Supplementary Figures 1~5

Glycoproteomics: the Identification of Different Glycosylation of the Prostate Specific Antigen at Normal and High Isoelectric Point by LC-MS/MS

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Keywords: Prostate specific antigen, PSA, N-linked glycosylation, glycopeptide, glycoproteomics, LC-MS/MS.

Supplementary Figure 1. Manual annotations of CID MS/MS of 38 glycopeptides from PSA or PSAH.

HexNAc2Hex3dHex1

PSAH_300ng_5B_masslists_071312_01 #584 RT: 17.99 AV: 1 NL: 5.83E3 F: ITMS + c NSI d Full ms2 680.01@cid35.00 [175.00-2000.00]



1

HexNAc2Hex6s/p1

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 PSAH_300ng_5B_masslists_071312_01 #721
 RT: 19.32
 AV: 1
 NL: 3.70E3

 T: ITMS + c NSI d Full ms2
 820.72@cid35.00 [215.00-2000.00]
 +3, 82b 0306



HexNAc3Hex4NeuAc1

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HexNAc3Hex4dHex1NeuAc1

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HexNAc3Hex7NeuAc1

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PSAH_300ng_5B_masslists_071312_01 #646 RT: 18.70 AV: 1 NL: 1.29E3 T: ITMS + c NSI d Full ms2 1013.26@cid35.00 [265.00-2000.00]





HexNAc4Hex3dHex1NeuAc1



PSA_2ug_5B_063012_01 #1048 RT: 19.74 AV: 1 NL: 3.21E4 T: ITMS + c NSI d Full ms2 912.76@cid35.00 [240.00-2000.00] +3, 912, 4279, 2134.262.







HexNAc4Hex4NeuAc1

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HexNAc4Hex4dHex1

PSAH_300ng_5B_masslists_071312_01 #552 RT: 17.63 AV: 1 NL: 5.19E4 T: ITMS + c NSI d Full ms2 869.41@cid35.00 [225.00-2000.00]



HexNAc4Hex4dHex1NeuAc1







HexNAc4Hex5dHex1



5. TO.



2000

HexNAc4Hex5NeuAc1



HexNAc4Hex5NeuAc2





HexNAc4Hex5dHex1NeuAc1

10 5

366.20

400

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, ope

922

41500

1048.22

1000

+2

800

776.54

657.24

(602.3)1

600

528.03

1200

1847.99

1400

1525.96

1600

1711.64

1877.01

1800

2000

1303.98

HexNAc4Hex5dHex2NeuAc1

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PSAH_300ng_5B_masslists_071312_01 #693 RT: 19.12 AV: 1 NL: 7.32E3 T: ITMS + c NSI d Full ms2 1069.15@cid35.00 [280.00-2000.00]



HexNAc4Hex5dHex1NeuAc2

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HexNAc4Hex6dHex1NeuAc1

PSAH_300ng_5B_masslists_071312_01 #663 RT: 18.91 AV: 1 NL: 8.58E3 T: ITMS + c NSI d Full ms2 1074.48@cid35.00 [285.00-2000.00]



9+2

HexNAc4Hex6dHex2NeuAc1



HexNAc5Hex3dHex1NeuAc1

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HexNAc5Hex4dHex1

PSAH_300ng_5B_masslists_071312_01 #535 RT: 17.46 AV: 1 NL: 5.35E4 T: ITMS + c NSI d Full ms2 937.10@cid35.00 [245.00-2000.00]



12

4

HexNAc5Hex4dHex1s/p1

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HexNAc5Hex4dHex1NeuAc1

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.

+2

1121.4529

1065.8358

985.4290

1000

بهنه مؤذيميا بالبونية بالمربع

800

+2

PCP 1275.4535 \$-

16

1200

m/z

DEP

1522.6531 Pep

1585.8938

1600

1859.3726

2000

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1800

0880

-1040

per

BM

1400

1304

494.4244

400

يعالم الجذب واجرالهم وبالز فترباخ فمج ترغ التنافي وتبطر عامد وتلاس

600

80

70-

0-

PSAH_300ng_5B_masslists_071312_01*#*962 RT: 21.47 AV: 1 NL: 2.08E5 F: ITMS + c NSI d Full ms2 1131.14@cid35.00 [300.00-2000.00]

HexNAc5Hex5NeuAc1

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HexNAc5Hex5dHex1NeuAc1

912

HexNAc5Hex5NeuAc2

6/30/201 24:11 PM

HexNAc5Hex6dHex1NeuAc2

PSAH_300ng_masslists_071312_01 #916 RT: 21.08 AV: 1 NL: 5.42E3 T: ITMS + c NStu Full ms2 1239.20@cid35.00 [330.00-2000.00]

m/z

HexNAc6Hex3NeuAc1

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psa 2ug 5b 063012 01 #1010 RT: 19.55 AV: 1 NL: 4.77E4 T: ITMS + c NSI d Full ms2 999.47@cid35.00 [265.00-2000.00]

2000

HexNAc5Hex6dHex1NeuAc1

HexNAc6Hex7dHex1NeuAc1

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Supplementary Figure 2. HCD MS/MS of Sulfated/phosphorylated (*s/p*) Glycopeptide HexNAc5Hex4dHex1*s/p*1. No diagnostic ions of sulfated/phosphorylated glycan residue was observed.

Supplementary Figure 3. Comparison of identified glycoforms between PSA and PSA at High pl

Supplementary Figure 4A. Peak areas of glycoforms associated with 3 peptide backbones identified from PSA at normal pl.

Supplementary Figure 4B. Peak areas of glycoforms associated with 3 peptide backbones identified from PSA at high pl.

Supplementary Figure 5A. Comparisons of total identified glycoforms with the 2012 ABRF study and the published study by Behnken *et al.*

Supplementary Figure 5B. Comparisons of quantitative values of 18 major/intermediate glycoforms identified from the 2012 ABRF study and this study.

