Core richness of N-glycans of *Caenorhabditis elegans:*
a case study on chemical and enzymatic release

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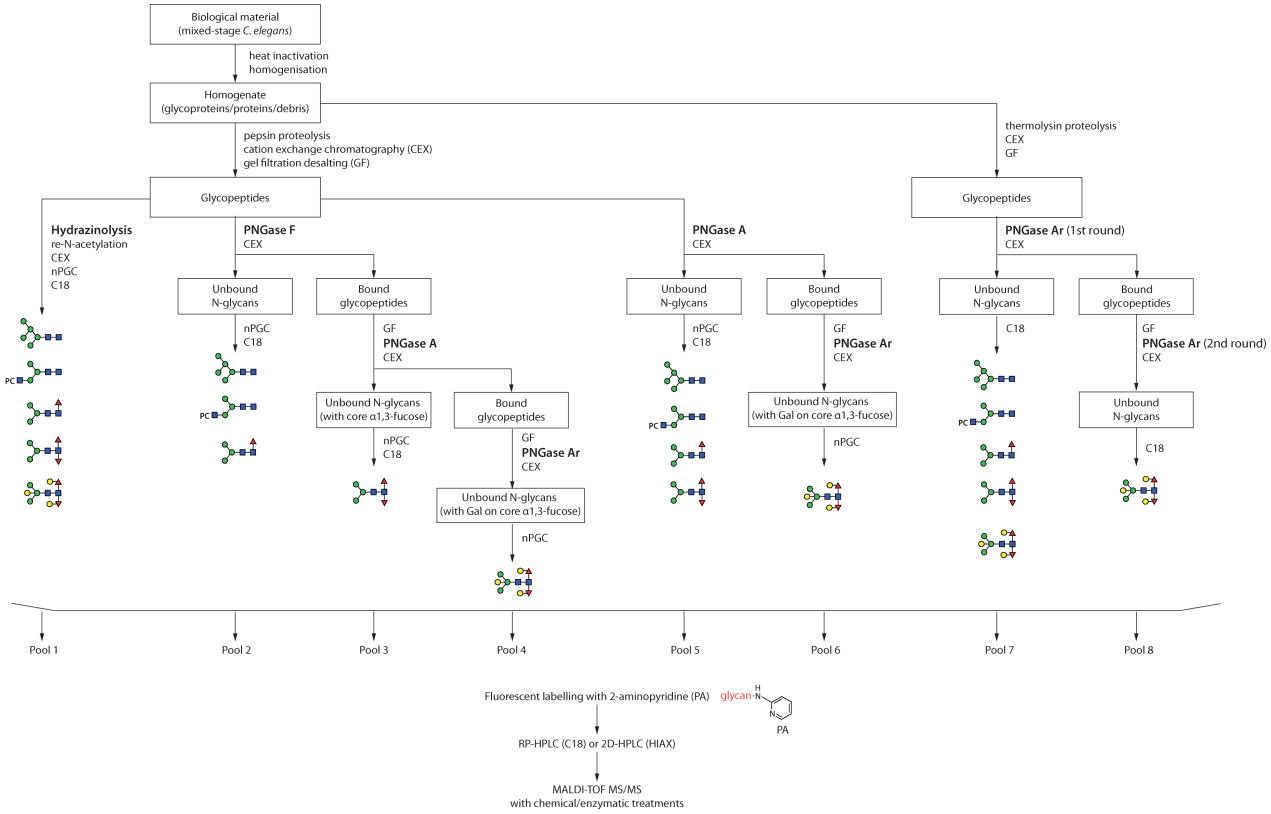
Scheme: ***Glycomic workflow employed in this study.*** Summary of the experimental procedures indicating glycan release by (i) hydrazinolysis, (ii) serial treatments with PNGase F, PNGase A and PNGase Ar, (iii) serial treatments with PNGase A and PNGase Ar or (iv) two rounds of PNGase Ar digestion followed by solid-phase extraction and labelling steps. Example N-glycans in different pools are shown. CEX, cation exchange chromatography; C18, Lichroprep C18 RP-HPLC material; GF, gel filtration; nPGC, non-porous graphitised carbon; PA, 2-aminopyridine. In the case of PNGase F/A/Ar, A/Ar and hydrazine release, pepsin (rather than, e.g., trypsin) was used to proteolyse worm material; on the other hand, thermolysin was used (in line with our latest procedures) to prepare glycopeptides for the two rounds of release with PNGase Ar alone. The RP-HPLC chromatograms of the eight different pools of PA-labelled glycans are shown in **Supplementary Figure 1**.



Supplementary Methods

The preparation, enzymatic release, labelling, separation and chemical/enzymatic treatments of N-glycans are based on previously-published procedures (presented in protocol format in "*Analysis of Invertebrate and Protist N-Glycans",* Hykollari A, Paschinger K , Eckmair B and Wilson IBH, *Methods Mol. Biol.* 2017, vol. 1503, pp. 167-18). An extended version of parts of the methods section in the main text is as follows:

Glycopeptide preparation:
Harvested worms were boiled in water for 10 minutes to heat-inactivate glycosidases prior to homogenisation. Nematode homogenates were transferred into glass flasks and treated either with pepsin (Sigma) in 5% formic acid overnight at 37 °C or with thermolysin (Promega) in 50 mM ammonium bicarbonate buffer (pH 8) supplemented with 0.5 mM $CaCl_2$ for 2 hours at 70 °C. Typically, 2 g (wet weight) of worms were proteolysed with 2 mg of protease.
After proteolysis, the mixtures were centrifuged and the glycopeptides were purified by cation exchange chromatography (Dowex 50W×8; BioRad): in the case of a thermolysin digest, the proteolysate was acidified, while the pepsin digests required no pH adjustment prior to incubation with 10 ml of the pre-washed chromatography medium. After 1 hr, the resin and supernatant were poured into a polypropylene column and unbound material washed with 2% acetic acid; glycopeptides were eluted with 0.5 M ammonium acetate, pH 6. The orcinol-positive fractions were pooled, lyophilised and glycopeptides were desalted on a Sephadex G25 column (GE Healthcare; column volume of 80 ml) using 0.5% acetic acid as eluent; again orcinol-positive fractions were pooled, lyophilised and remaining protease activity heat-inactivated at 95 °C for 10 minutes.

N-glycan release with peptide:N-glycosidases:
Enzymatic release of N-glycans from worm peptic glycopeptides was done using three different peptide:N-glycosidases: (i) recombinant bacterial PNGase F (from *Flavobacterium [Elizabethkingia] meningosepticum*, Roche; 3 U) under alkaline conditions overnight (50 mM ammonium bicarbonate, pH 8), (ii) native almond PNGase A under acidic conditions overnight (from *Prunus amygdalus*, Roche; in 50 mM ammonium acetate, pH 5, 0.25 mU) and (iii) recombinant rice PNGase Ar (from *Oryza sativa* expressed in *Pichia pastoris* and Endo H treated, New England Biolabs; also at pH 5 overnight, 15 U).
In the first experiments, digestion was either with PNGase F followed by PNGase A then PNGase Ar *or* with PNGase A followed by PNGase Ar of peptic peptides. In another experiment, thermolysin was used for proteolysis followed by two rounds of PNGase Ar release (each overnight with 25 U) without the use of the other two PNGases. Chemical release of glycans from peptic glycopeptides by hydrazine was performed as described below. Thereby, all three enzymes (PNGase F, A and Ar) as well as hydrazine were tested with peptic peptides, but a newer proteolysis protocol with thermolysin was followed when using PNGase Ar alone.
Between each enzymatic step, the released glycans were separated from the remaining glycopeptides Dowex 50W×8 (5 ml resin, with glycans in the 2% acetic acid 'filtrate' and glycopeptides in the 0.5 M ammonium acetate eluate). When the remaining glycopeptides were to be treated with another peptide:N-glycosidase, these were generally desalted by gel filtration on G25 as described above.

The glycans were then further purified by solid-phase extraction using non-porous graphitised carbon (nPGC; 250 mg ENVI-Carb, Supelco). The glycans were dissolved in water, the nPGC material washed with water and the glycans eluted with 40 % acetonitrile; in the case of *C. elegans*, a subsequent step with 40% acetonitrile and 0.5% trifluoroacetic acid resulted in elution of no further glycans as judged by MALDI-TOF MS. Finally, the glycans were purified by solid-phase extraction on C18 material (100 mg LiChroprep® RP-18, Merck; elution with water) prior to pyridylamination as previously described. In case of glycans released by two rounds of PNGase Ar digestion, solely C18 purification was performed after Dowex chromatography prior to pyridylamination.
In general, it can be noted that two rounds of any enzymatic release (regardless of the PNGase) are necessary to release all oligomannosidic and paucimannosidic structures; such 'pre-removal' of the majority of N-glycans then means that the final round of release by PNGase Ar results in isolation of the more unusual structures.

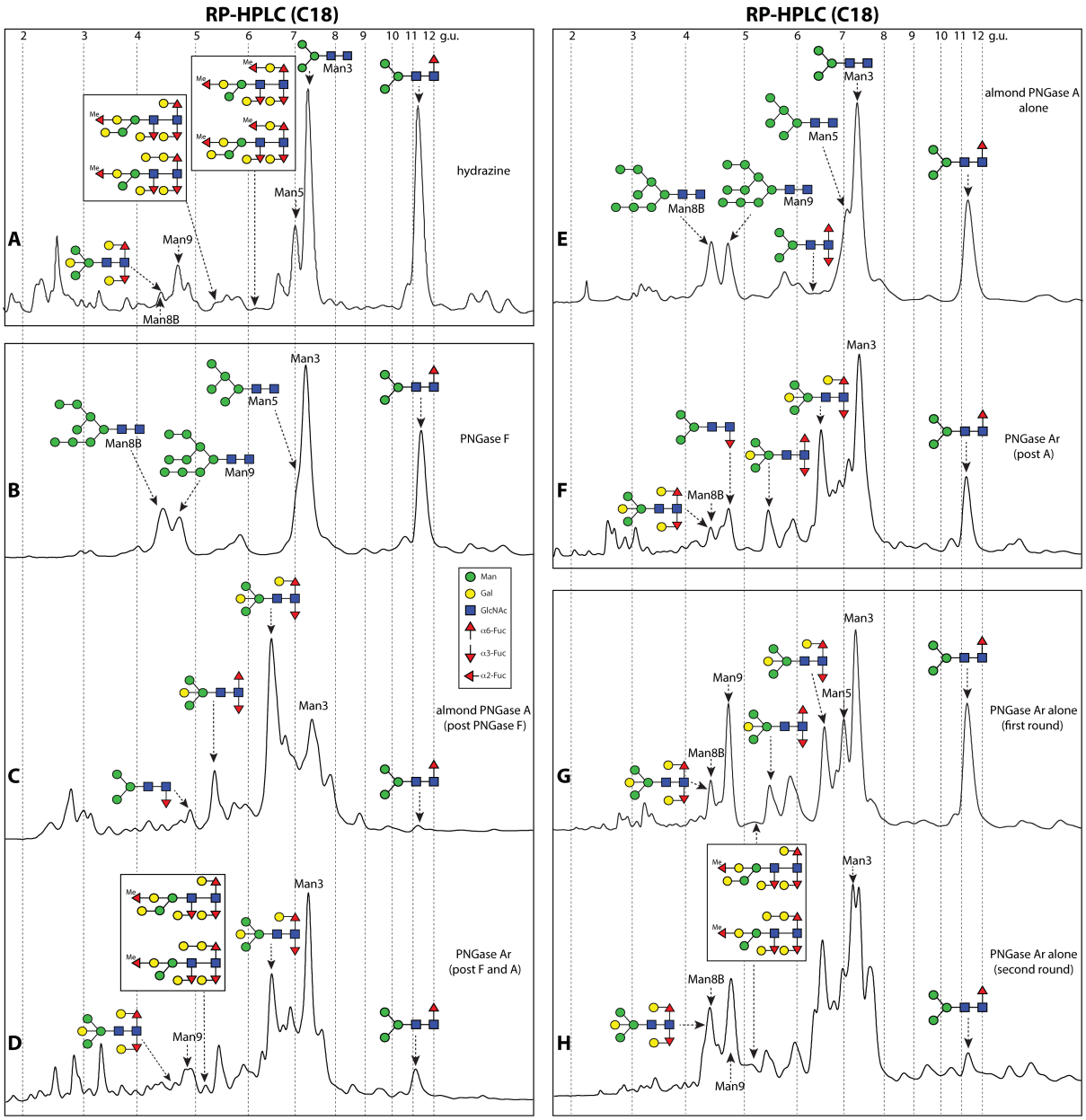
N-glycan release with hydrazine:
For chemical release, 10 mg peptic glycopeptides were transferred into a glass reaction tube and dried overnight prior to adding 500 µL of anhydrous hydrazine (prepared from monohydrate hydrazine; Sigma-Aldrich) and incubated at 100 °C for 5 h. Unreacted anhydrous hydrazine was removed by centrifugal evaporation. Samples were cooled to 0 °C and re-*N*-acetylated by the addition of 1 M sodium bicarbonate solution (450 µl) and acetic anhydride (21 µl) and incubated at 0 °C for 60 min. The samples were then acidified by addition of 5% (v/v) trifluoroacetic acid (600 µl) to the samples and incubated at 4°C for 60 min in order to liberate the reducing end of the glycans, followed by another round of Dowex 50W×8 chromatography. The glycans were then further purified by solid-phase extraction (as described above for enzymatic release) prior to pyridylamination. Note that hydrazine is a hazardous reagent and must only be used when applying relevant safety procedures. Hydrazinolysis was performed twice on two aliquots of the same glycopeptide preparation and both times yielded a similar set of glycans with evidence for only a minor degree of peeling of the reducing terminus.

Glycan labelling:
Pyridylamination was performed basically as described by Hase. In brief, 100 mg 2-aminopyridine (Sigma-Aldrich) was dissolved in 76 µl concentrated HCl and 152 µl water; 80 µl of this solution was added to the dried glycan sample, prior to incubation in boiling water for 15 minutes. Then a solution of 4.4 mg of sodium cyanoborohydride (Sigma-Aldrich) in a mixture of 9 µl of the aforementioned 2-aminopyridine solution and 13 µl water was prepared; 4 µl of this cyanoborohydride-aminopyridine solution was added to the sample and the incubation was continued overnight at 90 °C prior to gel filtration (Sephadex G15; GE Healthcare, 1 × 50 cm). Fluorescence (excitation/emission 320/400 nm) of the fractions was measured using a Tecan microtitre plate reader.

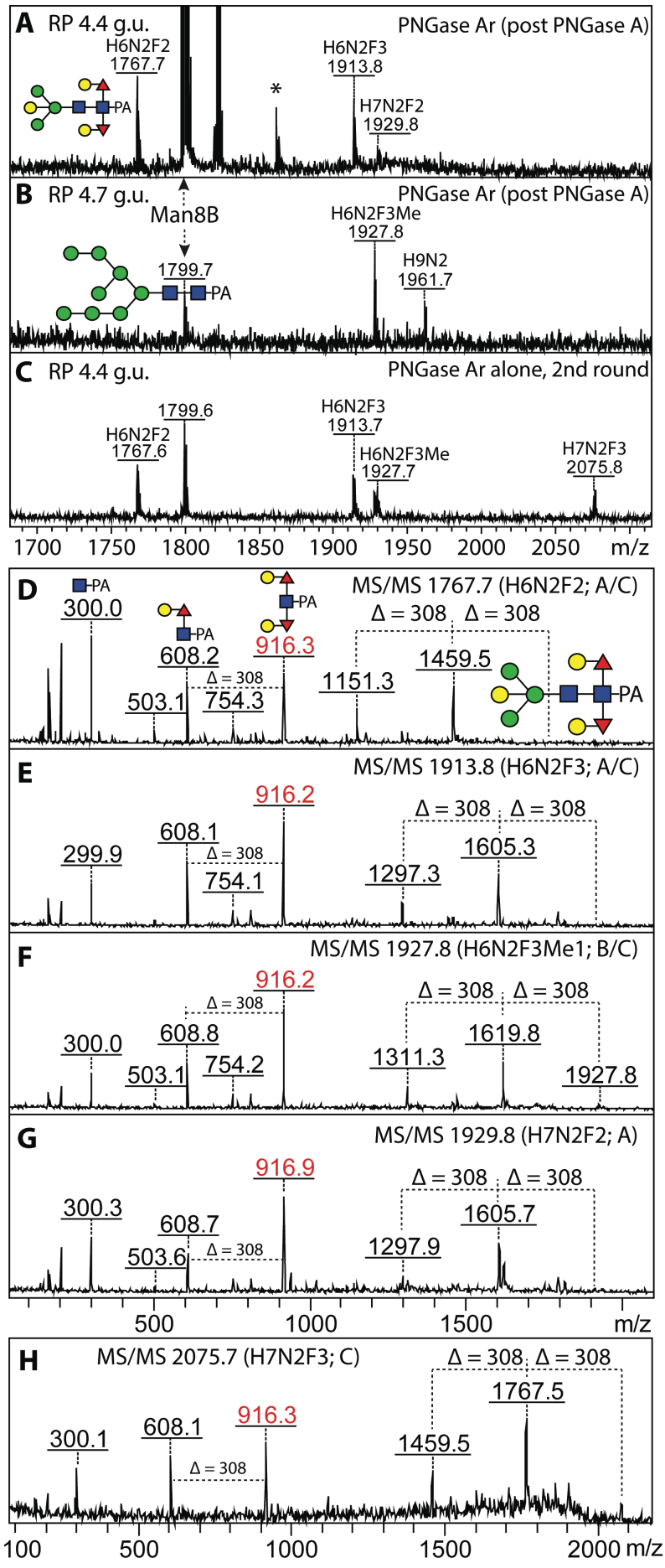
HPLC purification of N-glycans
Separation of PA-labelled glycans was carried out on a Shimadzu HPLC system equipped with a fluorescence detector (RF 20AXS; excitation/emission 320/400 nm). In case of RP-HPLC, a Hypersil ODS column (C18; Agilent, 4 mm × 250 mm, 5 µm, with guard cartridge; stored in 30 % MeOH) was used with 100 mM ammonium acetate, pH 4.0 (buffer A) and 30% (v/v) methanol (buffer B); a gradient of increasing buffer B (1% per minute) was

programmed. The column was calibrated daily in terms of glucose units (g.u.) with a pyridylaminated partial dextran hydrolysate.
For 2D-HPLC, selected fractions were re-applied to a combined hydrophilic-interaction anionic-exchange HPLC (HIAX, Dionex IonPac AS11; Thermo Scientific, 4 × 250 mm, with guard column; stored in 95 % acetonitrile) with an inverse gradient of acetonitrile in 800 mM ammonium acetate, pH 3.85: 0–5 min, 99 % of 80 % acetonitrile; 5–50 min, 99–90 % of 80 % acetonitrile; 50–65 min; 90–80 % of 80 % acetonitrile; 65–85 min, 80–75 % of 80 % acetonitrile. The HIAX column was calibrated with a set of oligomannosidic N-glycans derived from white beans.

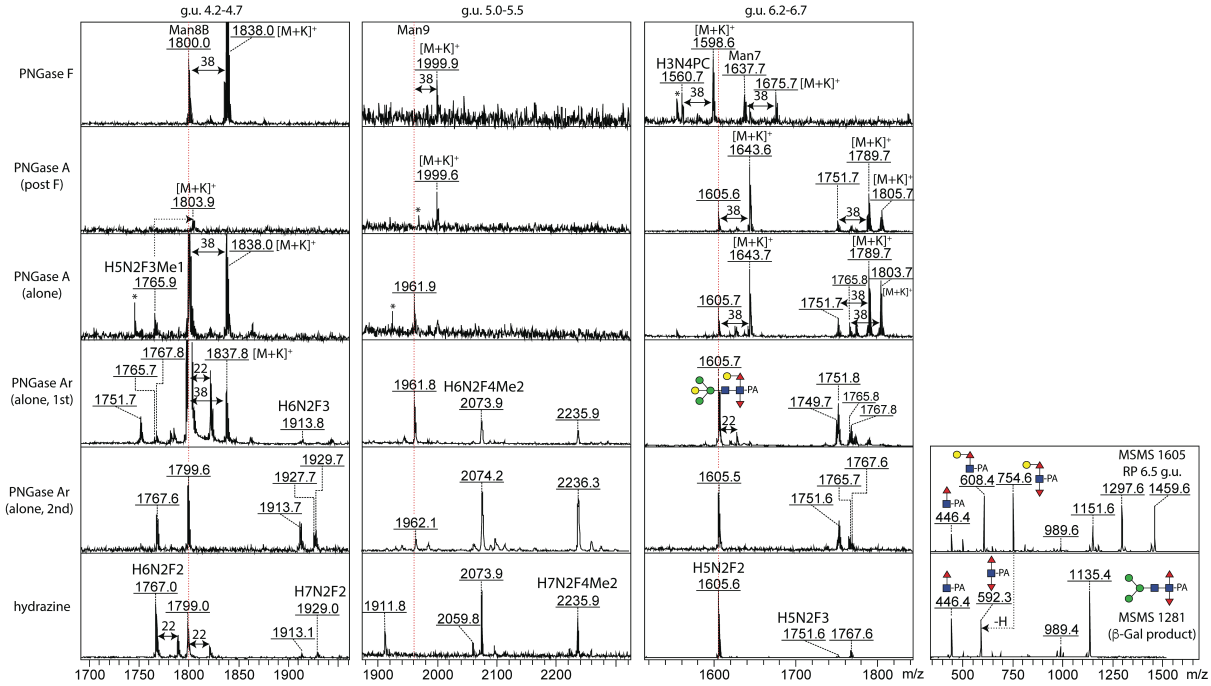
Treatments using exoglycosidases or hydrofluoric acid
In general, a 1 µl aliquot of a HPLC fraction (generally equivalent to 5-50 mV in terms of fluorescence which corresponds approximately to 2 - 20 pmol of glycan) was mixed with 0.2 µl exoglycosidase and 0.8 µl 100 mM ammonium acetate solution, pH 5.0; after an overnight incubation at 37 °C, a 0.5 µl aliquot of the mixture was analysed by MALDI-TOF MS. Exoglycosidases employed were: α-galactosidase from green coffee beans (Sigma, 11 mU), β-galactosidase from either *Aspergillus niger* (recombinant, produced in-house, 144 µU) or *Aspergillus oryzae* (native, 27 mU; Sigma), α-L-fucosidase from bovine kidney (Sigma-Aldrich, 10 mU), jack bean α-mannosidase (Sigma-Aldrich, 6.25 mU) or recombinant *Xanthomonas manihotis* α1,2/3-mannosidase (New England Biolabs, 6 - 8 U). In the latter case, $CaCl_2$ was added to a final concentration of 5 mM.
For removal of α1,2/3-linked fucose or methylfucose, glycan samples were dried in a SpeedVac and then incubated with 3 µl of 48% (w/v) hydrofluoric acid (HF) on ice for 24 hours. The HF was immediately removed in a SpeedVac. Chemically or enzymatically treated glycans were generally reanalysed, unless otherwise stated, by MALDI-TOF MS and MS/MS without further purification.



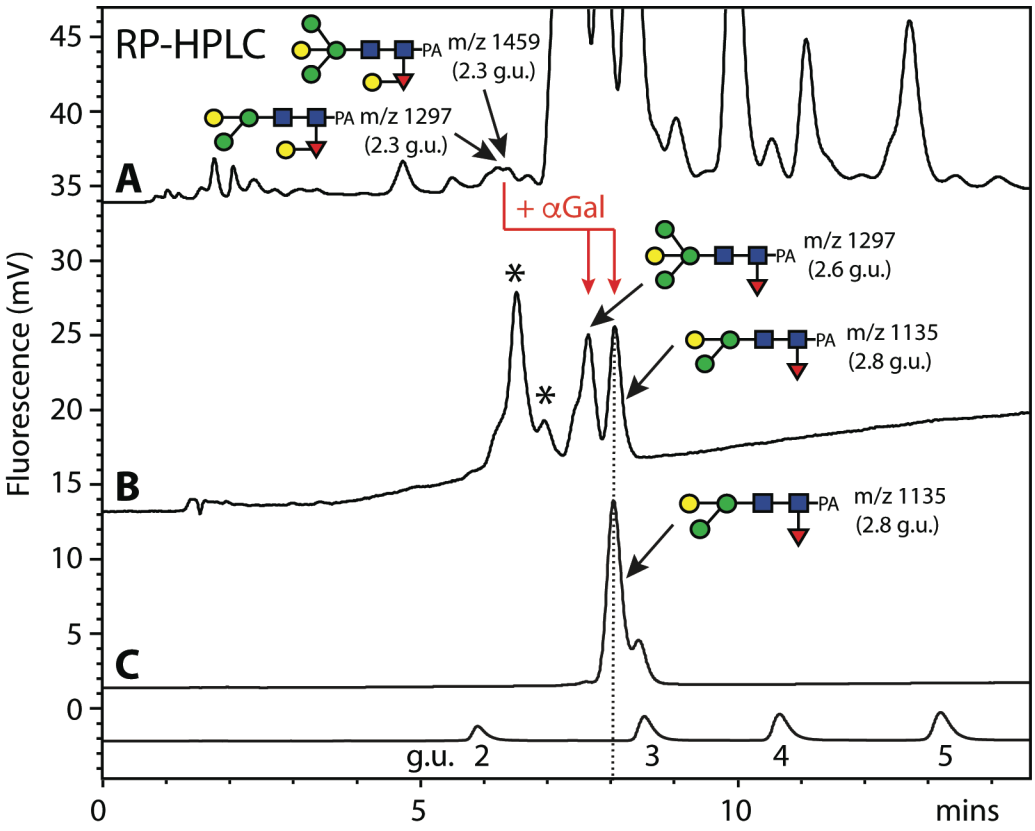
Supplementary Figure 1. RP-HPLC chromatograms of N-glycans released by PNGases and hydrazine. N-glycans were released from glycopeptides of the N2 wild type *C. elegans* (i) hydrazine (**A**), or (ii) PNGase F, PNGase A and PNGase Ar (**B-D**), (iii) PNGase A and PNGase Ar (**E, F**), (iv) two rounds of PNGase Ar (**G, H**) resulting in eight different glycan pools (see also **Scheme**) which were labelled with 2-aminopyridine and separated on RP-HPLC; the fluorescence intensities are normalised to the highest peak in each chromatogram. A partially-hydrolysed dextran standard was used to calibrate the column and the glucose units are indicated on the chromatograms (some minor day-to-day shifts occur). Dominant glycan structures, including $Man_{3,8,9}GlcNAc_2$ (Man3, Man8B, Man9) are annotated and used as "landmarks" to align the chromatograms run on different days from different nematode cultures. For the two rounds of PNGase Ar release, it is estimated (based on fluorescence intensities) that the second digestion step released less than 5% of the glycan amount as compared to the first.



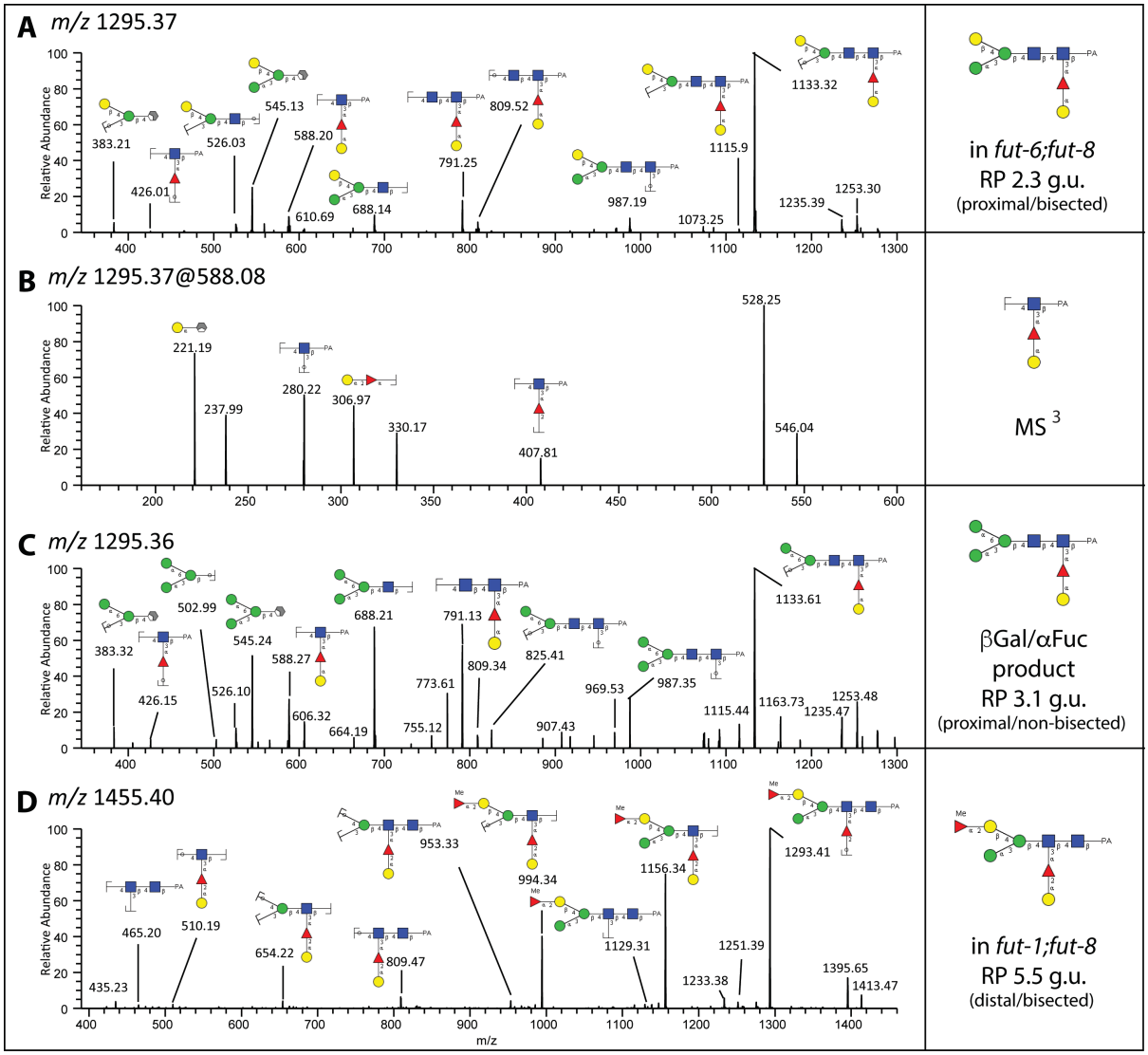
Supplementary Figure 2. Detection of two HexFuc modifications on the proximal core GlcNAc of enzymatically-released wild-type N-glycans. Five N-glycans ($Hex_{6-7}HexNAc_2Fuc_{2-3}Me_{0-1}$), released by PNGase Ar from glycopeptides of the wild type *C. elegans*, co-eluted with the oligomannosidic Man8B structure on RP-HPLC between 4.4 to 4.7 g.u. (**A-C**). Novel fragment ions at m/z 916 ($Hex_2HexNAc_1Fuc_2$-PA) were observed on the MS/MS spectra of these glycans (**D–H**, shown in red); the loss of two 308 (HexFuc) from their parent ions was indicative of a di-substitution on the proximal GlcNAc residue, occurring at the 3-OH and the 6-OH position. The major *m/z* 1799 glycan in the 4.4 g.u. fraction is the Man8B isomer of $Man_8GlcNAc_2$ as defined in previous studies.



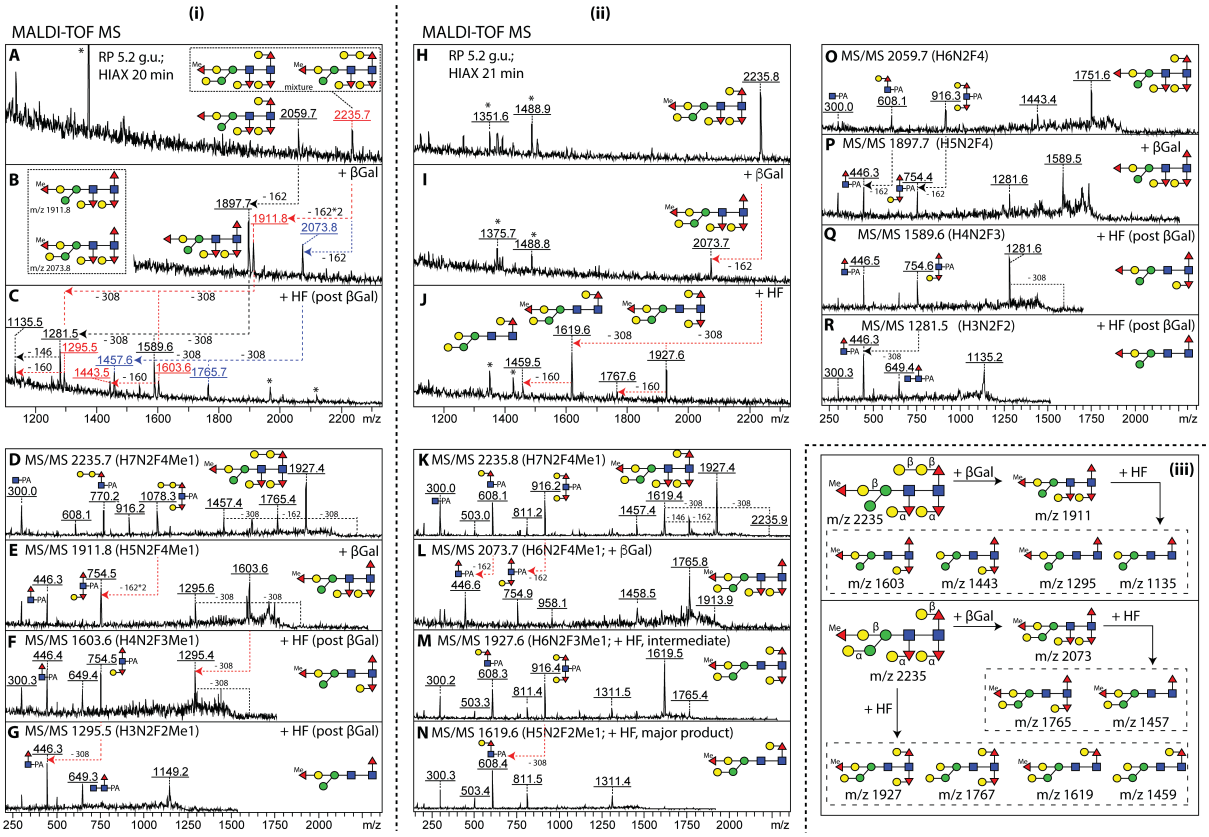
Supplementary Figure 3. MALDI-TOF MS comparison of RP-HPLC fractions in selected regions. Different sets of glycans were released from wild type *C. elegans* using PNGase F, PNGase A post F, PNGase A alone, PNGase Ar alone (first and second rounds) or hydrazine and identified by MALDI-TOF mass spectrometry. Examples are given for three different RP-HPLC regions: (i) glycans substituted with two GalFuc motifs on the proximal core GlcNAc ($Hex_{6-7}HexNAc_2Fuc_{2-3}Me_{0-1}$) eluted between 4.2 to 4.7 g.u., e.g., the *m/z* 1767 glycan, which was only observed in fractions from PNGase Ar or hydrazine but neither from PNGase F nor native PNGase A; (ii) tetra-fucosylated structures carrying three core GalFuc motifs eluted between 5.0 to 5.5 g.u., e.g., $Hex_{6-7}HexNAc_2Fuc_4Me_1$ (*m/z* 2073 and 2235), which were only observed in fractions from PNGase Ar or hydrazine; (iii) between 6.2 to 6.7 g.u. eluted one of the predominant structures $Hex_5HexNAc_2Fuc_2$ (*m/z* 1605), which was only observed after treatment with PNGase A or Ar or hydrazine, but was most pronounced in the PNGase Ar alone pool (see S**upplementary Figure 1**); its sensitivity to β-galactosidase treatment (loss of two hexoses to *m/z* 1281) and the associated changes in the core Y-fragments (*m/z* 608/754 to *m/z* 446/592) indicated that the structure contains β1,4-galactose both on the α1,6-fucose and the β1,4-mannose. The pyridylaminated glycans were detected as either $[M+H]^+$, $[M+Na]^+$ or $[M+K]^+$ or mixtures thereof; MS/MS was performed on the protonated parent ion of *m/z* 1605. Non-glycan impurities are indicated with asterisks.



Supplementary Figure 4. RP-HPLC chromatograms of N-glycans from a *fut-6;fut-8* double knockout. After treatment with a α-galactosidase, glycans from an early RP-HPLC fraction (at 2.3 g.u., **A**; see also **Figure 3** in the main text) were re-separated on RP-HPLC and collected fractions were re-analysed by MALDI-TOF MS/MS. Two products were observed in two peaks eluting at 2.6 g.u. and 2.8 g.u. (**B**) as judged by the glucose units of a PA-labelled dextran standard; the latter product (*m/z* 1135) co-eluted with a previously published structure of this mutant ($Gal_1Man_2GlcNAc_2Fuc_1$-PA) at 2.8 g.u. (**C**), whereas the former (*m/z* 1297) displayed the same elution time (2.6 g.u.) and MS/MS pattern as a bisected structure ($Gal_1Man_3GlcNAc_2Fuc_1$-PA) also previously found in this mutant. Asterisks indicate fractions containing non-glycan impurities.



Supplementary Figure 5. LC-MS^n analyses of galactosylated core fucose containing N-glycans from mutant *C. elegans*. **(A)** An enriched RP-HPLC fraction (2.3 g.u., also see in **Figure 3A**) containing an N-glycan from the *fut-6;fut-8* strain with the composition of $Hex_4HexNAc_1Fuc_1$-PA (*m/z* 1295, $[M-H]^-$) was analysed by negative-ion mode LC-MS/MS; **(B)** the MS^3 spectrum of fragmentation ions *at m/z* 588 suggests the presence of a galactosylated core fucose linked to the proximal GlcNAc-PA; **(C)** MS^2 of an exo-glycosidase product (*m/z* 1295, $[M-H]^-$) of the wild-type *m/z* 1767 glycan (see **Figure 2**); this isomer shows different intensities of some ions due possessing the normal trimannosyl core rather than a dimannosyl bisected galactose core as is the case for the glycan from the *fut-6;fut-8* strain; **(D)** MS^2 of an $Hex_4HexNAc_2Fuc_2Me_1$-PA N-glycan (*m/z* 1455, $[M-H]^-$) from the *fut-1;fut-8* mutant with a galactosylated fucose on the distal GlcNAc showing a different fragmentation pattern.



Supplementary Figure 6: Characterisation of tetrafucosylated N-glycans. (**i**) A 2D-HPLC purified fraction (5.2 g.u. on RP-HPLC, ≈20 min on HIAX; see **Figure 4** in the main text) contained two *m/z* 2235 isomers ($Hex_7HexNAc_2Fuc_4Me_1$) as judged by the MS/MS fragmentation; upon treatment with native *A. oryzae* β-galactosidase, these were converted into two final products of either *m/z* 2073 and 1911 (**A, B**), whereby the latter no longer presented key ions at *m/z* 770 and 1078, indicating removal of two β1,4-linked galactose residues from the core α1,6-fucose (compare **D and E**). Subsequent hydrofluoric acid treatment (**C**) resulted in major products at *m/z* 1295 ($Hex_3HexNAc_2Fuc_2Me_1$) and 1457 ($Hex_4HexNAc_2Fuc_2Me$) as well as intermediate ones of 1603 and 1765, which together with the shifts of key ions (from *m/z* 754 to 446) indicated the loss of both distal and proximal 3-linked GalFuc units (**F and G**). **(ii)** A second 2D-HPLC fraction (≈21 min on HIAX) contained only one isomer of *m/z* 2235, whose sensitivity to *A. oryzae* β-galactosidase (loss of one hexose; **H** and **I**) was accompanied by shifts of diagnostic ions from *m/z* 916 to 754 and from *m/z* 608 to 446 (**K** and **L**). Hydrofluoric acid treatment alone resulted in a sequential loss of two GalFuc units of 308 Da as evidenced by major products at *m/z* 1927 and 1619 (**J**) and a shift of the diagnostic ions from *m/z* 916 to 608 (**M** and **N**). The *m/z* 2059 glycan in the 20 min fraction was converted by serial treatment to structures of *m/z* 1897 (loss of galactose) and then of 1589 or 1281 (loss of two GalFuc units) concomitantly with the conversion of diagnostic ions at *m/z* 916 to 754 and *m/z* 608 to 446 **(A-C** and **O-R**). Asterisks indicate non-glycan impurities; red and blue dashed lines indicate the digestion pathway for minor and major *m/z* 2235 isomers and black for the glycan of *m/z* 2059, while partial removal of methylfucose from the bisecting galactose is shown by the loss of 160 Da. **(iii)** The proposed isomeric structures as well as structures of products upon galactosidase or hydrofluoric acid treatments; for data on α-galactosidase digestion as a further structural proof see **Figure 4**.

Further information regarding the glycomic analyses

Definition of the level of the glycan structural analysis: The goal was to compare different release methods (hydrazine vs. peptide:N-glycosidases) with wild-type *Caenorhabditis elegans* and to resolve unusual core modifications. The well-established N-glycan structures (paucimannosidic, oligomannosidic and phosphorylcholine-modified) were detected as determined by MS and MS/MS, but were not analysed in more detail.

Search parameters and acceptance criteria

a. **Peak lists:** As stated in the methods section: typically 1000 shots were summed for MS and 3000 for MS/MS. Spectra were processed with the manufacturer's software (Bruker Flexanalysis 3.3.80) using the SNAP algorithm with a signal/noise threshold of 6 for MS (unsmoothed) and 3 for MS/MS (four-times smoothed).
b. **Search engine, database and fixed modifications:** All glycan data were manually interpreted and no search engine or database was employed; the fixed modification is the pyridylamine label at the reducing end ($GlcNAc_1$-PA Y-fragments of *m/z* 300).
c. **Exclusion of known contaminants and threshold:** All glycan data were manually interpreted; only peaks with an MS/MS consistent with a pyridylaminated chitobiose core were included – the 'threshold' for inclusion was an interpretable MS/MS spectrum (at least in terms of composition).
d. **Enzyme specificity:** A description of the release methods (chemical or enyzmatic) is given in the methods section. Enzymes used during the analysis (glycosyl hydrolases) are defined in the methods by species name and supplier. Citations for in-house purified recombinant enzymes are also given. As previous experience with normalizing exoglycosidase amounts based on units of activity towards *p*-nitrophenyl sugars reduced digestion efficiency towards native oligosaccharides, aliquots of glycans (2 - 20 pmol) were incubated with 0.2 µl of the various enzyme preparations (whether commercial or in-house produced) overnight These conditions result in no obvious unspecific removal of residues as defined by shifts in mass, MS/MS or retention times, although steric hindrance in some glycans leads to a requirement for longer incubation times (48 hours). Ammonium acetate buffers were used (supplemented with $CaCl_2$ where required) as suppliers' buffers interfere with MALDI-TOF MS analysis; generally one-quarter of any glycosidase digest was applied directly to the target plate prior to drying and addition of matrix. Hydroflouric acid treatment (3 µl of 48% HF added to the dried glycan) was 24 or 48 hours on ice in the cold room prior to drying under vacuum; expected release of α1,3-fucose (also partial release in the case of α1,2-fucose), but not of other sugars, was observed under these conditions.

   Peptide:N-glycosidases: PNGase F, PNGase A and PNGase Ar (from respectively *Flavobacterium meningosepticum*, *Prunus amygdalus* and *Oryza sativa*) are, as shown by this and other studies, enzymes with different restrictions as to their de-N-glycosylation capability; recombinant PNGase F cannot release glycans with core α1,3-fucose (although it can release structures with core β1,3-mannose), while native PNGase A can release core α1,3-fucosylated glycans from glycopeptides (but not those with a core α1,3-fucose substituted by another monosaccharide). Information from the commercial supplier and our own data indicate that PNGase Ar can deglycosylate N-glycoproteins and can remove glycans with substituted core α1,3-fucose residues from glycopeptides.

Fucosidases: Bovine α-fucosidase (Sigma) removes core α1,6-fucose and α1,2-fucose, but not core α1,3-fucose; hydrofluoric acid was effective for the removal of α1,3-fucose and, to a lesser extent, α1,2-(methyl)fucose. Neither α1,2- nor α1,3/4-fucosidases were employed in this study.

Galactosidases: Coffee bean α-galactosidase (Sigma or Prozyme) was effective for removal of the α-galactose attached to either the proximal core α1,3-linked fucose or the α1,3-linked mannose. As previously reported, recombinant *Aspergillus niger* β-galactosidase (produced in house) removes the bisecting β1,4-galactose as well as the β1,4-galactose directly attached to the core α1,6-fucose residue. On the other hand, only native *Aspergillus oryzae* β-galactosidase could remove the digalactosyl Galβ1,4Galβ1,4 unit from the core α1,6-fucose.

Mannosidases: *Xanthomonas* α1,2/3-mannosidase (New England Biolabs) was used for the specific removal of the α1,3-mannose attached to the core β-mannose. Neither non-specific jack bean nor α1,6-mannosidases were used in this study.

e. **Isobaric/isomeric assignments:** For isomeric species, 2D-HPLC elution and/or digestion data were used for the assignment (as described in the text).

Glycan or glycoconjugate identification

a. **Precursor charge and mass/charge (*m/z*):** All glycans detected were singly-charged. For the positive mode, the *m/z* values are for protonated forms, whereas in negative mode the ions are $[M-H]^-$. Depending on the glycan amount or presence of buffers in exoglycosidase preparations, the relative amounts of the H^+ and Na^+ adducts varied. Maximally two decimal places are used for the *m/z* consistent with the accuracy of MALDI-TOF MS; in the figures and due to space limitations, only one decimal place is presented. Previous data indicate an average +0.03 Da (+ 22 ppm) deviation between the measured and the calculated *m/z* values on the instrument used.
b. **All assignments:** For the glycans present in each pool, see the HIAX and RP-amide-HPLC chromatograms annotated with structures shown according to the Standard Nomenclature for Glycans. Downwardly- and upwardly-drawn core fucose and mannose residues are respectively α1,3- and α1,6-linked.
c. **Modifications observed:** Listed are the *m/z* values for glycans carrying a reducing terminal pyridylamine group as judged by presence of an *m/z* 300 $GlcNAc_1$-PA fragment. As the glycans are otherwise chemically unmodified, *Δm/z* of 146, 160, 162, 176 and 203 correspond to deoxyhexose (presumed to be fucose), methylated fucose, hexose (mannose or galactose), methylated hexose or *N*-acetylhexosamine (here only *N*-acetylglucosamine).
d. **Number of assigned masses:** Glycan assignments were not just based on measured mass only, but on at least MS/MS, in most cases corroborated by digest and elution data.
e. **Spectra:** Representative annotated spectra (MS and MS/MS) defining structural elements are given in various figures.
f. **Structural assignments:** As noted in the results section, the typical oligomannosidic structures are assigned based on elution time and fragmentation pattern; it is otherwise assumed that the glycans contain a mannosyl core consistent with typical eukaryotic N-glycan biosynthesis. Assignments of antennal and core fucose residues are based on RP-HPLC retention time, fragmentation pattern and/or susceptibility to digestions. Example structures were also analysed by LC-ESI-MS^n.