

# Glycoprotein characterization using infusion chip technology combined with FT-ICR mass spectrometry and ECD/IRMPD fragmentation

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## Overview

**Potential and limitations of glycoprotein analysis based on MS infusion experiments alone, using TriVersa™-NanoMate chip technology coupled to LTQ-FT™ ICR MS.**

## Introduction

- Post translational modifications (PTMs) affect protein folding, function and localization
- Glycosylation is the most common PTM
- Glycoprotein analysis is a major analytical challenge usually requiring a combination of different MS technologies (LC-ESI-MS, MALDI-MS etc.)
- MCMV-m153 is a challenging example of glycoprotein analysis due to its high degree of glycosylation

## Materials and Methods

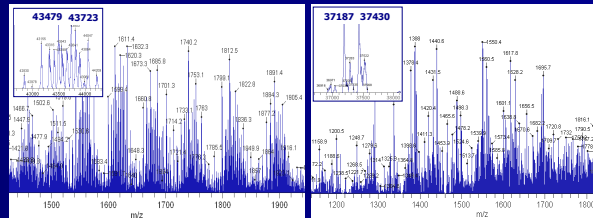
Histidine tagged MCMV-m153 was expressed in stable *Drosophila* S2 transfectants, purified with nickel-affinity, size-exclusion and anion-exchange chromatography consecutively and finally stored in PBS at -20 °C (c = 1 µg/µL). For MS infusion experiments, samples were further diluted with water, heated to 65 °C and desalted using Centri-Sep™ desalting spin columns (Princeton Separation, NJ) or ZipTip™ (Millipore, MA). After enzymatic or chemical treatment with Trypsin, PNGaseF, TCEP or DTT the sample was diluted in spraying buffer (Water/ACN/Isopropanol/Formic Acid = 60:20:20:0.2) and electro-sprayed using the TriVersa™-NanoMate chip technology (Advion Biosystems, NY) coupled to an LTQ-FT™ ICR mass spectrometer (Thermo Electron, Germany). 8 µL solution (= 1-5 pmol/µL) were sufficient for ca. 45 min analysis time (1.5 kV, 0.3 bar pressure assistance). MS and MS<sup>n</sup> data were recorded applying CID (35 % energy), ECD (5 x e<sup>3</sup> energy, 10 ms delay, 70 ms duration) or IRMPD (90 %, 10 ms delay, 90 ms duration) fragmentation of indicative precursor ions. All spectra recorded were the average of three FT-MS<sup>n</sup> scans (max. ion injection time 3000 ms, 5 Da window for precursor ion selection, resolution 100,000).

## MCMV-m153

1	EVVRPEV	GTVDI	QGPM	ELIFSVSR	30
31	SGATGER	IVTL	SIVSME	NGKPGTYEW	60
61	SFPANES	WPE	IQQLQNEF	VSRYADVWQ	90
91	TPGELVVEYR	CPVPQ	FNCIT	THRWKSETIM	120
121	SFDGAIQTI	SVTSEY	TTKIN	EDTLVWYRIG	150
151	LVNILLTDNA	KSIEH	WTEI	CKKLDADRP	180
181	DDNQYLTLEDD	ILED	DIEMDI	VQIQMTTQVP	210
211	LKYHMTVWSA	GRDS	RAALS	ADYYTDEIVA	240
241	SYLPVMEFQI	LNTT	CEITSS	SGWTVRLIFS	270
271	EEMVAASKAR	QAQK	RPLLPV	EPHGFMSDEH	300
301	GPAPVQR	ITIN	DSRL	TLVPRG	SHHHHHH

**Figure 1:** MCMV-m153 amino acid sequence (M<sub>r</sub>(av)=37,187.8)  
red: potential N-linked glycosylation sites  
green: potential cysteine binding sites  
[:]: theoretical tryptic cleavage sites

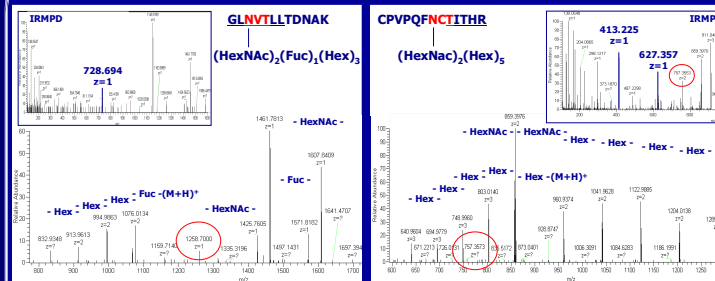
## Top-Down Protein Analysis



**Figure 2:** FT-ICR-MS spectrum of untreated (left) and PNGaseF treated (right) MCMV-m153 (ProMass software, Novatia, Princeton, NJ); inserts show spectrum deconvolution.

MCMV-m153 shows a complex set of signals around 43.5 kDa; proposed protein was detected after PNGaseF treatment, additional protein is caused by expression vector (N-terminal addition of RS)

## Glycopeptide characterization using IRMPD and CID fragmentation

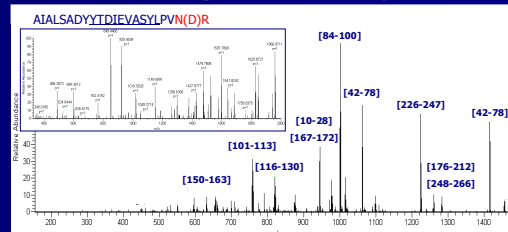


**Figure 3:** FT-ICR-MS<sup>n</sup> analysis of glycopeptides. Hex: Hexoses (Fru, Gal, Glc or Man); HexNAc: N-acetyl-hexosamines (GalNAc or GlcNAc); Fuc: Deoxyhexoses (Fuc or Rha); AA sequence tags detected with IRMPD are marked by underscores

Left: MS<sup>2</sup>-CID fragment spectra of tryptic peptide m/z 1149.084 (z=2); insert shows the IRMPD fragment spectrum  
Right: MS<sup>2</sup>-CID fragment spectra of tryptic peptide m/z 910.717 (z=3); insert shows the IRMPD fragment spectrum

CID and IRMPD fragmentation gives complementary information that together allows amino acid and glycan analysis.

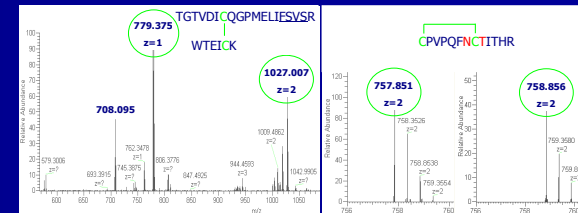
## AA sequence coverage



**Figure 4:** FT-ICR-MS survey scan after PNGaseF and Trypsin treatment (insert shows MS<sup>2</sup>-CID fragmentation spectrum of m/z 1223.119 (z=2))

FT-ICR-MS<sup>n</sup> peptide sequencing leads to an AA sequence coverage of 97 % (based on FT-MS data only) and 78 % (based on FT-MS<sup>2</sup>-CID data)

## Cys-Cys bond analysis using ECD fragmentation and/or reduction mass shifts



**Figure 5:** Left shows FT-ICR-MS<sup>n</sup> ECD-fragment spectrum of the tryptic peptide m/z 708.097 (z=4; AA sequence tag marked by underscore) and right shows FT-ICR-MS spectra of a peptide before and after DTT addition to the spray solution (Δ m/z 1.005 (z=2))

ECD can reduce C-C bonds and is an alternative to classical reduction mass shift experiments with TCEP or DTT

## Conclusions

MS infusion experiments reveal valuable information for glycoprotein characterization when using electrospray chip technology combined with accurate mass spectrometry capable of diverse fragmentation techniques.

Top-Down protein mass measurement:

M<sub>r</sub>(av) glycosylated protein: complex mixture at 43.5 kDa

M<sub>r</sub>(av) de-glycosylated protein: 37,187 and 37,430 Da

Cys-Cys binding sites: [16]-[171]; [101]-[108]; [203]-[255]

AA sequence coverage: 97 % (FT-MS); 78 % (FT MS<sup>2</sup>-CID)

N-linked glycosylation sites:

[8] NRT not occupied

[65] NES occupied (HexNAc)<sub>2</sub>(Fuc)<sub>1</sub>(Hex)<sub>3</sub>

[107] NCT occupied (HexNAc)<sub>2</sub>(Hex)<sub>1-7</sub>

[152] NVT occupied (HexNAc)<sub>2</sub>(Fuc)<sub>0,1</sub>(Hex)<sub>3-5</sub>

[246] NRS occupied not detected

[252] NTT occupied (HexNAc)<sub>2</sub>(Hex)<sub>3</sub>

[310] NDS occupied (HexNAc)<sub>2</sub>(Fuc)<sub>1</sub>(Hex)<sub>3</sub>

## Acknowledgements

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