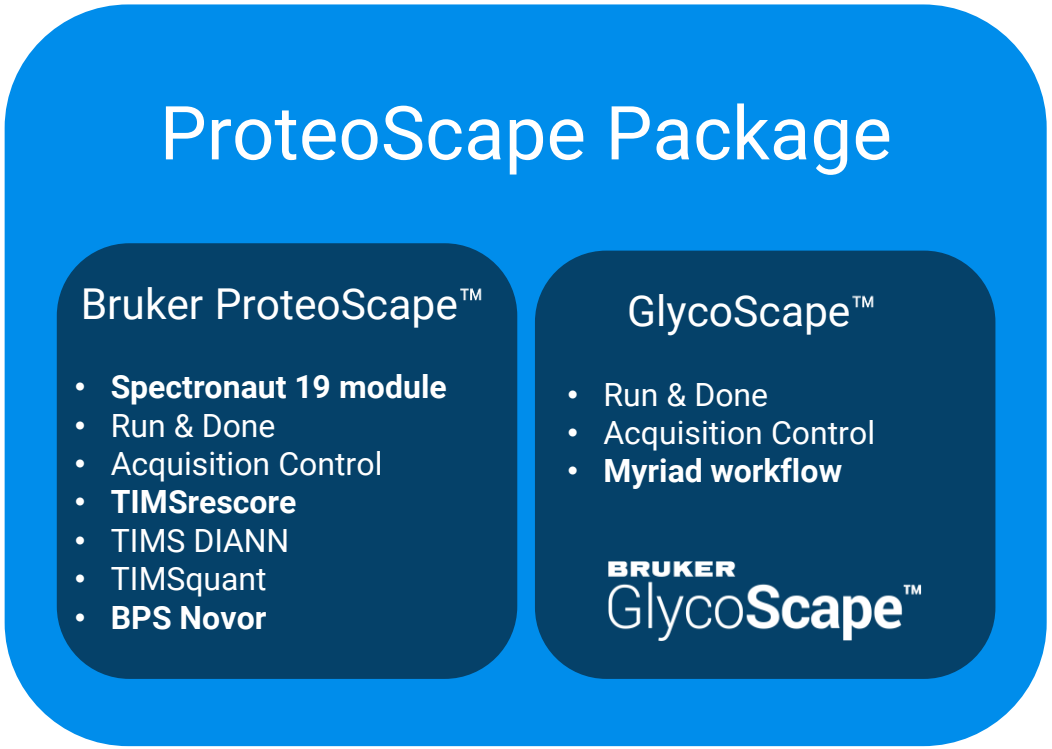
What’s new in Bruker ProteoScape Package 2025b: GlycoScape

Bruker ProteoScape Package

- Bruker ProteoScape Package includes:
  - HP Z8 workstation with 2x GPU cards
  - Ethernet switch
  - Bruker ProteoScape application
    - Including Spectronaut Core module
    - TIMSrescore, TIMS DIANN, TIMSquant etc.
      - Optional module: BPS Novor
  - GlycoScape application
    - Myriad workflow



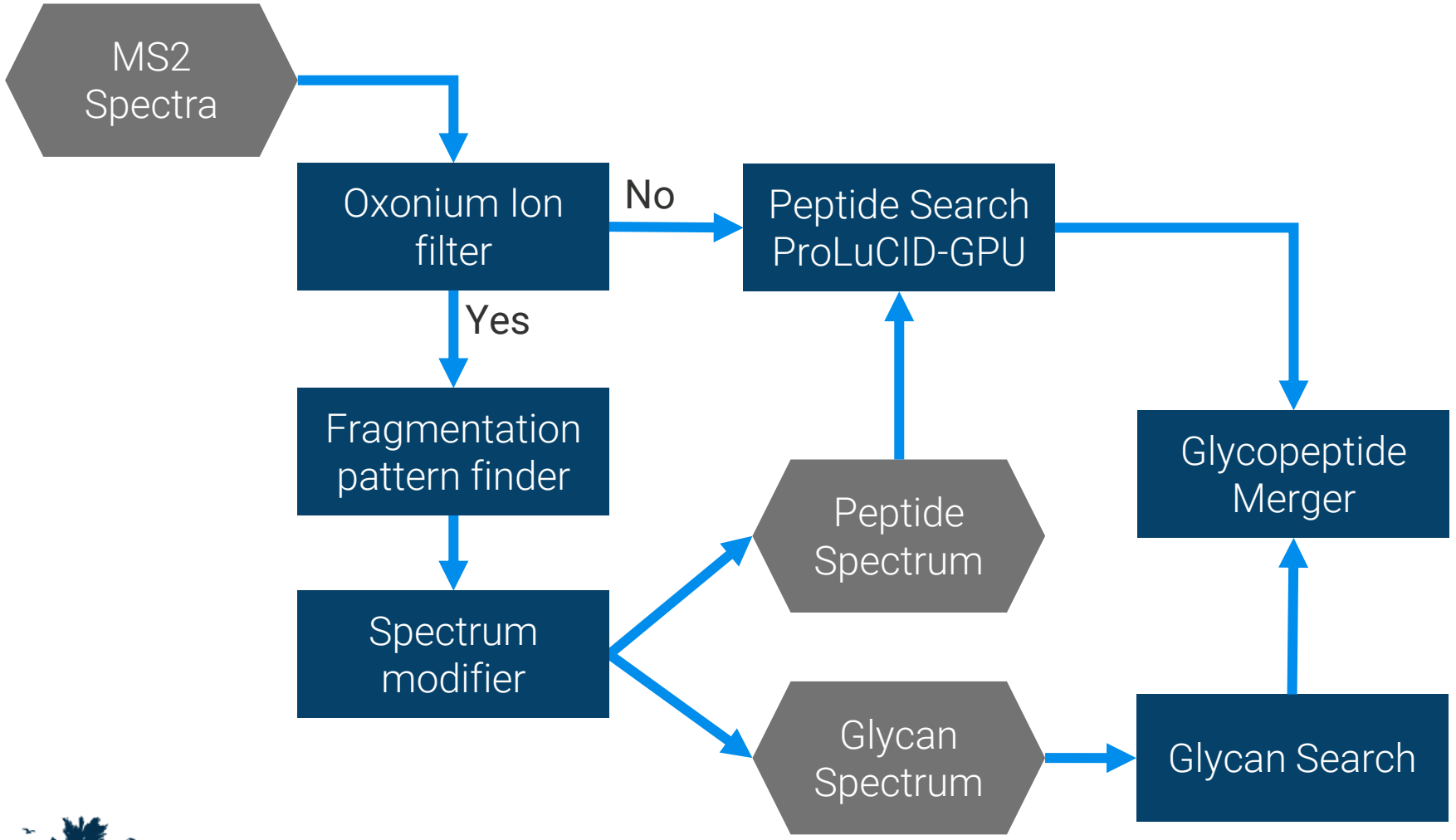
What’s new in Bruker ProteoScape 2025b

- GlycoScape utilizing the Myriad workflow for glycopeptide analysis
- Reminder of what’s new in 2025:
  - Spectronaut module updated to v19.0
    - Includes diagonal-PASEF support
    - Improved deep learning models for increased identification
  - TIMSrescore workflow
    - AI/ML models for increased identification
    - Dedicated quantitation workflow
  - UI/UX improvements
    - Most columns are sortable
    - First search functionality within table (case sensitive)
  - Others …
    - Robustness and stability improvements

GLYCOPEPTIDE ANALYSIS AT THE SPEED OF PASEF

GlycoScape™

GlycoScape: real-time glycopeptide analysis workflow



BRUKER GlycoScape™ BRUKER

NL Health~Holland

- GlycoScape utilizes the Myriad workflow originally described by Armony et al., 2023
- Each spectrum is analyzed for the presence of oxonium ions, if found then the spectrum is separated into two components
  - The peptide spectrum component is analyzed by ProLuCID-GPU
  - The glycan spectrum component is analyzed by Myriad's composition generator **without any glycan databases**

https://doi.org/10.3390/ijms24097869

(HH Public Private Partnership Grant # LSHM21032)

BRUKER GlycoScape™ BRUKER

"A sister-product to Bruker's ProteoScape, GlycoScape now opens up the analysis of glycoproteomic mass spectrometry data from the timsTOF platform for on-the-fly processing without glycan database restrictions."

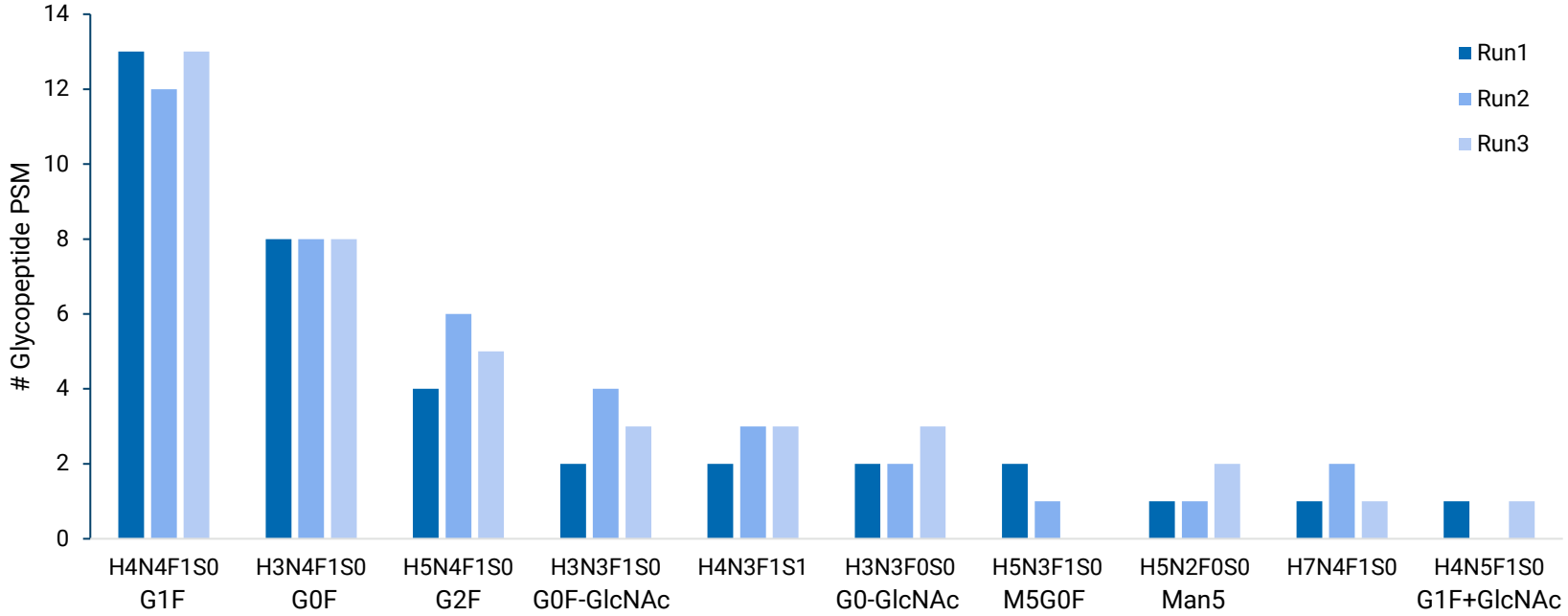
Dr. Hans Wessels
RadboudUMC

Myriad Workflow Publication

Glyco-PASEF Optimization Publication

GlycoScape: real-time identification of glycopeptides from NIST mAB reference standard

BRUKER GlycoScape™ BRUKER



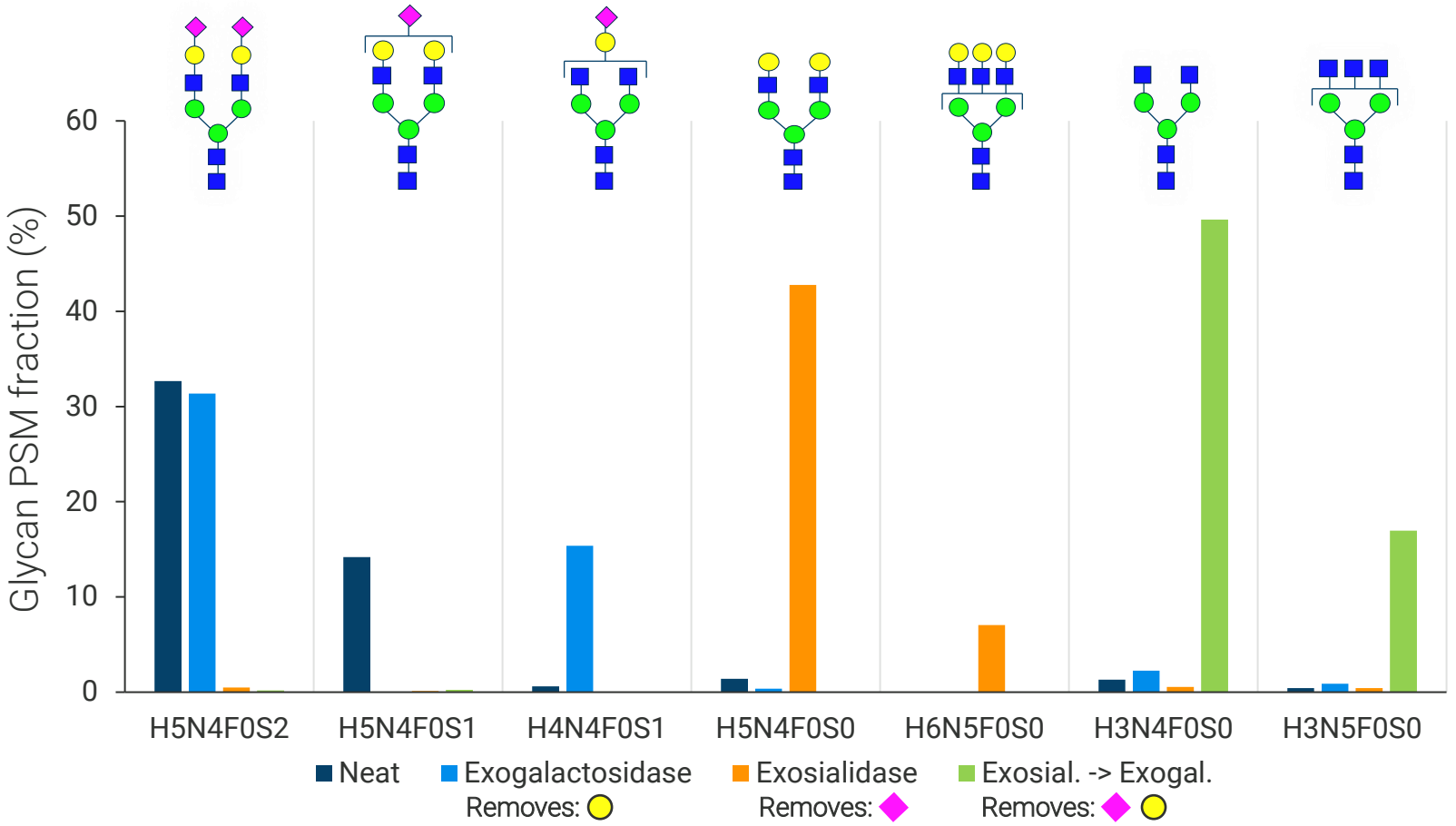
- GlycoScape, utilizing the Myriad workflow (Armony et al., 2023), identifies 12-20 glycan compositions (depending on acquisition method) on the IgG heavy-chain of the NIST mAB reference standard, in line with the interlaboratory study by Leoz et al., 2020
- Shown above, top 10 most abundant glycan compositions identified

- 50ng Trypsin digested NIST mAB analyzed with 35min nLC gradient on timsTOF Pro2
- With GlycoScape:
  - Identify glycopeptides
  - In real-time
  - With results available seconds after acquisition ends

Data provided by:
Ho-Tak Lau and Richard Rogers
Umoja Biopharma, Seattle, WA USA
Acquisition methods based on:
https://doi.org/10.1021/acs.analchem.3c05874

https://doi.org/10.3390/ijms24097869
https://doi.org/10.1074/mcp.RA119.001677

GlycoScape shows great specificity for the identified glycan compositions

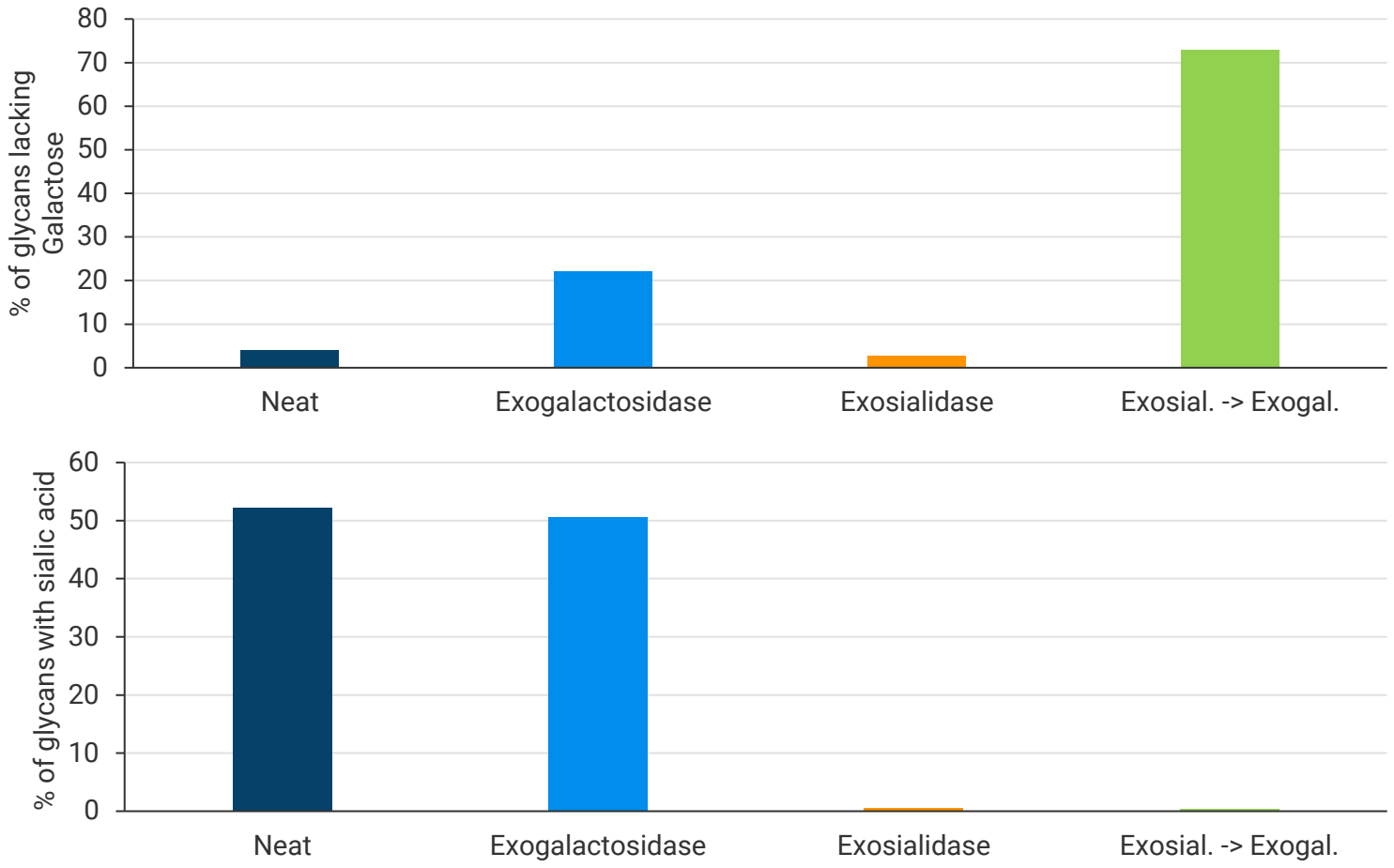


For simplicity, the average of 2 replicates is shown for each condition.

Data provided by: Fokje Ziljstra, Gad Armony, Alain van Gool, Dirk Lefeber and Hans Wessels.
RadboudUMC, the Netherlands

- In HILIC enriched Human plasma samples:
  - Treatment with Exogalactosidase shows only the unprotected Galactose residues are removed
  - Treatment with Exosialidase shows only sialic acids residues are removed
  - Sequential treatment of Exosialidase followed by Exogalactosidase shows de-protection and removal of Galactose residues.

GlycoScape shows great specificity for the identified glycan compositions



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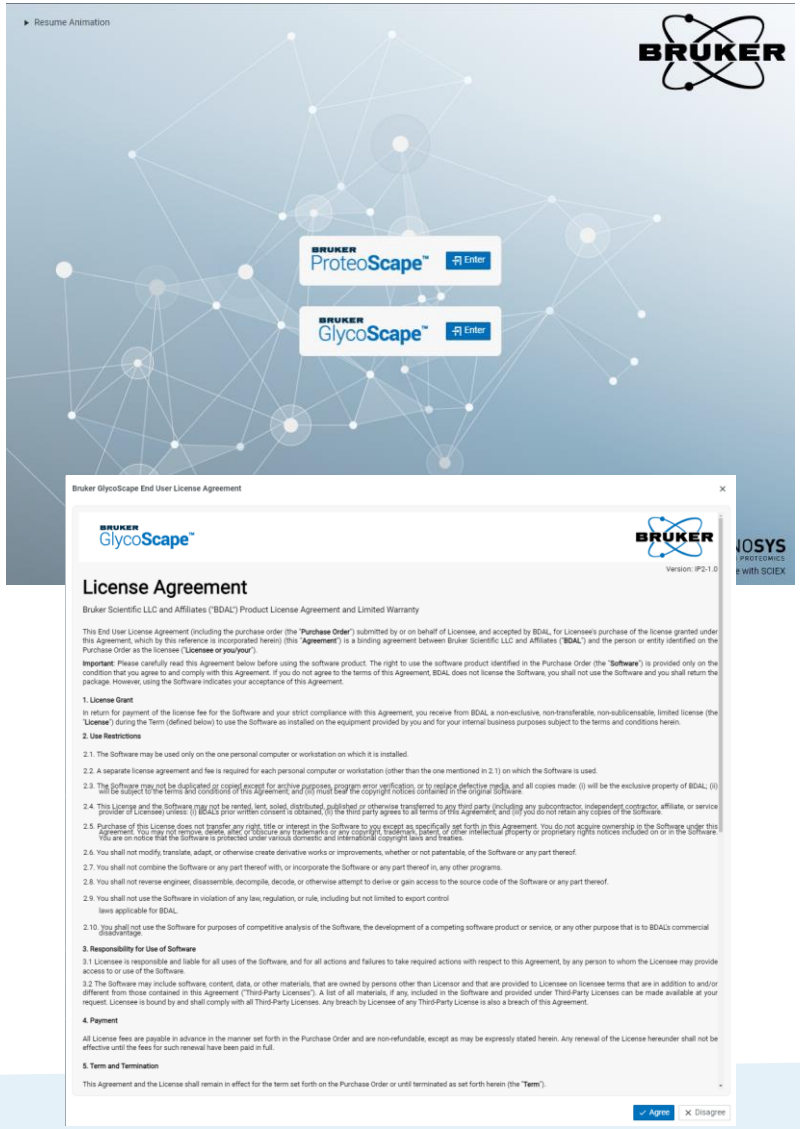
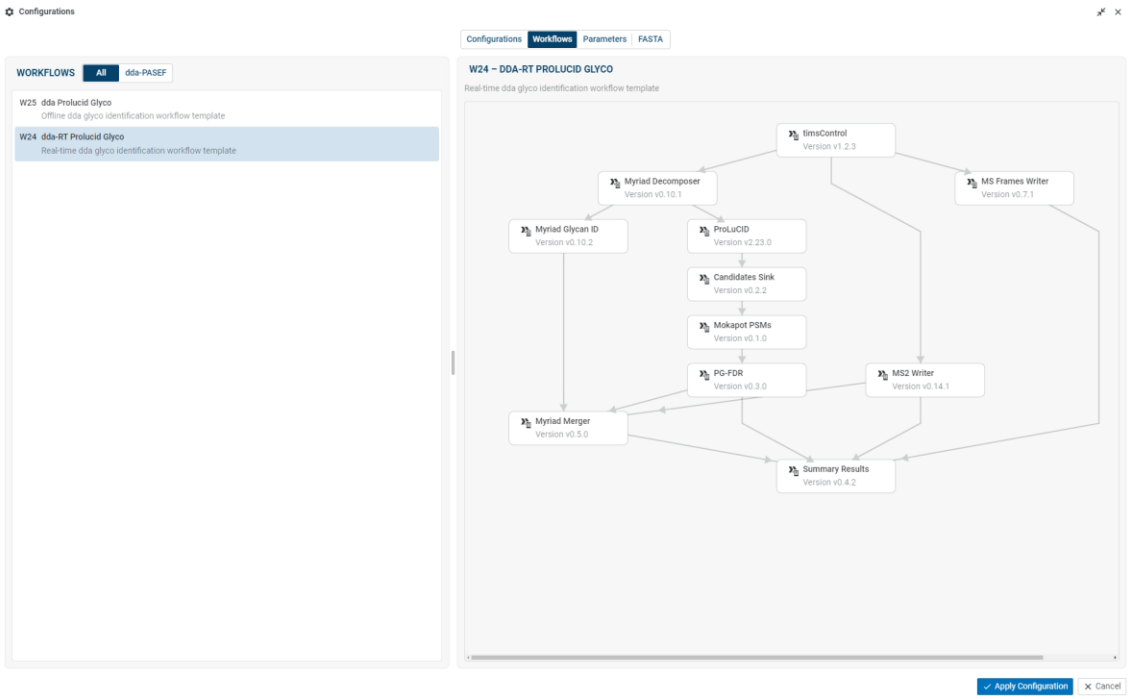
Using GlycoScape

Open the GlycoScape Application



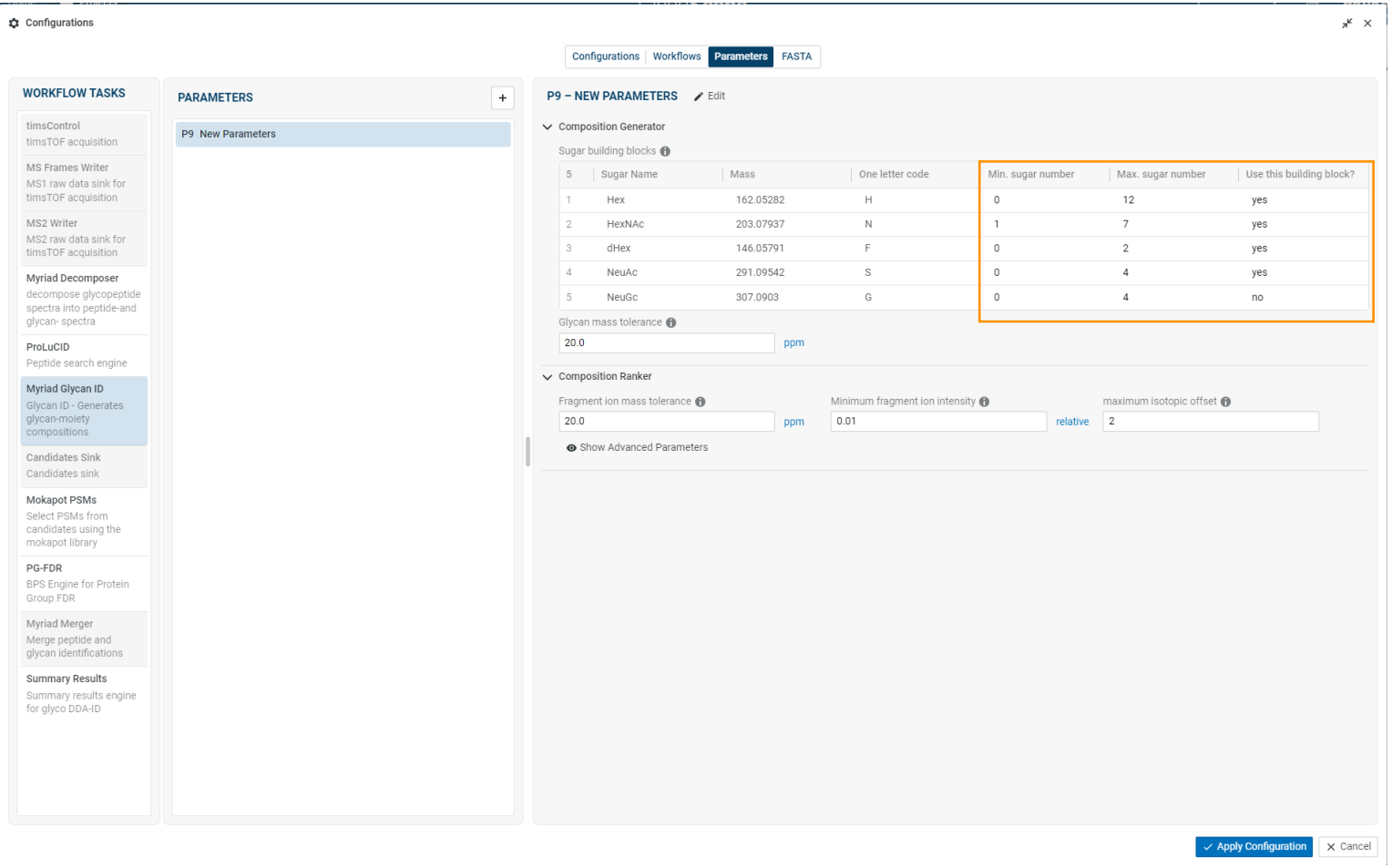
- Login as usual, then the idle screen will present the choice of application to enter
- Choose GlycoScape
  - On first use, EULA will be presented for acceptance.

Create a configuration for real-time or offline re-analysis

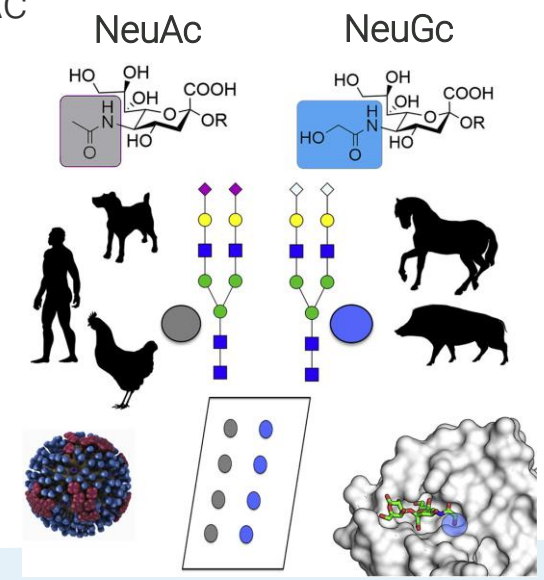


- Default parameters should work for human derived samples

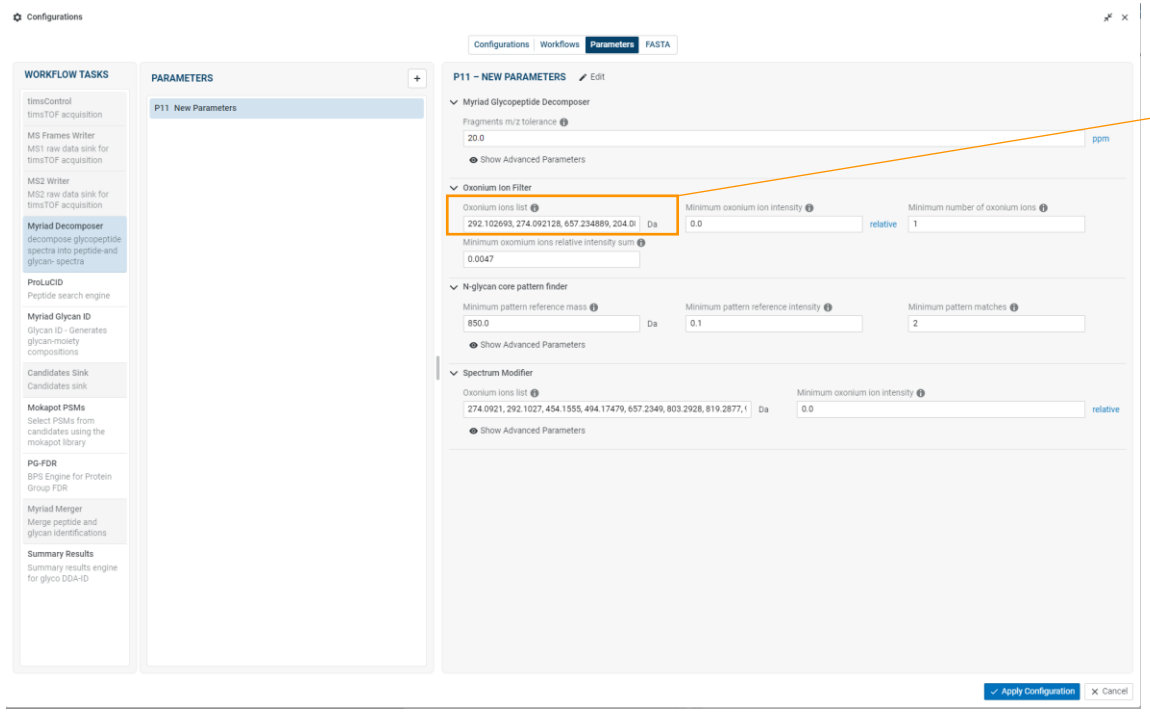
Myriad – GlycanID Parameters



- Currently limited to the 5 sugar building blocks shown
  - Typical human samples utilize NeuAc but some cancer tissues utilize NeuGc
  - Most other species utilize NeuGc and not NeuAc

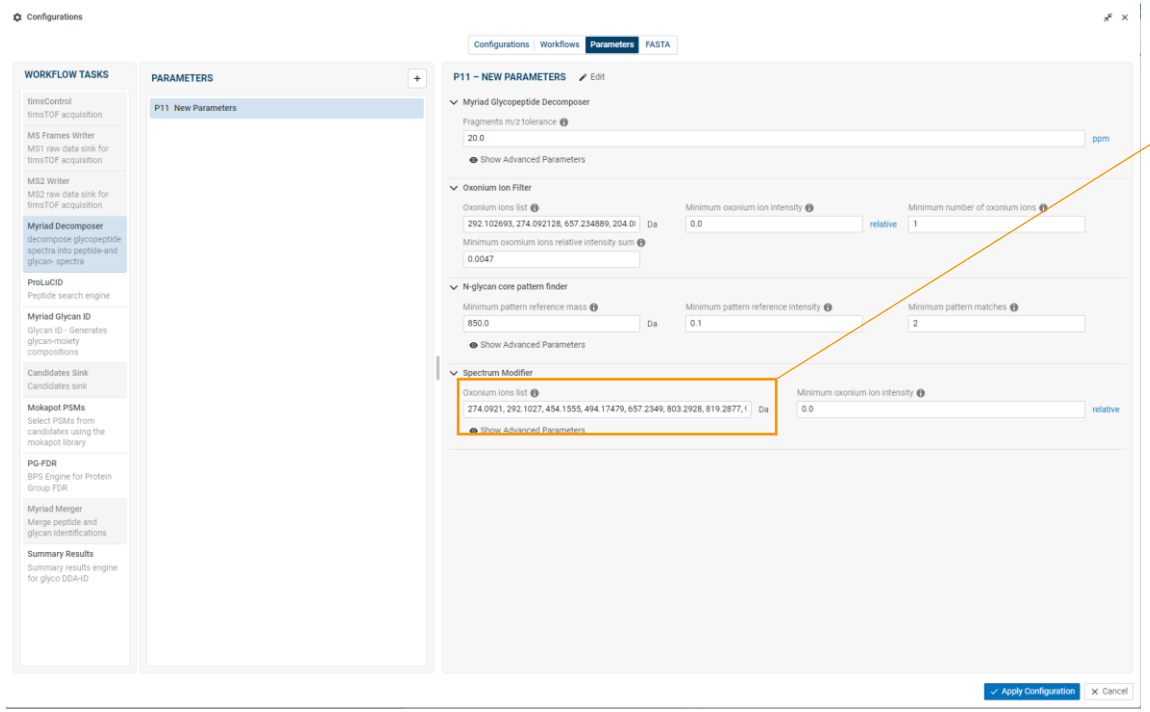


Myriad Decomposer – Oxonium Ion Filter



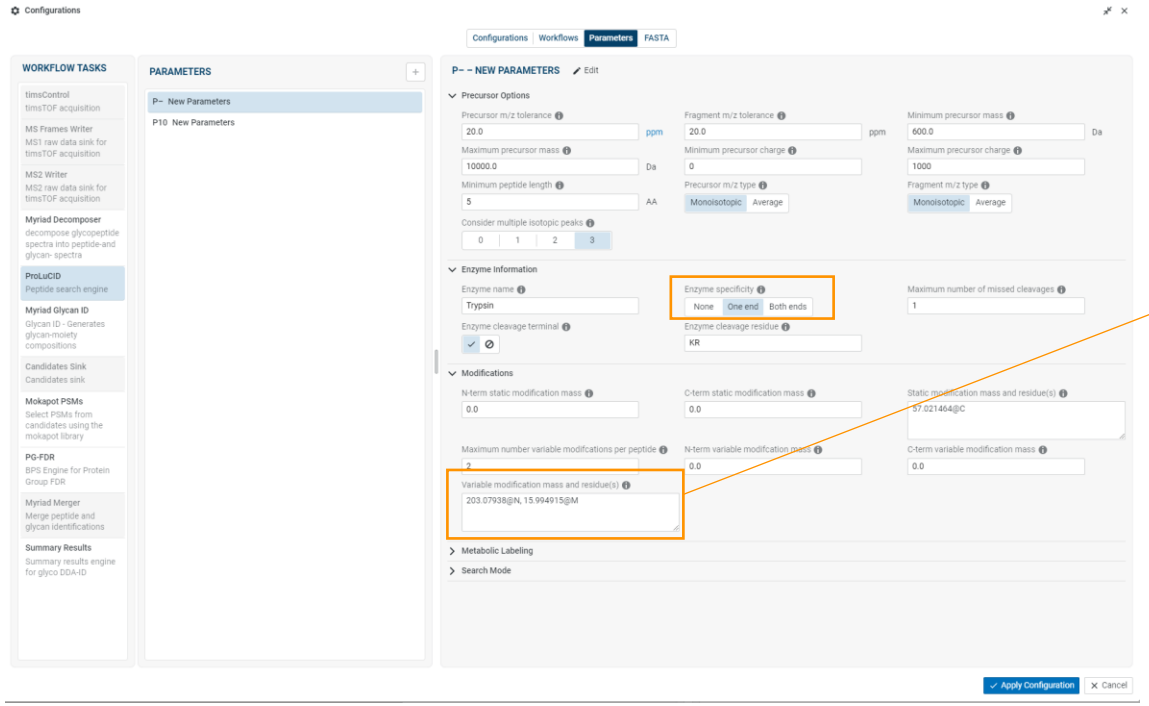
- If NeuGc is selected in the GlycanID, then the default Oxonium Ion Filter and Oxonium ions list needs to be modified:
- Oxonium Ion filter:
  - Ions considered: S, S-H2O, HNS, N, HN, HNF
  - Default NeuAc: 292.102693, 274.092128, 657.234889, 204.0867, 366.139472, 512.19793
  - NeuGc: 308.0976, 290.087, 673.2298, 204.0867, 366.139472, 512.19793
  - NeuAc & NeuGc: 292.102693, 274.092128, 657.234889, 308.0976, 290.087, 673.2298, 204.0867, 366.139472, 512.19793

Myriad Decomposer – Oxonium Ion list



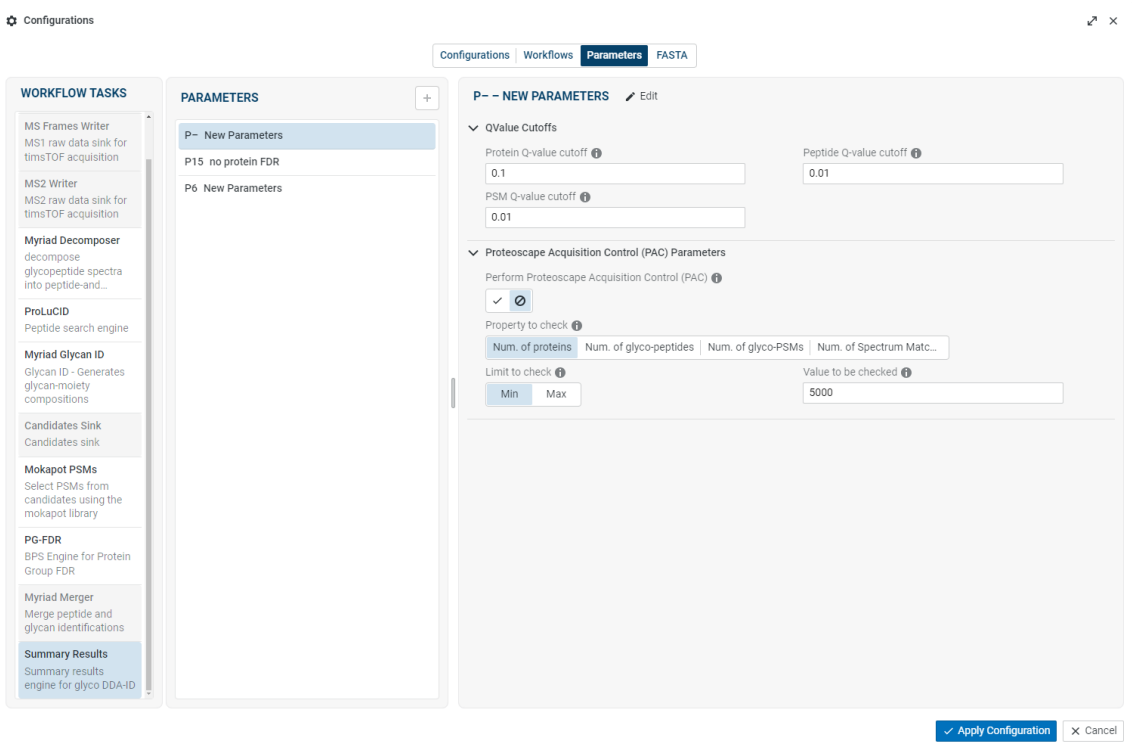
- If NeuGc is selected in the GlycanID, then the default Oxonium Ion Filter and Oxonium ions list needs to be modified:
- Oxonium Ion List:
  - Ions considered: S-H2O, S, HS, NS, HNS, HNFS, H2NS, H2NFS, F, H, N, HF, H2, NF, HN, N2, H3, HNF, H2N, N2F, HN2, H4, H2NF, H3N, N2F2, HN2F, H2N2, H5, H3NF, HN2F2, H2N2F, H3N2, H6, H2N2F2, H3N2F, H3N3, H7, H3N2F2, H3N3F, H8, H3N4, H3N3F2, H3N4F, H9, H3N4F2, H10, H11, H12
  - Default NeuAc: 274.0921, 292.1027, 454.1555, 494.17479, 657.2349, 803.2928, 819.2877, 965.3456, 147.0652, 163.0601, ….
  - NeuGc: 290.0870, 308.0976, 470.1504, 510.16969, 673.2298, 819.2877, 835.2826, 981.3405, 147.0652, 163.0601, ….
  - NeuAc & NeuGc : 274.0921, 292.1027, 454.1555, 494.17479, 657.2349, 803.2928, 819.2877, 965.3456, 290.0870, 308.0976, 470.1504, 510.16969, 673.2298, 819.2877, 835.2826, 981.3405, 147.0652, 163.0601, ….

ProluCID



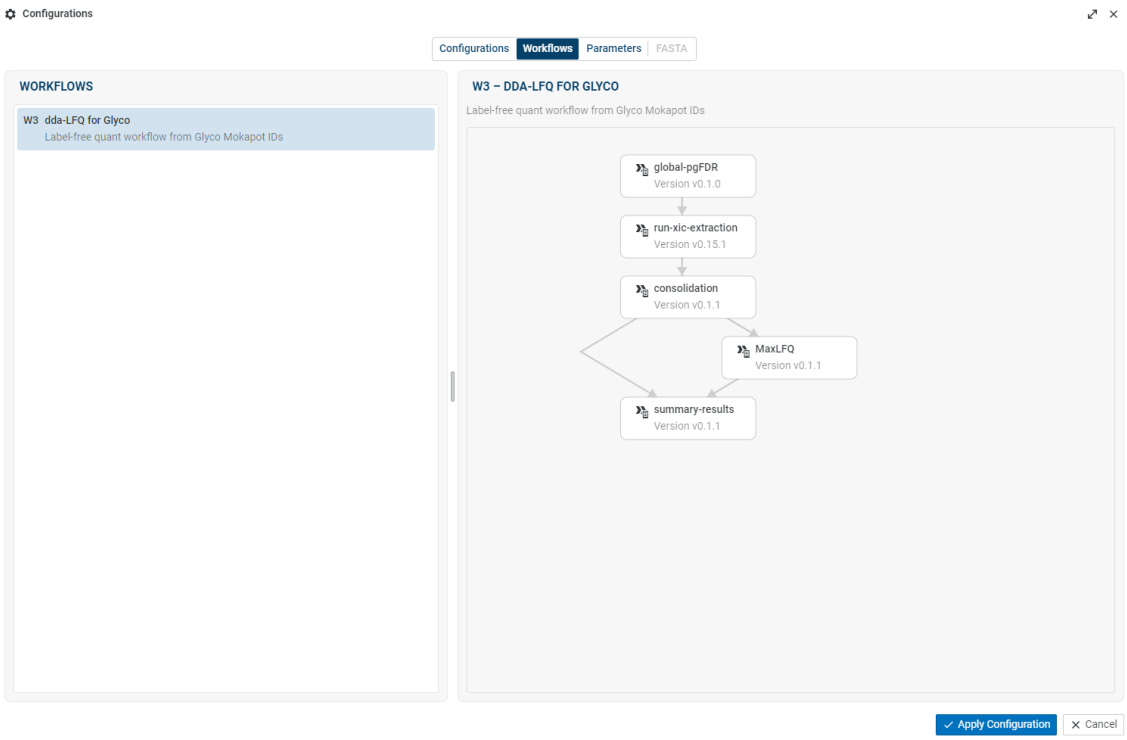
- By default, it semi-tryptic searches are recommended. Typically, this is done in conjunction with a smaller FASTA file (secreted proteins only) or some such, if not a longer than acquisition search is likely.
- Variable modification of by N-Acetylhexosamine on Asparagine must be specified for the Myriad workflow to function correctly = 203.07938@N

Summary results



- Often times when a small database is utilized for protein identification, it's desirable to set the Protein Q-value cutoff in the Summary Results to a value greater that 0.01. By default, results upto 0.1 are shown.
  - For very small FASTA files, such as NIST mAB with only contaminant proteins, the Summary Results protein Q-value filter can be set 1 so that visual elements can be utilized in GlycoScape

Quant with GlycoScape



- After single run analysis, >2 samples can be selected for quantitation
  - Global FDR is calculated across selected samples
  - Then XIC extracted for all (glyco)peptides above user specified q-value
  - maxLFQ based protein quantitation is provided

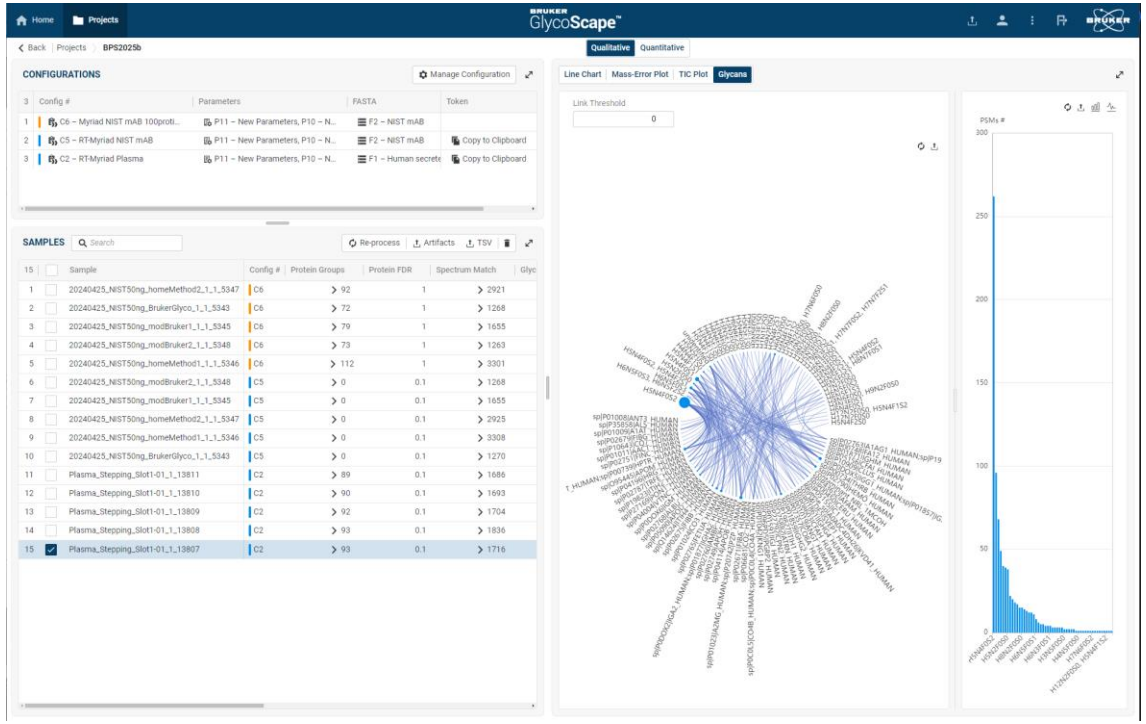
- Currently missing match-between-runs type functionality … only quantified precursors identified in the run (no transfer between runs)

GlycoScape Specific Views

Mass Error Plots

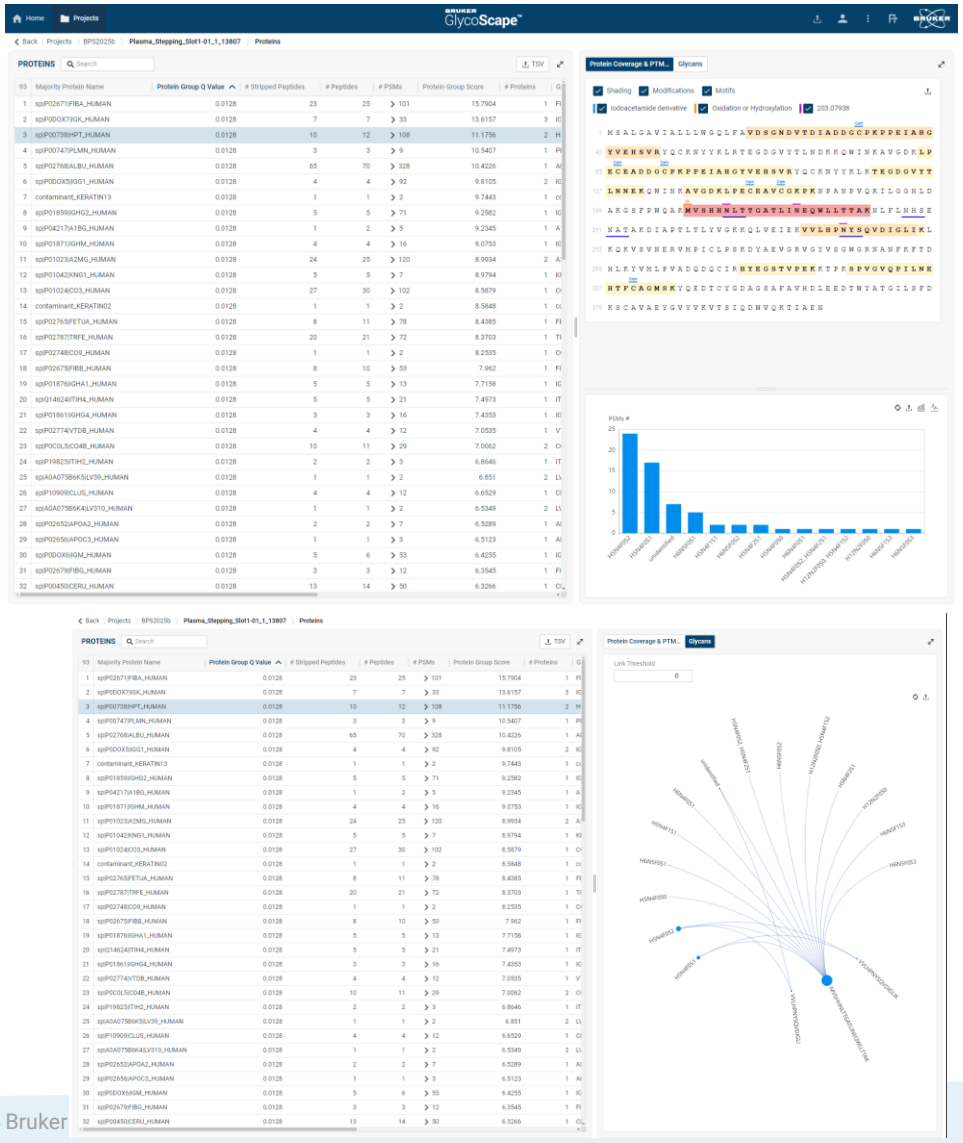
- Glycan and Peptide mass errors are plotted independently

Chord Diagrams – Sample view



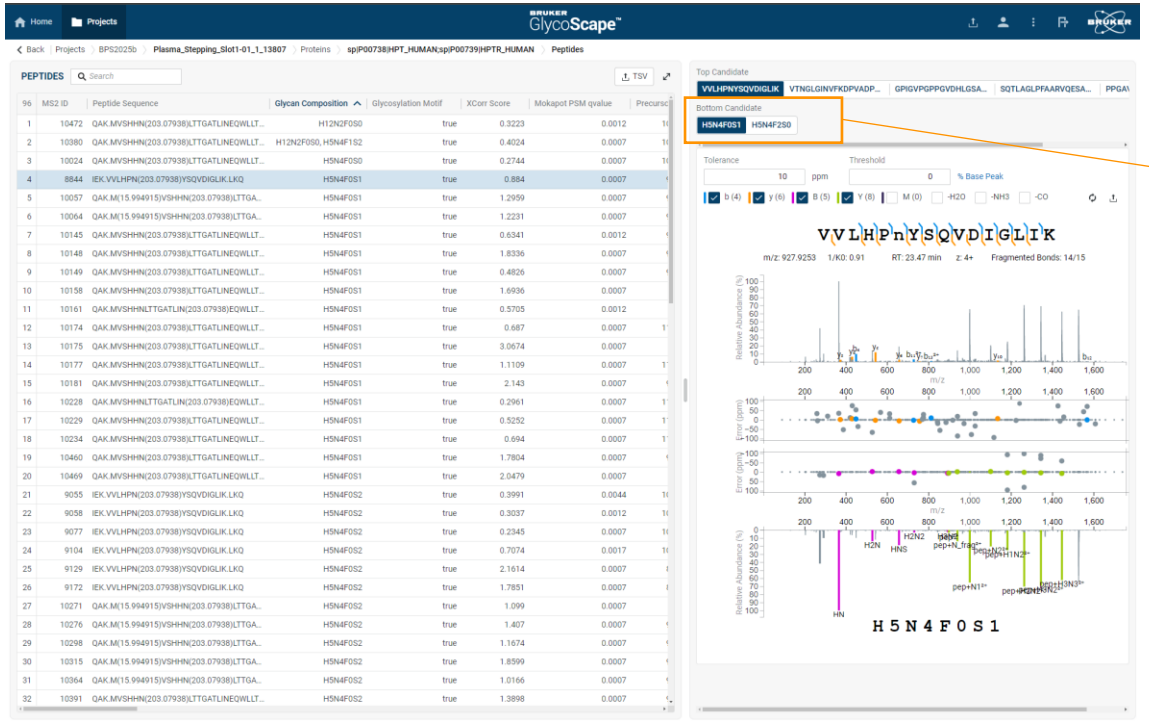
- From Samples view, a chord diagram can be accessed for each sample (only one sample can be shown at a time)
  - Diagram shows all identified glycan compositions and which protein groups they were found on
    - Node size = PSM counts
- Additional, bar graph of counts of each Glycan composition

Protein View



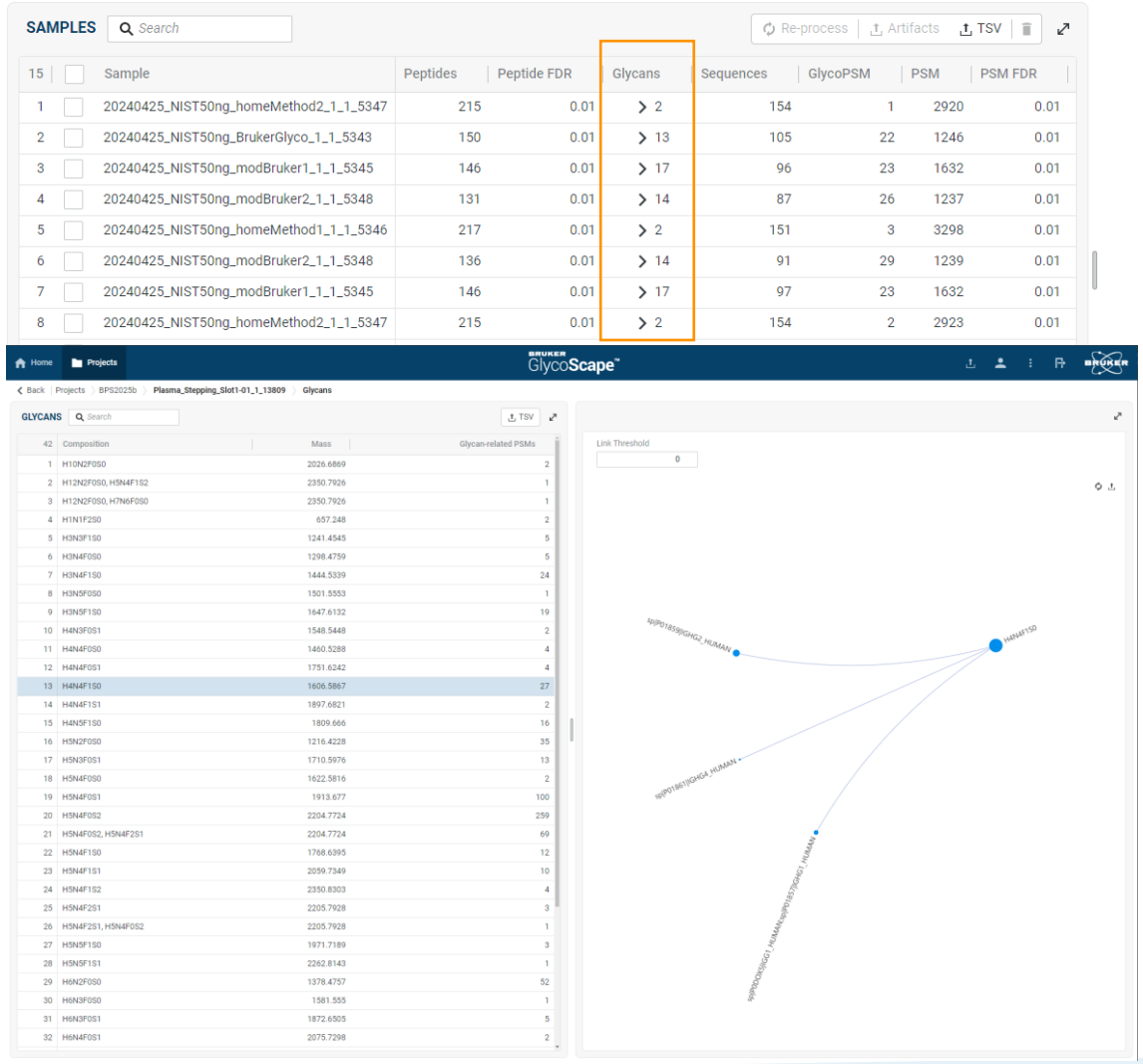
- Any potential glycosylation motifs are underlined in the sequence
- Chord diagram for glycan compositions mapping to peptides is accessible

PSM View



- PSM View
  - Top show peptide candidate
  - Bottom show glycan candidate compositions
    - Can choose candidate to be viewed if more than one is possible

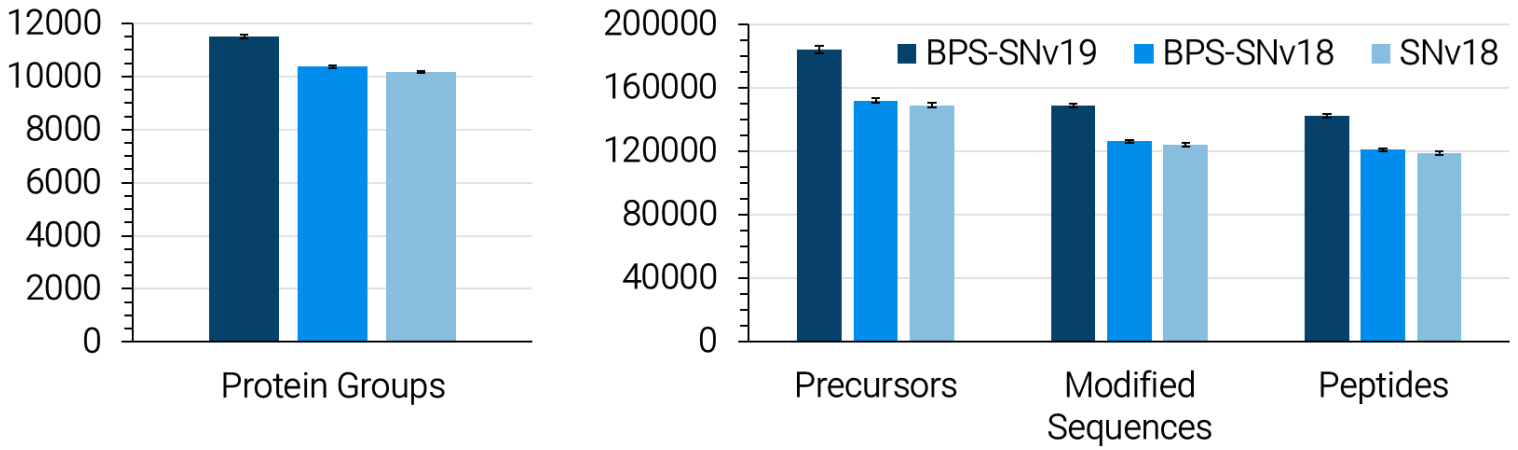
Glycan View



- Glycan view can be accessed from the sample table for each sample
  - Provides chord diagrams for each glycan composition

Spectronaut 19 module in BPS

Increased identifications with Spectronaut 19 module in Bruker ProteoScape



A three species mix of human, E.Coli and Yeast analyzed with a 45min active LC gradient on timsTOF Pro 2 was re-analyzed in BPS using the directDIA workflow.

Reminder: The Spectronaut module in BPS uses directDIA+ (Fast) by default, where the Spectronaut standalone uses directDIA+(Deep) by default. For comparison, be sure to uses the same settings. In the examples above, directDIA+(Fast) was utilized.

- On average a 13% gain in identifications can be observed between SN18 and SN19
- New deep-learning models are the largest contributors.
  - DeepCCS
  - DeepFrag
  - DeepiRT
  - DeepQuant

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diagonal-PASEF support in BPS with SN v19

- BPS 2025 now supports the analysis of diagonal-PASEF data, including synchro-PASEF and midia-PASEF, with the Spectronaut v19 module's directDIA workflow

[MiDIA-PASEF https://doi.org/10.1101/2023.01.30.526204](https://doi.org/10.1101/2023.01.30.526204)
[synchro-PASEF https://doi.org/10.1016/j.mcpro.2022.100489](https://doi.org/10.1016/j.mcpro.2022.100489)

MCP TECHNOLOGICAL INNOVATION AND RESOURCES

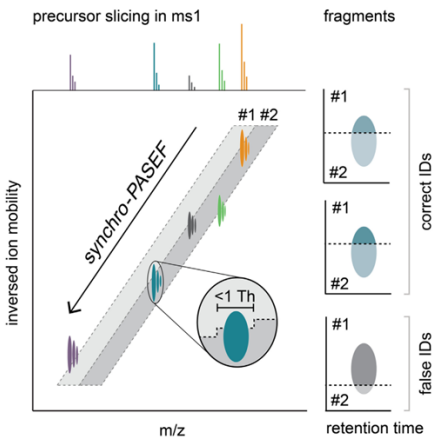
Synchro-PASEF Allows Precursor-Specific Fragment Ion Extraction and Interference Removal in Data-Independent Acquisition

Authors
Patricia Skowronek, Florian Krohs, Markus Lubeck, Georg Wallmann, Ericka C. M. Itang, Polina Koval, Maria Wahle, Marvin Thielert, Florian Meier, Sander Willems, Oliver Raether, and Matthias Mann

Correspondence
sander.willems@bruker.com;
oliver.raether@bruker.com;
mmann@biochem.mpg.de

In Brief
The novel scan mode synchro-PASEF efficiently follows the natural shape of the precursor cloud in the m/z and the trapped ion mobility space. This manifests in short cycle times and high sampling frequency of the eluting peptides and fragment signal amplification. Additionally, the seamlessly movement of the quadrupole nearly universally slices the precursor in the ion mobility dimension. The slicing position adds tremendous specificity, allowing deconvolution of the fragment space to 'pure fragmentation spectra' reminiscent of data-dependent acquisition spectra.

Graphical Abstract



Highlights

- Synchro-PASEF is a highly efficient scan mode for MS-based proteomics.
- It allows very fast cycle times, amplifying the fragment signal three-fold.
- Fragments are directly linked to precursors *via* precursor slicing.
- Precursor slicing enables deconvolution to pure fragmentation spectra.
- Synchro-PASEF unites the benefits of data-dependent and data-independent acquisition.

2023, Mol Cell Proteomics *22*(2), 100489

midiaPASEF maximizes information content in data-independent acquisition proteomics

Ute Distler[1]*, Mateusz Krzysztof Łącki[1#]*, Michał Piotr Startek[1,2], r[3], Sven Brehmer[4], Jens Decker[4], Thilo Schild[1], Jonathan Krieger[5], s[4], Oliver Raether[4], Andreas Hildebrandt[3] and Stefan Tenzer[1,6#]
nology, University Medical Center of the Johannes-Gutenberg University Mainz, Germany
Mathematics, Informatics, and Mechanics, University of Warsaw, Warsaw, Poland
nstitute for Informatics, Johannes-Gutenberg University Mainz, Germany
[4] Bruker Daltonics GmbH & Co. KG, Bremen, Germany
[5] Bruker Ltd, Milton, Canada
elmholtz institute for Translational Oncology (HI-TRON) Mainz, Germany
* contributed equally
[#] correspondence should be addressed to
M.L. (matteo.lacki@uni-mainz.de) and S.T. (tenzer@uni-mainz.de)

2023/01/30

Abstract

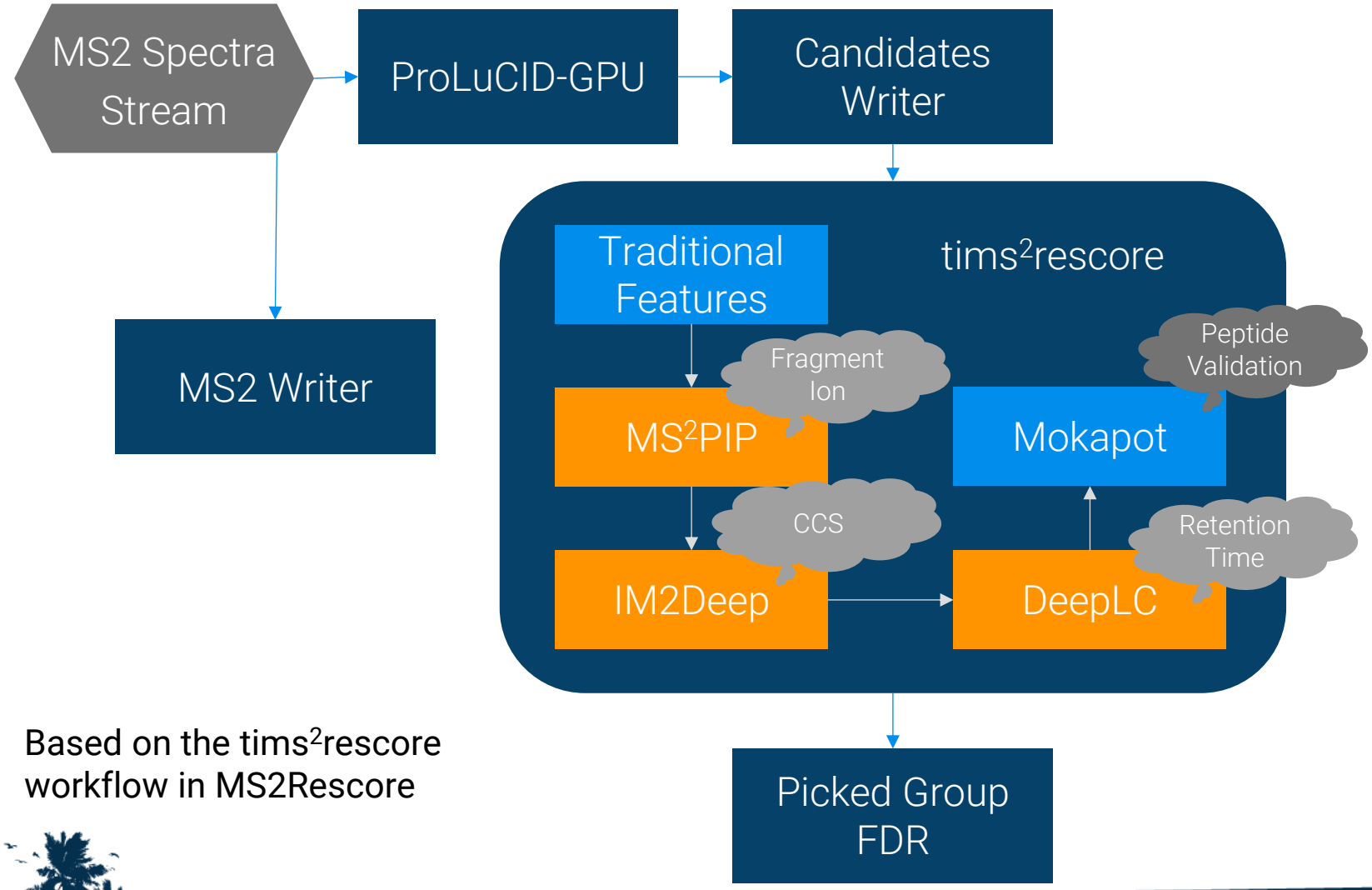
dent acquisition (DIA) approaches provide comprehensive records of all detectable pre-nent ions. Here we introduce midiaPASEF, a novel DIA scan mode using mobility-coding of overlapping quadrupole windows to optimally cover the ion population in nass to charge plane. Using overlapping ion mobility-encoded quadrupole windows, imizes information content in DIA acquisitions which enables the determination of the each fragment ion with a precision of less than 2 Th. The Snakemake-based MIDIAID s algorithms for multidimensional peak detection and for machine-learning-based clas-rsor-fragment relationships. The MIDIAID pipeline enables fully automated processing onal deconvolution of midia-PASEF files and exports highly specific DDA-like MSMS e suitable for *de novo* sequencing and can be searched directly with established tools , FragPipe and Mascot. midiaPASEF acquisition identifies over 40 unique peptides per ides powerful library-free DIA analyses including phosphopeptidome and immunopep-

, DIA, DDA, PASEF, Scan Mode, scanning quadrupole, TOF

TIMSrescore

BRUKER ProteoScape™ BRUKER

The TIMSrescore workflow



Based on the tims²rescore workflow in MS2Rescore

- The TIMSrescore workflow:
  - Identify peptides with database search
  - For each peptide compare:
    - Predicted fragment ion intensity
    - Predicted CCS
    - Predicted retention time
  - Validate peptides based on all these extended metrics
  - Create protein groups from validated peptides

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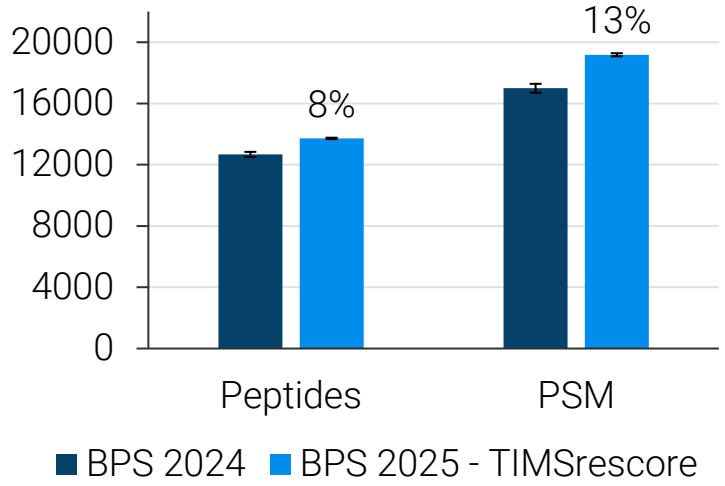
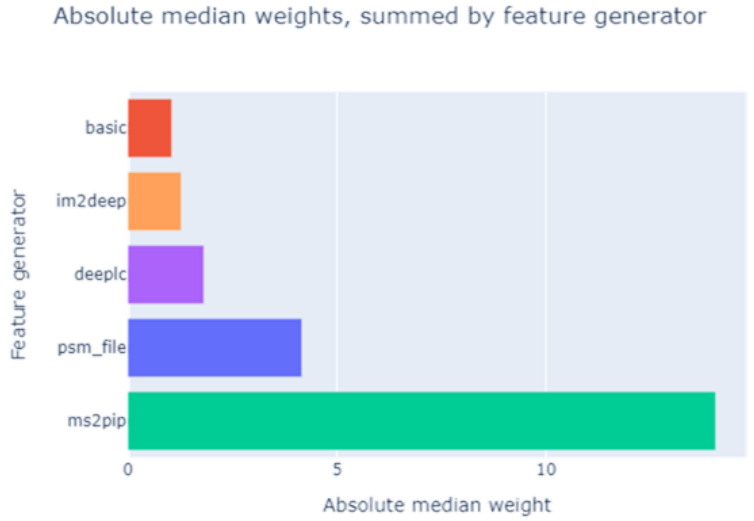
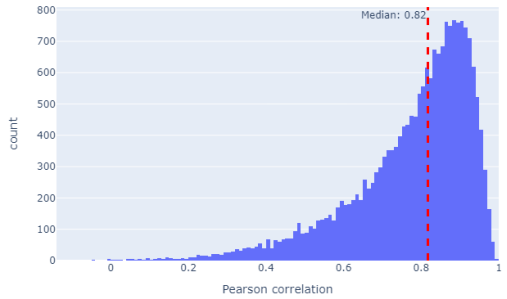
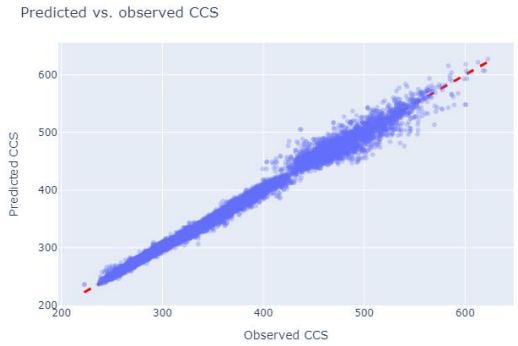
TIMSrescore increases confident identifications



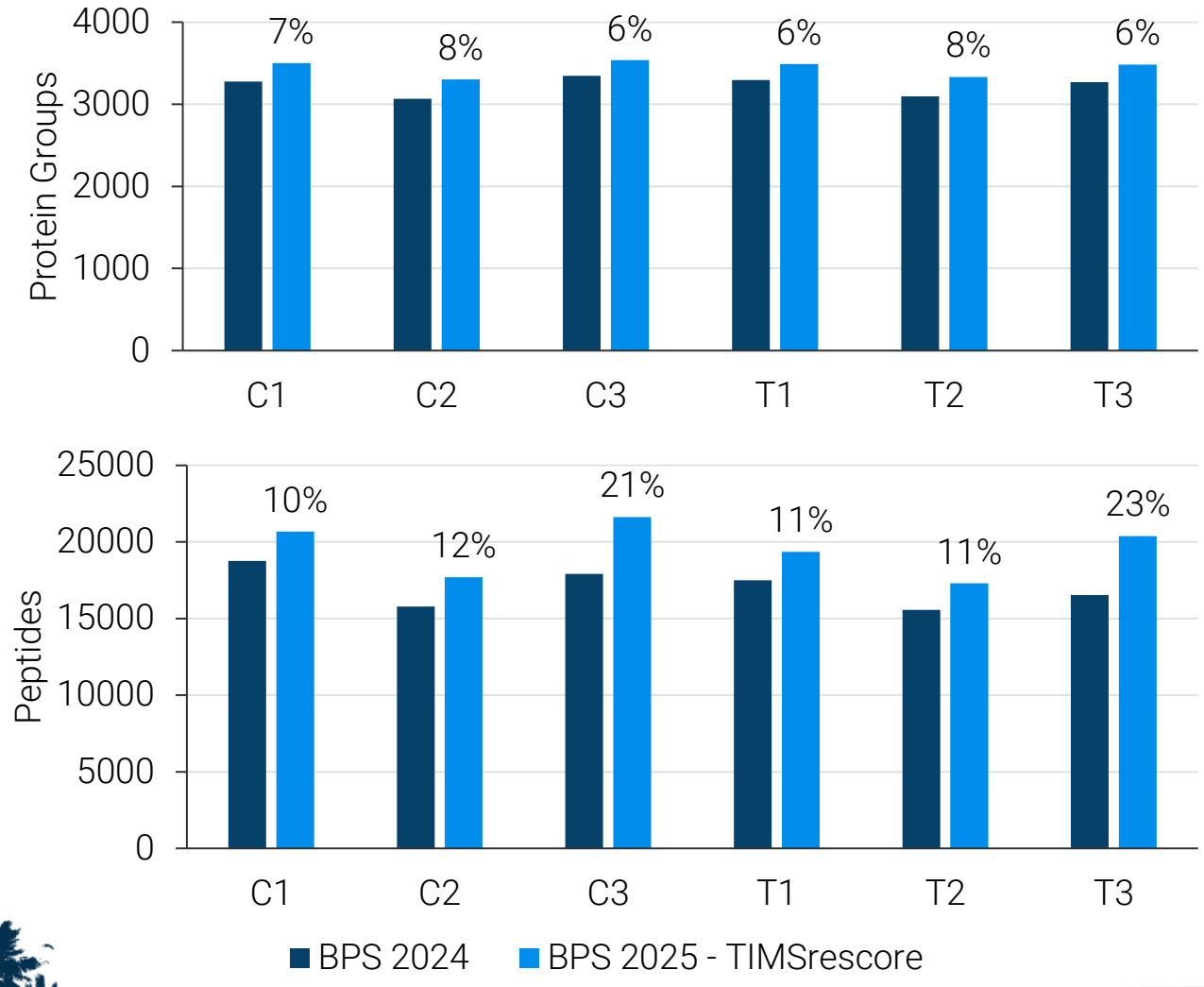






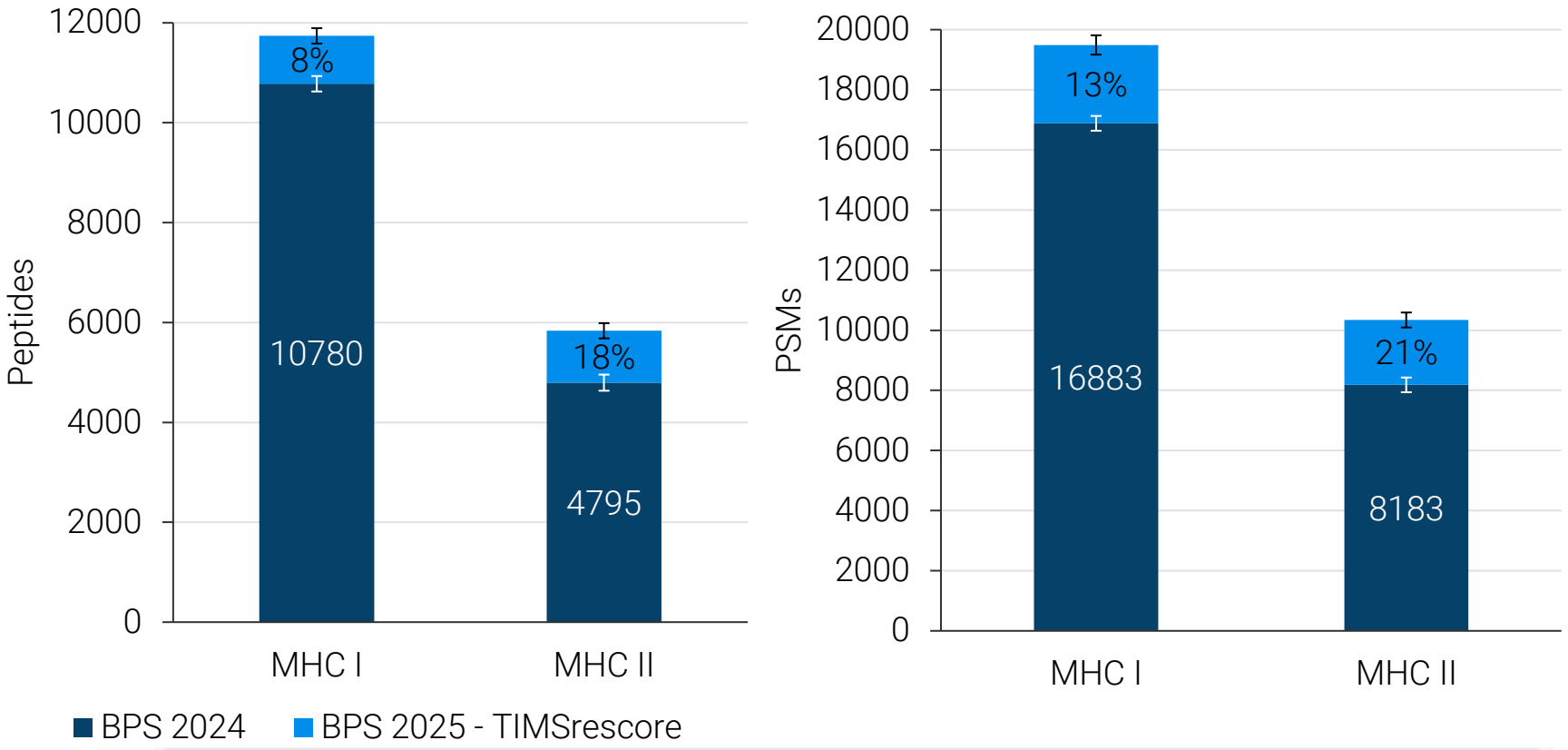
- In this HeLa Elastase digested triplicate measurement:
  - Peptides increased 8%
  - PSMs increased 13%
- Primary contribution for the gain was the timsTOF optimized fragment ion intensity prediction by ms^{2}pip

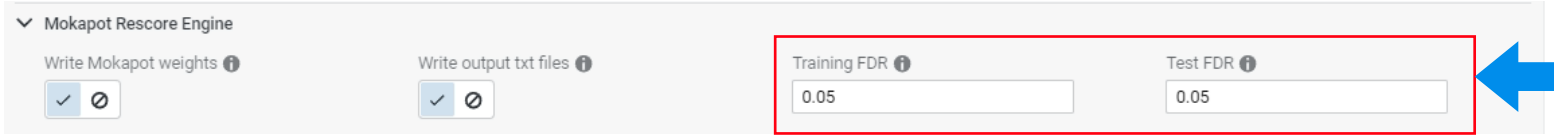
TIMSrescore increases confident identifications for phosphorylation enriched data



- Phospho-enriched dataset shows:
  - Protein groups increased ~7%
  - Peptides increased ~15%
- TIMSrescore consistently increases phosphorylated peptides and protein groups

TIMSrescore increases confident identifications for immunoproteomics data





- TIMSrescore increased confident MHC-I peptides by 8% and MHC-II peptides by 18%.
- timsTOF optimized fragmentation ion intensities and CCS predictions were largest contributors

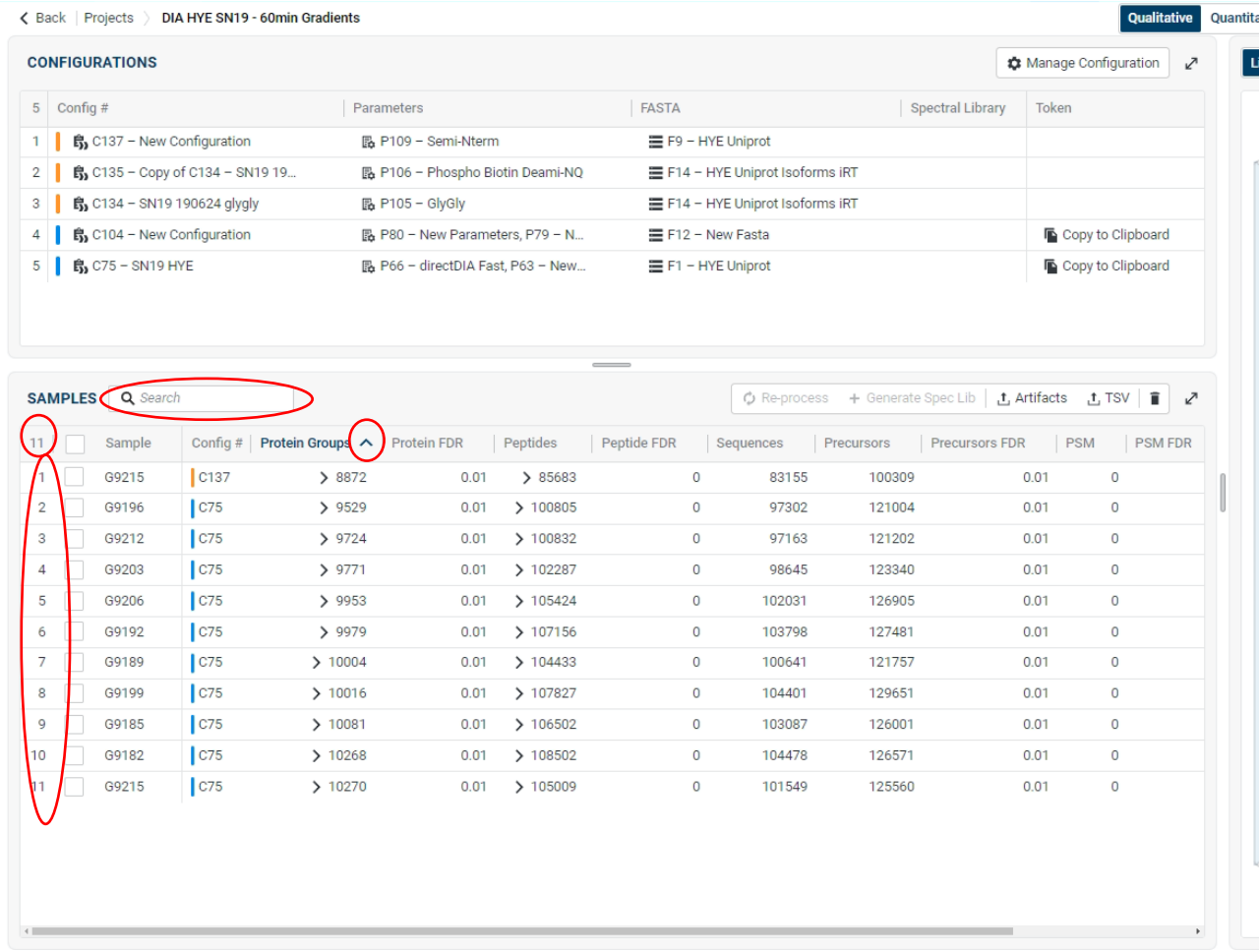
For large search spaces, such as immunopeptidomics it is necessary to adjust the Training and Test FDR to be less stringent (0.01 -> 0.05)

Raw data from: Hoenisch Gravel, N. *et al.* TOFIMS mass spectrometry-based immunopeptidomics refines tumor antigen identification. *Nat Commun* 14, 7472 (2023).

UI/UX improvements

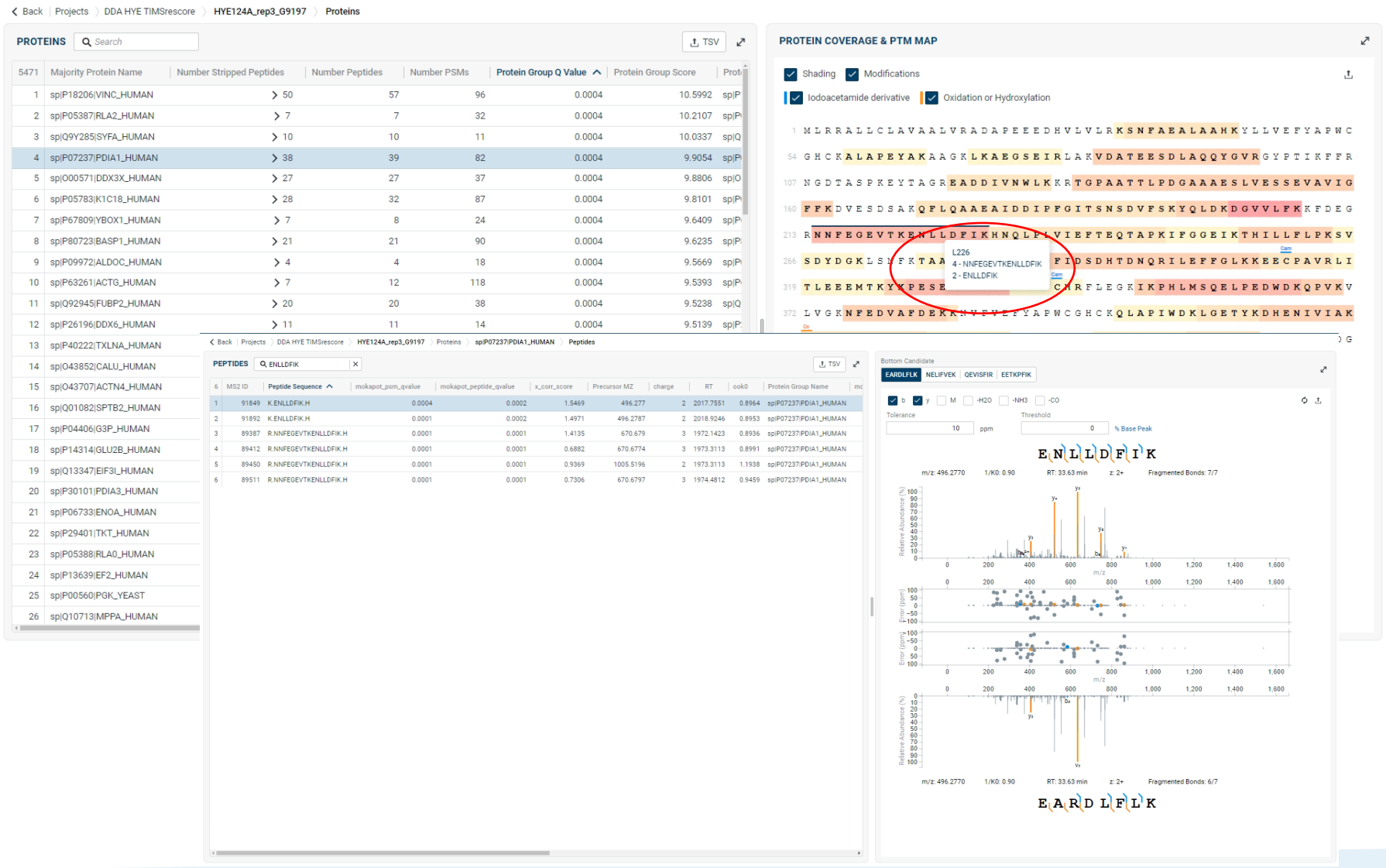
UI/UX improvements in BPS 2025

- Samples, Proteins and peptides tables have a case sensitive search bar
- Samples, Proteins and peptides tables can be sorted by user desired columns
- Columns in protein and peptide tables have been re-arranged to provided most queried information to the front
- All Tables have row number and top right shown total # rows in the table.



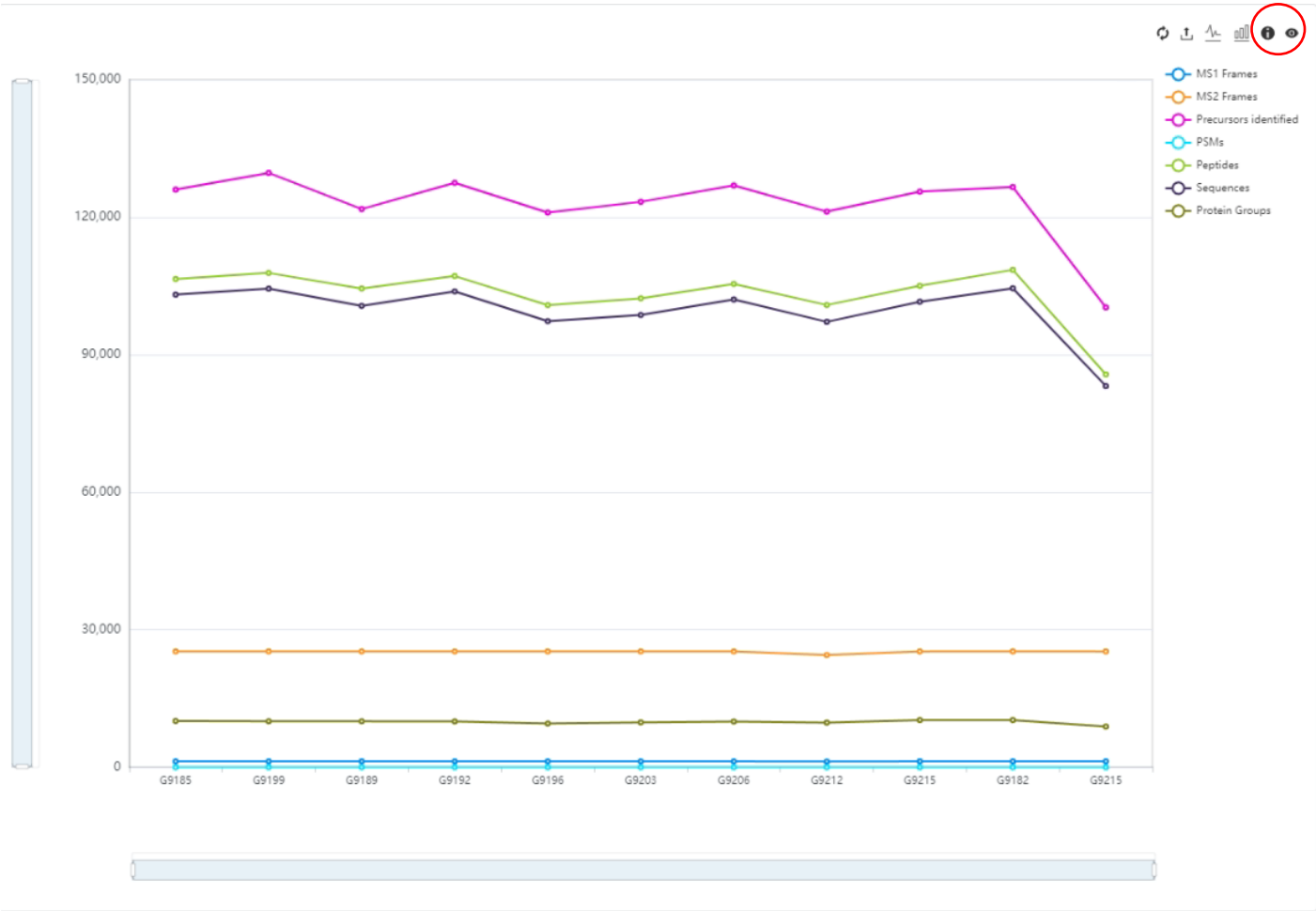
UI/UX improvements in BPS 2025

- Clicking on protein coverage maps, jumps to peptide view
- Peptide view table simplified by showing modification in the peptide sequence
- Prolucid ppm mass error corrected for amplitude and isotope mismatches



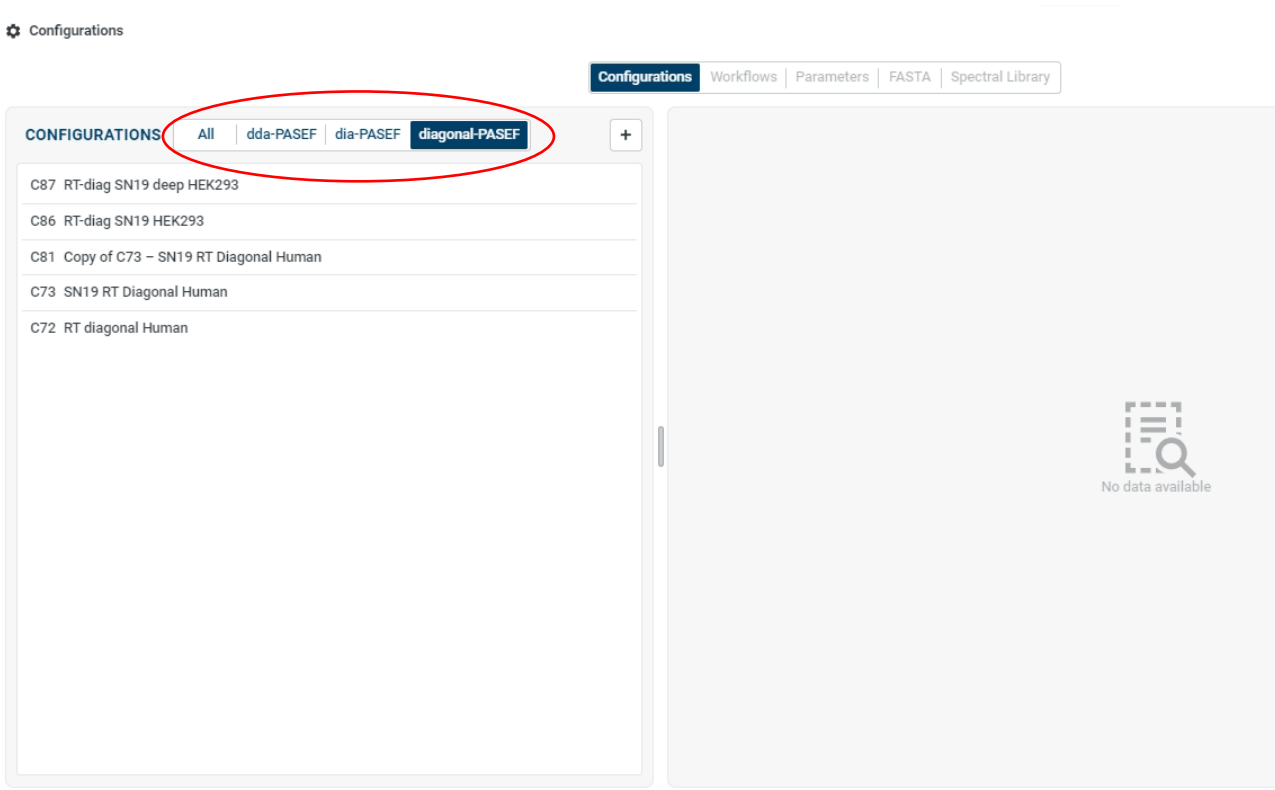
UI/UX improvements in BPS 2025

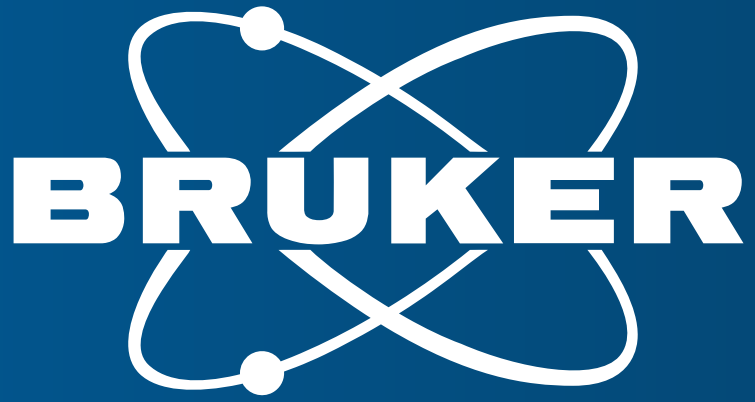
- Updates to most graph type visualizations
  - Legends always on the right-hand side
  - Show\hide legends and x-axis labels
  - Zoom bars for both axis



UI/UX improvements in BPS 2025

- Scan mode-based filtering of configurations and workflows





Innovation with Integrity