Developing a High-Throughput Host Cell Protein Analysis Workflow with Novel SpotMap MS Software
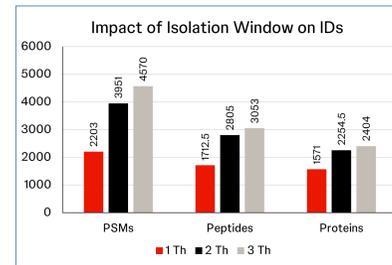
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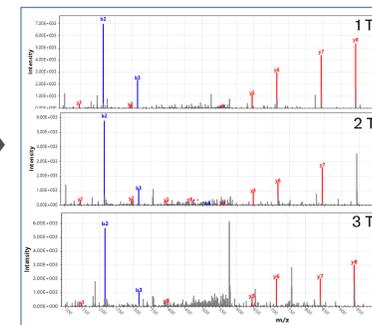
Introduction

- Host cell proteins (HCPs) are a major concern for biotherapeutic development with impacts to drug efficacy, product shelf life, and adverse reactions.
- The identification and quantitative analysis of host cell proteins is made difficult by the large dynamic range of analytes, especially compared to the biotherapeutic.
- Here we use high speed, narrow window data-independent acquisition (DIA) on a Thermo Orbitrap Astral mass spectrometer to profile HCPs with greater sensitivity, higher throughput, and reduced co-fragmentation of interfering precursor ions.
- By applying HCP analyses earlier in the drug development pipeline, we aim to better understand HCP persistence/clearance and to mitigate risks for downstream purification.

Cell Engineering and Analytical Sciences

Cell Engineering and Analytical Sciences (CEAS) sits at the entry point for biotherapeutics entering the manufacturing pipeline from lead engineering.

- Diverse portfolio of mAbs, multi-specifics, and ADCs

Current Sample Workflow

Figure 1: Native digest is used to minimize interference from the biotherapeutic while still digesting the HCPs
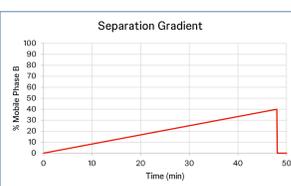
Mobile Phase A: 0.1% FA in H_2O
Mobile Phase B: 0.1% FA in ACN
Flow Rate: 0.3 mL/min

Analytical flow RPLC coupled to Thermo Orbitrap Astral Mass Spectrometer

Proteome Discoverer with Chimerys for improved DIA searching

Orbitrap Astral HCP Optimization

Figure 2: Number of unfiltered identifications from different isolation windows. Increasing isolation window width allows more scans and PSMs, but those PSMs are less confident and require more validation.
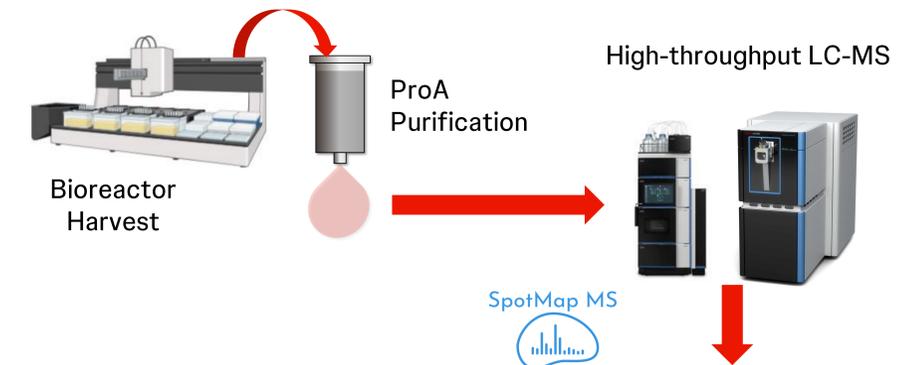

Figure 3: MS/MS data from different isolation windows. Increasing isolation window allows more scans, but loses confidence in IDs due to co-isolation.

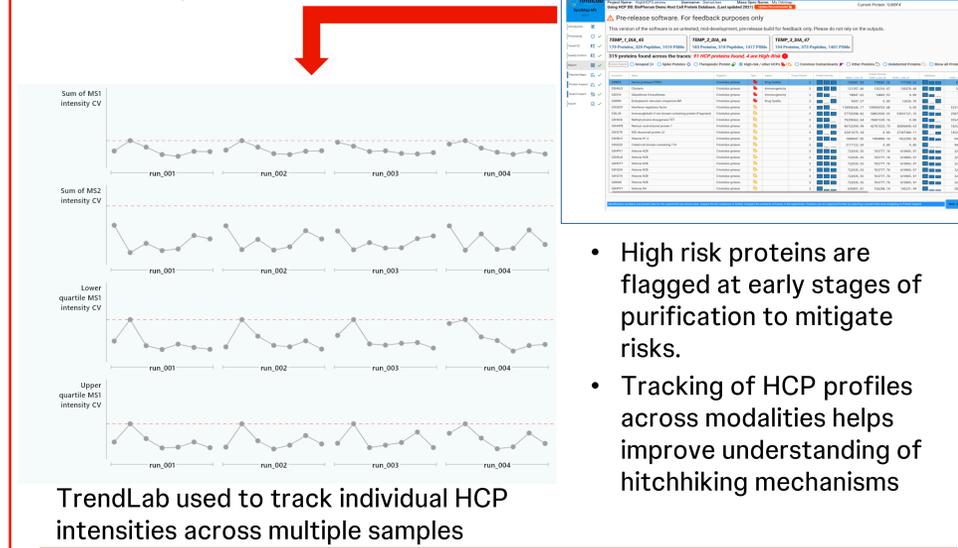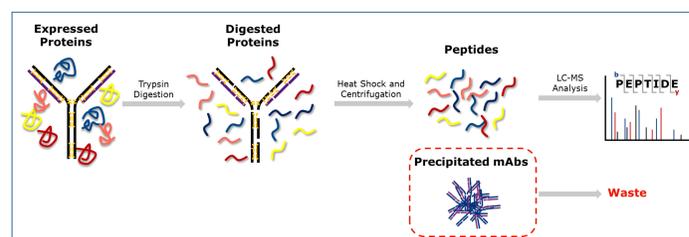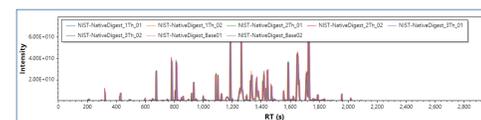**Table 1:** Finalized Parameters for Astral MS

RF Lens (%)	40
AGC Target (%)	500
MS1 Detector	Orbitrap (240k)
MS1 Scan Range	350-1600
Precursor Mass Range	350-1200
MS2 Detector	Astral
MS2 Scan Range	100-1600
HCD Collision Energy (%)	25
Isolation Window	2 Th

- Host cell proteins were analyzed in NIST 8671 reference material.
- Decreasing the scan range and isolation window had the greatest impact on the number of useful spectra collected.
- Methods were optimized to improve **high-quality** identifications.

SpotMap MS Software

SpotMap MS from TotalLab

- Quickly visualize TIC from multiple samples to ensure data reproducibility.
- Automatically flag high risk proteins based on published data.
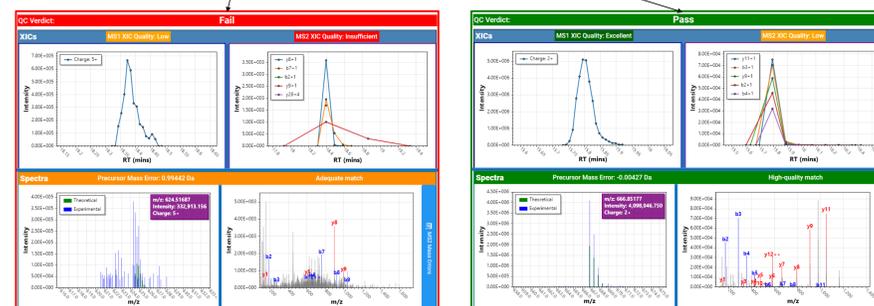
- Designed to rapidly validate identified peptides
- Manual Pass/Fail system based on XIC quality, mass error, and MS/MS fidelity.

Implementation of Routine HCP Analysis

High-throughput LC-MS

ProA Purification

Bioreactor Harvest

SpotMap MS

DIA data is analyzed, and high-risk proteins are reported

Longitudinal Study of HCP Profiles

TrendLab used to track individual HCP intensities across multiple samples

- High risk proteins are flagged at early stages of purification to mitigate risks.
- Tracking of HCP profiles across modalities helps improve understanding of hitchhiking mechanisms

Conclusions

✓ Native digest followed by LC-MS analysis using high-resolution Orbitrap Astral allows for highly sensitive HCP analyses.
✓ Methods were optimized to improve the number of useful spectra collected and subsequent HCPs identified.
✓ SpotMap MS is used for data processing as it is fast and gives the user more control in evaluating potential peptide identifications.
✓ Future goals are to scale down to nano-flow to improve sensitivity and generate HCP profiles during all cell selection campaigns.

References

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2. Damoc E, Tabiwang NA, Pashkova A, Denisov E, Scheffler K, Srzentic K. Increasing sensitivity of high throughput host cell protein analysis on a novel high-resolution accurate mass platform.
3. Kiyonami R, Melani R, Chen Y, Leon AD, Du M. Applying UHPLC-HRAM MS/MS Method to Assess Host Cell Protein Clearance during the Purification Process Development of Therapeutic mAbs. International Journal of Molecular Sciences. 2024; 25(17):9687. https://doi.org/10.3390/ijms25179687

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