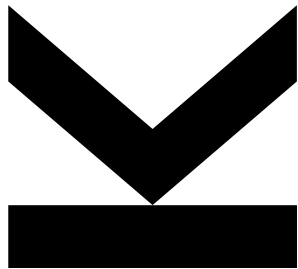


NEW BIOINFORMATIC ALGORITHMS FOR PROTEOME-WIDE CROSS-LINKING MASS SPECTROMETRY



PhD Pre-Defense – Micha J. Birklbauer

27th February 2025



BIOINFORMATICS
RESEARCH GROUP
H A G E N B E R G

Supervised by FH-Prof. PD DI Dr. Stephan Winkler



Der Wissenschaftsfonds.

in close cooperation with



PROTEOMICS

- ❑ Proteins are the core components of life
- ❑ They are integral to controlling cellular function and state
- ❑ Ultimately they determine the characteristics of any organism
- ❑ Proteomics is the study of all proteins

MOTIVATION

- ❑ Research of native protein structures and their interactions is crucial for understanding diseases and pathological processes (vital for drug development)
- ❑ In this regard Cross-Linking Mass Spectrometry (XLMS) has become the tool of choice

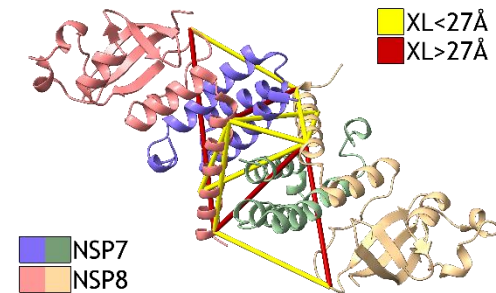


Figure 1

CROSS-LINKING MASS SPECTROMETRY

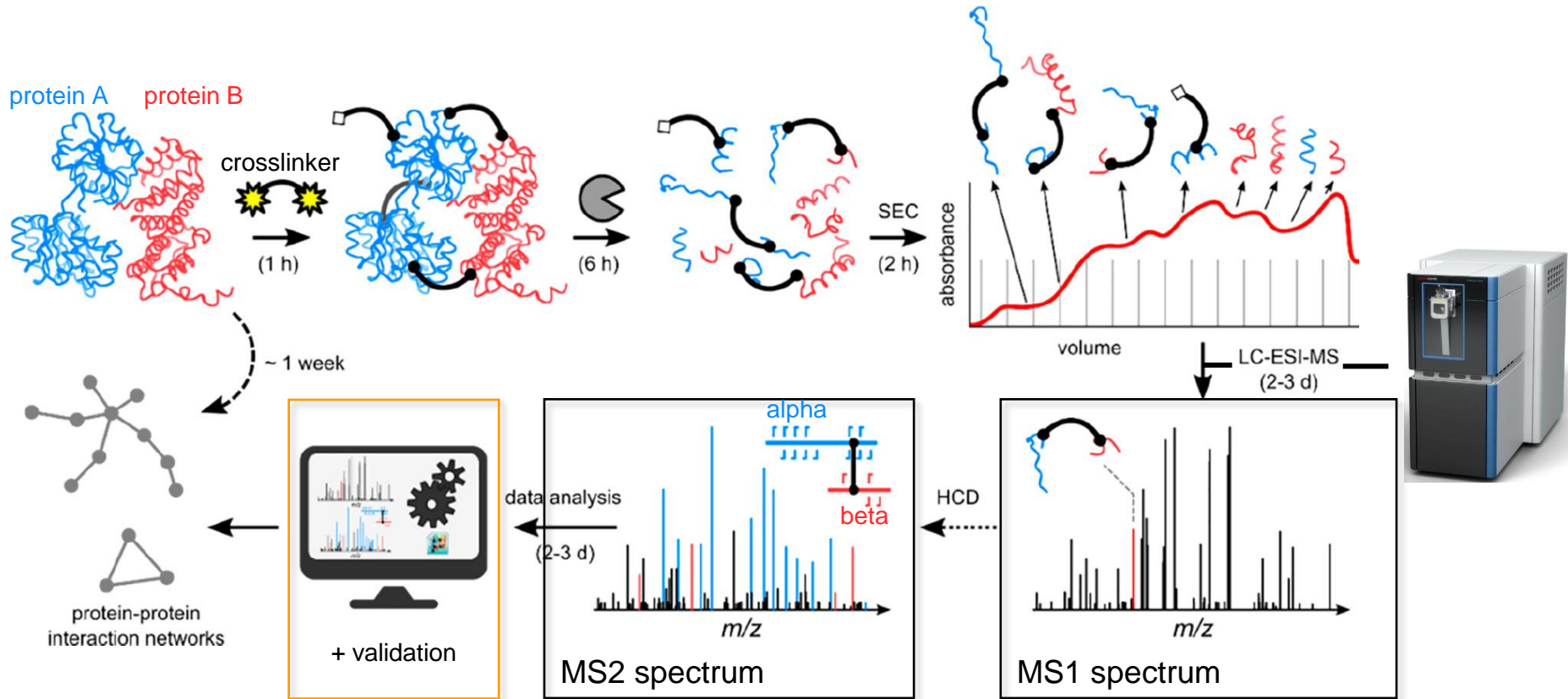


Figure 2*

MY RESEARCH:

- ☐ Identification of crosslinks from MS2-MS3 acquisition workflows
- ☐ Identification of crosslinks from non-cleavable reagents

*Figure adopted from Piersimoni et al., *Chemical Reviews*, 2021

MS2-MS3-BASED CROSSLINK IDENTIFICATION

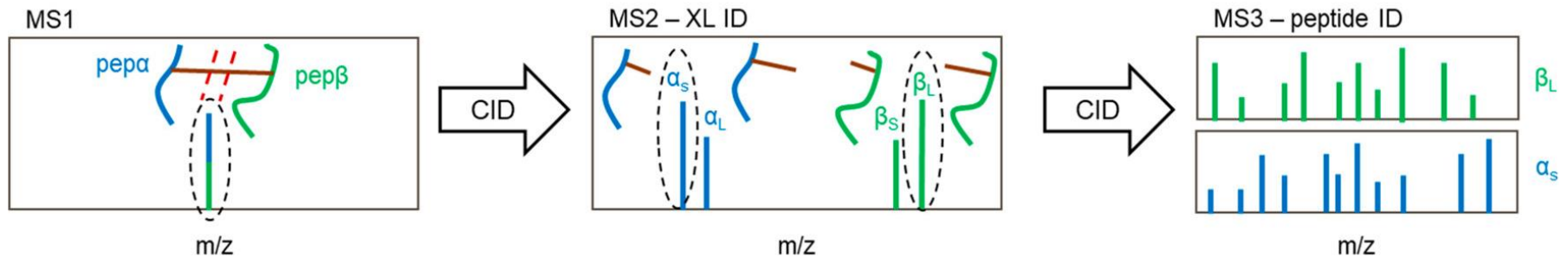


Figure 3*

MOTIVATION

- ❑ One of the most common workflows
- ❑ Software exist, but limited in sensitivity and specificity (crucial for biological application)

PROBLEM

- ❑ Algorithm of high sensitivity and specificity needed

CHALLENGES

- ❑ Incorrect precursor isolation (1)
- ❑ Incorrect C12 assignment from isotope distribution (2)
- ❑ Matching and combining MS2 and MS3 information

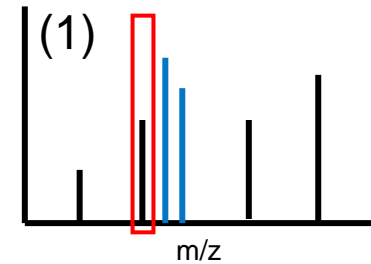


Figure 4

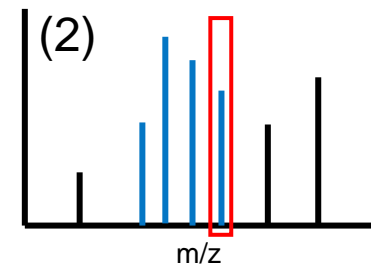


Figure 5

*Figure adopted from Matzinger et al., *Journal of Proteome Research*, 2021

MS2-MS3-BASED CROSSLINK IDENTIFICATION

INCORRECT PRECURSOR ISOLATION

- ☐ Verify precursor ion by comparison to MS2 spectrum
- ☐ If MS3 spectrum originates from non-crosslinked precursor → discard spectrum

INCORRECT C12 ASSIGNMENT

- ☐ Re-calculate C12 mass from MS2 spectrum based on known rules for isotope distributions

MATCHING AND COMBINING MS2 / MS3

- ☐ Match via precursor and product ion mass considering mass tolerance and retention time frame
- ☐ Combination via novel scoring function

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

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

The score is a measurement of similarity between peptide and experimental spectrum and should reflect match quality

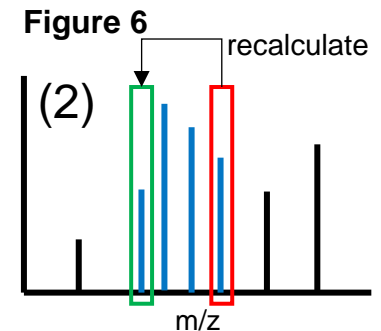
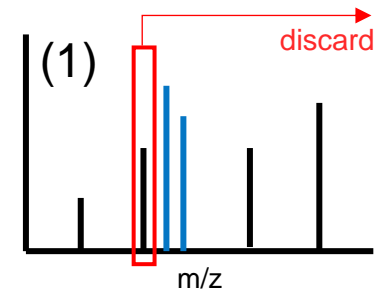


Figure 7

MS2-MS3-BASED CROSSLINK IDENTIFICATION

INCORRECT PRECURSOR ISOLATION

- ❑ Verify precursor ion by comparison to MS2 spectrum

- ❑ **Annotation of precursor ion is needed**
- ❑ **Match of MS2 and MS3 spectrum is prerequisite**

INCORRECT C12 ASSIGNMENT

- ❑ Re-calculate C12 mass from MS2 spectrum based on

- ❑ **Pattern matching problem**
- ❑ **Need to consider rules from chemistry and physics**
- ❑ **Dependent on used mass spectrometry instrument**
- ❑ **Isotopic envelopes often ambiguous**
- ❑ **Interference from noise**
- ❑ **Whole subfield dedicated to identifying isotope patterns**
- ❑ **Multi-hundred LOC algorithm**

When

p is a user-defined boost parameter (default 20)

n is the number of unique scan numbers that peptide has been identified in

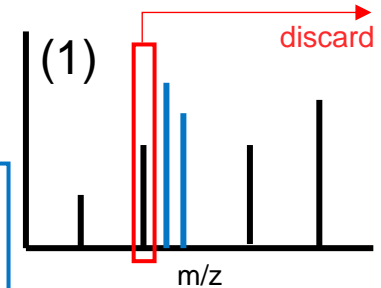


Figure 6

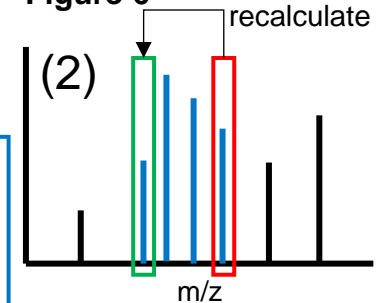


Figure 7

MS2-MS3-BASED CROSSLINK IDENTIFICATION

❑ Algorithms implemented in MS Annika 2.0 [1]

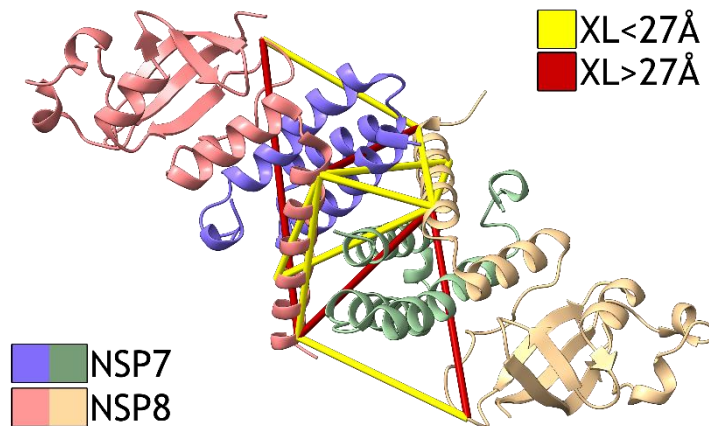
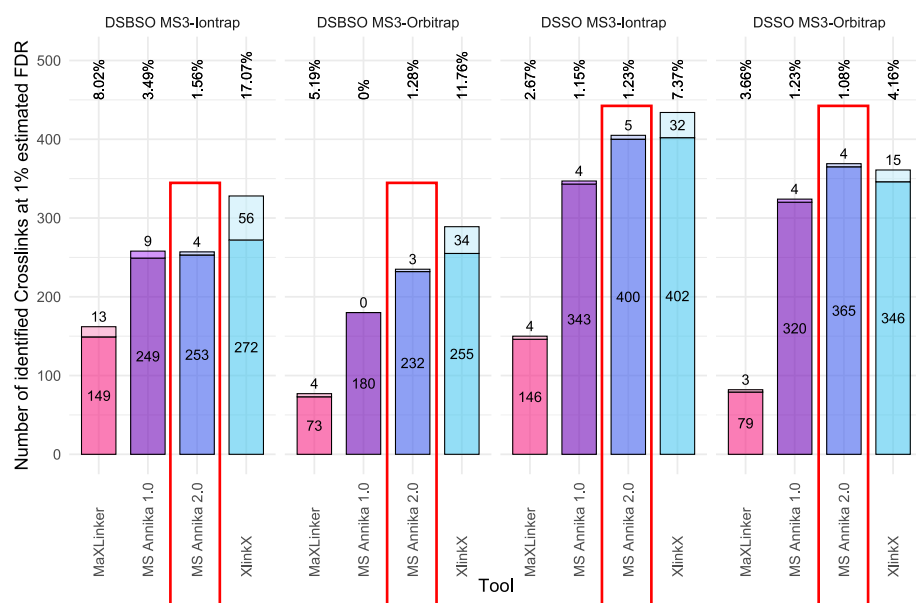
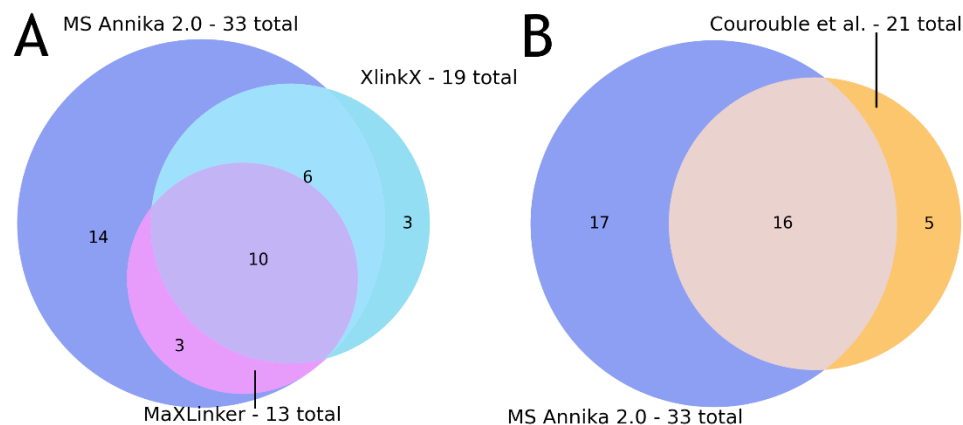
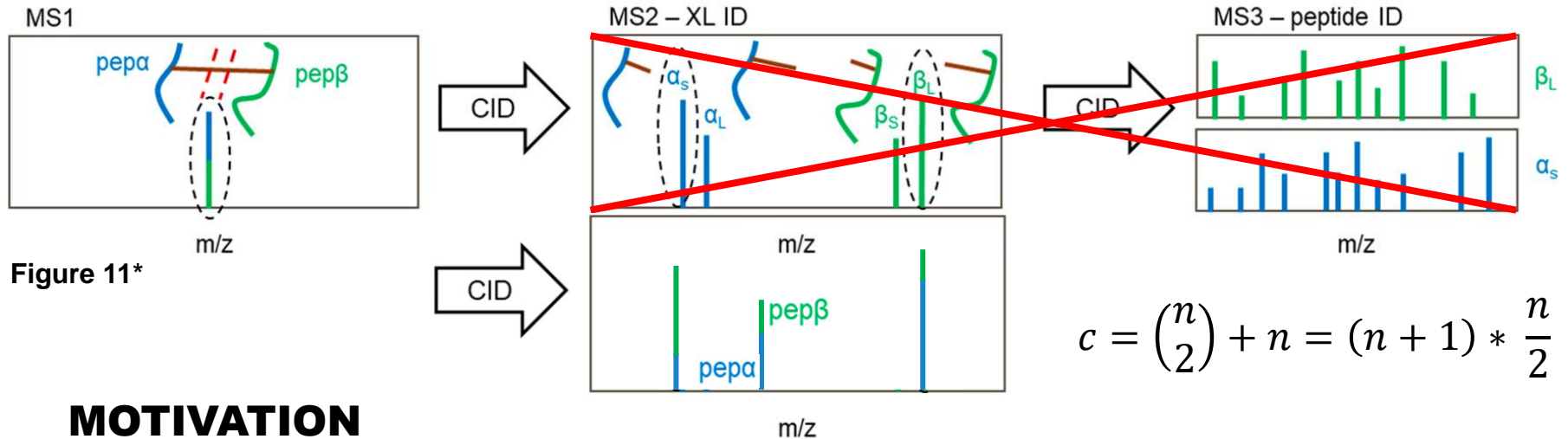


Figure 8, 9, 10

[1] M. J. Birklbauer, M. Matzinger, F. Müller, K. Mechtler, and V. Dorfer, “MS Annika 2.0 Identifies Cross-Linked Peptides in MS2-MS3-Based Workflows at High Sensitivity and Specificity”, *Journal of Proteome Research*, vol. 22, no. 9, pp. 3009-3021, Aug. 2023



NON-CLEAVABLE CROSSLINK IDENTIFICATION



MOTIVATION

- ❑ Non-cleavable crosslinkers are the most common crosslinker reagent
- ❑ Limited software support for data analysis, especially for complex samples

PROBLEM

- ❑ An efficient algorithm for proteome-wide searches is needed

CHALLENGES

- ❑ Reduction of search space
- ❑ Efficient search algorithm for considering potentially millions of peptide candidates and tackling the n -squared problem

*Figure adopted from Matzinger et al., *Journal of Proteome Research*, 2021

NON-CLEAVABLE CROSSLINK IDENTIFICATION

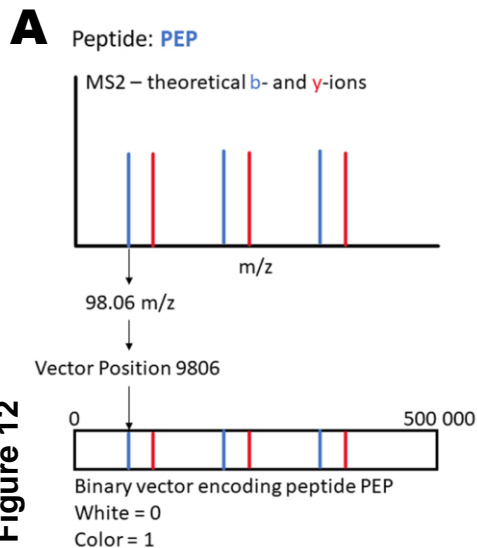
REDUCTION OF SEARCH SPACE

- ❑ Divide and conquer approach: identify one of the two cross-linked peptides first, the second peptide can then be more easily inferred (*linear problem*)

EFFICIENT SEARCH ALGORITHM

- ❑ Encode peptides and spectra as sparse vectors
- ❑ Fast scoring by matrix multiplication

Peptide Encoding



Mass Spectrum Encoding

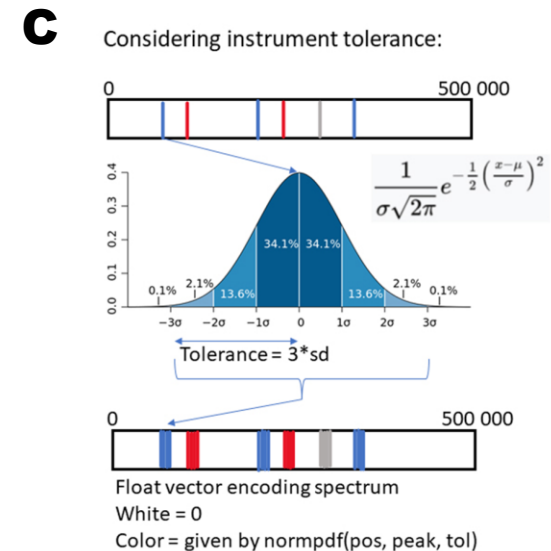
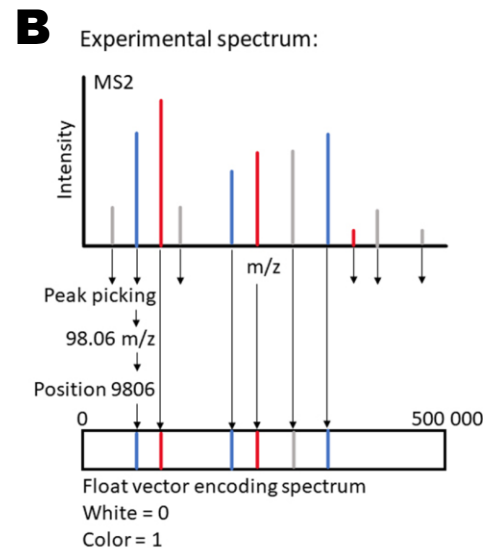


Figure 12

NON-CLEAVABLE CROSSLINK IDENTIFICATION

□ Algorithms implemented in MS Annika 3.0 [2]

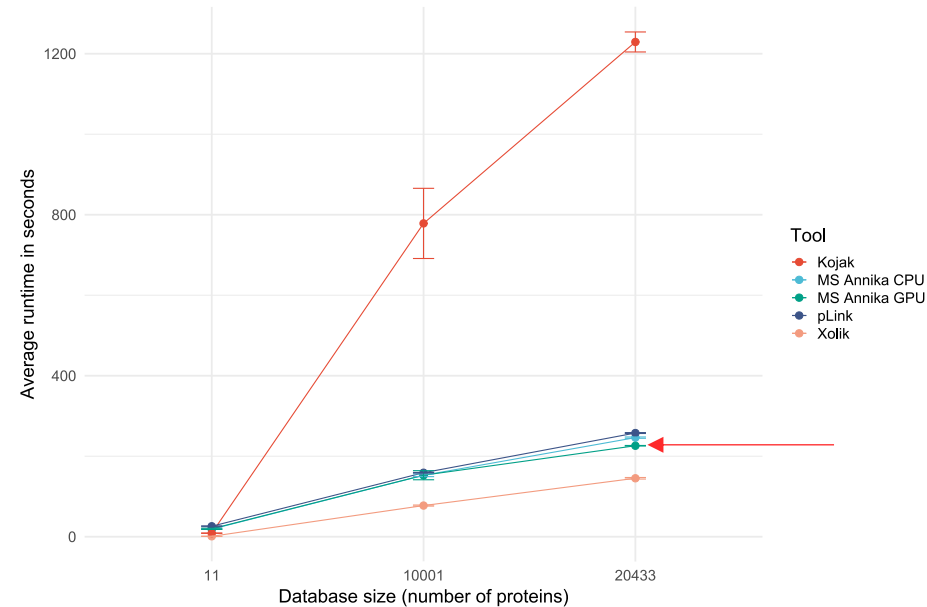
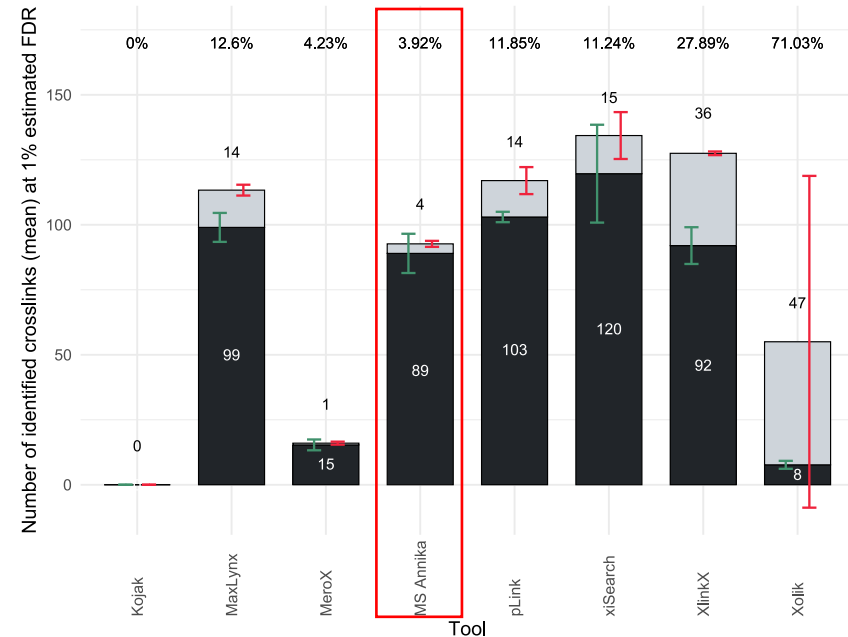
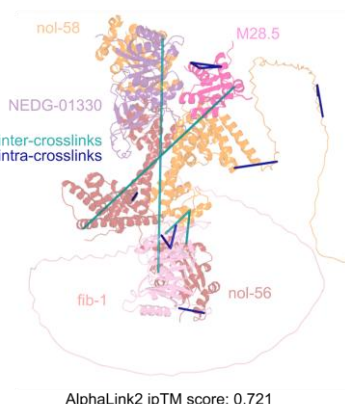


Figure 13, 14, 15

[2] M. J. Birklbauer, F. Müller, S. S. Geetha, M. Matzinger, K. Mechtler, and V. Dorfer, "Proteome-wide Non-Cleavable Crosslink Identification with MS Annika 3.0 Reveals the Structure of the *C. elegans* Box C/D Complex", *Communications Chemistry*, vol. 7, no. 1, Dec. 2024



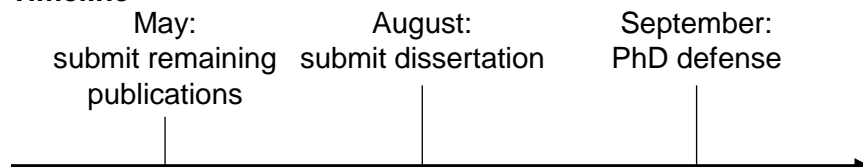
Improved structure of the *C. elegans* Box C/D RNP complex
Database size: ~26 000 proteins

RESEARCH PROGRESS

Cross-linking Publications (Published or in Preparation)

- **M. J. Birklbauer**, M. Matzinger, F. Müller, K. Mechtler, and V. Dorfer, “MS Annika 2.0 Identifies Cross-Linked Peptides in MS2-MS3-Based Workflows at High Sensitivity and Specificity”, *Journal of Proteome Research*, vol. 22, no. 9, pp. 3009-3021, Aug. 2023
- **M. J. Birklbauer**, F. Müller, S. S. Geetha, M. Matzinger, K. Mechtler, and V. Dorfer, “Proteome-wide Non-Cleavable Crosslink Identification with MS Annika 3.0 Reveals the Structure of the *C. elegans* Box C/D Complex”, *Communications Chemistry*, vol. 7, no. 1, Dec. 2024
- F. Müller, B. R. Brutiu, I. Saridakis, T. Leischner, **M. J. Birklbauer**, M. Matzinger, T. Lendl, S. Shaaban, V. Dorfer, N. Maulide, and K. Mechtler, “A Journey Towards Developing a New Cleavable Crosslinker Reagent for In-Cell Crosslinking”, in review at *Communications Chemistry*
- **M. J. Birklbauer**, F. Müller, M. Matzinger, K. Mechtler, and V. Dorfer, “Unified Down-Stream Analysis of Crosslink Results with pyXLMS”, is a planned journal article to be submitted in 2025 to *Bioinformatics*
- F. Müller, **M. J. Birklbauer**, D. Hollenstein, V. Dorfer, and K. Mechtler, “Optimized Crosslink Quantification using Data-Independent Acquisition”, is a planned journal article to be submitted in 2025 to *Nature Communications*

Timeline



Other Publications (Published or in Preparation)

- A. Grimaud, **M. J. Birklbauer**, L. Levitsky, L. Buur, V. Gorshkov, Z. Udvardy, C. Lennartsson, and V. Schwämmle, “Making Sense of Internal Ions” is a planned journal article to be submitted in 2025 to the *Journal of Proteome Research*

Software

- **MS Annika** is a cross-linking search engine capable of identifying crosslinks from both cleavable and non-cleavable reagents, and with support for a variety of acquisition workflows including multi-stage tandem acquisition. MS Annika is available at <https://github.com/hgb-bin-proteomics/MSAnnika>

Conference Contributions

- 21/03/2022 Poster at the EuBIC-MS Winter School
- 13/09/2022 Talk at APRMS 2022
- 15/01/2023 Talk and poster at the EuBIC-MS Developers Meeting
- 27/09/2023 Poster at APRMS 2023
- 15/01/2024 **Co-Organizer** and poster at the EuBIC-MS Winter School
- 25/09/2024 Talk, chair, and „**Best Presentation**“ winner at APRMS 2024

Other Research Activities

- Reviewer for *Analytical Chemistry*
- Member of the APMA junior board since 2023