Supporting information

Title of manuscript: Site-specific glycoprofiling of *N*-linked glycopeptides using MALDI-TOF MS: strong correlation between signal strength and glycoform quantities

Authors: Morten Thaysen-Andersen, Simon Mysling and Peter Højrup.

Affiliation: Department of Biochemistry and Molecular Biology, University of Southern Denmark, Campusvej 55, Odense M, DK-5230, Denmark

Contents

Legends for supporting figures

Figure S1

A) Relative abundances of RNase B glycoforms determined using different ionization techniques (MALDI-TOF MS and ESI-LTQ MS) of various products (free glycans, glycopeptides, and intact protein) (see insert for color coding). Data from five replicates were averaged and standard deviations are shown. These profiles were all recorded in positive ionization mode. The data were compared to a reference profile (black outline). For simplicity no standard deviations are shown for the reference profile. Examples of glycoprofiling of intact RNase B using **B)** ESI-LTQ MS and **C)** MALDI-TOF MS are presented.

Figure S2

GELoader tip micro-columns and the standard curve used to determine the chromatographic bead volume as a function of column length. **A)** HILIC (left) and graphite (right) micro-column in Eppendorf GELoader Tips. HILIC columns were constricted 1 mm from the tip to retain the column material. For graphite columns, a C_{18} plug was used as an alternative to constricting the tip to allow uniform packing of the larger and heterogeneous-sized graphite particles. **B)** Standard curve relating the length of a micro-column to the volume, when measured 1 mm from the tip. For graphite column volume determination, the volume from the plug to the tip was determined and subtracted from total graphite column volume. Standard deviations were determined from n=5 measurements of column volume using water.

Figure S1

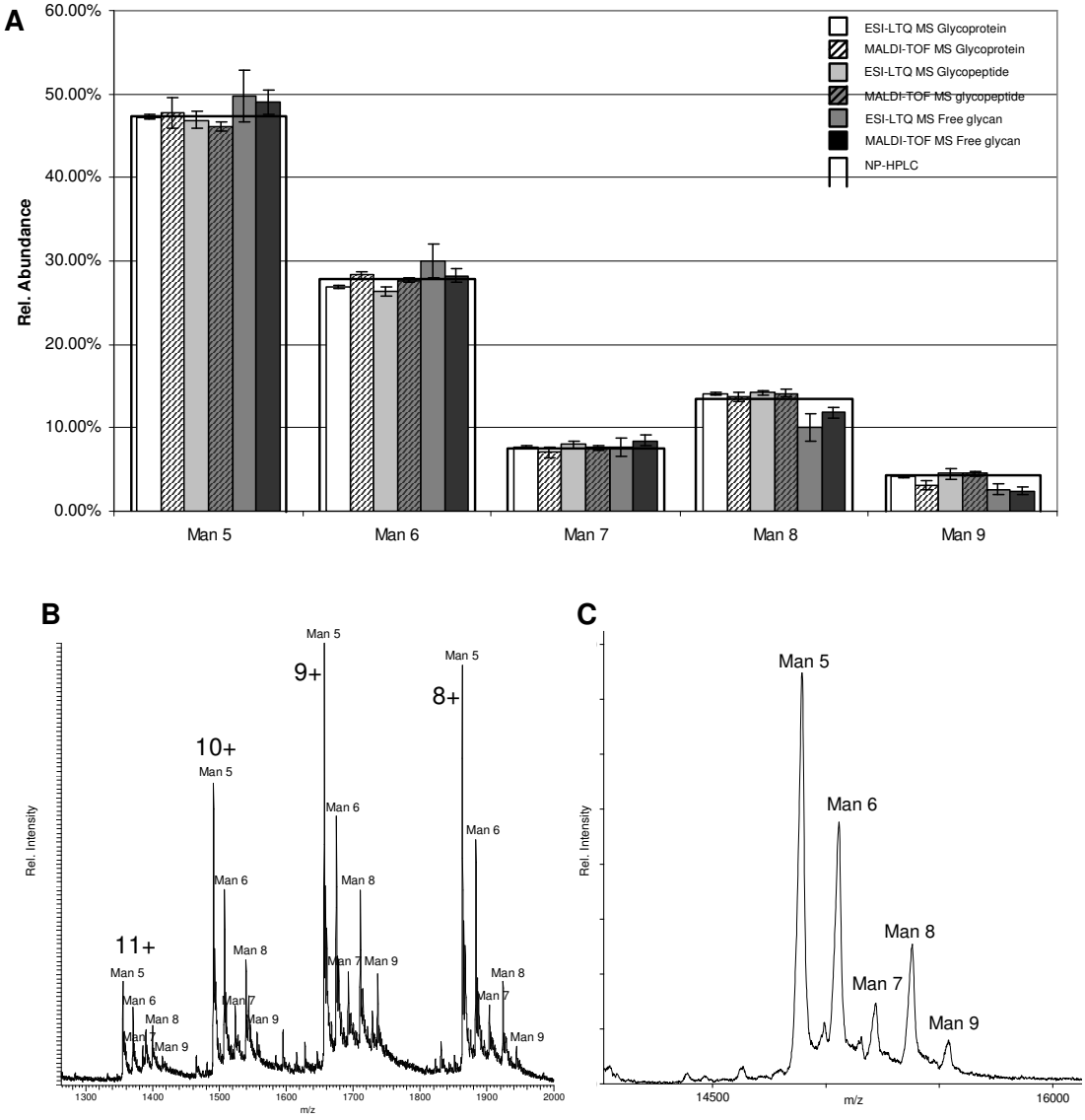
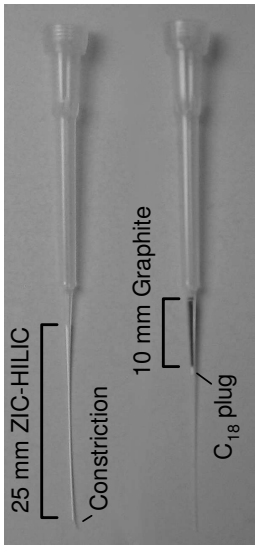

A
60.00%
50.00%
40.00%
30.00%
20.00%
10.00%
0.00%
Rel. Abundance
ESI-LTQ MS Glycoprotein
MALDI-TOF MS Glycoprotein
ESI-LTQ MS Glycopeptide
MALDI-TOF MS glycopeptide
ESI-LTQ MS Free glycan
MALDI-TOF MS Free glycan
NP-HPLC
Man 5
Man 6
Man 7
Man 8
Man 9
B
Rel. Intensity
11+
10+
9+
8+
Man 5
Man 6
Man 7
Man 8
Man 9
1300
1400
1500
1600
1700
1800
1900
2000
m/z
C
Rel. Intensity
Man 5
Man 6
Man 8
Man 7
Man 9
14500
16000
m/z

Figure S2

A. Micro-columns



B. Standard curve (column length vs volume)