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Unravel Glycoprotein Complexity Under Native Condition Using Proton Transfer Charge Reduction and Direct Mass Technology mode

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ABSTRACT

Purpose: Glycoprotein characterization using native mass spectrometry remains challenging as its heterogeneity leading to complex spectra. This study leverages proton transfer charge reduction (PTCR) and Direct Mass Technology mode (charge detection mass spectrometry, CDMS) on Orbitrap platforms to unravel glycoprotein complexity under native condition.

Methods:

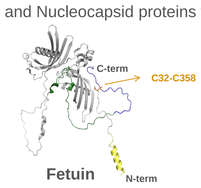
1. The complex glycoproteins were quadrupole-isolated in narrow windows to minimize interference from various glycoform signals.
2. Subsequently, the isolated ion packets were charge reduced in the ion trap and scanned in the Orbitrap.
3. Data independent acquisition-PTCR (DIA-PTCR) streamlined the stepped isolation window through a wide m/z range followed by PTCR, facilitating the capture of comprehensive MW profiles.

Results: Five-Th isolation of Fetuin followed by PTCR could reduce the charge +12 to +4. Moreover, we successfully separated two distinct charge envelopes originating from precursors, each representing unique PTMs. Stepping through m/z 3300-4500 with 10 Th isolation prior to PTCR unveiled numerous glycoforms from 38 kDa to 46 kDa. The ultra-high resolution at 480K enables the isotope resolution of each glycoform, resulting in unambiguous monoisotopic MW. A full MS scan of 49 kDa nucleocapsid protein displayed partially resolved peaks atop the elevated baseline. Employing the DIA-PTCR across the entire m/z range could not only resolve the charge states of 49 kDa species decorated with various covalent or noncovalent interactions, but also identify their dimers near 100 kDa. Surprisingly, we also observed a cluster of peaks ~70 kDa. These findings are consistent with complementary Direct Mass Technology analyses.

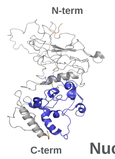
MATERIALS AND METHODS

Sample Preparation

Human Fetuin and Nucleocapsid proteins were buffer exchanged into ammonium acetate.



Fetuin



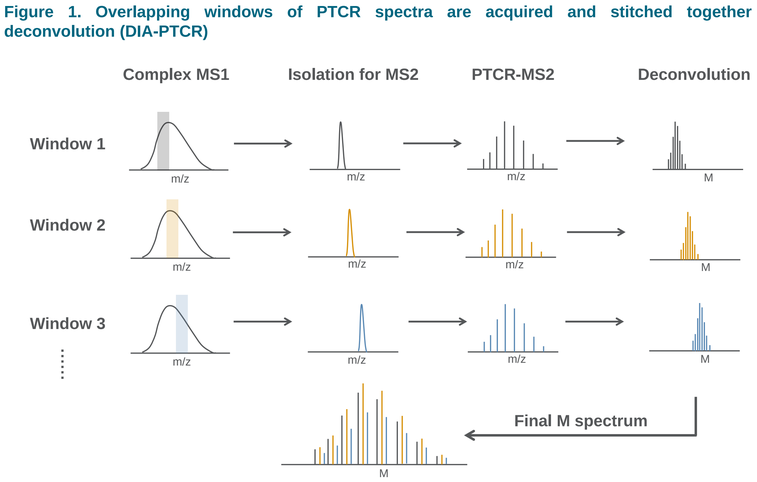
Nucleocapsid protein

Test Methods

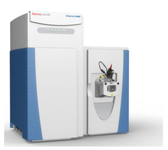
1. PTCR analyses were performed on Thermo Scientific™ Orbitrap Eclipse™ & Ascend™

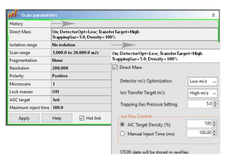
- Native MS
- Native top-down
- PTCR
- ETD
- UVPD

Figure 1. Overlapping windows of PTCR spectra are acquired and stitched together for deconvolution (DIA-PTCR)



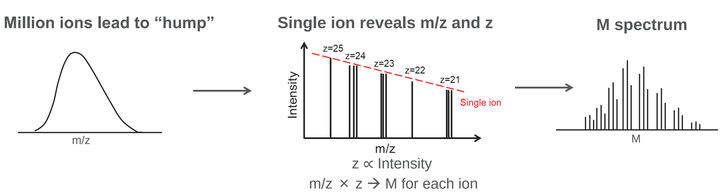
2. Direct Mass Technology mode analyses were performed on Thermo Scientific™ Q Exactive™ UHMR.





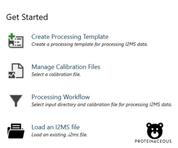
- Native MS
- Native top-down
- Direct Mass Technology

Figure 2. Direct Mass Technology mode analysis revealing m/z and z simultaneously



Data Analysis

Data were analyzed using Thermo Scientific™ BioPharma Finder™ 5.0 and STORIboard (Proteinaceous).



RESULTS

Data Independent Acquisition - Proton Transfer Charge Reduction

Challenges:

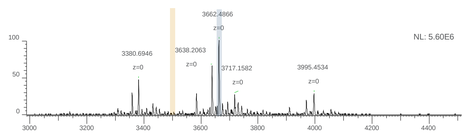
Unresolved charge states due to heterogeneity

DIA-PTCR:

Automated sliding window of gas phase charge reduction separates species overlapped in m/z

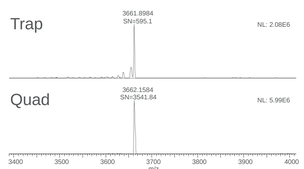
1. Ion trap vs. Quadrupole isolation followed by PTCR

➢ **MS1 scan of Fetuin**

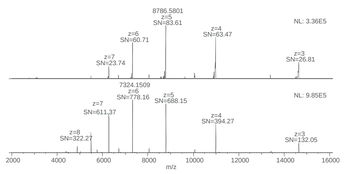


➢ **Trap vs. Quad isolation of m/z 3662 - Better S/N**

MS2 isolation



MS2 PTCR

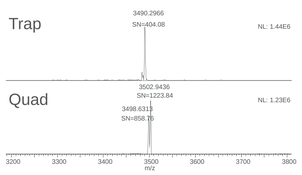


✓ Better S/N ratio using quad isolation

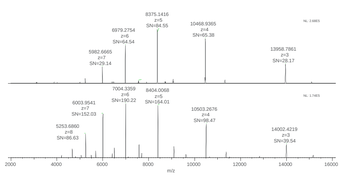
✓ Better S/N ratio post PTCR using quad isolation

➢ **Trap vs. Quad isolation of m/z 3500 - Better isolation accuracy**

MS2 isolation

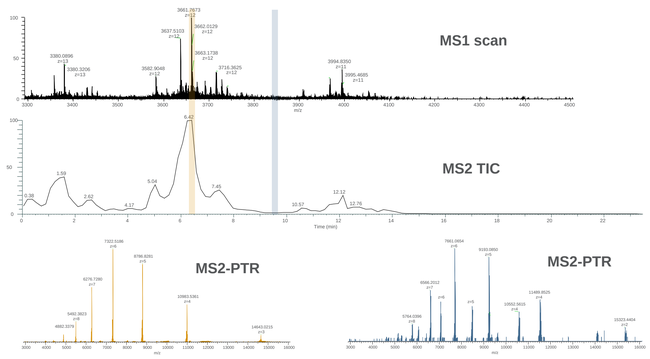


MS2 PTCR



✓ More accurate isolation using quad isolation

2. DIA-PTR of m/z 3300-4500 with 20 Th quad isolation



✓ The ultra-high resolution at 480K enables the isotope resolution of each glycoform

✓ Separate overlapped signals in MS1

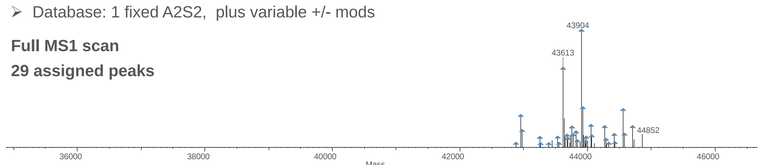
✓ Unravel meaningful information from nearly signal baseline

3. Fetuin glycoform assignment from MS1 vs. DIA-PTR in BPF

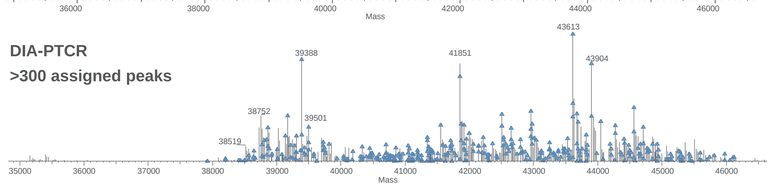
➢ Assigned proteoforms via BPF with 20 ppm sequence matching, 2 min detected intervals

➢ Database: 1 fixed A2S2, plus variable +/- mods

Full MS1 scan
29 assigned peaks

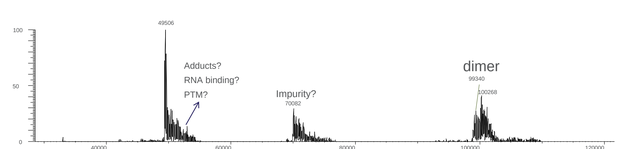


DIA-PTCR
>300 assigned peaks

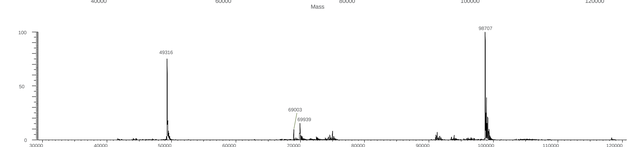


4. Application of DIA-PTCR on Nucleocapsid protein

SID100



SID120



✓ DIA-PTCR discloses Nc protein decorated with either covalent modification or noncovalent interaction

✓ Mild and strong desolvation condition reveals different extent of noncovalent and covalent interactions

Direct Mass Technology mode

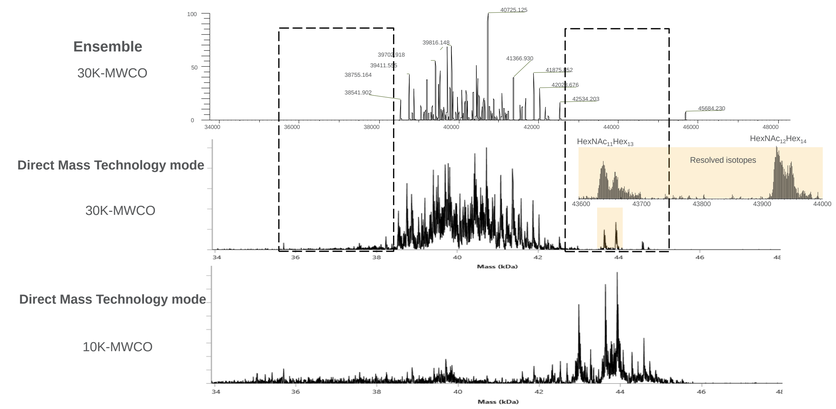
Challenges:

Heterogeneity causes loss of charge state resolution – particularly for large or heavily PTM molecules

Direct Mass Technology mode:

m/z and z are simultaneously measured for single ion

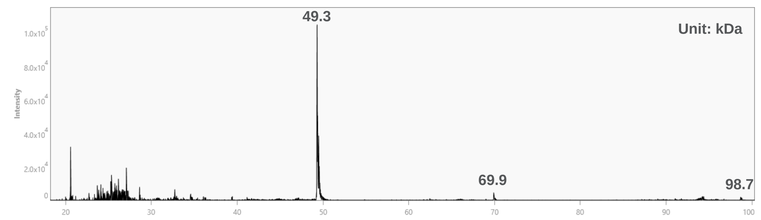
1. Comparison of ensemble measurement and Direct Mass Technology analysis of Fetuin



✓ Fetuin cleaned by 30K-MWCO indicate main glycoforms distributing from 38.5 kDa to 42.5 kDa

✓ Results demonstrate exceptional sensitivity and dynamic range of Direct Mass Technology mode

2. Direct Mass Technology analysis of Nucleocapsid protein

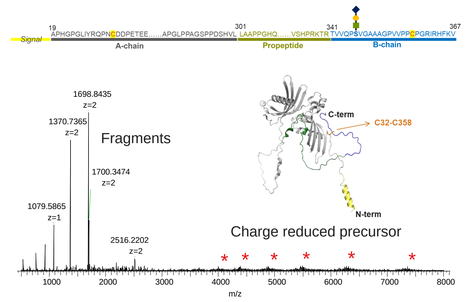


✓ DMT analysis shows monomer, dimer and impurities, resonating with PTCR results

Native top-down for structure analysis

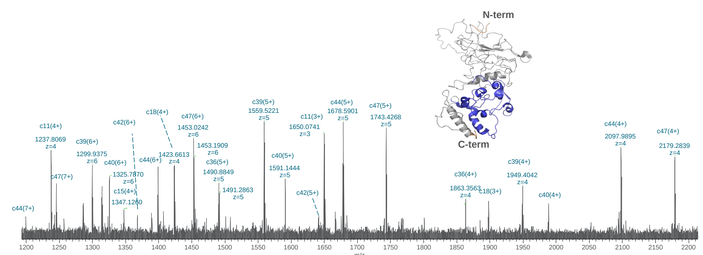
➢ Native top-down analyses using Hybrid fragmentation confirm sequence, resolve chain connection, identify glycosylation site, and providing structure information

1. EChcD spectrum and sequence map to resolve chain connection of Fetuin



✓ Although N terminal of B-chain is connected to the C-terminus of A-chain through a propeptide, EThcD fragments demonstrate the B-chain is disulfide bonded to the N-terminal of A-chain through cys32-cys358

2. EChcD spectrum to identify dimerization interface of Nucleocapsid protein



✓ ETD fragments are c-ions predominately reflecting N-terminus is exposed

✓ It correlates with the structure of exposed N-terminus and dimerized C-terminus

CONCLUSIONS

✓ High resolution/accurate mass DIA combined with PTCR empowers the elucidation of multiple proteoforms of both fetuin and Nucleocapsid proteins, while also revealing sample contaminants.

✓ Direct Mass Technology mode analysis works as complementary technique for resolving complex biomolecules

✓ Native top-down analysis provides structure information such as chain linkage and interaction interface.

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