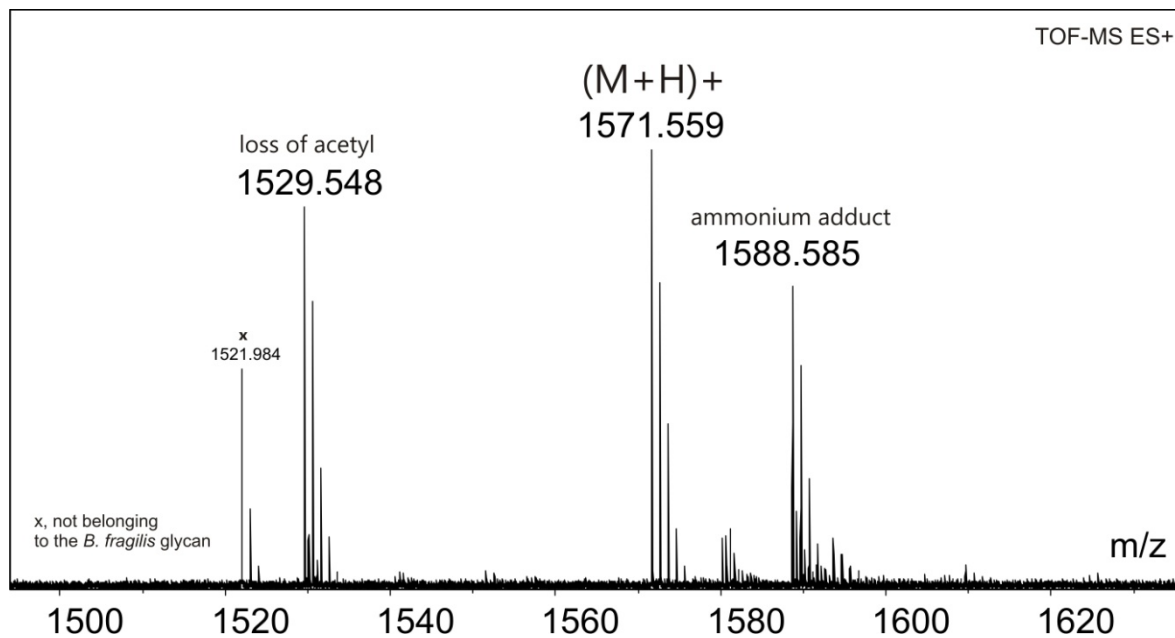
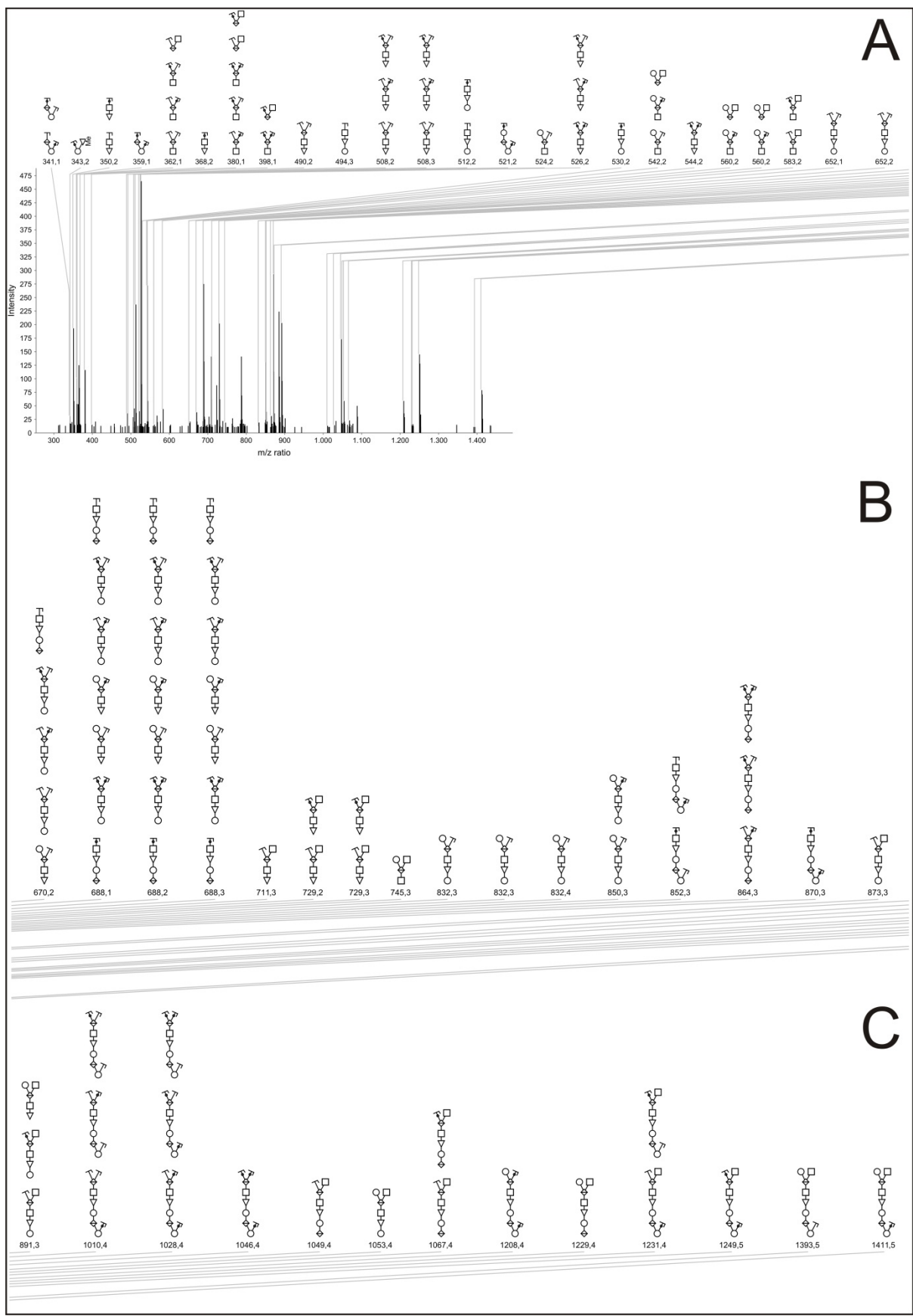


## SUPPLEMENTARY MATERIAL



**Fig S1.** High resolution ESI-TOF-MS spectrum of the Borohydride-reduced *B. fragilis* protein *O*-glycan showing a parent mass of 1571.56 Da. A partial loss of acetylation was observed. Mass analysis was performed on the Bruker Maxis 4G Q-TOF instrument.



**Fig. S2.** ABC. Annotation of fragment ions derived from the *B. fragilis* O-glycan according to GlycoWorkbench (Ceroni et al. 2008) and the potential *B. fragilis* glycan structure as assumed by ESI-MS/MS experiments.

Ceroni A, Maass K, Geyer H, Geyer R, Dell A, Haslam SM. 2008. GlycoWorkbench: A tool for the computer-assisted annotation of mass spectra of glycans. *J Proteome Res.* 7:1650-1659.

**Table S1.** Exact masses evaluated for sugar residues of the *B. fragilis* O-glycan after MS/MS experiments of the free reduced glycan and glycopeptides. Mass deviation to the supposed corresponding sugar residue is expressed in milli Dalton (mDa).

<b>possible sugar</b>	<b>measured</b>	<b>calculated</b>	<b>delta mDa</b>
<b>O-linking hexose**</b>	162,0534	162,0528	0,60
<b>methyl-deoxyhexose**</b>	160,074	160,07355	0,45
<b>„inner“ hexuronic acid*</b>	176,0311	176,0321	-1,00
<b>„middle“ hexose**</b>	162,0540	162,0528	1,20
<b>deoxyhexose**</b>	146,0584	146,0579	0,50
<b>„inner“ HexNAc**</b>	203,0873	203,0794	7,90
<b>„outer“ hexuronic acid*</b>	176,0326	176,0321	0,50
<b>„outer HexNAc“ */***</b>	203,0938	203,0794	14,40
<b>outer hexose*</b>	162,0569	162,0528	4,10

\*calculated from borohydride reduced oligosaccharides

\*\*calculated from glycopeptides

\*\*\*The presence of HexNAc is uncertain; the residue shows a partial loss of 42 Da, rather indicative of O-acetylation

**Table S2.** Summary of all glycopeptides as determined by heterologous glycosylation experiments.

**TfsA in *B. fragilis*, log<sub>e</sub> = -146.5, identified peptides:32**

Glycopeptide	Peptide mass [M+H] <sup>+</sup>	Glycopeptide mass (measured)	Deconvoluted glycopeptide mass / attached glycan portion	Position
NQT <u>DSAR</u>	791.36	1171.5 (M+2H) <sup>2+</sup>	2341/1550.64	939-945
TCGAS <u>DSVR</u>	952.40	1252.0 (M+2H) <sup>2+</sup>	2502/1550.6	191-199
LSFVPAIHAG <u>DTL</u> YILNGRPIEAFIQK	2983.64	1512.1 (M+3H) <sup>3+</sup>	4533.3/1550.66	968-994
<u>DTL</u> YVLK	964.57	n.d.*	-	602-609
TYFQLVIP <u>DSL</u> R	1451.79	n.d.*	-	613-624
LVYYPSE <u>DSL</u> VINVR	1766.93	n.d.*	-	332-346
FNTTDESAIGSDNP <u>DTL</u> KFHR	1924.87	n.d.*	-	804-821
HLDTDWAIYV <u>DTA</u> YVNR	2051.98	n.d.*	-	881-897
IDLSNFHYTDTITGLPIT <u>DTVR</u>	2554.28	n.d.*	-	727-748
FLAMDEFGYITLV <u>DSA</u> FIYGASVPADSSYR	3305.57	n.d.*	-	60-89
EDGGIAEAT <u>DSL</u> NWFAYTFQYLSGLSGNHYMGFDEK	4033.79	n.d.*	-	562-597

**TfsB in *B. fragilis*, log<sub>e</sub> = -137, identified peptides: 20**

FAT <u>DSVVR</u>	894.46	1223.06 (M+2H) <sup>2+</sup>	2444.12/1550.66	469-476
LMV <u>DTL</u> PR	944.5	1248.09 (M+2H) <sup>2+</sup>	2494.18/1550.68	489-496
TAIYV <u>DTA</u> YVNR	1385.70	1468.69 (M+2H) <sup>2+</sup>	2935.38/1550.68	1063-1074
AIT <u>DSTR</u>	763.39	n.d.*	-	462-468
GMYLINAT <u>DSA</u> K	1283.63	n.d.*	-	1114-1125
<u>DTA</u> TVNLYAFNYR	1547.75	n.d.*	-	751-763
<u>IDTV</u> NVDAGHYKPYGYGVK	2096.04	n.d.*	-	803-821
<u>DSL</u> IYVQGTNCFDIAYFR	2182.03	n.d.*	-	785-802
YLYV <u>DTV</u> YNSGNSQFLK	2174.04	n.d.*	-	397-414
EGANDGYYYLQV <u>DSM</u> VAYDK	2301.00	n.d.*	-	53-72
GVTLYMG <u>PSA</u> GQNALWIDVMGR	2451.18	n.d.*	-	104-126
GQYLHVPLYSAH <u>DSA</u> VWVFLDK	2545.29	n.d.*	-	610-631
GGRPLVSYLDET <u>MDTV</u> AVLVG <u>DTL</u> NK	2763.42	n.d.*	-	232-257
MVYYPSG <u>DSI</u> YINPFSATYLPYDPK	3002.42	n.d.*	-	436-461
GTGHKQPQYMLMIRPTIVE <u>DTL</u> GCCDDNGDLTIPLPGYR	4257.11	n.d.*	-	1075-1112

**Bf2494 in *T. forsythia*, log<sub>e</sub> = -217.8, identified peptides: 32\*\*\***

KPY <u>DTL</u> K	864.48	861.03 (M+3H) <sup>3+</sup> (- terminal Fuc, - Me**)	2580.09/1716.61	76-82
<u>GDTAK</u>	491.25	n.d.*	-	222-226
<u>DTIL</u> PQVAYYATLAADR	1880.98	n.d.*	-	170-186

**Bf3567 in *T. forsythia*, log<sub>e</sub> = -338.6, identified peptides: 24\*\*\***

LGDISL <u>DTV</u> AVR	1258.70	1041.14 (M+3H) <sup>3+</sup> (-Me**)	3120,4/1862.6	200-211
<u>GDS</u> INAK	704.36	807.65 (M+3H) <sup>3+</sup> (- terminal Fuc, - Me**)	2419.96/1716.61	138-144
IEIPATAVL <u>DSI</u> IYDNR	2016.10	n.d.*	-	323-340

\*neither free peptide nor glycosylated form was detected, possibly due to chromatographic properties

\*\*methyl group is missing on ManNAcCONH<sub>2</sub> residue

\*\*\* IonTrap MS/MS data were used for calculating X! TANDEM log<sub>e</sub> values