

Crosslinked peptide support in Skyline

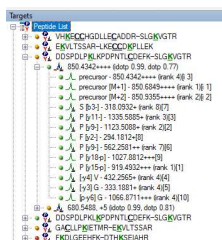
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Overview

- Skyline is a freely-available, open-source Windows client application for analyzing and viewing many types of mass spectrometry data including SRM, DIA/SWATH, DDA and PRM
- Skyline has traditionally been used to predict m/z values of peptide fragment ions in order to extract chromatograms from LC-MS/MS data.
- The next release of Skyline, version 20.2, will have support for crosslinked peptides.
- Crosslinked peptides are two or more ordinary peptides linked together by a chemical crosslinker

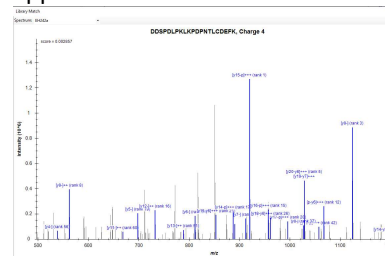
Displaying crosslinked peptides in Skyline



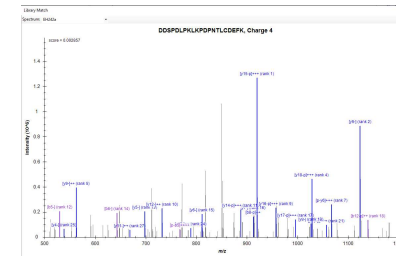
Skyline can predict the masses of ions that involve fragmentation of any or all of the linked peptides.

Spectral library support

BiblioSpec (.bib) spectral libraries can be made from Protein Crosslinking (proxl) search results.



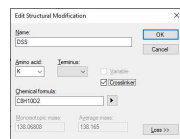
Spectrum annotated with all y ions fragments from either or both linked peptides.



Spectrum annotated with b and y ions from either but not both linked peptides.

Defining crosslinkers in Skyline

Crosslinked peptides in Skyline are modeled as a main peptide with a special "crosslinker" modification on it.

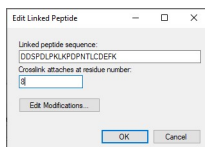


A crosslinker modification has a chemical formula which describes the set of atoms that are added to (or subtracted from) the chemical formulas of the two unlinked peptides.

The crosslinker modification can be applied to an appropriate residue using the Edit Modifications dialog



When a crosslinker modification is chosen, the Edit Linked Peptide dialog appears and allows the user to specify the amino acid sequence of the linked peptide and the location that the crosslinker attaches



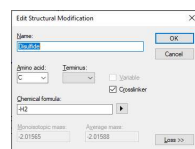
Adding multiple crosslinked peptides to Skyline at once

Position 1	Peptide 1	Position 2	Peptide 2	Classifier
3	VHKECHDGLLECAQDR	4	SLKVGTR	DSS
2	EVKLTSSAR	7	LKECCDPLLEK	DSS
8	DOSPFLKLPDPNTLCDEFK	4	SLKVGTR	DSS
10	DOSPFLKLPDPNTLCDEFK	4	SLKVGTR	DSS
7	QALLKPEYTRK	3	EVKLTSSAR	DSS
2	INDLGEHFK	4	DTHANSEIARR	DSS

Multiple simply crosslinked peptides can be added to a Skyline document by specifying the two peptide sequences, the position that the crosslinker attaches at, and the name of the previously defined crosslinker. For more complicated crosslinks, the **String representation of complex crosslinked peptides** can be used.

Disulfide bonds

Disulfide bonds can exist between Cysteine residues and result in a net loss of two hydrogens



Syntax for representing complex crosslinked peptides

The Skyline user interface currently allows defining peptides that are linked together in a tree structure



PKEPTIKDEA-PEKPTIDKEB-PEPKTIDEKC[+57.021464][+138.06808@2,3,*][+138.06808@7,*,4]

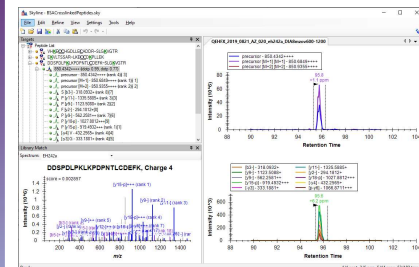
These are represented inside of BiblioSpec files with the flat peptide sequences followed by the crosslinker masses, and the ordinals at which the crosslinker attaches to each peptide sequence. Asterisks are placeholders where a crosslinker does not attach to a particular peptide sequence in the list.



PKEPTIKDEA-PEKPTIDKEB-PEPKTIDEKC[+57.021464][+138.06808@2,3,*][+138.06808@*,8,4]

Skyline 20.2 will only be able to deal with tree structures of peptides where a crosslinker attaches to only two peptides, and there are no cycles. It is hoped that this crosslinker syntax will be able to represent all crosslinking scenarios that are of interest to scientists, even though they are beyond the capabilities of Skyline. If a crosslinker attaches to a single peptide in multiple places, the multiple positions will be able to be specified separated by hyphens. For example: [+138.06808@2-7,8,4] would mean that the crosslinker attaches to the first peptide at positions 2 and 7.

Conclusions



Skyline 20.2 will provide extensive support for crosslinked peptides. This will make it much easier to use Skyline to study protein interactions where a chemical crosslinker reagent was used, as well as to study disulfide bonds that form.

Skyline 20.2 will be released in the latter half of 2002. These features will be available in Skyline-Daily before then. The latest versions of Skyline and Skyline-Daily can be downloaded from: <https://skyline.ms>