Glycan Analysis of Prostate Specific Antigen (PSA) Directly from the Intact Glycoprotein by HR-ESI/TOF-MS

*Henning N. Behnken, Alexandra Ruthenbeck, Jan-Mirco Schulz and Bernd Meyer**

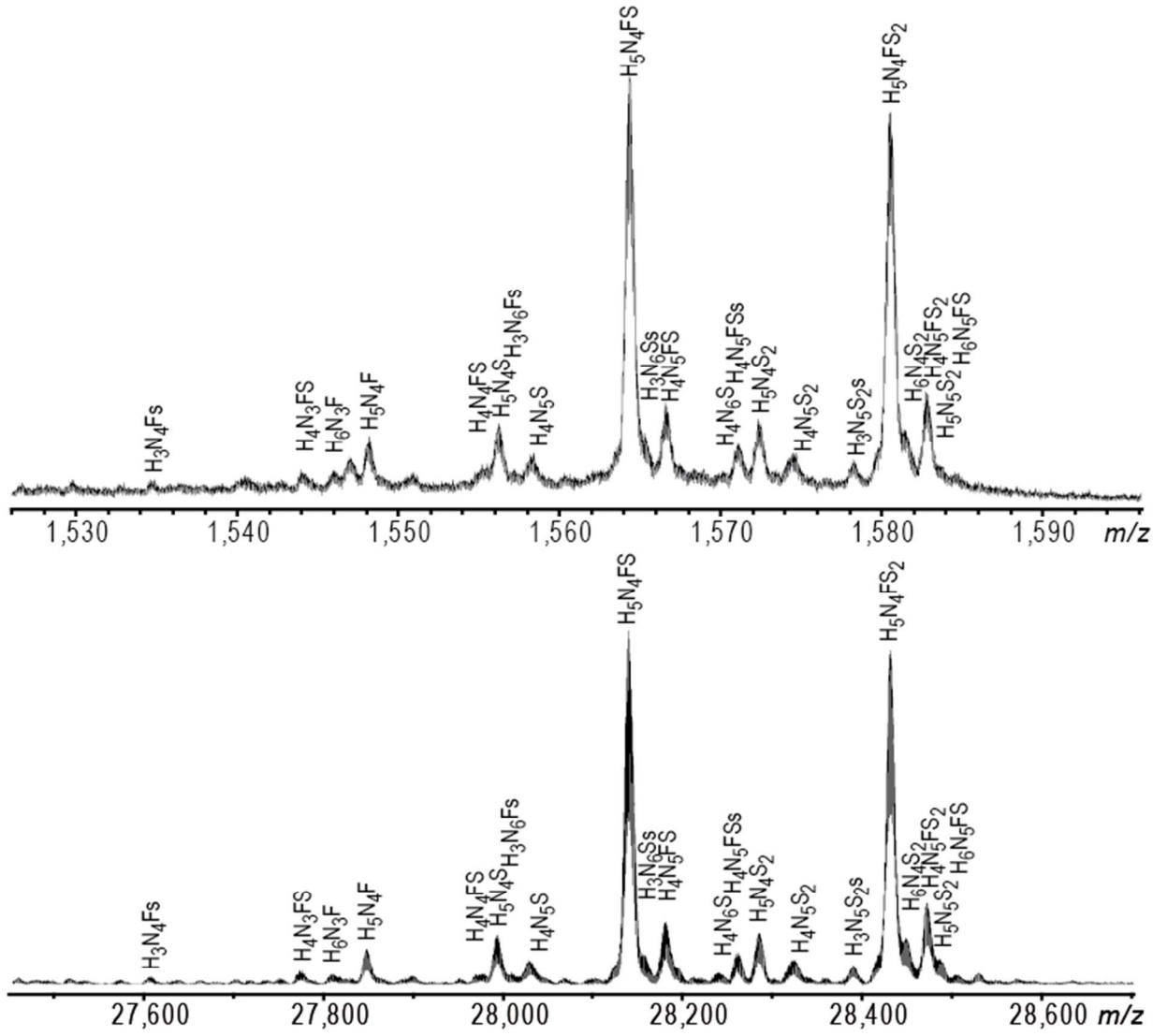

Figure S1. MS spectra of PSA-2 (18.9-19.7 min). Shown are an extension of the MS spectrum at the position of the 18+ charged glycoforms (Top) of PSA-2 and the deconvolved spectrum (Bottom). The M+18H+ spectrum and the deconvolved spectrum are annotated with glycan structures that exemplify the determined glycan composition.

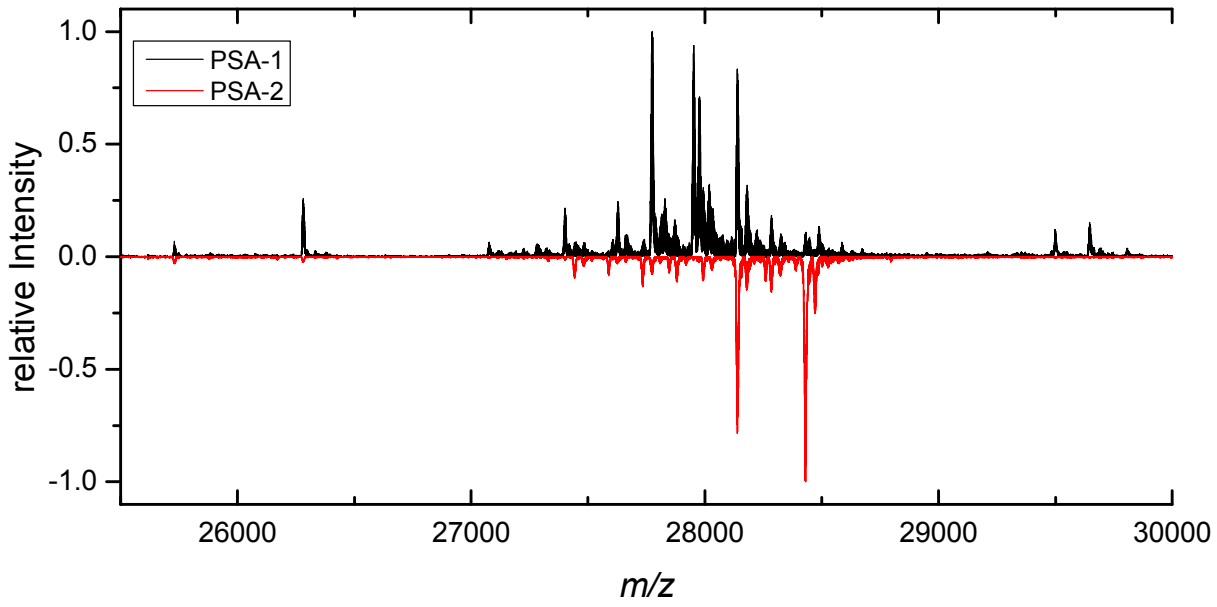

Figure S2. Deconvolved spectra of two PSA samples. PSA-1 is shown at the top and PSA-2 at the bottom with an inversed intensity. Striking differences in the glycosylation patterns exist. Analyzing the intact protein spectra of the samples allows a fast determination of the differences. In order to optimize quantification the spectra were baseline corrected. The ions in the high mass range of the spectrum of PSA-1 at *m/z* 29481.362 and *m/z* 29628.385 correspond to the *m/z* of a PSA SNP (Asp78Asn; dbSNP: 61752561)[1] carrying a second glycosylation site that exhibits dominantly glycans of the high mannose type (H_5N_2 and H_6N_2). The glycoforms on the *N*-glycosylation site at Asn^{45} are $H_5N_4FS_2$ and $H_5N_4FS_2$. The SNP is not present in high pI PSA-2. (Kristina Neue et. al., Bruker Application Note LCMS-76).

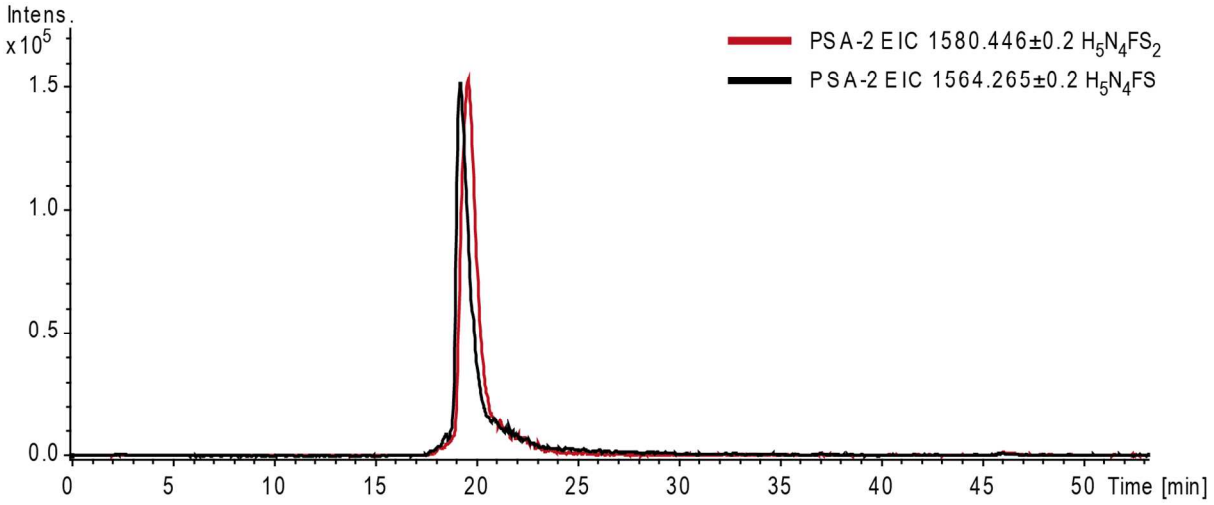

Figure S3. EICs of the chromatographic profiles of intact PSA glycoforms on a C8-column in a 60 min LC run. The PSA glycoform at *m/z* 1564.32 (complex type glycan, composition H_5N_4FS) elutes at 19.2 min and the glycoforms at *m/z* 1553.92 (complex type glycan, composition $H_5N_4FS_2$) at 19.6 min.

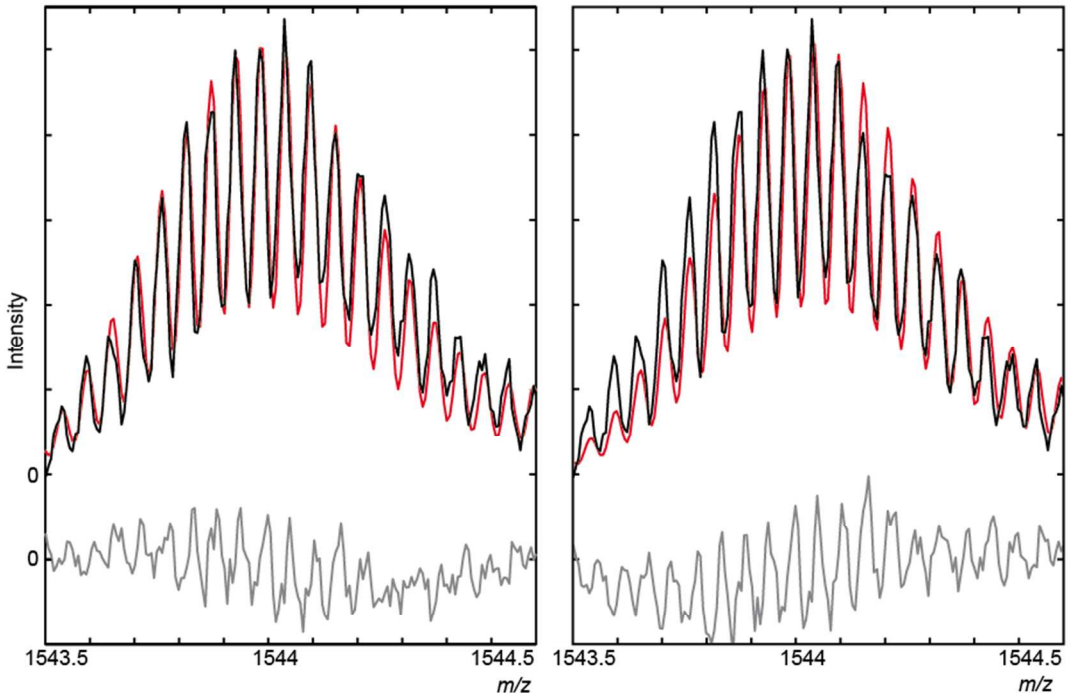

Figure S4. Isotopic resolved peaks (black trace in each panel) allow discrimination of glycoforms that differ only by a mass of 1 Da. The red trace in each panel shows the calculated isotopic distributions of PSA at charge state 18+ with a H_4N_3FS (left) and $H_4N_3F_3$ (right). The difference of these two glycoforms is 1.020 Da (cf. Supplementary Table 5). The grey trace at the bottom of each panel represents the difference of the calculated and experimental spectrum. The best fit was obtained for the situation shown in the left.

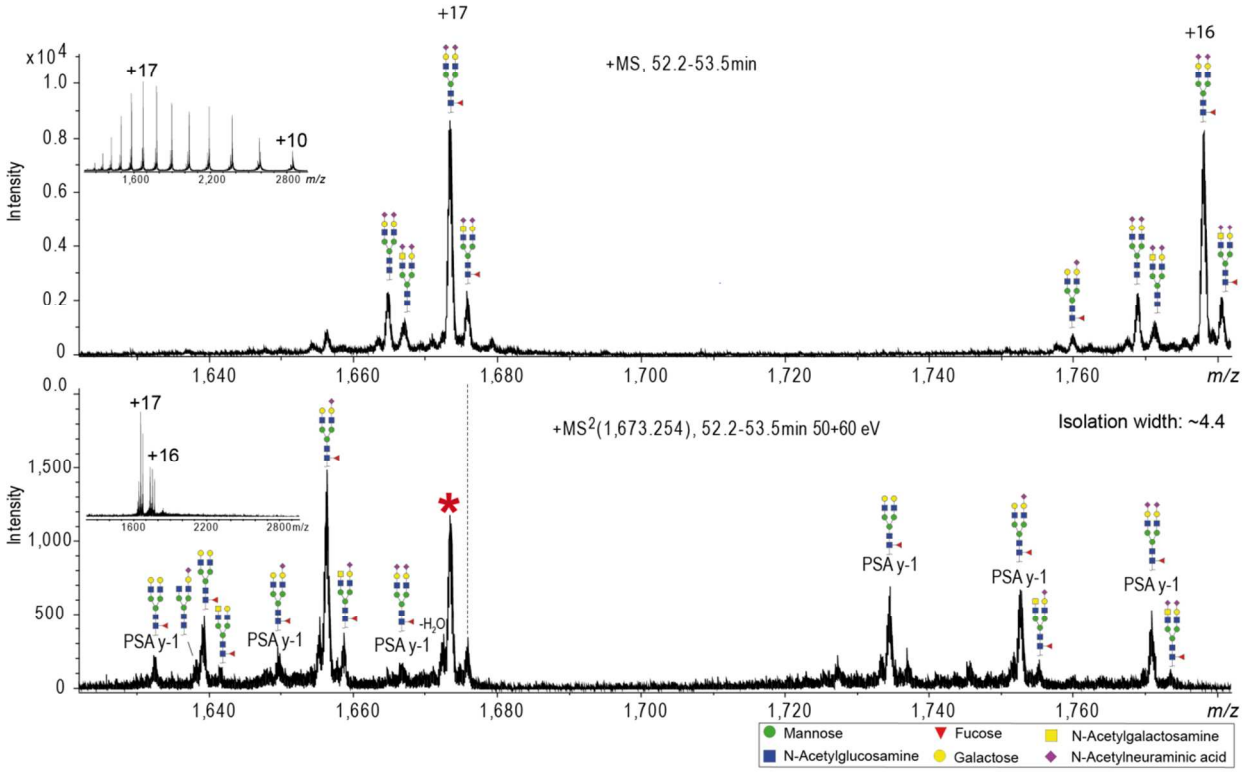

Figure S5. MS and MS/MS spectrum of a different sample of prostate specific antigen. Top: experimental LC-MS spectrum (inset shows full charge state envelope spectrum covering charge states from 10+ to 22+). Bottom: The fragment spectrum of the isolated glycoform PSA-$H_5N_4FS_2$ (peak at *m/z* 1672.37 in the top spectrum) gives information on the glycan composition at the termini. Fragments that correspond to a loss of the *N*-terminal isoleucine (Y-1 ions) were observed as well. The Y-1 ions were found at the charge state 16+ whereas initially the charge state at 17+ was isolated (see inset top left in bottom panel). A complete isolation in quadrupole of the PSA glycoform was not achieved with the experimental set up. To achieve a proper signal-to-noise ratio the isolation width was 4.4. This resulted in the presence of the acetylated glycoform PSA- $H_4N_5FS_2$ in the fragment spectra as well. At the charge state 17+ the isoform has a distance of only 2.4 Da and could therefore not be separated.

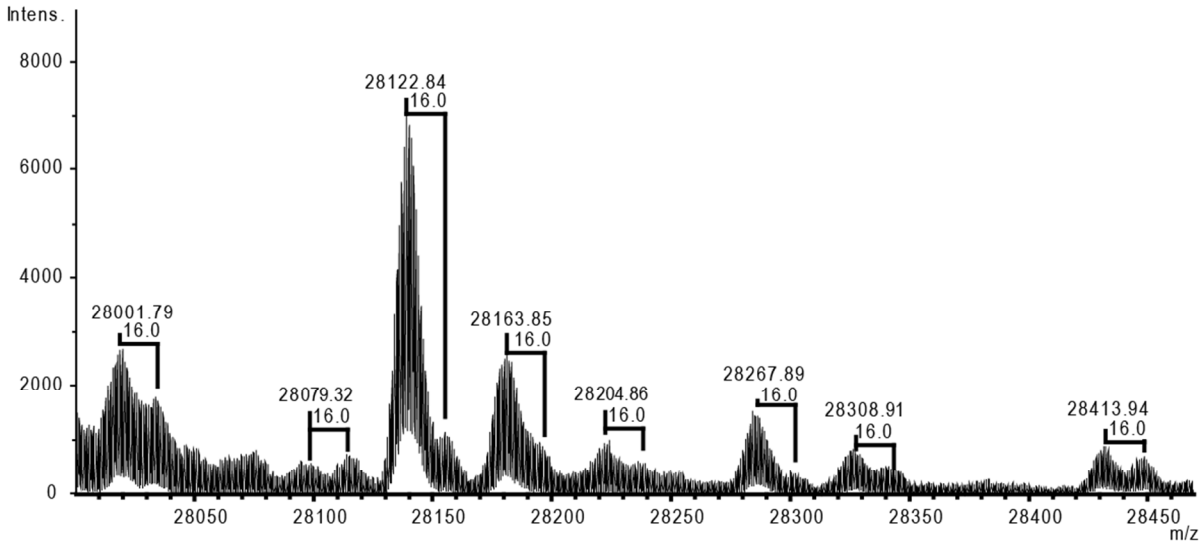

Figure S6. Section of the deconvolved MS spectrum of PSA-1 from an LC-ESI-MS run in the intact protein mode. Pairs of peaks are annotated which arise from protein oxidation and/or glycoforms of PSA.

Table S1. Glycoforms of prostate specific antigen samples PSA-1 and PSA-2. Spectra were deconvolved using the maximum entropy tool. The monoisotopic masses were determined utilizing the SNAPTM algorithm.

	PSA-1			PSA-2		
Glycan Composition	M+H+	δ [Da]	Relative Intensity[b]	Relative Intensity[b]	M+H$^+$	δ [Da]
$H_6N_5FS_2$				0.5%	28,779.12	0.10
$H_4N_6FS_2$	28,655.01	-2.98	0.5%			
H_4N_7FS	28,568.99	-0.99	0.7%			
H_6N_5FS	28,486.96	-0.96	0.5%	0.9%	28,486.94	-0.99
$H_5N_5S_2$	28,470.95	0.04	1.7%	1.7%	28,470.94	0.03
$H_4N_5FS_2$				7.7%	28,453.98	-0.93
$H_6N_4S_2$	28,430.94	1.06	0.9%	3.5%	28,430.93	1.05
$H_5N_4FS_2$	28,413.94	0.05	1.2%	31.4%	28,412.96	-0.92
$H_3N_5S_2s$				1.7%	28,372.92	-0.91
H_5N_5FS	28,324.91	-0.96	0.6%			
$H_4N_5S_2$	28,308.91	0.05	1.2%	2.6%	28,306.90	-1.96
H_6N_4FS	28,283.87	-0.98	0.3%			
$H_5N_4S_2$	28,267.89	0.06	2.1%	4.6%	28,266.89	-0.94
H_4N_5FSs				2.9%	28,242.86	-1.93
H_4N_6S	28,219.83	-0.79	0.6%	0.7%	28,238.90[a]	0.21
H_3N_6FS	28,204.86	0.01	1.2%			
H_5N_5S	28,177.84	-1.98	0.9%			
H_4N_5FS	28,163.85	0.04	4.1%	4.2%	28,162.85	-0.97
H_3N_6Ss	28,139.84	0.09	1.0%	2.5%	28,138.83	0.99
H_5N_4FS	28,122.84	0.04	10.8%	23.7%	28,121.86	-0.93
H_4N_5Ss	28,097.82	-0.91	0.9%			
H_6N_3FS	28,079.32	-2.44	0.6%			
H_3N_6S	28,058.81	0.02	0.9%			
H_4N_5S	28,016.79	-0.97	2.6%	1.6%	28,012.76	-5.00[c]
H_3N_5FS	28,001.79	0.03	3.5%			
H_3N_6Fs	27,992.78	-1.93	1.5%	0.4%	27,992.73	0.95
H_5N_4S	27,976.76	0.03	3.7%	2.9%	27,975.76	-0.97
H_4N_4FS	27,959.77	-0.97	10.1%	0.5%	27,957.71	-3.02
H_6N_3S	27,935.75	0.04	12.7%			
H_3N_5S	27,855.74	0.03	1.7%			
H_5N_4F	27,830.70	-0.99	1.0%	1.9%	27,831.72	0.03
H_4N_4S	27,815.71	1.03	3.2%			
H_3N_4FS	27,798.72	0.03	2.2%			
H_6N_3F	27,808.70*	0.41	0.9%	0.6%	27,791.63	0.96
H_5N_3S	27,773.70	0.05	1.5%			
H_4N_3FS	27,757.71	0.05	13.9%	2.4%	27,757.63	-0.03
H_6N_3p	27,724.61	-0.97	0.7%			
H_6N_3	27,645.62	1.01	1.5%			
H_4N_3S	27,611.64	0.04	3.1%			
H_3N_4Fs	27,588.58	0.02	0.9%	0.3%	27,588.52	-0.04
H_4N_3F	27,466.55	-0.01	0.5%			
N	26,266.19	0.06	4.0%	0.7%	26,267.22	1.09

[a]: only average mass could be determined, #: quantification was obtained from the MATLAB script (cf. Methods). [c]: automatic peak picking algorithm failed due to signal overlap or low signal-to-noise ratio. The abbreviations of the glycan composition are as follows. H: hexose, N: *N*-acetyl-hexosamine, F: fucose, S: *N*-acetyl-neuraminic acid, G: *N*-glycolyl-neuraminic acid, s:

sulfate and p: phosphate. A summary of alternative glycan compositions is shown in Table S2. A single nucleotide polymorphisms (SNP) of PSA is present with ~5% in PSA-1 (dbSNP Build ID: 61752561, published frequency 0.04)[1, 2] that gives rise to a second glycosylation site (Asp78Asn) carrying dominantly high mannose glycans (H5N2 and H6N2 at Asn78; H5N4FS2 and H5N4S2 at Asn45).

Table S2. Summary of mass similar and mass isobaric glycan compositions. The abbreviations of the glycan composition are as follows. H: hexose, N: N-acetylhexosamine, F: fucose, S: N-acetylneuraminic acid, G: N-glycolylneuraminic acid, s: sulfate and p: phosphate. N-Glycolylneuraminic acid cannot be synthesized by humans but was repeatedly found in human glycoproteins. It is assumed that N-glycolylneuraminic acid is taken up by humans from the food and is then processed to end up in glycoproteins in small amounts.[3]

Composition	Mass [Da]	Alternative Composition	Mass [Da]	Δ [Da]
S	291.10	F_2	292.12	-1.020
G	307.09	HF	308.11	-1.020
H_2p	405.08	N_2	406.16	-1.079
SH	453.15	GF	453.15	-
H_3	486.16	N_2s	487.12	-0.965
GH_2	631.20	N_2Fs	633.18	-1.985
S_2Fs	809.21	H_5	810.26	-1.051

References

1. Sherry, S. T.; Ward, M. H.; Kholodov, M.; Baker, J.; Phan, L.; Smigielski, E. M.; Sirotkin, K., dbSNP: the NCBI database of genetic variation. *Nucleic Acids Res* **2001,** 29, (1), 308-11.

2. Gallagher, D. J.; Vijai, J.; Cronin, A. M.; Bhatia, J.; Vickers, A. J.; Gaudet, M. M.; Fine, S.; Reuter, V.; Scher, H. I.; Hallden, C.; Dutra-Clarke, A.; Klein, R. J.; Scardino, P. T.; Eastham, J. A.; Lilja, H.; Kirchhoff, T.; Offit, K., Susceptibility loci associated with prostate cancer progression and mortality. *Clin Cancer Res* **2010,** 16, (10), 2819-32.

3. Tangvoranuntakul, P.; Gagneux, P.; Diaz, S.; Bardor, M.; Varki, N.; Varki, A.; Muchmore, E., Human uptake and incorporation of an immunogenic nonhuman dietary sialic acid. *Proc. Natl. Acad. Sci. U. S. A.* **2003,** 100, (21), 12045-12050.