How Well Did You Capture that Ion? Find Out with PeptidePrisoner!

A New Tool in the Trans-Proteomic Pipeline

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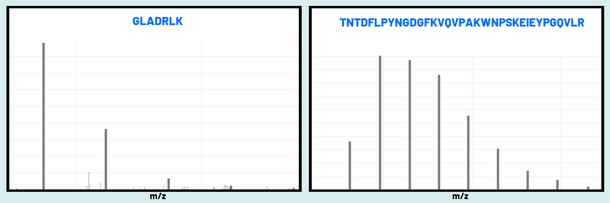
Overview

- Establish a quantitative metric for adherence of isotopic envelope of precursor ion to theoretical
- Compute a precursor isolation metric that takes the above fit into account
- User interface for the visualization, exploration, and interrogation of precursor signal quality
- Integrated into the Trans-Proteomic Pipeline (**TPP**)
  www.tppms.org

Introduction

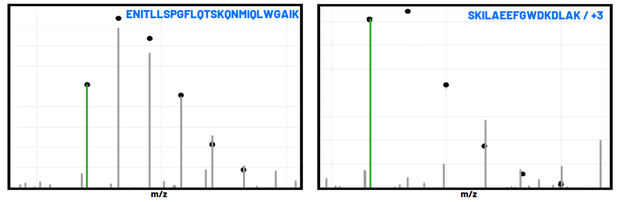
- Amino acids in natural or synthetic proteins are composed of atoms with a mixture of **isotopes**.
- This creates an isotopic **envelope** in the MS1 signal of the parent ion.



- A **good** fit against the **theoretically-predicted** distribution might provide corroborating evidence for the peptide match.
- A **bad** fit could be indicative of an **incorrect identification**; the **wrong charge state** reported by the instrument software; **overlapping ions** from other peptides, whether isobaric or not; **noise**; or a combination of these.
- This fit can also be used to estimate the amount of **signal** in the isolation window **attributable** to selected precursor ion.
- A low value is suggestive of co-fragmented species, leading to complex **chimeric** spectra.

Methods: Precursor Isotopic eNvelope Quality score

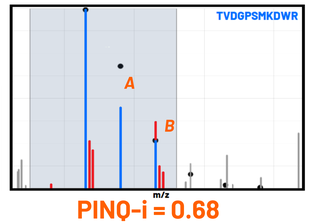
- The *PINQ* score is computed as the best **Chi-squared fit** of observed m/z and intensities of isotopic peaks to those **predicted** by theory
- Data are extracted from **pepXML** and **mzML** files



Methods: *PINQ-i* (isolation) score

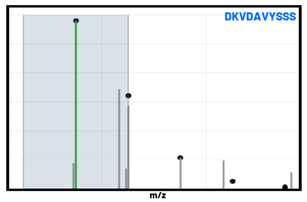
- Computed by adding the peak intensities of all **isotopic peaks** within the **selection window**, and dividing by the **total signal** in the window
- The intensity used is the **lower** of:
  - observed signal [A]
  - intensity attributable via *PINQ* fit [B]

- ***PINQ-i = 0.00*** : none of the signal in selection window can be attributed to the identified peptide
- ***PINQ-i = 1.00*** : all of the signal was likely generated by the peptide ion



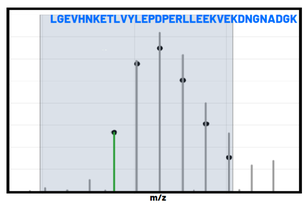
Scores are written back to the source **pepXML** file.

Results (examples)



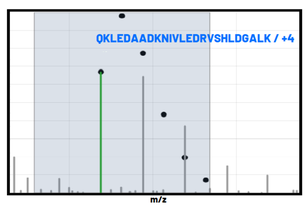
- PINQ = 3.52
- PINQ-i = 0.61
- Prob = 0.15

Very good isotopic pattern fit, but low probability and marginal isolation score suggest **co-fragmentation** of another ion.



- PINQ = 2.44
- PINQ-i = 0.92
- Prob = 0.00

Excellent isotopic pattern fit and isolation score. Poor probability suggests this is a real peptide that was **not in the search database** – perhaps a variant, contaminant, or from another organism.



- PINQ = 98.0
- PINQ-i = 0.83
- Prob = 0.00

Very poor isotopic pattern fit and probability, and good isolation score would indicate that the **charge state might be incorrect**.
Quick examination via the interface suggests a charge of +2 instead of +4.

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User Interface

Precursor isotopic envelope explorer

- Peptide and scan information, signal strength
- Relative intensities of detected isotopic peaks as well as those expected from theory
- *PINQ/-i* scores and other TPP confidence metrics
- Links to display the original spectral data along with the expected intensities and best fit
- Pan, zoom, search and filter functionality

Future Work

- Incorporate the *PINQ* and *PINQ-i* scores as extra discriminants for PSM validation in **PeptideProphet**
- Flag potentially **chimeric** spectra
- Consider **fragment** ion isotopes

Conclusions and Availability

- The TPP is a widely used and well-validated **free and open source** suite of software tools that facilitates and standardizes proteomics analysis.
- These various updates will allow TPP users to analyze, validate, and visualize precursor isolation quality results from **any** supported search engine.
- These features will be available as of the next release of TPP, version **6.4.0**, planned for Summer 2023, or in preview mode in an upcoming dev build.