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Benchmarking A Visual Acuity Ion Classifier for MS/MS Deconvolution and Identification of Native Membrane Proteins In Vitro

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Introduction

Advances in sample preparation continue to enable the use of native and top-down mass spectrometry (TDMS) to characterize increasingly sophisticated targets, such as membrane proteins. Due to the complexity of the spectra produced, accurate deconvolution is an essential foundation of robust TDMS data analysis platforms. The unbiased deconvolution method in ExDViewer software shows excellent performance on TDMS/native TDMS (nTDMS) data from an array of peptides and proteins ranging from 1.4-150 kDa acquired on different instruments. Notably, the method is "parameter-free," using only a small set of comprehensible input parameters whose broadly applicable default settings yield robust, reproducible results. Furthermore, the uniquely intuitive interface helps to accelerate manual validation, which remains a necessary yet time-consuming reality for many TDMS experiments. ExDViewer 4.6 is available as Freeware at ***exdviewer.agilent.com*** [3]

Experimental

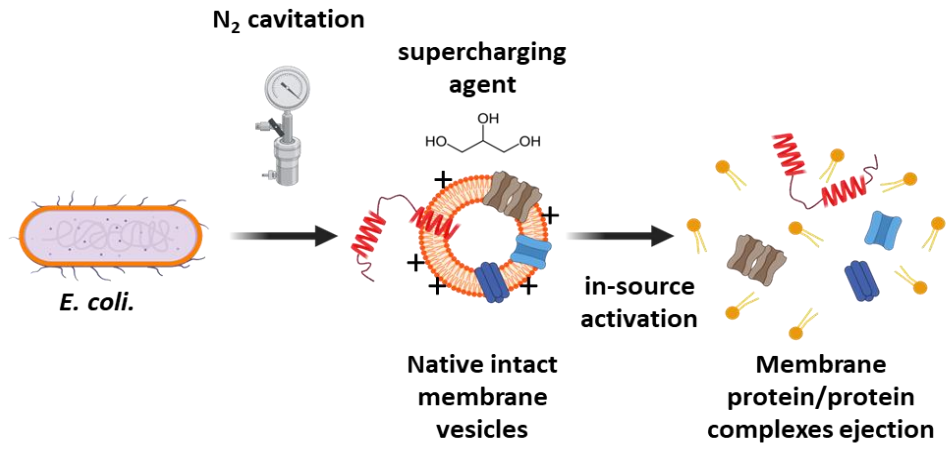
Methods

Download and Install ExDViewer 4.6 Freeware [3] at ***exdviewer.agilent.com***

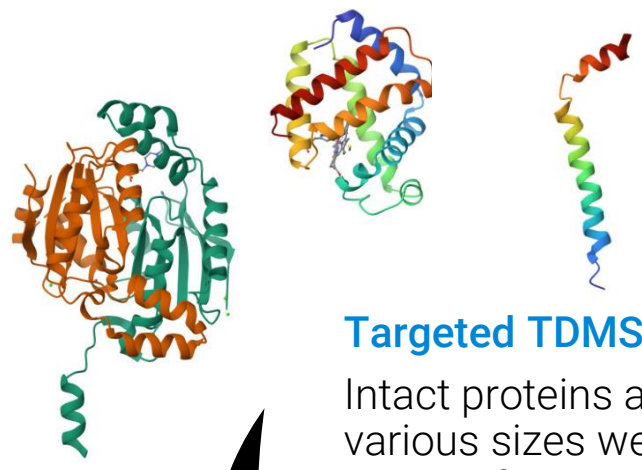
TDMS data of peptides and model proteins including carbonic anhydrase and NIST monoclonal antibody (mAb) were collected from targeted analysis on Agilent 6545XT AdvanceBio LC/Q-TOF equipped with an ExD cell to enable electron-based fragmentation. Three nMS-ready proteoliposomes (VAMP2, Aquaporin Z, and semiSWEET) were prepared using a prior protocol [1]. These proteoliposomes were directly subjected to nTDMS analysis on Thermo QE UHMR upgraded with ExD (e-MSion, Inc., now a part of Agilent). Deconvolution in ExDViewer assigns an IonScore of 0-15 to each predicted isotopic cluster based on how well observed data matches theoretical isotopic cluster. It considers profile peak data, centroid m/z error, hydrogen transfer, and overlap of neighboring peaks

In previous work [2] we benchmarked v4.3 using targeted CID+ECD TDMS data of a diverse set of peptides and proteins from multiple vendors. Over 10,000 fragment ions were automatically identified and hand-annotated by expert users to benchmark deconvolution. With a scoring threshold of 1.5, over 90% of all ions detectable by expert users were captured, with less than 2% having incorrect *m/z* and charge assignments.

Experimental

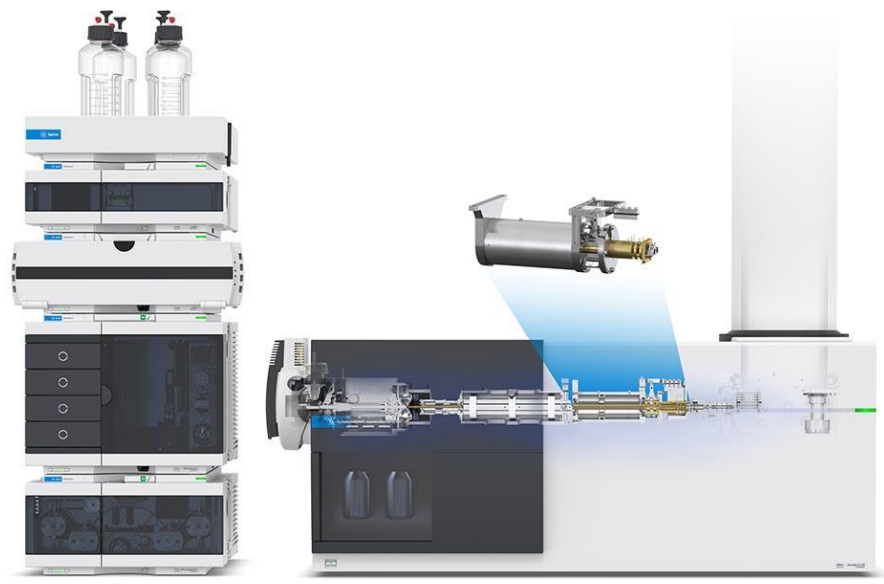


Samples analyzed on Thermo QE UHMR mass spectrometer with Legacy ExD upgrade (e-MSion, Inc., now a part of Agilent) [1].



Targeted TDMS on Q-TOF+ExD

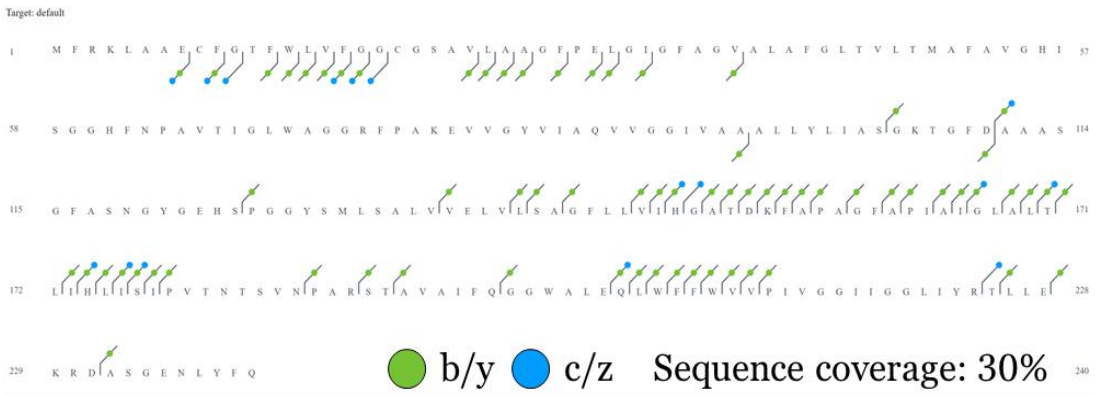
Intact proteins and peptides of various sizes were analyzed by direct infusion or Liquid Chromatography

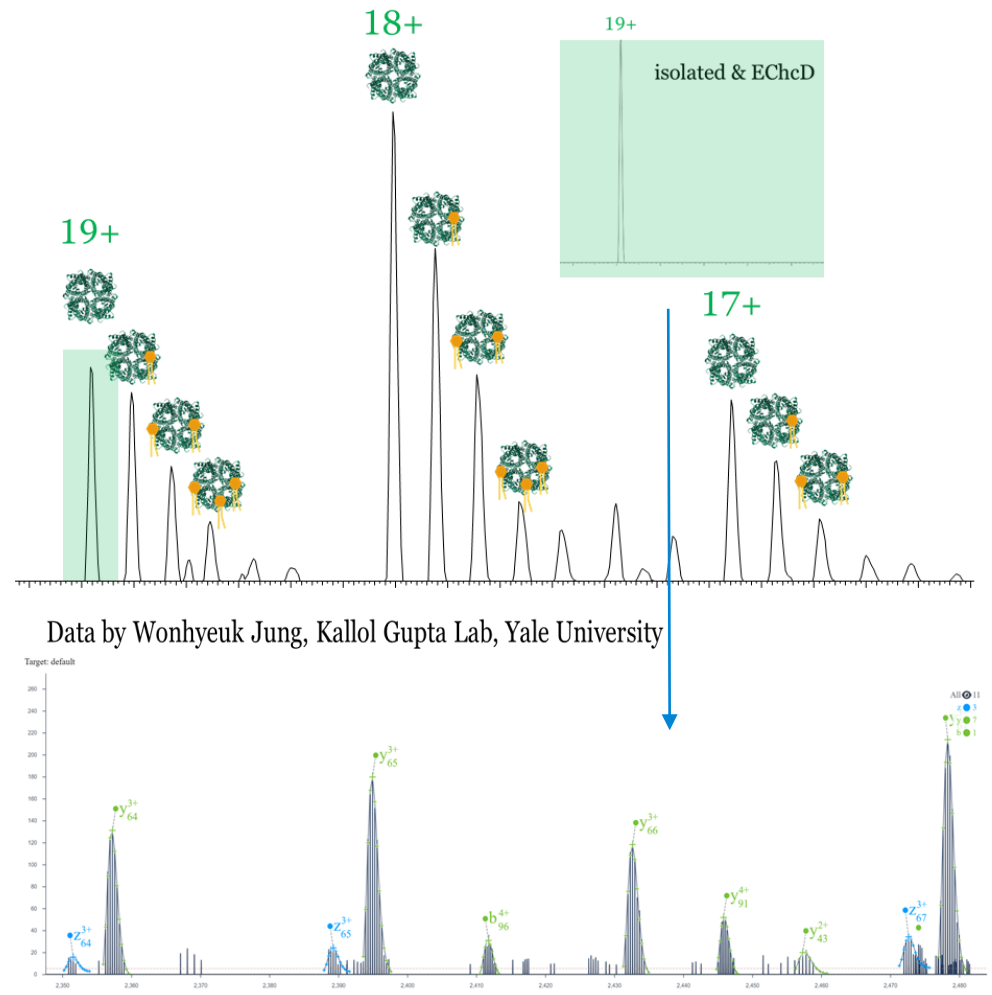


Agilent 6545XT Q-TOF mass spectrometer with ECD cell.

In Vitro Membrane Protein TDMS

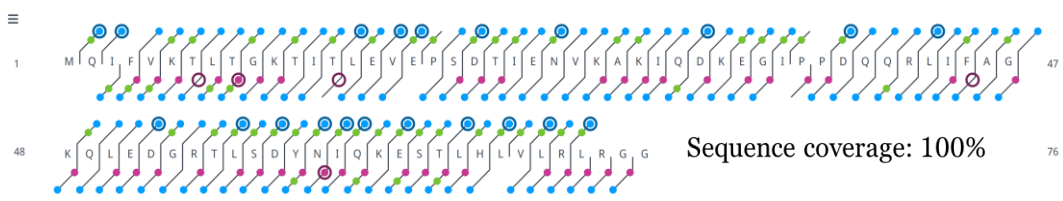
We have analyzed nTDMS data of intact oligomeric MPs directly from *E. coli* polar lipid extract. The respective physiological oligomeric state of each MPs was isolated and then subjected to nTD-MS analysis. Robust fragmentation was observed for all three proteins, reaching 44% sequence coverage for VAMP2, 70% for semiSWEET, and 30% for AqpZ.

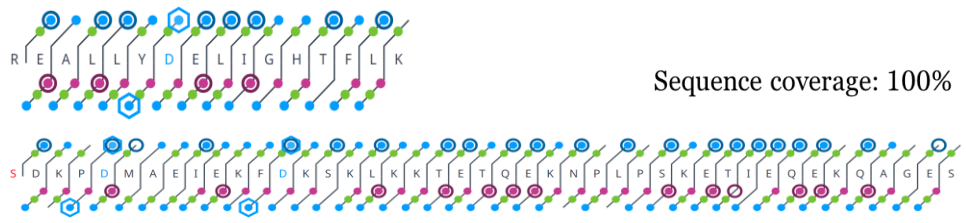


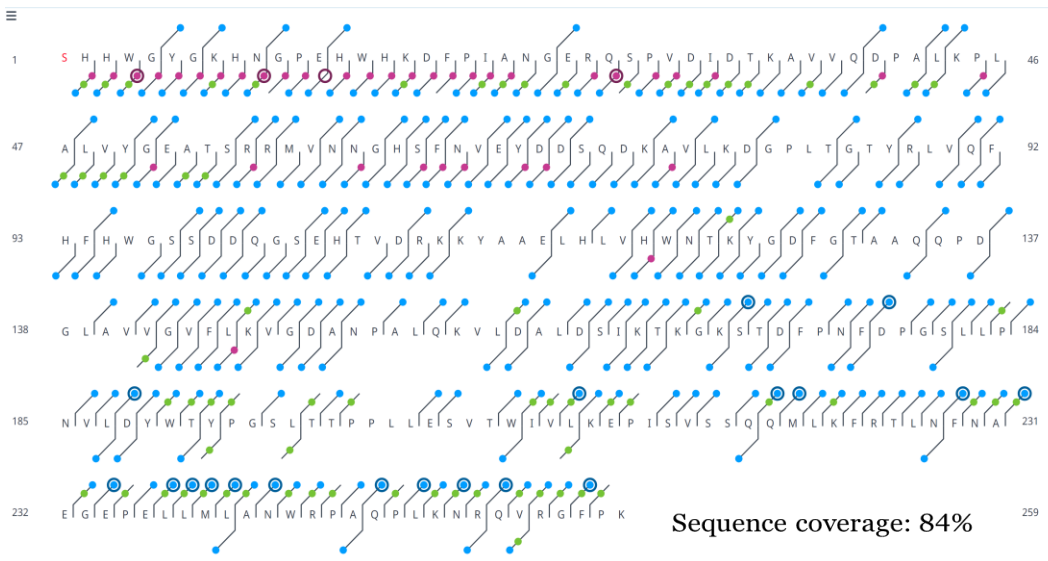


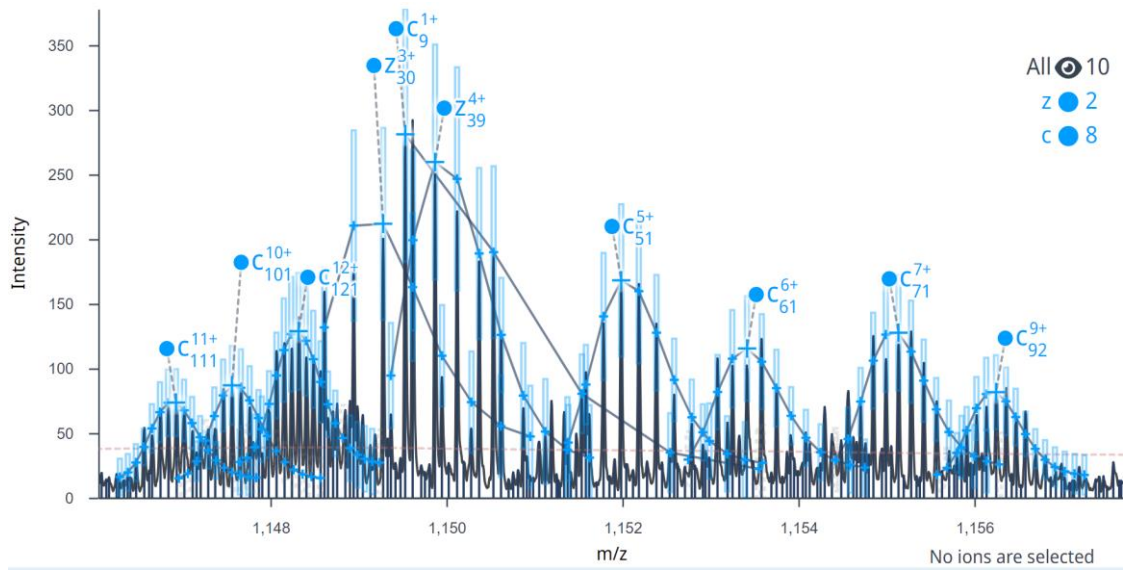
Targeted TDMS

ExDViewer effectively adapts to unfiltered TOF signals, yielding robust performance on small peptides and large proteins acquired with the Agilent Q-TOF platform. Optimum performance is achieved when processing raw profile data.

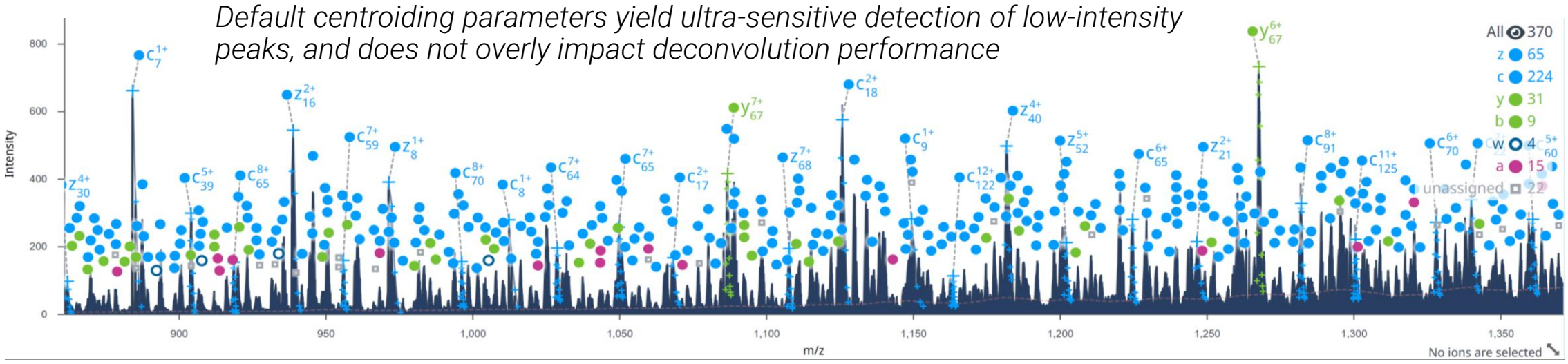




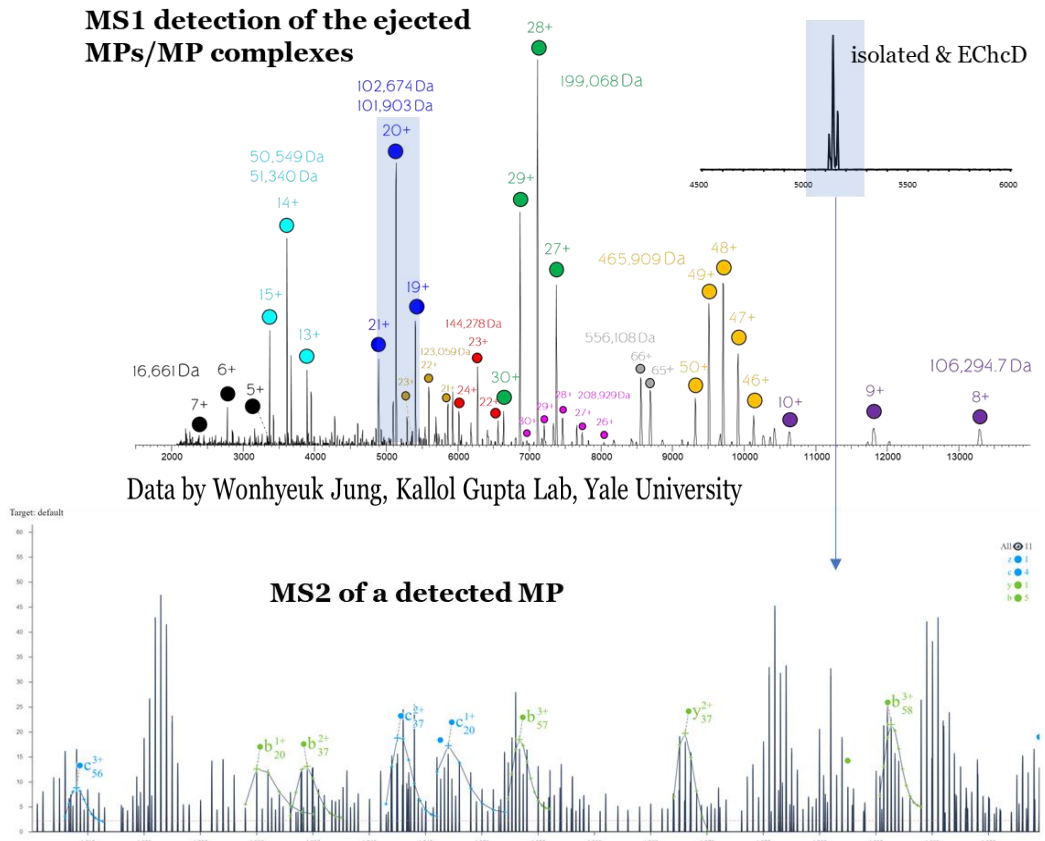




Default centroiding parameters yield ultra-sensitive detection of low-intensity peaks, and does not overly impact deconvolution performance



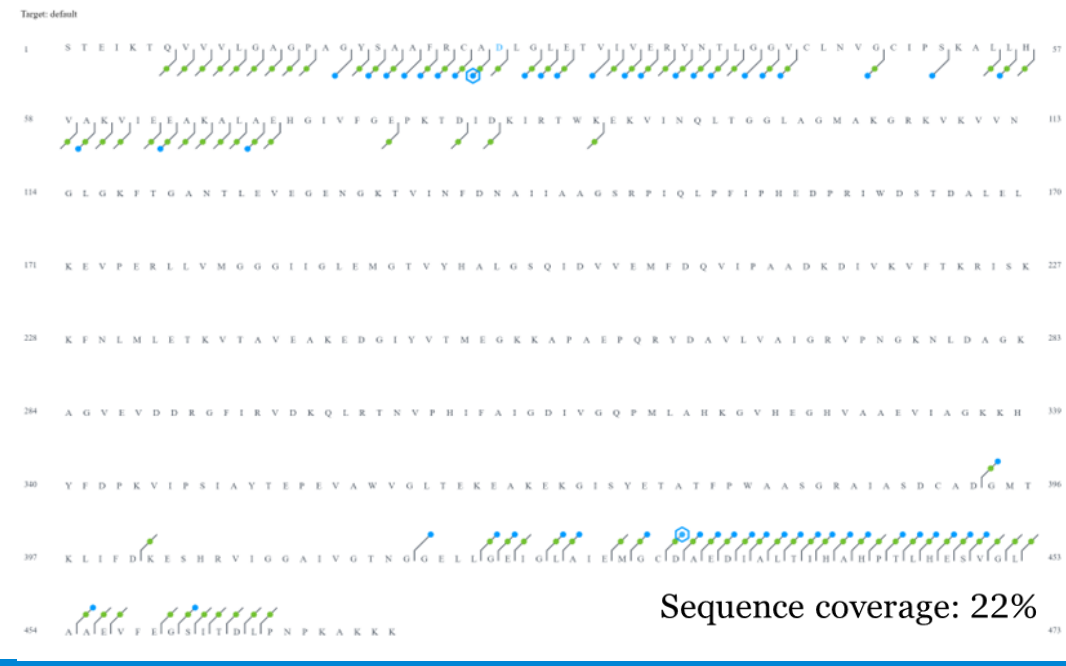
Results and Discussion



For majority of the users, default "Parameter-free" deconvolution and quick manual verification afterwards produces robust, reproducible data.

In Vitro Membrane *Complex* TDMS

Here we could directly identify different multimeric membrane protein complexes in their native organization states from their physiological environment. Deconvolution within ExDViewer was sensitive enough to detect low intensity ions in a sample-limited environment, yet accurate enough to facilitate quick manual verification without sifting through many false ion matches.



Conclusions

- Deconvolution performance was benchmarked previously and further investigated here on challenging membrane proteins isolated *in vitro*, where sensitivity to detect low-intensity ions was key for robust identification. The follow-up work will focus on benchmarking performance with other tools across a larger set of users.
- ExDViewer accommodates a wide range of users regardless of their experience level and experimental goals. "Parameter-free" deconvolution allows naïve users to confirm a sequence in complex top-down spectra with the same ease as in bottom-up spectra without changing default parameters. The wide array of parameters is adjustable for advanced users as well.
- ExDViewer now supports the incorporation of custom amino acids (AA) and custom post-translational modifications (PTMs) into the Target Editor, enabling deconvolution with non-standard AAs.

References

[1] Panda A, Giska F, Duncan AL, Welch AJ, Brown C, McAllister R, Hariharan P, Goder JND, Coleman J, Ramakrishnan S, Pincet F, Guan L, Krishnakumar S, Rothman JE, Gupta K. Direct determination of oligomeric organization of integral membrane proteins and lipids from intact customizable bilayer. Nat Methods. 2023 Jun;20(6):891-897. doi: 10.1038/s41592-023-01864-5. Epub 2023 Apr 27. PMID: 37106230; PMCID: PMC10932606.

[2] Guthals A, Sturgeon D, Gavrilenko A, Hakkila B, Sturgeon S, Gavrilenko J, Franklin R, Vasil'ev Y, Meeuwsen J, Voinov V, Beckman J; Parameter-free Deconvolution and Visualization of Peptide and Protein Fragmentation Mass Spectra. ASMS 2023

[3] ExDViewer is Agilent proprietary software that is distributed at no monetary cost to users under specific terms of use defined in the End User License Agreement. ExDViewer is not open-source software. Download at exdviewer.agilent.com.

Conflict of Interest Disclosure

Authors Derrill Sturgeon, Stelios Gkekas, Panos Iatrou, and Alex Gavrilenko are employees of Devicepros, who work under contract of Agilent Technologies, which sells the instrumentation used in this analysis.

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