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

PhD Pre-Defense – Micha J. Birklbauer

27th February 2025

BIOINFORMATICS
RESEARCH GROUP
HAGENBERG

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

in close cooperation with

JOHANNES KEPLER
UNIVERSITÄT LINZ

UNIVERSITY
OF APPLIED SCIENCES
UPPER AUSTRIA

Der Wissenschaftsfonds.

IMP
Research Institute of
Molecular Pathology

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

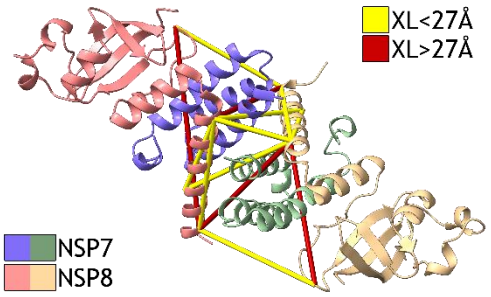


Figure 1

CROSS-LINKING MASS SPECTROMETRY

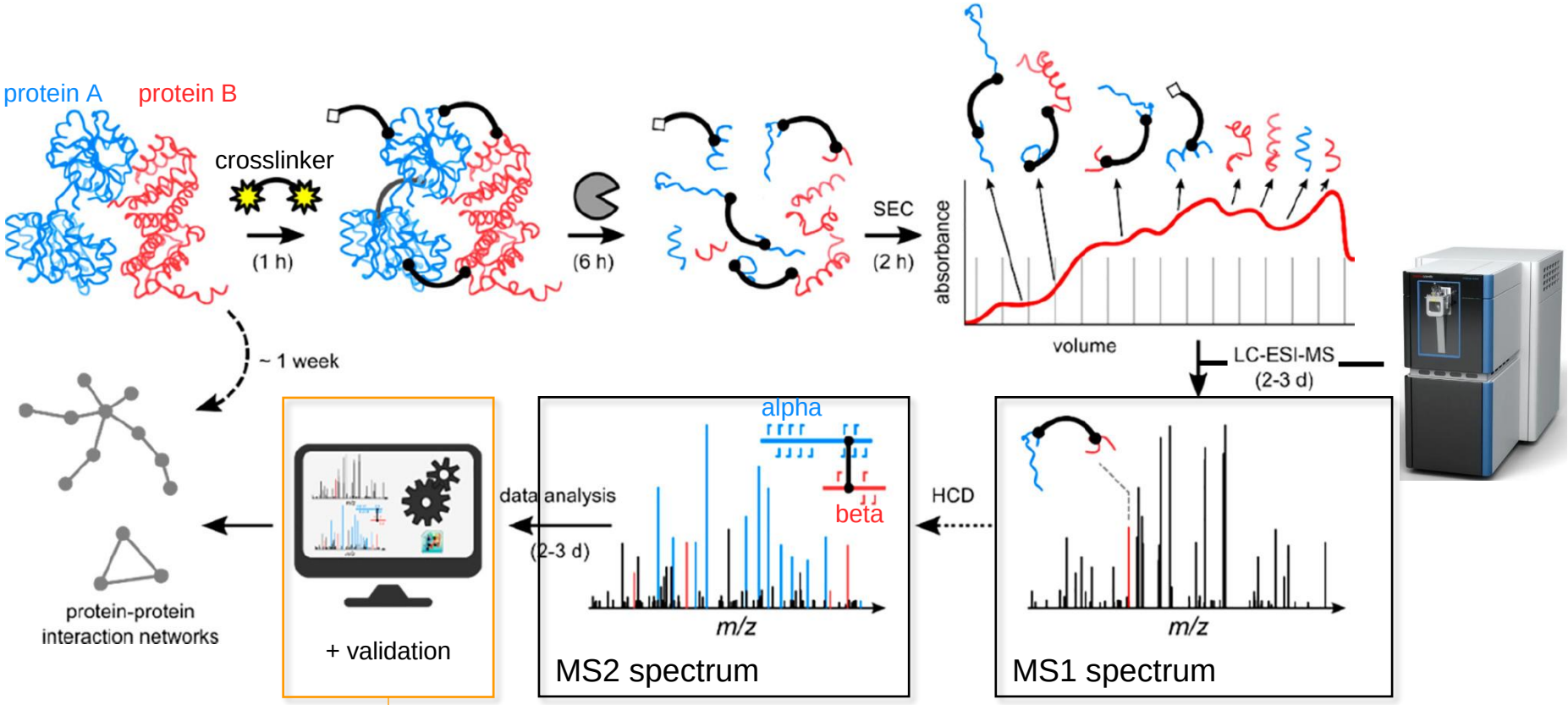


Figure 2*

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

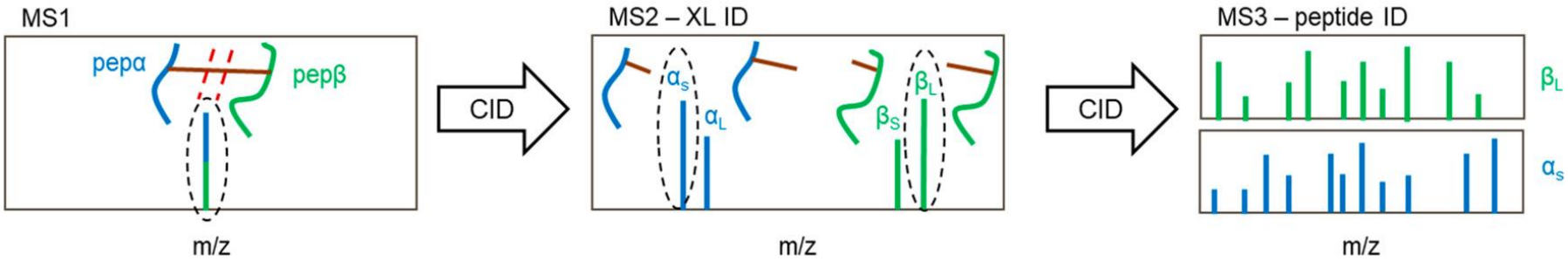


Figure 3*

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

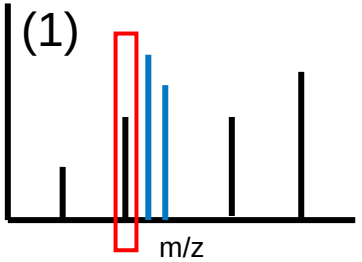


Figure 4

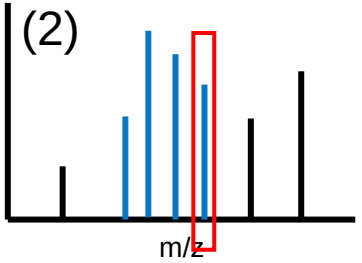


Figure 5

*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

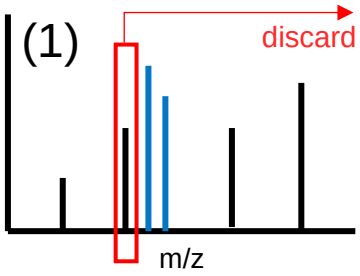


Figure 6

INCORRECT C12 ASSIGNMENT

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

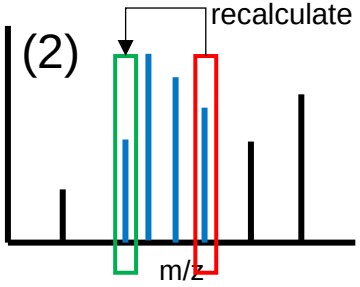


Figure 7

MATCHING AND COMBINING MS2 / MS3

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

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

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

MS2-MS3-BASED CROSSLINK IDENTIFICATION

INCORRECT PRECURSOR ISOLATION

- ❑ Verify precursor ion by comparison to MS2 spectrum
- ❑ If MS2 [illegible]

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

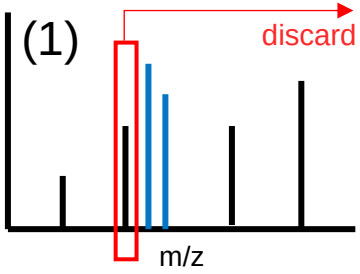


Figure 6

INCORRECT C12 ASSIGNMENT

- ❑ Re-calculate C12 mass from MS2 spectrum based on [illegible]

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

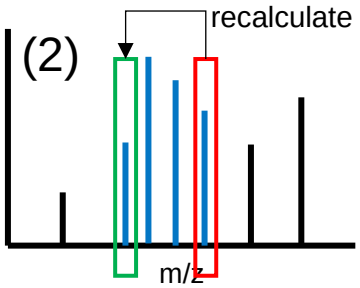


Figure 7

MA

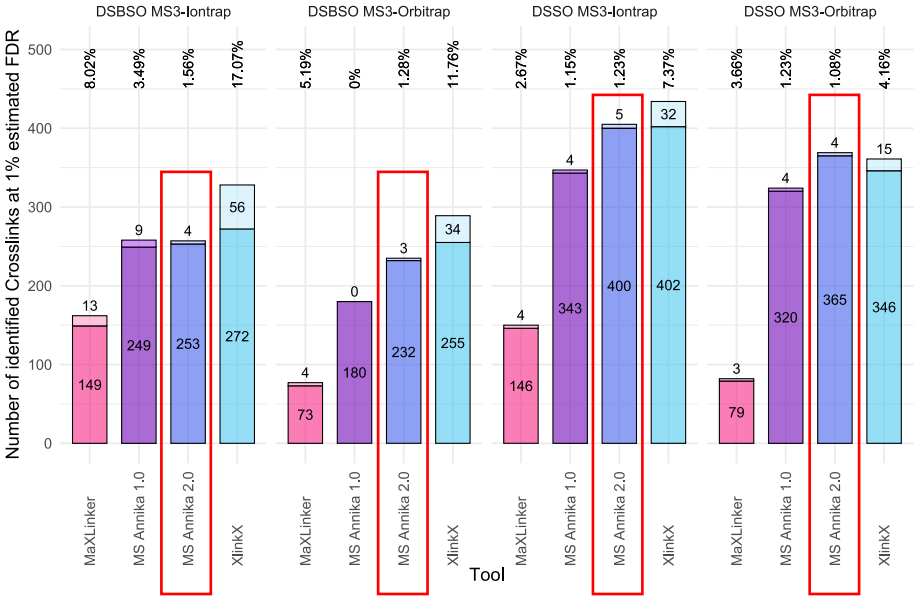
- ❑ [illegible]
- ❑ [illegible]

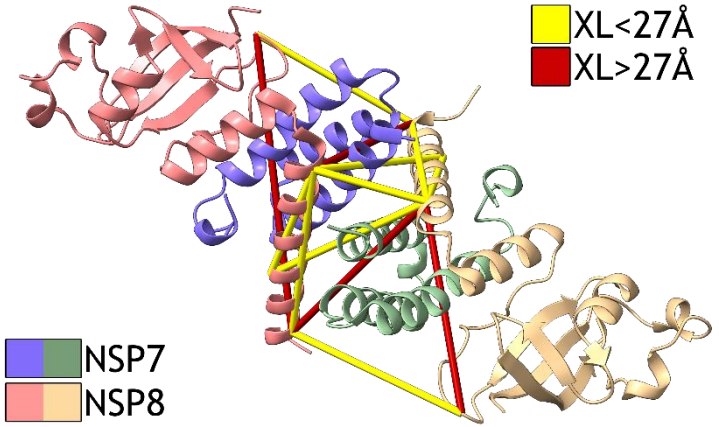
Whe[illegible]

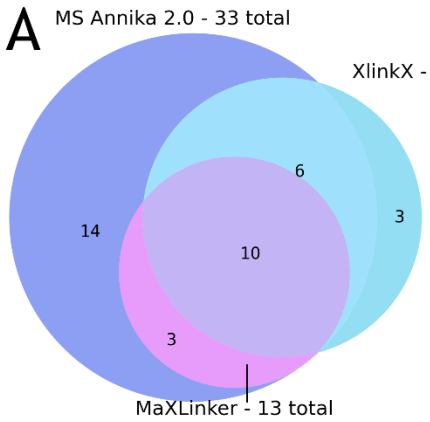
p is a user-defined boost parameter (default 20)
n is the number of unique scan numbers that peptide has been identified in

MS2-MS3-BASED CROSSLINK IDENTIFICATION

- Algorithms implemented in **MS Annika 2.0** [1]







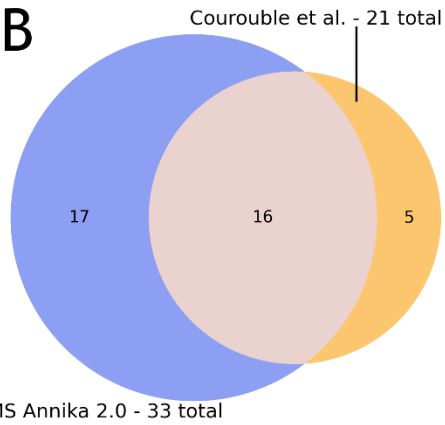


Figure 8, 9, 10

[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

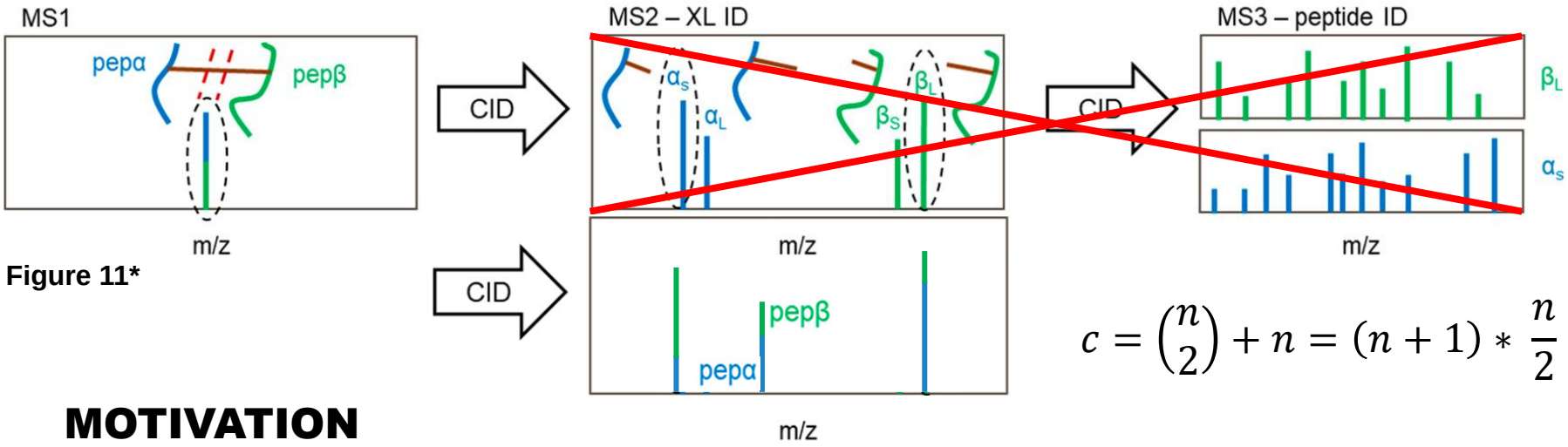


Figure 11*

$$c = \binom{n}{2} + n = (n+1) * \frac{n}{2}$$

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

EFFICIENT SEARCH ALGORITHM

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

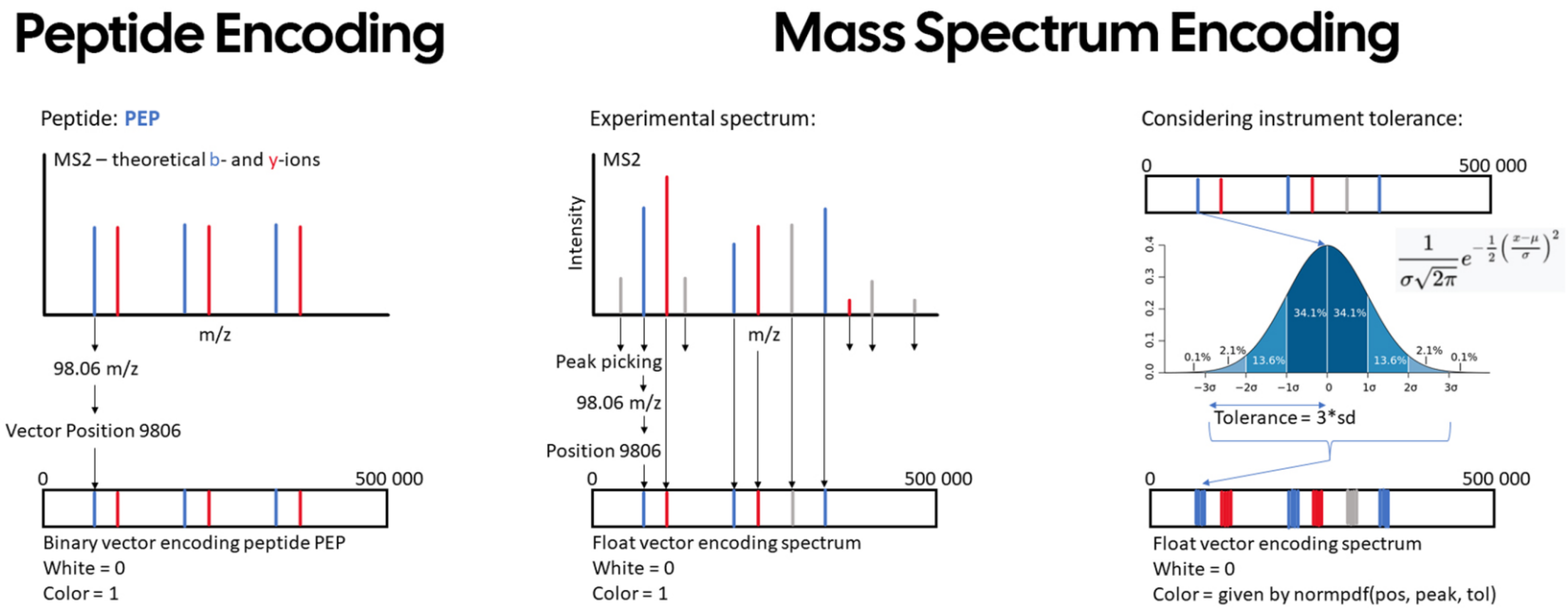
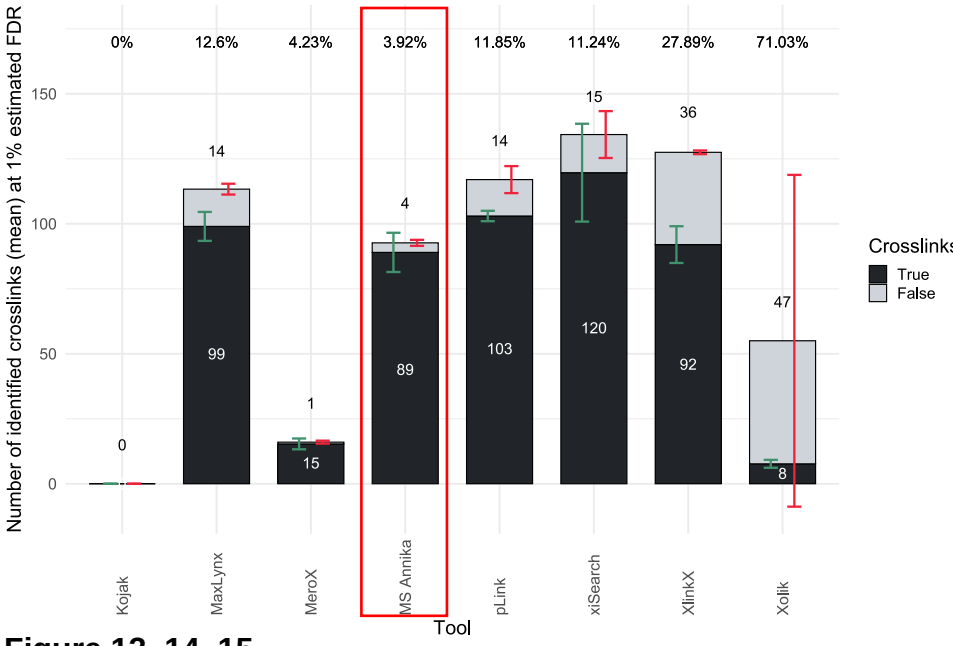


Figure 12

NON-CLEAVABLE CROSSLINK IDENTIFICATION

- Algorithms implemented in **MS Annika 3.0** [2]



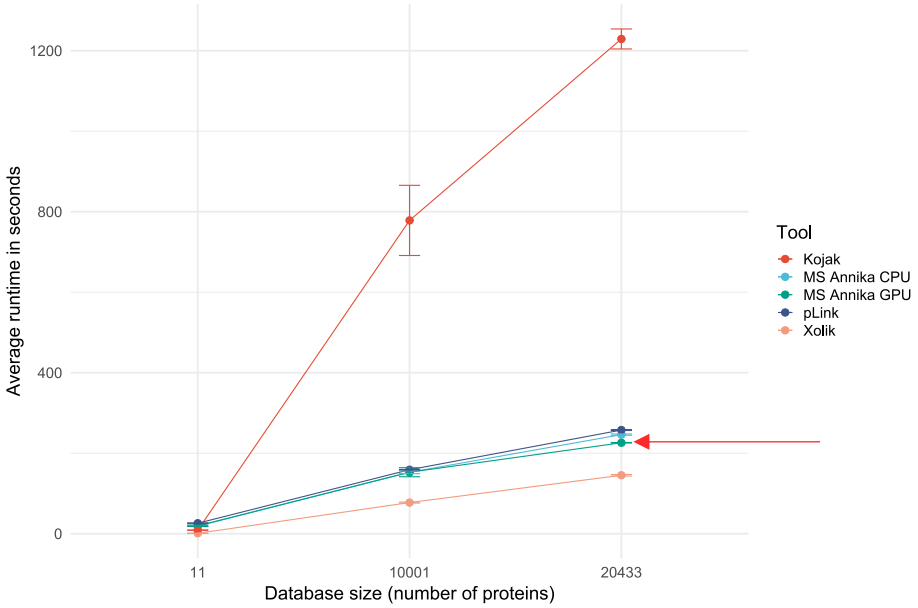
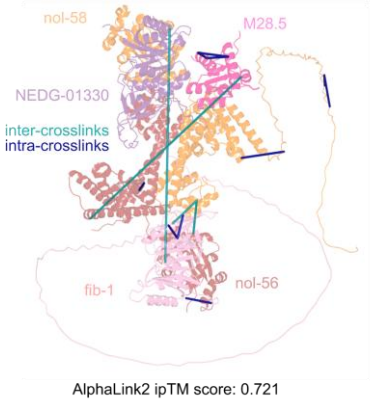


Figure 13, 14, 15



Improved structure of the *C. elegans* Box C/D RNP complex

Database size: ~26 000 proteins

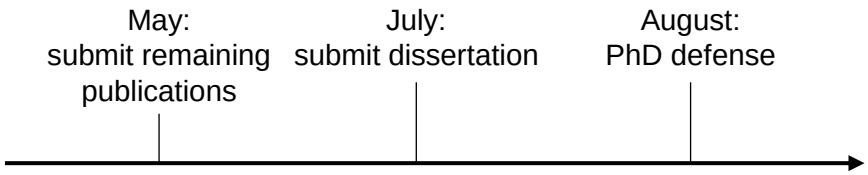
[2] **M. J. Birklbauer**, F. Müller, S. S. Geetha, M. Matzinger, K. Mechtler, and V. Dorfer, "Proteome-wide Non-Cleavable Crosslink Identification with MS Annika 3.0 Reveals the Structure of the C. elegans Box C/D Complex", *Communications Chemistry*, vol. 7, no. 1, Dec. 2024

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