Development of immunopeptidomics as one of the most promising tool for immunology and cancer research

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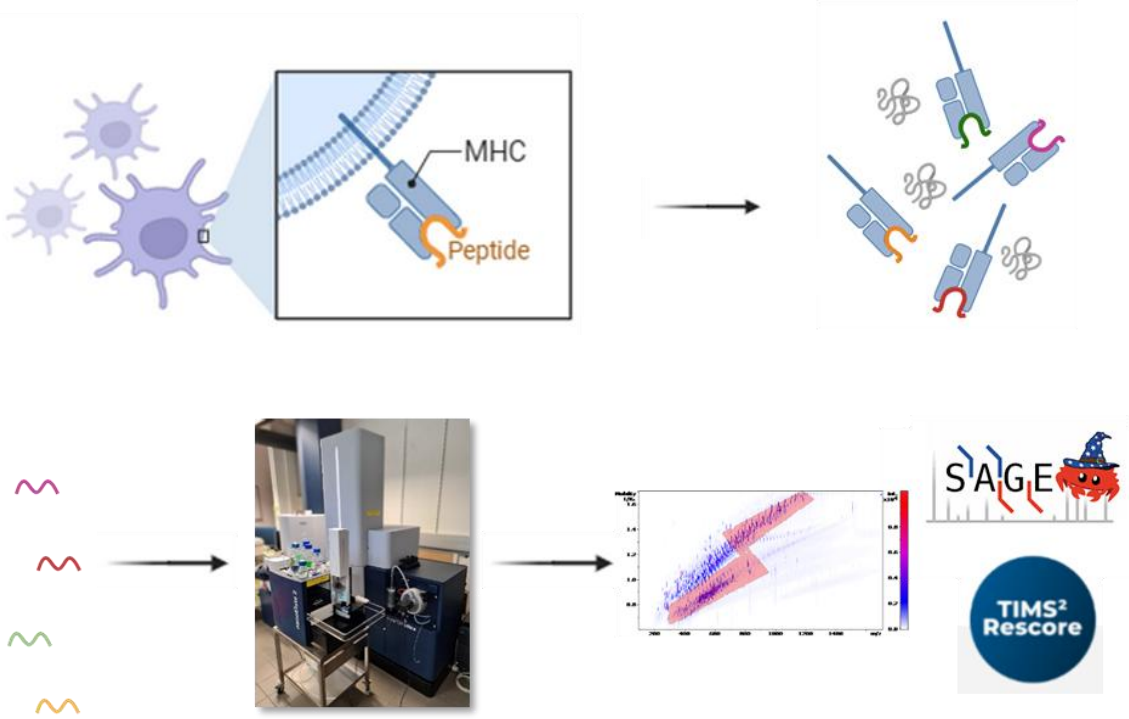
Jeewan Babu Rijal, Christine Carapito

Équipe LSMBO, IPHC

Journée scientifique et technique de l'IPHC

9th December 2025

From bredeles to immunopeptidomics



Immunopeptidomics

- Why immunopeptidomics?
- Challenges of immunopeptidomics analysis
- Sample preparation optimisation using magnetic beads
- Fine tuning of LC and MS methods

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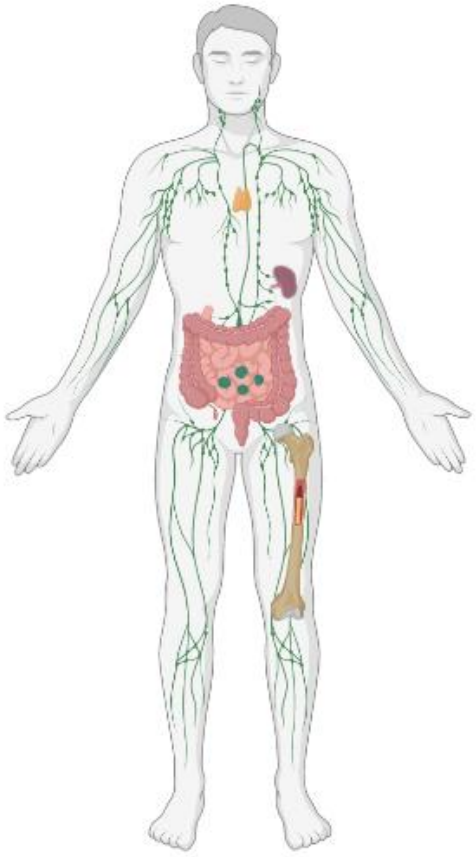
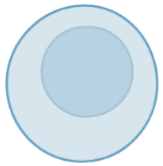
The immune system protects and defends us

with the ability to distinguish between:



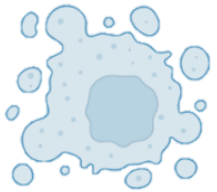


Self

Cells, proteins

Non-self

Bacteria, virus, transplanted tissues



Self-modified

Mutated cells, cancer cells

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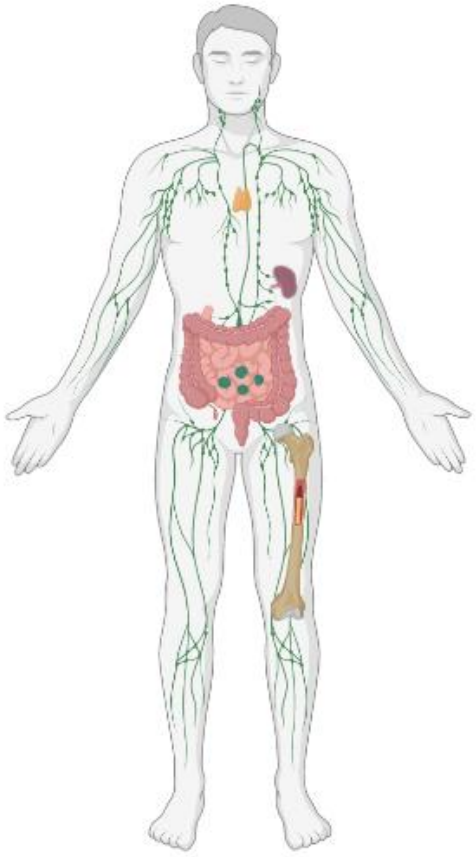

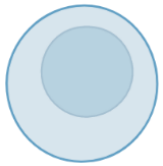

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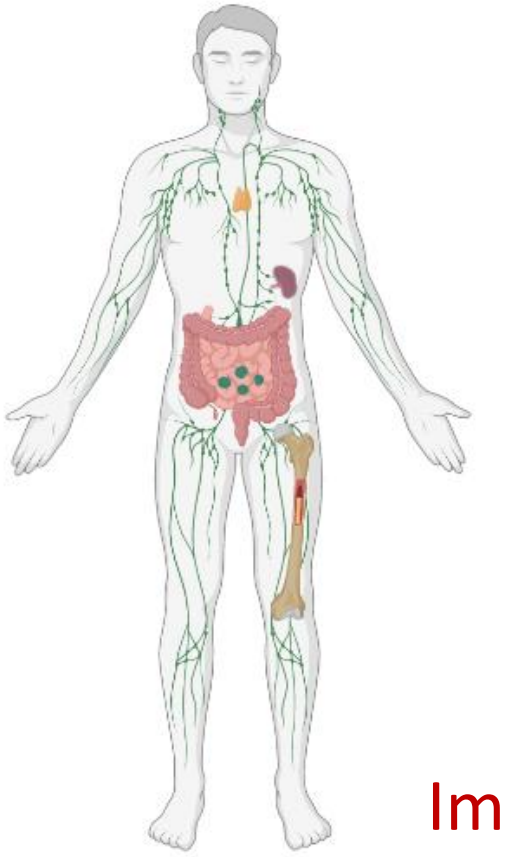

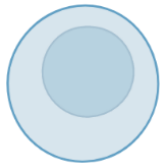

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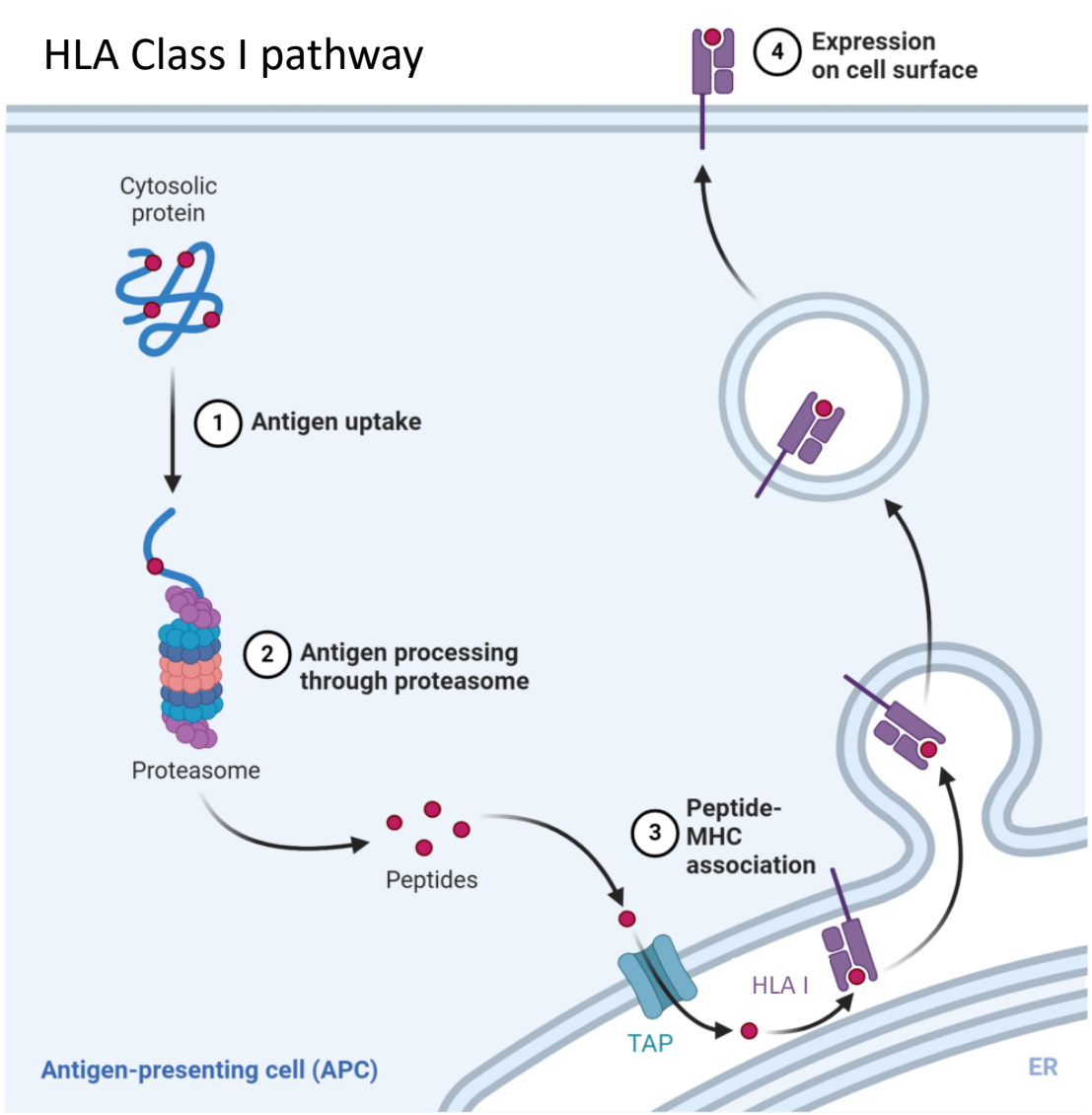
Implication of **M**ajor **H**istocompatibility **C**omplex (**MHC**) system,

Human **L**eukocyte **A**ntigen (**HLA**) system for human

What is HLA ?



Created with Biorender

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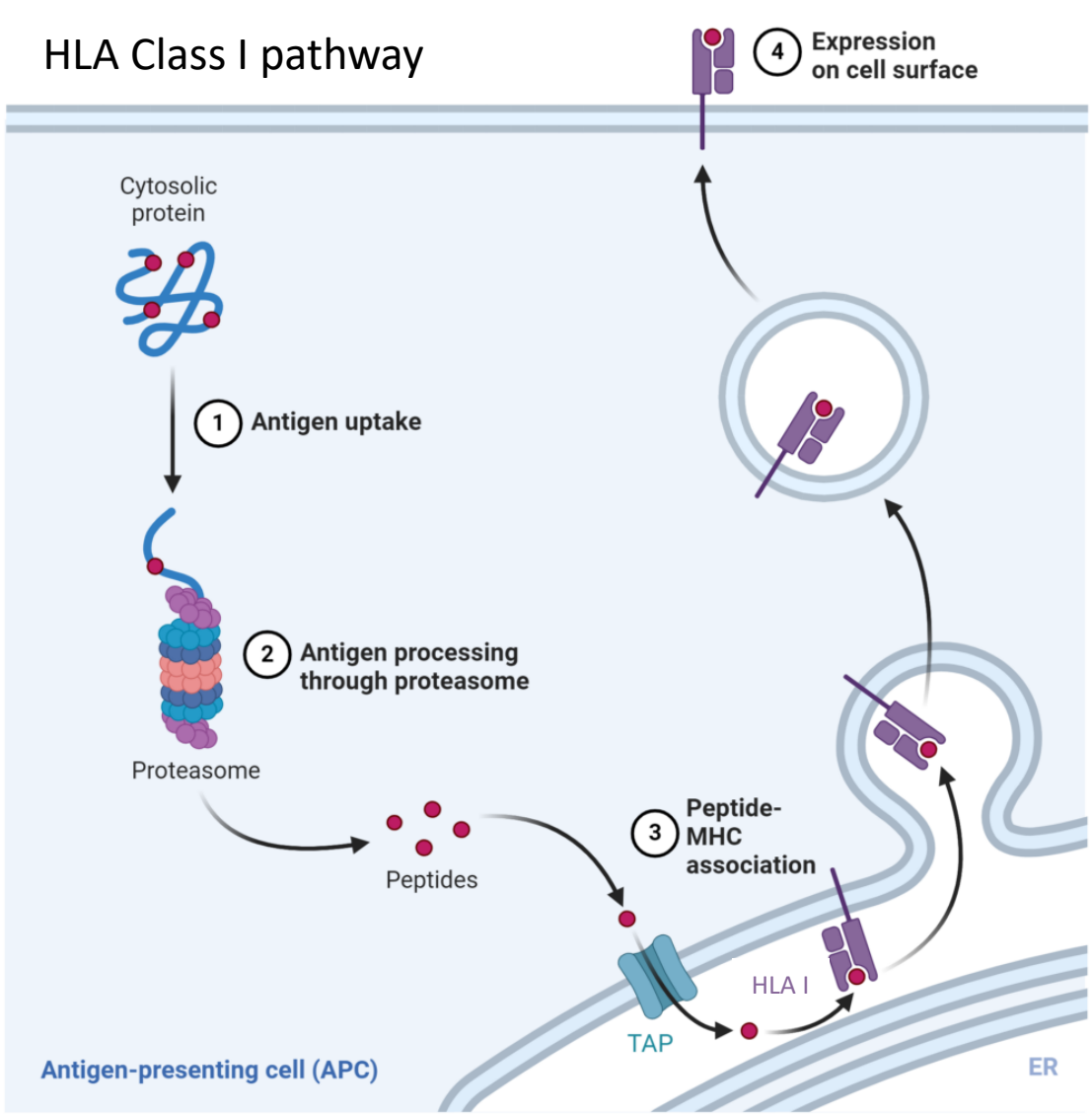

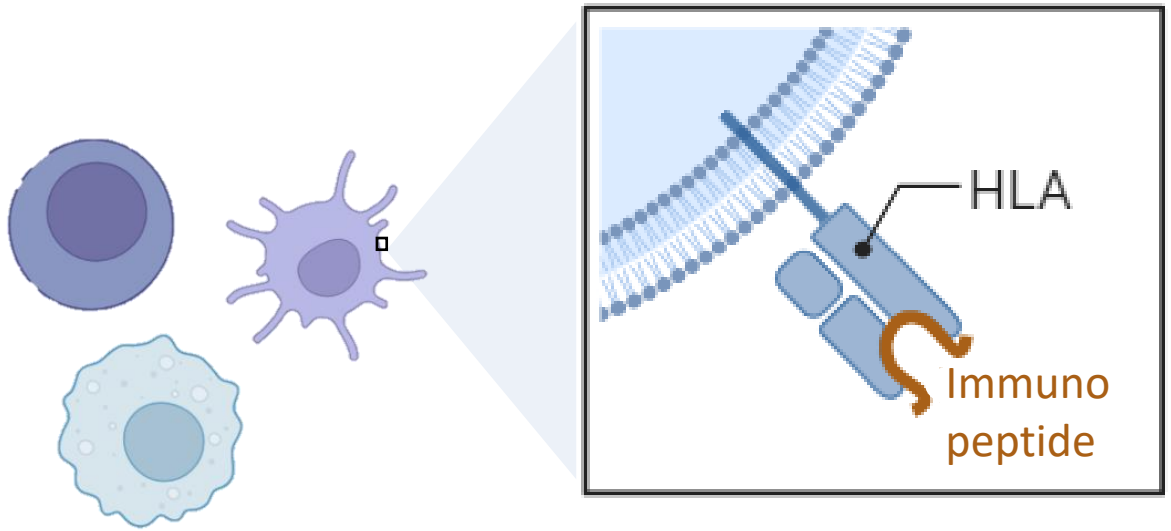

- Complex system of genes that encodes for surface membrane glycoproteins (**HLA molecules**).
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- For HLA class I, peptides are 8-12 amino acids long

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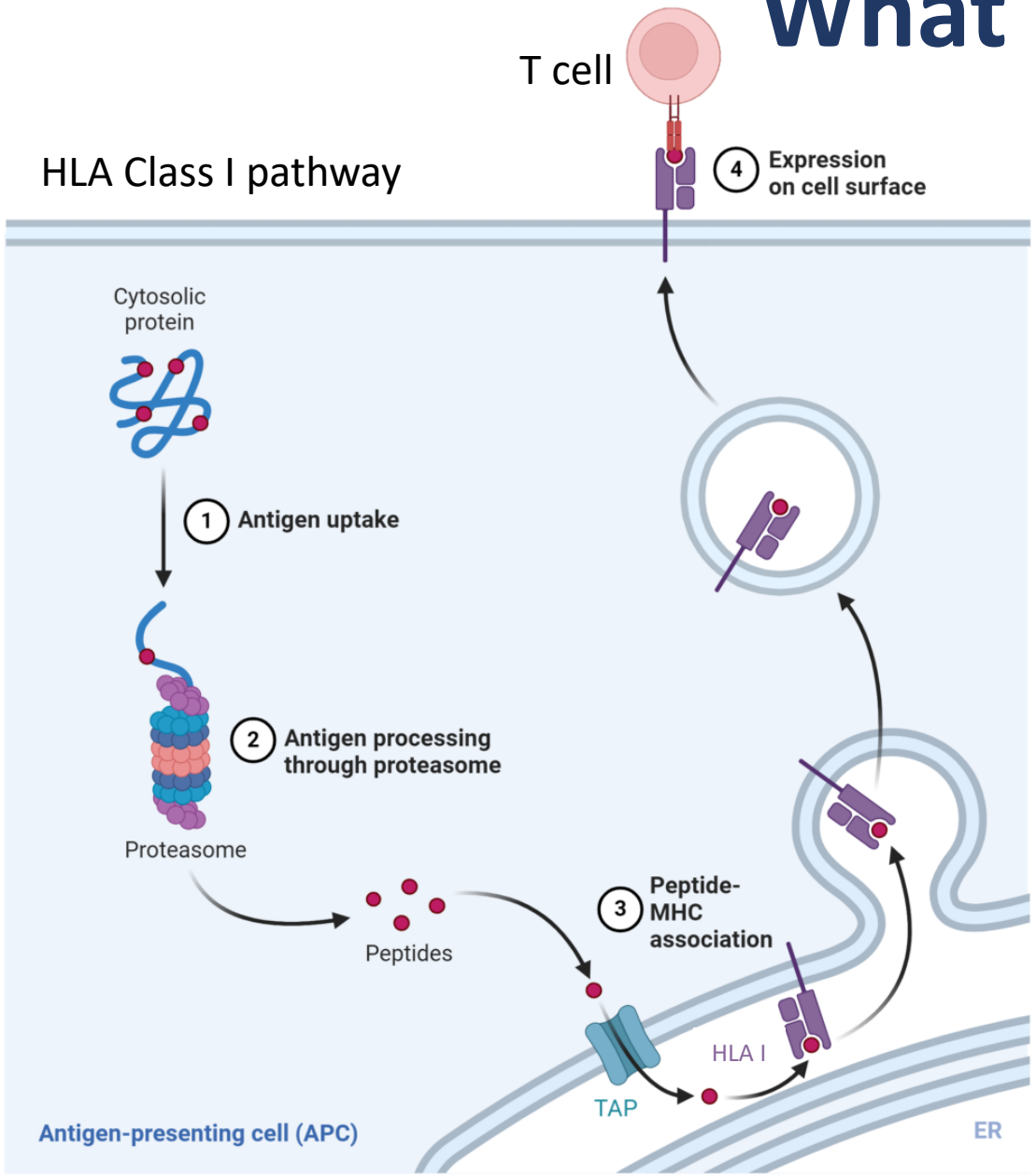

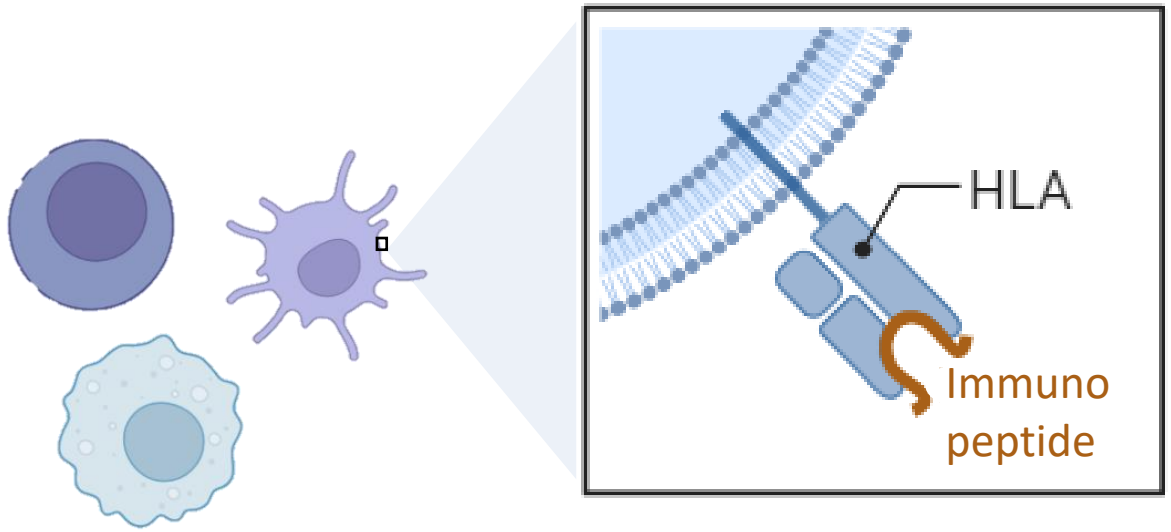

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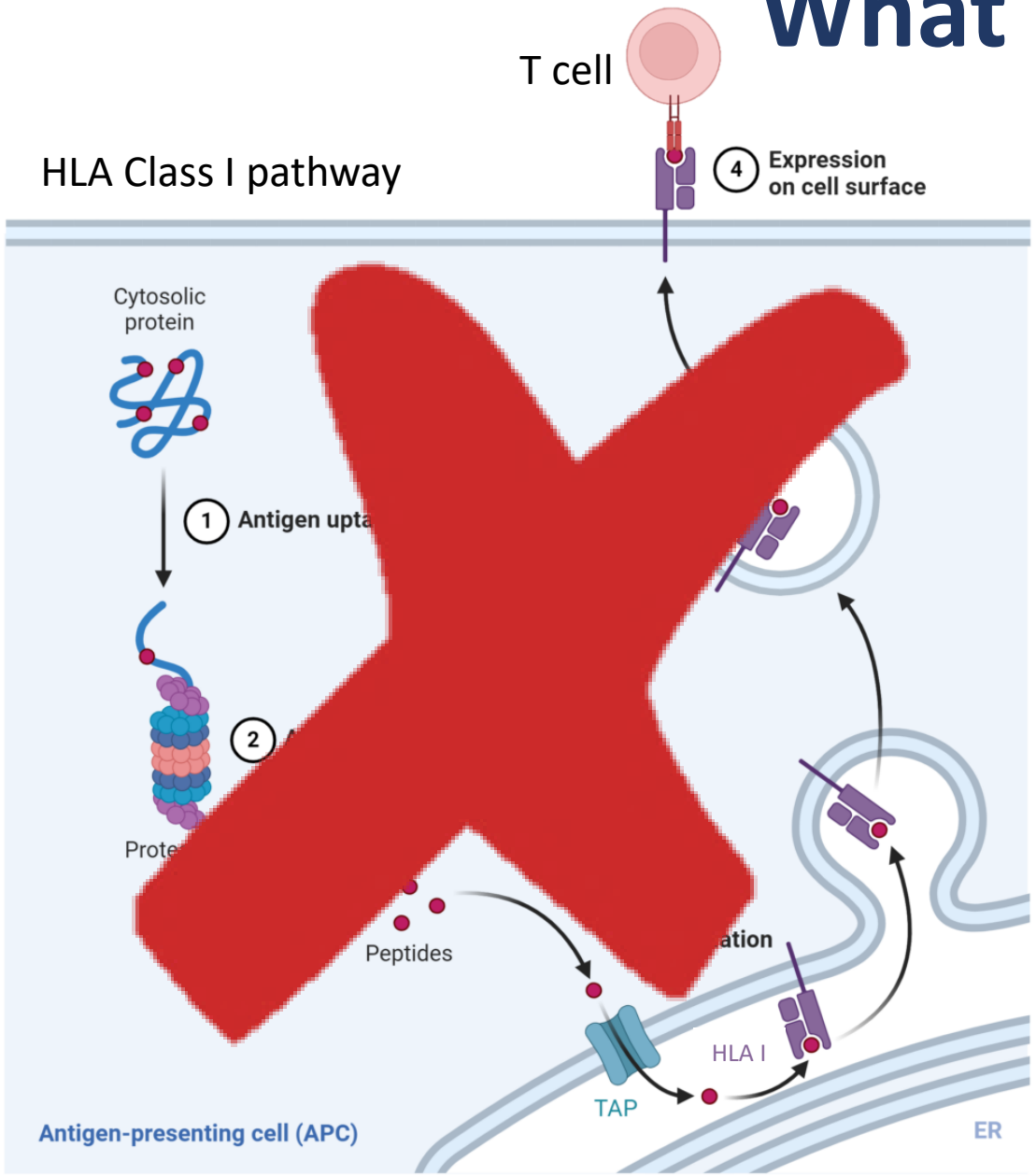

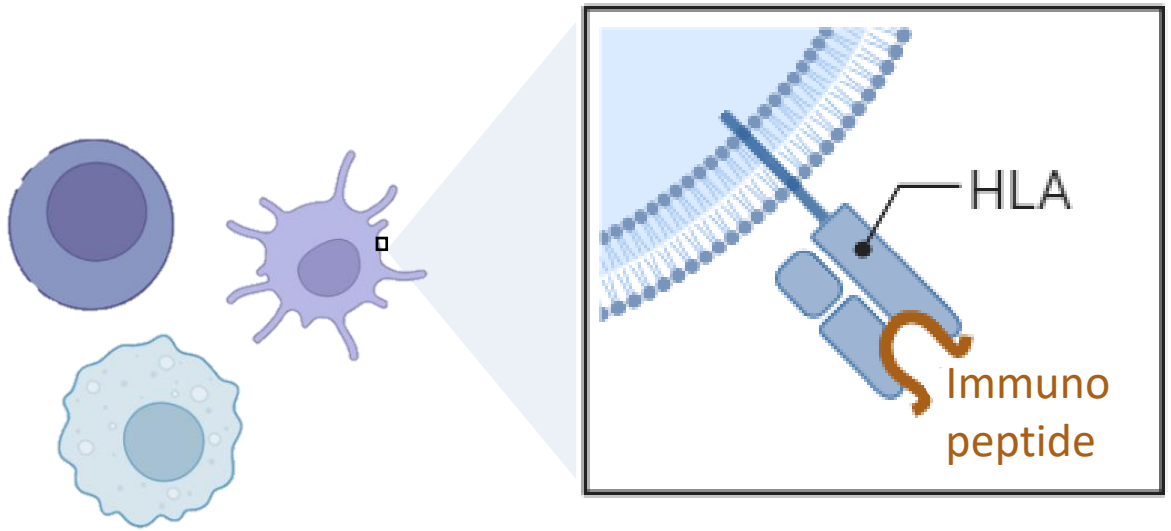

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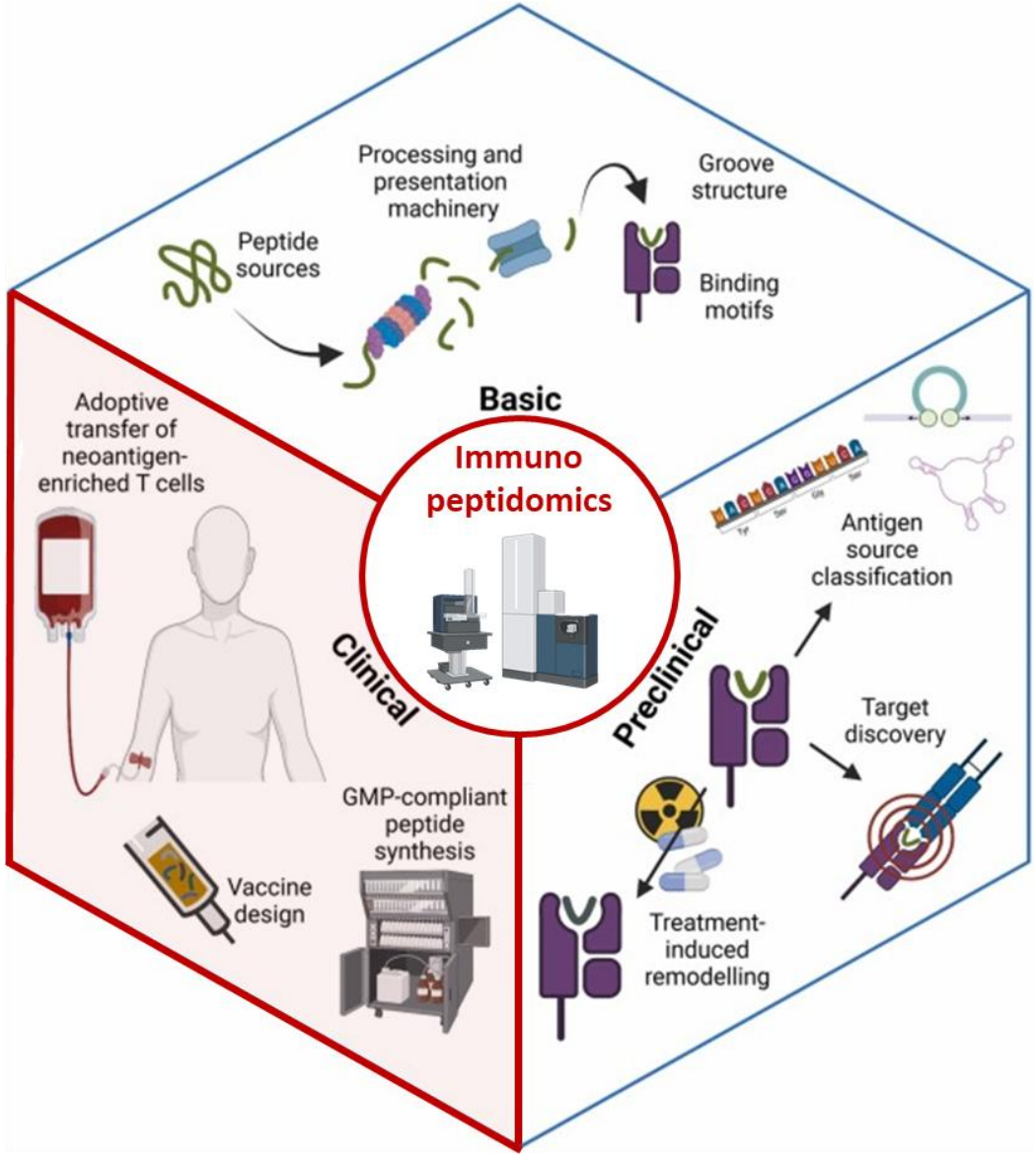
From discovery to therapy: The multiple applications of immunopeptidomic

Cancer immunotherapy
Neoantigen* discovery, personalized cancer vaccines, T-cell therapies

Vaccine development
Identification of pathogen-derived peptides for targeted vaccines

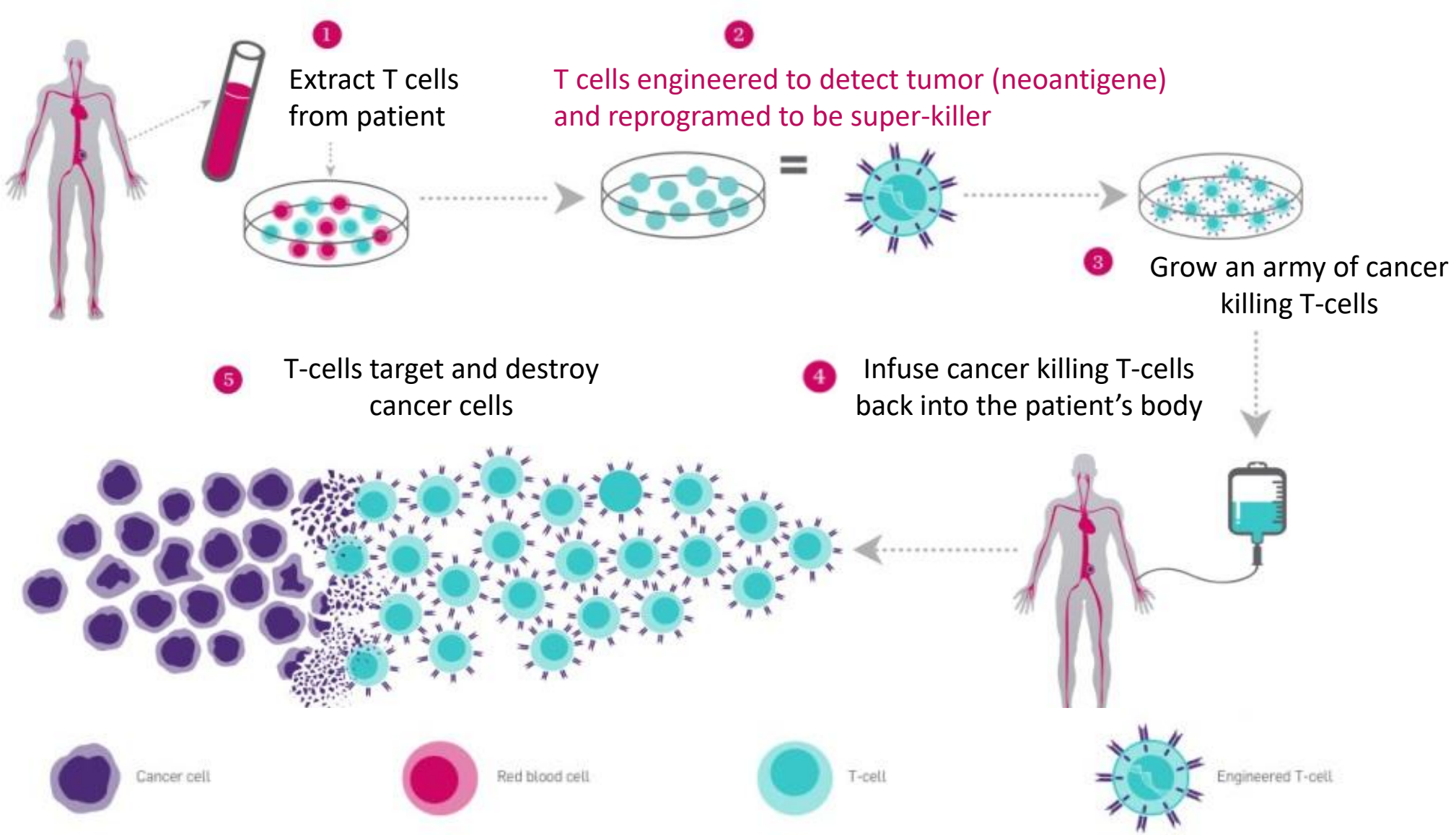
Infectious disease research
Understanding immune responses to viruses, bacteria, parasite



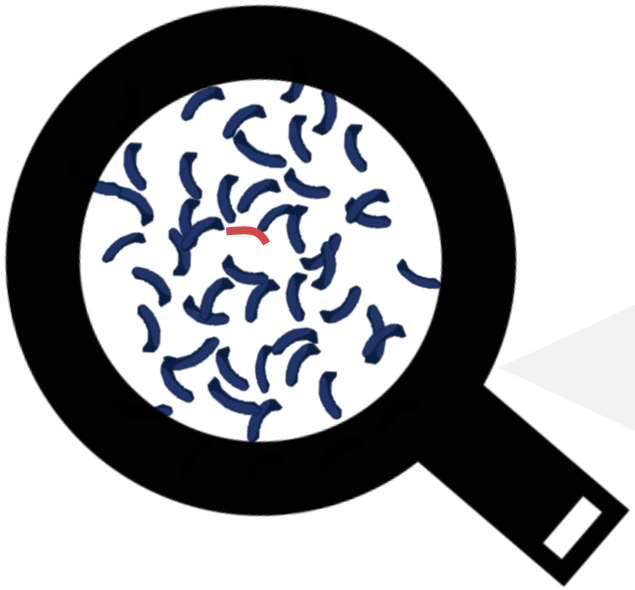
*mutated cancer peptides

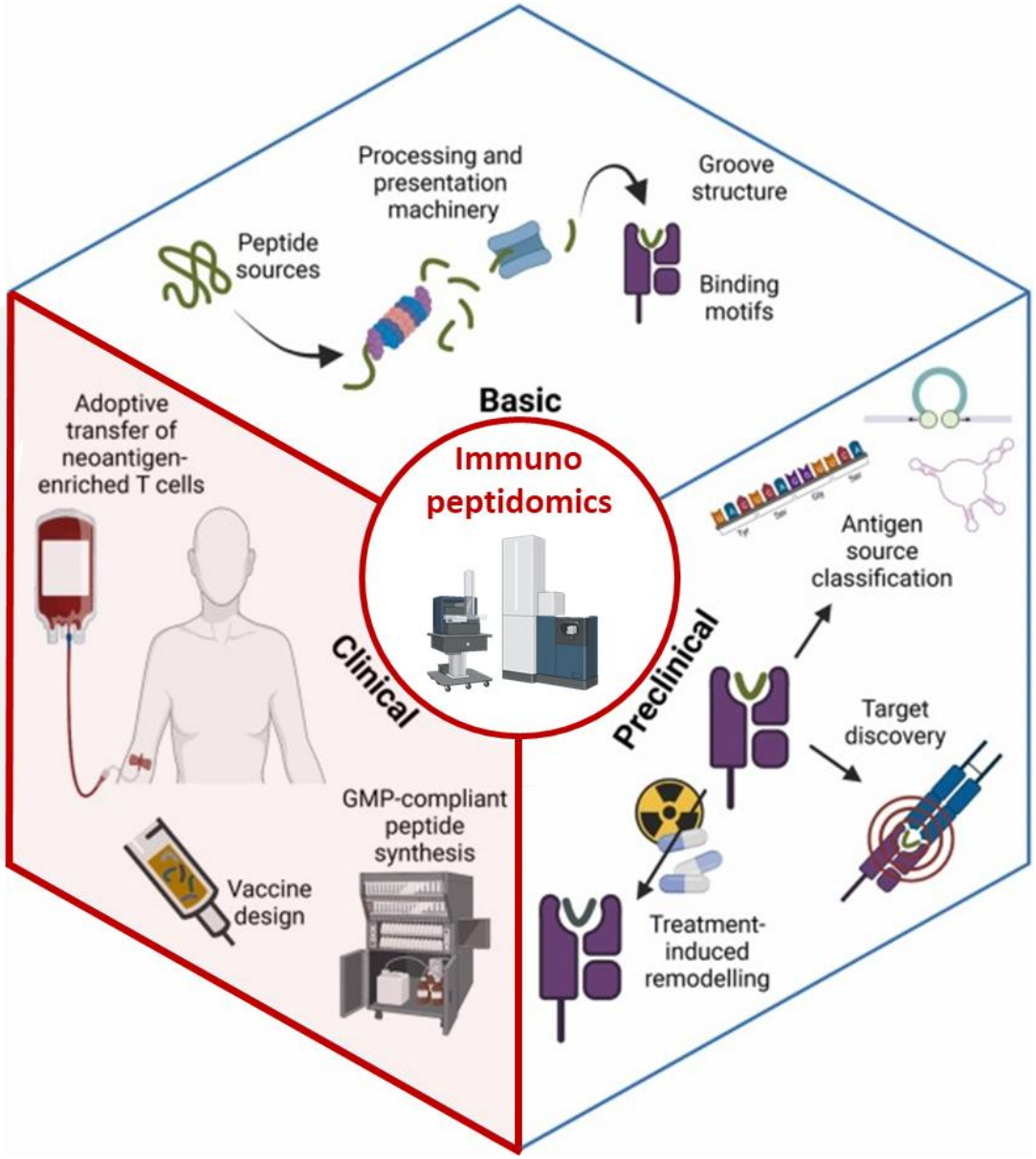
Shapiro IE. *et al*., 2023, Seminars in Immunology

Personalized therapy as an example for immunopeptidomics application



Identifying neoantigen requires extreme sensitivity



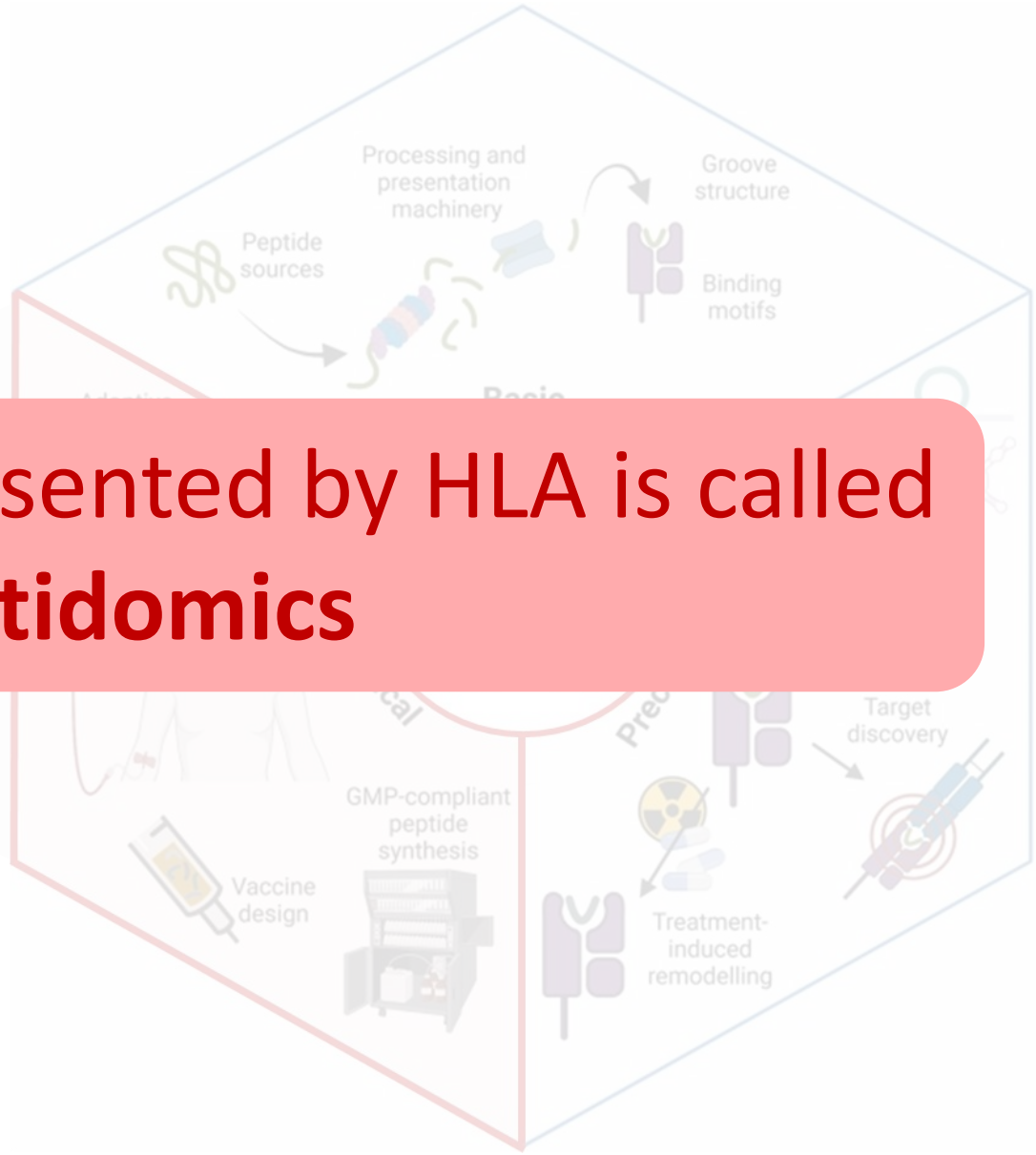


Neoantigens: promising targets to develop personalized therapy

Shapiro IE. *et al*., 2023, Seminars in Immunology

Identifying neoantigen requires extreme sensitivity

Analysis of all peptides presented by HLA is called **Immunopeptidomics**



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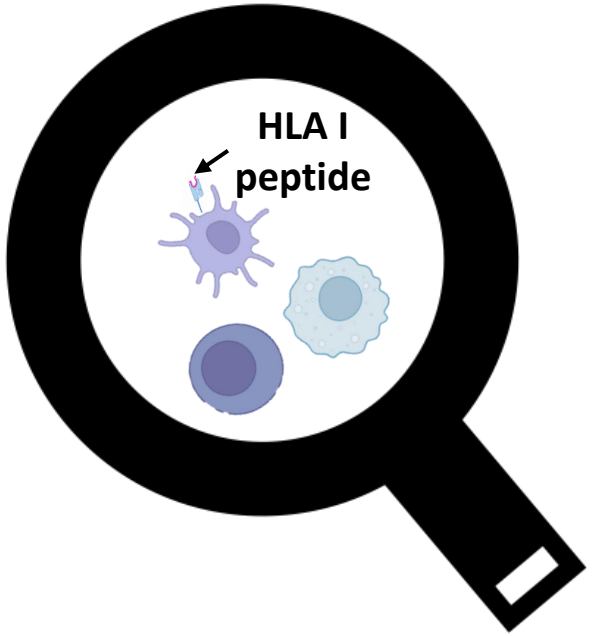
Immunopeptidomics analysis is challenging



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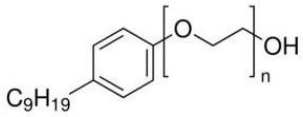
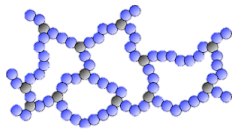
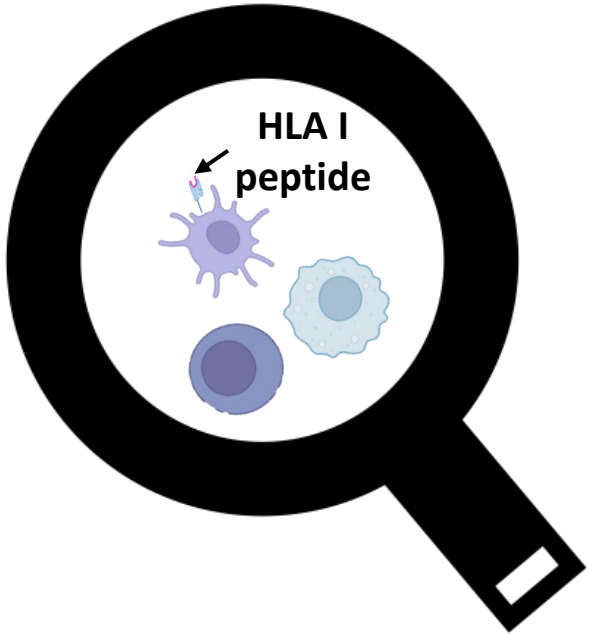
Specific and tricky sample preparation







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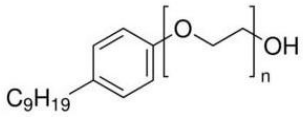
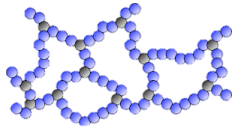
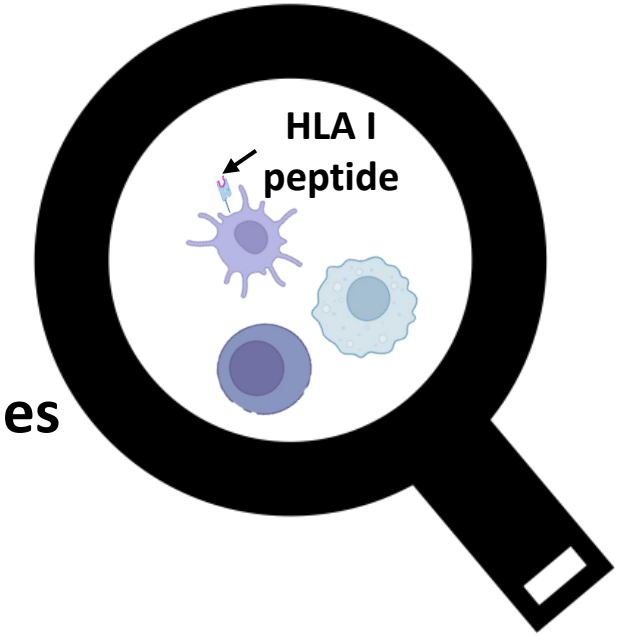
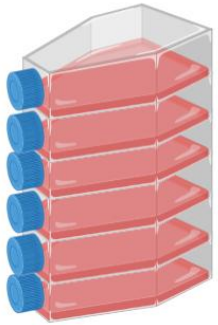






Low abundance of immunopeptides



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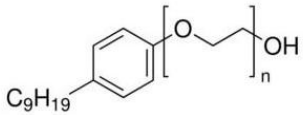

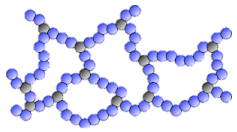

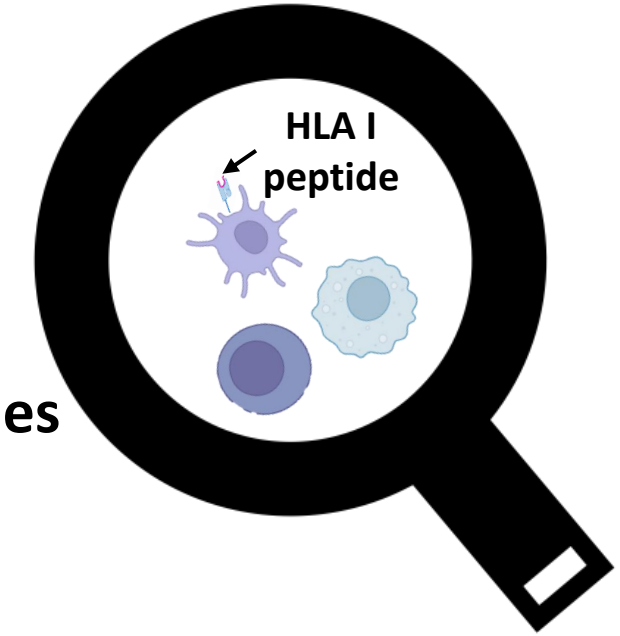

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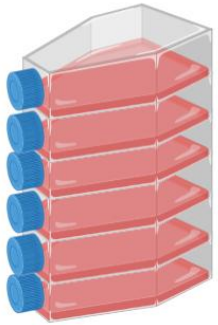
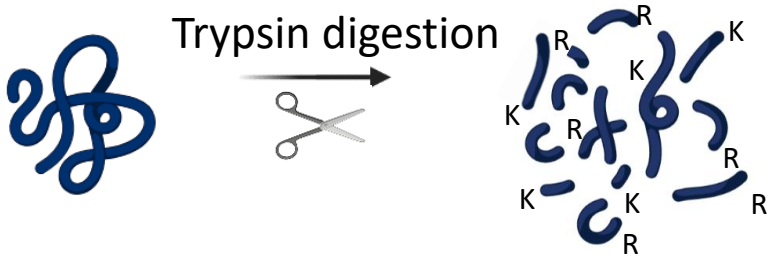


Non tryptic nature of immunopeptides

Classical proteomics digestion



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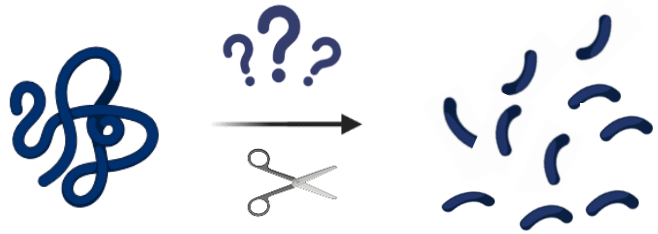

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Non tryptic nature of immunopeptides

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Immunopeptides

Low abundance

sensitivity

All steps of the workflow still benefit from thorough optimization

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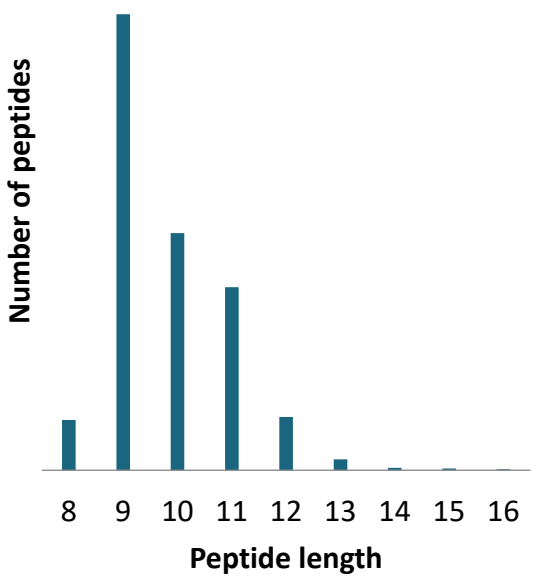
Sample preparation → **LC-MS/MS analysis** → **Bioinformatic data treatment**

Fortunately we have tools to confirm HLA ligand peptides

Peptide length



Majority of 9 amino acids

M. Nielsen, NAR (2020)

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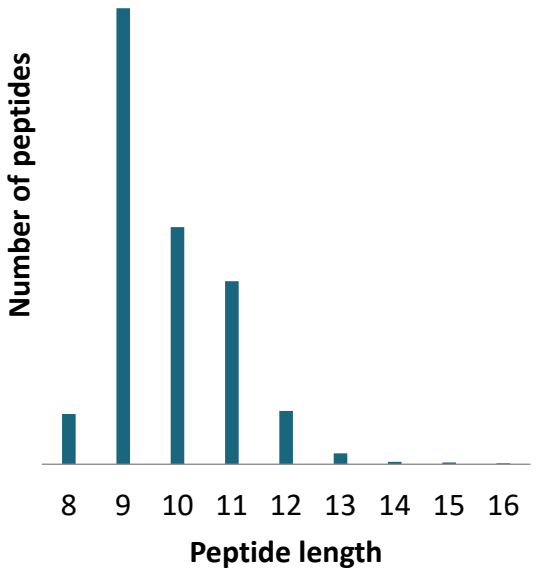
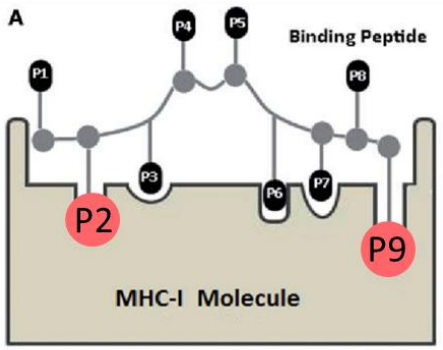
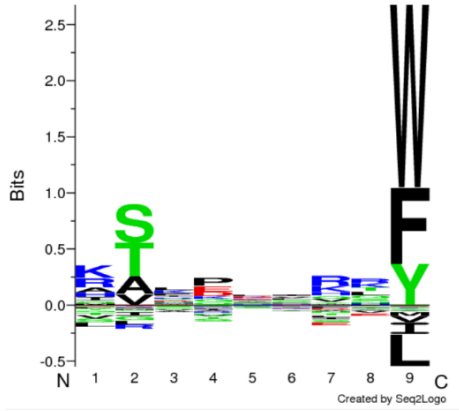


Majority of 9 amino acids

Amino acid repartition

GibbsCluster–v2.0





Information on peptide specificity

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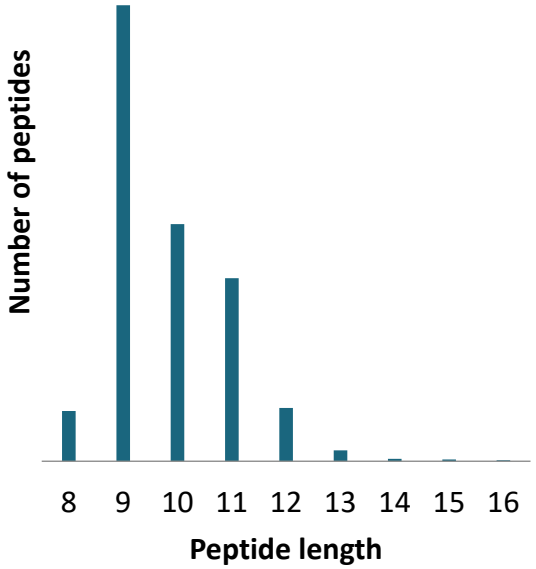

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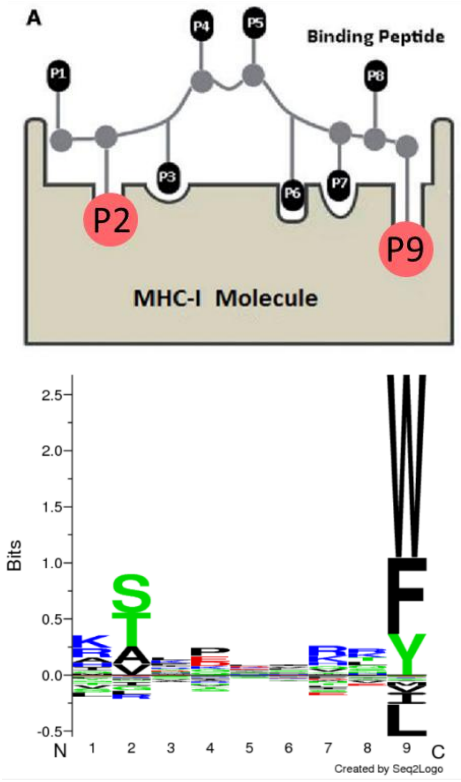
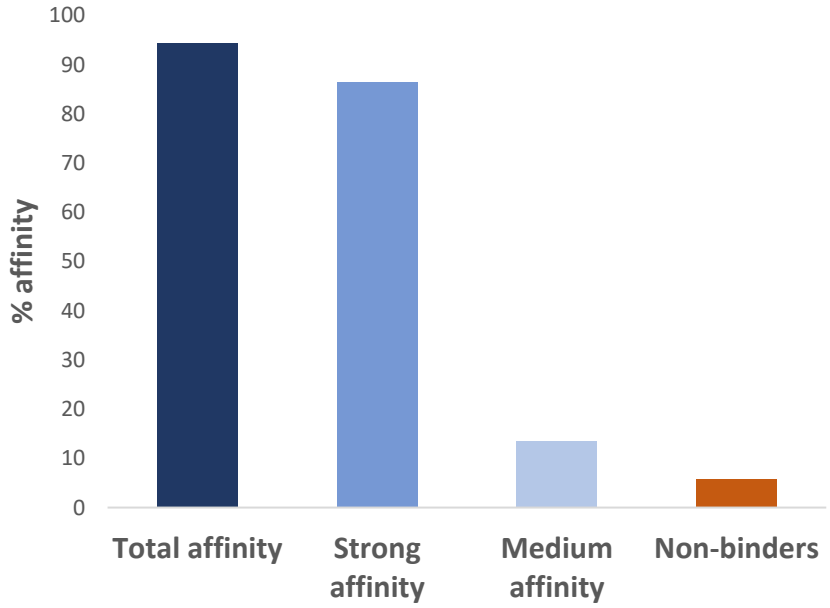


Information on peptide specificity

Bioinformatic prediction binding affinity

NetMHCpan–v4.1



Information of binding specificity of the peptides for HLA-I

M. Nielsen, NAR (2020)

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Implementation of a new immunopeptidomic sample preparation protocol

Lim Kam Sian *et al*, 2023, Frontiers in Immunology

Implementation of a new immunopeptidomic sample preparation protocol

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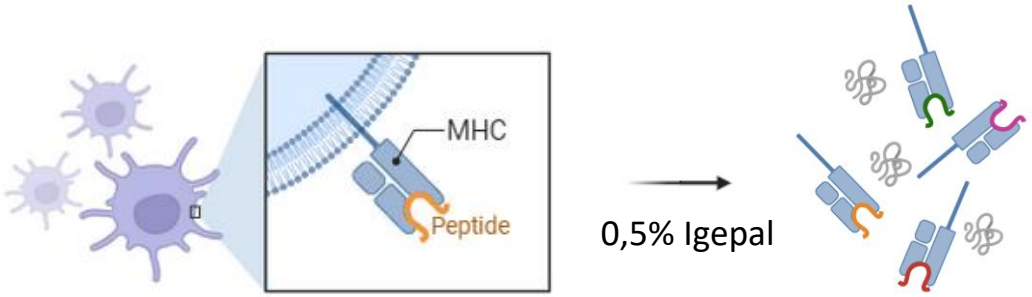
Cell lysis



60 x10^6 HL60 cells

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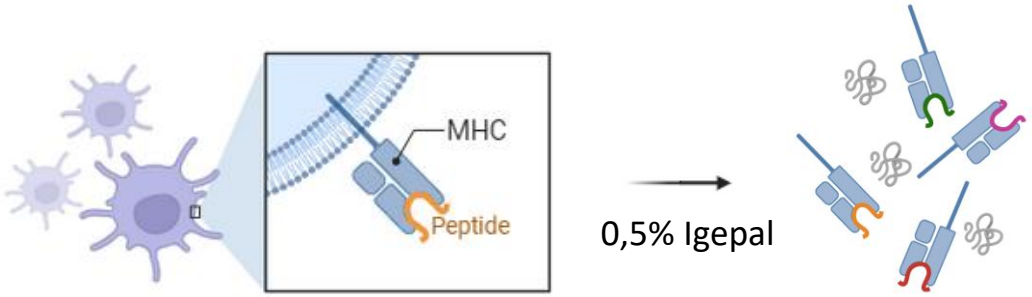

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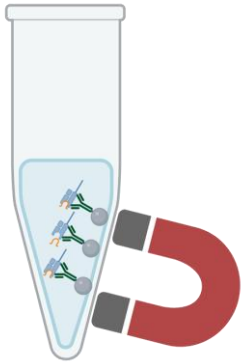
Step 2:
HLA immunoprecipitation



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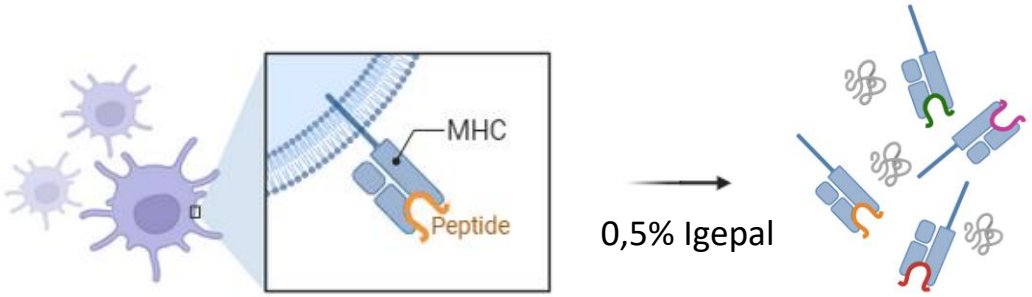

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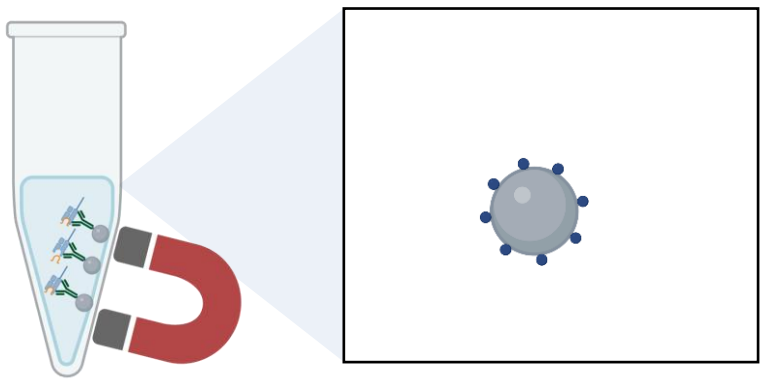

Magnetic bead + Protein A

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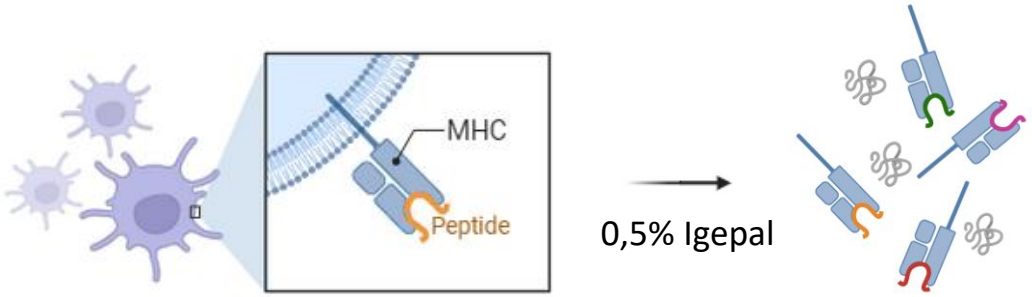

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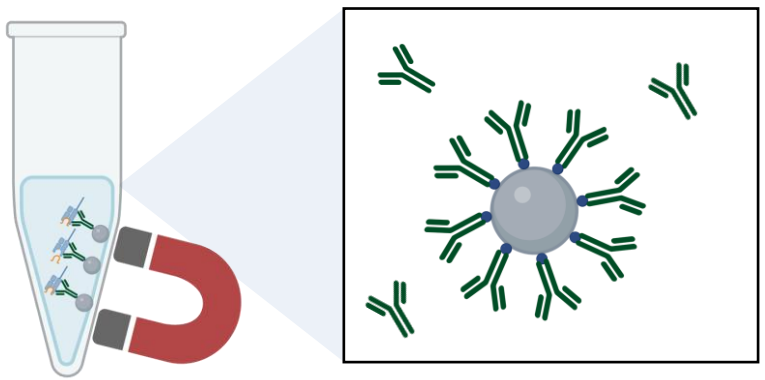

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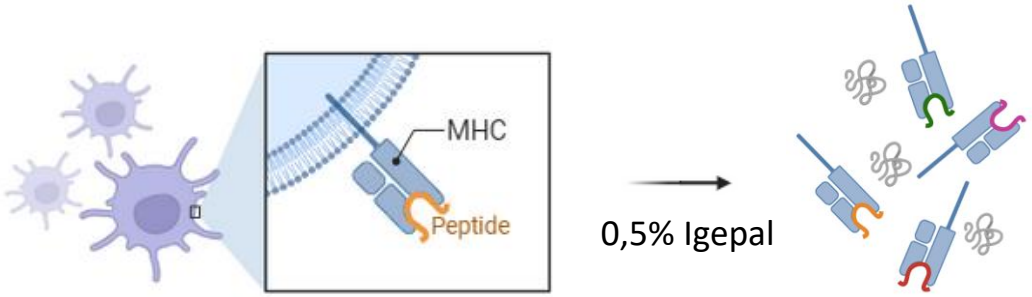

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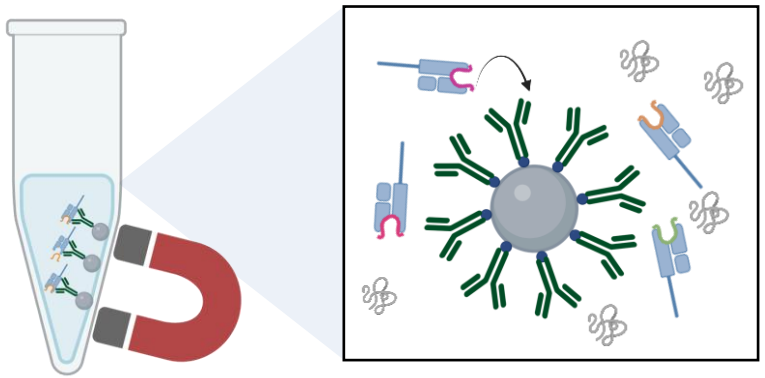


Magnetic bead + Protein A

Anti HLA I antibody

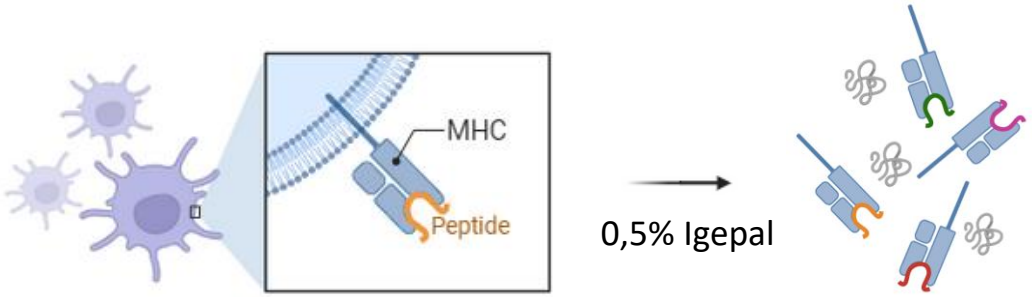
HLA class I + immunopeptide

Other proteins

Lim Kam Sian *et al*, 2023, Frontiers in Immunology

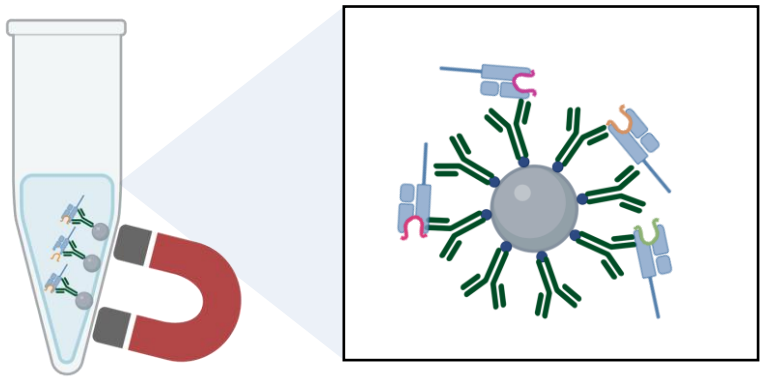
Implementation of a new immunopeptidomic sample preparation protocol

Step 1:
Cell lysis

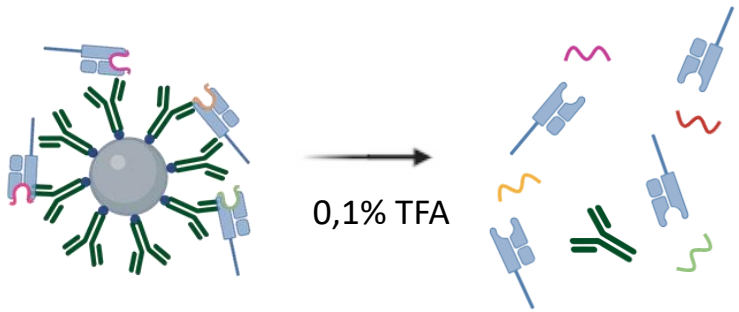


60 x10^6 HL60 cells

Step 2:
HLA immunoprecipitation



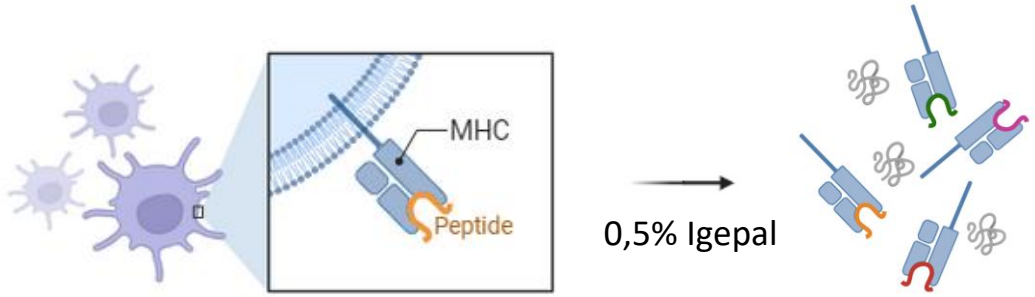
Step 3:
HLA peptide elution and cleanup



Lim Kam Sian *et al*, 2023, Frontiers in Immunology

Implementation of a new immunopeptidomic sample preparation protocol

Step 1:
Cell lysis



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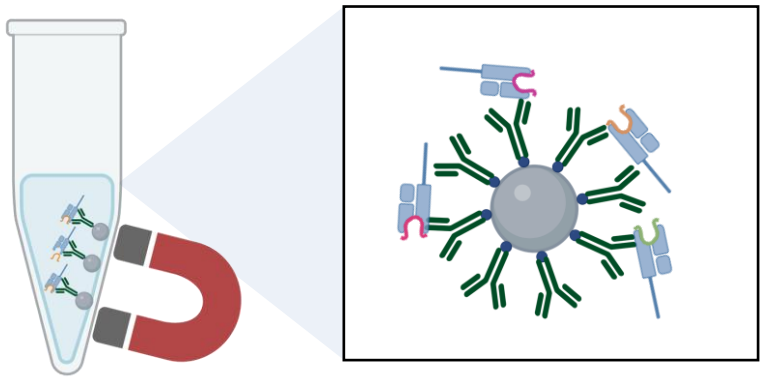

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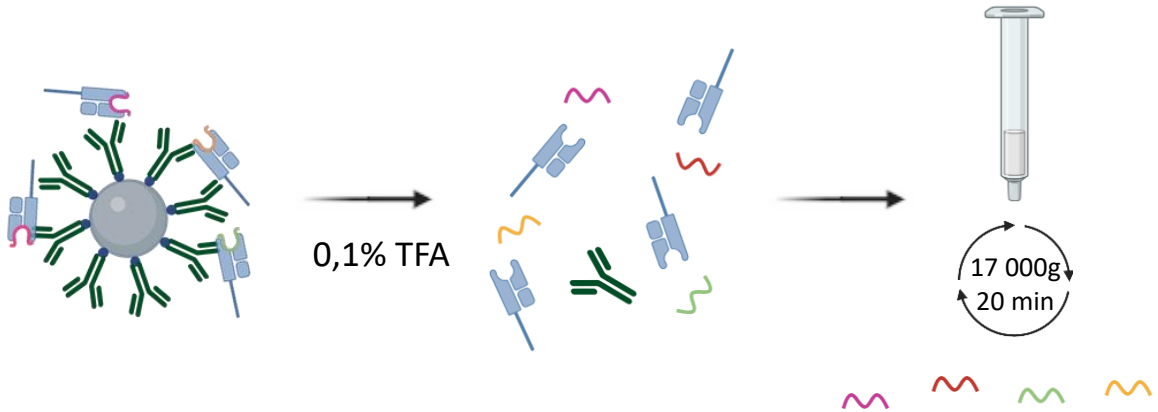
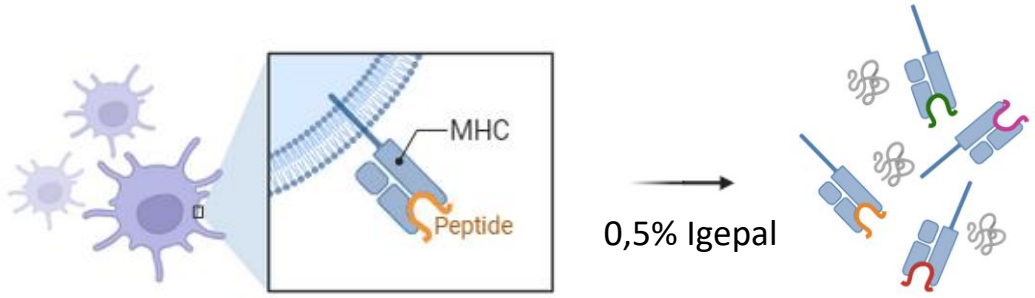


Lim Kam Sian *et al*, 2023, Frontiers in Immunology

Implementation of a new immunopeptidomic sample preparation protocol

Step 1:
Cell lysis



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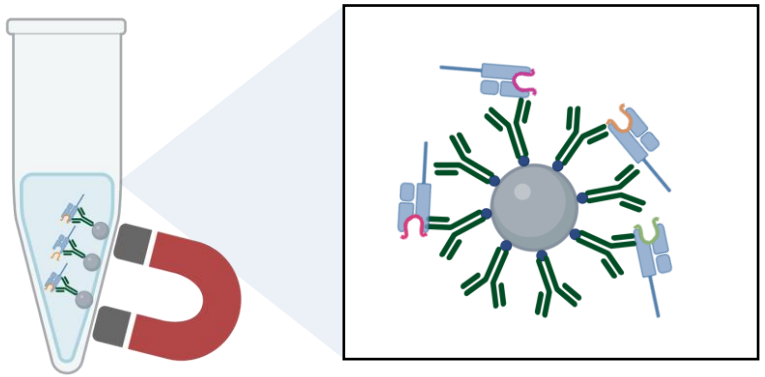
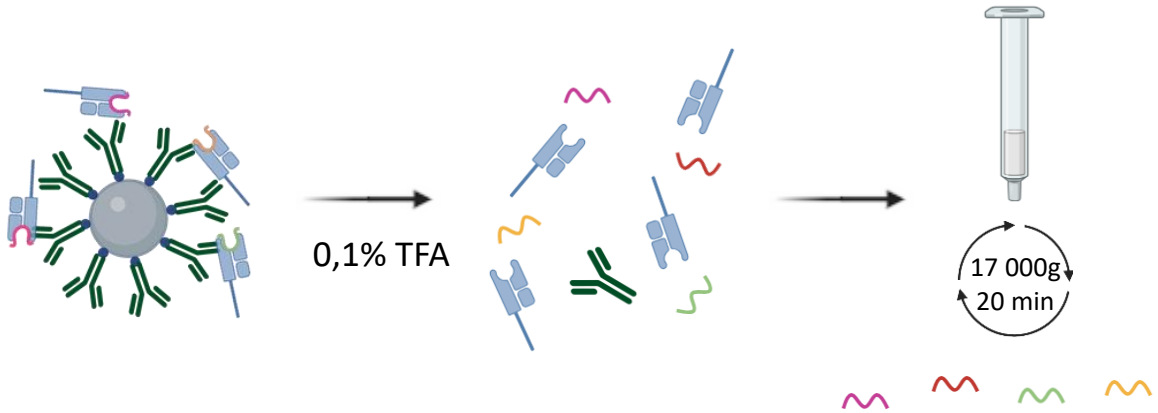
Step 2:
HLA immunoprecipitation



Step 3:
HLA peptide elution and cleanup

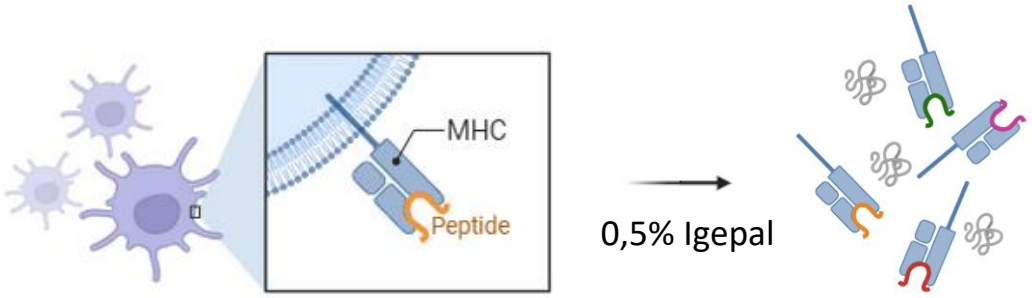


- **10x less starting material (60 million vs 600 million cells)**
- **2x faster**
- **Automation on the Bravo Assay Map plateform**

Lim Kam Sian *et al*, 2023, Frontiers in Immunology

Implementation of a new immunopeptidomic sample preparation protocol

Step 1:
Cell lysis



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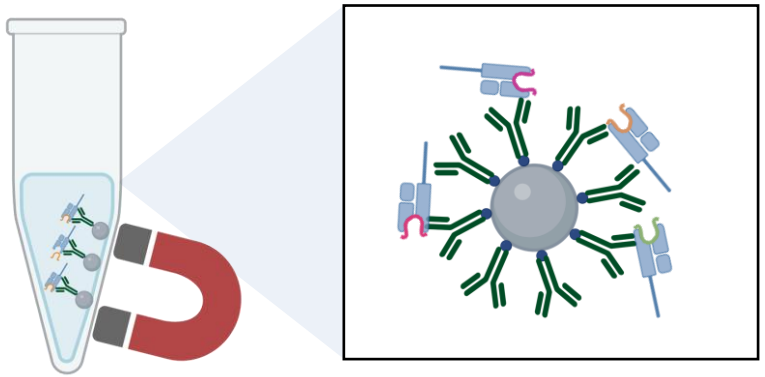

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HLA class I + immunopeptide

Other proteins

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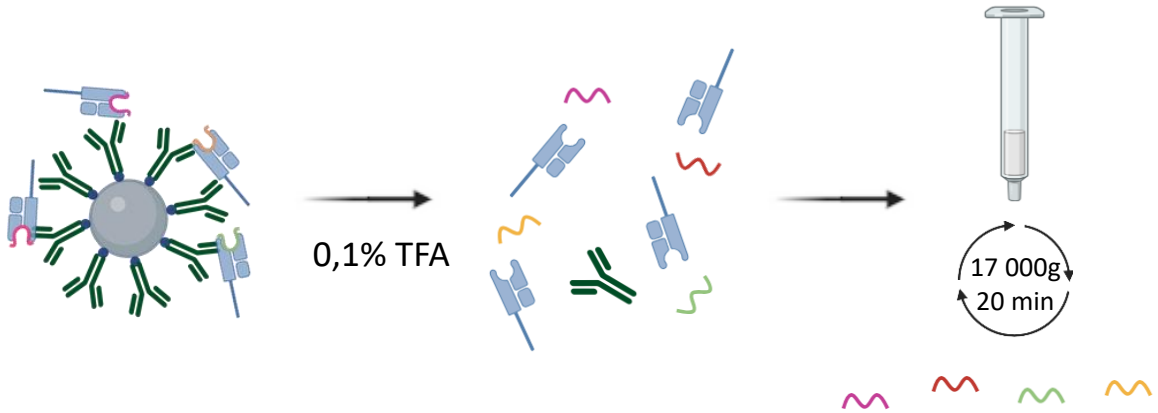
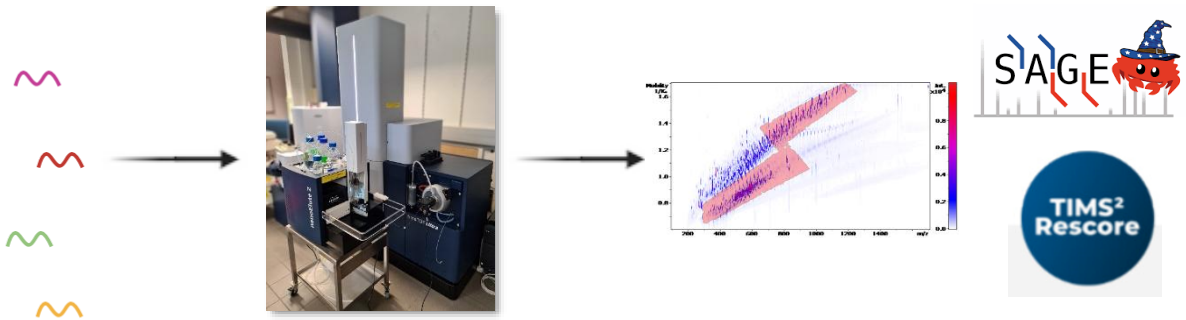
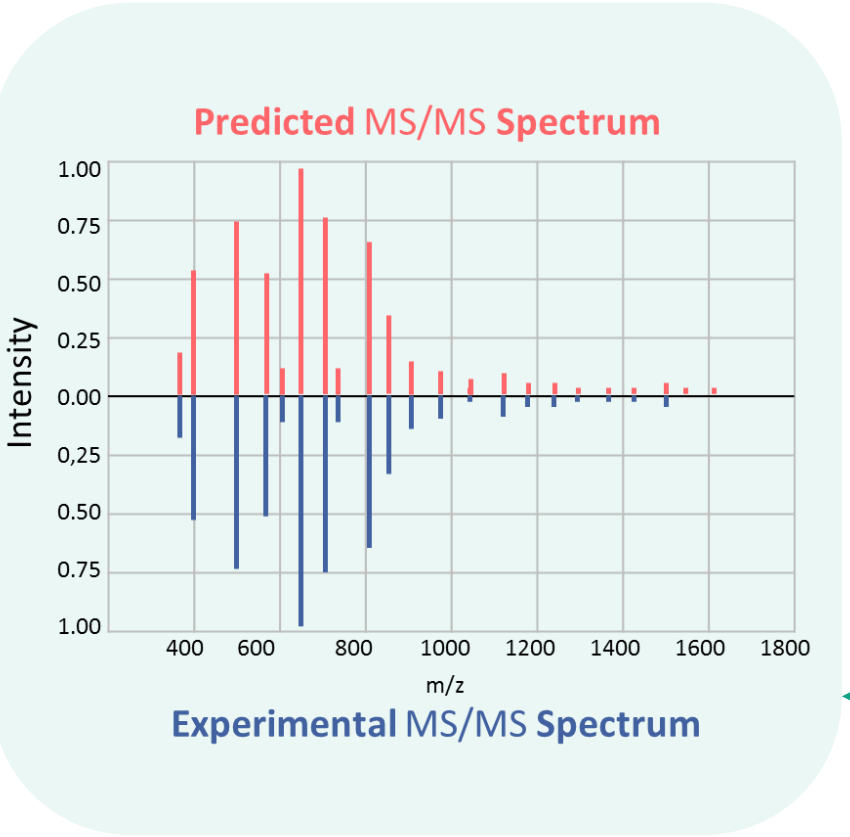


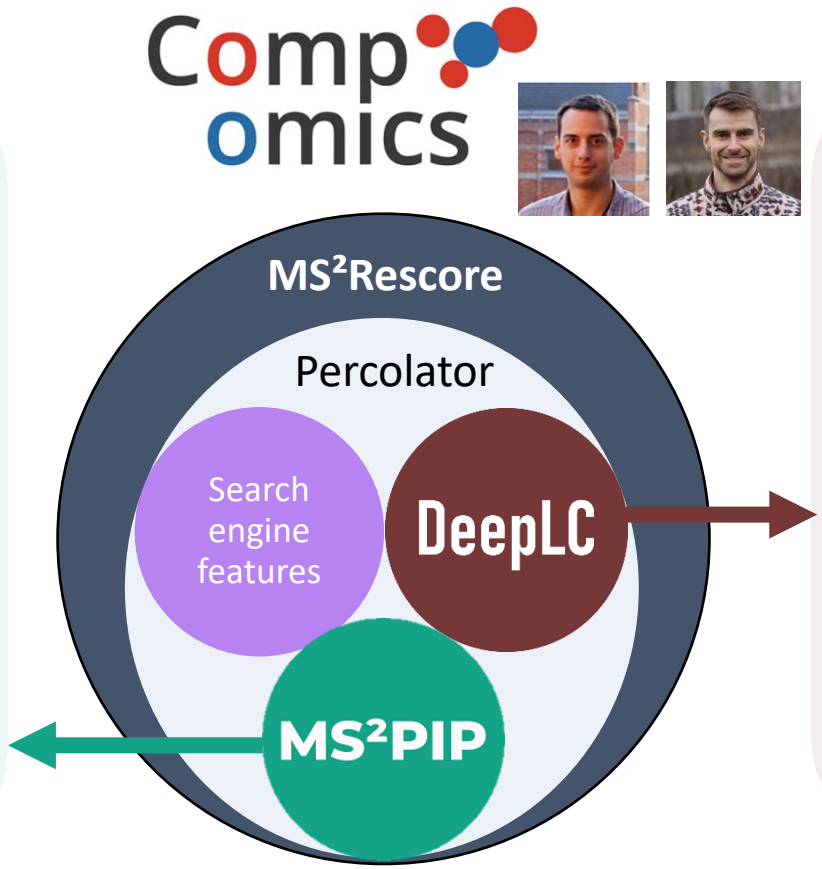
Step 4:
nanoLC-MS/MS analysis

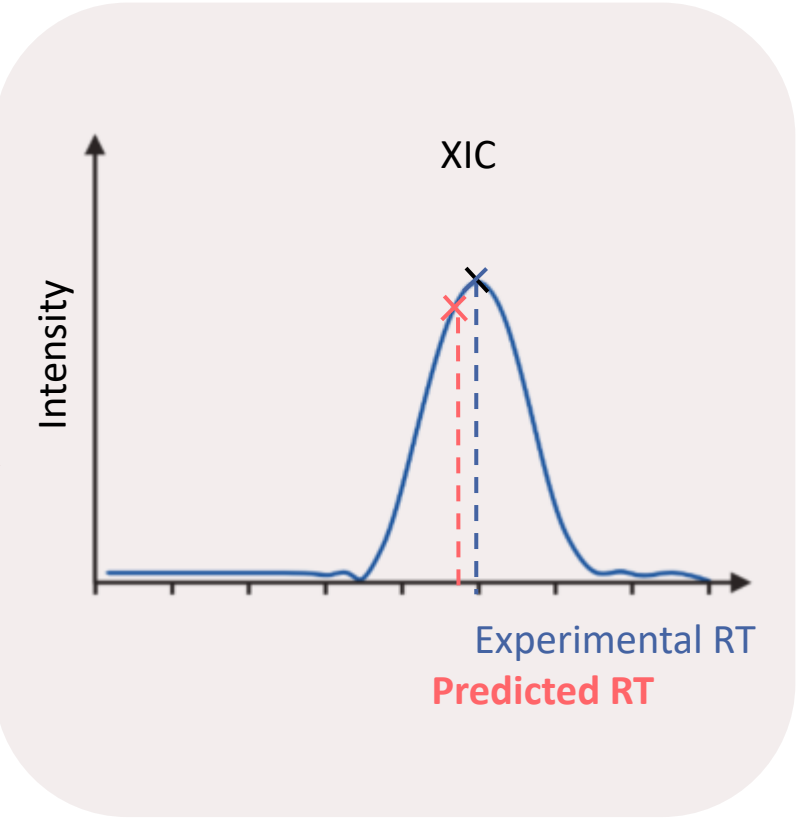


Lim Kam Sian *et al*, 2023, Frontiers in Immunology

AI tool, MS²Rescore, leverages peak intensity and retention time prediction to increase identification





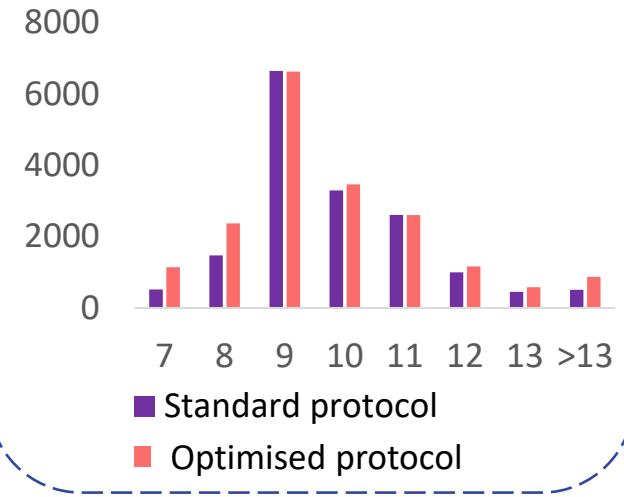


MS²Rescore: Declercq A. *et al*, 2022, MCP ; Declercq A. *et al*, 2023, NAR ; Buur L.M. *et al*., 2024, JPR
DeepLC: Bouwmeester R. *et al*, 2021, Nature Methods
MS²PIP: Gabriels R. *et al*, 2019, Nucleic Acids Research

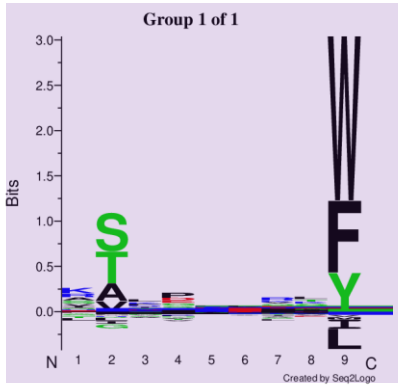
Optimised protocol outperforms previous protocol

Jeewan Babu Rijal

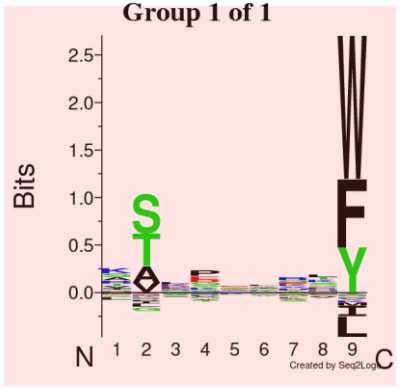
Peptide length



Amino acid repartition

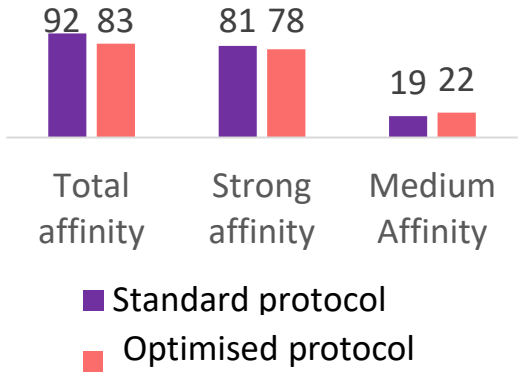


Standard protocol



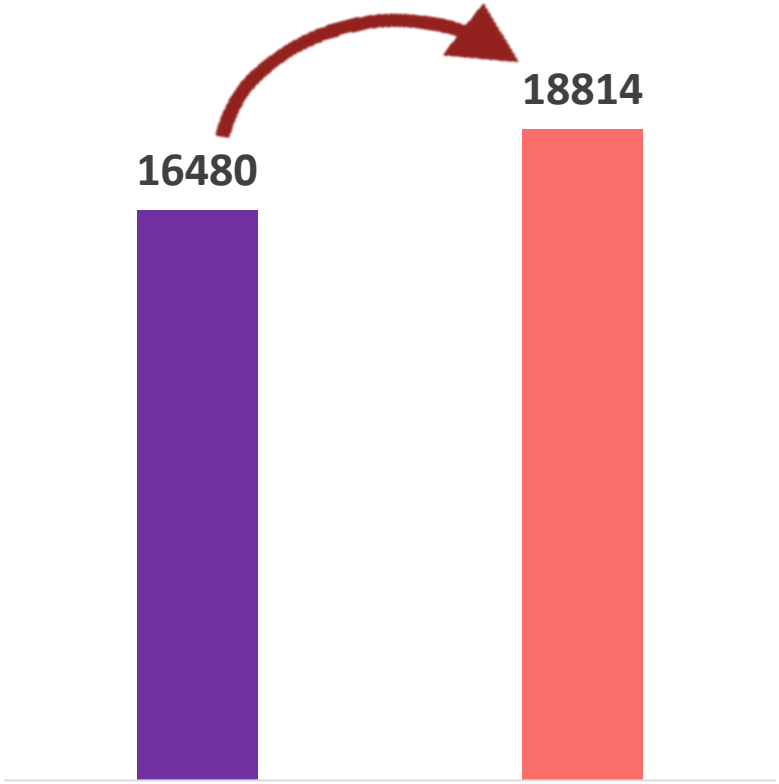
Optimised protocol

Prediction binding affinity



Peptides number

10x less starting material
2x less injected cells



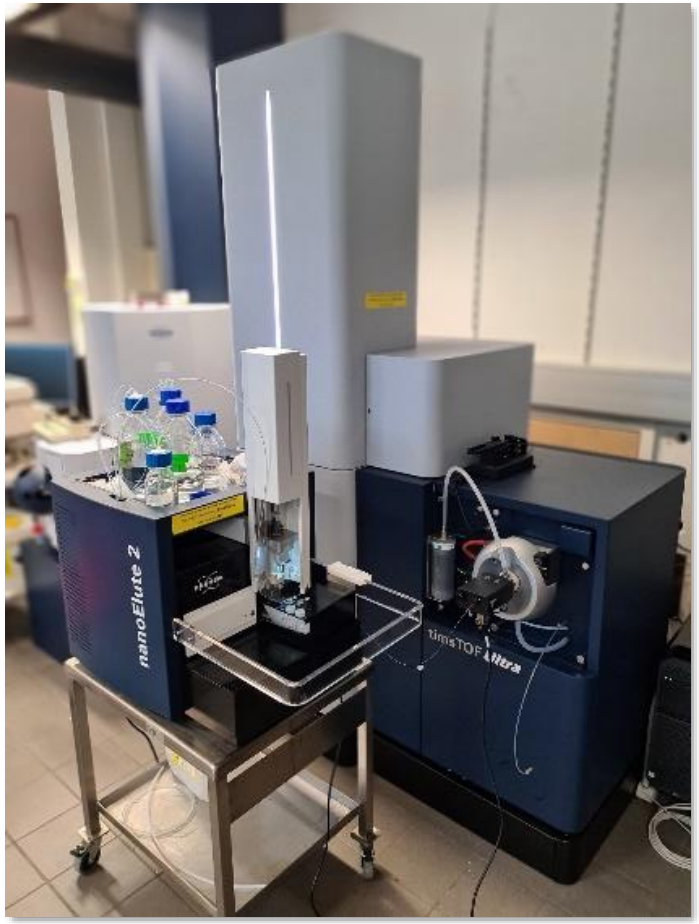
30 million cells | **15 million cells**

Immunopeptidomics

- Why immunopeptidomics?
- Challenges of immunopeptidomics analysis
- Sample preparation optimisation using magnetic beads
- Fine tuning of LC and MS methods

Ultra sensitive instruments used for immunopeptidomics

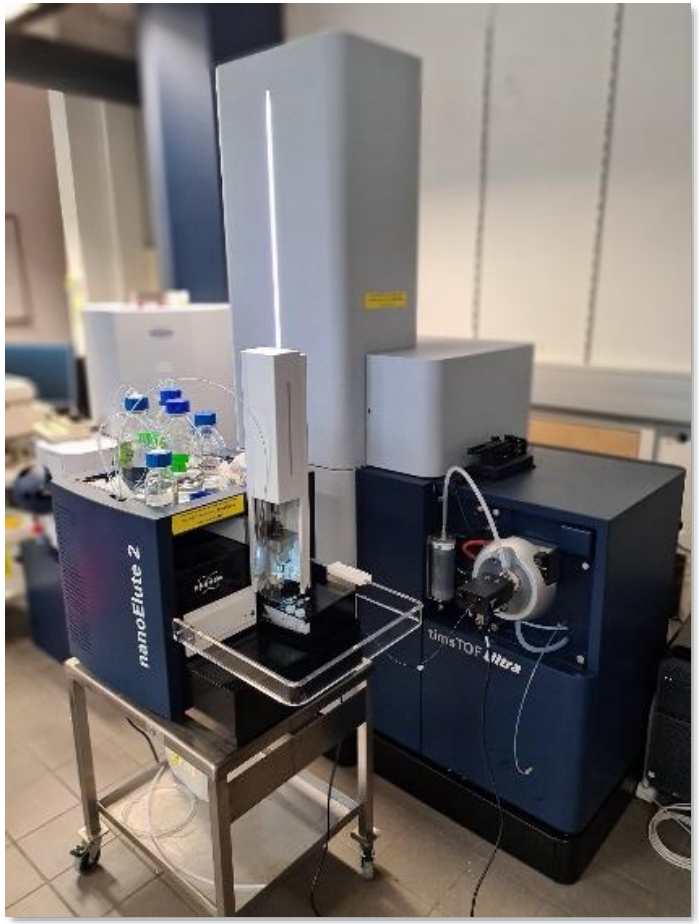
nanoElute 2 +
timsTOF Ultra 2 (Bruker)



Vanquish Neo +
Orbitrap Astral (Thermo Fisher)

Immunopeptidomics on timsTOF Ultra 2 intrument

nanoElute 2 +
timsTOF Ultra 2 (Bruker)



Vanquish Neo +
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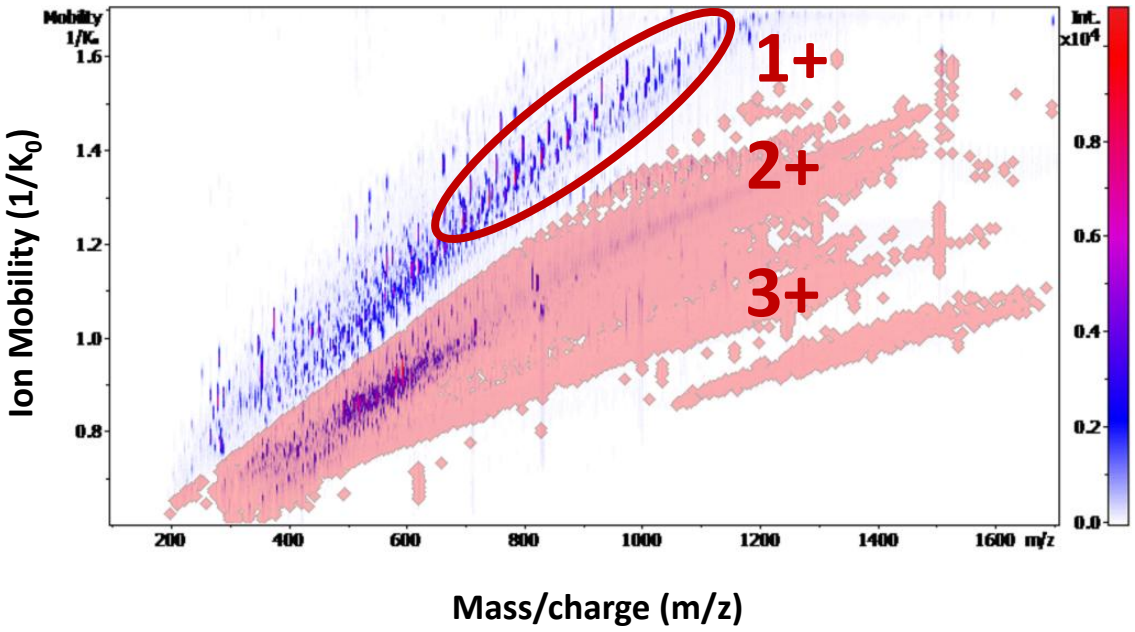
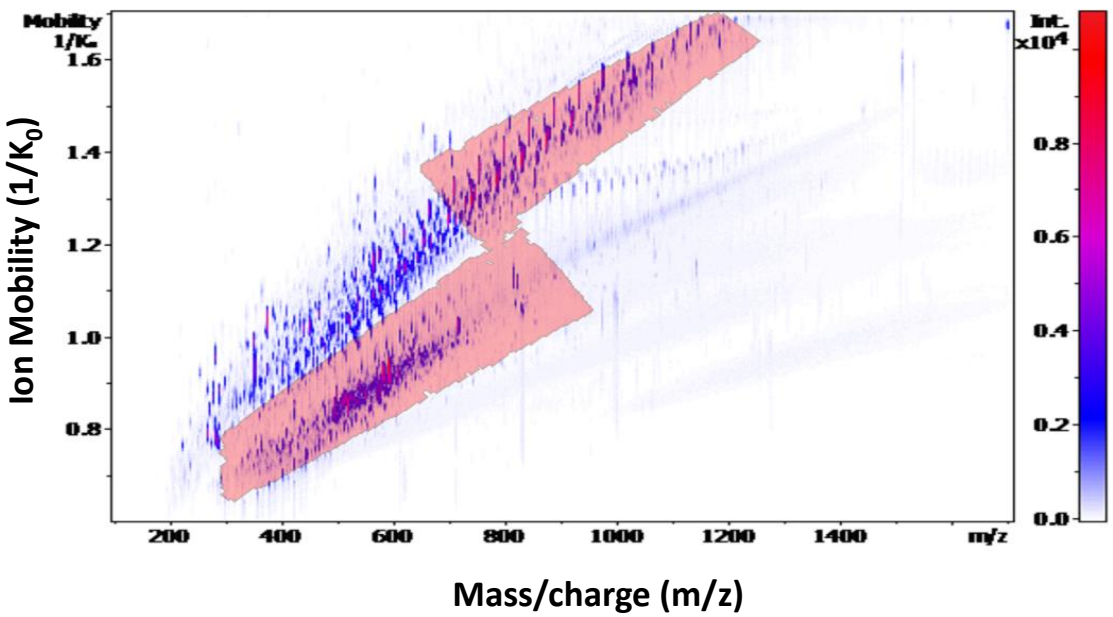
Immunopeptidomics tailored method is crucial

Standard polygon in classical proteomics



HLA tailored polygon



✗ Fragmentation of **singly charged peptide ions** are missing in standard proteomics due to the filter polygon shape.

✓ **A tailored polygon** method is needed to acquire singly charged peptides.

✓ The shape of the polygon should be narrow to avoid noise.

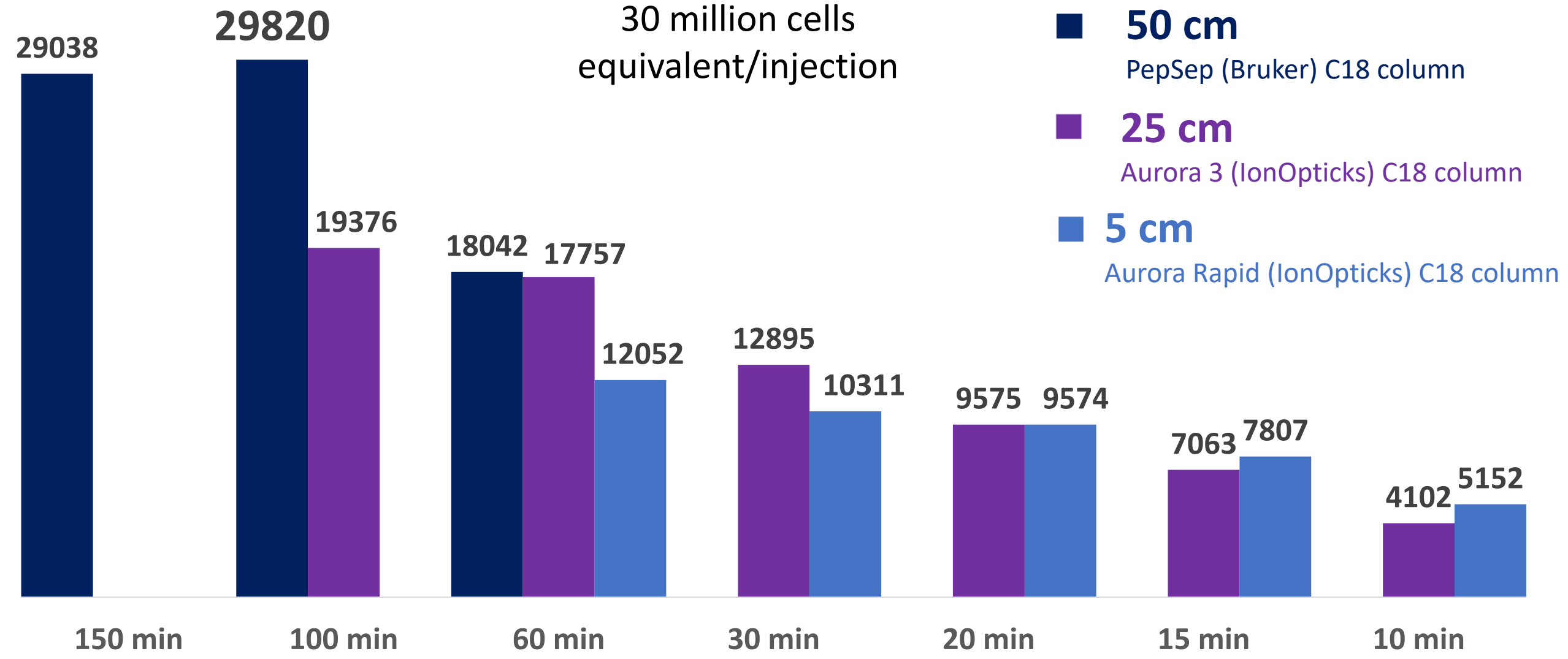
Gomez- Zepeda D., *et al*, 2024, Nature Communication

Up to 30 000 IDs on timsTOF Ultra!

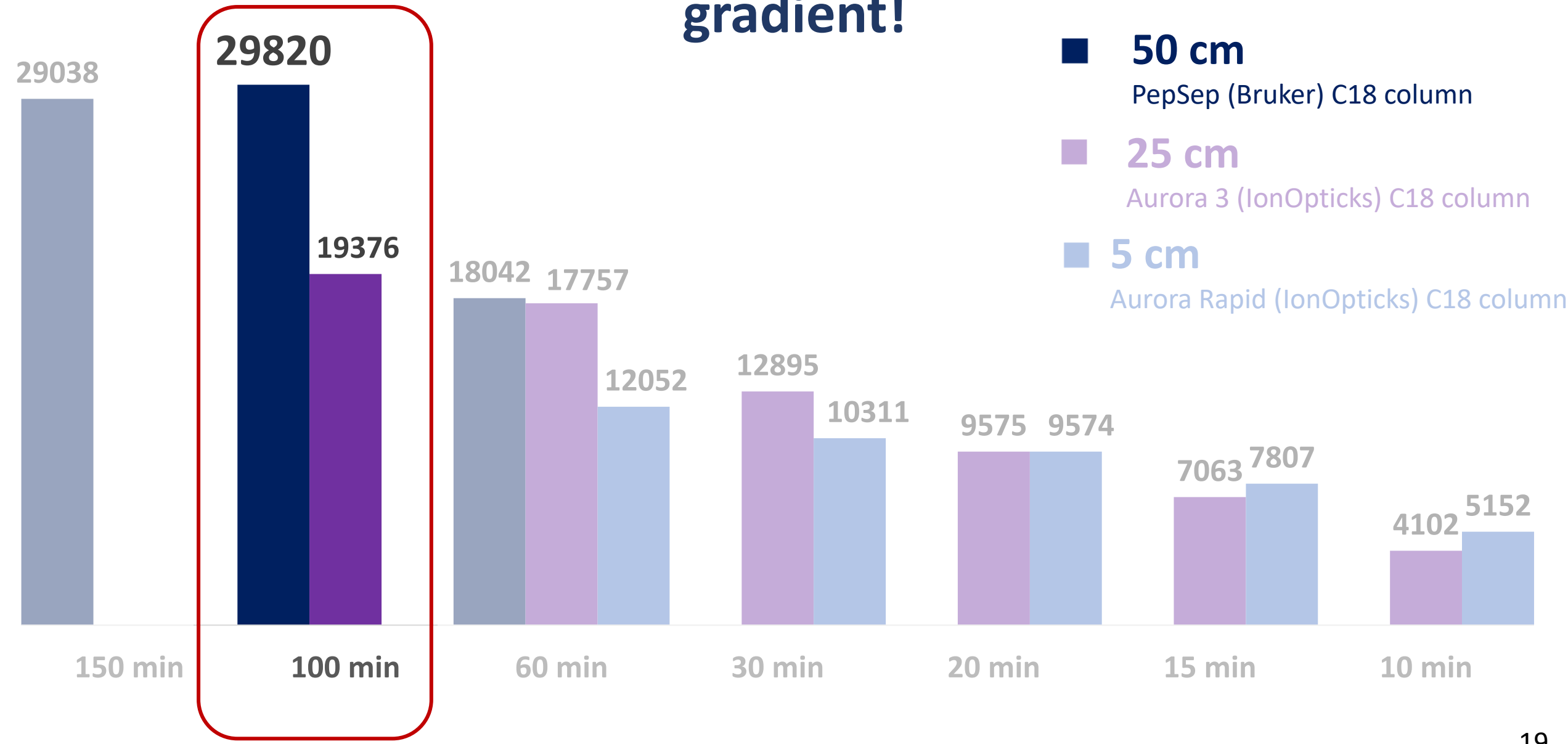
30 million cells
equivalent/injection

50 cm
PepSep (Bruker) C18 column

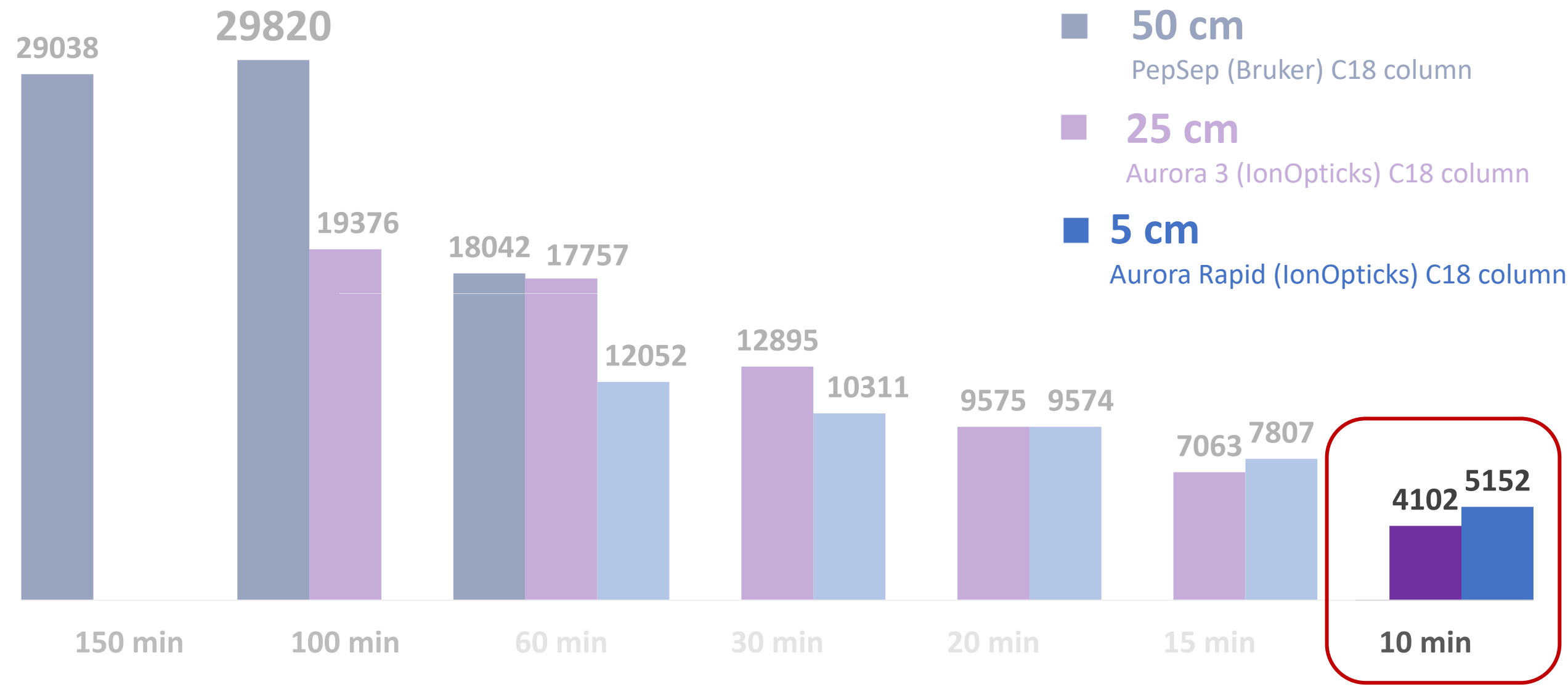
Impact of column length and gradient time!



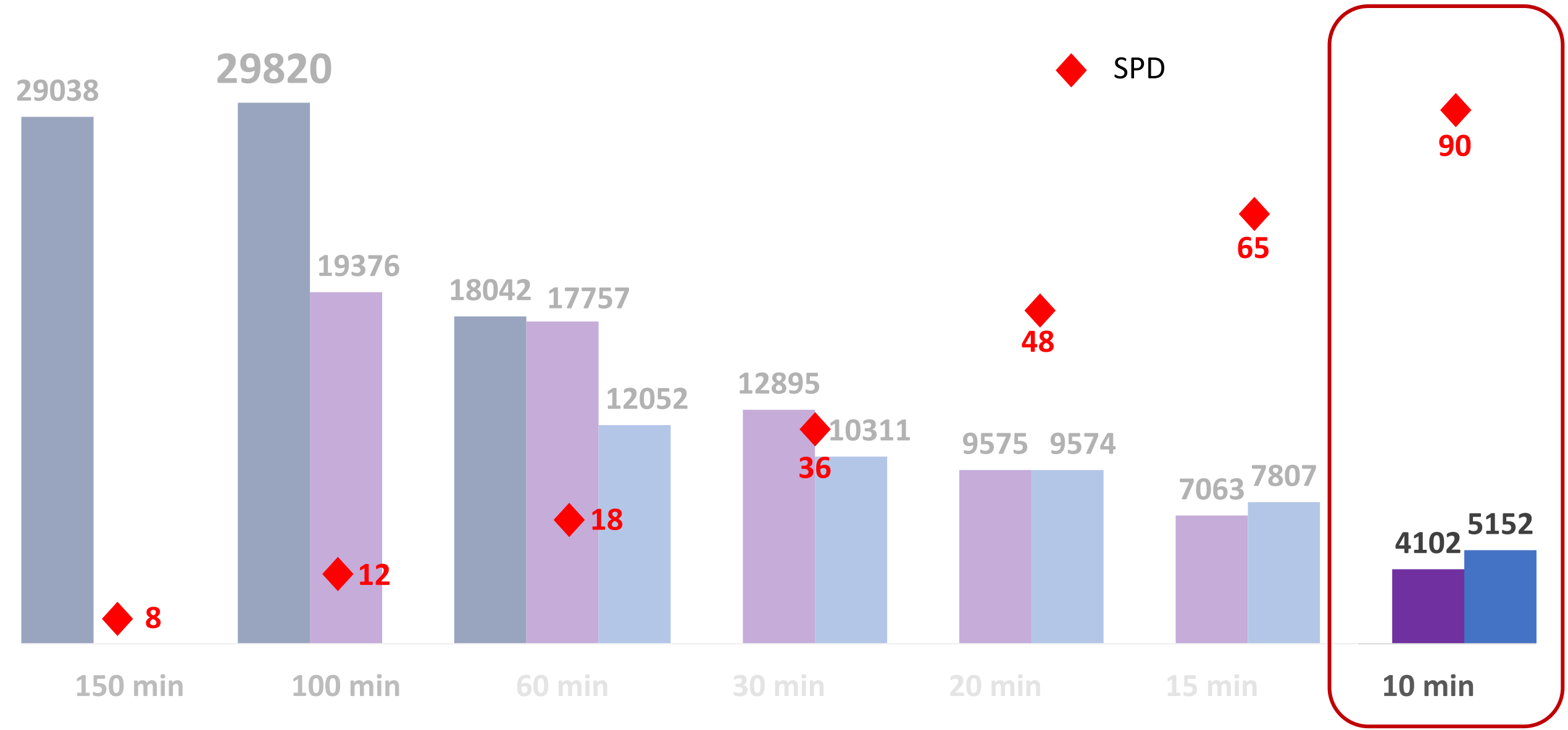
Highest sensitivity with 50 cm column using 100 minute gradient!



More than 5000 IDs using 5 cm column in 10 minutes



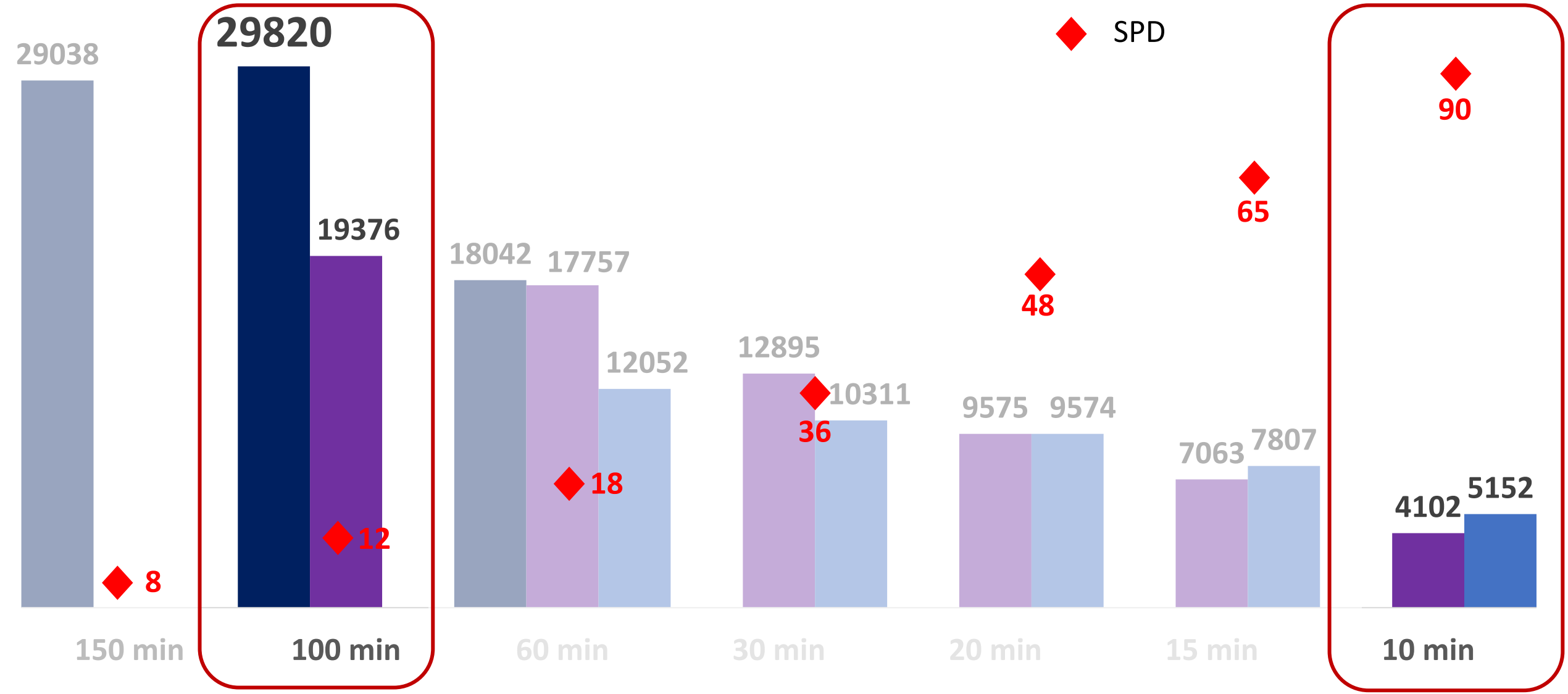
Highest throughput with 5 cm column



Dedicated workflows

Highest coverage

Highest throughput



Immunopeptidomics on Orbitrap Astral intrument

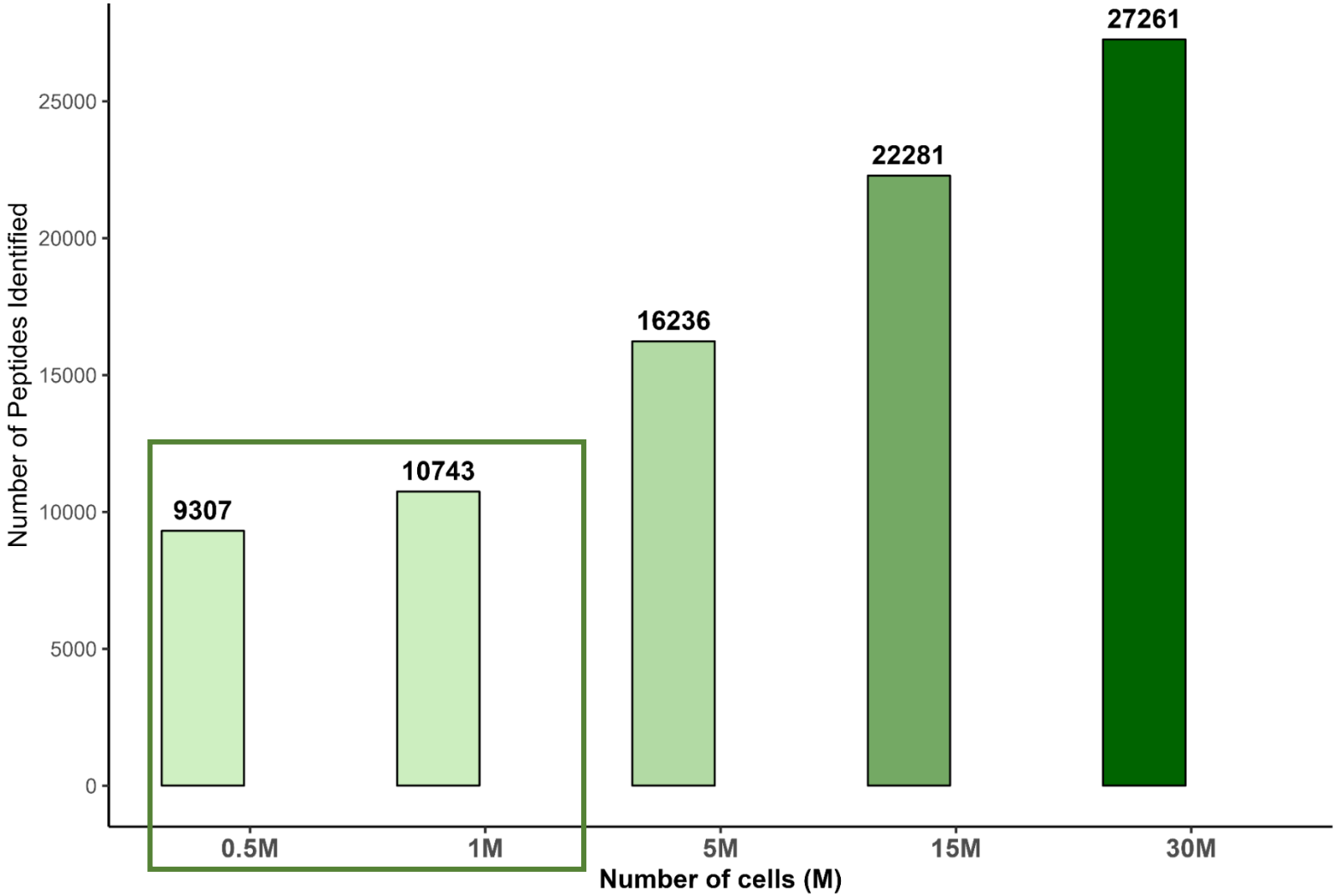
nanoElute 2 +
timsTOF Ultra 2 (Bruker)

Vanquish Neo +
Orbitrap Astral (Thermo Fisher)

Excellent coverage even with lowered input

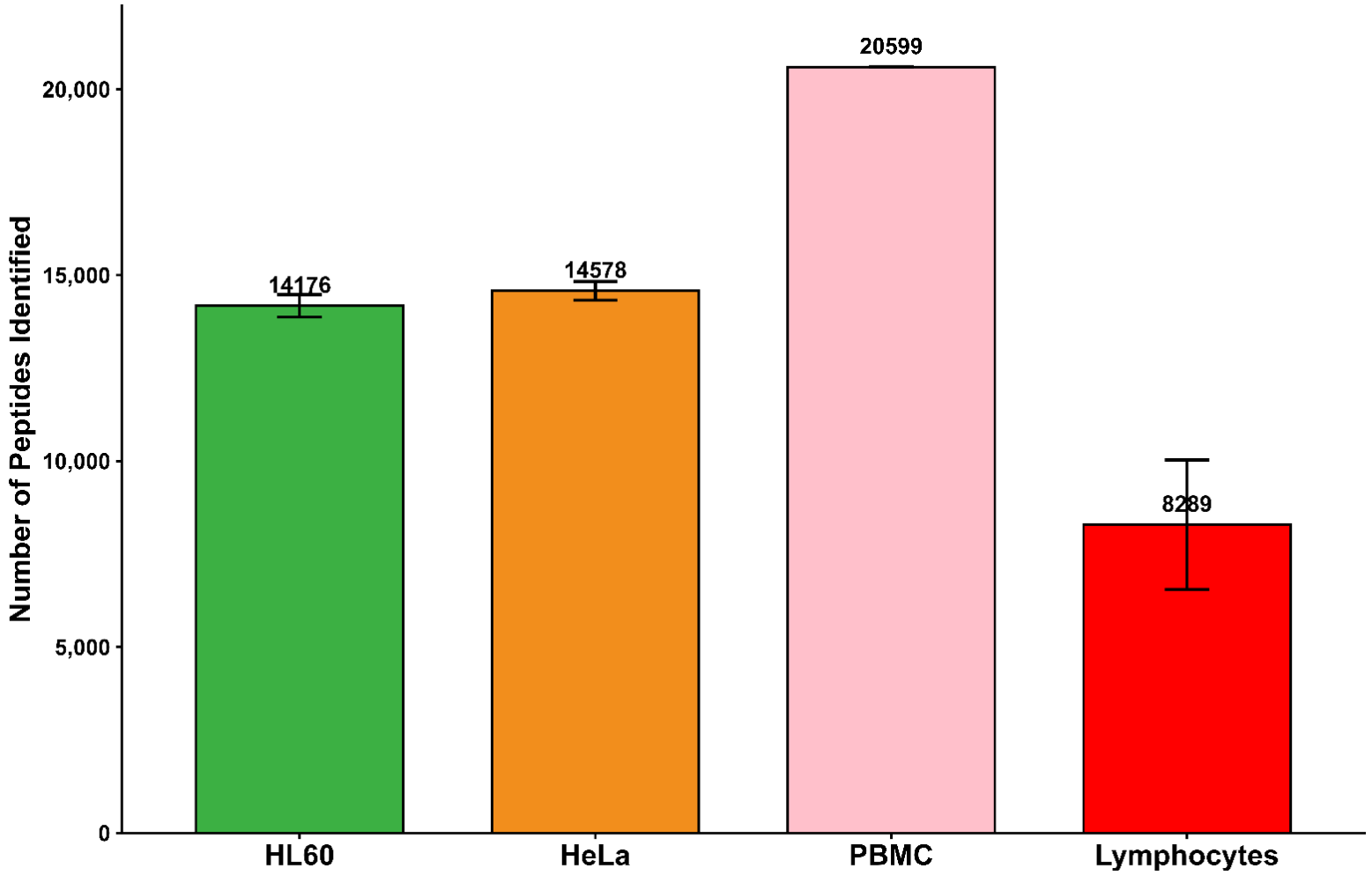
Jeewan Babu Rijal

Farida Salimova



• *µPAC neo 2.0 column • 120-minutes acquisition • Data analysis: MS Fragger*

This optimised workflow is also applicable on other cell types



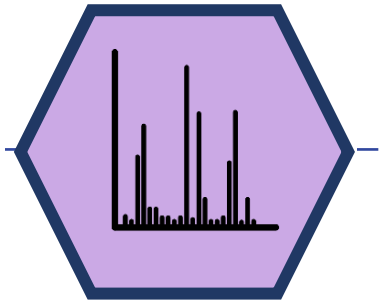
Conclusion

Fine-tuning of all steps of immunopeptidomic workflow

Sample preparation

LC-MS/MS method aquisition

Data processing



High throughput and sensitivity

Up to 30,000 immunopeptides identified

+10,000 immunopeptides identified from <1M cells

Ultra-sensitive workflow ideal for limited input

Mature and accessible technology

Unlocking new ideas to other research projects (astrocytes, B lymphocytes, mouse HLA class II) and clinical samples

Acknowledgments

LSMBO

All LSMBO team and especially,
Christine Carapito
Jeewan Babu Rijal
Charline Keller
Farida Salimova
Delphine Rolland

CompOmics (Ghent, Belgium)

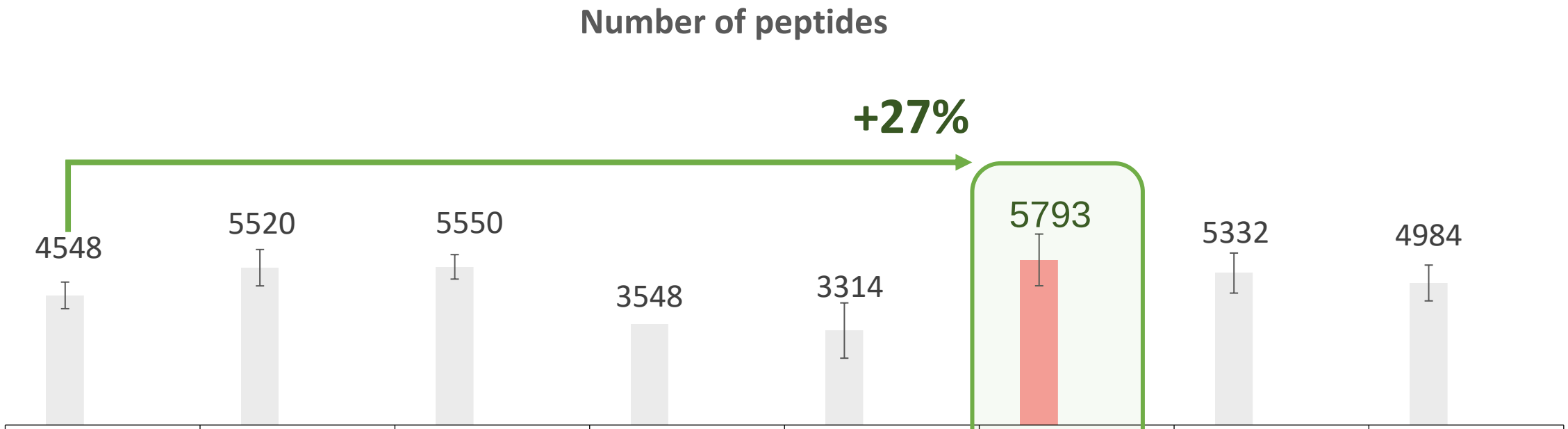
Arthur Declercq

Lennart Martens

INSERM UMR_S 1109 (Strasbourg, France)

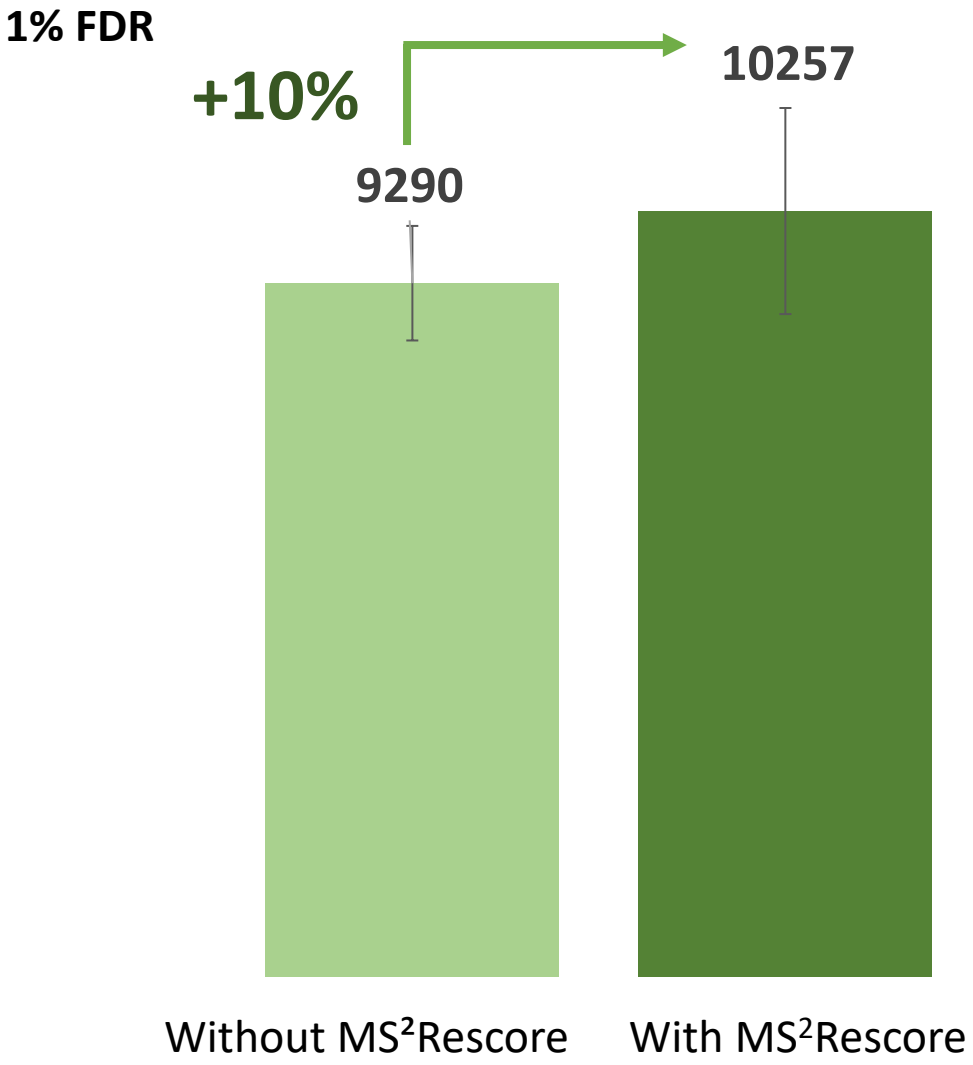
Perrine Spinnhirny
Seiamak Bahram
Raphael Carapito

Optimised MS parameters on timsTOF Pro 2 instrument



IM range (1/K0)	0.6 – 1.6	0.6 – 1.6	0.6 – 1.6	0.6 – 1.6	0.6 – 1.7	**0.6 – 1.7**	0.6 – 1.7	0.65 – 1.75
Accumulation time (ms)	100	200	166	200	200	**200**	166	200
PASEF scans	10	6	10	6	6	**6**	6	6
CE ramp (eV)	20-59	20-59	20-59	10-55	10-55	20-59	20-59	20-59

Identification improved with MS²Rescore even more at 0.1% FDR



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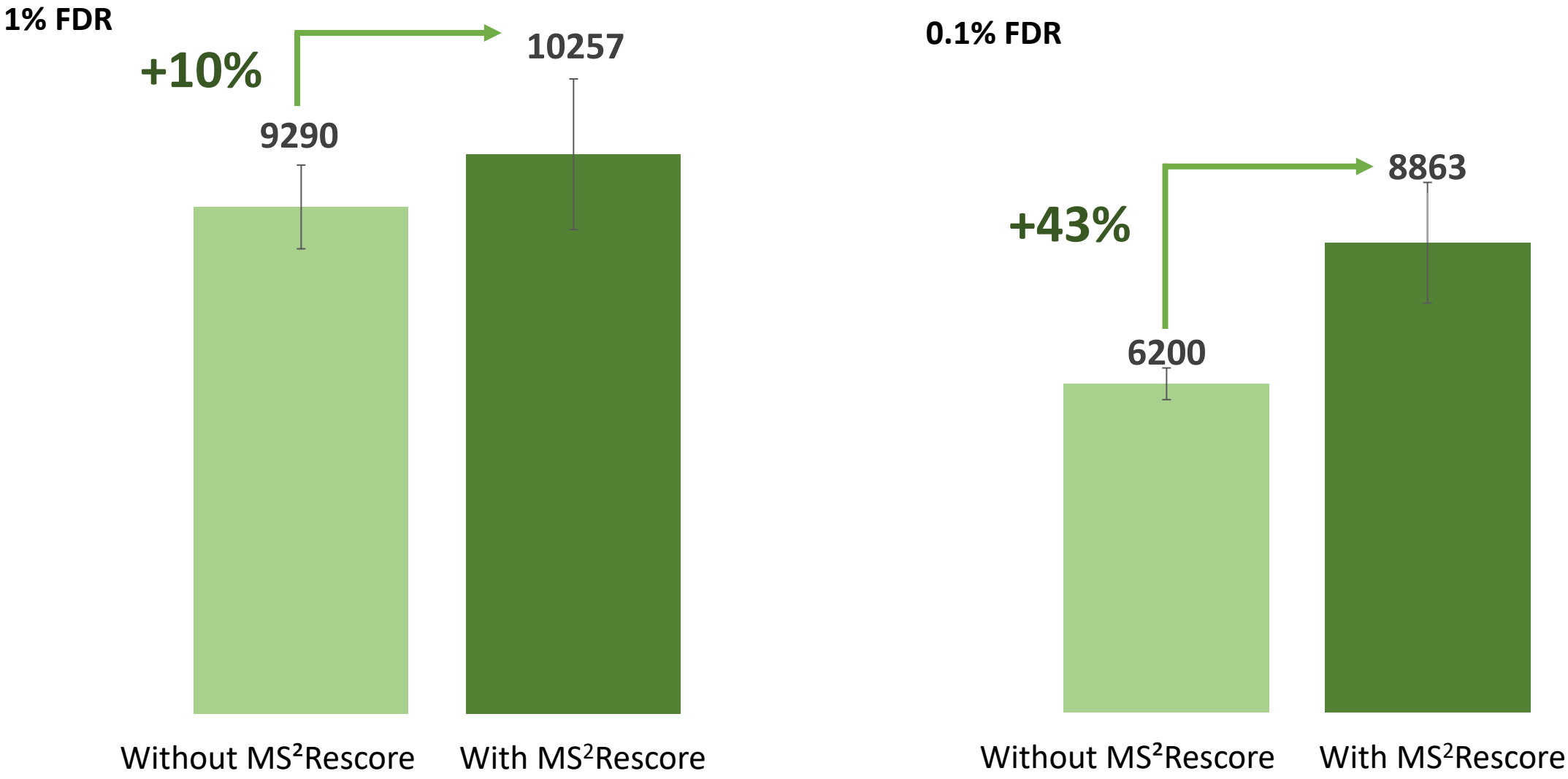
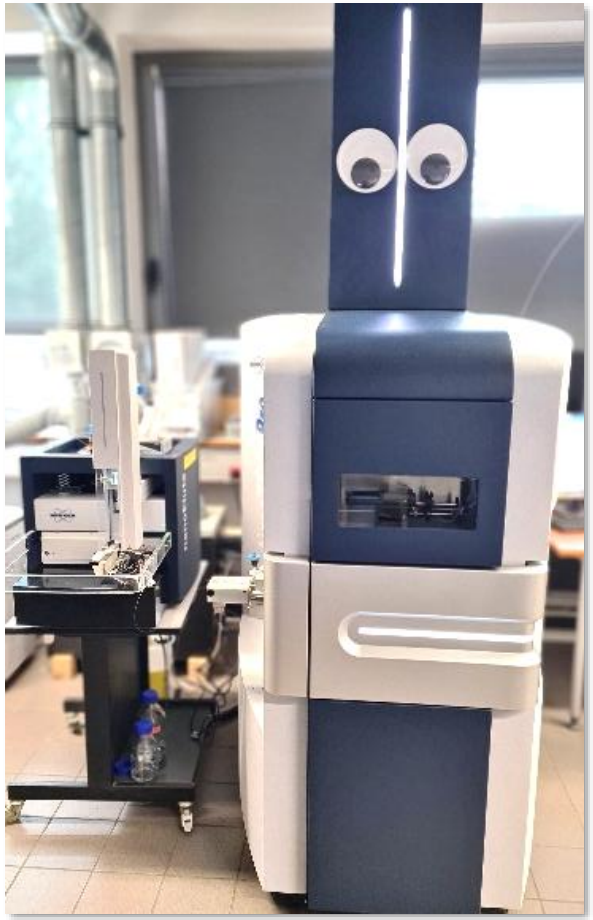
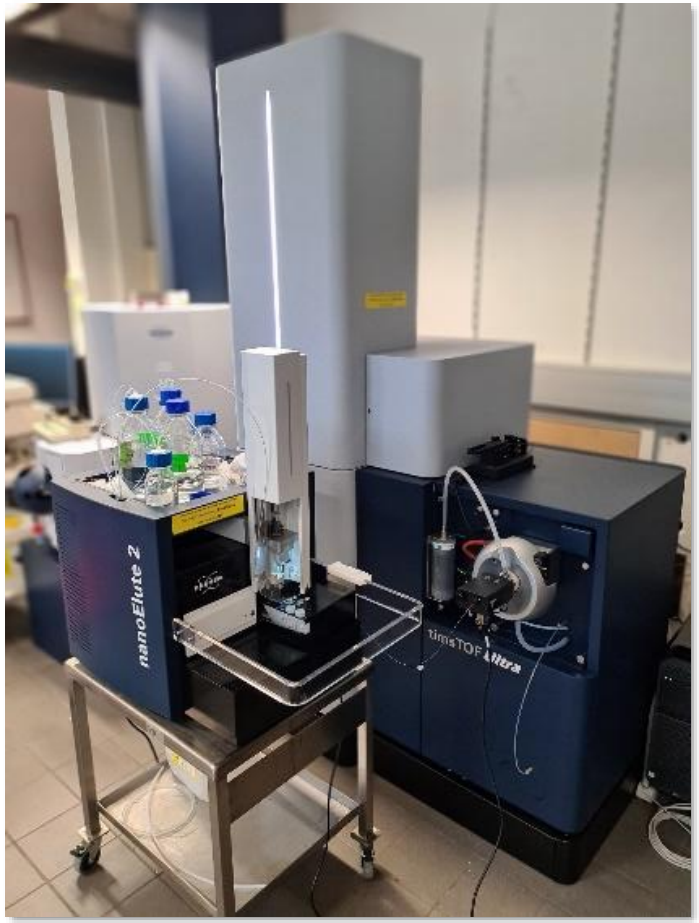


Different instruments used for immunopeptidomics

nanoElute +
timsTOF Pro 2 (Bruker)



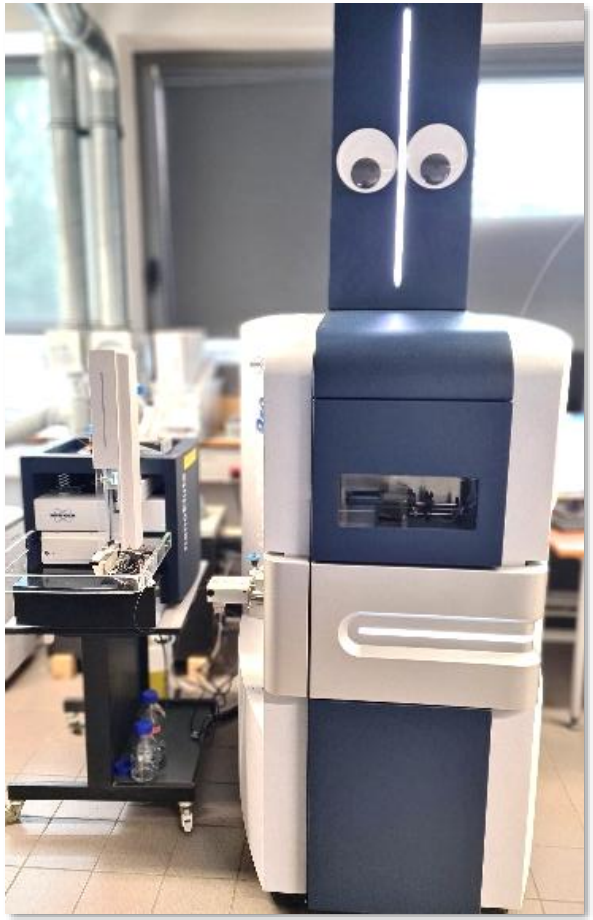
nanoElute 2 +
timsTOF Ultra (Bruker)



Vanquish Neo +
Orbitrap Astral (Thermo Fischer)

Immunopeptidomics on timsTOF Pro 2

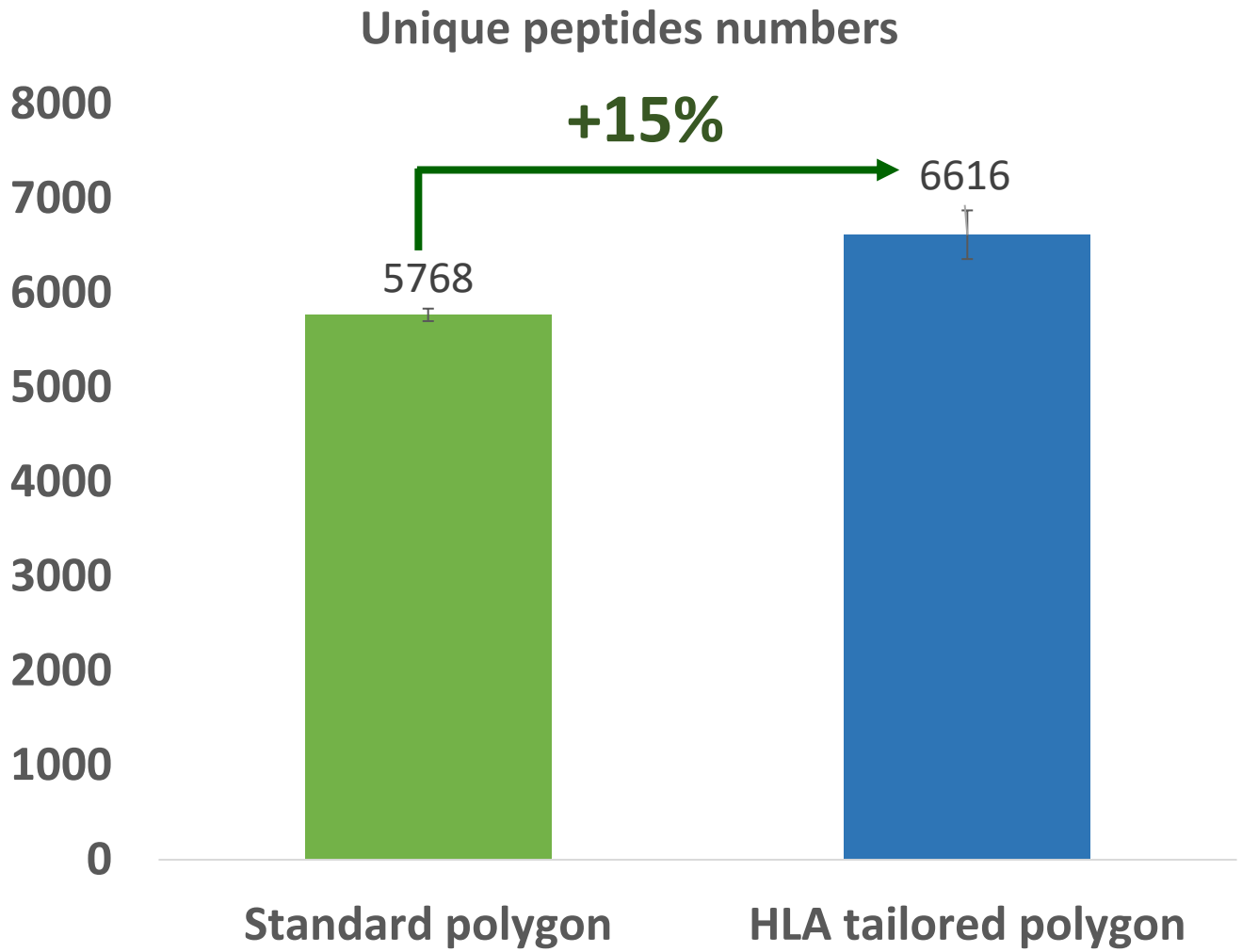
nanoElute +
timsTOF Pro 2 (Bruker)



nanoElute 2 +
timsTOF Ultra (Bruker)

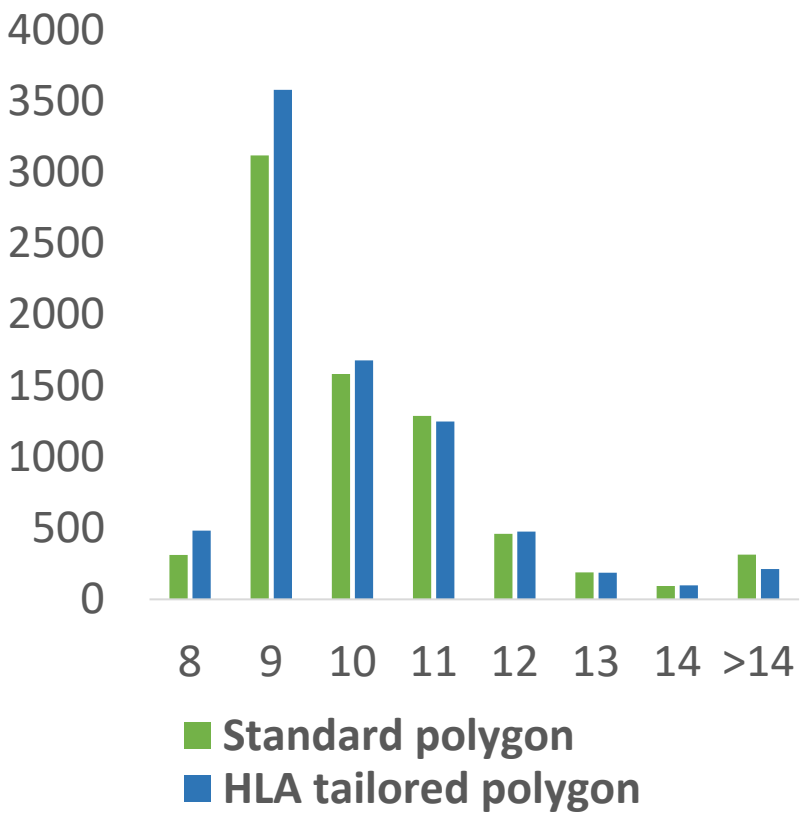
Vanquish Neo +
Orbitrap Astral (Thermo Fischer)

Filter polygon optimisation increased IDs by 15%

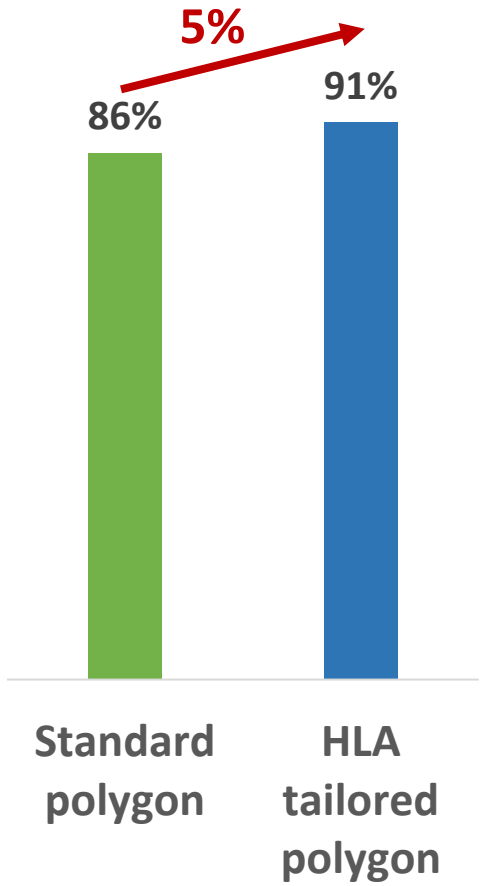


Polygonal filter optimisation on singly charged IDs is valuable

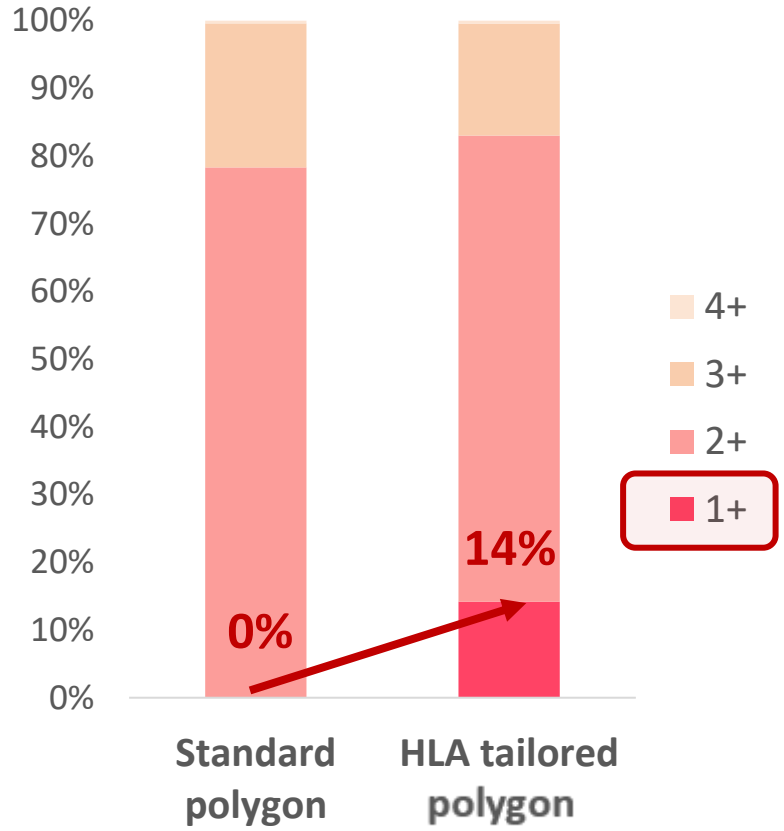
Peptide length



Total binding affinity prediction



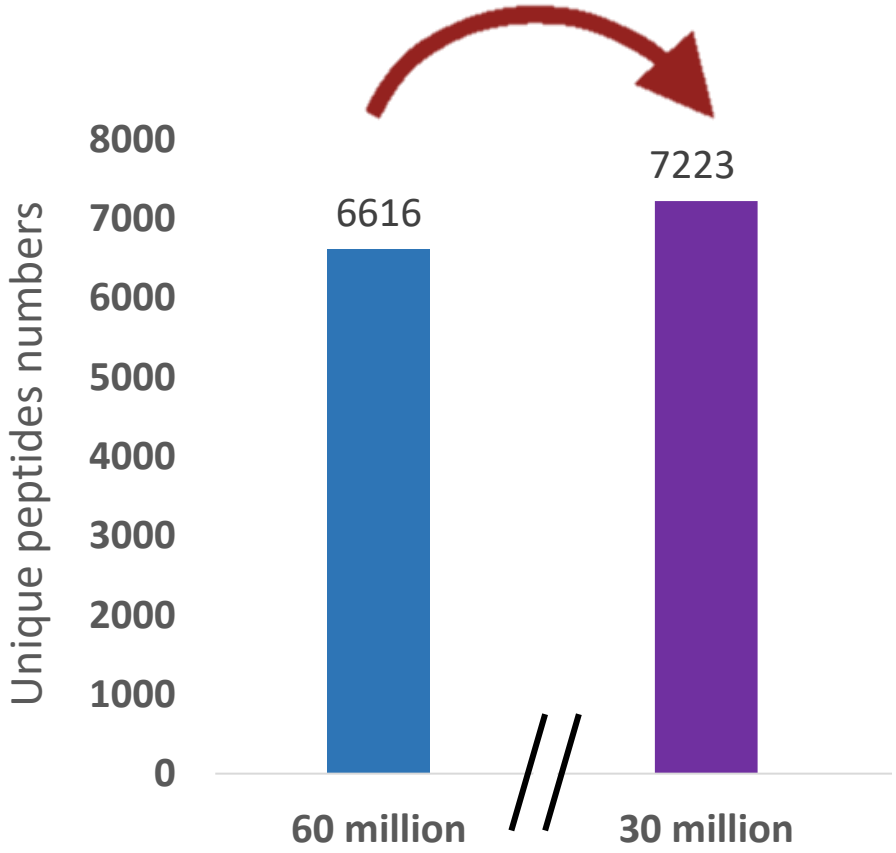
Unique peptides charges



timsTOF Ultra increases sensitivity

Jeewan Babu Rijal

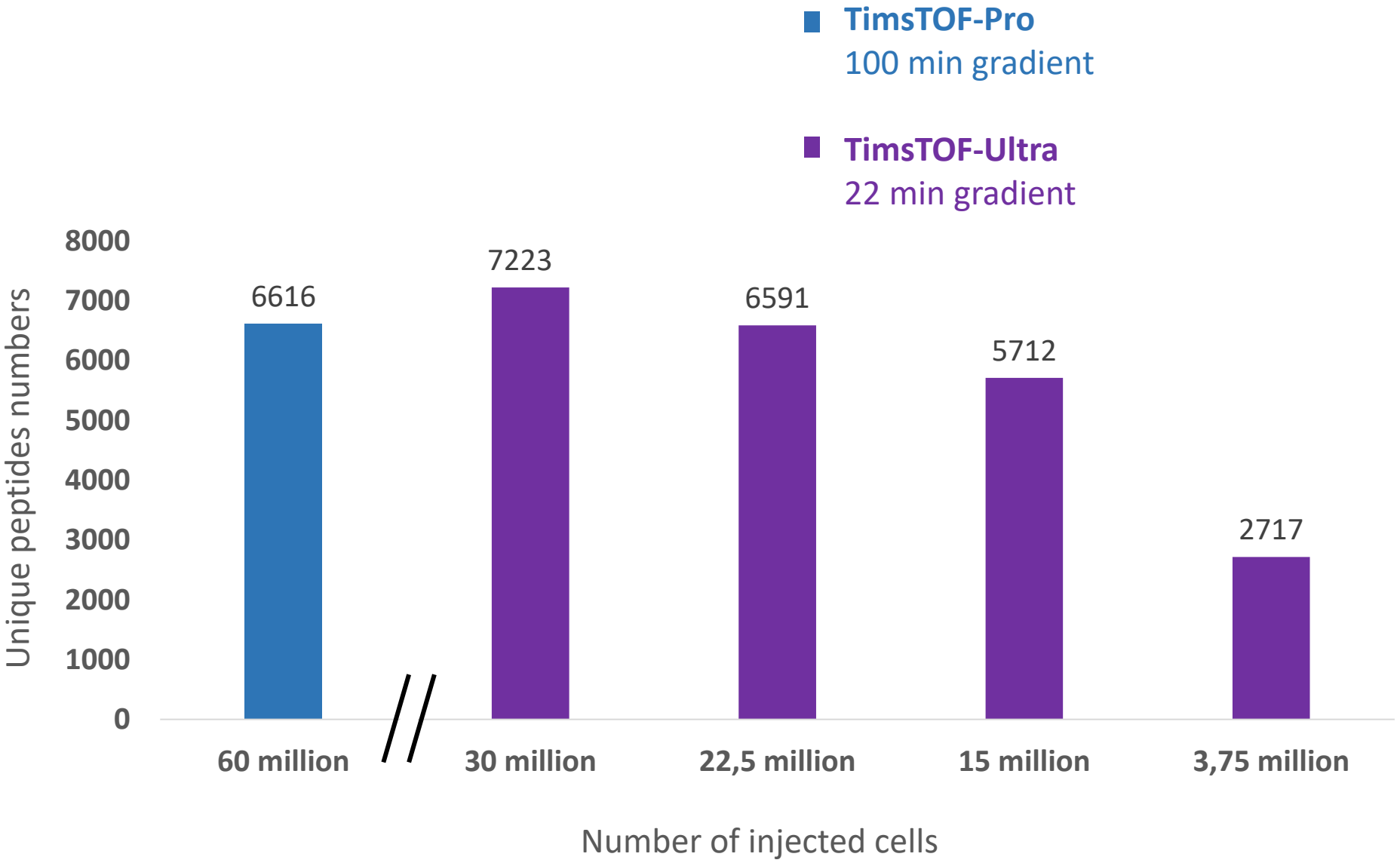
5x shorter gradient time
2x less injected material



- **TimsTOF-Pro**
  100 min gradient
- **TimsTOF-Ultra**
  22 min gradient

timsTOF Ultra increases sensitivity

Jeewan Babu Rijal



Large search space lowers the confidence of peptides identification and decreases the number of identifications

Classical trypsin digestion

1 000 amino acid protein

Tryptic digestion

115 tryptic peptides

Search space

Peptides IDs

LKDIKAQWK
GTDGHPFGWK
LAAKLLAKYR
VVVPAEWIKNWR

Large search space lowers the confidence of peptides identification and decreases the number of identifications

Classical trypsin digestion

1 000 amino acid protein

Tryptic digestion

115 tryptic peptides

Search space

Peptides IDs

LKDIKAQWK
GTDGHPFGWK
LAAKLLAKYR
VVVPAEWIKNWR

X100

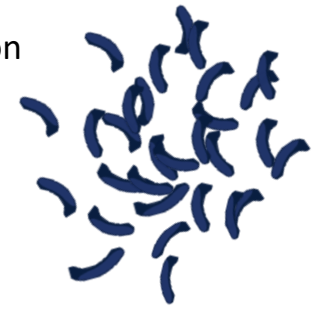
Immunopeptides

1 000 amino acid protein

Non-specific digestion

9 aa

991 non-tryptic peptides



Variable length

8-12 aa

15 768 non-tryptic peptides

Big search space

Immunopeptide IDs

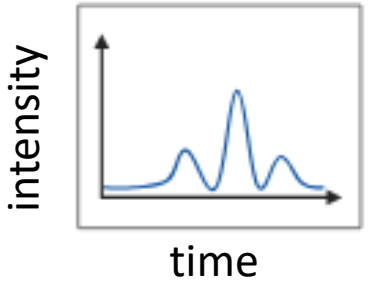
RLKDIKAQW
~~GTDGHPFGW~~
LAAKLLAKY
~~VVVPAEWIKNW~~

Low identification rate

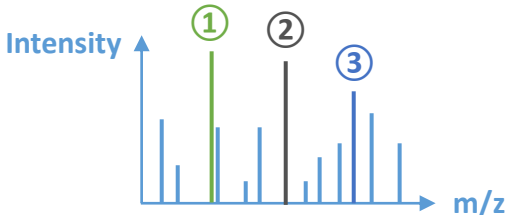
Faridi P. *et al*., 2018, Proteomics

How does LC-MS/MS work?

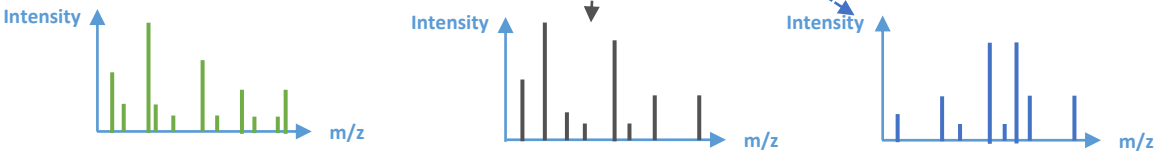
LC (liquid chromatography)

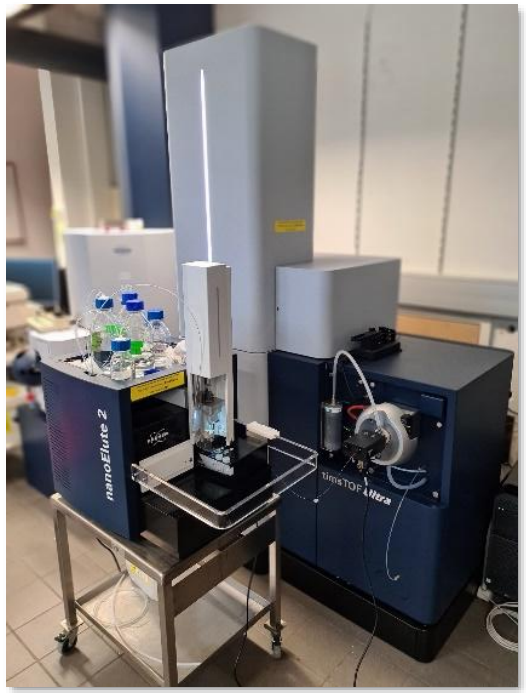


MS (mass spectrometry)



MS/MS
(tandem mass spectrometry)





LC

MS
MS/MS

Identification of the peptide sequence using MS/MS signals

Global protein identification