Advancements in Multi-Protein Quantitation for Host Cell Protein Discovery

Lawrie Veale, Antony Harvey, Michael Georgoulopoulos, Lukas Tvrdy, Claire Bramwell, Yong Kil

Protein Metrics, LLC, Boston, MA

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Summary:

Aim: Introduce the new multi-protein quantitation mass spectrometric analysis workflow tools for discovery-phase protein scientists.

These workflows will help scientists in every phase of biopharmaceutical discovery and development from early cell line development to final drug purity verification and profiling of cell therapy samples.

These workflows produce auto-curated analysis for quantification of thousands of proteins from sample replicates, quickly processing gigabytes of input data.

The Byosphere® cloud platform rapidly compares data across samples, projects, and geographic sites leveraging Deep Query and Dashboard capabilities in a GxP compliance-ready environment.

Introduction

Vendor-Neutral, Application-Specific Workflows Identify and Quantify Thousands of Proteins:

Multi-Protein Quantitation
Identification and Quantification of Protein Mixtures

Multi-Protein Identification
Proteomics ID, characterization and annotation of PTMs

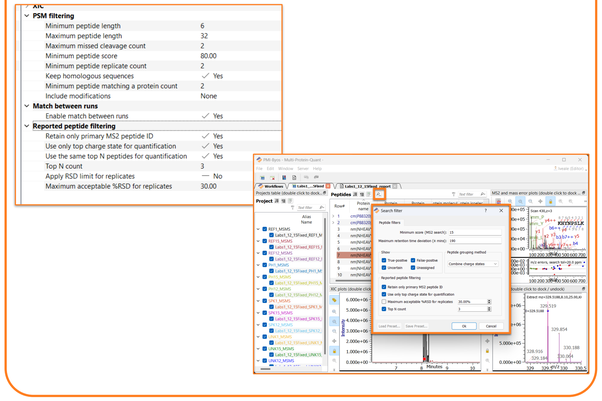
Multi-Protein Preview
Preview MS2 Data Quality and Search Parameters

Enhanced Performance & Time Saving Solutions

- Multi-Protein Quantitation:
  - Rapid analysis that immediately shows you the relative abundance of a complex mixture of proteins
  - Label-free Quantitative analysis of thousands of proteins at a time
  - Protein-centric analysis
- Multi-Protein Preview to automatically assess sample status:
  - Mass spectrometer calibration/performance
  - Enzymatic digestion specificity
  - Alkylation efficiency
  - Sample quality/unexpected artifacts
- Multi-Protein search and identification of thousands of proteins:
  - Protein-centric layout
  - Focused review
  - Quicker result verification
  - Built by the same team that created the ground-breaking Byonic algorithm

Dynamic Filtering :

Filtering allows analyst control over peptide quantitation during (left) and after (right) project creation.



Identification and Quantitation Dynamic Inspection

Two Case Studies: NIST MAM Comparison Studies

Figures 1(a&b). Multi-Instrument Comparison: Identification and Quantitation of low level host cell proteins.

Normalized XIC Area Summed Comparison of All HCPs

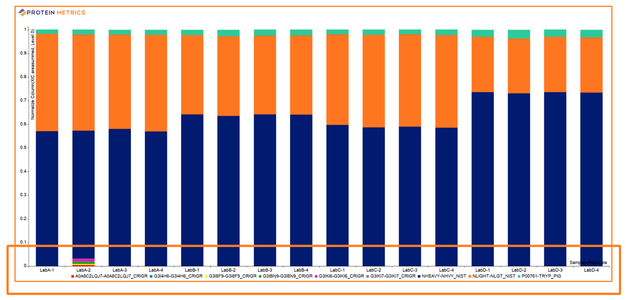


Figure 1a (left): Normalized XIC area summed of all proteins identified in samples from four different instruments in four different labs with four different conditions [(1) reference, (2) pH, (3) spiked, and (4) unknown]. Data suggests relatively similar amounts of NIST mAb heavy chain (blue) and light chain (orange) along with trypsin (green) for each instrument. Notably, a visually apparent amount of HCPs were found in the instrument Lab A-2 pH sample.

Normalized XIC Area Summed Comparison of Low Level HCPs

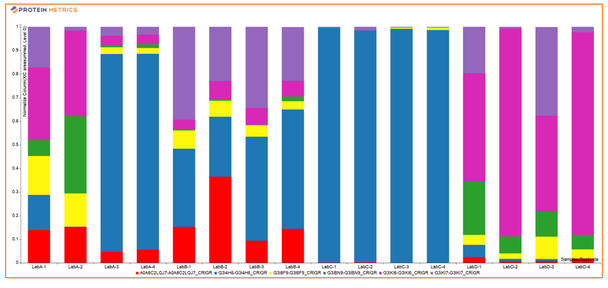


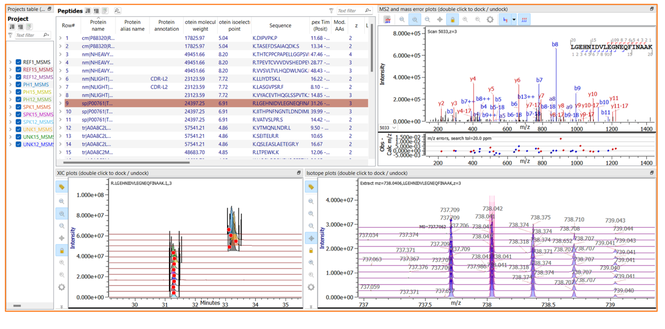
Figure 1b (right): Zoomed in stacked bar chart of normalized XIC area summed amounts of low level HCPs that are identified in the samples.

Automatically generated reports enable proteome-wide protein identification with a simple, protein-centric review capability.

Figure 2. Multi-Lab Comparison with NIST

Figure 2 (right): Inspection view of data sets from 3 labs containing 4 different conditions show integrated XICs using Match Between Runs to align peptide identifications across 12 datasets and differing retention times.

The inspection view along with the automatically generated reports offers a rapid assessment of sample preparation quality via analysis of mass spectrometer precision and resolution and enzyme digestion efficiency.



Dashboards Monitor HCPs With Deep Query

Dashboards allow for the monitoring of Host Cell Protein levels across every phase of biopharmaceutical discovery and development from early cell line development to bioprocess optimization or antibody purification to FDA New Drug Application (NDA) submissions, through final drug purity verification and profiling of cell therapy samples.

Dashboards Update with Most Up-To-Date Information and Automatically Alert Out of Range Proteins

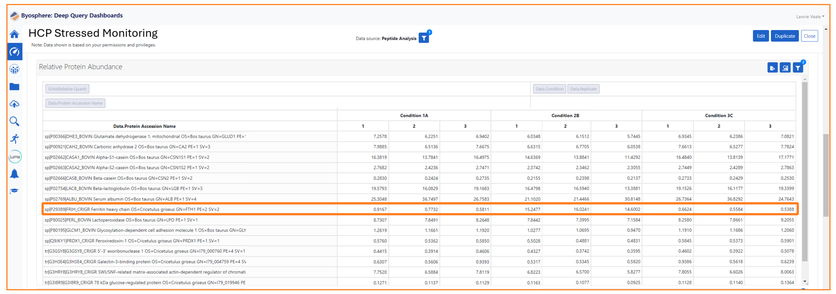
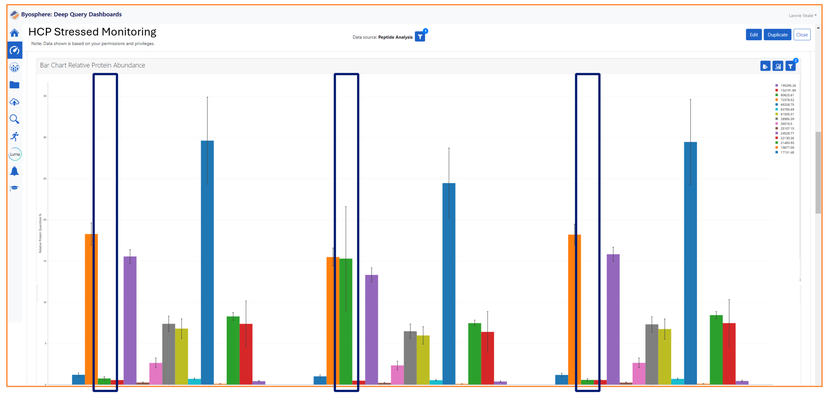


Figure 3a (above): The HCP ferritin heavy chain has an elevated level for the condition 2B (orange box) versus the levels that were detected in stress conditions 1A and 3C. Byosphere Dashboards are enabled with the ability to set and notify with automatic alerts to notify if proteins of interest falls out of range.

Figure 3b (below) Byosphere dashboards allow for the configuration of replicate comparison and different visulization of data sets. In the blue boxes, is the replicate analysis of the ferritin HCP that has increased relative protein abundance in the stressed condition 2B vs 1A and 1C samples.



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Conflict of Interest Statement
Some of the authors are employees and/or shareholders of Protein Metrics, LLC, which has commercialized the software described here.