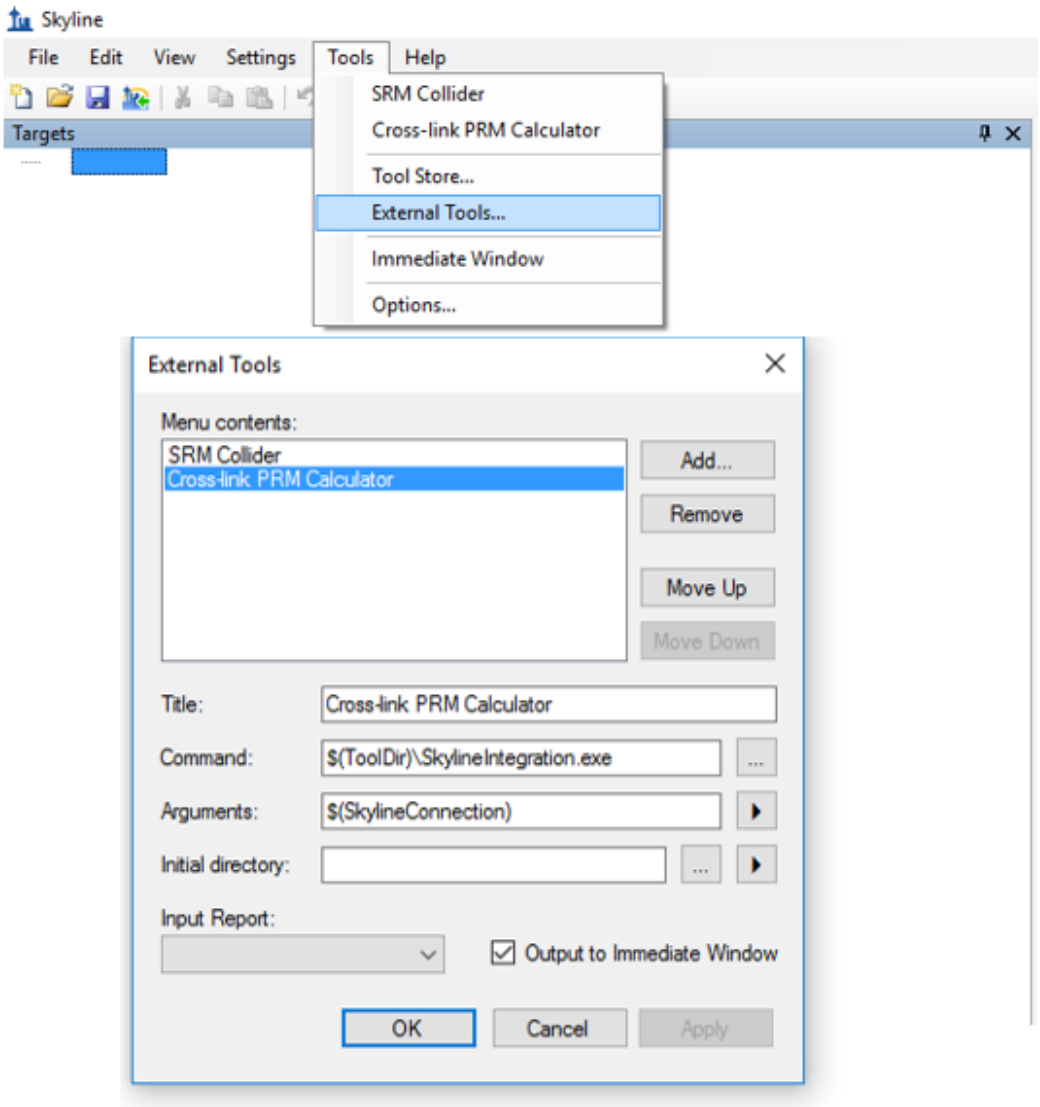
Developed by Jimmy K. Eng, Devin K. Schweppe, Juan D. Chavez, and James E. Bruce.
Implemented as a Skyline plugin by Yuval Boss.

Program Goal:

The Cross-link (XLink) PRM transition calculator generates parallel reaction monitoring (PRM) transitions for cross-linked peptides in Skyline. The calculator is compatible with both cleavable and non-cleavable cross-linkers.

Installation:

The XLink PRM transition calculator plugin for Skyline can be installed through the Skyline tool store interface. Tools -> Tool Store -> XLink PRM. After downloading the zip file to your computer, it can be installed within Skyline as follows: Tools -> External Tools -> XLink PRM ->Add and select downloaded zip file.



Use of the XLink PRM calculator:

Cross-linked peptide information is input as tab delimited text file. The format of the input files is as follows:

```
neutral_reporter_mass
peptideA  peptideB  modificationA  modificationB  precursor_charge
peptideA  peptideB  modificationA  modificationB  precursor_charge
peptideA  peptideB  modificationA  modificationB  precursor_charge
```

A cleavable cross-link example:

```
751.40508
QKAEADKNDK  QKAEADKNDK   197.03@2  197.03@2   4
MEESKAK     KVEKVTISNR   197.03@5  197.03@4   4
MKETQK      IMKAQALR     197.03@2  197.03@3   4
IMKAQALR    KVEKVTISNR   15.99@2,197.03@3  197.03@4 4
```

The first line of the input file is the reporter ion neutral mass of the cleavable cross-linker. For PIR, this would be 751.40508 Da and for DSSO, the reporter neutral mass would be 47.96699 Da. For a non-cleavable cross-linker, this mass should be set to 0.0 (zero). The format of the modification string is “mass@residue_position”. The modification mass is the mass added from the modification to the mass of the respective residue. In the above example, the PIR cross-linker attaches to lysine residues and after dissociation leaves a stump mass of 197.032422 Da on the lysine residues. Once loaded into Skyline the modification mass will be displayed as the total mass of the cross-linker modification plus the residue mass 325.13 in this case mass is the mass of lysine (128.094963 Da) plus the cross-link stump mass (197.032422 Da). The string “325.13@2” specifies the 2nd residue has a total modification mass of 325.13 Da. Additionally, the last entry above shows a modification string “15.99@2” which corresponds to oxidation of methionine on the second residue of the first peptide.

Here is an example of an input file for two peptides linked with DSS non-cleavable cross-linker with a linker mass of 138.07 Da and a precursor charge state of 6:

```
0.0
DFNKVPNFSIR  IVQSKSGLNMENLANHEHLLSPVR  2823.47@4  1473.76@5  6
```

The “0.0” on the first line indicates a non-cleavable cross-linker. The modification mass is the new mass of the modified residue. In the case of this cross-link, 2823.47@4 corresponds to the modification of the 4th residue lysine where 2823.47 is the mass of the peptideB (2685.402068) plus mass of the cross-linker (138.07). “1473.76@5” is derived as the mass of peptideA (1335.693535) plus mass of cross-linker (138.07).

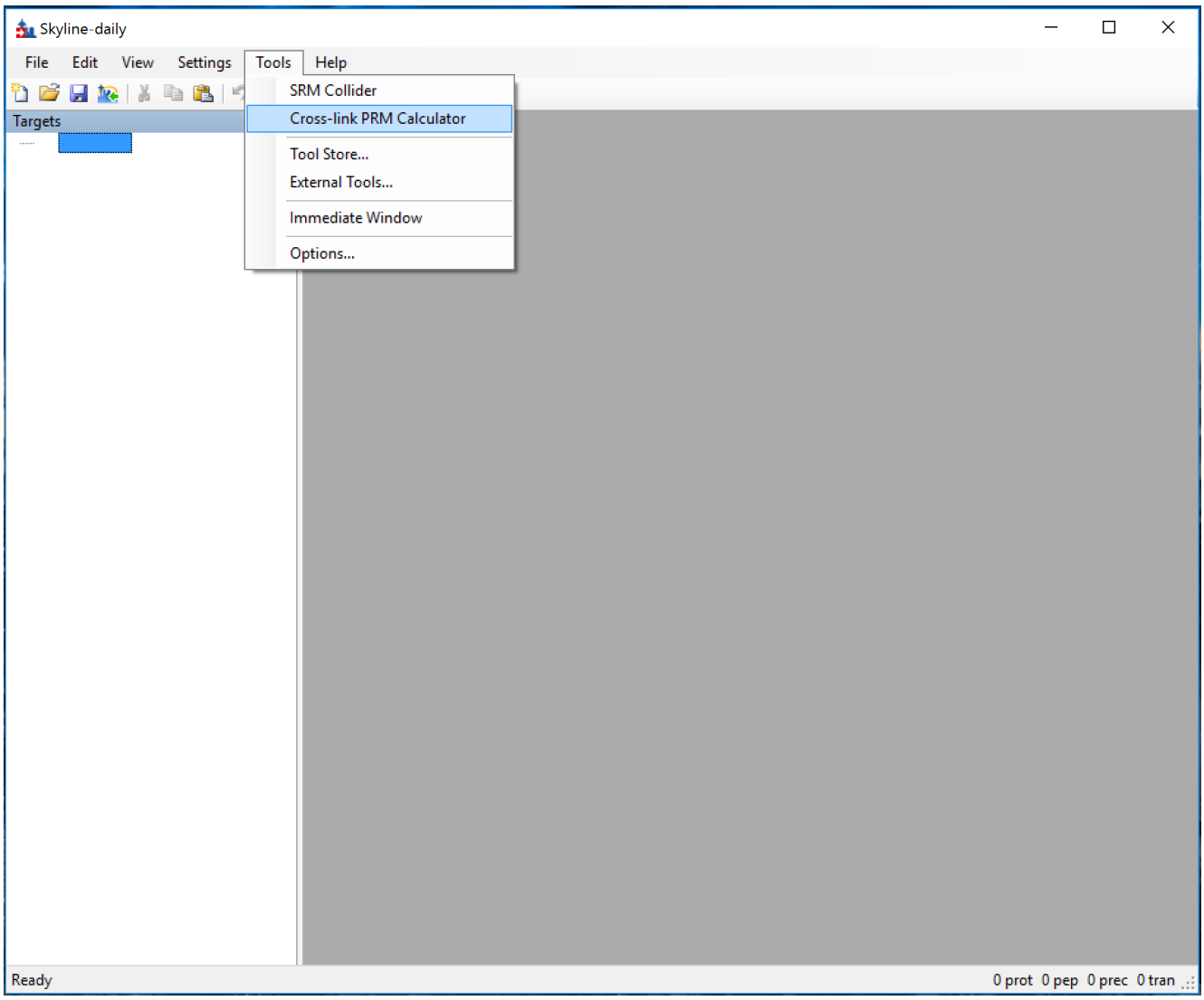
When a non-cleavable cross-linker is used, the masses that are calculated and exported as PRM transitions are:

- Intact precursor m/z for all charge states from 1 to precursor_charge
- b- and y-ions for both peptides, from charges 1 to 3

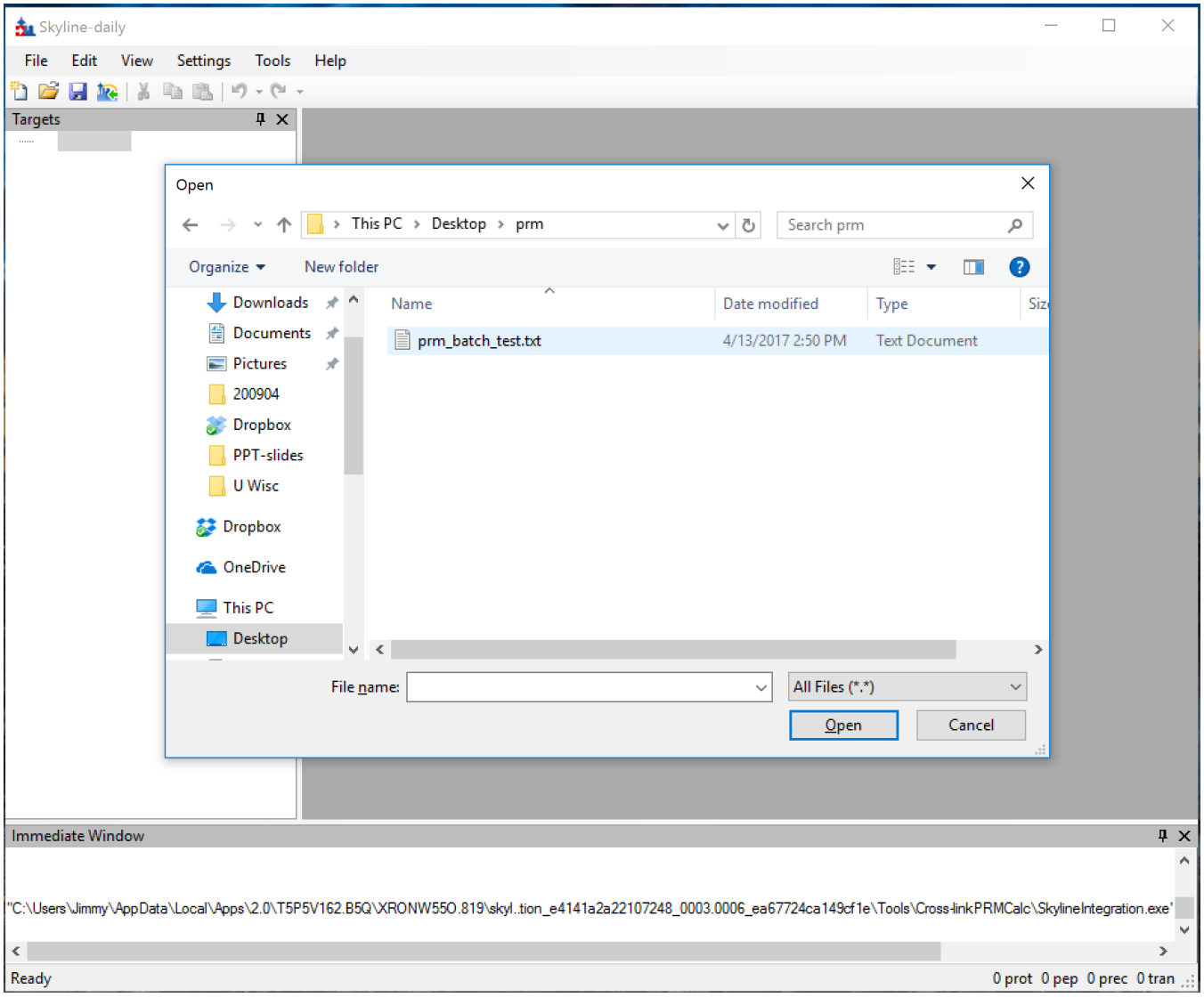
For cleavable cross-linkers, the calculated masses are:

- released precursor m/z for charge states 1 and 2 for each released peptide
- long arm m/z for charge states 1 to 3 for each released peptide. The long arm is an ion resulting from cleavage of a single cleavable bond within the cross-linker resulting in a peptide with the reporter and stump portions of the cross-linker attached.
- b- and y-ions for both peptides of charge 1+

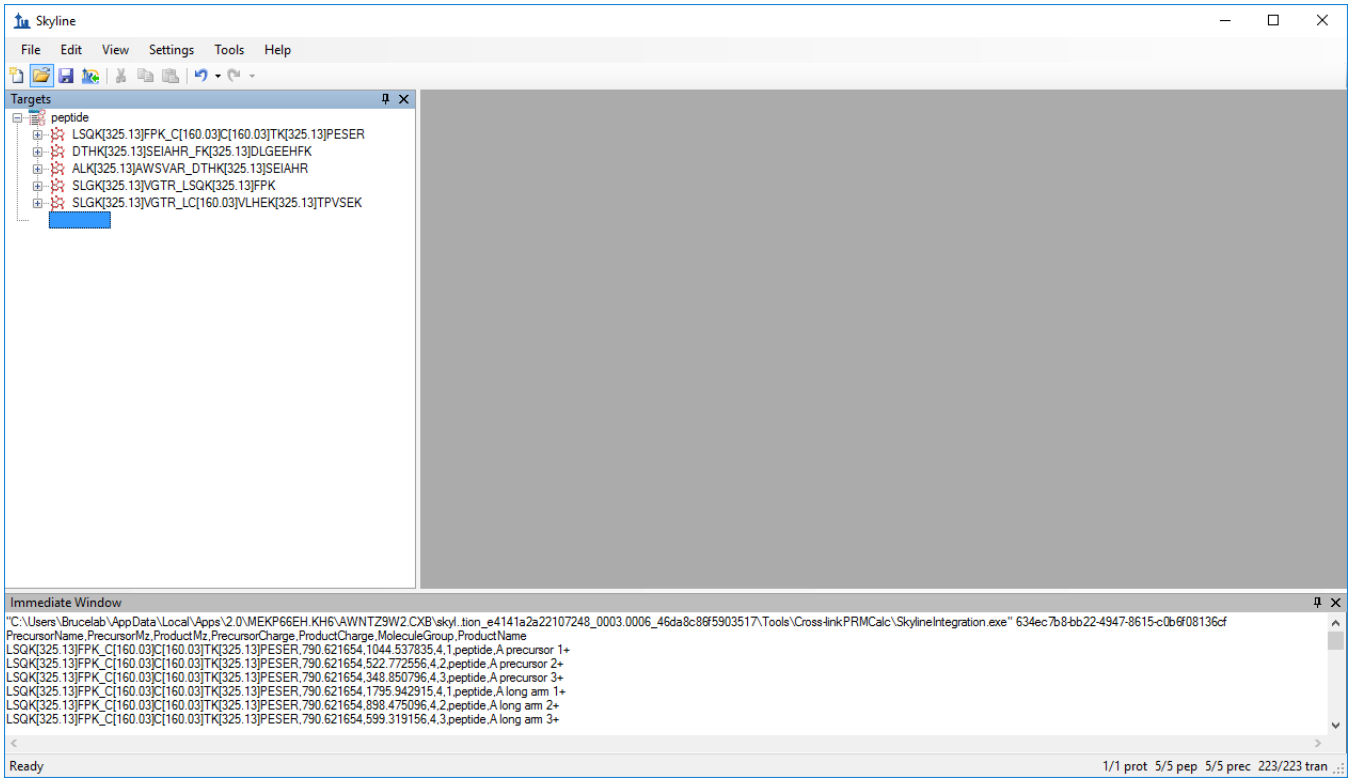
To run this plug-in in Skyline, select the “Cross-link PRM Calculator” menu item from the “Tools” menu.



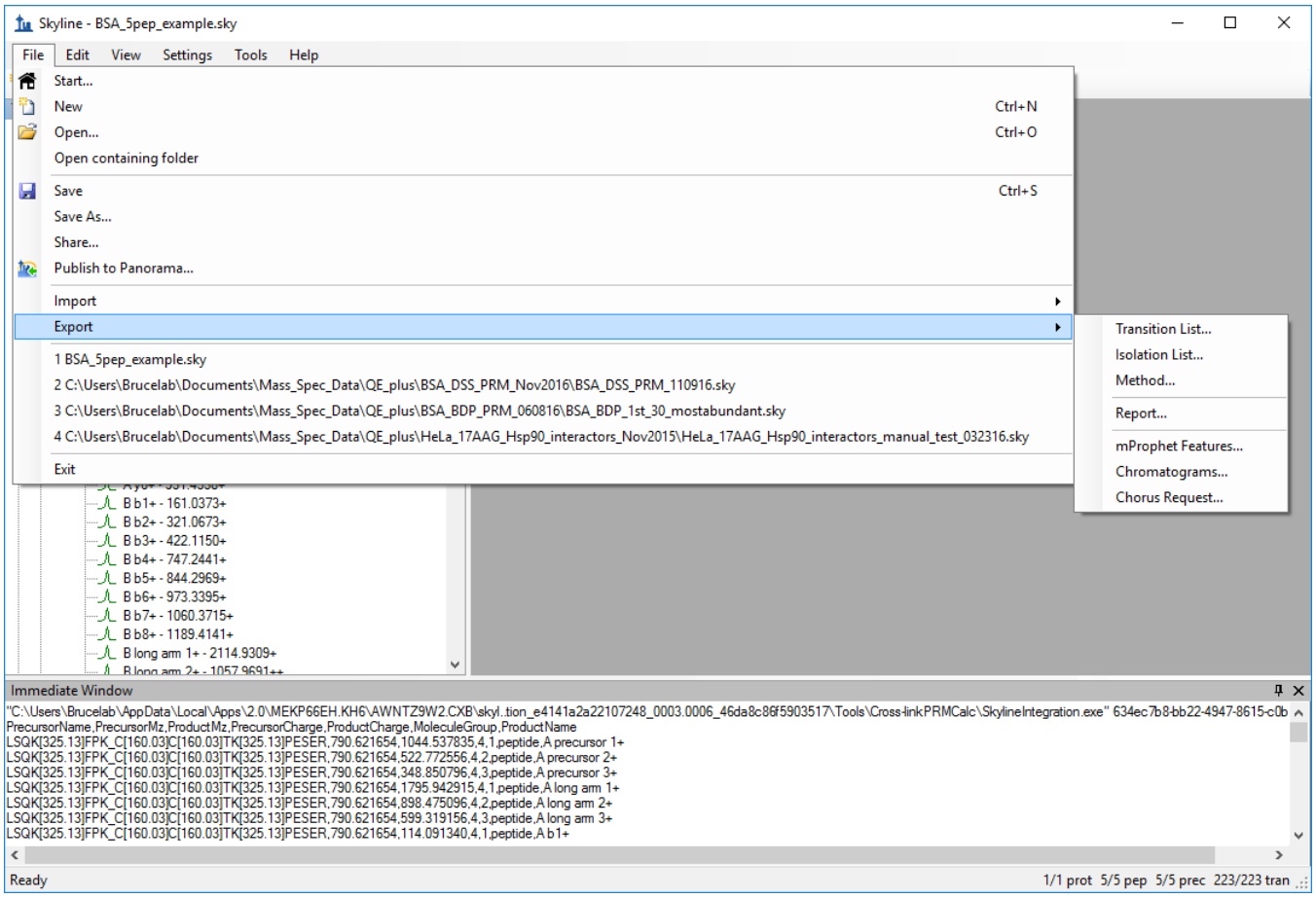
This immediately brings up a file selection box. Choose the appropriate input file and hit the “Open” button.

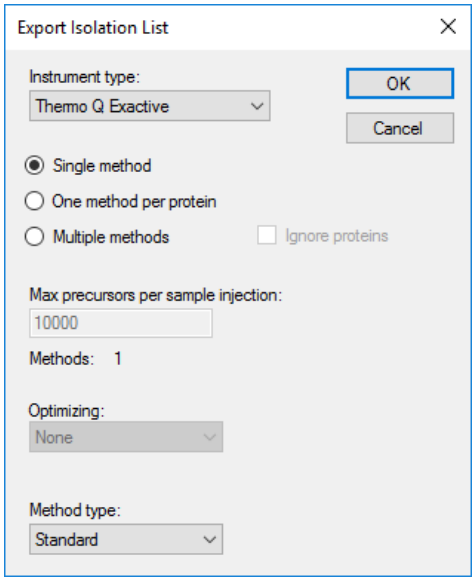


If the right input file is selected and the entries in the file are formatted correctly, you will see a list of cross-linked peptides as below. Note: modification masses when loaded into Skyline are displayed in the format of residue mass + modification mass (i.e K[325.13] = 128.094963 Da for Lys plus the cross-link stump mass 197.032422 Da).

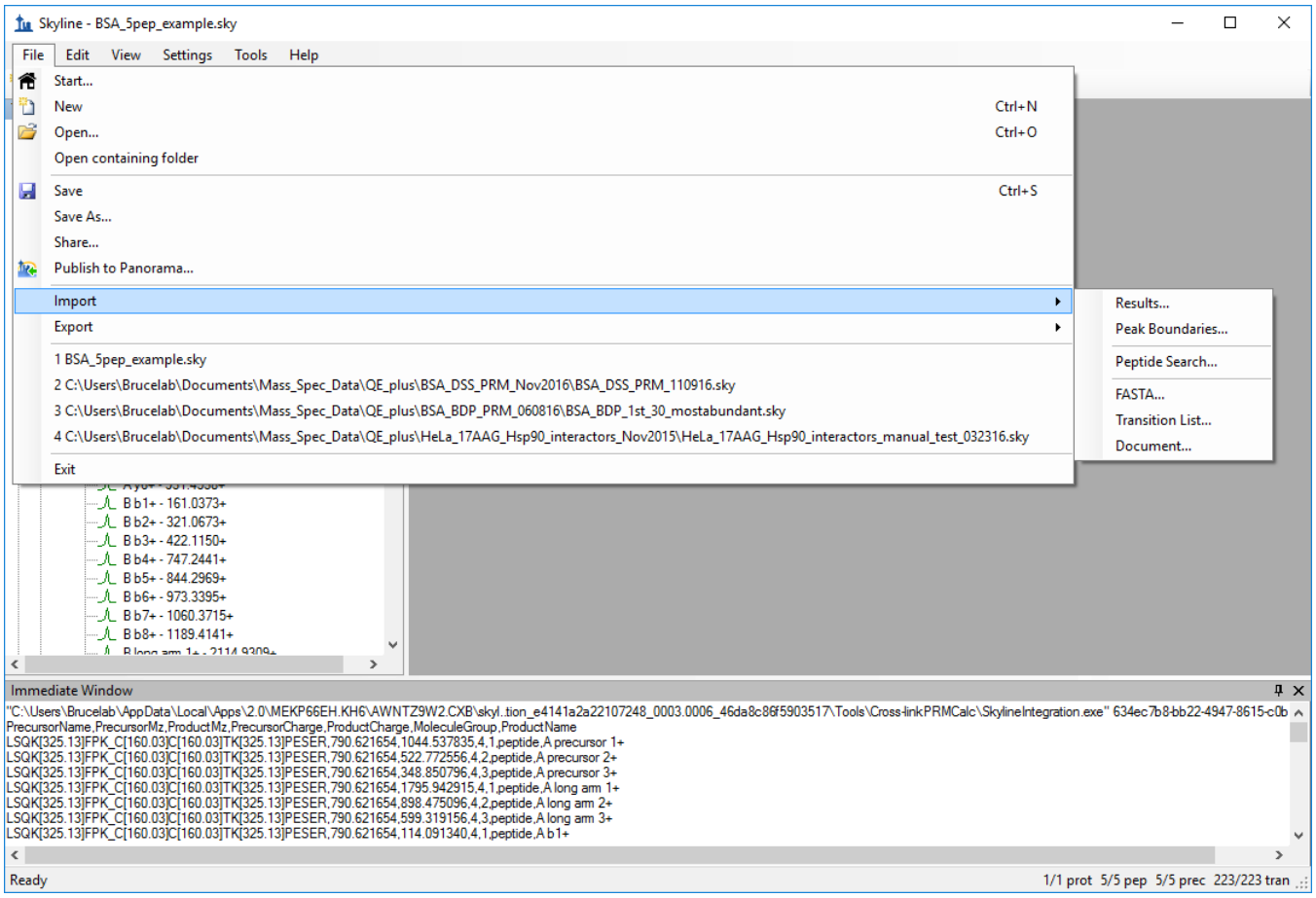


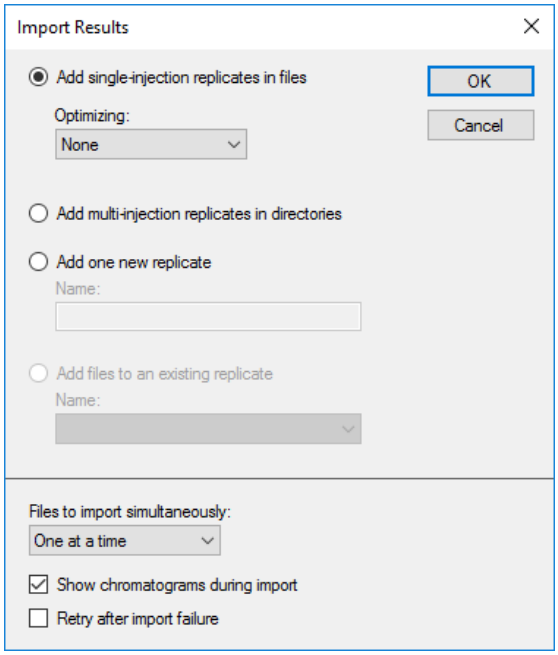
At this point Skyline can be used to export a precursor isolation list for use in generating a PRM method by selecting File->Export->Isolation List then selecting the appropriate instrument type.



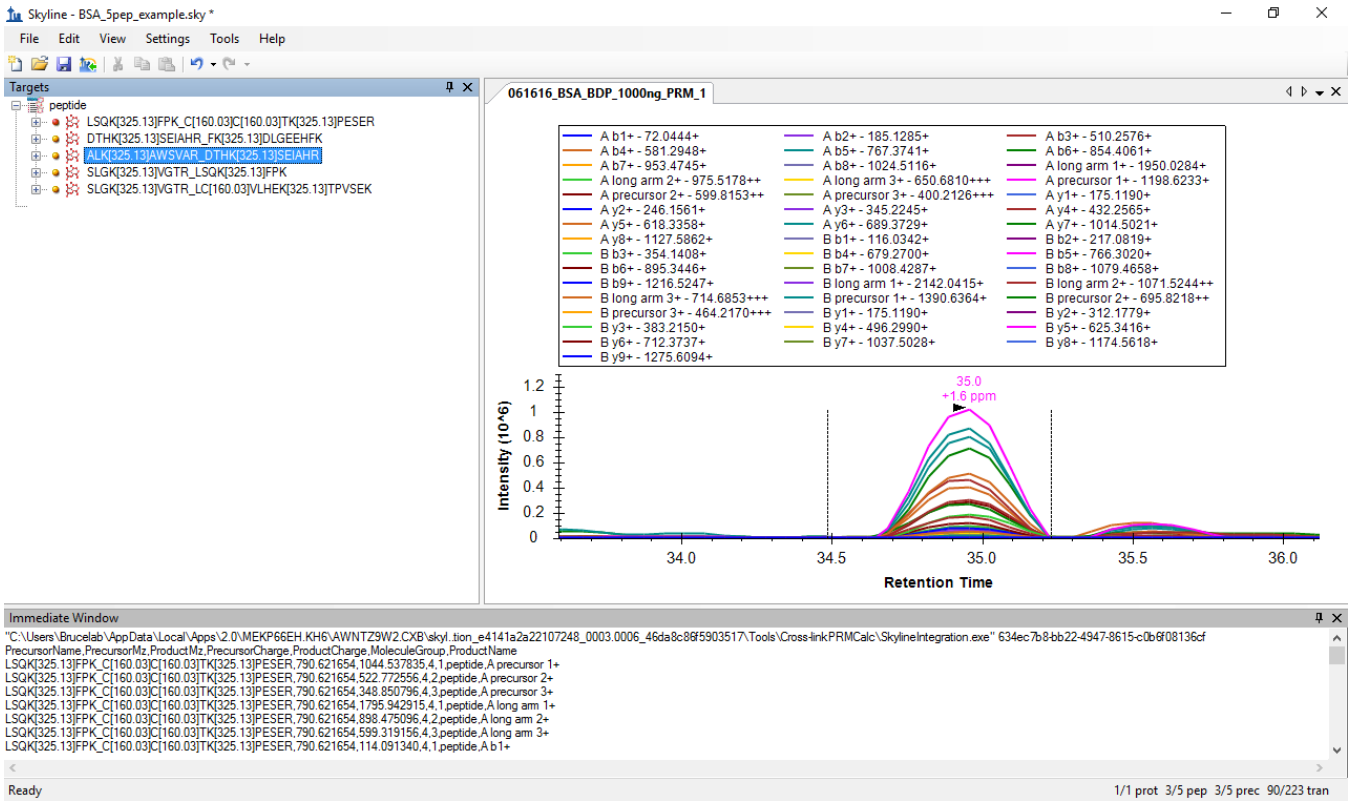


Mass spec data can now be loaded by selecting File->Import->Results and selecting appropriate mass spec data file.

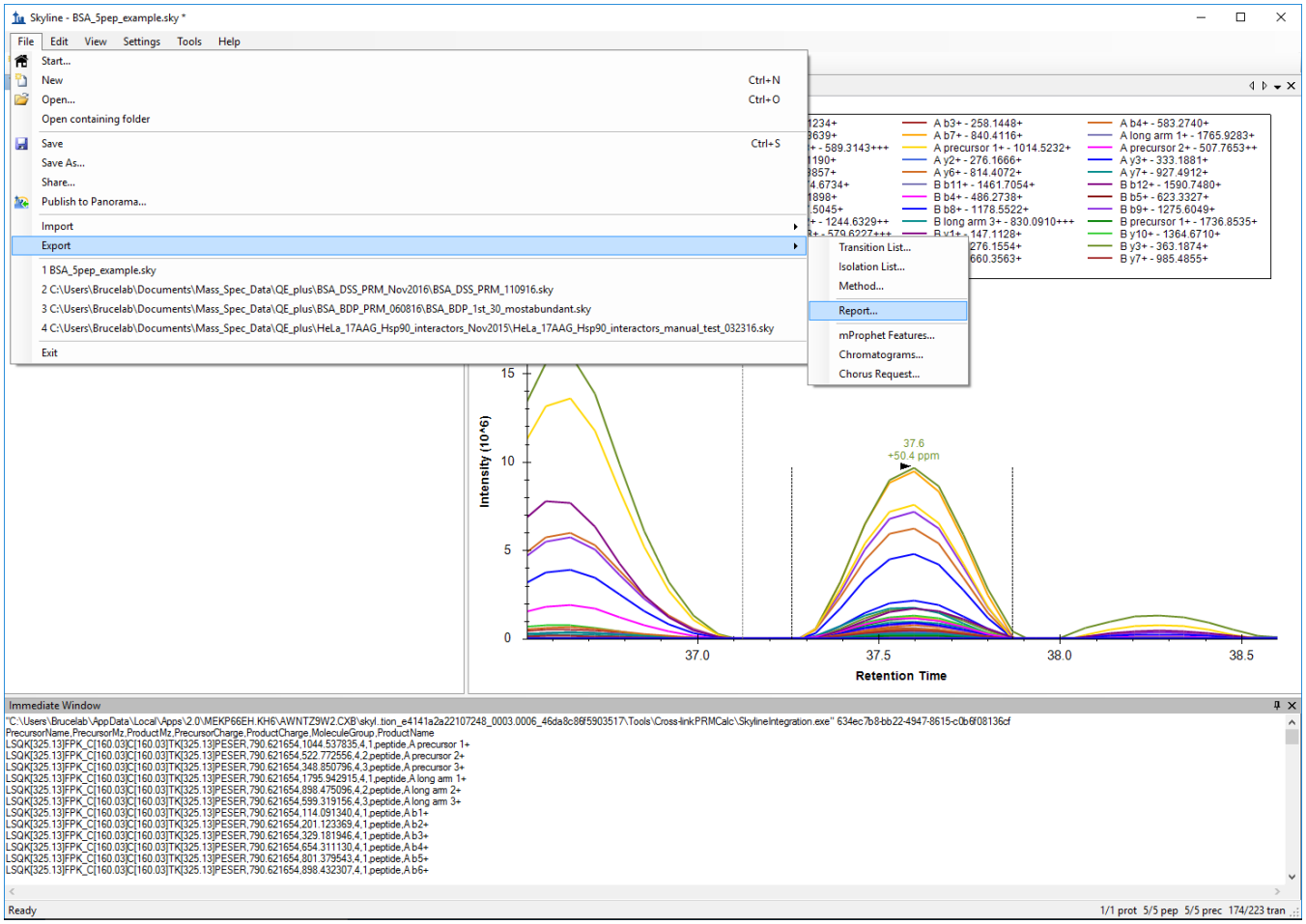




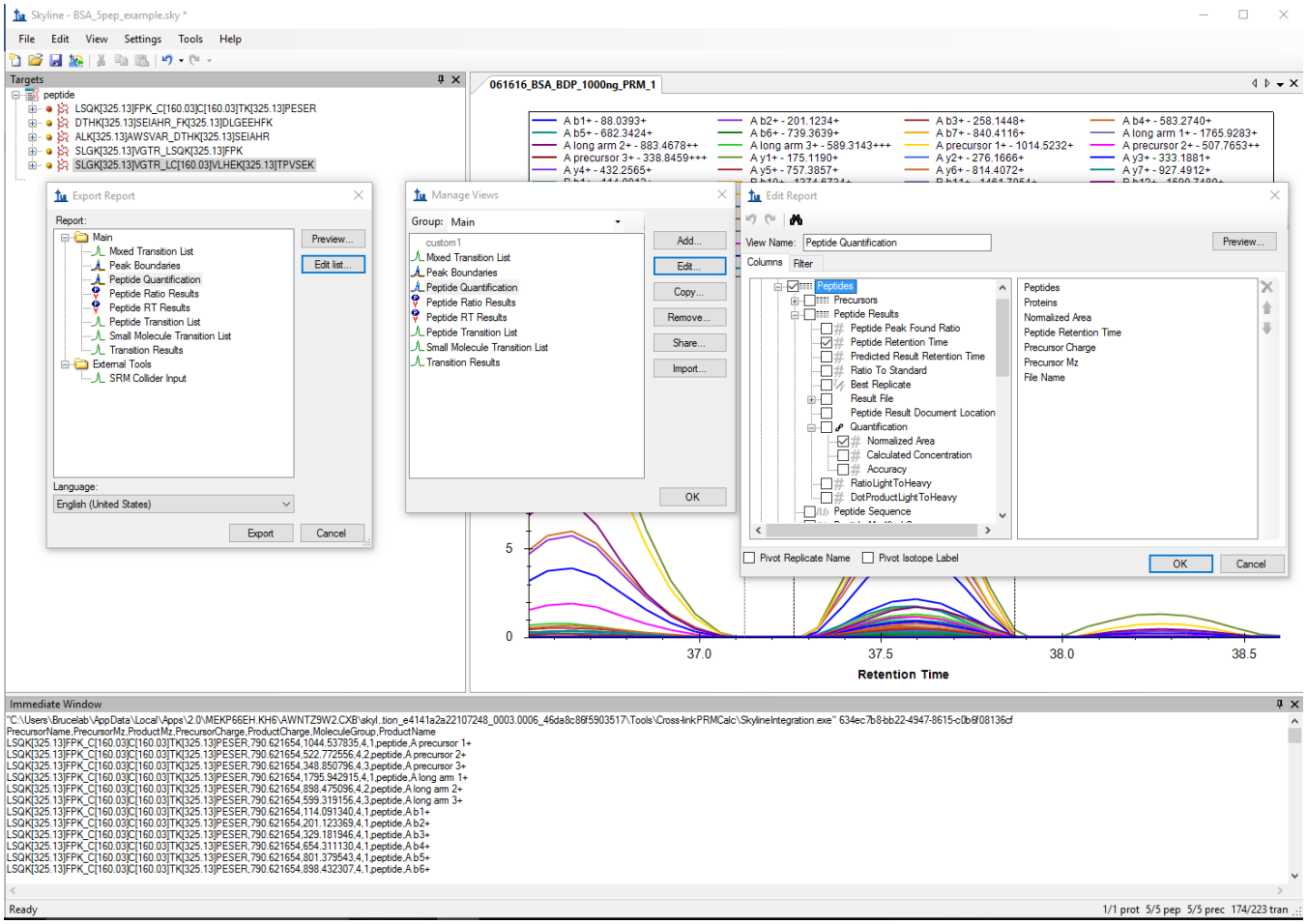
Once mass spec data is loaded PRM transition extracted ion peak areas can be viewed.



The results can be exported from Skyline by clicking File→Export→Report.

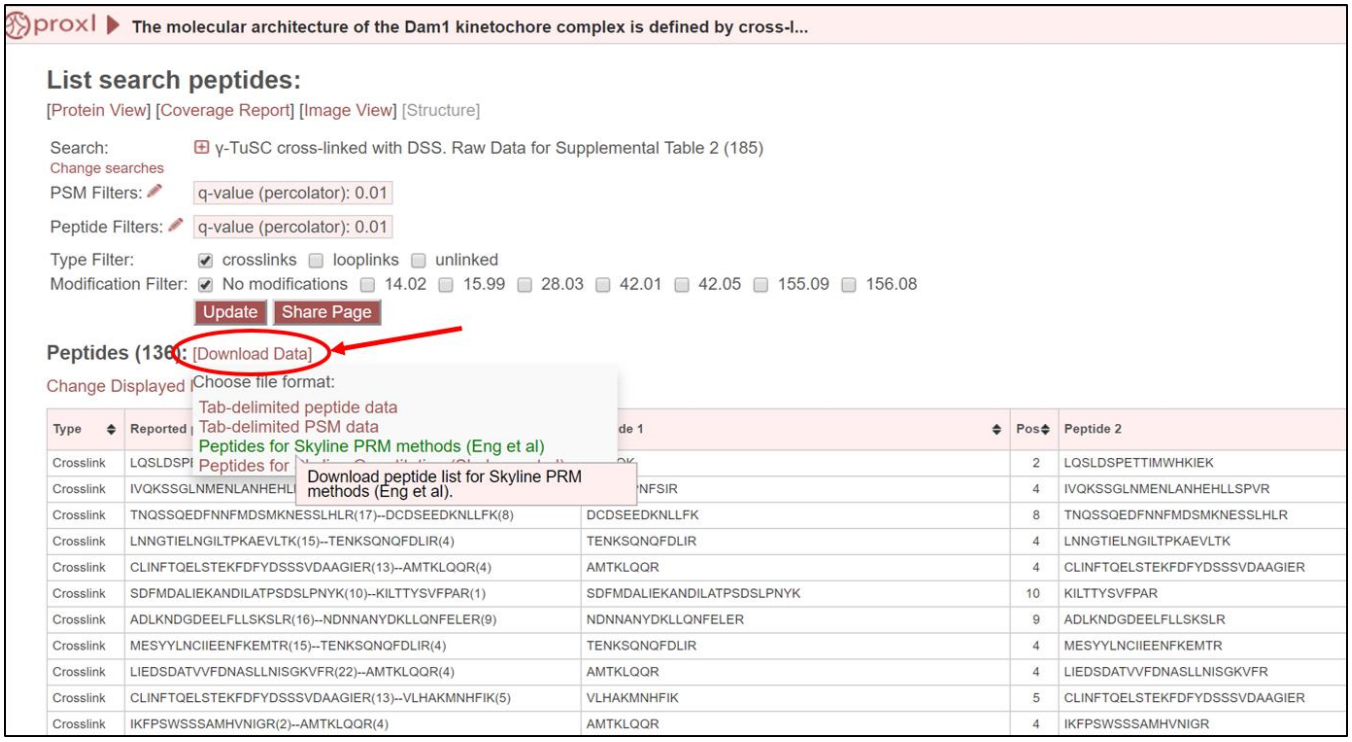


Select Peptide Quantification. Note: before exporting the report first click on Edit List->Peptide Quantification and expand the dropdown menu to make sure that the Peptide Results->Quantification->Normalized Area check box is selected.



Data export from ProXL:

Cross-link peptides in ProXL (proxl.yeastrc.org) can be exported into the right input format for this plugin. Look for the “Download Data” link and select “Peptides for Skyline PRM methods” option. This will generate a tab-delimited text file of the selected cross-linked peptides. You can further edit this file by deleting any rows of cross-link pairs that are unnecessary or redundant such as the same cross-linked peptide pairs identified in different precursor charge states.



Possible reasons for failure:

If you open an input file and no transitions appear in the Target window, this could happen for various reasons. Unfortunately, the mechanism by which the Cross-link PRM transition tool is implemented as a Skyline plugin does not allow the software to report why a failure might have occurred nor can Skyline currently pass information, like transition mass ranges, to the plugin to mitigate possible problems. In testing, we have observed the program to fail for these reasons:

- Incorrect format of the input file. Please confirm your input file is of the right format and has all required fields.
- Precursor m/z of the intact cross-link molecule is larger than the transition settings allow in Skyline. Check your Skyline settings and make sure the precursor m/z range is set to accommodate the data in the input file.