MS in Structural biology - Native MS

Glycoprotein characterization combining native Mass Spectrometry and Direct Mass Technology

Weijing Liu, Ping Yip, Christopher Mullen, Claire Dauly, Rosa Viner
Thermo Fisher Scientific, San Jose, CA, United States, 95134

Abstract

Purpose:

Using proton transfer charge reduction, hybrid fragmentation, and Direct Mass Technology to unravel glycoprotein complexity

Methods:

1. Proton transfer charge reduction (PTCR)
2. Electron transfer dissociation supplemented with HCD
3. Direct Mass Technology mode

Results:

Complementary mass spectrometry analyses including Native, PTCR, Direct Mass Technology mode, and EThcD could comprehensively characterize heavily glycosylated proteins.

Introduction

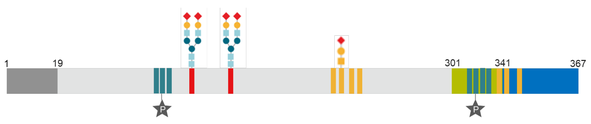
Glycoproteins are actively involved in physiological functions as well as certain diseases. However, glycoprotein characterization using native and native top-down mass spectrometry remains challenging as its heterogeneity leading to complex spectrum.

In this study, we evaluated a few novel techniques including Direct Mass Technology mode and proton transfer charge reduction (PTCR) to unravel proteoforms of Human Fetuin A, a heavily glycosylated and phosphorylated protein, comprehensively under native condition. We further expand the application to heavily glycosylated Spike protein variants for determining their MW and oligomeric states.

Materials and methods

Sample preparation

- Human Fetuin A from human plasma was purchased from Sigma-Aldrich. Fetuin was buffer exchanged into ammonium acetate with Amicon 10K-MWCO or 30K-MWCO.



Average MW 37343.23 (w/o signal peptide and PTMs)

Signal Peptide: AA1-18

A-chain: AA19-300

Propeptide: AA301-340

B-chain: AA341-367

N-glycosylation: N156, and N176

Phosphorylation: S134, S135, S138, T319, S325, S328, S330

O-glycosylation: S256, T270, S280, S293, T339, T341, S346

Cys-Cys: 12 disulfide bonded cysteines

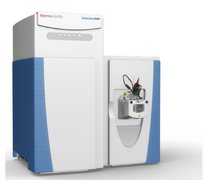
R340 can be missing

- Spike protein samples expressed in HEK 293 cells were provided by Dr. Ganesh Anand, Penn State University.

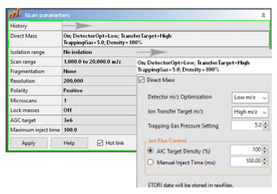
Test methods

Native MS and Direct Mass Technology mode were performed on Thermo Scientific™ Q Exactive™ UHMR mass spectrometer. PTCR and EThcD analyses were performed on Thermo Scientific™ Orbitrap Eclipse™ mass spectrometer.

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

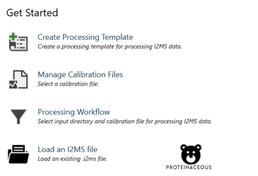


- Native MS
- Native top-down
- Direct Mass Technology



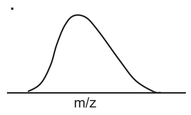
Data Analysis

Data were analyzed using Thermo Scientific™ BioPharma Finder™ 4.1 and STORIboard (Proteinaceous) software.



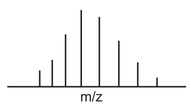
Results

Proton transfer charge reduction



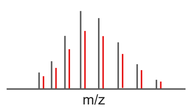
Denatured MS

- Unresolved charge states due to heterogeneity
- Unable to obtain MW without knowing charge states



Native MS

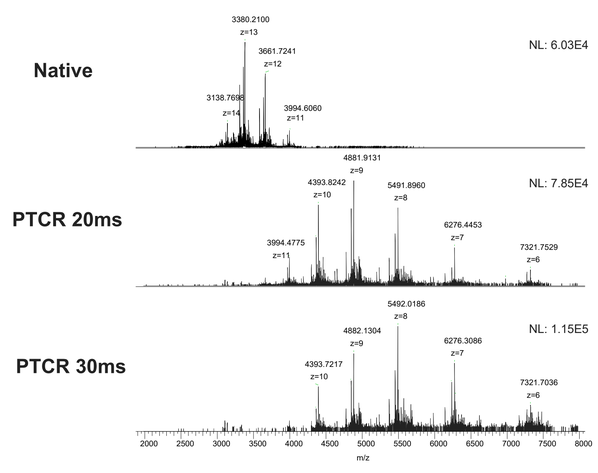
- Native MS provides relatively low charge states
- Wider spacing between adjacent z may decipher charge states



PTCR

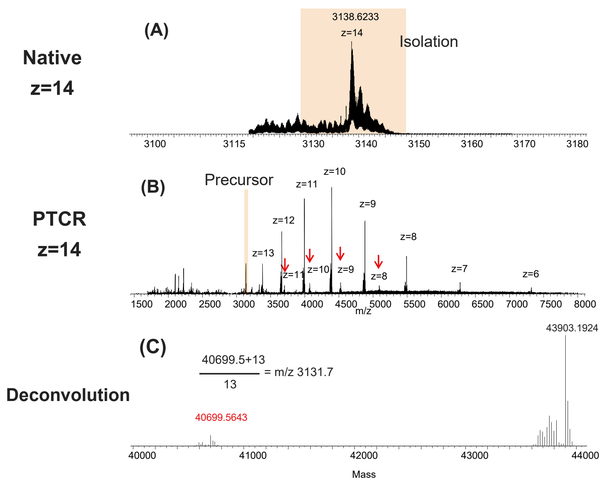
- Gas phase charge reduction by PTCR may separate species overlapped in m/z better
- Increase the number of identified species

Figure 1. Comparison of native MS spectrum and PTCR MS spectra at 20 and 30 ms reaction time.



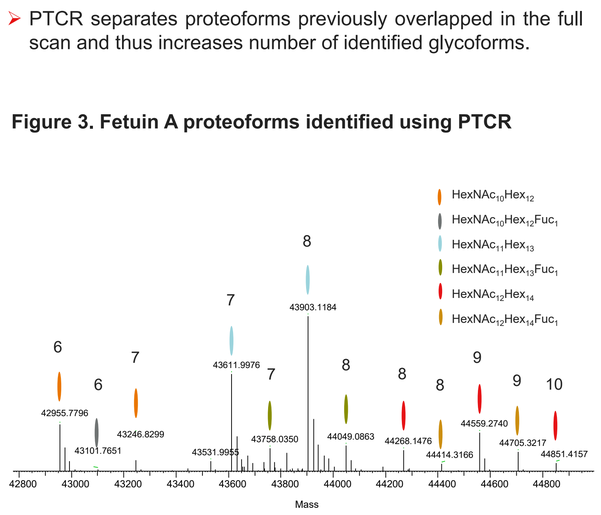
- PTCR[1] pushes the charge envelope to higher m/z and lower charge states via charge reduction
- Spectra reflects the sample complexity

Figure 2. (A) Zoom-in of z=14+ in native MS spectrum; (B) PTCR MS spectrum with 20 Th isolation width on z=14+; (C) Deconvoluted spectrum from PTCR scan.



- PTCR separates proteoforms previously overlapped in the full scan and thus increases number of identified glycoforms.

Figure 3. Fetuin A proteoforms identified using PTCR



*All proteoforms contain 1 phosphorylation and the number of sialic acids as labelled[2]

- Accurate monoisotopic mass unambiguously deciphers different numbers of phosphorylation, HexNAc, Hex, and Fucose on each glycoform.

Direct Mass Technology

- **Conventional MS:**

Charge must be deduced from spacing between adjacent peaks of charge envelope or isotopic peaks

- **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

- Frequency provides m/z
- Amplitude provides charge

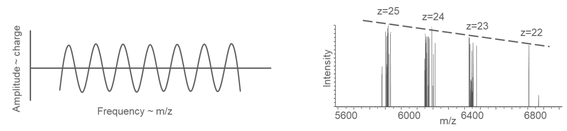
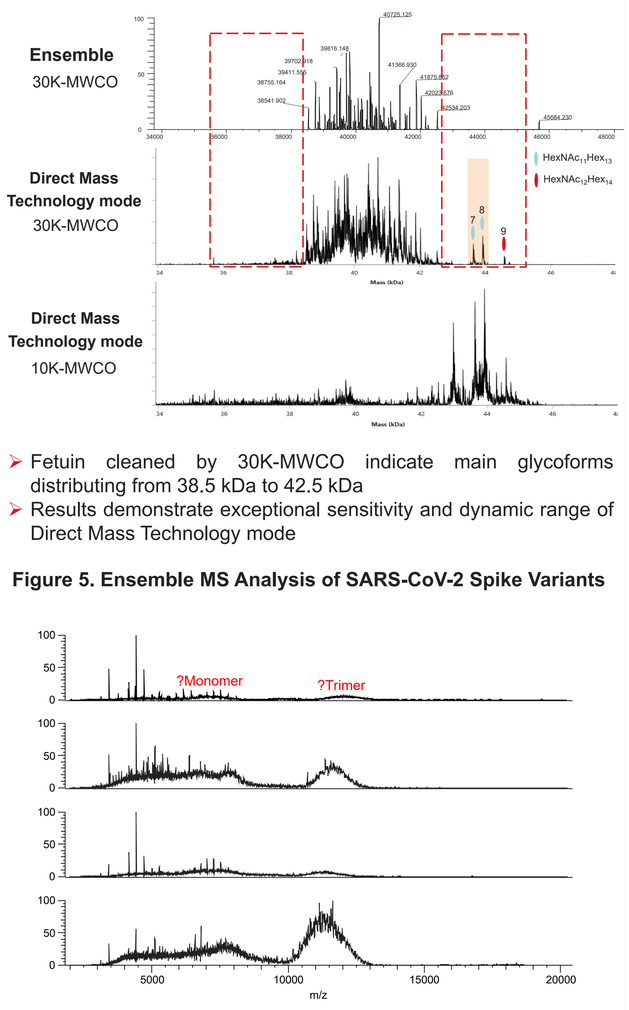
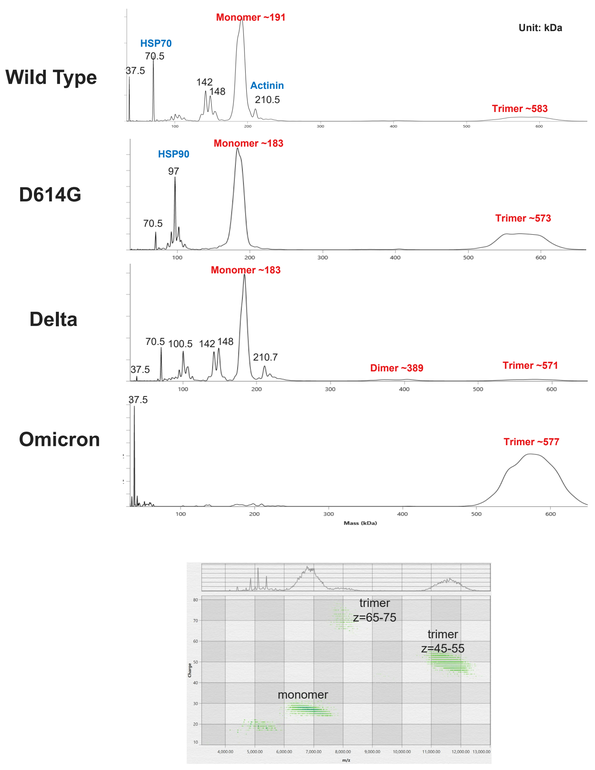


Figure 4. Comparison between spectra from ensemble measurement and Direct Mass Technology mode



- 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

Figure 5. Ensemble MS Analysis of SARS-CoV-2 Spike Variants



- Ensemble measurement shows hump above and below m/z 10,000 potentially indicating trimer monomer respectively

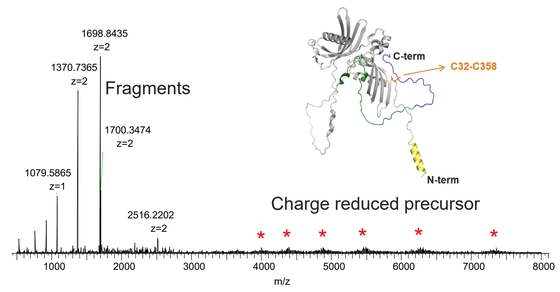
Figure 6. Direct Mass Technology mode Analysis of SARS-CoV-2 Spike Variants

- Direct Mass Technology mode provides the charge states and calculation of the molecular weight profile of each variant
- Multiple forms of HSP70, 90 and cell matrix proteins were detected
- Multiple confirmations of Spike trimers were observed

Native top-down

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

Figure 7. EChcD spectrum and sequence map to resolve chain connection of Fetuin A

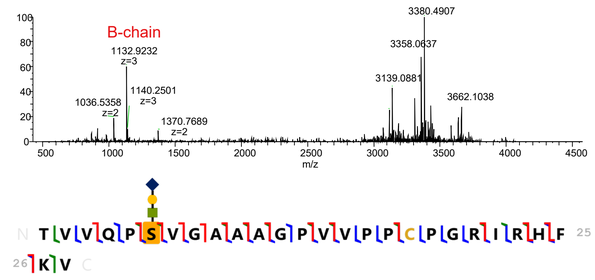


TVVQPSVGAAAGPVVPPCPGRIRHFKV

N	A	P	H	G	P	G	L	I	Y	R	Q	P	N	C	D	D	P	E	T	E	E	A	L	V	25
26	A	I	D	Y	I	N	Q	N	L	P	W	G	Y	K	H	T	L	N	Q	I	D	E	V	K	V	50
51	W	P	Q	Q	P	S	G	E	L	F	E	I	E	I	D	T	L	E	T	T	C	H	V	L	D	75
76	P	T	P	V	A	R	C	S	V	R	Q	L	K	E	H	A	V	E	G	D	C	D	F	Q	L	100
101	L	K	L	D	G	K	F	S	V	V	Y	A	K	C	D	S	S	P	D	S	A	E	D	V	R	125
126	K	V	C	Q	D	C	P	L	L	A	P	L	N	D	T	R	V	V	H	A	A	K	A	A	L	150
151	A	A	F	N	A	Q	N	N	G	S	N	F	Q	L	E	E	I	S	R	A	Q	L	V	P	L	175
176	P	P	S	T	Y	V	E	F	T	V	S	G	T	D	C	V	A	K	E	A	T	E	A	A	K	200
201	C	N	L	L	A	E	K	Q	Y	G	F	C	K	A	T	L	S	E	K	L	G	G	A	E	V	225
226	A	V	T	C	M	V	F	Q	T	Q	P	V	S	S	Q	P	Q	P	E	G	L	A	N	E	A	V	250
251	P	T	P	V	V	D	P	D	A	P	P	S	P	P	L	G	A	P	G	L	P	P	A	G	S	275
276	P	P	D	L	S	H	V	L	L	A	A	P	P	G	H	Q	L	H	L	R	A	H	Y	D	L	R	H	300
301	T	L	F	M	G	V	V	L	S	L	G	S	P	S	G	E	V	L	S	H	P	L	R	K	T	L	R	C

- 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

Figure 8. UVPD spectrum and fragments map for Fetuin B-chain sequencing and glycosylation site confirmation



N T V V Q P S V G A A A G P V V P P C P G R I R H F K V C

25 26

- UVPD top-down analysis could provide 95% sequence and identify O-glycosylated site at Ser346.

Conclusions

- By combining different MS methods such as I2MS, PTCR and EThcD, we could successfully characterize heavily glycosylated Fetuin.
- Native MS combined with PTCR could resolve proteoforms overlapping in m/z to identify more proteoforms.
- Top-down analyses using hybrid fragmentation comfirmed the sequence and PTM site.
- Direct Mass Technology not only isotopically resolved numerous proteoforms of Fetuin but also determine charge states, thus MW of Spike proteins

References

1. Huguet R et al, Anal.Chem.2019,15732-15739
2. Lin, Yu-Hsien, Vojtech Franc, and Albert JR Heck. Journal of proteome research 17.8 (2018): 2861-2869

Acknowledgements

1. Christopher Mullen, Mike Senko, Kristina Srzentić, Daniel Hermanson, Kyle Fort, Florian Grosse-Goosmann from Thermo Fisher Scientific

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Jared Kafader, Prof. Neil Kelleher from Northwestern University

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