Analytical and Bioanalytical Chemistry

Electronic Supplementary Material

N-linked glycosite profiling and use of Skyline as a platform for characterization and relative quantification of glycans in differentiating xylem of *Populus trichocarpa*

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MISearch.py - 216_2016_9776_MOESM2_ESM.py
MIsearchTemplate - 216_2016_9776_MOESM3_ESM.xlsx
Supplementary_Data - 216_2016_9776_MOESM4_ESM.xlsx

Content

ESM 1

Supplementary Figures

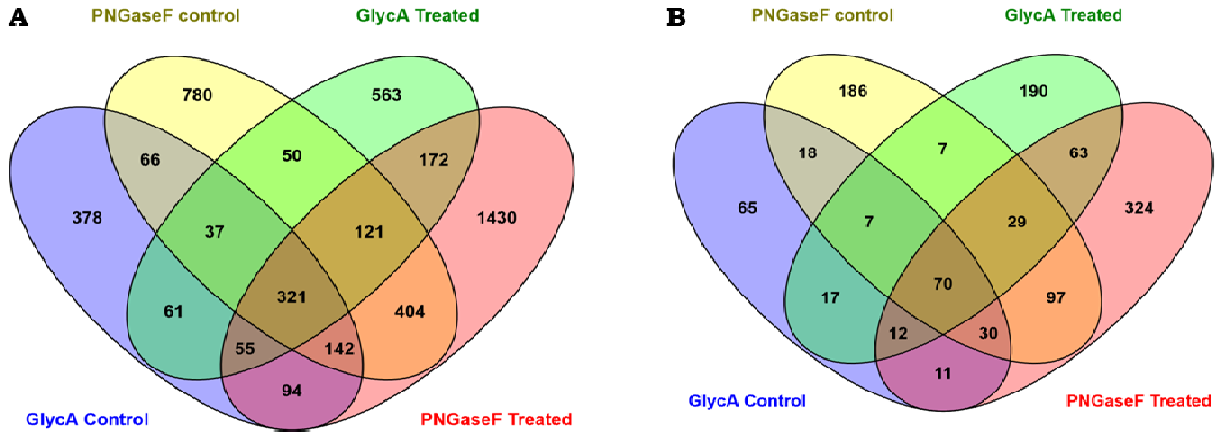


Fig. S1 (**A**) Venn diagrams showing the overlap of deamidated peptides identified by control (no enzymatic treatment) samples and treated (PNGase F or Glycosidase A) samples. (**B**) The number of deamidated peptides in the treated is further reduced by filtering out peptides which do not contain the NXS/T glycosylation motif
Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html

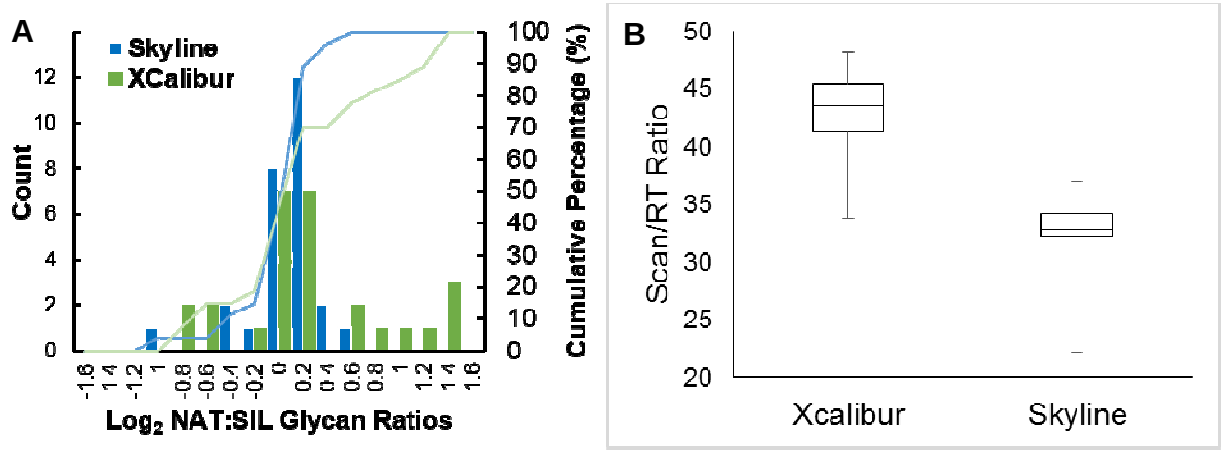


Fig. S2 (A) The distribution of the glycan ratios calculated in Skyline or XCalibur are compared. (B) For eight glycans with mean abundance ratios greater than 0.98, the scan/RT ratios were calculated for each method. When pooled, the mean scan/RT ratio was 42.9 and 32.9 for XCalibur and Skyline method, respectively ($p = 5.4 \times 10^{-14}$)

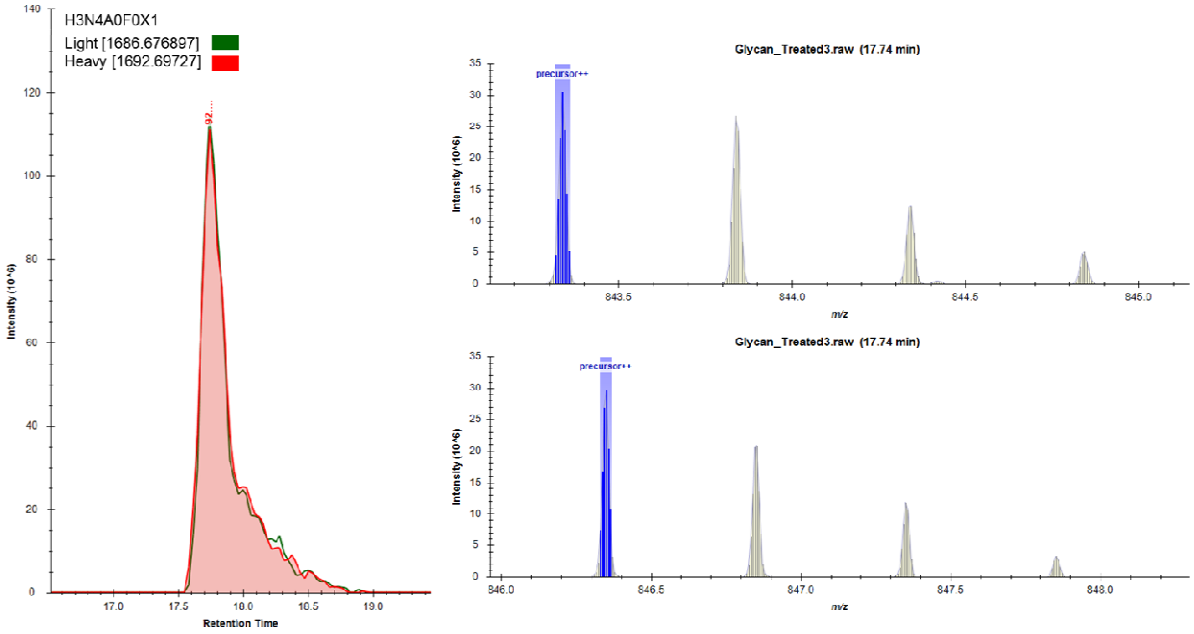
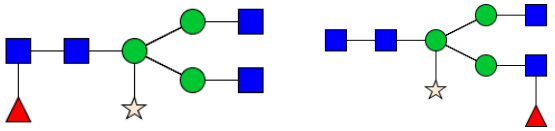
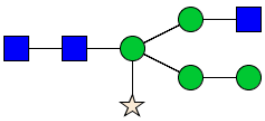


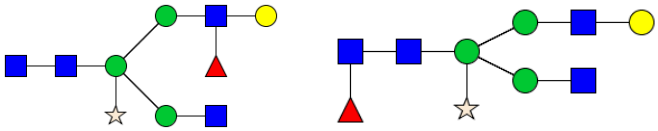
Fig. S3 Confirmation of Glycans by Co-elution of Native/SIL Species in 1-to-1 Abundance, along with accurate mass and isotopic distribution in Skyline

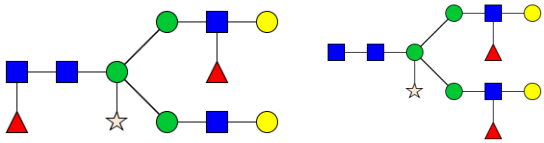
Supplementary Tables

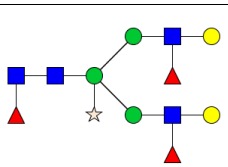
Table S1 For any complex-type glycan, selected diagnostic fragments are reported. In each MS/MS, the associated NAT or SIL tag was observed. For the sake of brevity, small saccharide ions in the MS/MS spectra that reveal little about complex structure were emitted, as were tables for glycans with whose structure may be completely inferred by biology (*e.g.* di- and tri- saccharides, *N*-glycan core structures). $H_{10}N_2$, $H_4N_2F_1X_1$, $H_6N_5F_2$, and $H_5N_4F_1X_1$ were not selected for MS/MS

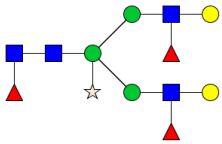
H3N4F1X1			
Composition of Fragment	Theoretical m/z (neutral)	m/z observed	Mass Accuracy (ppm)
N1	185.07	186.08	-2.134
N1X1	335.12	336.13	-2.283
H1N1	365.13	366.14	-2.287
H1N1X1	383.14	384.15	4.32
H1N1X1	497.17	498.18	-10.03
H2N1	527.19	528.19	3.917
H2N1X1	659.23	660.23	-3.36
H1N2F1/H3N1	689.24	690.24	-7.291
H3N1X1	821.28	822.28	-14.07
H2N2X1	862.31	863.31	-4.61
H3N2X1	1024.4	1025.4	-0.249

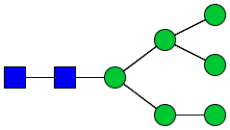
H4N3X1			
Composition of Fragment	Theoretical m/z (neutral)	m/z observed	Mass Accuracy (ppm)
H2	324.1056	325.1123	-3.471
H1N1	365.1322	366.1394	-1.712
H2N1X1	659.2273	660.2302	-7.532
H3N1	689.2378	690.2455	-0.123
H3N1X1	821.2801	822.2781	-11.987
H3N1X1	839.2907	840.2988	0.363
H4N1	851.2907	852.2993	0.863
H4N1X1	983.3329	984.3406	-0.147
H4N1X1	1001.3435	1002.3402	-11.160

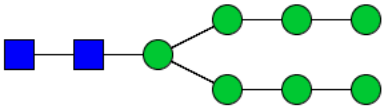
H4N4F1X1			
Composition of Fragment	Theoretical m/z (neutral)	m/z observed	Mass Accuracy (ppm)
N1X1	335.1216	336.1254	-2.819
N1F1	349.1373	350.1476	7.203
H1N1	365.1322	366.1391	-2.533
H1N1	383.1428	384.1473	-8.574
N2	406.1587	407.1649	-4.099
H1N1X1	453.1482	454.1655	20.909
H1N1X1	497.1745	498.1803	-4.133
H1N1F1	511.1901	512.1964	-2.964
H2N1	527.1850	528.1921	-1.299
H2N1	545.1956	546.2027	-1.330
H2N1X1	659.2273	660.2341	-1.509
H3N1	689.2378	690.2458	0.239
H2N2	730.2644	731.2670	-7.114
H3N1X1	821.2801	822.2851	-3.44
H3N2	892.3172	893.3095	-17.455
H4N2F1X1	1332.4702	1333.4915	10.083
$H3N3X_{Fuc}X1$/ $H3X_{GAL}N3X1$/ $H4X_{GLCNAC}N2X1$	1333.4655	1334.4636	-7.278

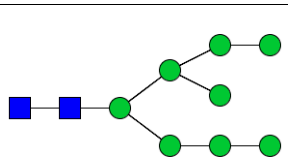
H5N4F2X1			
Composition of Fragment	Theoretical m/z (neutral)	m/z observed	Mass Accuracy (ppm)
N1F1	349.1373	350.1434	-4.941
H1N1	365.1322	366.1393	-2.123
H1N1	383.1428	384.1486	-5.311
H1N1F1	511.1901	512.1971	-1.536
$N1H2/N1^{1,5}X_{GAL}F1X1$	527.185	528.1896	-6.042
H2N1	545.1956	546.2030	-0.780
Tag-N2	642.2901	643.2935	-6.889
H2N1X1	659.2273	660.2343	-1.251
H2N1F1	673.2429	674.2501	-0.988
H3N1	689.2378	690.2393	-9.177
H2N1F1X1	805.2852	806.2849	-10.090
H3N1X1	821.2801	822.2877	-0.323
H3N1F1	835.2958	836.3033	-0.389
H2N2X1	862.3067	863.3024	-14.061
H3N2	892.3172	893.3499	27.877
H3N1F1X1	967.338	968.3274	-19.047
H3N2X1	1024.3595	1025.3641	-3.109
Tag-H2N2X1	1116.4486	1117.4547	-1.545
H3N2F1X1	1170.4174	1171.4301	4.122
H4N2X1	1186.4123	1187.4274	6.107
Tag-H3N2X1	1279.5092	1279.5028	-5.025
H4N2F1X1	1332.4702	1333.4742	-2.841
H4N2F1X1	1350.4808	1351.4796	-6.675
H3N2F1X1	1424.5593	1425.5626	-3.176
H4N3F1X1	1535.5496	1536.5320	-16.571
Tag-H4N3F1	1643.6336	1644.6244	-10.358
Tag-H4N3F2	1789.6915	1790.7008	0.824

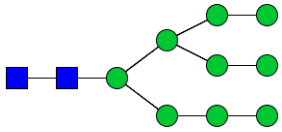
H5N4F3X1			
Composition of Fragment	Theoretical m/z (neutral)	m/z observed	Mass Accuracy (ppm)
N1F1	349.1373	350.1426	-7.289
H1N1	365.1322	366.1379	-5.792
H1N1	383.1428	384.1476	-7.947
H1N1X1	497.1745	498.1797	-5.24
H2N1/ X_{Gal}N1F1X1	527.1850	528.1898	-5.814
H1N1F1X1	643.2324	644.2386	-2.542
H2N1X1	659.2273	660.2332	-2.890
H2N1F1	673.2429	674.2438	-10.227
H1N1X_{Gal}F1X1	689.2378	690.2402	-7.827
H2N1F1X1	805.2852	806.2749	-22.508

H5N5F2X1			
Composition of Fragment	Theoretical m/z (neutral)	m/z observed	Mass Accuracy (ppm)
N2	406.1587	407.1650	-3.730
H2N1	527.1850	528.1939	2.058
H3N1	689.2378	690.2440	-2.416
H3N1	689.2378	690.2440	-2.416
H4N1	851.2907	852.2996	1.286
H4N1	869.3012	870.3088	-0.213
H2N4(2,5XGal)	1176.4181	1177.4171	-7.493
H3N4(2,5XGal)	1338.4709	1339.4541	-18.398
H3N2F1(1,4XGlcNac)(0,2XGal)	1355.4862	1356.4587	-26.031
H4N2F2(1,4XGal)(2,5XGlcNac)	1517.5390	1518.5060	-26.915
H4N4(2,5XFuc)	1518.5343	1519.5117	-20.023
H4N4(2,5XFuc)	1518.5343	1519.5117	-20.023
H5N2(1,3XGlcNac)(2,5XFuc)F1	1540.5285	1541.5287	-4.956
H4N5	1703.6031	1704.5687	-24.768
H5N4(1,3XFuc)2	1794.6552	1795.7025	22.001
H3N4F1(0,4XGal)(1,5XFuc)	1795.6504	1796.6636	2.977
H3N5F1(0,1XGal)(3,5XFuc)	1795.6504	1796.6637	3.049

H6N2			
Composition of Fragment	Theoretical m/z (neutral)	m/z observed	Mass Accuracy (ppm)
Tag-H1N1	822.3534	823.3454	-19.244
H3N1	689.2378	690.2409	-6.841
Tag-N2	660.3006	661.30664	-2.703
Tag-N1	457.2212	458.22791	-2.439
H2N1	527.1850	528.1920	-1.641
Tag-H3(Xman)N2	1152.4485	1153.4834	23.493

H7N2			
Composition of Fragment	Theoretical m/z (neutral)	m/z observed	Mass Accuracy (ppm)
Tag-H2N2	984.4063	985.40277	-11.535
Tag-H3N2	1146.4591	1147.45386	-11.396
Tag-H1N1	822.3534	823.35358	-9.296
H4N1	851.2907	852.2924	-7.171
Tag-N2	660.3006	661.30383	-6.959
Tag-N1	457.2212	458.2287	-0.711
H2N1	527.1850	528.1925	-0.616
H3	486.1585	487.1674	2.129
H3N1	689.2378	690.2484	4.041

H8N2			
Composition of Fragment	Theoretical m/z (neutral)	m/z observed	Mass Accuracy (ppm)
H3	486.1585	487.1662	-0.257
H2N1	527.1850	528.1903	-4.884
H3N1	689.2378	690.2449	-1.008
H4N1	851.2907	852.2925	-7.031
Tag-H2(Xman)N2	990.3957	991.4317	28.448
Tag-H3(Xman)N2	1152.4485	1153.4694	11.311
Tag-H4(Xman)N2	1314.5013	1315.5206	8.752
Tag-H4(Xman)N2	1378.5174	1379.5309	4.102
H7(Xman)N1	1379.4597	1380.5153	34.604

H9N2			
Composition of Fragment	Theoretical m/z (neutral)	m/z observed	Mass Accuracy (ppm)
H1	144.0423	145.0497	-2.742
H1	162.0528	163.0604	-1.697
N1	185.0688	186.0761	-3.107
N1	203.0794	204.0871	-0.566
N1	221.0899	222.0959	-8.119
H2	324.1056	325.1131	-1.003
H1N1	365.1322	366.1414	3.656
H3	486.1585	487.1653	-2.088
H2N1	527.185	528.1924	-0.844
H2N1	568.2116	569.2189	-0.871
H4	648.2113	649.2166	-3.880
Tag-N2	660.3006	661.3021	-9.579
H3N1	689.2378	690.2432	-3.475
Tag -H1N1	822.3534	823.3530	-10.038
H4N1	851.2907	852.2946	-4.658
Tag -H2N2	984.40635	985.4129	-1.290
H5N1	1013.3435	1014.3428	-8.442
Tag -H3N2	1146.4591	1147.4616	-4.728
Tag -H4N2	1308.512	1309.5016	-13.890

ESM 2: In-house program to refine expansive glycan theoretical peak list.

The following program (MIsearch) was written to parse monoisotopic masses detected in the glycan elution window. It operates under the assumption that glycans are labeled equally with NAT and SIL co-eluting tags and therefore is present in both channels. The following supplementary material described the necessary steps to employ the workflow described in the main manuscript (Figure A).

MI Search Template (File ESM2_MIsearchTemplate) Preparation:

1. Generate a theoretical composition database for the targeted species.
2. Calculate multiple *m/z* values per composition, corresponding to multiple charge states. Note: if known, the singular dominant charge state per glycan species may be used to further reduce false positives.
3. For each glycan *m/z* and for each label, calculate the neutral mass range corresponding to the MMA of the data (*e.g.* 5 ppm). This may be calculated with the following equation:

$$Lower\ bound = \left(\frac{[M+H_n]^{n+}}{n} - \frac{[M+H_n]^{n+}}{n} \times MMA)\right) \times n - n \times H$$

$$Upper\ bound = \left(\frac{[M+H_n]^{n+}}{n} - \frac{[M+H_n]^{n+}}{n} \times MMA)\right) \times n - n \times H$$

4. For each theoretical composition, input the associated information. The NAT low (column E), NAT high (column F), SIL low (column J), SIL High (column K) correspond to the neutral bounds generated in step three. Likewise, the charge state (n) should be inputted in column D and I. The ID column may be filled with any text or commentary to be associated with the glycan; it is used for reporting purposes and not for searching. The NAT and SIL theoretical columns are also used for reporting and not for searching; for convenience, the theoretical *m/z*, versus the neutral mass, may be inputted here so it is easily available.

Peak Picking Method:

1. Convert MS^1 data to an mzXML file (msconvert, ProteoWizard).
2. Download and execute Hardklor to generate a mass list, using either the recommended parameters (methods section, main manuscript) or parameters tailored to the system. Specify the output file to .txt.
3. Open the Hardklor output .txt file in Excel and copy the neutral monoisotopic masses of the peaks and charge states (column B and C)
4. Paste Hardklor columns B and C into MI search template columns A and B. This moves the neutral monoisotopic masses into the “experimental” column and the charge states into the “charge” column.
5. Save the MI search template as desired file name.

MI Search (File ESM2_MISearch.py) Execution:

1. Download Python 2.7: https://www.python.org/downloads/
2. Set Python Path and Path variables:
    a. Go to Control Panel > System> Advanced System Settings > Environmental Variables
    b. Add user variable (PATH) and value (C:\python27)
    c. Add system variable (PYTHONPATH) and value (C:\python27)
3. Download setuptools: https://pypi.python.org/pypi/setuptools
    a. Unzip and then go into directory and run python setup.py install
4. Download openpyxl: https://pypi.python.org/pypi/openpyxl
    a. Unzip and then go into directory and run python setup.py install
5. Open the command line and navigate to the directory where the file prepared above was stored
6. Run the program by typing: python MIsearch.py [file.xlsx]
7. Multiple files may be run simultaneously by typing: python MIsearch.py [file.xlsx] [file.xlsx] [file.xlsx] …
8. Within the directory, a new file with name: file_output.xlsx will be produced. It will output the ID, theoretical charge for the NAT and SIL species, and their associated charge states.