IMPROVED IMMUNOPEPTIDOME ANALYSIS USING TIMSTOF FRAGMENT ION INTENSITY PREDICTION

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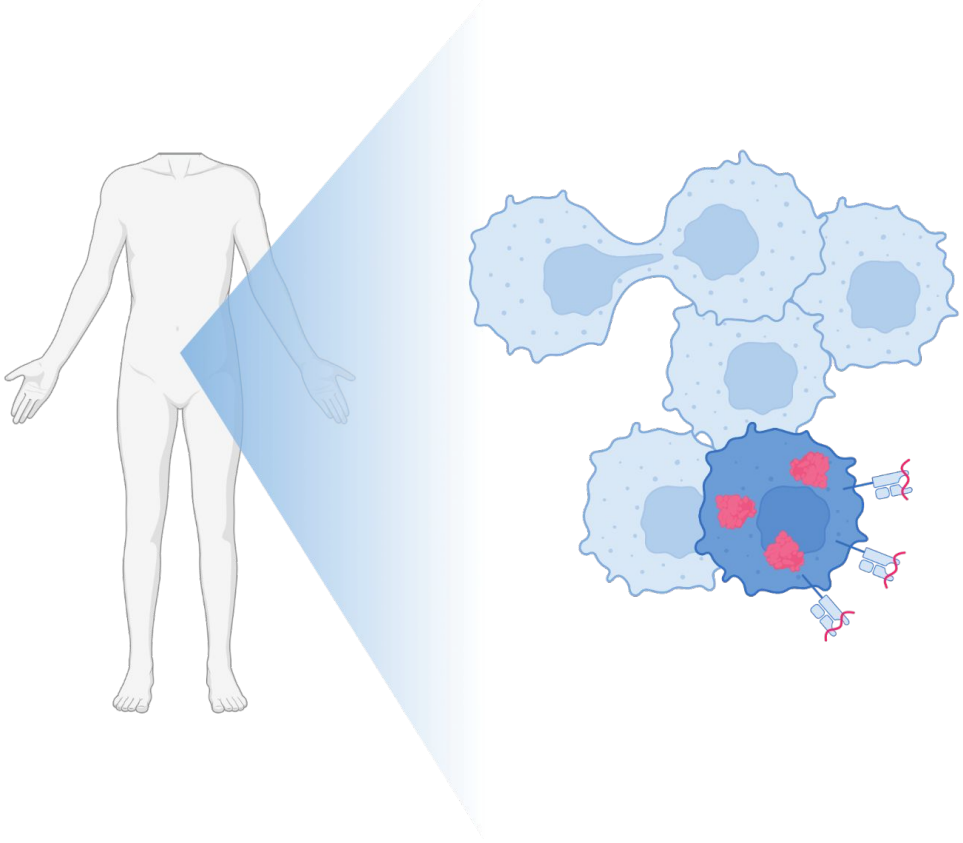
WHY INVESTIGATE THE IMMUNOPEPTIDOME?



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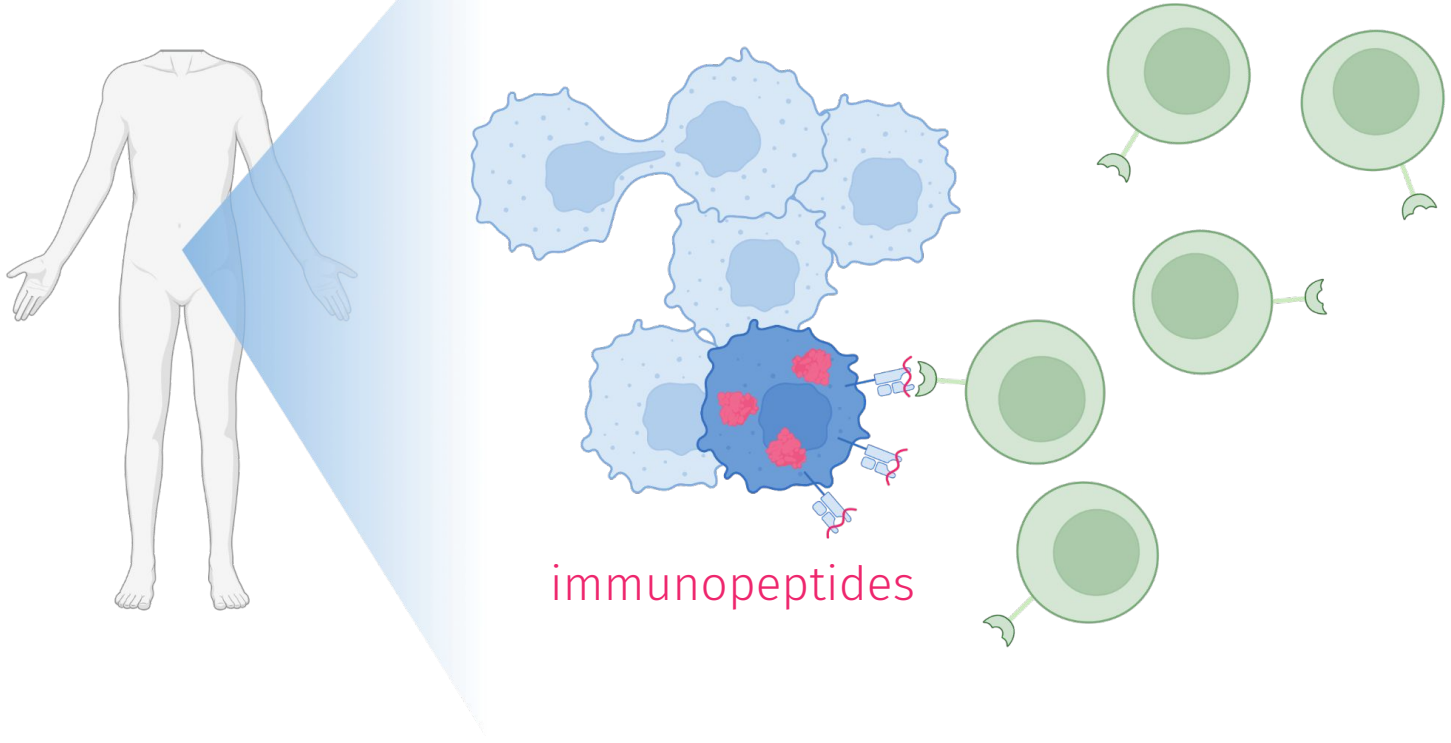

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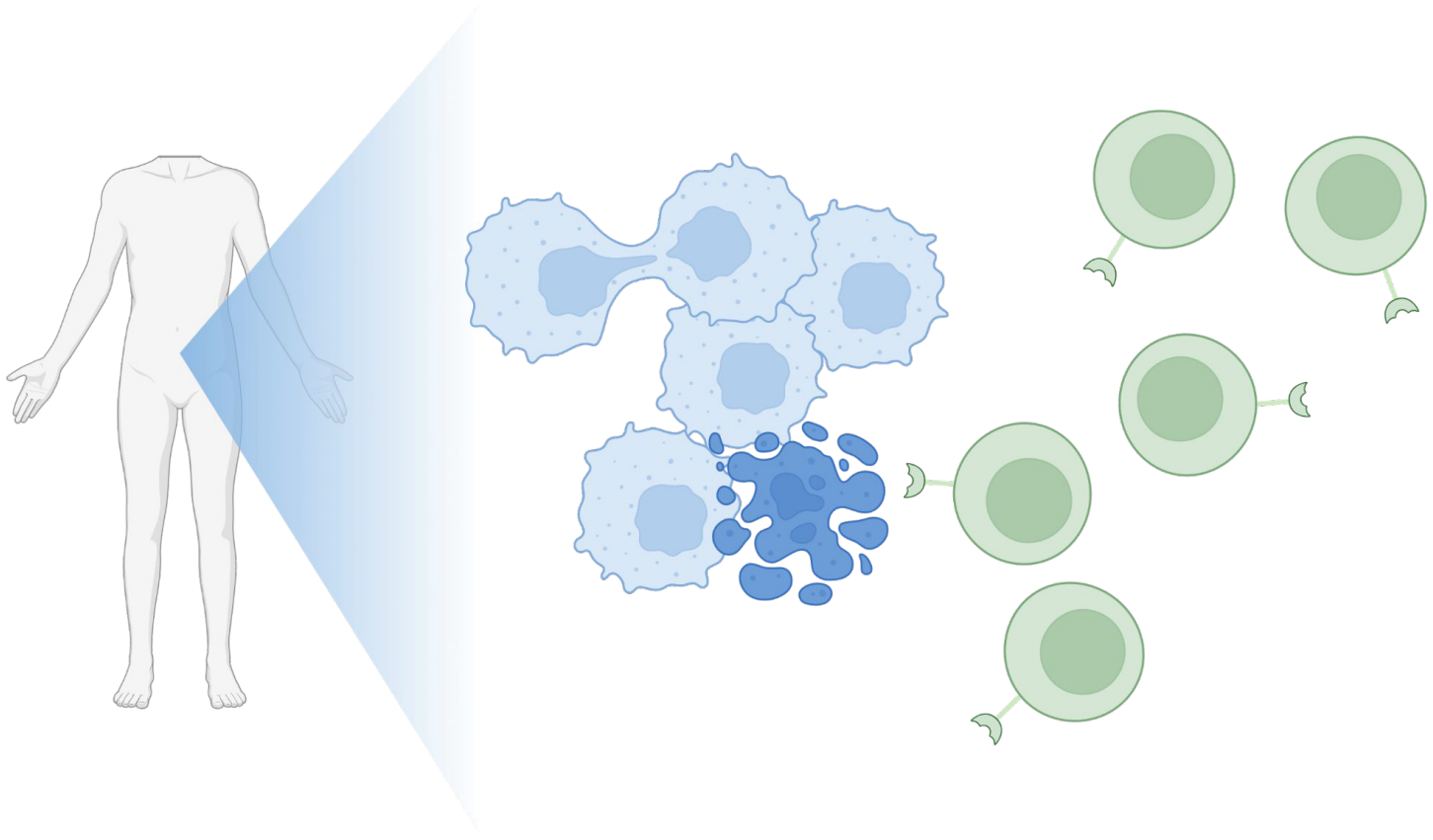

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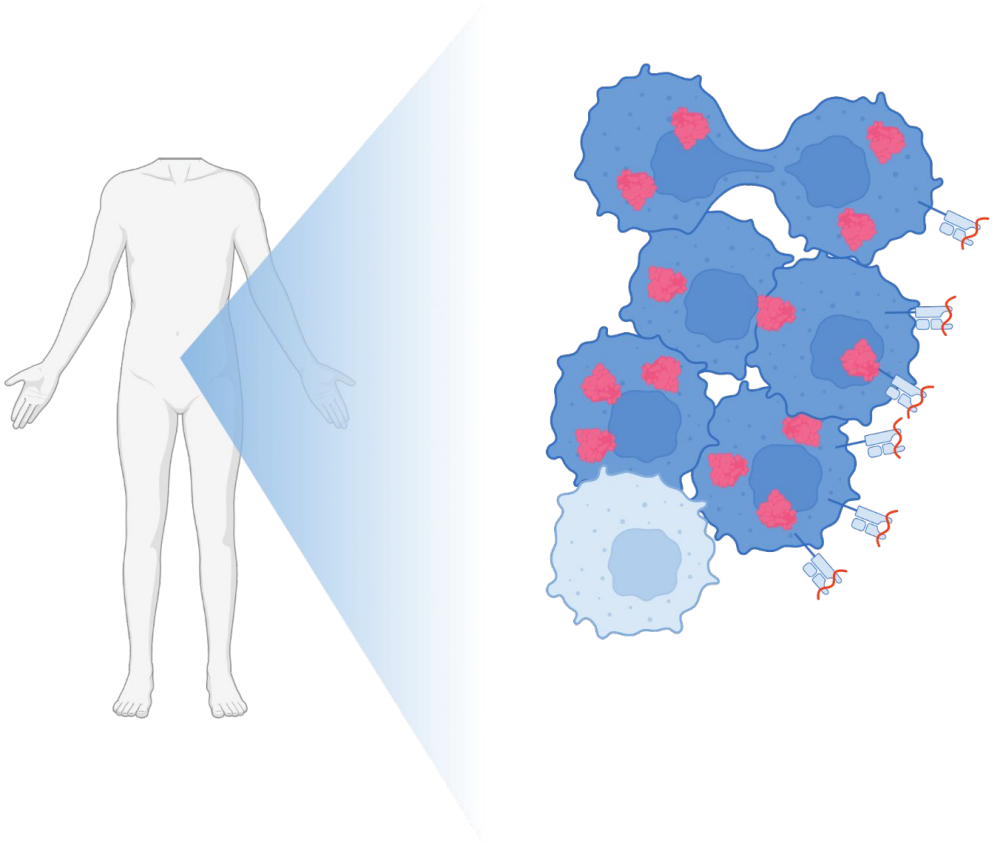

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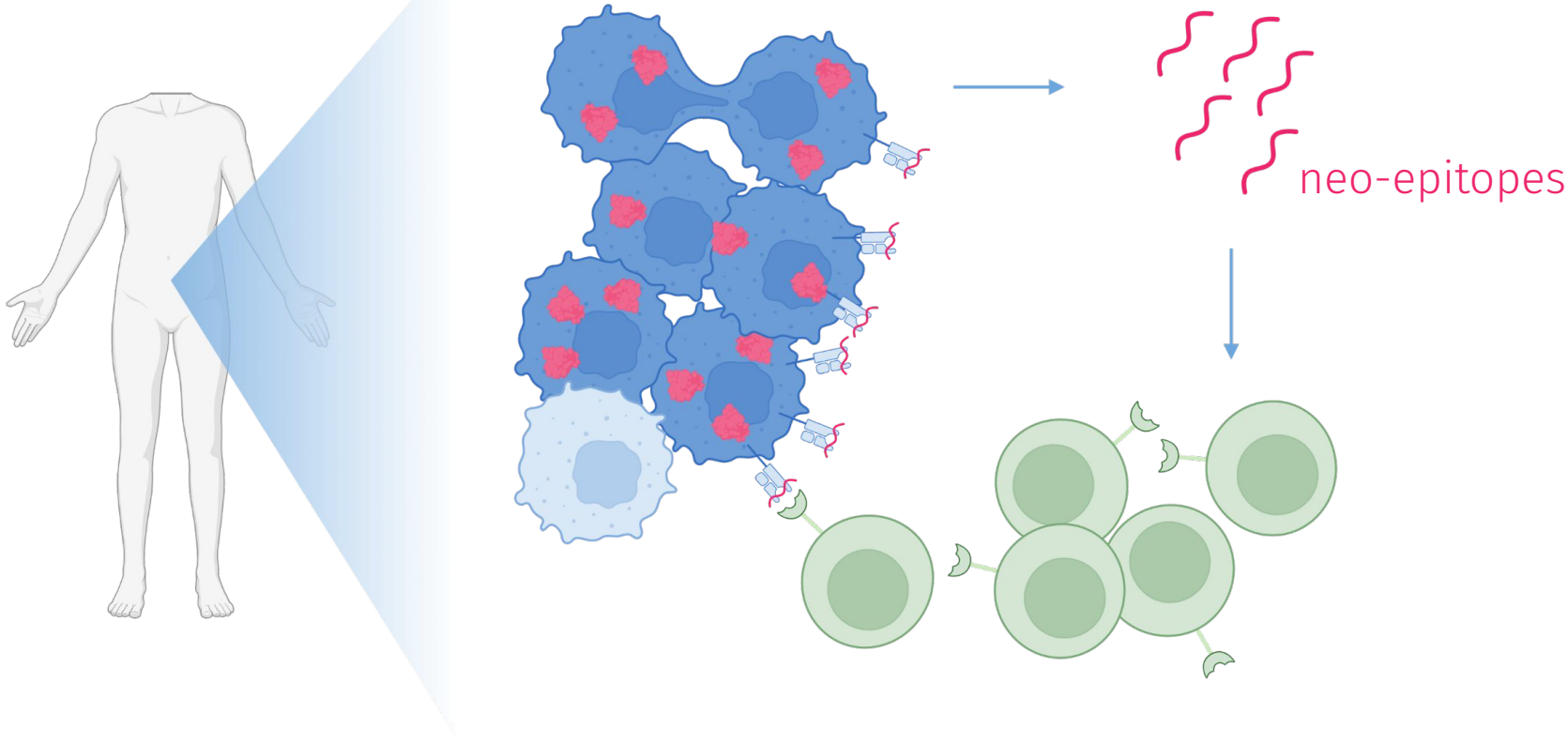
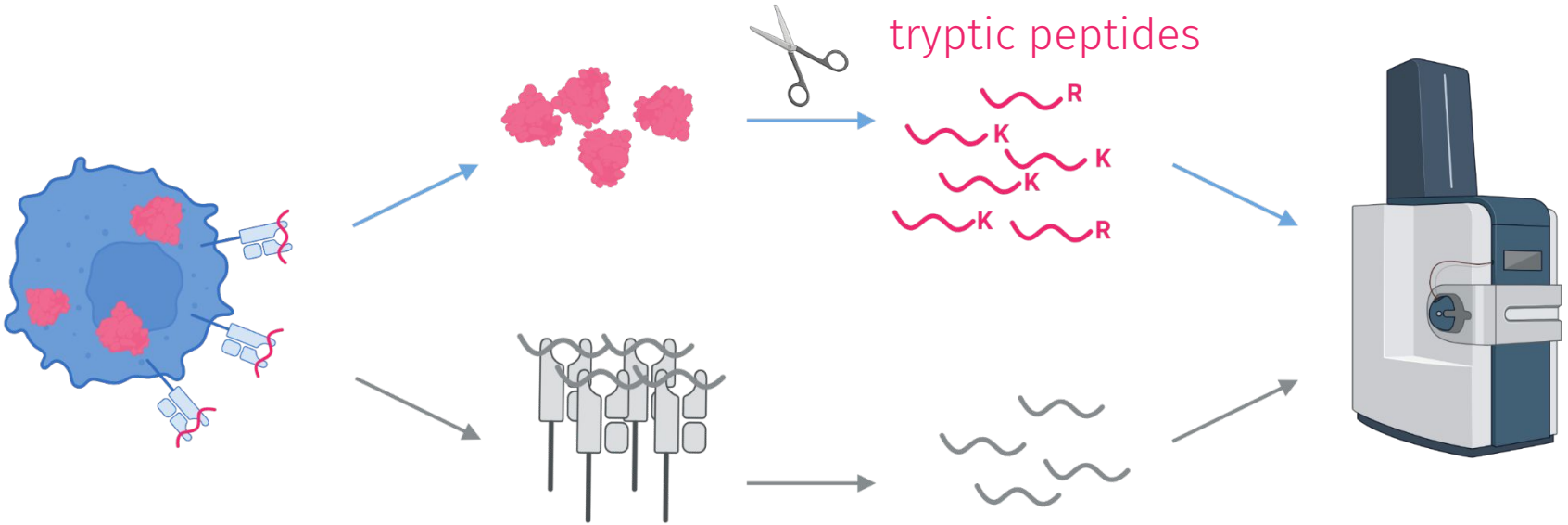


IMMUNOPEPTIDOMICS



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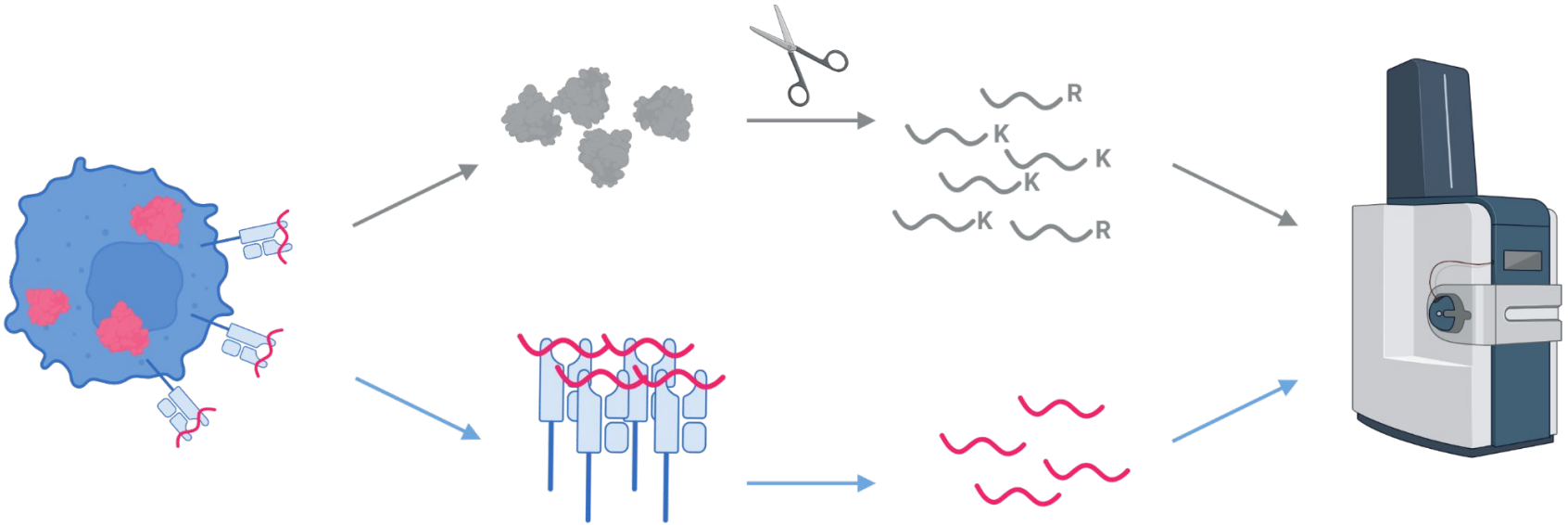
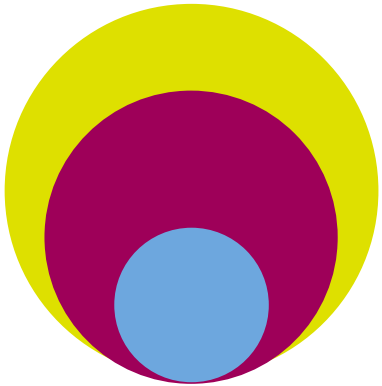
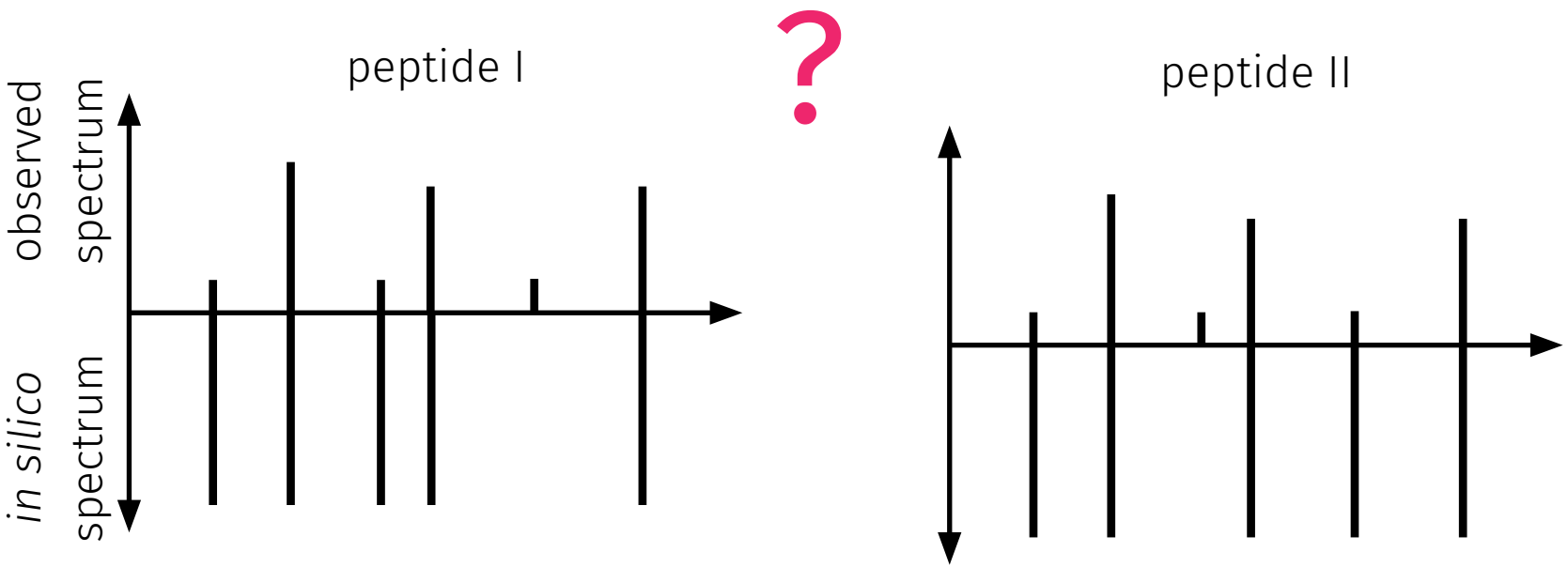


SEARCH SPACE IN IMMUNOPEPTIDOMICS

- Massive search space: all protein subsequences have to be considered
- Increased probability of identifying high-scoring decoys
- Reduced identification rate at a fixed FDR



SPECTRUM ANNOTATION IS CHALLENGING



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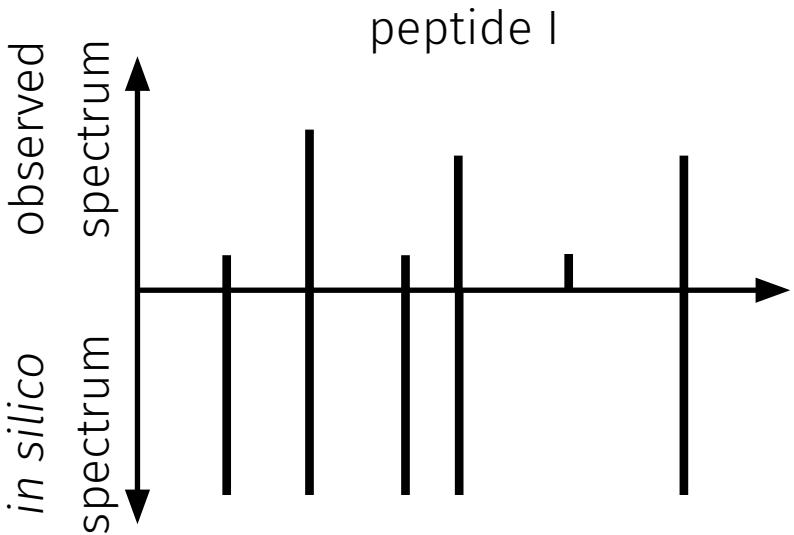
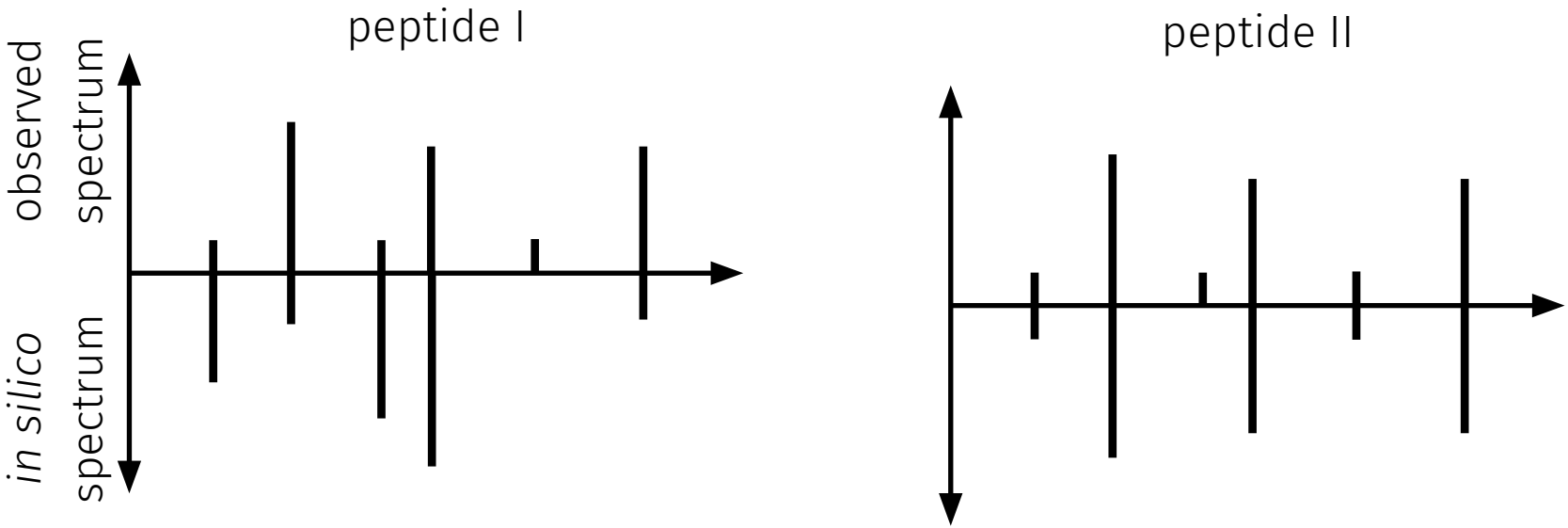


INTENSITY INFORMATION AVOIDS FALSE POSITIVES



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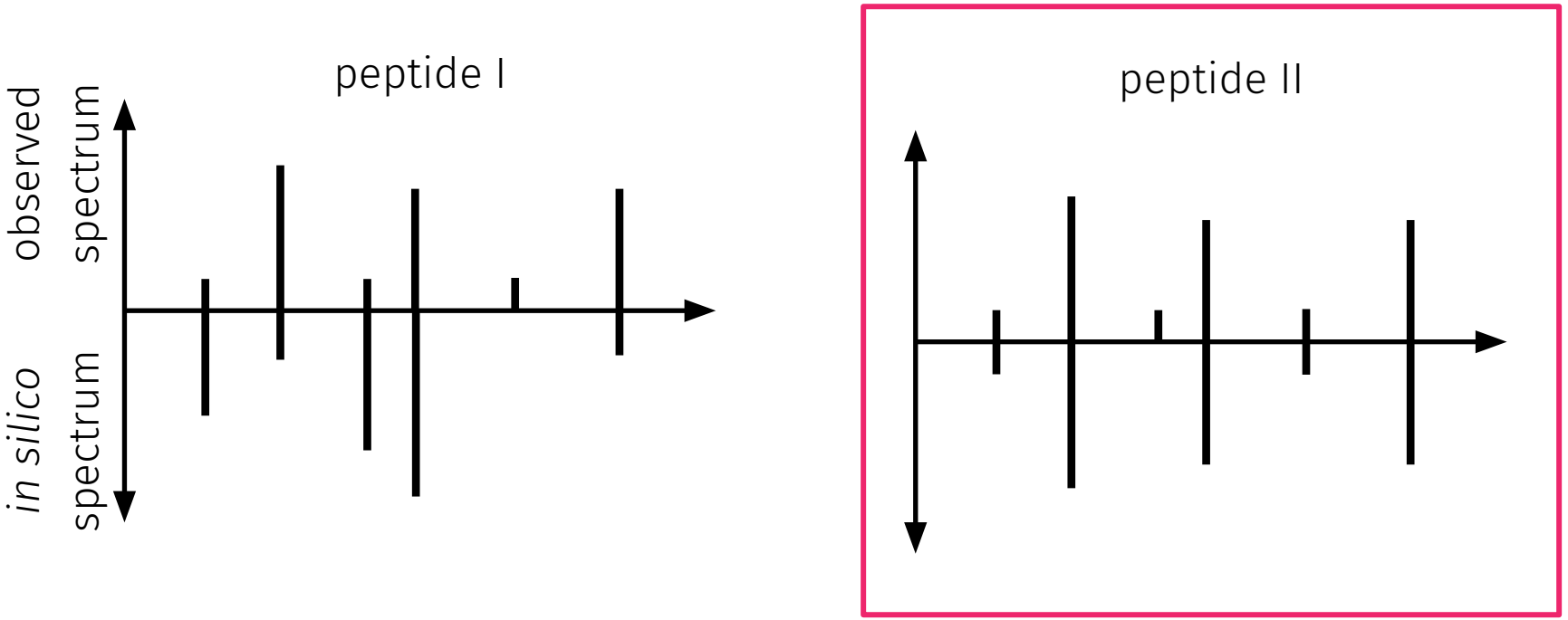
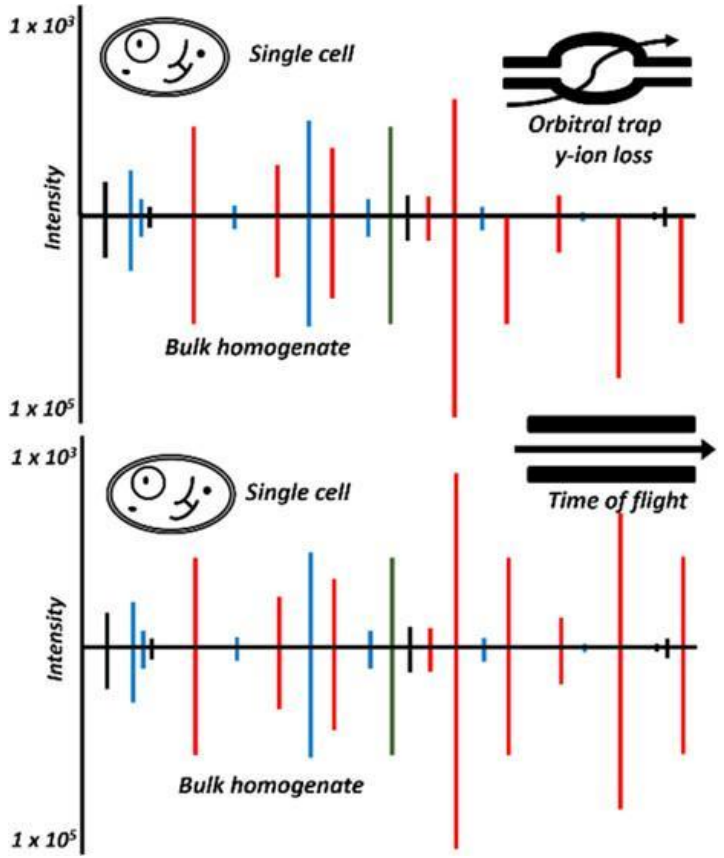


VALUE OF TIMSTOF FOR IMMUNOPEPTIDOMICS

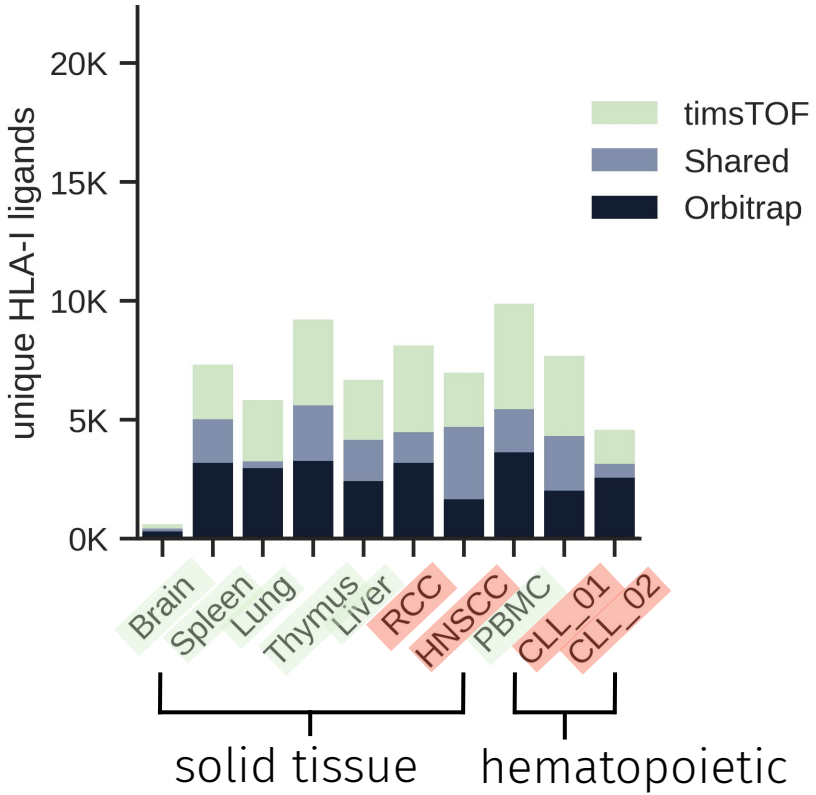
- timsTOF stays stable at low abundances
- A few immunopeptides can elicit an immune response



Orsburn, B. *Journal of Proteome Research* (2023).

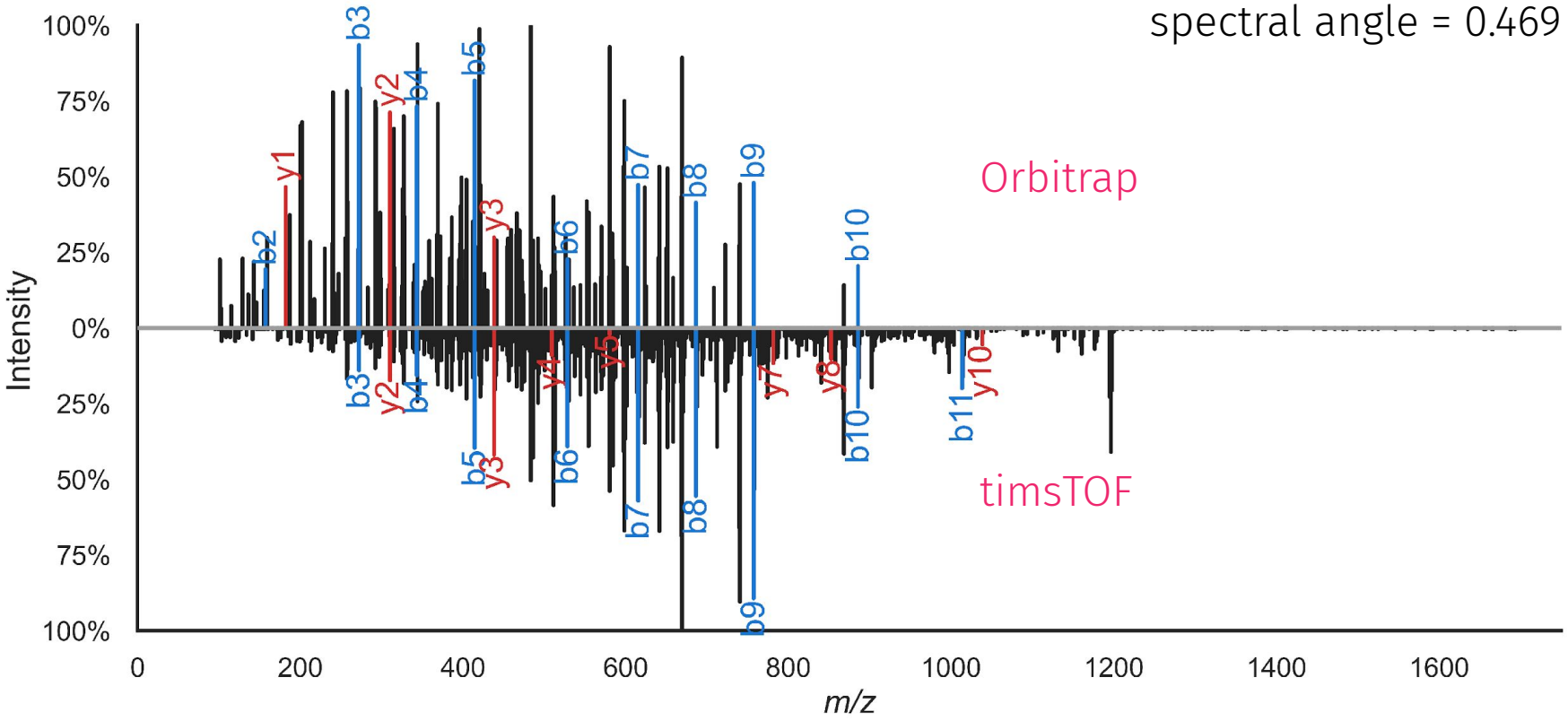
1.5-FOLD PEPTIDE INCREASE ON TIMSTOF

- Benign and malignant samples
- Measured on both Orbitrap and timsTOF



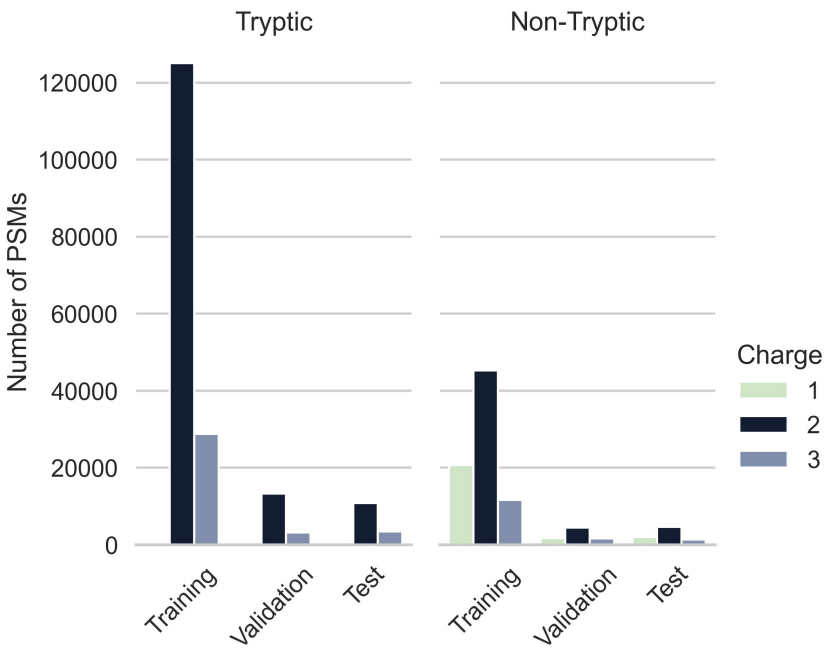
Gravel, N.H., *et al. Research Square* (2023).

NEED FOR A TIMSTOF INTENSITY PREDICTION MODEL



ORIGIN OF THE TRAINING DATA

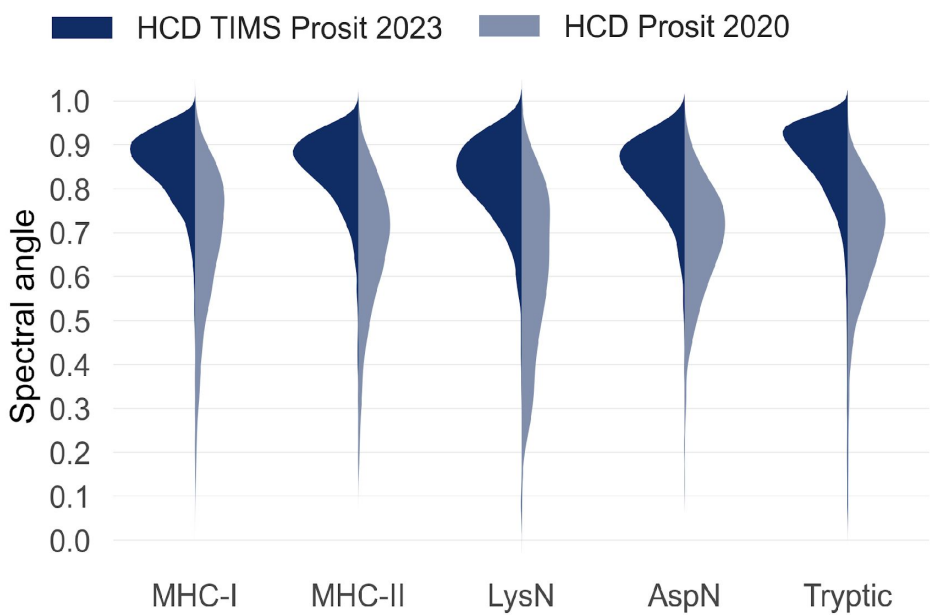
- Measured >300,000 non-tryptic synthesized peptides
- >120,000 previously acquired tryptic synthesized peptides



Meier, F., *et al. Nature Communications* (2021).

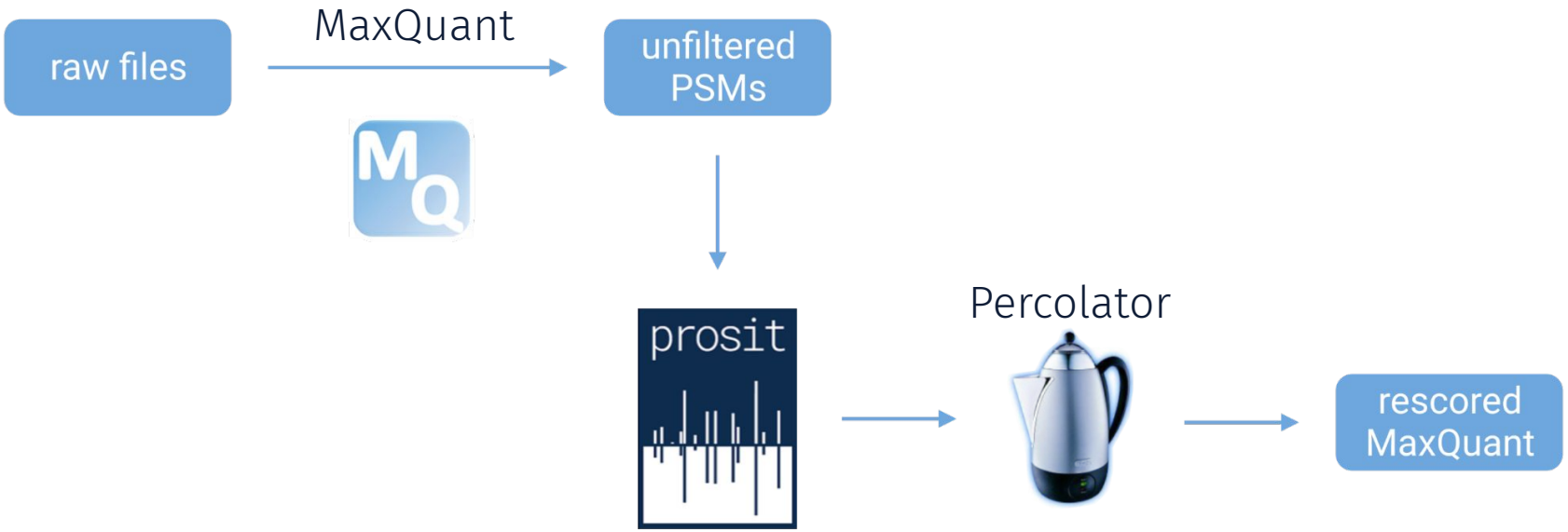
FINE-TUNING THE PROSIT HCD MODEL

- Prosit HCD 2020 was trained on ~30 million spectra (9 million non-tryptic spectra)
- ~280,000 timsTOF spectra
- Improved spectral angle between predicted and experimental spectra

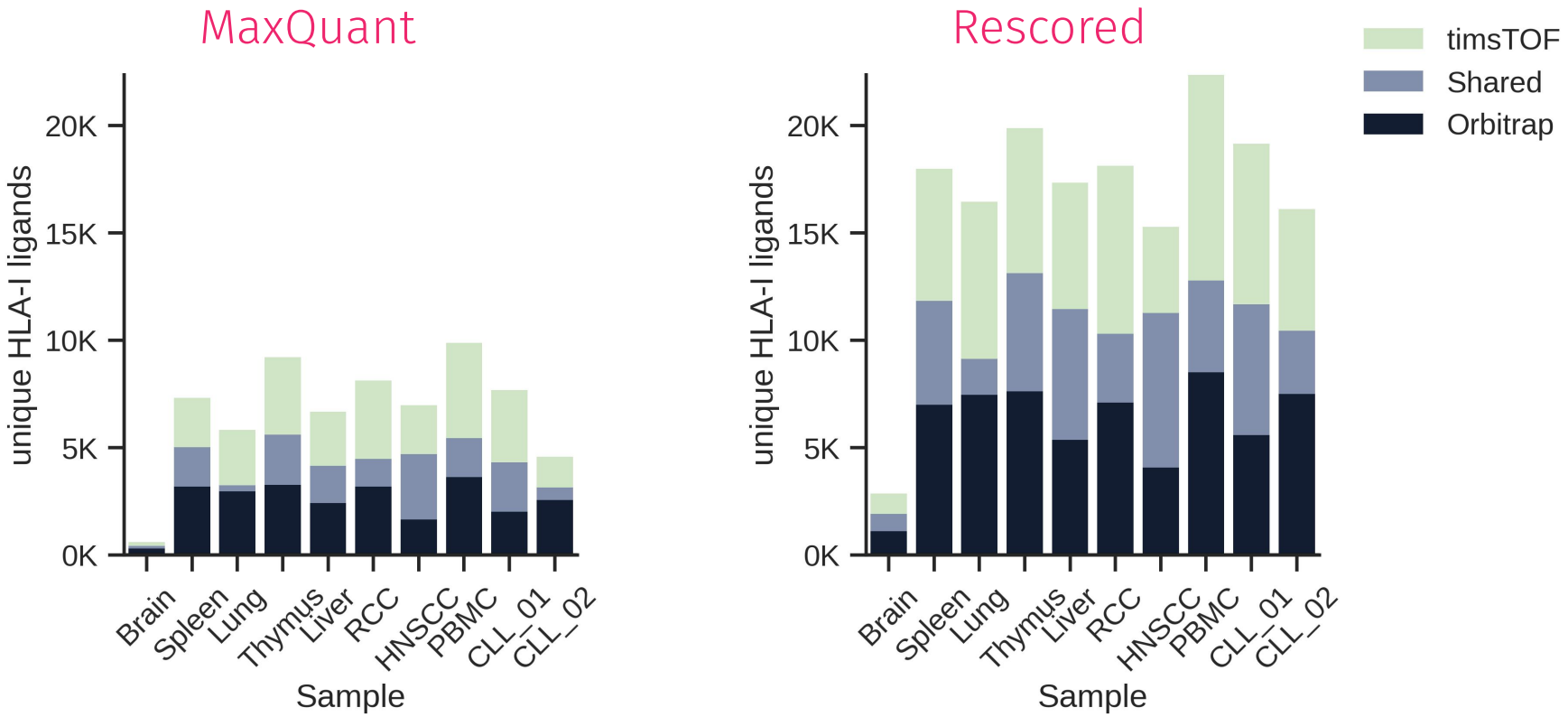


Wilhelm, M., *et al. Nature Communications* (2021).

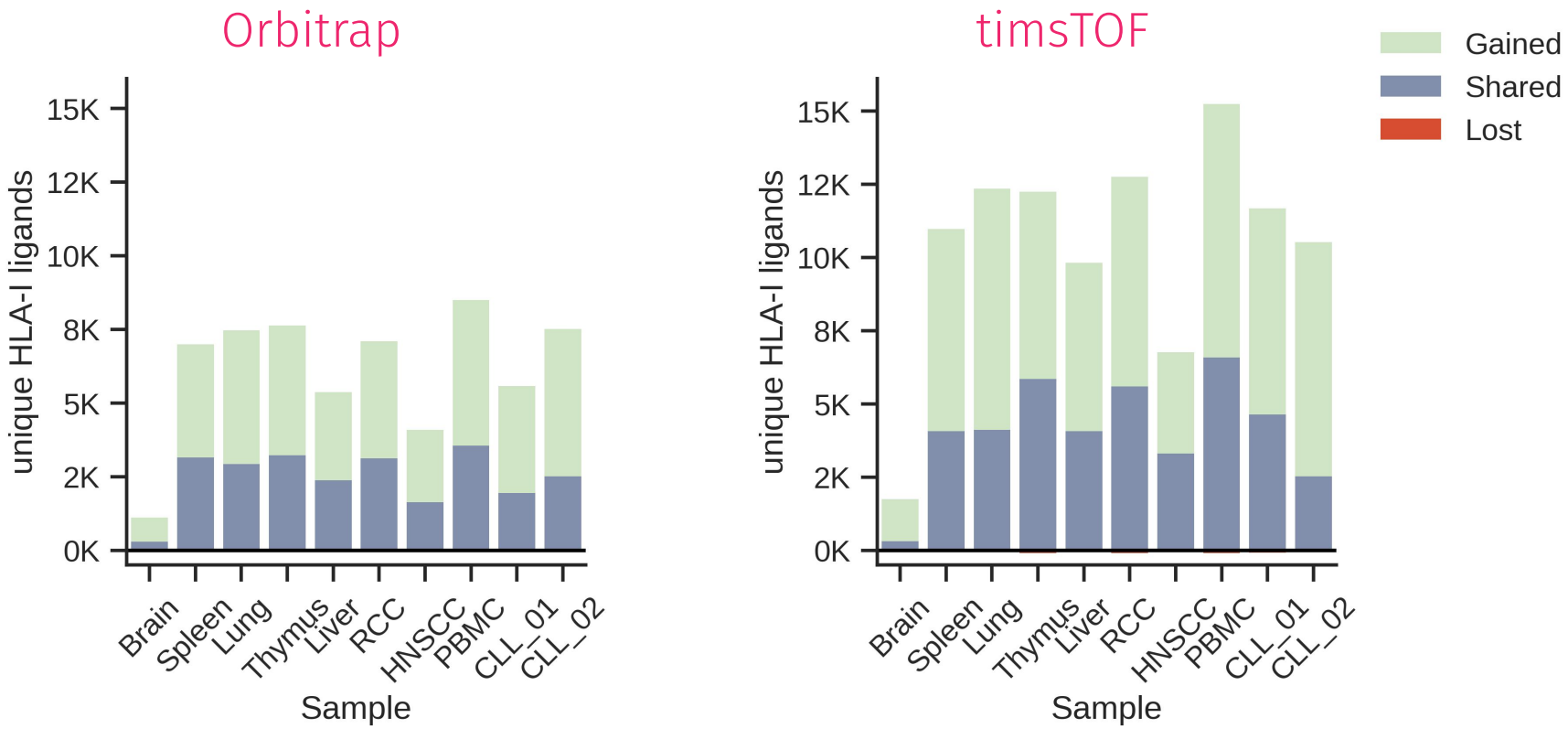
OUR RESCORING PIPELINE



2.4-FOLD PEPTIDE INCREASE AFTER RESCORING



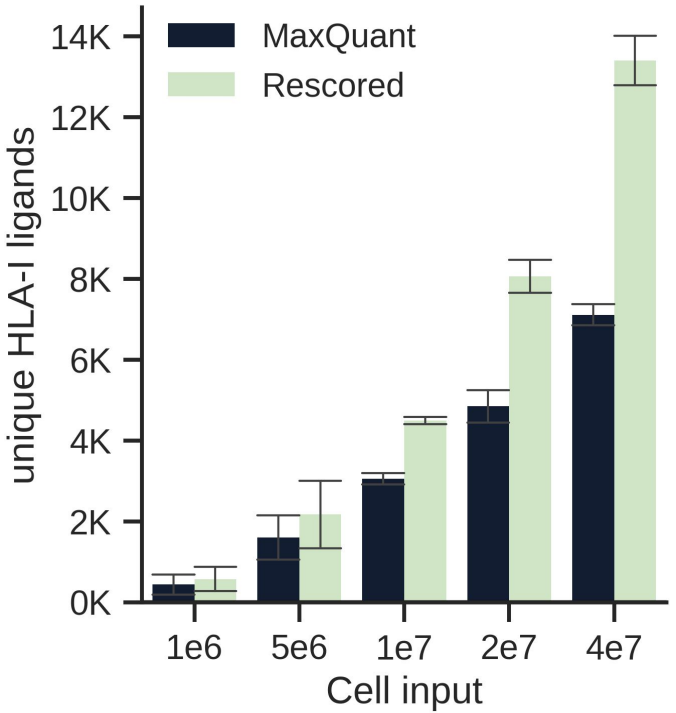
INCREASED PERFORMANCE ON TIMSTOF VS ORBITRAP



IMMUNOPEPTIDOMICS ON MELANOMA CELLS

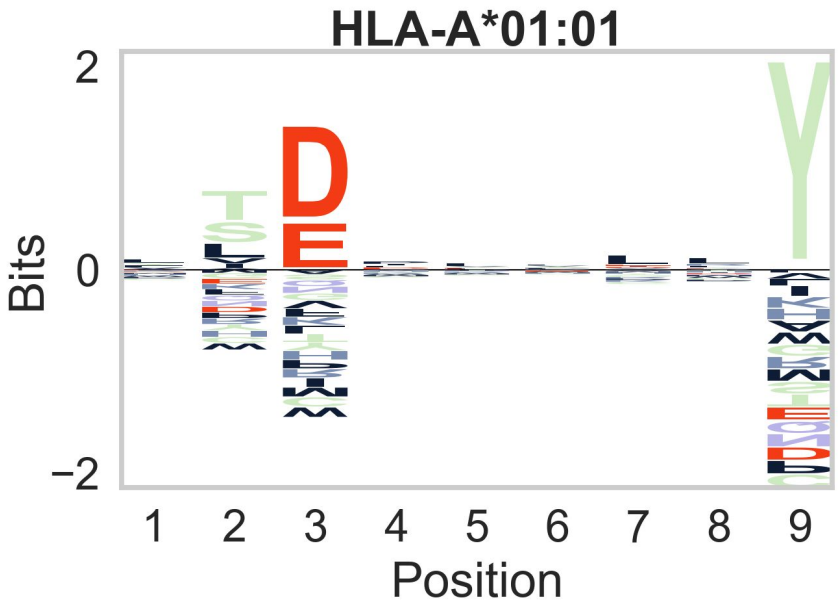
- A375, a melanoma cell line
- Measured in triplicate
- Missense SNPs were added to the FASTA file
- HLA types

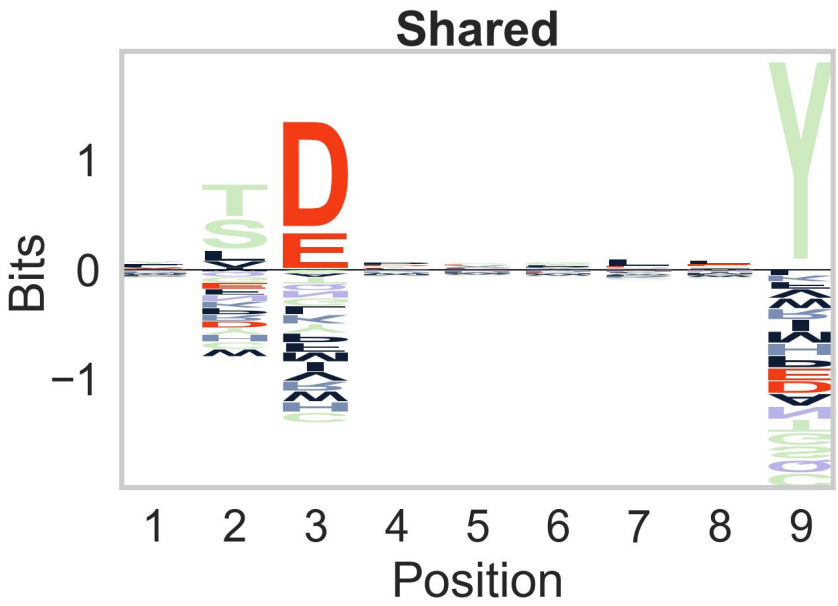
HLA-A	HLA-B	HLA-C
01:01 + 02:02	57:01 + 44:03	16:02 + 06:02



Phulphagar, K. M., *et al. Molecular & Cellular Proteomics* (2023).

MOTIFS OF IDENTIFIED PEPTIDES MATCH HLA TYPES





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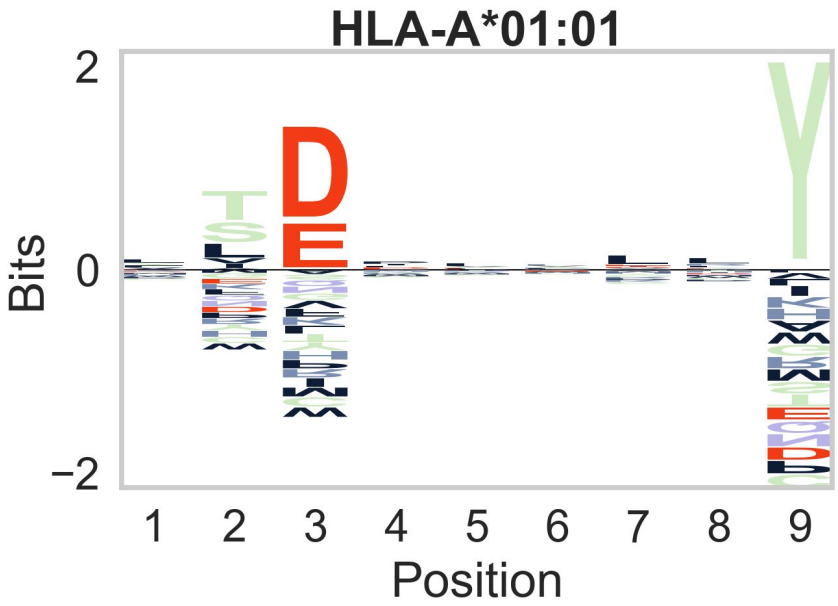

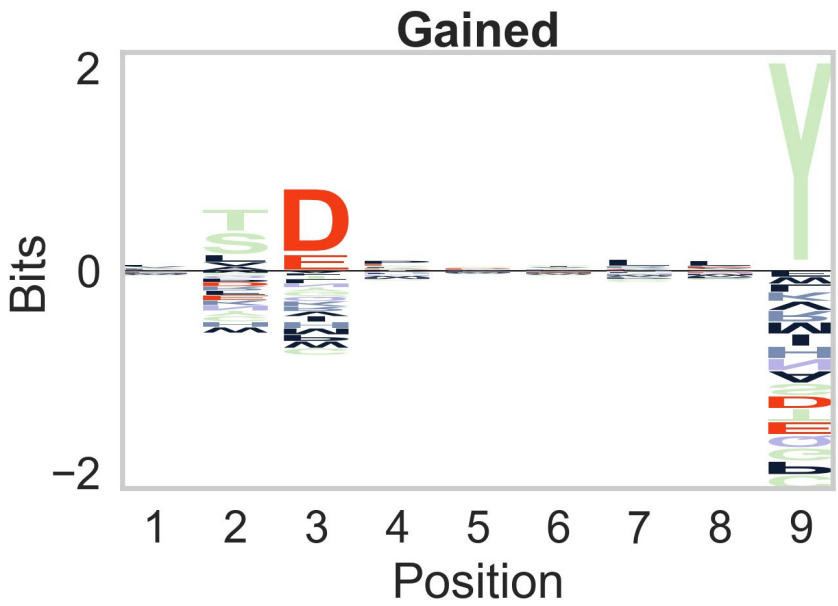

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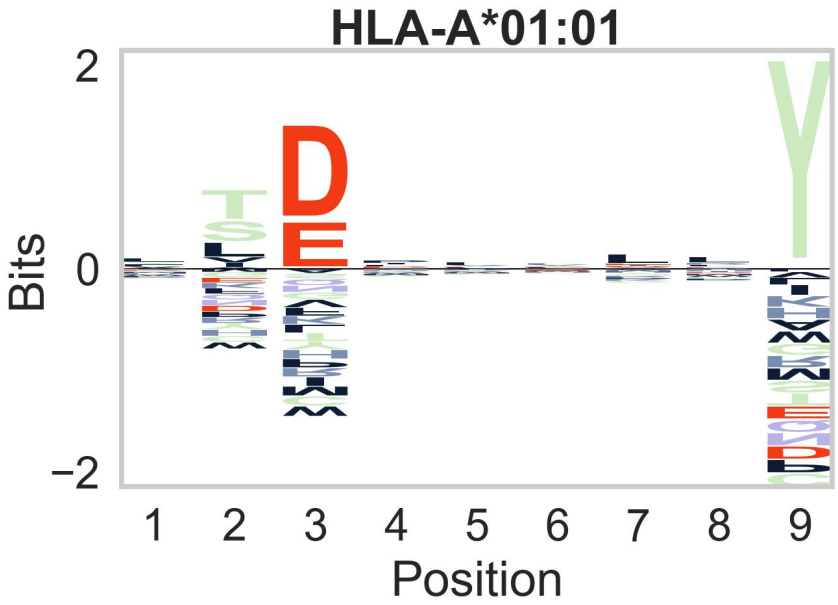

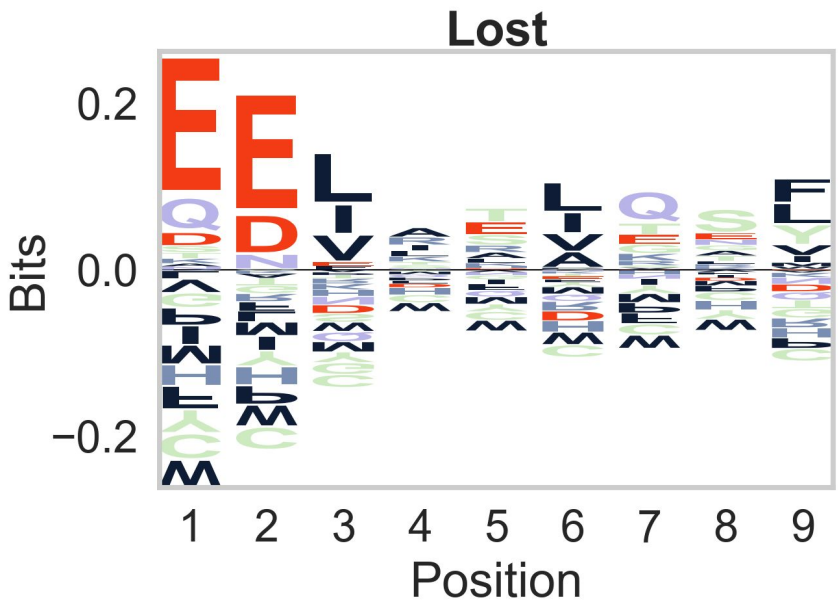

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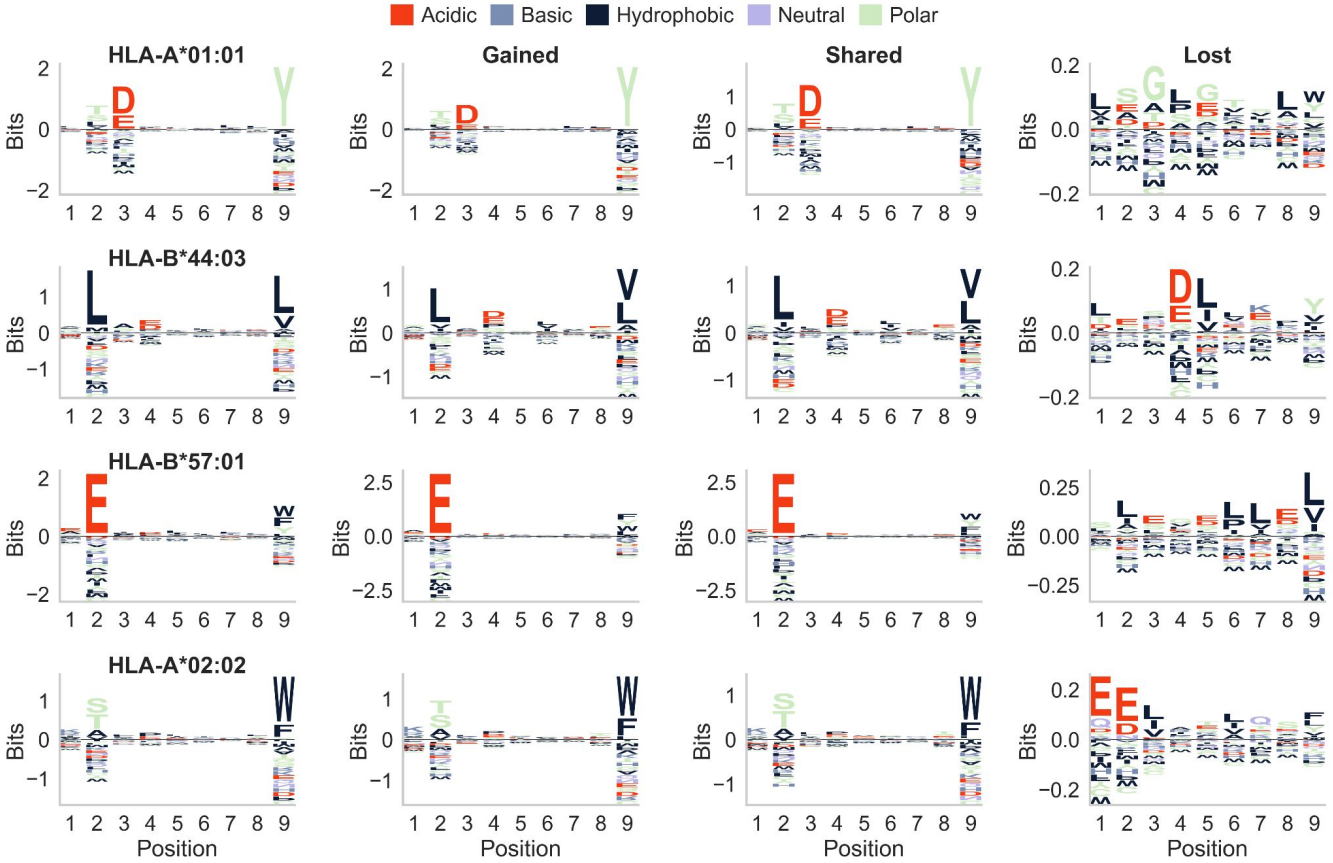
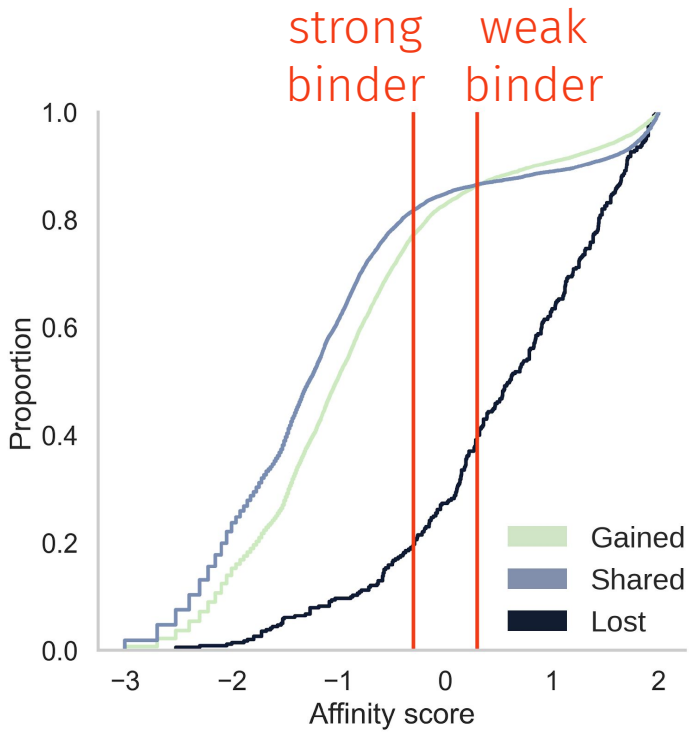


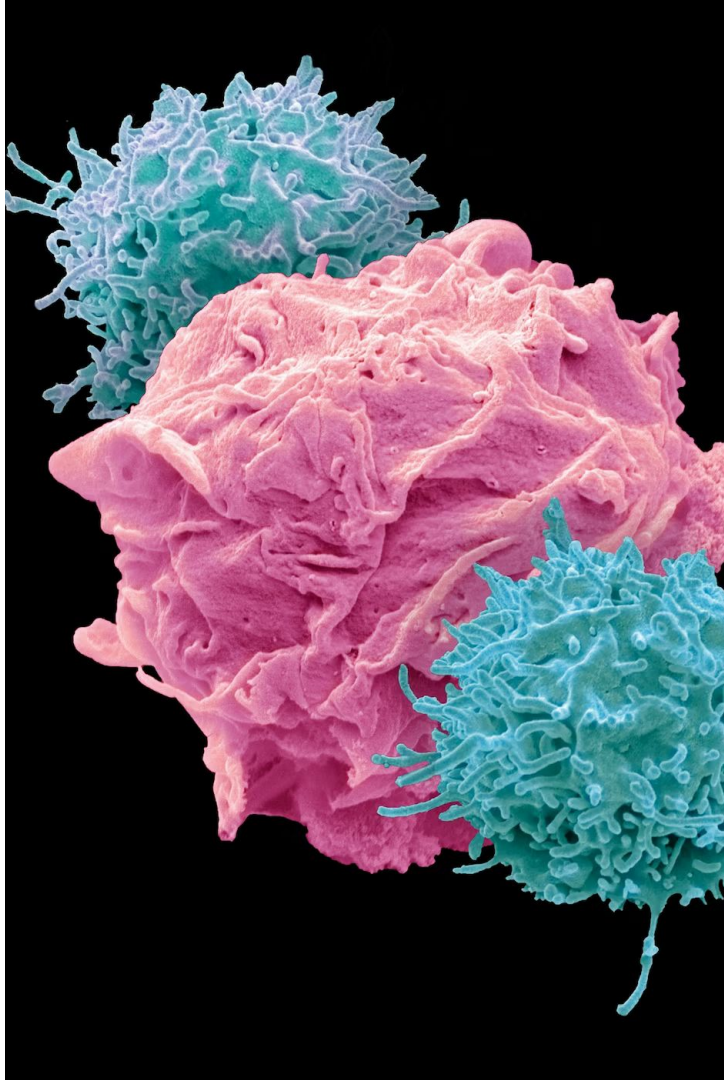
IDENTIFIED PEPTIDES HAVE STRONG HLA BINDING

- NetMHCpan 4.1 to predict the HLA binding affinity
- Best (=lowest) score selected for each peptide against HLA types
- 86% of peptides after rescoring are at least weak binders



CONCLUSION

- Identifying immunopeptides is challenging
- Fragment ion intensity prediction for rescoring on timsTOF data
- Discover neo-epitopes that could be used to develop immunotherapies



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