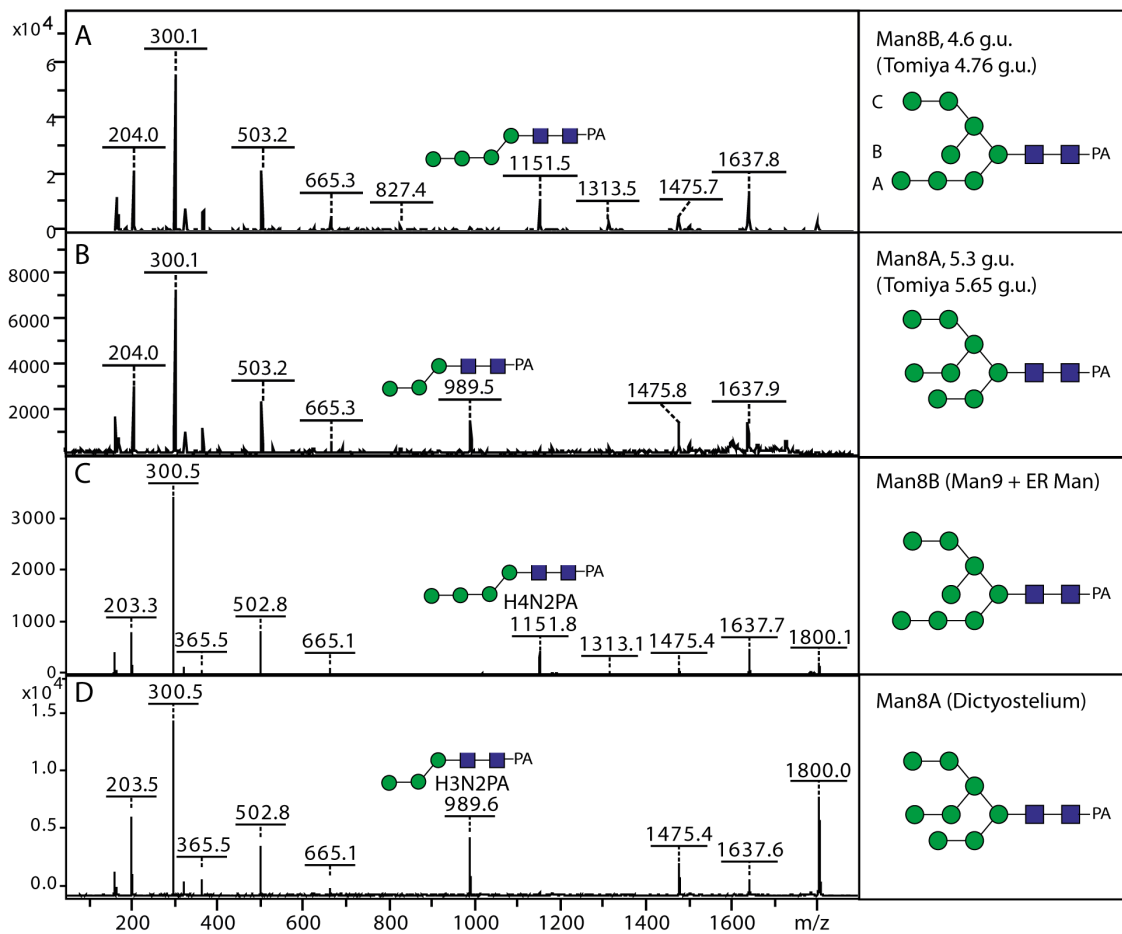


COMPARISON OF RP-HPLC MODES TO ANALYSE THE N-GLYCOME OF THE FREE-LIVING NEMATODE *PRISTIONCHUS PACIFICUS*

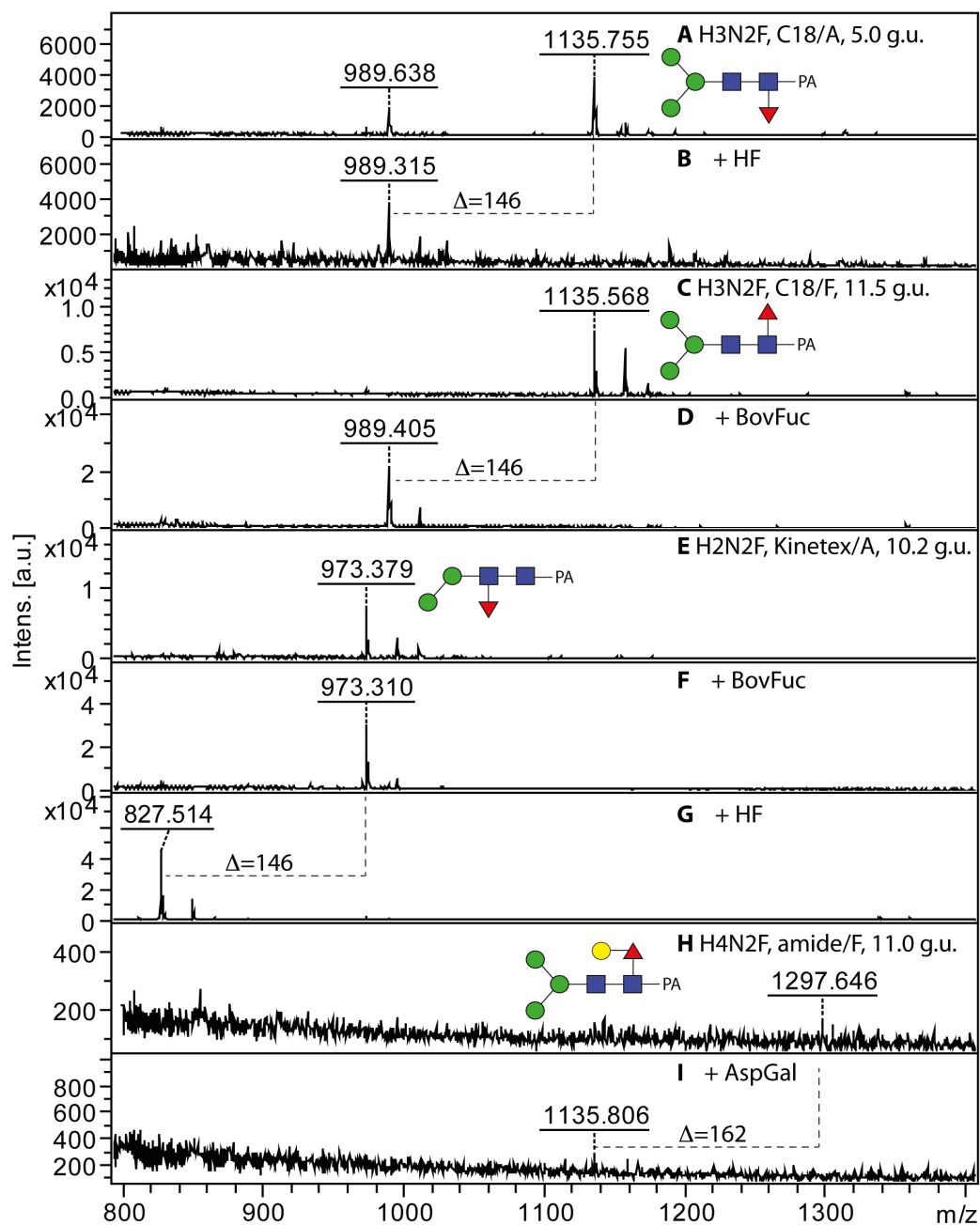
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Supplementary Data

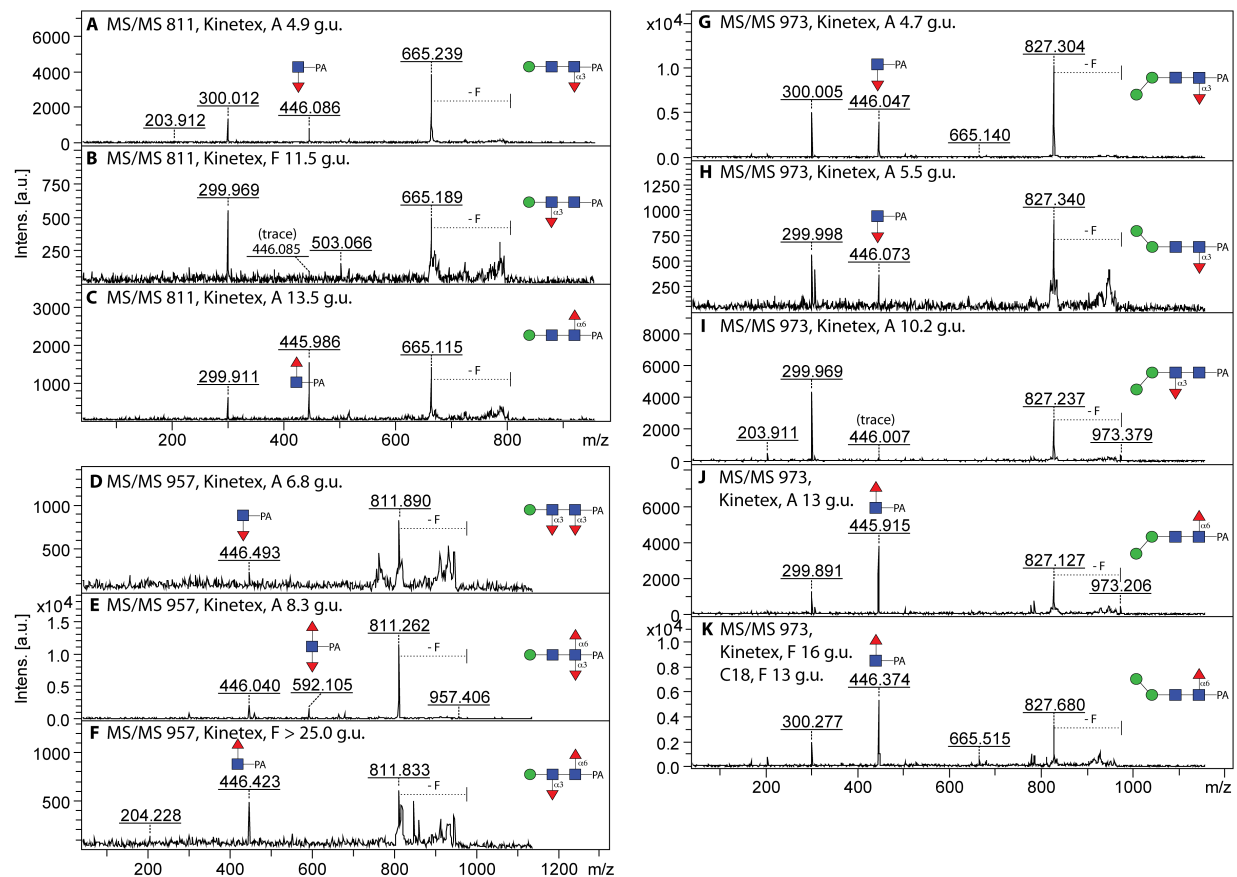
Supplementary Figure 1. Fragmentation patterns of Man₈GlcNAc₂ isomers: The MALDI-TOF MS/MS spectra for the two isomers of Man₈GlcNAc₂ found in different RP-HPLC fractions of the pyridylamino-labelled pool of the *Pristionchus* PNGase F released N-glycans (A, B) are compared to a standard Man₈B (generated by the action of *Caenorhabditis elegans* ER mannosidase on commercial Man₉GlcNAc₂; C) and the Man₈A isomer isolated from *Dictyostelium discoideum* (D). The latter two spectra are also presented in Supplementary Figure 4 of Ref. 32. The different branches (A, B and C) of the Man₈ structure are also annotated as are the RP-HPLC glucose unit values given by Tomiya *et al* in their 1991 paper (Ref. 41). Note the absence of the *m/z* 1151 fragment from Man₈A, which instead has an *m/z* 989 fragment; this pattern is interpreted as indicating loss of the α 1,6-antenna (B and C branches) during MS/MS.



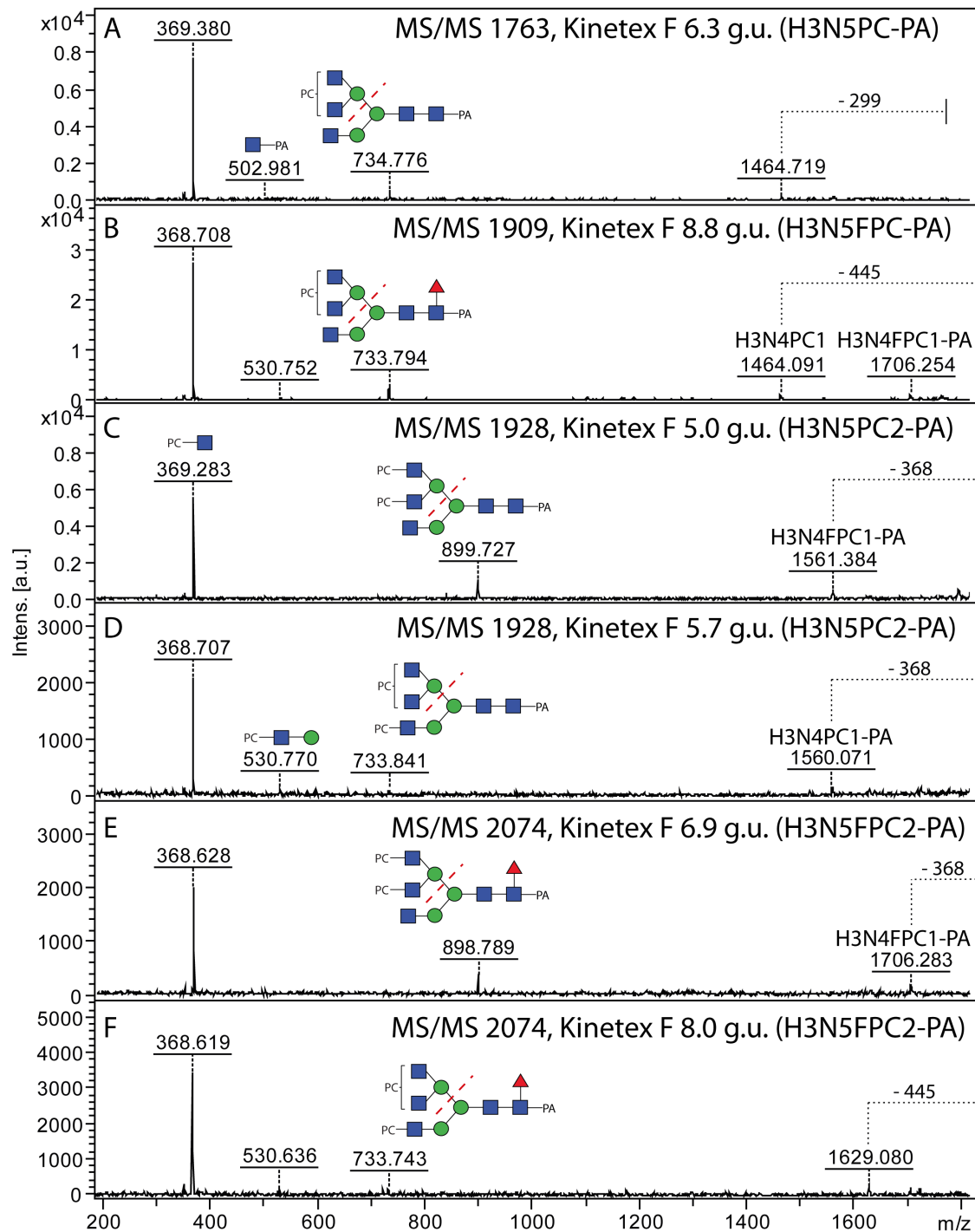
Supplementary Figure 2. Exoglycosidase and chemical treatments of monofucosylated N-glycans: The effect of hydrofluoric acid (*B* and *G*), bovine α -fucosidase (*D* and *F*) or *Aspergillus* β -galactosidase (*I*) on four different monofucosylated pyridylamino-labelled *Pristionchus* N-glycans ($\text{Hex}_{2-4}\text{GlcNAc}_2\text{Fuc}_1$) as shown by MALDI-TOF MS. Note that the $\alpha 1,3$ -fucose on the proximal or distal GlcNAc residues is sensitive to HF, whereas $\alpha 1,6$ -fucose on the proximal GlcNAc is sensitive to bovine α -fucosidase (the relevant panel *D* is the non-magnified version of Figure 6E) and the galactose attached to core fucose is lost upon *Aspergillus* galactosidase treatment; loss of the relevant m/z 446 or 608 core fragments was confirmed. Other data on hydrofluoric acid, fucosidase and galactosidase sensitivities of related structures are shown in Figure 5 and 6 of the main text. The annotated MS/MS spectra of the original glycans *A* (m/z 1135), *C* (m/z 1135), *E* (m/z 973) and *H* (m/z 1297) are shown in Figure 4 (panels A, B, C and G) of the main text.



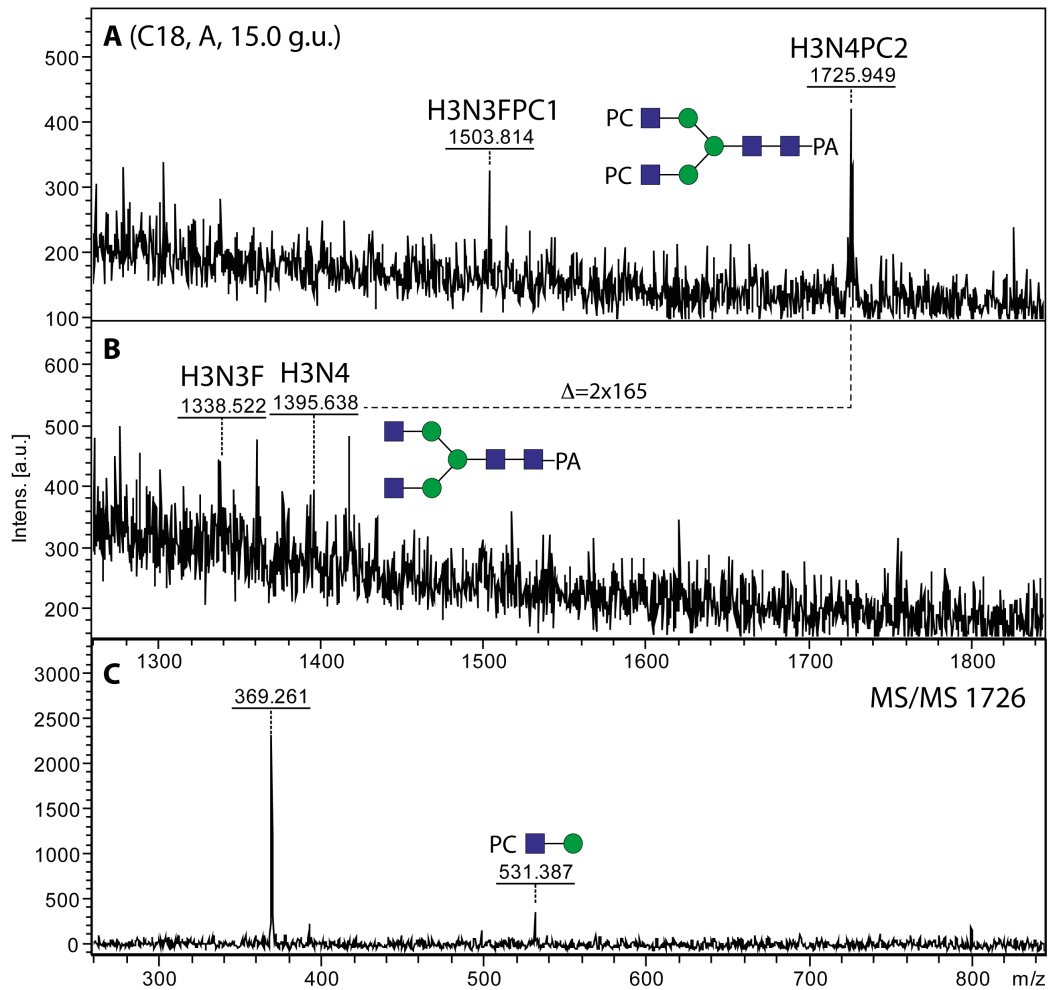
Supplementary Figure 3. Fragmentation patterns of fucosylated pyridylamino-labelled N-glycans: MS/MS spectra for the three differently-eluting isomers of $\text{Man}_1\text{GlcNAc}_2\text{Fuc}_1$ (m/z 811; A-C), three isomers of $\text{Man}_1\text{GlcNAc}_2\text{Fuc}_2$ (m/z 957; D-F) and five isomers of $\text{Man}_2\text{GlcNAc}_2\text{Fuc}_1$ (m/z 973; G-K) from *Pristionchus* (panel I is also as shown in Figure 4C of the main text). As mentioned in the main text: α 1,6-fucosylation of the reducing-terminal proximal GlcNAc results in strong m/z 446 fragments (C, F, J, K), α 1,3-fucosylation of the proximal GlcNAc results in m/z 300 and 446 fragments of approximately equal intensity (A, G, H), difucosylation of the proximal GlcNAc results in m/z 446 and 592 fragments of approximately equal intensity (E) and α 1,3-fucosylation of the distal (second core) GlcNAc is often associated with weak (trace) m/z 446 fragments resulting from a low level of fucose rearrangement (B, D). Loss of one fucose from the parent ion is indicated by ‘F’.



Supplementary Figure 4. MALDI-TOF MS/MS analysis of triantennary N-glycans modified with one or two phosphorylcholine residues. Further examples of the fragmentation patterns of phosphorylcholine-modified pyridylamino-labelled N-glycans from *Pristionchus*, including two isomers each of Hex₃HexNAc₅Fuc₀₋₁PC₂. The overall predicted structures are shown with an indication of the predicted fragmentation position for the diagnostic *m/z* 734 and 899 ions (panel A is a further version of Figure 8E in the main text). Losses of either the core GlcNAc-PA or GlcNAcFuc-PA or an antennal GlcNAc-PC is indicated by the loss of 299, 445 or 368.

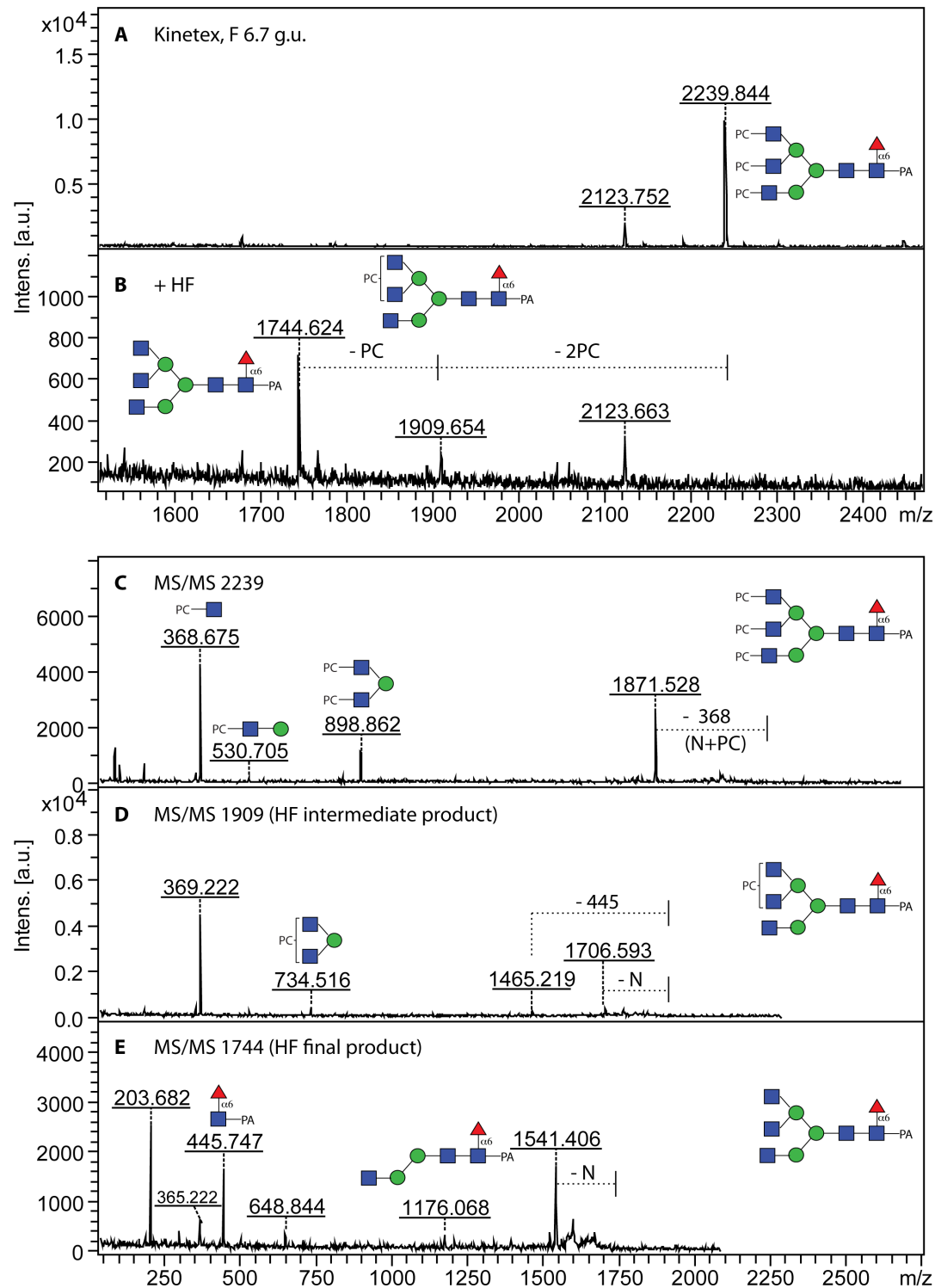


Supplementary Figure 5. MALDI-TOF MS and MS/MS of Hex₃HexNAc₄PC₂: A biantennary N-glycan predicted to carry two phosphorylcholine residues (*m/z* 1725; A) was treated with hydrofluoric acid prior to MALDI-TOF MS (B); the *m/z* 1503 glycan is carried over from the neighbouring fraction (for its analysis see Figure 9 D-F of the main text). The products are primarily present as sodiated adducts, but the protonated ions are annotated. MS/MS of the original glycan (C) shows phosphorylcholine-containing fragments of *m/z* 369 and 531 GlcNAc (Hex₀₋₁HexNAc₁PC₁) compatible with phosphorylcholine modification of non-reducing terminal.



Supplementary Figure 6. Chemical treatment of a phosphorylcholine-modified N-glycan:

A triantennary core fucosylated N-glycan predicted to carry three phosphorylcholine residues (A) was treated with hydrofluoric acid prior to MALDI-TOF MS (B). MS/MS of the original glycan (C) and of the intermediate (D) and final products (E) show the dominance of the phosphorylcholine-containing fragments (m/z 369, 530, 734 and 898); only with the final product is the fragment diagnostic of the core fucose (m/z 446) observed.



Supplementary Table. Summary of evidence for the proposed structures of *Pristionchus* N-glycans. An extended version of Table 1 (see main text) is presented with brief summaries of the evidence for the proposed structures referring also to relevant figures, supplementary figures and citations. Retention times for the three glucosylated N-glycans (Glc₁₋₃Man₉GlcNAc₂; not shown for reasons of space in Table 1) are also included.

Structure	<i>m/z</i>	Retention time			Evidence
		RPC18	RP-amide	Kinetex	
	665.28	A/F 6.4	A/F 6.2	F 7.8	Based on biosynthetic pathways Tomiya, <i>et al.</i> (1991) <i>Anal Biochem</i> 193 , 90; 6.60 g.u.
	811.35	A 4.3	A 4.3	A 4.9	Only found in PNGase A released pool. Key fragment: 446 [suppl. Fig 3A]
	811.35	A/F 7.7	F 7.5	A/F 11.5	Trace amount of 446 fragment due to rearrangement [suppl. Fig 3B]
	811.35	A/F 10.3	A/F 8.2	A/F 13.5	[Fig 3E and F] Key fragment: 446 [suppl. Fig 3C]
	827.34 (0M)	A/F 5.9	A/F 6.1	A/F 7.5	[Fig 3B, sensitive to alpha-1,2/3 mannosidase] Tomiya, <i>et al.</i> (1991); 6.59 g.u.
	827.34 (M0)	A/F 7.7	A/F 7.2	A/F 9.5	Tomiya, <i>et al.</i> (1991); 7.40 g.u. Sensitive to alpha-1,6 mannosidase
	957.40	A 5.9	n.d.	A 6.8	Only found in PNGase A released pool. Key fragment: 446 [suppl. Fig 3D]
	957.40	A 7.0	A 6.3	A 8.3	Only found in PNGase A released pool. Key fragment: 592 [suppl. Fig 3E] [also Fig 3I]
	957.40	n.d.	A 8.7	F > 25.0	Key fragment: 446 [suppl. Fig 3F]
	971.42	A 19.0	A 9.5	A >25	Only found in PNGase A released pool. Key fragments: 446 and 606 [Fig 5E and F, sensitive to bovine fucosidase and HF]
	973.40 (0MF ³)	A 4.2	A 4.5	A 4.7	Only found in PNGase A released pool. Key fragment: 446 [suppl. Fig 3G]
	973.40 (M0F ³)	A 5.0	A 5.0	A 5.5	Only found in PNGase A released pool. Key fragment: 446 [suppl. Fig 3H] Sensitive to alpha-1,6 mannosidase
	973.40	A/F 7.7	F 7.2	A/F 10.2	[Suppl. Fig 2 F and G, resistant to bovine fucosidase but sensitive to HF] Sensitive to alpha-1,2/3 mannosidase Key fragment: trace 446, due to re-arrangement [suppl. Fig 3I]
	973.40 (0MF ⁶)	A/F 9.8	A/F 8.2	A/F 13	Key fragment: 446 [suppl. Fig 3J] [Fig 3D, sensitive to alpha-1,2/3 mannosidase] Sensitive to bovine fucosidase
	973.40 (M0F ⁶)	A/F 13.0	A/F 9.0	A/F 16	Key fragment: 446 [suppl. Fig 3K] Sensitive to bovine fucosidase

	989.39 (MM)	A/F 7.2	A/F 7.2	A/F 9.5	Tomiya, <i>et al.</i> (1991); 7.39 g.u. [Fig 3J, sensitive to α 1,2/3 mannosidase] Trimannosyl core structure as found in many invertebrates
	1030.42 (0Gn)	A/F 6.5	A/F 6.1	A/F 7.5	[Fig 7B, sensitive to FDL]
	1119.46	A 5.2	A 5.4	A 6.8	Only found in PNGase A released pool. Key fragment: 446 Sensitive to α 1,2/3 mannosidase
	1119.46	A 7.0	A 6.3	A 8.3	Only found in PNGase A released pool. [Fig 3J, sensitive to α 1,2/3 mannosidase] Key fragment: 592 [Fig 4D]
	1119.46	F 13.0	n.d.	F >25.0	Key fragments: 446 [Fig 4E] Sensitive to bovine fucosidase
	1135.45 (MMF ³)	A 5.0	A 5.1	A 5.5	Only found in PNGase A released pool. Key fragment: 446 [Fig 4A] HF sensitive [Suppl. Fig. 2B]
	1135.45 (MMF ⁶)	A/F 11.5	A/F 9.5	A/F 16.0	Key fragment: 446 [Fig 4B] Tomiya, <i>et al.</i> (1988) 10.2 g.u. [Fig 6D and E, sensitive to α 1,2/3 mannosidase and bovine fucosidase; also Suppl. Fig. 2D]
	1176.48	n.d.	F 8.2	F 13.0	Based on structure of m/z 1030 Key fragment: 446 Elution shift as compared to 1030 caused by core α 1,6-fucose
	1151.45	A/F 7.2	A/F 7.2	A/F 9.5	[Fig 6H, sensitive to α 1,2/3 mannosidase] Fits order of Golgi mannosidase II: Rose (2012) <i>Curr Opin Struc Biol</i> 22, 558 Elution: Tomiya, <i>et al.</i> (1991); 7.49 g.u. Key fragment: 827
	1192.47 (MGn)	A/F 7.0	A/F 6.8	A/F 8.4	[Fig 3J, resistant to α 1,2/3 mannosidase] [Fig 7B, sensitive to FDL] For elution see also: Gutternigg, <i>et al.</i> (2007) <i>J Biol Chem</i> 282, 27825
	1192.47 (GnM)	A/F 10.3	A/F 9.0	F/A 16.0	[Fig 3F and Fig 7D, sensitive to α 1,2/3 mannosidase] Resistant to FDL For elution see: Gutternigg, <i>et al.</i> (2007)
	1195.47	A/F 7.7	A/F 5.5	A/F 6.9	Based on structure of m/z 1030 Due to PC, C18 elution later and Kinetex/amide elution earlier Key fragments: 369; 531; HF sensitive
	1265.51	A 8.0	A 6.8	A 9.2	Only found in PNGase A released pool. Key fragments: 446 and 592 [Fig 4H] [Fig 6H and I, sensitive to α 1,2/3 mannosidase and HF (loss of 2 fucose residues)]
	1265.51	A 9.8	A 8.0	A 11.2	Only found in PNGase A released pool. Key fragments: 446; 592 [Fig 6J] [Fig 6K, sensitive to HF, loss of 308 and 146]
	1281.51 (MMF ³ F ⁶)	A 7.2	A 6.8	A 8.5	Only found in PNGase A released pool. [Fig 5B and C, sensitive to bovine fucosidase and HF, loss of 1 fucose residue] Key fragments: 446 and 592
	1297.50	A 5.0	A 5.0	A 5.8	Only found in PNGase A released pool. Resistant to α 1,6 mannosidase Based on structure of Man4GlcNAc2 Key fragments: 446

	1799.66 (Man8A)	A/F 5.3	F 5.8	A/F 6.0	Key fragment: 989 Tomiya, <i>et al.</i> (1991); 5.65 g.u. [Suppl. Fig. 1]
	1871.72	A/F >20	F 8.6	F 14.5	Key fragments: 369; 531 Based on 1725; later elution caused by core alpha1,6-fucose.
	1909.75	n.d.	F 6.8	F 8.8	Key fragments: 369; 531; 734 [Suppl. Fig. 4B] Elution shift as compared to 1763 caused by core alpha1,6-fucose
	1928.74	F 17.0	F 4.0	F 5.0	Key fragments: 369; 899 [Suppl. Fig. 4C]
	1928.74	n.d.	n.d.	F 5.7	Key fragments: 369; 531; 734 [Suppl. Fig. 4D]
	1961.71 (Man9)	A/F 4.9	A/F 5.5	A/F 5.3	Tomiya, <i>et al.</i> (1991); 5.33 g.u. Key fragment: 1151 Biosynthesis: typical Man9 resulting after action of glucosidases I and II on typical transferred Glc3Man9GlcNAc2
	2074.80	F > 20.0	n.d.	F 6.9	Key fragments: 369; 899 [Suppl. Fig. 4E] Elution shift as compared to 1928 caused by core alpha1,6-fucose
	2074.80	n.d.	F 6.3	F 8.0	Key fragments: 369; 734 [Suppl. Fig. 4F] Elution shift as compared to 1928 caused by core alpha1,6-fucose
	2093.80	A/F >20.0	A/F 4.0	F 4.8	Key fragments: 369; 899 [Fig 8F] Sensitive to HF, loss of three PC residues [Fig 9H]
	2123.76	F 6.0	F 6.6	F 6.7	Biosynthetic pathways Key fragment: 1313 Tomiya, <i>et al.</i> (1991); 6.29 g.u. Shift to later retention time as compared to Man9 observed for proven glycan from <i>Dictyostelium</i> M31 mutant (Hykollari <i>et al.</i> (2014) <i>Electrophoresis</i> 35, 2116
	2239.86	F >20.0	F 4.5	F 6.7	Key fragments: 369; 899 Sensitive to HF, loss of three PC residues; after HF, fragmentation as for 1744 with strong 446 fragment indicative of core alpha1,6-Fuc [Suppl. Fig 6]
	2285.81		F 6.3	F 6.6	Biosynthetic pathways (trace amount) Key fragment: 1475 Shift to later retention time as compared to Man9 observed for proven glycan from <i>Dictyostelium</i> M31 mutant (Hykollari <i>et al.</i> (2014) <i>Electrophoresis</i> 35, 2116
	2447.86		F 6.5	F 6.9	Biosynthetic pathways (trace amount) Key fragment: 1637