

1 **Supporting Information**

2 **On-plate Glycoproteins/Glycopeptides Selective Enrichment and Purification Based on**
3 **Surface Pattern for Direct MALDI MS Analysis**

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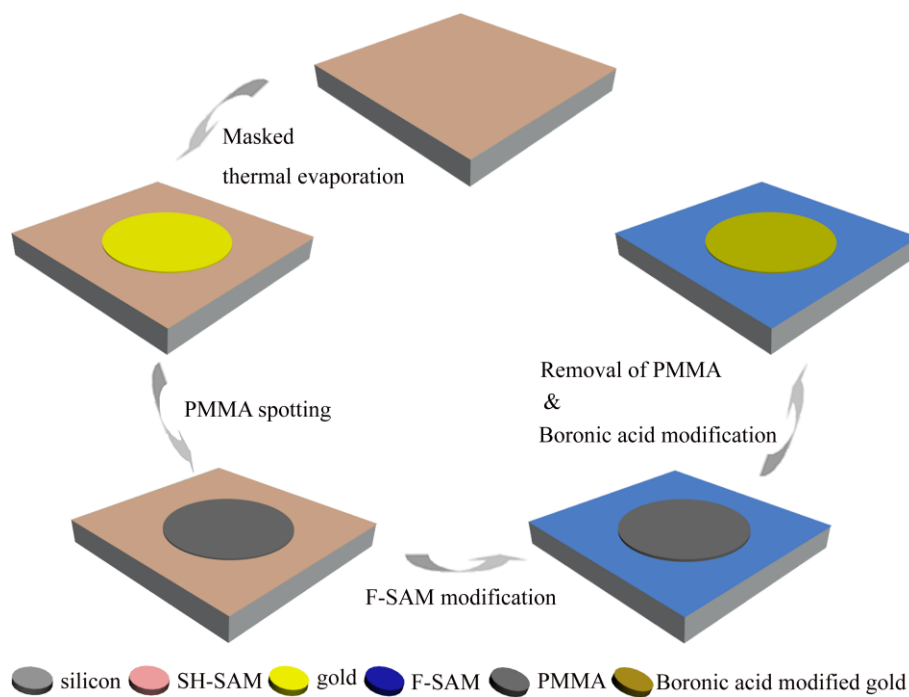
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Figure S1. Schematic illustration for the preparation of the boronic acid-modified gold microspot.

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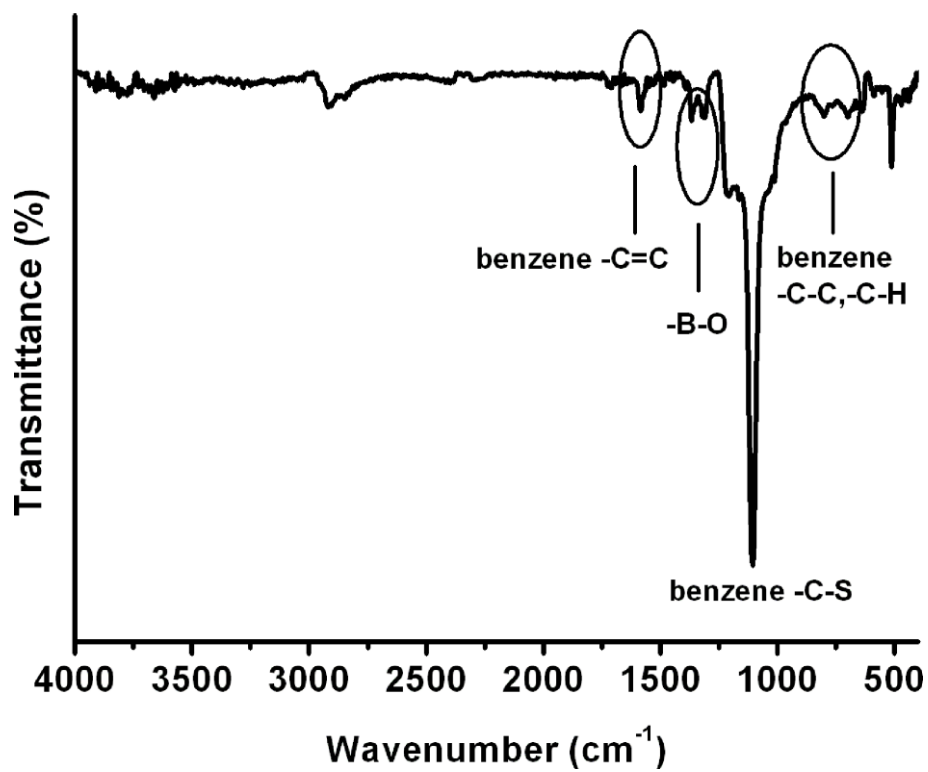
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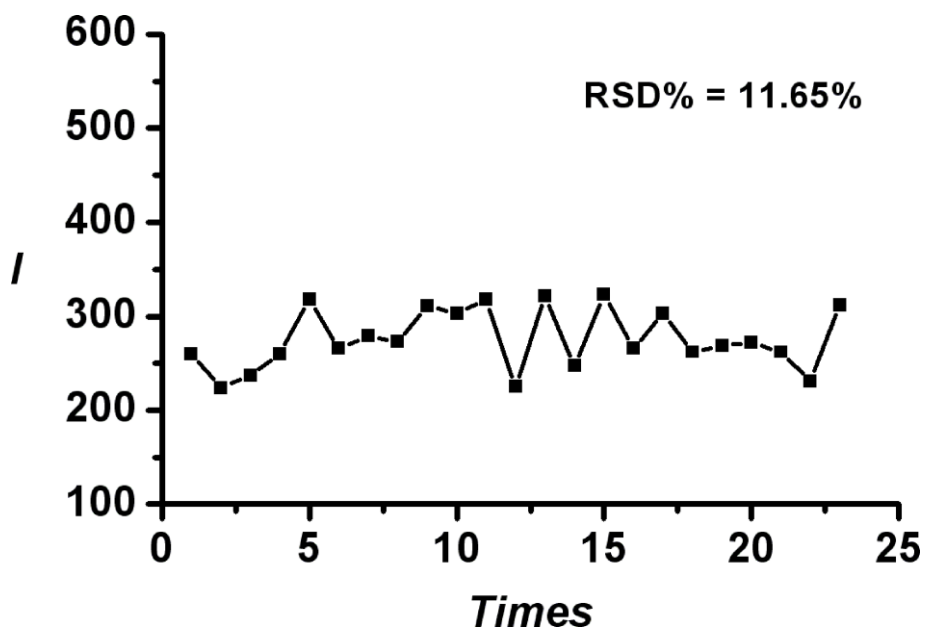
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Figure S2. FT-IR spectrum of 4-mercaptophenylboronic acid modified gold.



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2 **Figure S3.** The spot-to-spot reproducibility of HRP protein ($50 \text{ fmol } \mu\text{L}^{-1}$) with the surface patterned

3 sample support.

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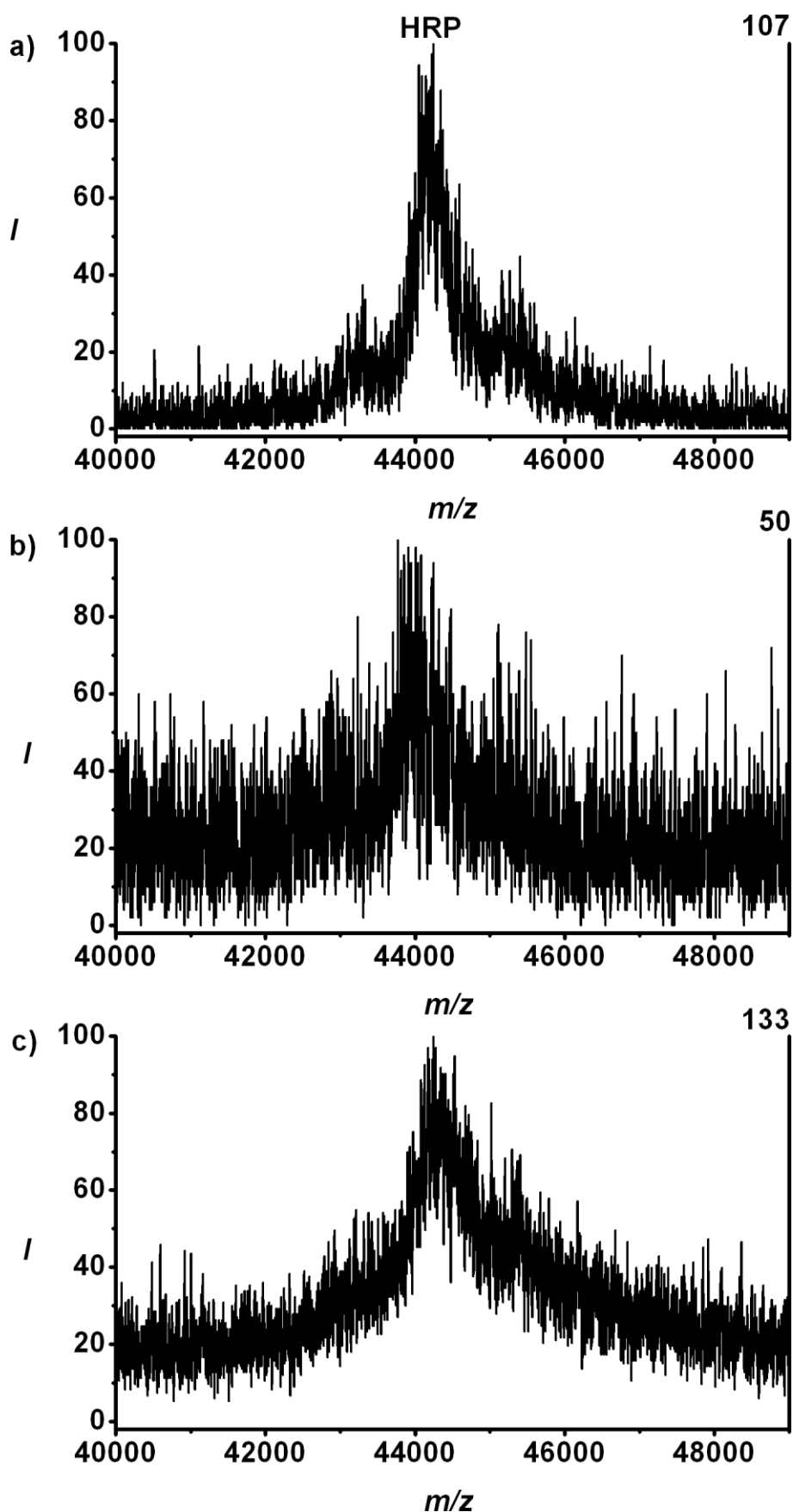
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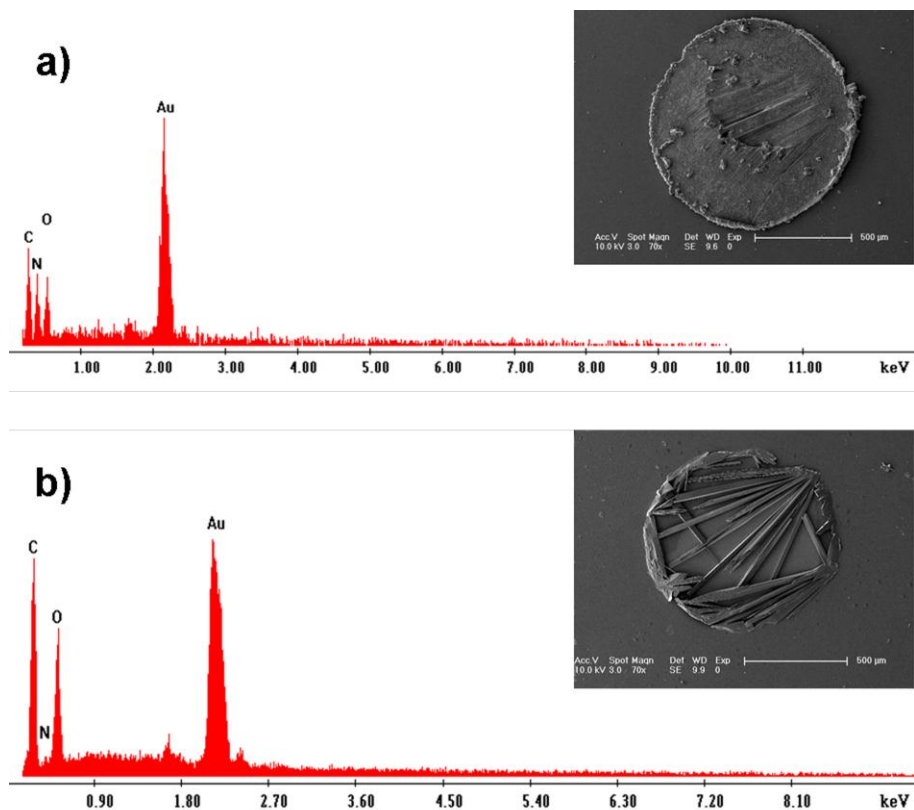
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2 **Figure S4.** The limit of detection of HRP protein: a) 50 $\text{fmol } \mu\text{L}^{-1}$, with the stainless steel MALDI

3 plate; b) 25 $\text{fmol } \mu\text{L}^{-1}$, with the phenylboronic acid monolayers; c) 5 $\text{fmol } \mu\text{L}^{-1}$, with the surface

4 patterned sample support.

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4 **Figure S5.** EDX/SEM images of salt-containing sample (urea 1M) prepared on the surface patterned
5 sample support: a) the salts-containing sample is dried in air; b) the salts are removed and
6 analyte-matrix co-crystallizations are formed. Table S-1: Glycopeptides detected in the HRP protein
7 digests solution by using the surface patterned sample support and the stainless steel MALDI plate.

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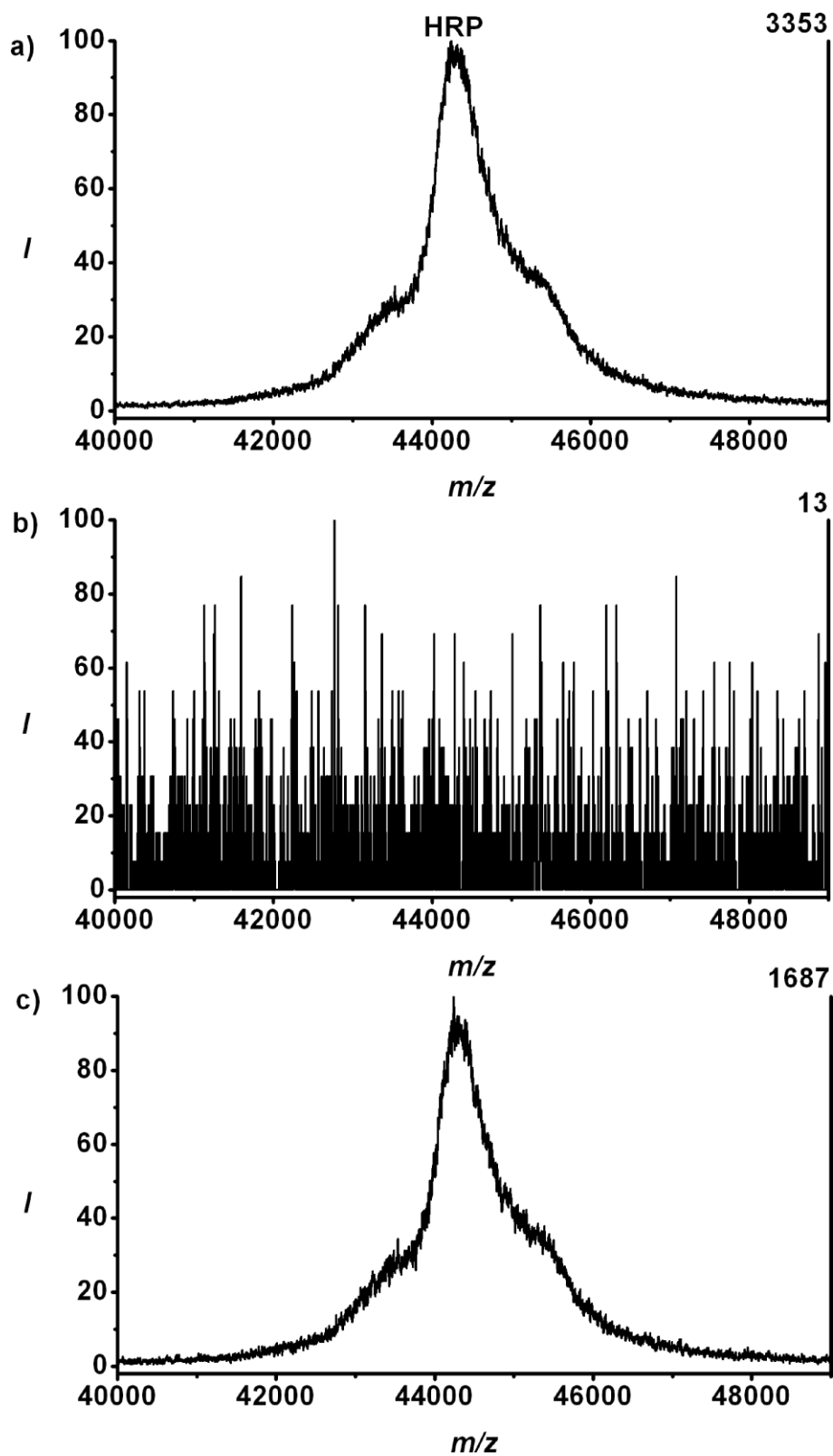
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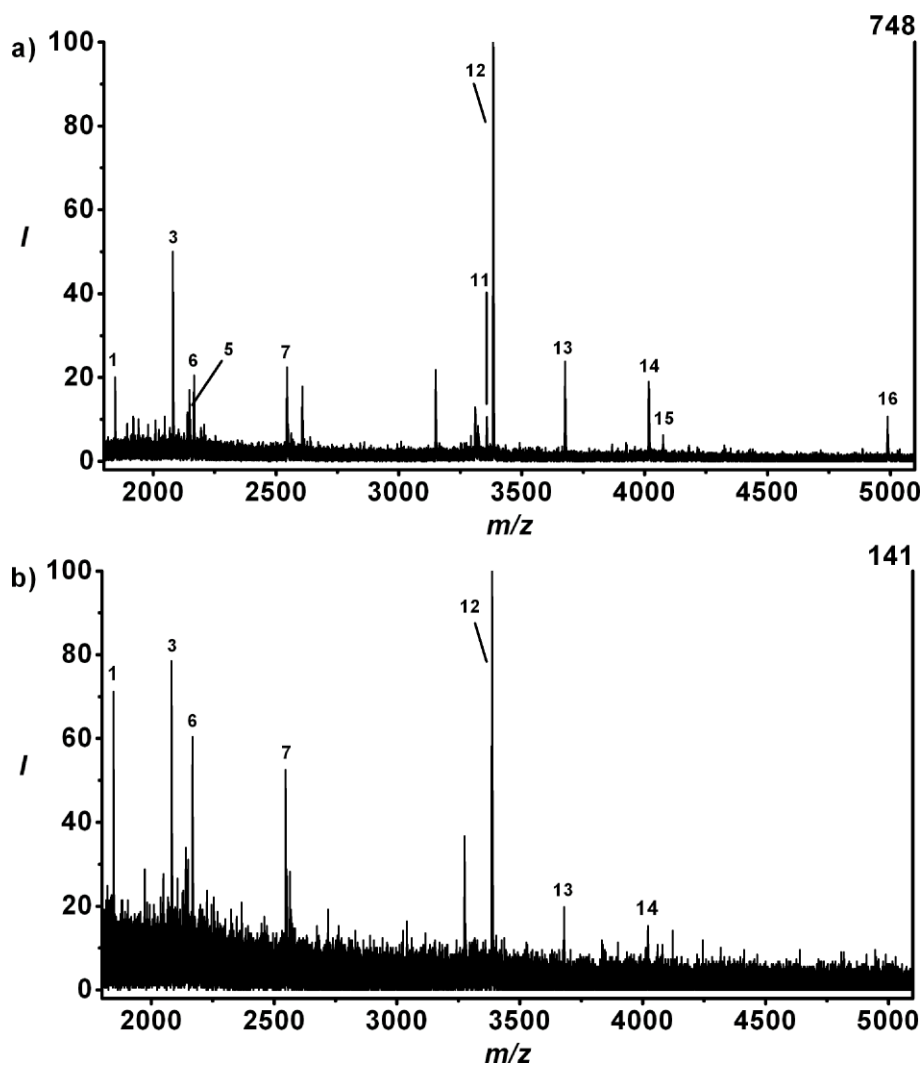
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2 **Figure S6.** Mass spectra for the reuse of the boronic acid-modified gold microspots: a) first use; b)
3 regeneration with 5 % TFA and 20 % ACN after first use; c) second use. The sample enrichment
4 steps are carried out using HRP at the concentration of 500 fmol μL^{-1} .

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2 **Figure S7.** Mass spectra of HRP digests ($230 \text{ fmol } \mu\text{L}^{-1}$) and BSA digests mixture after enrichment

3 and desalting on the surface patterned sample support: a) at the mol ratio of 1:1, b) at the mol ratio of

4 1:10.

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1 **Table S1.** Glycopeptides detected in the HRP protein digest solution by using the stainless steel
 2 MALDI plate, the phenylboronic acid monolayers and the surface patterned sample support.

| Peak number | Observed m/z | Glycan composition | Amino acid sequence ^[a] | A | B | C |
|-------------|--------------|--|------------------------------------|---|---|---|
| 1 | 1845 | Man ₃ GlcNAc ₂ FucXyl | NVGLN#R | | + | |
| 2 | 2068 | Man ₃ GlcNAc ₂ FucXyl | PN#VSNIVR | | + | |
| 3 | 2081 | Man ₃ GlcNAc ₂ | MGN#ITPLTGTQG | + | + | + |
| 4 | 2139 | Man ₂ GlcNAc ₂ FucXyl | LYN#FSN#TGLP | | + | + |
| 5 | 2149 | Man ₃ GlcNAc ₂ Xyl | LYN#FSN#TGLP | | + | + |
| 6 | 2164 | Man ₃ GlcNAc ₂ Fuc | LYN#FSN#TGLP | | + | + |
| 7 | 2543 | Man ₃ GlcNAc ₂ FucXyl | SSPN#ATDTIPLVR | | + | |
| 8 | 2612 | Man ₃ GlcNAc ₂ Xyl | MGN#ITPLTGTQGQIR | | + | |
| 9 | 3275 | Man ₃ GlcNAc ₂ FucXyl | SC(AAVESACPR)PN#VSNIVR | | + | + |
| 10 | 3323 | Man ₃ GlcNAc ₂ FucXyl | QLTPTFYDN SCPN#VSNIVR | | + | + |
| 11 | 3355 | Man ₃ GlcNAc ₂ FucXyl | SFAN#STQTFNFV EAMDR | | + | + |
| 12 | 3378 | Man ₂ GlcNAc ₂ Fuc | GLIQSDQELFSSPN#ATDTIPLVR | + | + | + |
| 13 | 3673 | Man ₃ GlcNAc ₂ FucXyl | GLIQSDQELFSSPN#ATDTIPLVR | + | + | + |
| 14 | 4018 | Man ₃ GlcNAc ₂ FucXyl GlcNAc | LYN#FSNTGLPDPTLN#TTYLQTLR | | + | + |
| 15 | 4076 | Man ₃ GlcNAc ₂ Xyl | QLTPTFYDN SC(AAVESACPR)PN#VSNIVR | | + | + |
| 16 | 4985 | Man ₃ GlcNAc ₂ FucXyl Man ₃ GlcNAc ₂ FucXyl | LYN#FSNTGLPDPTLN#TTYLQTLR | + | + | + |

3 [a] The N-glycosylation sites are marked with N#. GlcNAc = N-acetylglucosamine, Fuc = fructose,
 4 Man = mannose, Xyl = xylose. 'A' represents the peptides identified with the stainless steel MALDI
 5 plate; 'B' represents the peptides identified with the surface patterned sample support, 'C' represents
 6 the peptides identified with the phenylboronic acid monolayers. '+' represents positive identification.

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