

Crosslinked Peptides in Skyline

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Nicholas Shulman

Defining a crosslinker

- There is a new checkbox “Crosslinker” on the “Edit Structural Modification” dialog. Checking that will tell Skyline that this modification should get linked to another peptide.
- The Amino acid and Terminus restrictions, if specified, apply to both ends of the crosslinker
- If the two ends of your crosslinker can attach to different amino acids, then you can either leave “Amino acid” blank, or put in a comma separated list of all the amino acids that the crosslinker might attach to

The screenshot shows the "Edit Structural Modification" dialog box with the following fields and values:

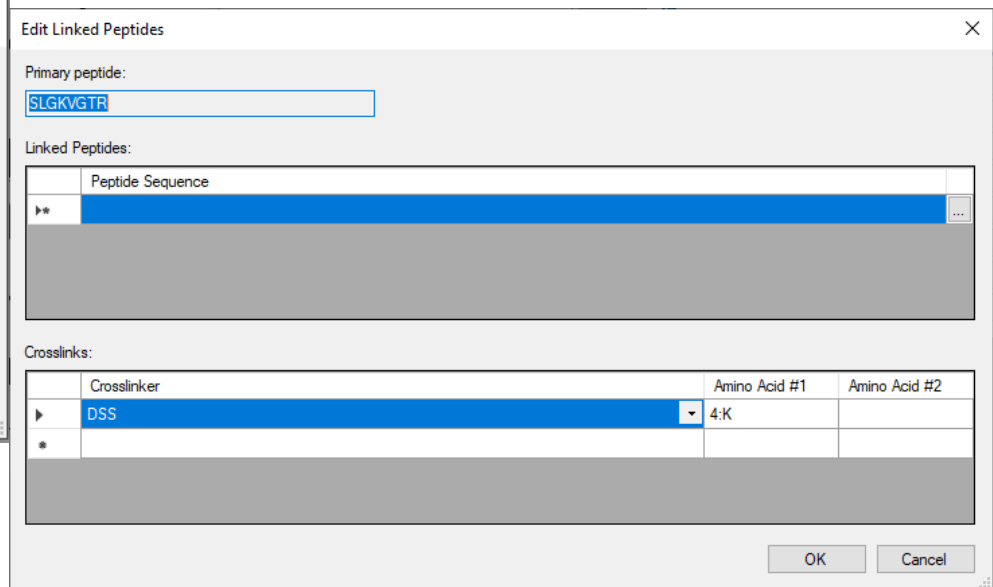
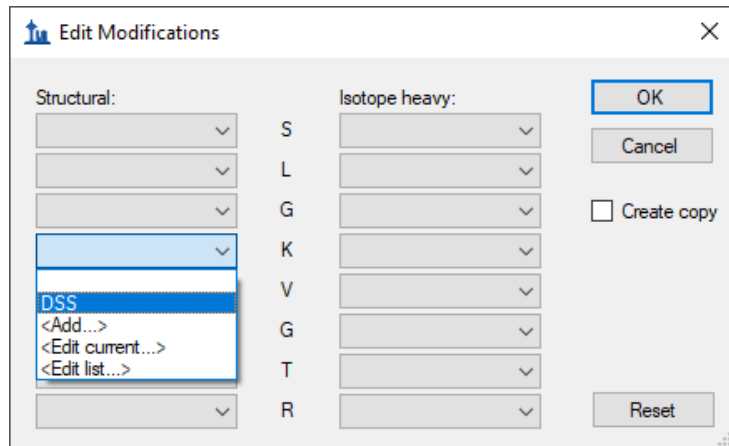
- Name: DSS
- Amino acid: K
- Terminus: (blank)
- Chemical formula: C8H10O2
- Monoisotopic mass: 138.06808
- Average mass: 138.165

Buttons: OK, Cancel, Loss >>

Options: Variable, Crosslinker

Adding a crosslinker to a peptide in a Skyline document

- A crosslinker can be added to a peptide using the “Edit Modifications” menu item.
- Selecting a crosslink modification brings up the “Edit Linked Peptides” dialog



Edit Linked Peptides Dialog

Primary peptide:
SLGKVGTR

Linked Peptides:

Peptide Sequence
DDSPDLPKLKPDPNTLCDEFK

Crosslinks:

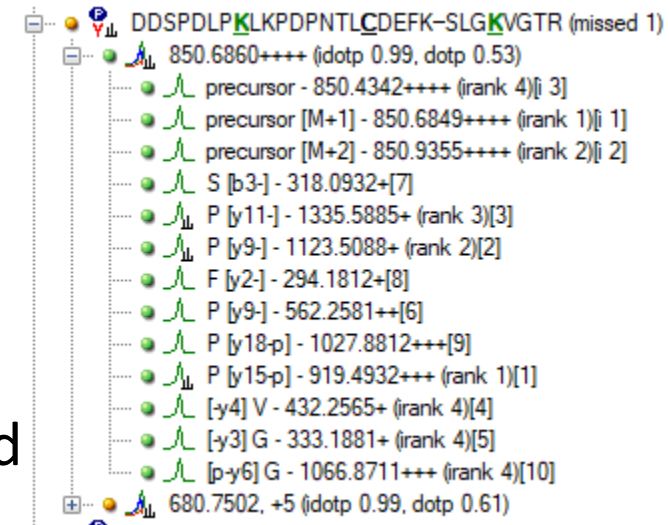
Crosslinker	Peptide #1	Amino Acid #1	Peptide #2	Amino Acid #2
DSS	1:SLGKVGTR	4:K	2:DDSPDLPKLKPDPNTLC...	10:K

OK Cancel

- In the upper grid, you can list zero or more peptide sequences to be linked to the primary panel.
- In the lower grid, you can list crosslinkers and specify which amino acids they attach to
- If the linked peptides have modifications of their own you can specify those by pressing the “...” button next to the linked peptide sequences in the upper grid

Display of linked peptides in Targets tree

- Linked peptides will be displayed in the Targets tree as two peptide sequences separated by a hyphen
- The crosslinked amino acid in each peptide is shown as bold, underline, and green
- Fragment ions are displayed as the type of ion from the left peptide, and the right peptide, separated by a hyphen surrounded by square brackets
- For non-precursor fragments, the amino acid corresponding to that fragment ion is displayed on the left or right side of the square brackets.



Control of which fragment ions you get

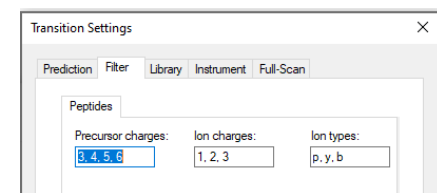
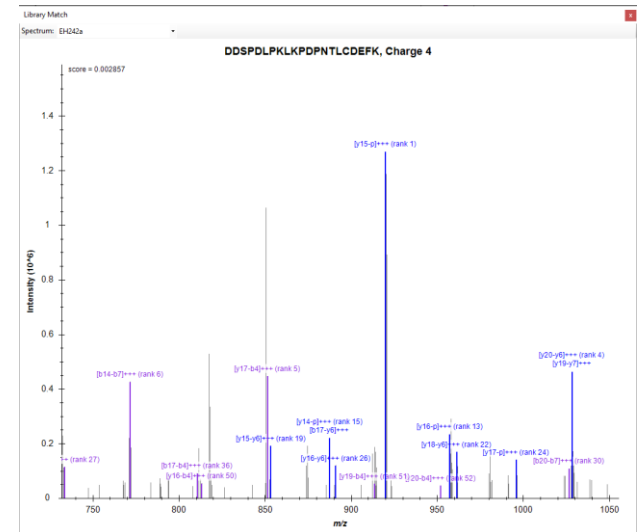
- The set of available transitions is controlled by the “Max losses” settings at “Settings > Peptide Settings > Modifications”
- If Max Losses is 1, then you only get ions where one or the other of the linked peptides fragmented. That is, you can get “precursor-y16” or “y7-precursor” but not “y16-y7”

Spectral Library support

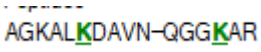
- BiblioSpec spectral libraries can be built from .proxl search results.
- .proxl is an XML format designed to represent results from crosslink-aware search engines including Kojak.
- In the future, BiblioSpec will be able to read some of these crosslink search results directly without having to first be converted to .proxl

Annotation of peaks in a library spectrum

- The Library Match window in Skyline annotates m/z values with the fragment names from the currently selected crosslinked peptide in the Targets tree
- Ion types have an ordering controlled by the “Ion Types” setting at Settings > Transition Settings > Filter
- When a fragment ion has two types (e.g. “y17-b4”), it is treated as being the “type” of the latest appearing ion type in that Transition Settings list. So, “y17-b4” is treated as a “b” ion in terms of hiding and showing it.



Modified Sequence for crosslinked peptides

- The Modified Sequence for crosslinked peptides consists of the two flat peptides separated by a hyphen, followed by the crosslinker with an @ sign and a comma-separated list of the residue numbers where the crosslinker attaches
- For example: 

In a .blib file, the crosslinker will be described using its mass:

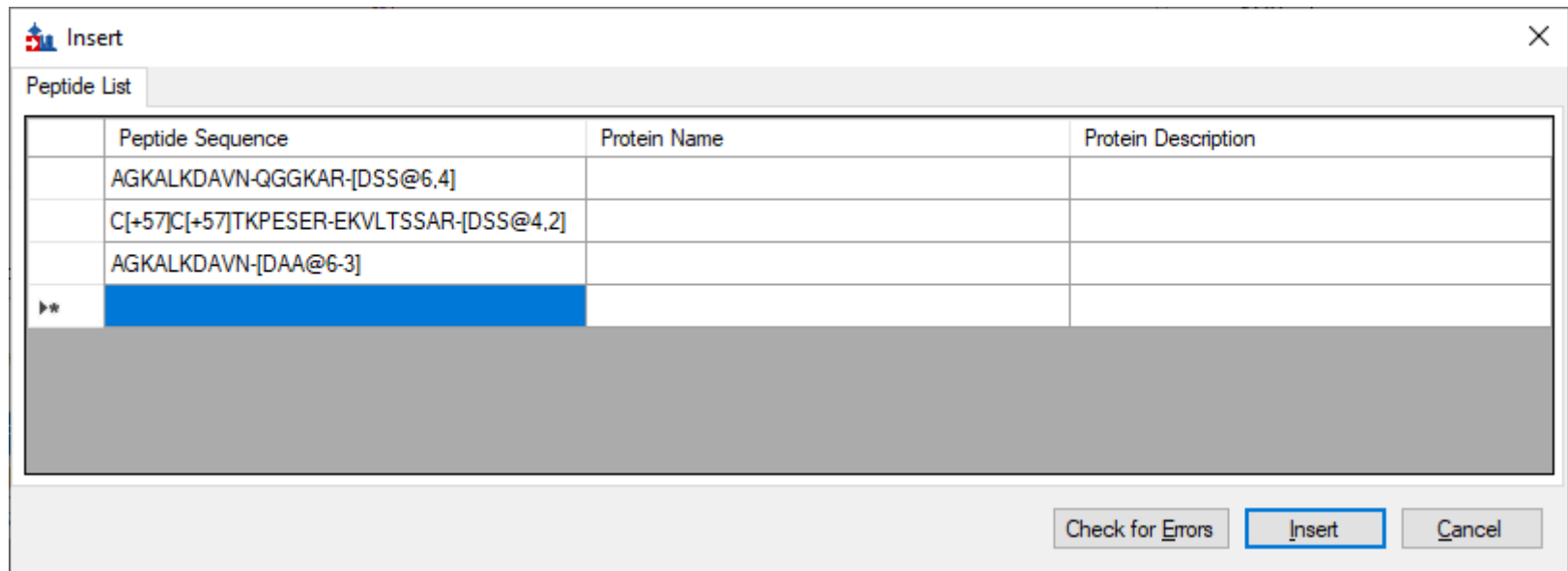
AGKALKDAVN-QGGKAR- [+138.1@6, 4]

In other places, users may find it more convenient to use the name of the crosslinker modification:

AGKALKDAVN-QGGKAR- [DSS@6, 4]

Using the Insert Peptides dialog to add crosslinked peptides to the Skyline document

- The Edit > Insert > Peptides menu item can be used to insert crosslinked peptides using the new Modified Sequence.



Cleavable crosslinks (Skyline 23.1)

- Defining a “neutral loss” for a crosslink modification tells Skyline that the crosslinker is cleavable.

Dialog box: Edit Structural Modification

Name: DSSO

Amino acid: K

Terminus:

Chemical formula: C6O3SH6

Monoisotopic mass: 158.003765

Average mass: 158.17594

Losses:

Buttons: OK, Cancel, Variable, Crosslinker, Loss <<, Add neutral loss

Dialog box: Edit Loss

Loss chemical formula: C3O2H4

Monoisotopic loss: 72.021129

Average loss: 72.06311

Include loss by default: Matching Library

Charge: 0

Buttons: OK, Cancel

The chemical formula of the neutral loss represents what needs to be subtracted from the modification formula to result in the cleaved crosslinker. Subtracting “C3O2H4” from “C6O3SH6” results in a cleaved crosslinker with the formula C3OSH2