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Identifying Crosslinks in MS2-MS3-based Workflows with MS Annika

Micha Johannes Birklbauer, University of Applied
Sciences Upper Austria, Hagenberg

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Cross-linking

- Crosslinker: Small molecule that covalently links amino acids in or between proteins
- Digestion
- Smaller connected fragments → α Peptide and β Peptide
- Analyzed with mass spectrometry
- Used for structure determination and protein-interaction networks

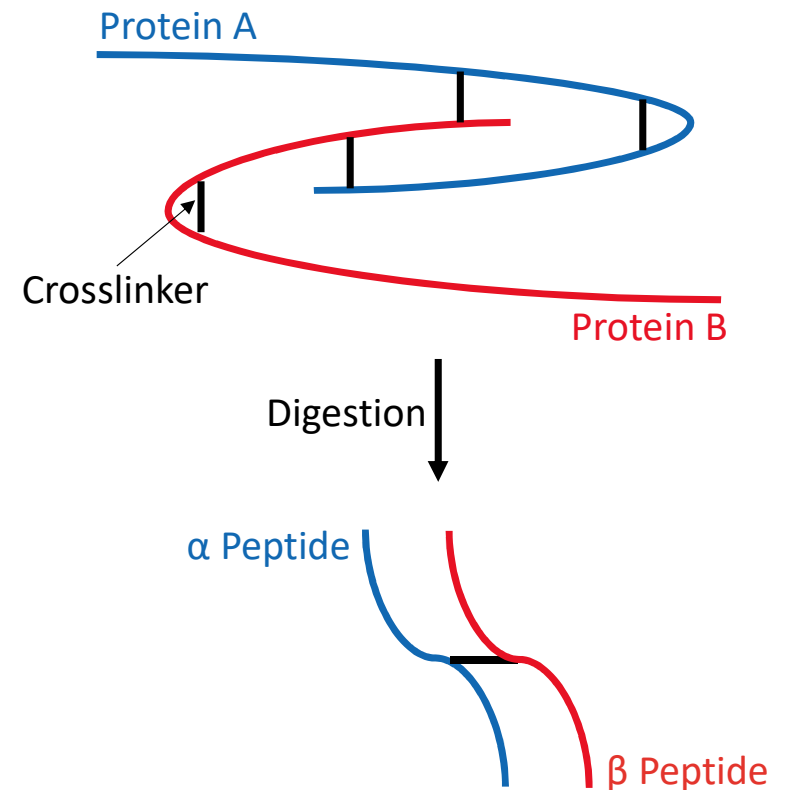


Fig. 1: Cross-linking basics.



MS2 vs. MS3-based Cross-linking Workflows

- In the context of MS-cleavable crosslinkers, identification of crosslinks can be done via:
 - MS2-based workflows
 - MS2-MS3-based workflows

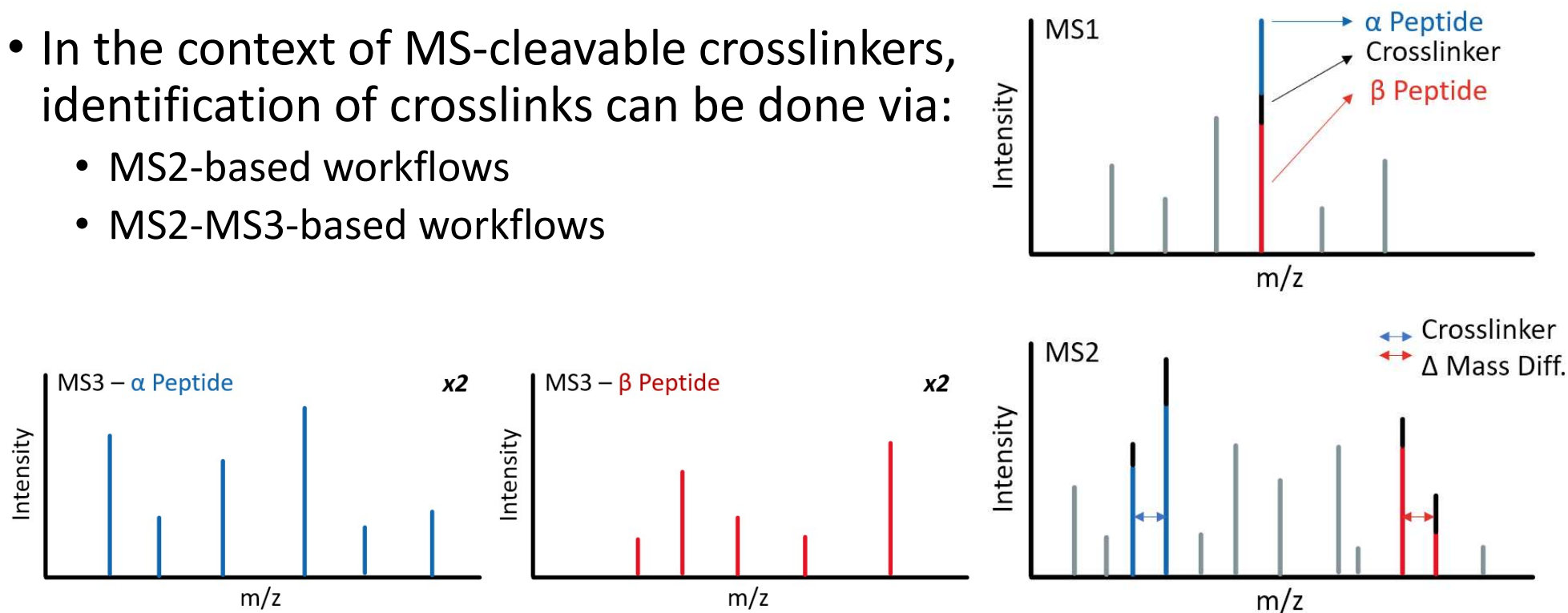


Fig. 2: Cross-linked peptides at different MSⁿ levels.





MS Annika

- We previously developed MS Annika¹ – a cross-linking search engine for MS2-based workflows
- MS Annika is implemented as nodes for Proteome Discoverer
- The updated version of MS Annika now also supports data from MS3-based workflows

1) Pirklbauer G. J., et al. (2021) MS Annika: A New Cross-Linking Search Engine. *Journal of Proteome Research* (20)

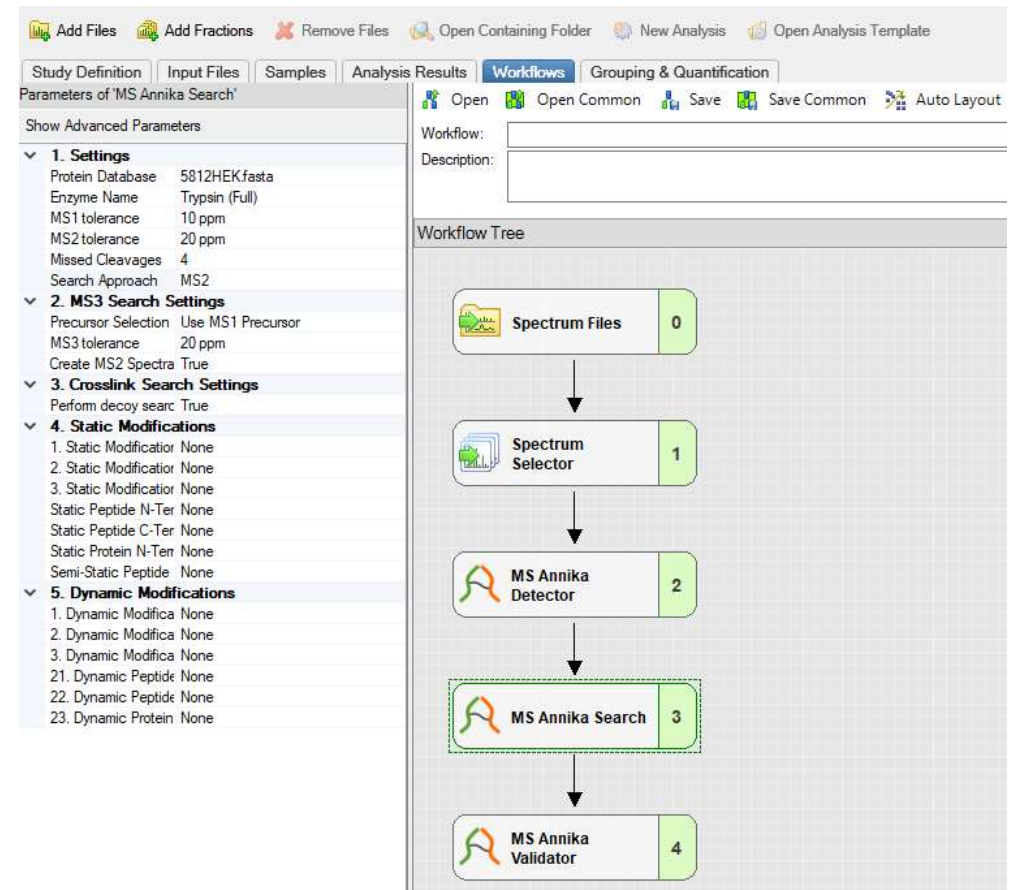


Fig. 3: A basic cross-linking workflow with MS Annika in Proteome Discoverer.



Identification of Crosslinks from MS3 Spectra

- Match MS3 spectra to MS2 crosslink peaks → modification, verification
- De-isotoping → mono-isotopic mass
- Calculating unmodified peptide masses
- Search

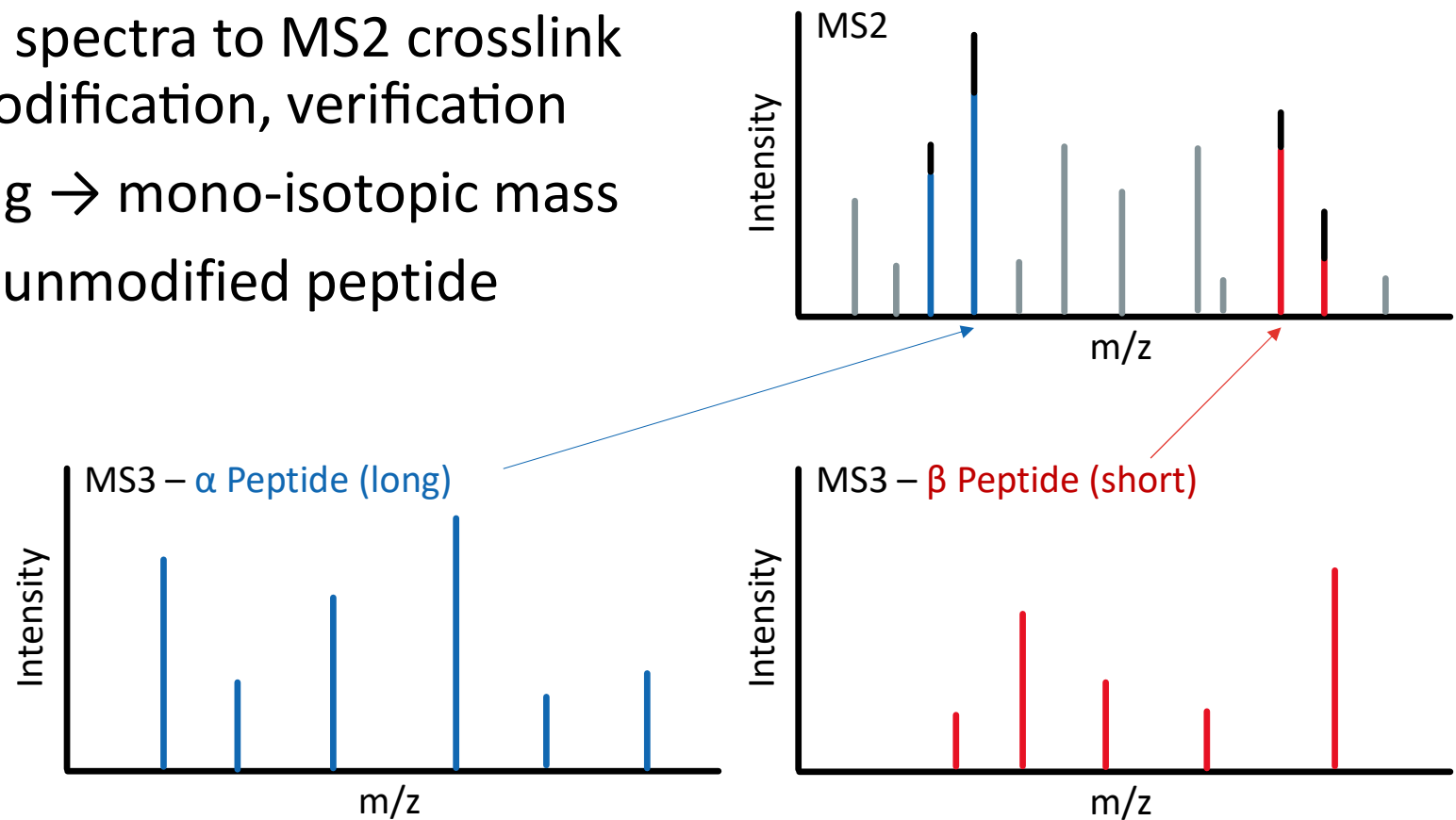


Fig. 4: MS3 spectra matched to precursor MS2.



Search

- Identification of peptides with MS Amanda² – our in-house developed search engine. *Example:*
- **Input:** Mass of α Peptide, MS3 Spectrum
- Database search with MS Amanda
- **Output:** Peptide Sequence, Score

2) Dorfer V., et al. (2014) MS Amanda, a Universal Identification Algorithm Optimized for High Accuracy Tandem Mass Spectra. *Journal of Proteome Research* (13)

Input:

α Peptide Mass + MS3 Spectrum

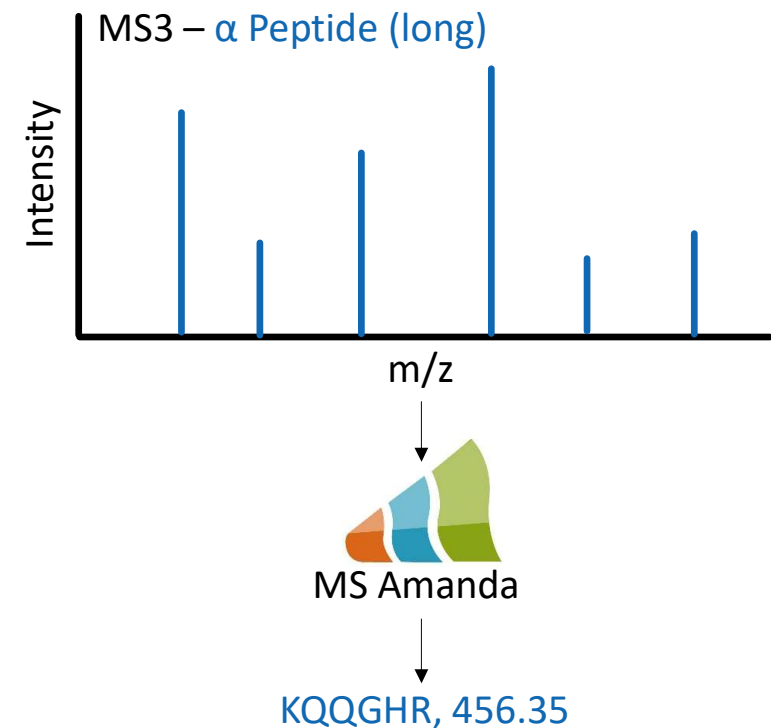


Fig. 5: Peptide search with MS Amanda.



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7

Boosting – Scoring Function

- New scoring function:

$$Score(peptide) = \max(scores_{peptide}) * (1 + \frac{p}{100})^{n-1}$$

- Where:
 - $scores_{peptide}$ is a list of all scores of that peptide
 - p is a user-defined boost parameter (default 20)
 - n is the number of unique scan numbers that peptide has been identified in



CSM and Crosslink Creation

- Having identified both α Peptide and β Peptide, the two results are combined to one Crosslink-Spectrum-Match (CSM), e.g.:

KQQGHR – AMEKAR (Spectrum 1337)

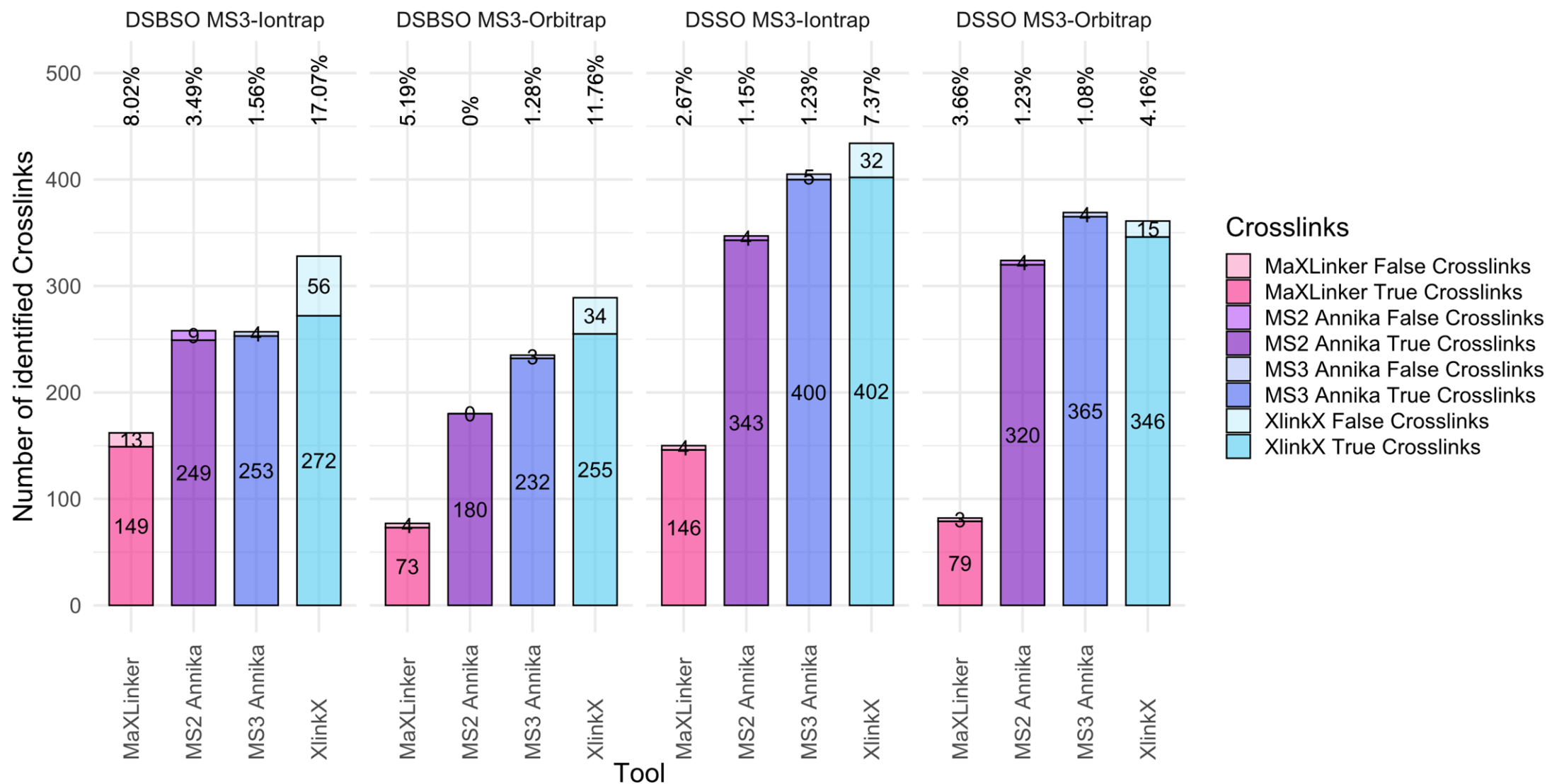
- The CSM score is the minimum score of the α Peptide and β Peptide
- Multiple CSMs (with the same sequence) are combined to one crosslink
- The crosslink score is the maximum score of all associated CSMs
- MS Annika does 2 step validation – at CSM level and crosslink level



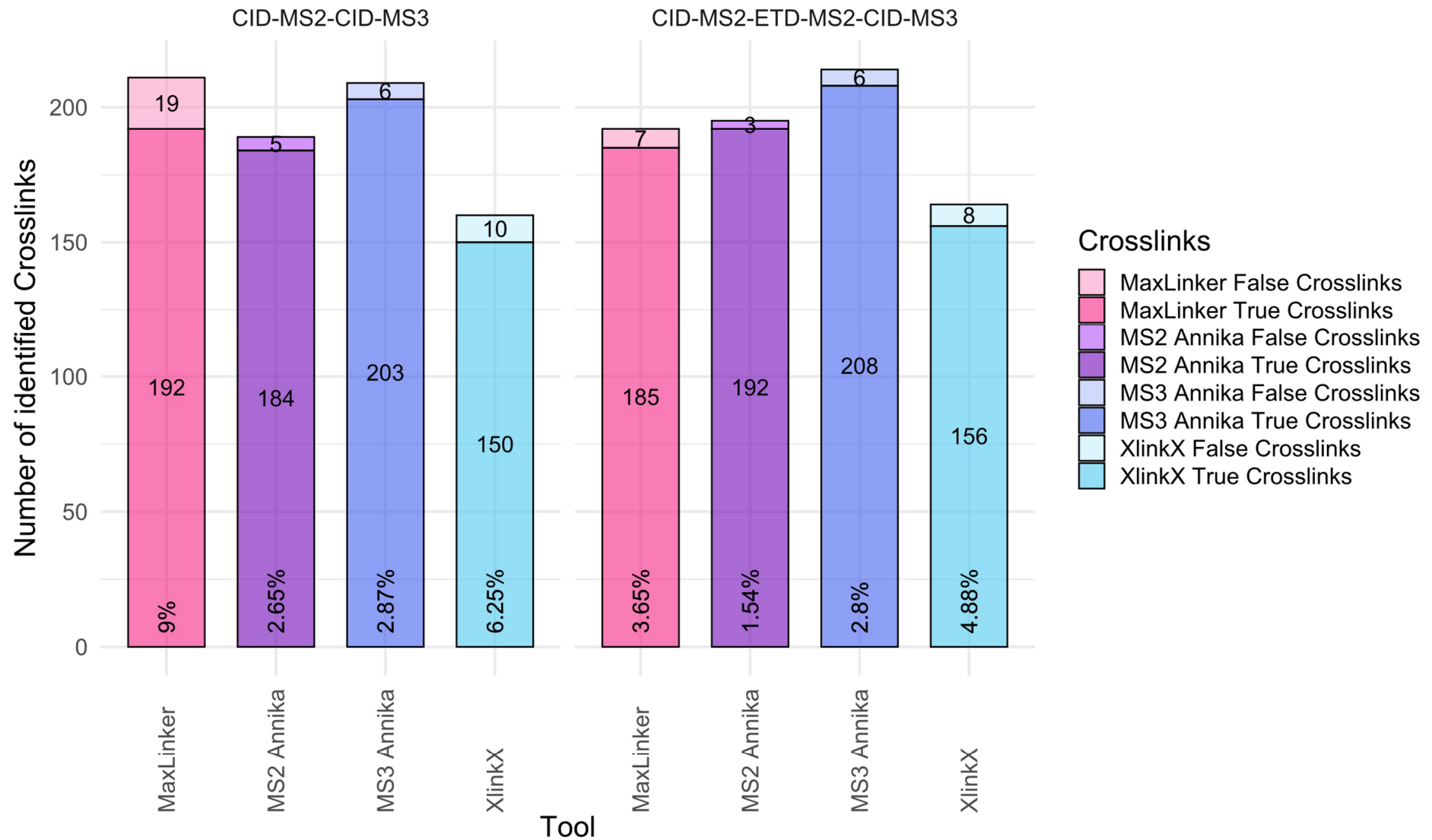
Results



Dataset of synthetic peptides by Matzinger et al., 2022: Number of identified crosslinks per tool, crosslinker and mass analyzer (1% FDR)



Dataset of synthetic peptides by Beveridge et al., 2020:
Number of identified crosslinks per tool and method (1% FDR, crosslinker: DSSO)

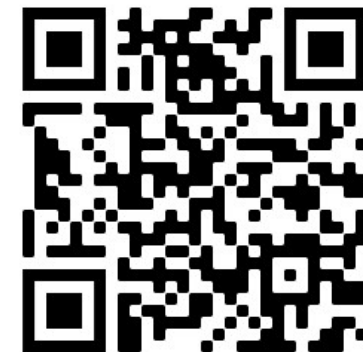


Conclusion

- We updated our cross-linking search engine MS Annika to support MS3-based workflows
- MS Annika yields accurate FDR estimates while reporting high numbers of unique crosslinks
- The MS2 version of MS Annika is available for free download at:
<https://ms.imp.ac.at/index.php?action=ms-annika>
- If you are interested in a beta version of the MS3 Annika, please drop me an email:
micha.birklbauer@fh-hagenberg.at



MS Annika



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Questions?

