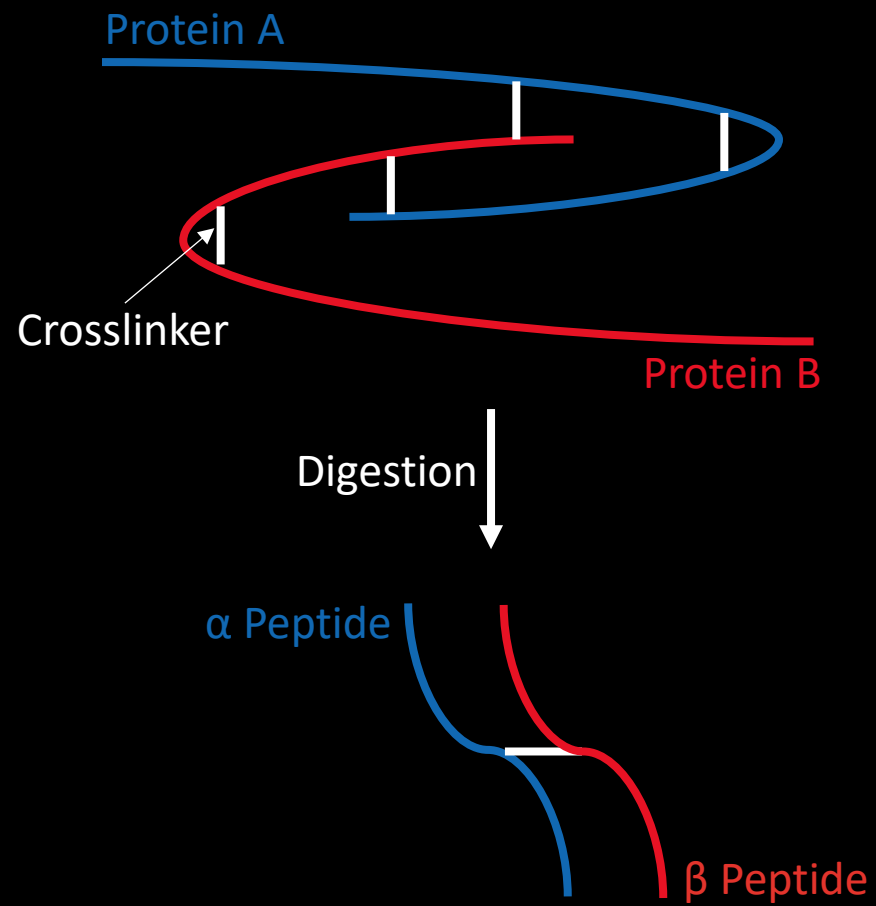
Solving big problems on small computers

alternative title:
Proteome-wide Non-Cleavable Crosslink Identification Using Sparse Matrix Multiplication with MS Annika 3.0

Micha Birklbauer, September 2024

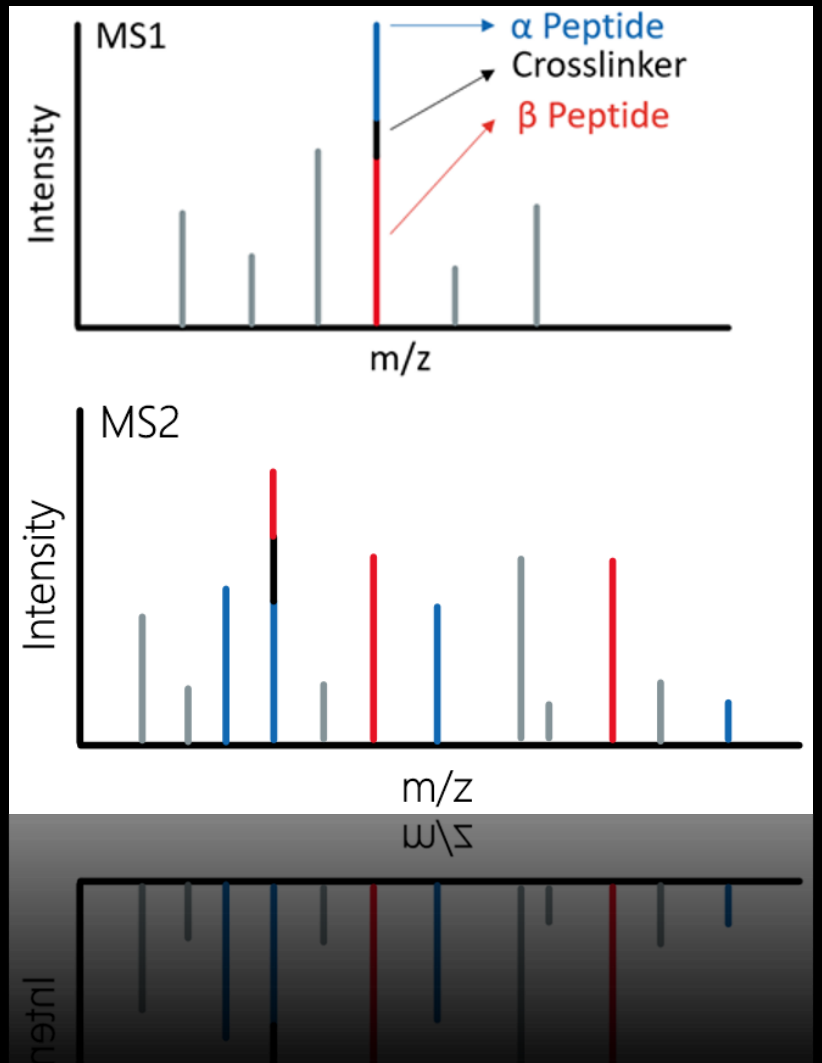
Introduction: Cross-linking

- 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 analyses and to study protein-interaction networks



The Problem

- In non-cleavable cross-linking experiments we cannot infer the masses of the two individual peptides
- We know the mass of the complete cross-linked entity (precursor)
- For identification we need to consider any combination of two peptides that make up the precursor mass



The n^2 Problem

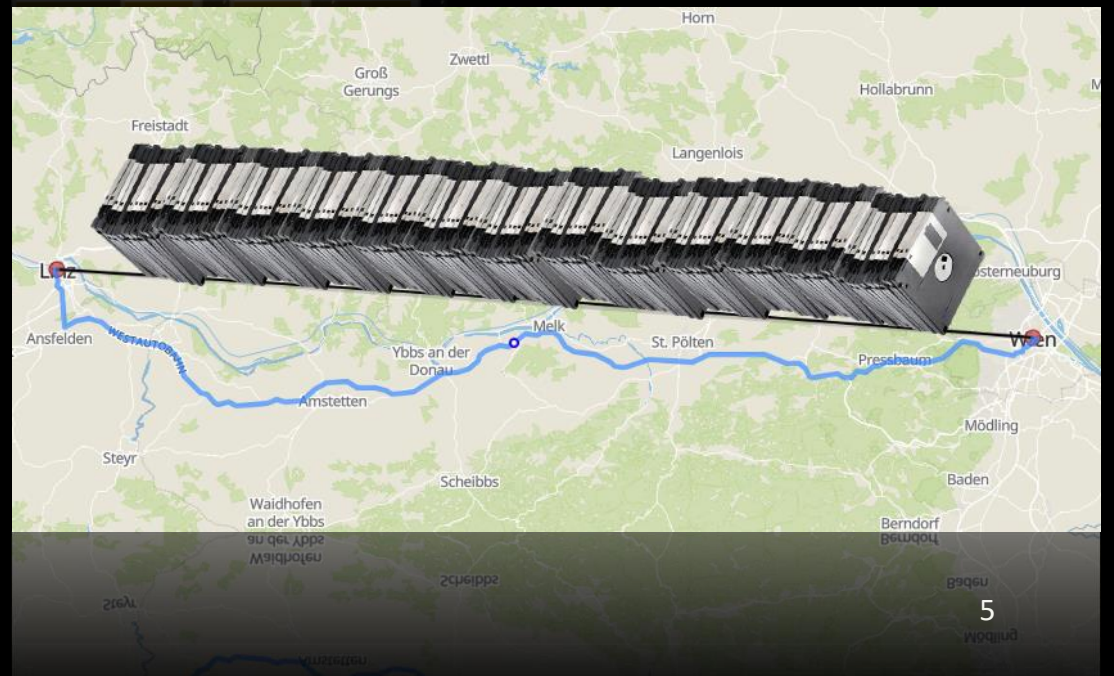
- Considering every combination of peptides leads to very large search spaces
- Consider a database of *n* peptides, the number of possible combinations *c* would be:
- $c = \binom{n}{2} + n = (n+1) * \frac{n}{2}$
- For big *n* this can be considered $O(n^2)$ complexity

- For the human proteome (SwissProt, 20 337 p), trypsin digest, maximum 3 missed cleavages, 5 – 30 peptide length:
- n = 2 749 058
- c = 3 778 661 318 211
- one combination = 16 bytes
- Total = ?

54.98676 TB

- This would roughly fit on three enterprise-level hard disks

- Or on enough 3.5“ floppy disks to cover the distance from Linz to Vienna



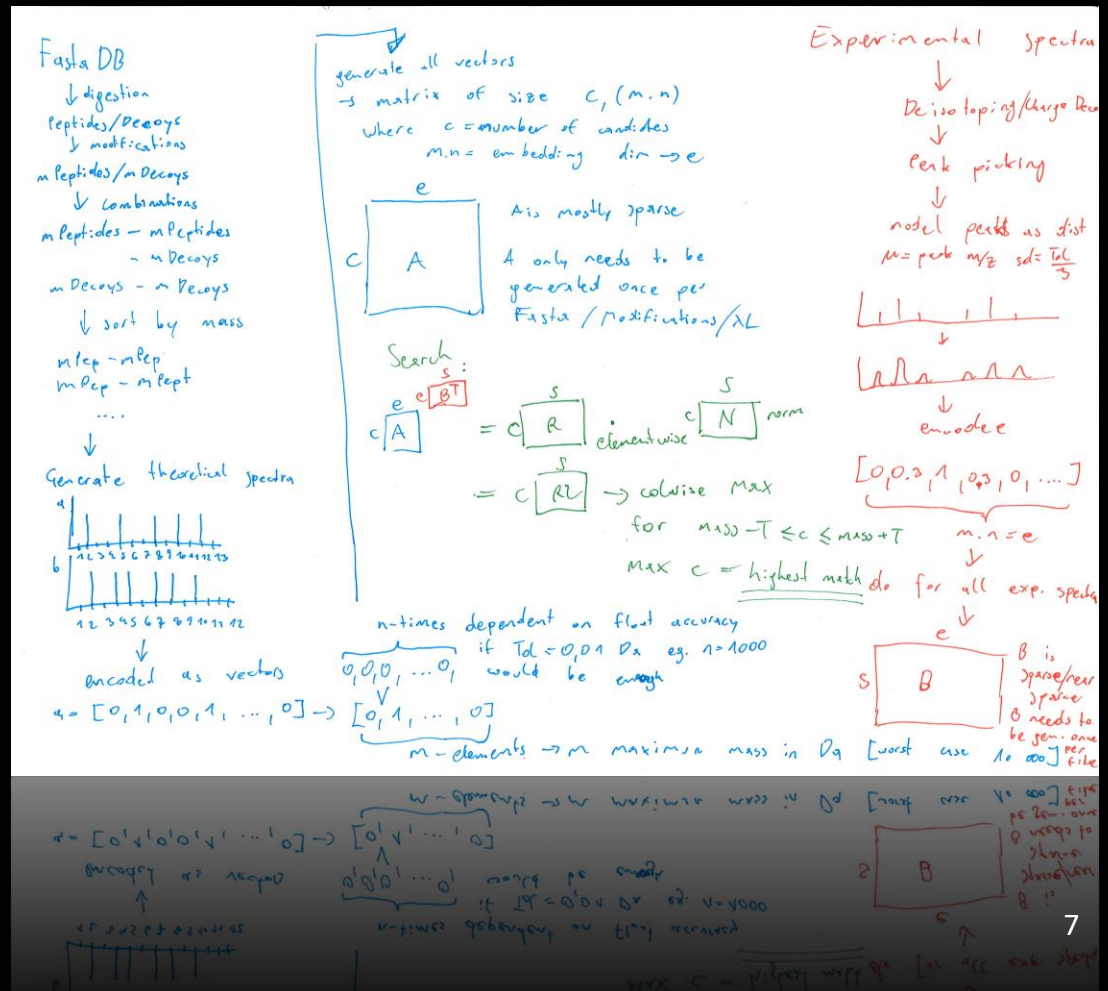
Tackling the n^2 Problem

- Three different options:
  - Cry
  - Reducing the search space
  - Speeding up the search process so more combinations can be considered

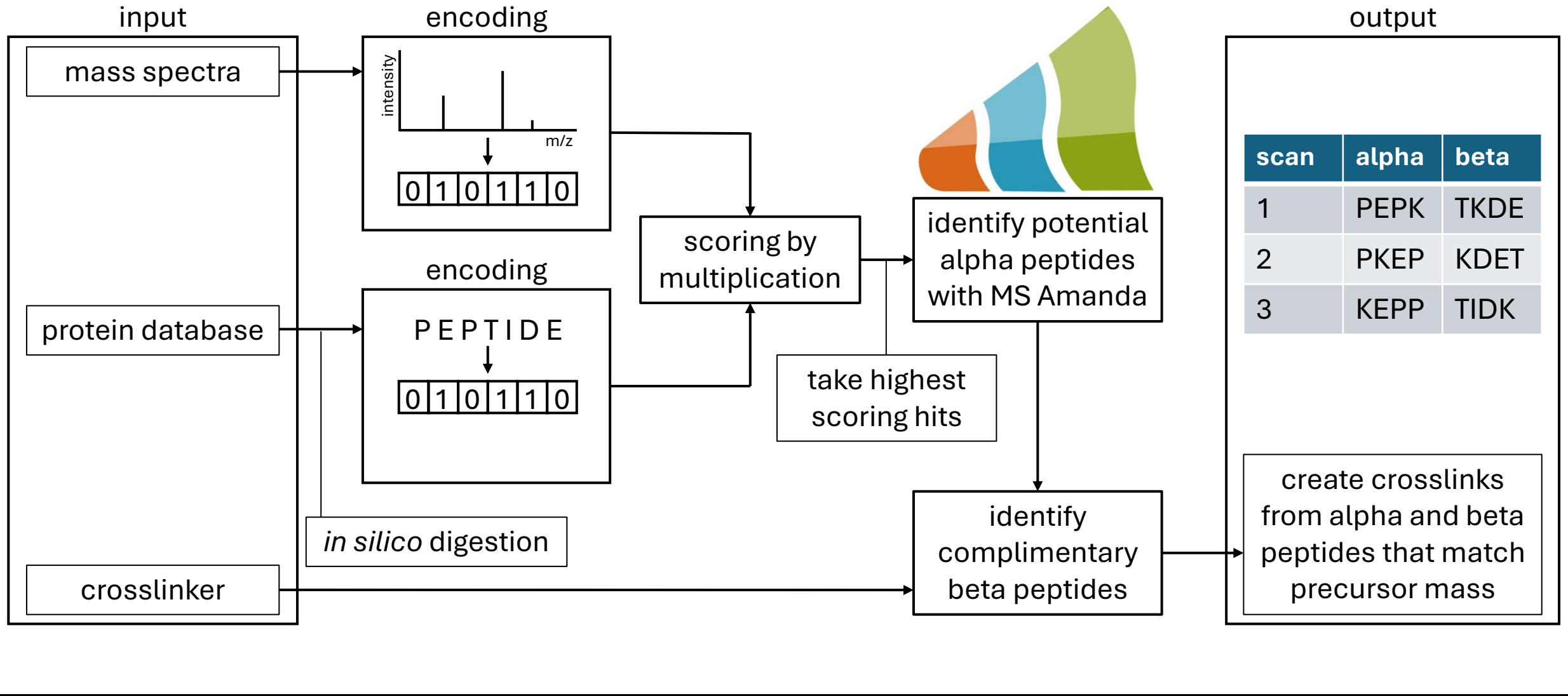
source: giphy.com

The Idea

- Reduction of search space:
  - Identify one of two peptides first
  - → Limits the number of combinations to consider
- Identification of the peptide by a fast approximate search:
  - Encoding of peptides and spectra as sparse vectors
  - Scoring purely based on addition and multiplication
  - Adhering to the SIMD paradigm



MS Annika 3.0

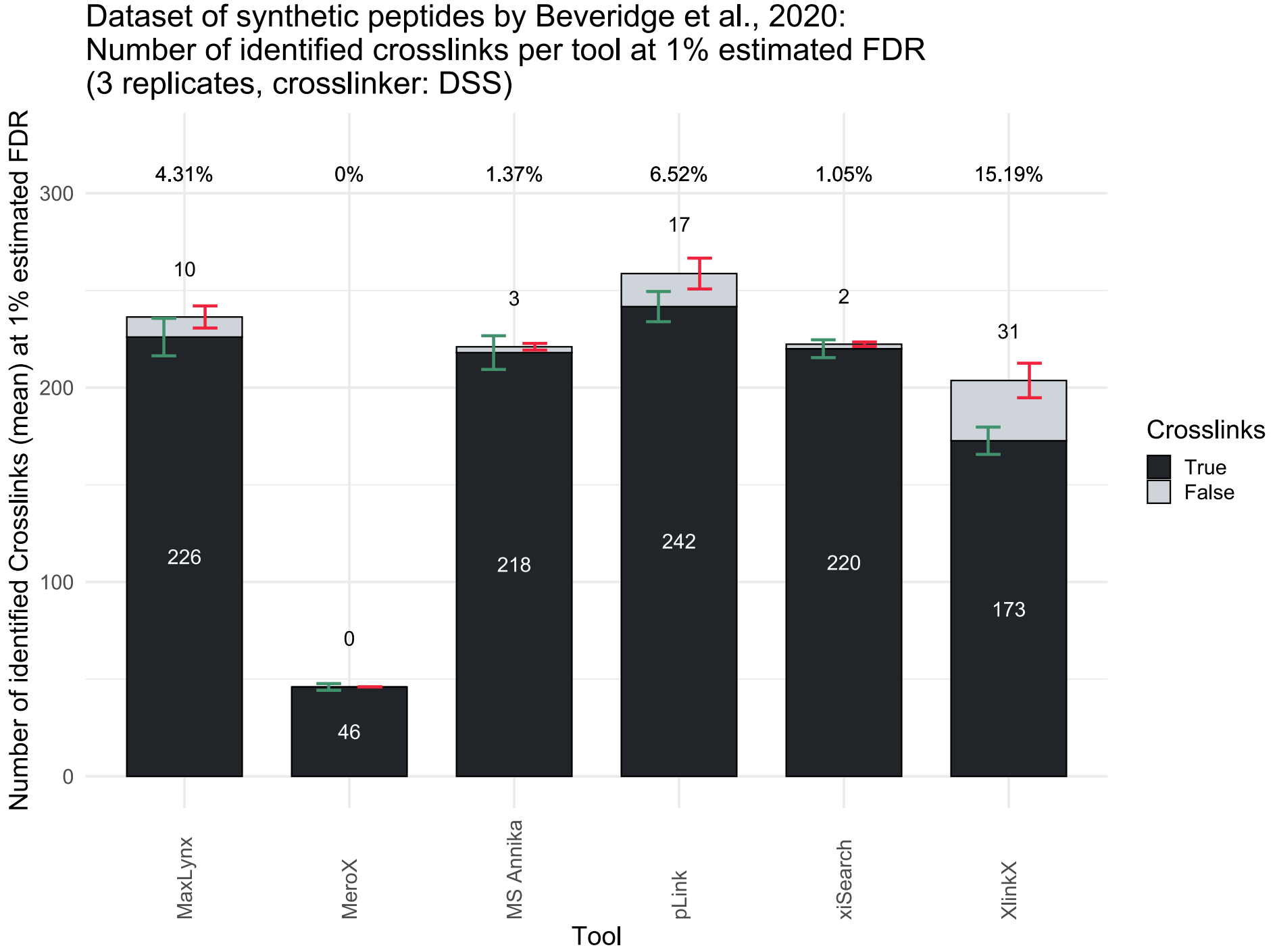

input
mass spectra
protein database
crosslinker
encoding
intensity
m/z
0 1 0 1 1 0
encoding
P E P T I D E
0 1 0 1 1 0
scoring by multiplication
identify potential alpha peptides with MS Amanda
take highest scoring hits
in silico digestion
identify complimentary beta peptides
output
scan alpha beta
1 PEPK TKDE
2 PKEP KDET
3 KEPP TIDK
create crosslinks from alpha and beta peptides that match precursor mass

The End Product

Quite literally…

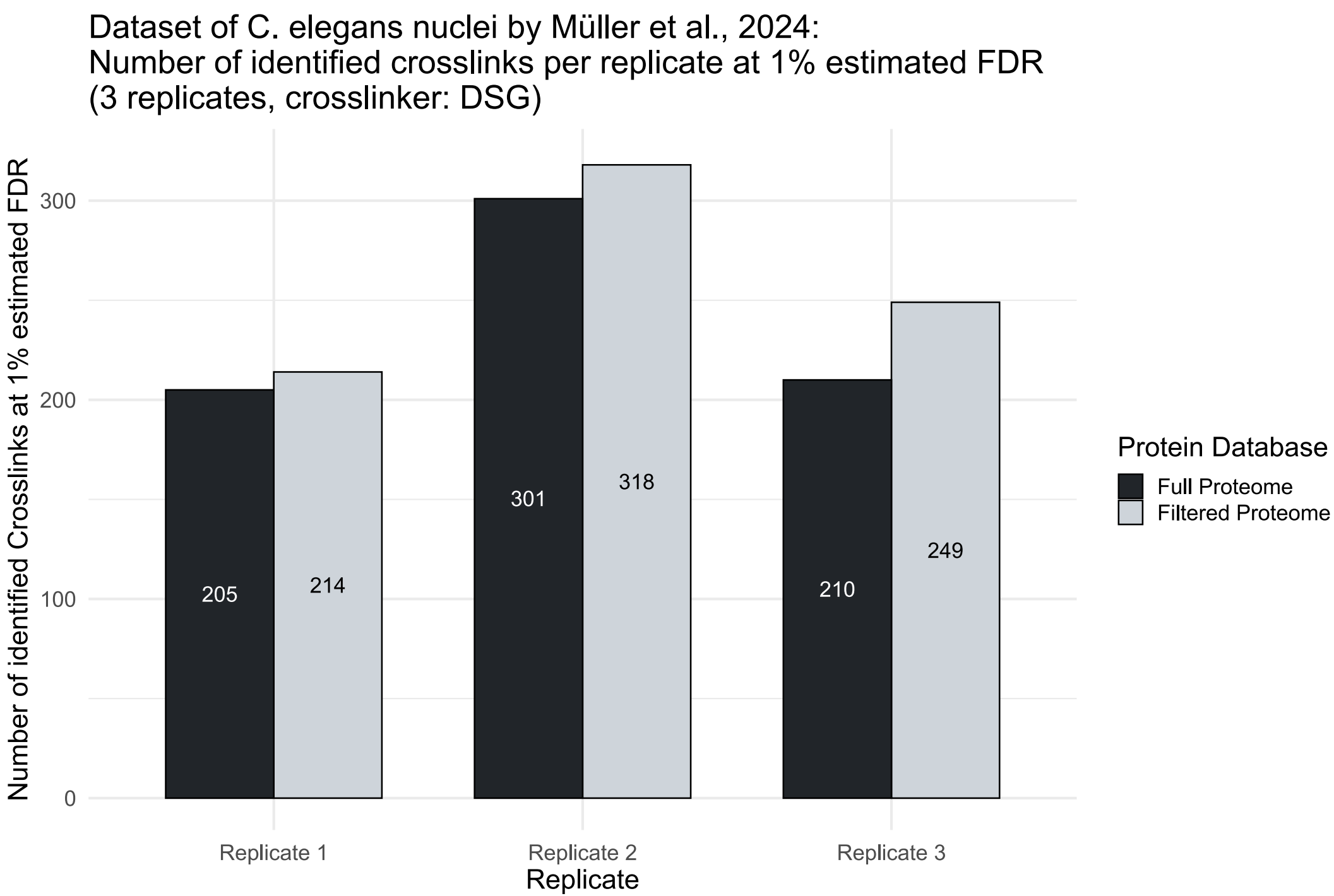
Comparison of MS Annika 3.0 to Other Search Engines

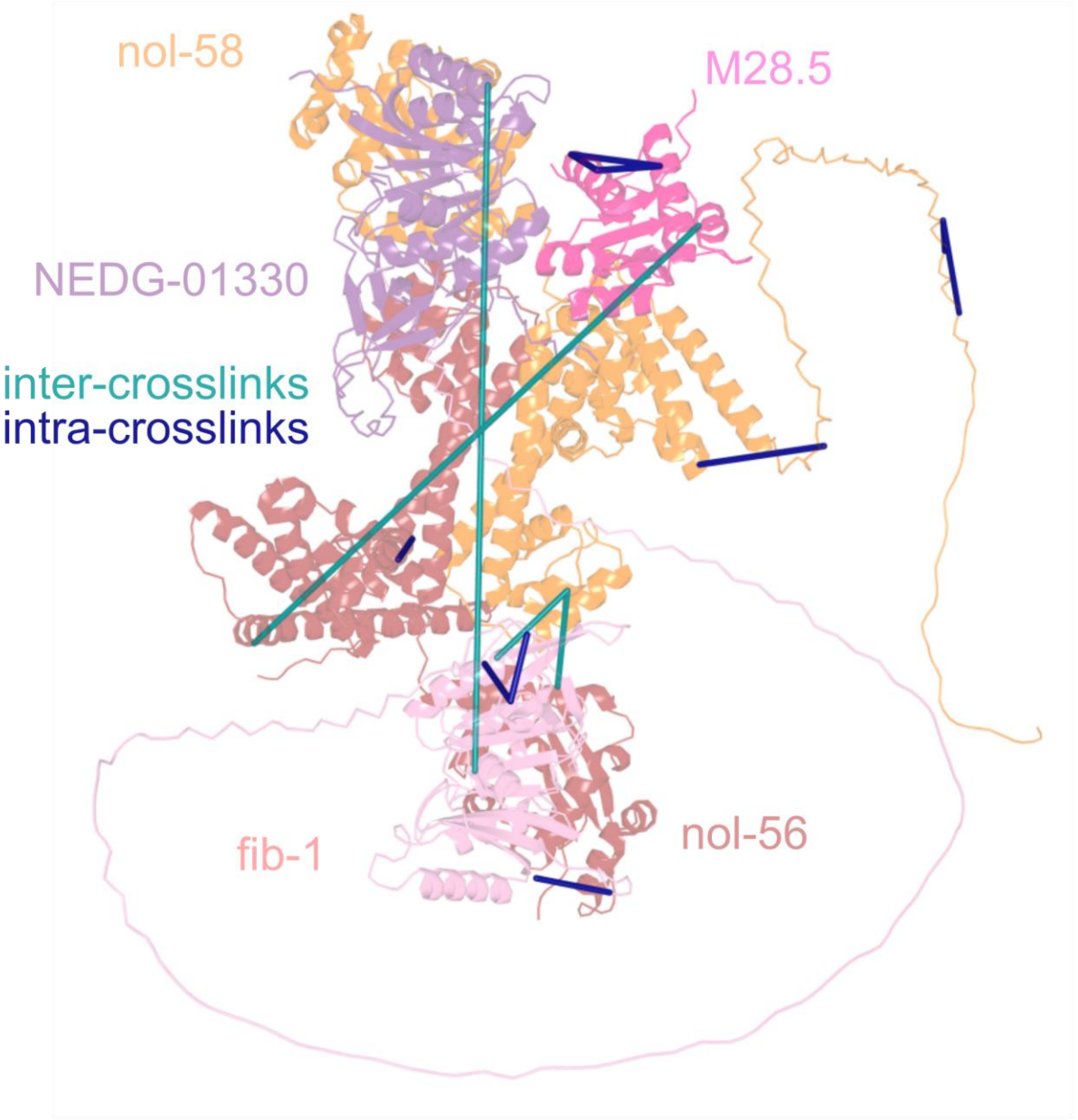
- Dataset by Beveridge *et al.* → PXD014337
- Synthetic peptides cross-linked with DSS
  → allows calculation of “true“ FDR
  → allows assessment of which crosslinks are true positive identifications and which are false positive identifications
- Comparison against MaxLynx (MaxQuant), MeroX, pLink, xiSearch and XlinkX
- **Goal:** Identify as many true positive crosslinks as possible while staying close to 1% FDR


Dataset of synthetic peptides by Beveridge et al., 2020:
Number of identified crosslinks per tool at 1% estimated FDR
(3 replicates, crosslinker: DSS)
Number of identified Crosslinks (mean) at 1% estimated FDR
300
200
100
0
4.31%
0%
1.37%
6.52%
1.05%
15.19%
10
17
3
2
31
226
0
46
218
242
220
173
MaxLynx
MeroX
MS Annika
pLink
xiSearch
XlinkX
Tool
Crosslinks
True
False

The Real Test: Large Scale Proteome-Wide Crosslink Identification

- Mass spectra from *C. elegans* nuclei samples
- Samples cross-linked with DSG
- Comparison of two searches:
  - Searched against database of most abundant proteins (n ≈ 3000)
  - Searched against the full *C. elegans* proteome using all sequences available in UniProt (n ≈ 26 000)
- Validated for 1% FDR with xiFDR


Dataset of C. elegans nuclei by Müller et al., 2024:
Number of identified crosslinks per replicate at 1% estimated FDR
(3 replicates, crosslinker: DSG)
Number of identified Crosslinks at 1% estimated FDR
300
200
100
0
205
214
301
318
210
249
Replicate 1
Replicate 2
Replicate 3
Replicate
Protein Database
Full Proteome
Filtered Proteome



AlphaLink2 ipTM score: 0.721

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

Conclusions

- MS Annika 3.0 can successfully tackle proteome-wide non-cleavable crosslink studies
- The implemented algorithm is super efficient, allowing proteome-wide searches on commodity hardware
- This will allow researchers to:
  - Perform non-cleavable crosslink studies of complex samples that were previously unfeasible
  - Re-analyze published crosslink data with bigger protein databases, potentially uncovering new biological insights
- The algorithm presents a transferable solution for big search space problems

Acknowledgements

- Fränze Müller, Sowmya Geetha, Manuel Matzinger & Karl Mechtler
- Viktoria Dorfer
- FWF
- The Eigen developers & community
- My research group 😊

UNIVERSITY OF APPLIED SCIENCES UPPER AUSTRIA

IMP Research Institute of Molecular Pathology

FWF Der Wissenschaftsfonds.

Discussion

source: giphy.com

Pre-Print:

MS Annika:
github.com/hgb-bin-proteomics/MSAnnika

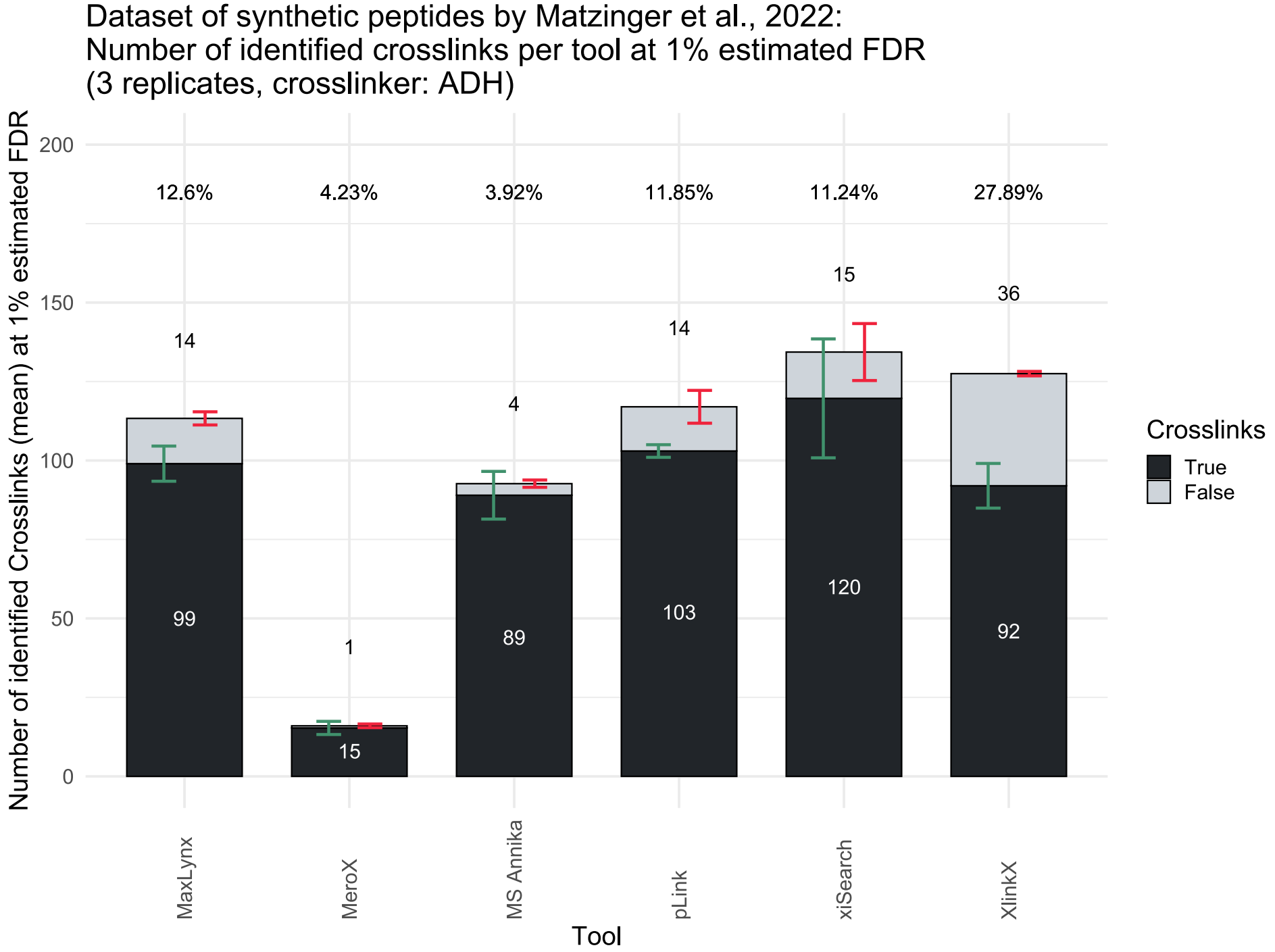
Contact:
micha.birklbauer@fh-hagenberg.at
github.com/michabirklbauer
michabirklbauer.me

Appendix A

More Comparisons

Comparison of MS Annika 3.0 to Other Search Engines

- Dataset by Matzinger *et al.* → PXD029252
- Synthetic peptides cross-linked with ADH
  → allows calculation of “true“ FDR
  → allows assessment of which crosslinks are true positive identifications and which are false positive identifications
- Comparison against MaxLynx (MaxQuant), MeroX, pLink, xiSearch and XlinkX
- **Goal:** Identify as many true positive crosslinks as possible while staying close to 1% FDR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Number of identified crosslinks per tool at 1% estimated FDR
(3 replicates, crosslinker: ADH)
Number of identified Crosslinks (mean) at 1% estimated FDR
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150
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50
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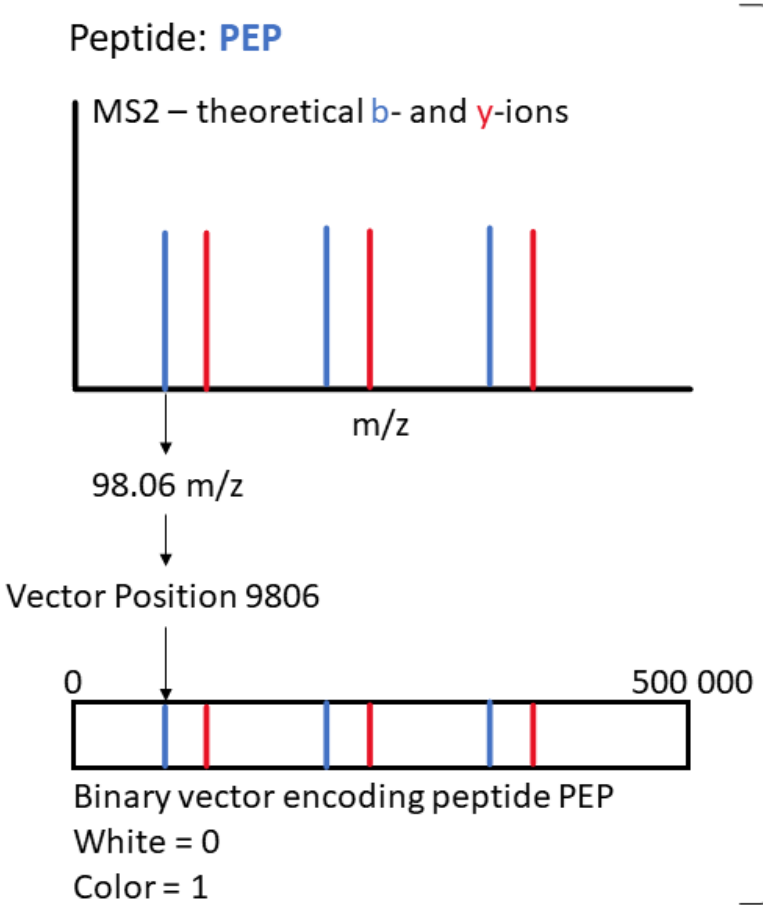
12.6%
4.23%
3.92%
11.85%
11.24%
27.89%
14
99
1
15
4
89
14
103
15
120
36
92
MaxLynx
MeroX
MS Annika
pLink
xiSearch
XlinkX
Tool
Crosslinks
True
False

Appendix B

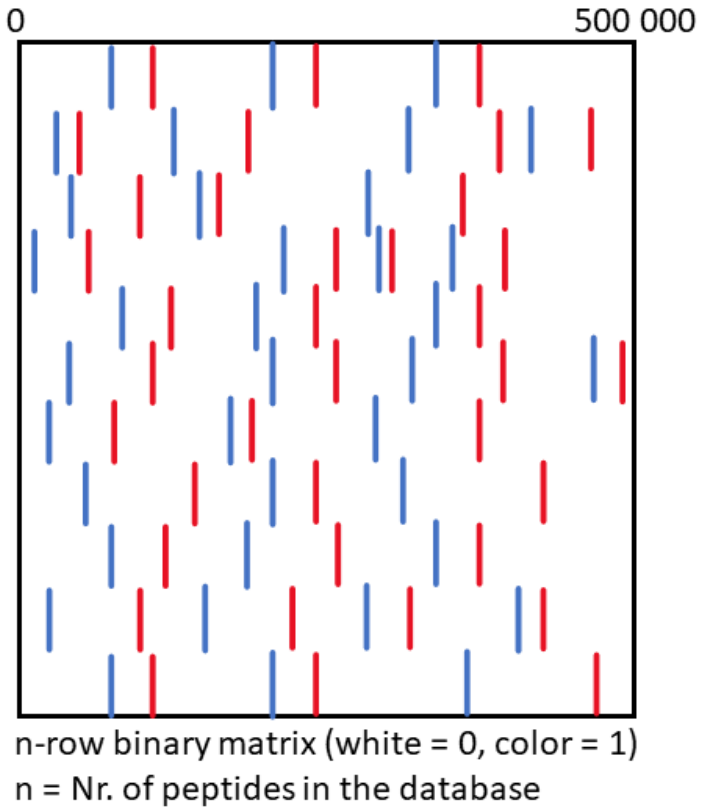
Matrix Encoding

Vectorizing example

Peptide: **PEP**

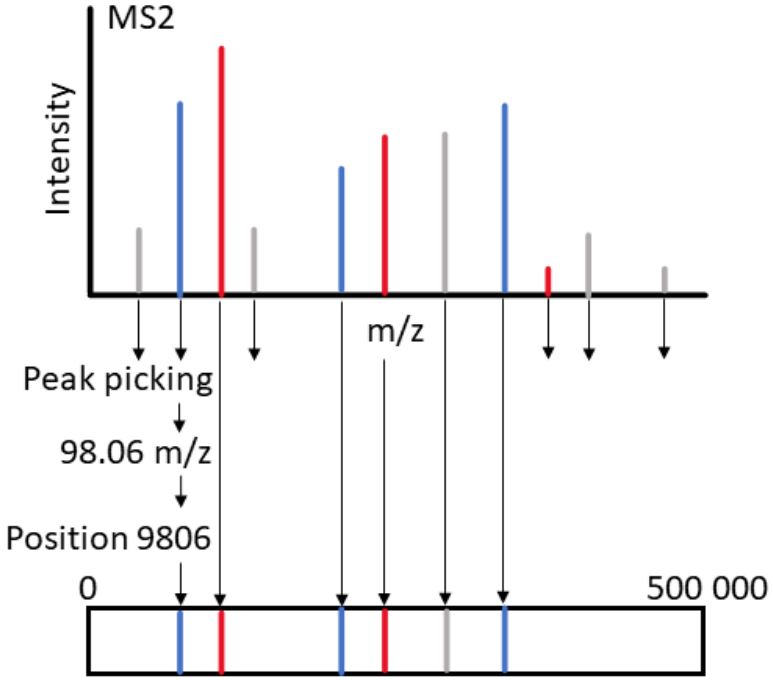


Do for every peptide in database:



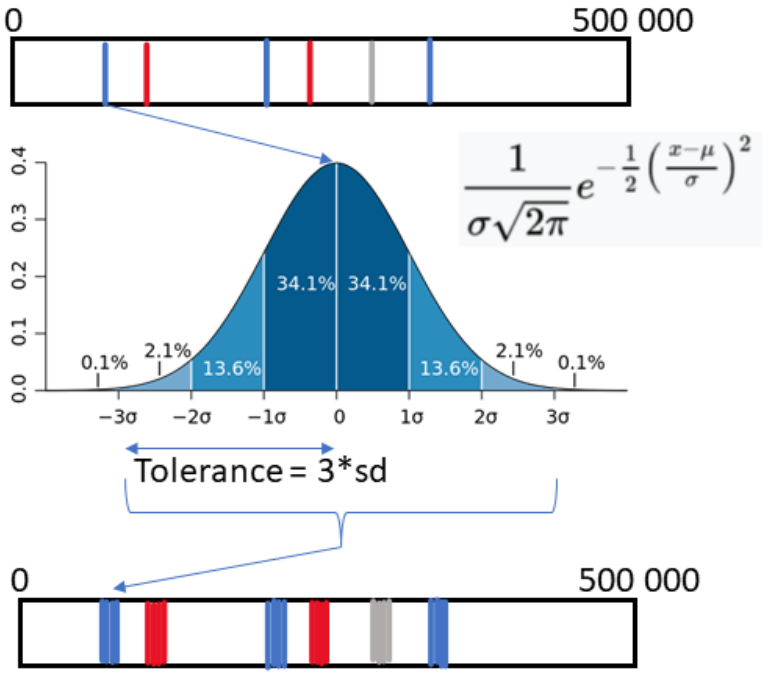
Vectorizing example

Experimental spectrum:



Float vector encoding spectrum
White = 0
Color = 1

Considering instrument tolerance:



Float vector encoding spectrum
White = 0
Color = given by normpdf(pos, peak, tol)

Vectorizing example - search

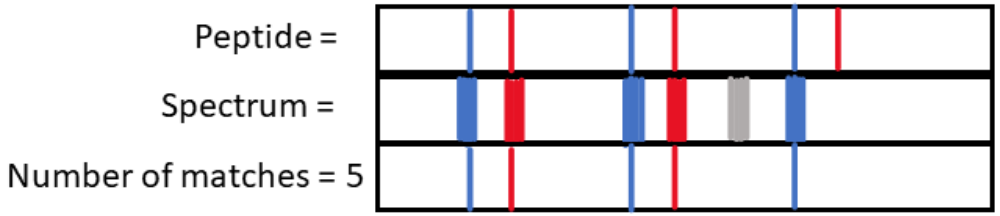
Peptide: **PEP**

Binary vector encoding peptide PEP

Spectrum:

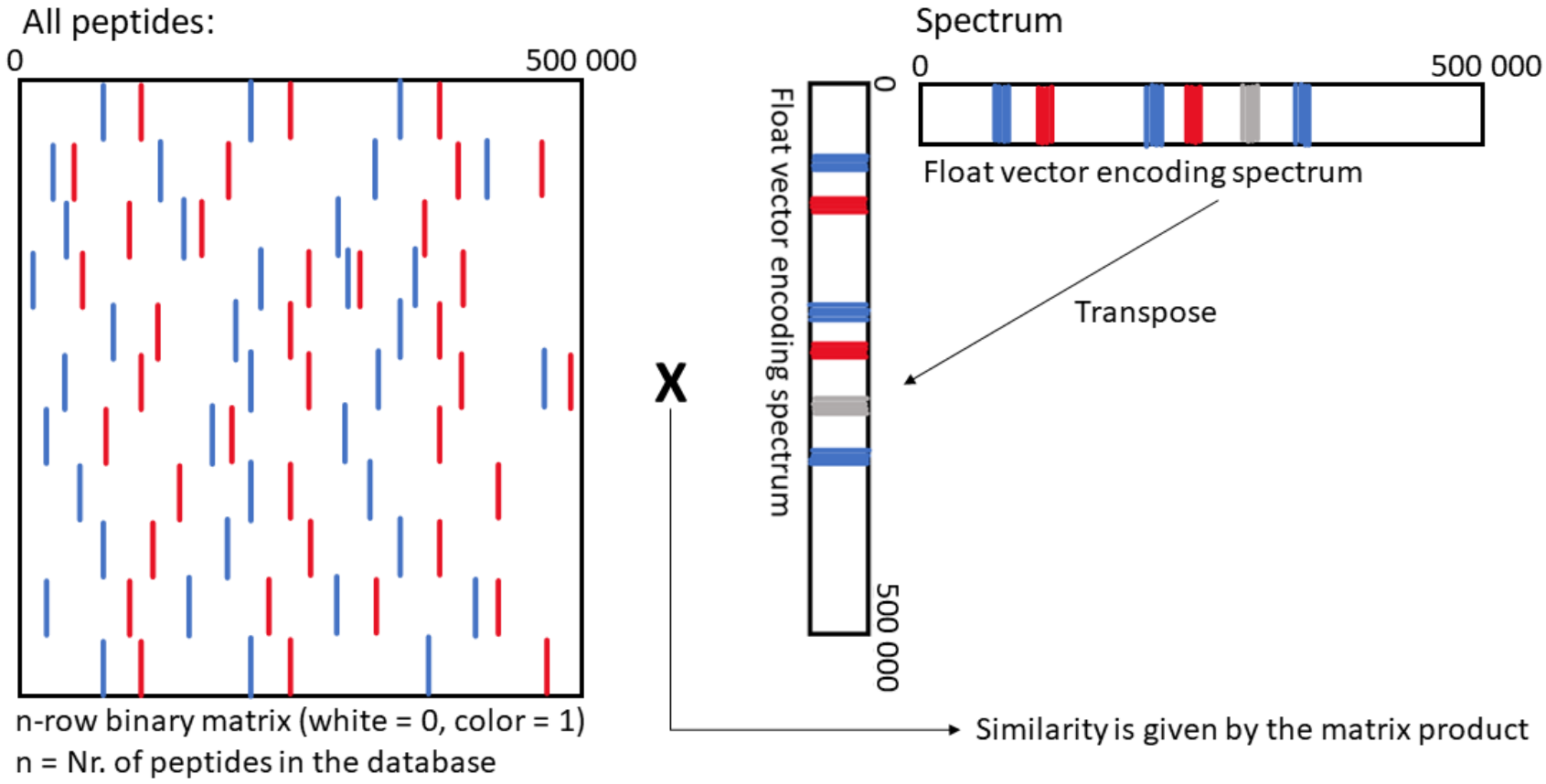
Float vector encoding spectrum

Similarity is given by the dot product of the two vectors
= the sum of overlapping positions
= the number of peaks that match

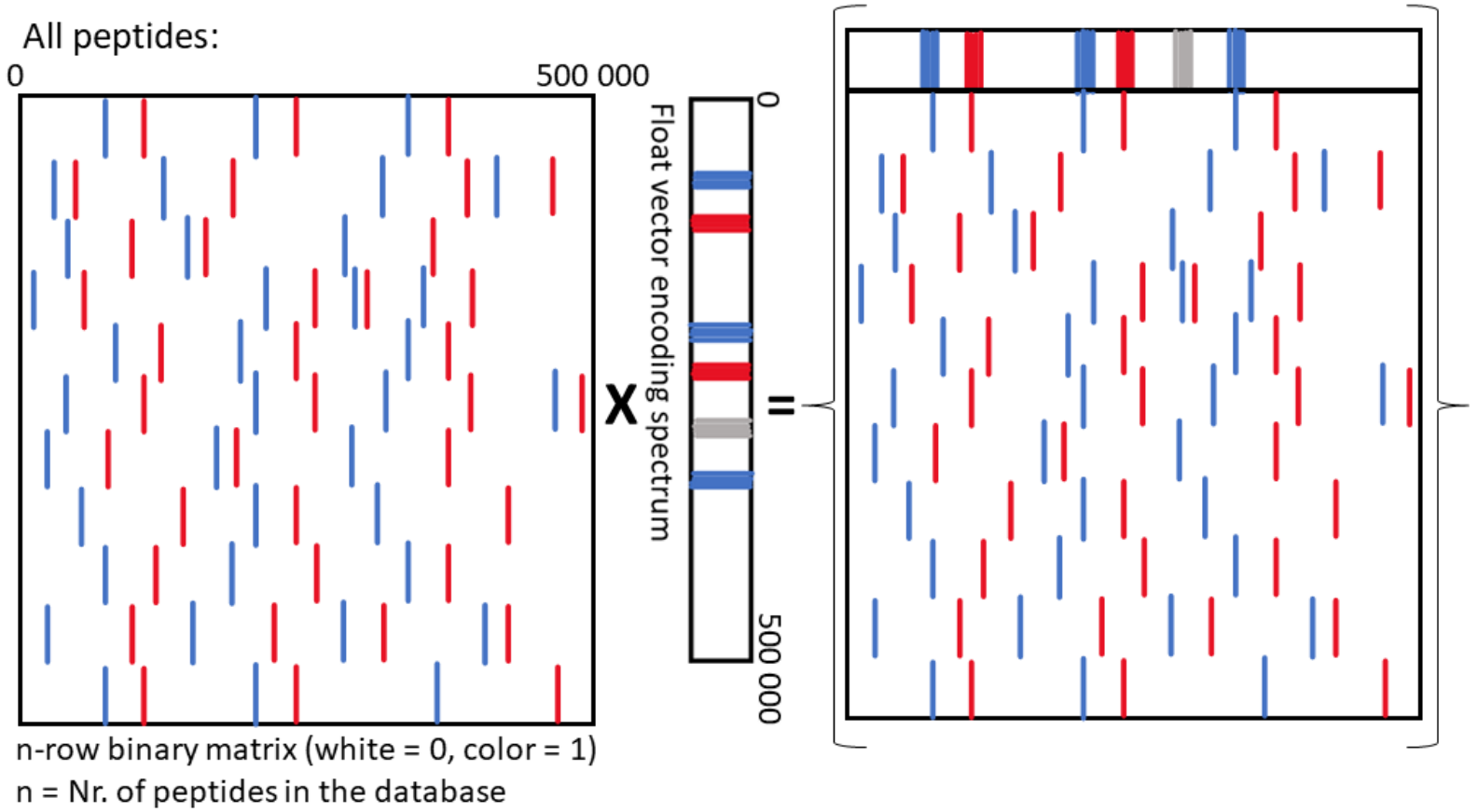


(Normalization = Number of matches / Number of ions of PEP = 5/6 = 0.8)

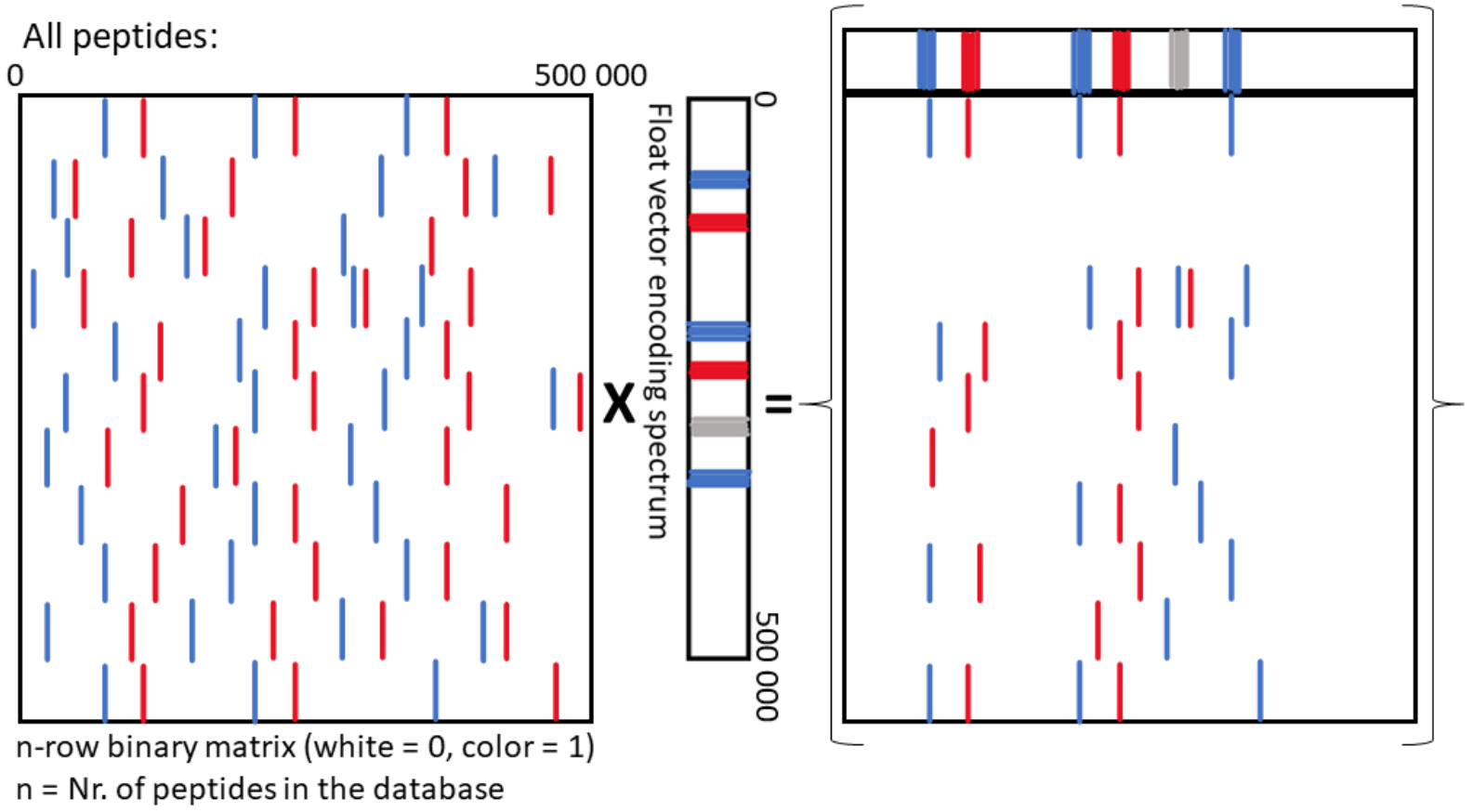
Vectorizing example - search



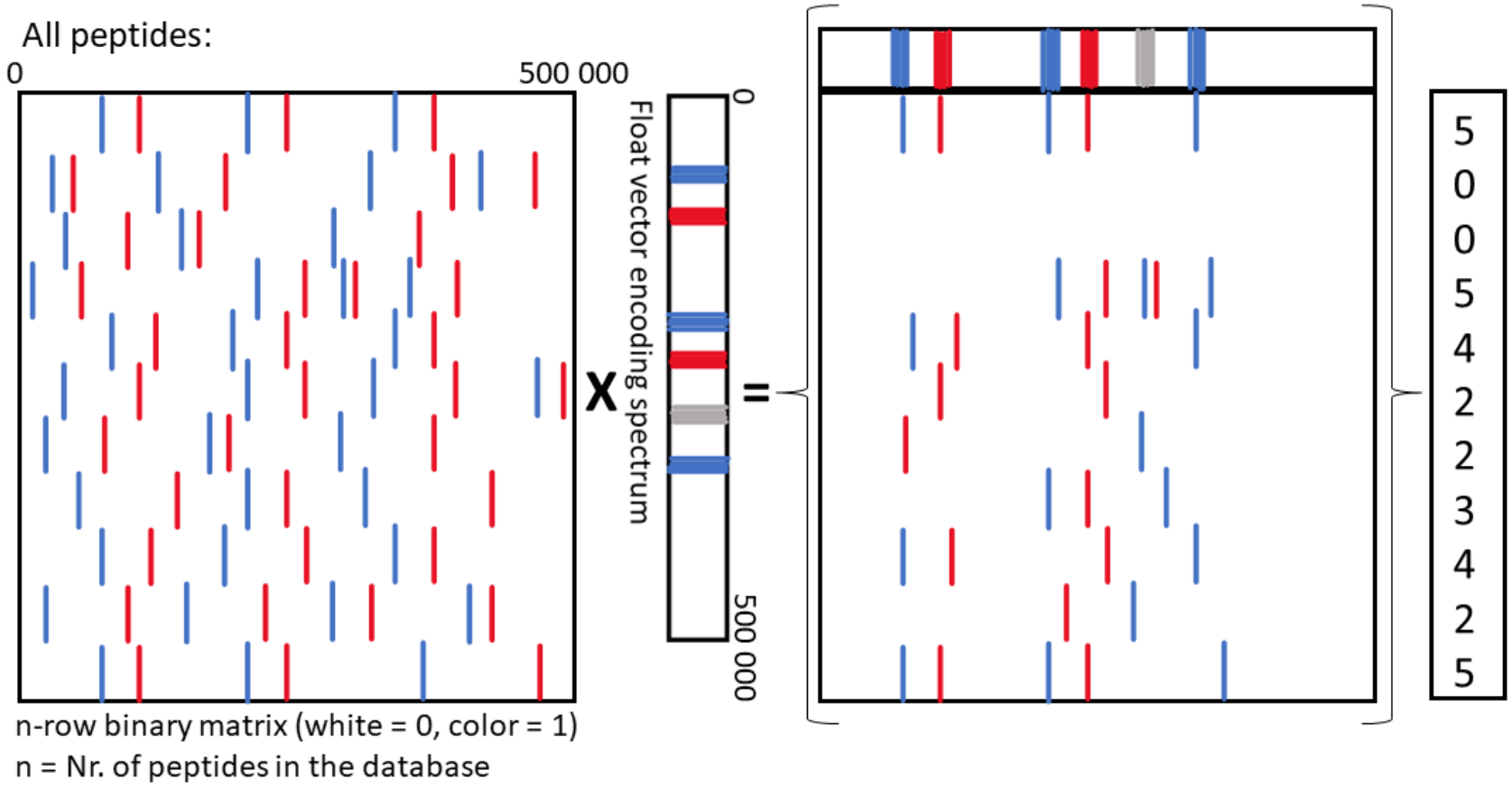
Vectorizing example - search



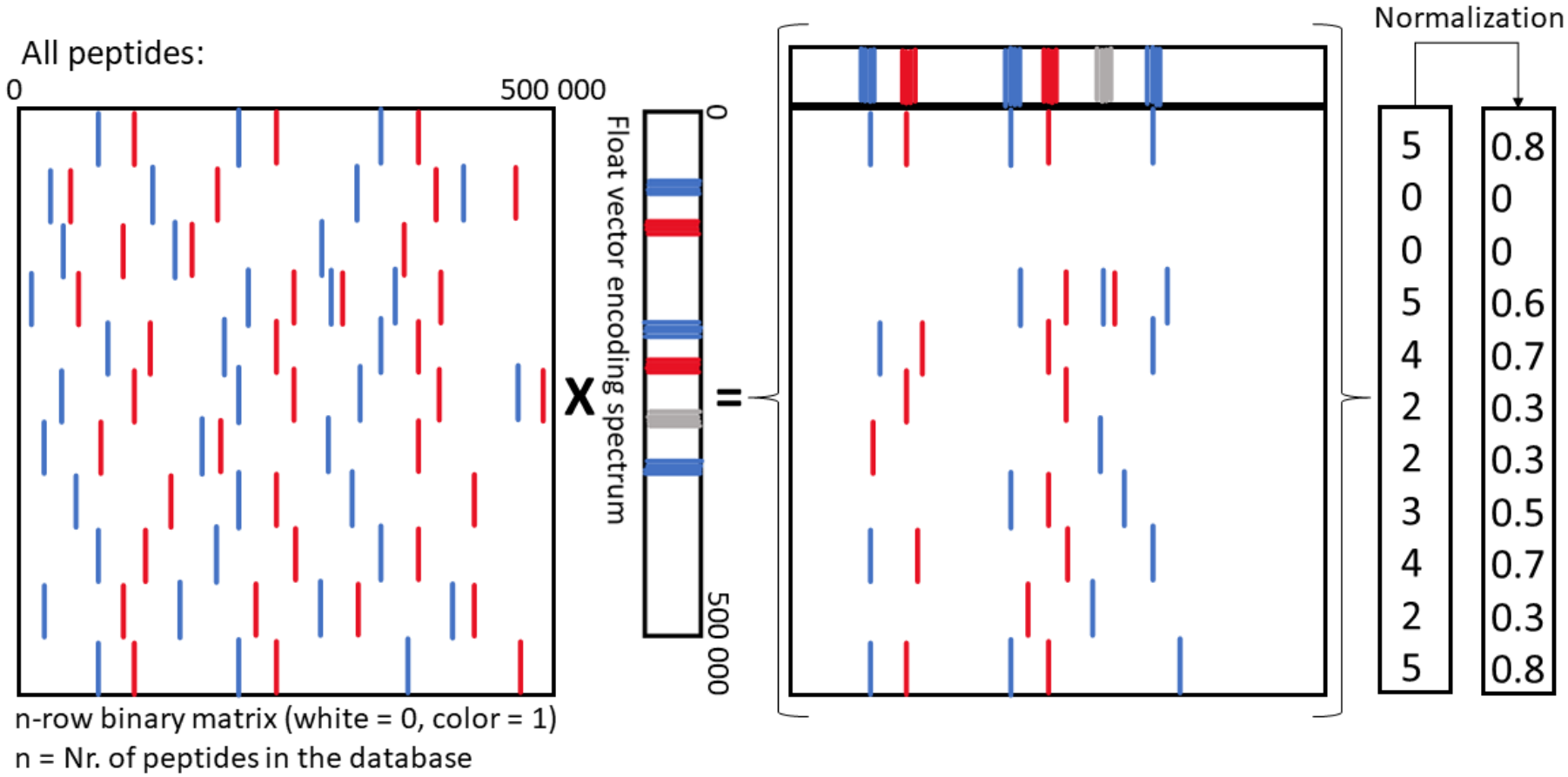
Vectorizing example - search



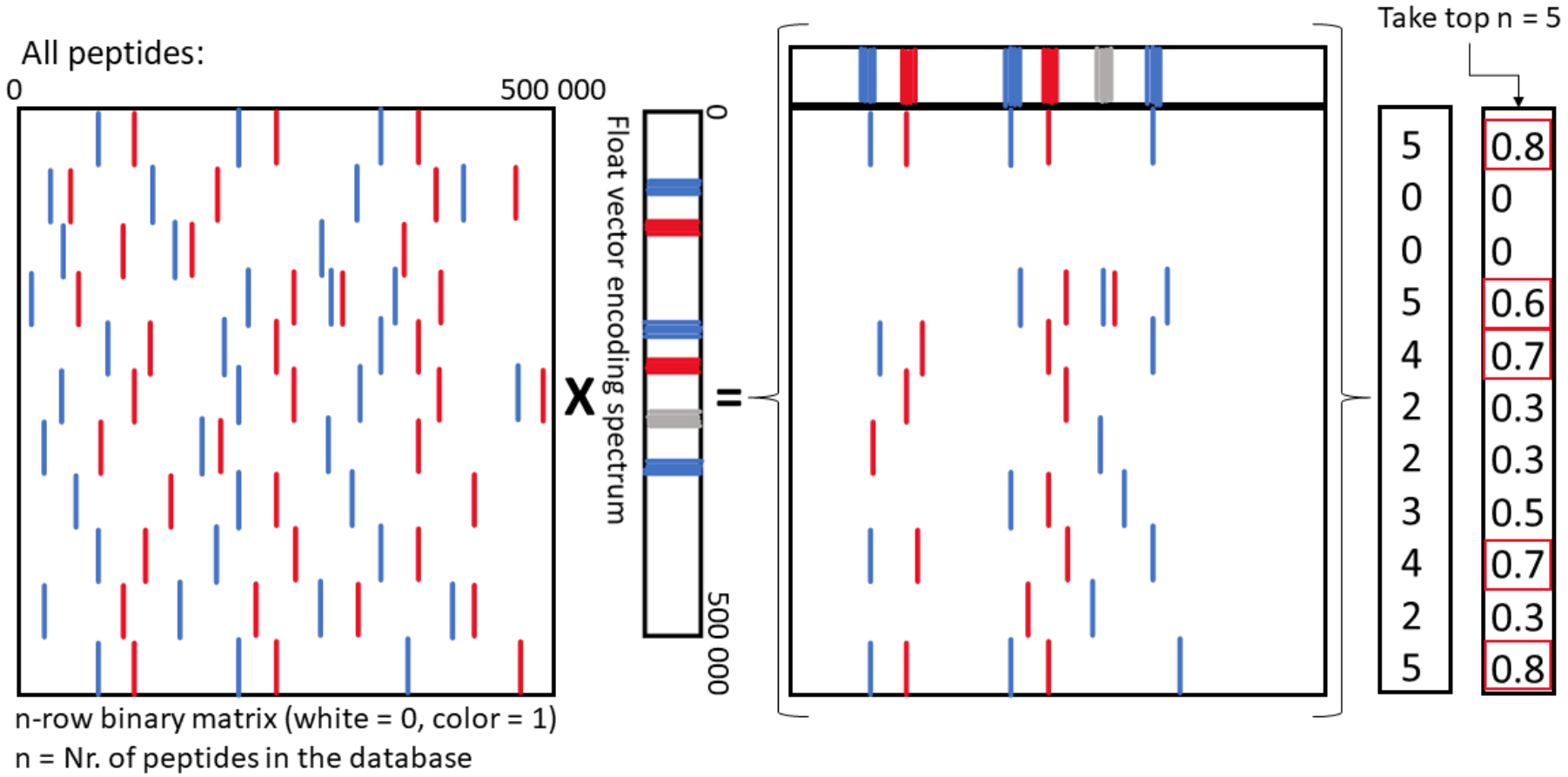
Vectorizing example - search



Vectorizing example - search



Vectorizing example - search



Vectorizing example - search