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Data Article

Glycan microarray analysis of the carbohydrate-recognition specificity of native and recombinant forms of the lectin ArtinM



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ABSTRACT

This article contains data related to the researc.h article entitled "Yeast-derived ArtinM shares structure, carbohydrate recognition, and biological effects with native ArtinM" by Cecílio et al. (2015) [1]. ArtinM, a D-mannose-binding lectin isolated from the seeds of *Artocarpus heterophyllus*, exerts immunomodulatory and regenerative activities through its Carbohydrate Recognition Domain (CRD) (Souza et al., 2013; Mariano et al., 2014 [2,3]). The limited availability of the native lectin (n-ArtinM) led us to characterize a recombinant form of the protein, obtained by expression in *Saccharomyces cerevisiae* (y-ArtinM). We compared the carbohydrate-binding specificities of y-ArtinM and n-ArtinM by analyzing the binding of biotinylated preparations of the two lectin forms using a neoglycolipid (NGL)-based glycan microarray. Data showed that y-ArtinM mirrored the specificity exhibited by n-ArtinM.

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Specification Table

Subject area More specific sub- iect area	Biology Glycobiology
Type of data	Graphs and table
How data was acquired	The data were generated from a NGL-based microarray system [4]. After binding analyses, the slide was scanned using ProScanArray microarray scanner (PerkinElmer) and the image files were quantified using ScanArray Express software (PerkinElmer).
Data format	A dedicated in-house-designed software suite was used for storing, retriev- ing and displaying carbohydrate microarray data [5], here as histogram charts (Fig. 1) and result table (Table 1).
Experimental factors	n-ArtinM and y-ArtinM forms were biotinylated and analyzed for binding using a NGL-based microarray (in-house designation 'Array Sets 18–22bis') containing 255 lipid-linked glycan probes (Table 1).
Experimental features	Glycan microarray analyses of an immunomodulatory lectin
Data source location	University of Sao Paulo, Brazil and Imperial College London, UK.
Data accessibility	The data are supplied with this article and will be online available at the Web Portal of Glycosciences Laboratory, Imperial College London: https://glycosciences.med.ic.ac.uk/data.html.

Value of the data

- The wide spectrum of glycans that constitute the glycan microarray makes this platform suitable to compare the carbohydrate-binding specificities exhibited by native and recombinant lectins.
- The data derived from the NGL-based microarray analyses provide important information on the carbohydrate binding specificities of y-ArtinM and n-ArtinM, and serve as the basis for further studies on the fine specificities of the lectins using other microarray systems or complementary techniques.

1. Data

In this study, we analyzed the native form of ArtinM and its yeast-derived counterpart, in terms of their ability to bind to 255 glycans distributed in a microarray platform, in order to identify whether n-ArtinM and y-ArtinM shared sugar-recognition specificity. Measurement of fluorescence intensity indicated that both preparations bound to *N*-glycan-related sequences (Fig. 1A and B), with a preference for probes having the core trimannoside Man α 1-3(Man α 1-6) Man. This binding intensity was enhanced when the probe contained a Fucose residue at the trimannoside core (Table 1 – probe 131); whereas binding was diminished when a similar position in the glycan was occupied by β 1-2-linked xylose (probes 130 and 132). Some differences between the two lectin forms were identified in the magnitude of binding to probes 129, 131, 133, 135, 147, 148, 149, 150, 152, 153, 158, 159 and 160. In general, y-ArtinM showed higher fluorescence intensity than n-ArtinM.



Fig. 1. Carbohydrate microarray analyses of n-ArtinM (*A*) and y-ArtinM (*B*). Numerical scores of the binding signals are means of duplicate spots at 7 fmol/spot (with *error bars*). The complete list of probes and their sequences and binding scores are in Table 1.

2. Materials and methods and data

2.1. Sample preparation

n-ArtinM was obtained from a saline extract of *Artocarpus heterophyllus* (jackfruit) seeds [6]. *Saccharomyces cerevisiae* BJ3501 was used to express y-ArtinM and the lectin was obtained by yeast lysis [1]. n-ArtinM and y-ArtinM were purified by affinity chromatography on a D-mannose column

Table 1

Oligosaccharide probes included in the microarray and the binding signals (means of the fluorescence intensity at 7 fmol/probe spot) of n-ArtinM and y-ArtinM.

			Fluoresce	nce signals
Pos. ^a	Probe ^b	Structure	n-ArtinM	y-ArtinM
1	Galactocerebrosides	Galβ-Cer	-	74
2	H-Di	Fuca-2Gal-DH	-	76
2	4 T.:	GalNAca-3Gal-DH		02
- 3	A-Iri	Fuca-2 Gala-3Gal-DH	-	83
4	B-Tri	Fuca-2	-	133
5	Sulfatide	SU-3Galβ-Cer	6	89
6	GSF-1	SU-3GalB-C30	-	-
7	Glucocerebrosides	GlcB-Cer	-	128
8	GSF-19	SU-6GlcB-C30	-	-
9	Lactocerebrosides	GalB-4GlcB-Cer	598	61
10	Lac	GalB-4Glc-DH	-	44
11	Lac-AO	Galβ-4Glc-AO	-	-
12	GalNAca-3Galß-4Glc	GalNAca-3Galβ-4Glc-DH	-	5
13	Ceramide trihexoside	Galα-4Galβ-4Glcβ-Cer	20	79
14	Globoside (P-antigen)	GalNAcB-3Gala-4GalB-4GlcB-Cer	136	106
15	Forssmann glycolipid	GalNAca-3GalNAcβ-3Gala-4Galβ-4Glcβ-Cer	258	321
16	NeuAcα-(3')Lac	NeuAca-3Gal8-4Glc-DH	-	25
17	NeuAcα-(3')Lac-AO	NeuAca-3GalB-4Glc-AO	-	-
18	Neu4,5Ac-(3')Lac	(4-OAc)NeuAcq-3Gal8-4Glc-DH	-	-
19	Neu4,5Ac-(3')Lac-AO	(4-OAc) NeuAcg-3Gal 8-4Gl c-AO	-	-
20	Neuα-(3')Lac	Neux-3Gal6-4Glc-DH	-	110
21	Neuα-(3')Lac-AO	Neug-3Ga16-4G1c-AO	-	-
22	NeuAca-(6')Lac	Neukow-66al6-46lc-DH	118	108
23	NeuAcα-(6')Lac-AO	Neukow-66a18-461c-A0	-	-
24	Neuα-(6')Lac	Neuro-6Ga18-4G1c-DH	-	-
25	Neuα-(6')Lac-AO	Neur-6Gal6-4Glc-AO	-	-
26	NeuAcß-(3')Lac	NeukoB-3GalB-4Glo-DH	-	-
27	NeuAcß-(3')Lac-AO	Neuach-3Galh-4Glc-AQ	-	-
28	NeuAcß-(6')Lac	NeuAcB-6GalB-4Glc-DH	88	96
29	NeuAcß-(6')Lac-AO	NeuAc6=66a18=461c=A0	-	-
30	LacNAc(1-3)	Gala-3Gl CNAC-DH	113	-
31	LacNAc(1-3)-AO	Gala-3GLONAC-AQ	-	-
32	LacNAc	GalR-4GI CNAC-DH	252	-
33	LacNAc-AO	GalR-4GI (NRC-RO	-	-
34	Gala-4Galß-4GlcNAc	Gala=4Gal6=4GlcNAc=DH	-	131
35	SU(3')-LN	SU-3Ga18-4G1 CNAC-DH	-	5
		Galβ-3GlcNAc-DH		
36	Lea-Tri	Fuca-4	-	-
37	Lea-Tri-AO	GAIB-3GLENAC-AO	_	_
57	Lea-III-AO	Fucα-4 Galβ-4GlcNAc-DH		
38	Lex-Tri	Fuca-3	-	-
		Galβ-4GlcNAc-AO		
39	Lex-Tri-AO	Fuca-3	-	-
40	Lex-Tri-(Me)AO	Burn 3	_	-
		SU-3Galβ-3GlcNAc-DH		
41	SU(3')-Lea-Tri	Fuca-4	-	-
	CU(2)) L T	SU-3Galβ-4GlcNAc-DH		
42	SU(3')-Lex-Iri	Fuca-3		-
43	NeuAcα-(3')LN	NeuAca-3Galβ-4GlcNAc-DH	72	-
44	NeuAcα-(3')LN-AO	NeuAca-3Galβ-4GlcNAc-AO	-	-
45	NeuAcα-(6')LN	NeuAca-6Galβ-4GlcNAc-DH	123	-
46	Neu5,9Ac-(6')LN	(9-OAc)NeuAca-6Galβ-4GlcNAc-DH	-	-

		NeuAca-3Galβ-3GlcNAc-DH		
47	SA(3')-Lea-Tri	Fuca-4	29	-
48	DLNN	GlcNAc8-3Gal8-4Glc-DH	414	203
49	LNT	Galß-3GlcNAcß-3Galß-4Glc-DH	-	124
50	Paragloboside	GalB-4GlcNAcB-3GalB-4GlcB-Cer	-	75
51	LNnT	Galß-4GlcNAcß-3Galß-4Glc-DH	236	107
52	B-like pentaosylceramide	Galα-3Galβ-4GlcNAcβ-3Galβ-4Glcβ-Cer	150	95
53	Klaus glycolipid	Galβ-3Galβ-4GlcNAcβ-3Galβ-4Glcβ-Cer	114	75
54	SU(3')-Tri	SU-3Galβ-4GlcNAcβ-3Gal-DH	-	72
55	Led-II pentaosylceramide	Fucα-2Galβ-3GlcNAcβ-3Galβ-4Glcβ-CerA	-	84
56	Led-I pentaosylceramide	Fucα-2Galβ-3GlcNAcβ-3Galβ-4Glcβ-CerB	146	-
57	LNFP-I	Fuca-2Galβ-3GlcNAcβ-3Galβ-4Glc-DH	-	111
FO	D have any learamide	Galα-3Galβ-4GlcNAcβ-3Galβ-4Glcβ-Cer	145	60
56	b-nexaosylceramide	Fucα-2 GalNAcα-3Galβ-3GlcNAcβ-3Galβ-4Glc-DH	145	60
59	A-Hexa	Fuca-2	-	139
		Fuca-4		
		GalNAca-3Galβ-3GlcNAcβ-3Galβ-4Glc-DH		
60	A-Hepta	Fucα-2	32	44
61	INFP-II	Galb-SGroweb-SGalb-AGIC-DH	284	129
01		Fuco-4 Galp-3GlcNAcp-3Galp-4Glc-DH	204	125
62	LNDFH-II	Fuca-4 Fuca-3	40	54
62	tale le constate de succestate	Fucα-2Galβ-3GlcNAcβ-3Galβ-4Glcβ-Cer		120
63	Leb-nexaosylceramide	Fucα-4 Fucα-2Galβ-3GlcNAcβ-3Galβ-4Glc-DH	-	139
64	LNDFH-I	Fuca-4	-	103
		Fuca-2Galβ-3GlcNAcβ-3Galβ-4Glc-DH		
65	LNIFH-I	Fucα-4 Fucα-2 GalR-4GLCMacR-3GalR-4GLC-DH	-	-
66	LNFP-III	Fucα-3	63	44
		Galβ-4GlcNAcβ-3Galβ-4Glc-AO		
67	LNFP-III-AO	Fucα-3 Fucα-2Gal8-4GlcNAc8-3Gal8-4Glc-DH	-	-
68	LNnDFH-I	Fucα-3	73	70
60		Galβ-4GlcNAcβ-3Galβ-4Glc-DH		70
69		Fuca-3 Fuca-3 Galβ-4GlcNAcβ-3Galβ-4Glc-DH	-	79
70	LNnDFH-V	Fuca-3 Fuca-2	-	4
71		Fucα-2Galβ-4GlcNAcβ-3Galβ-4Glc-DH	274	60
/1	LINN I FH-I	Fucα-3 Fucα-2 SU-3Galβ-3GlcNAcβ-4Galβ-4Glc-DH	374	69
72	SU(3')-LNFP-II	Fuca-4	141	-
		SU-6Galβ-3GlcNAcβ-3Galβ-4Glc-DH		
73	SU(6')-LNFP-II	Fucα-4	-	-
74	SU(3')-LNFP-III	Enco-3	209	51
		SU-6Galβ-4GlcNAcβ-3Galβ-4Glc-DH		
75	SU(6')-LNFP-III	Fuca-3	-	132
		50-6		
76		SU-3Galp-4GlCNACp-3Galp-4GlC-DH	52	
70		Fucα-3		30
	2010	weuAcα-sGaiβ-3GlcNAcβ-3Gaiβ-4Glc-DH Galβ-3GlcNAcβ-3Galβ-4Glc-DH	-	
78	LSTb	NeuAca-6	230	30
70	DELNT	NeuAcα-3Galβ-3GlcNAcβ-3Galβ-4Glc-DH		10
20	SialyInaraglobosido	NeuAca-6		43
00 Q1		NeuAca-3Galβ-4GlcNAcβ-3Galβ-4Glcβ-Cer	90	18
01	LU10	NeuAcα-bGaiβ-4GicNAcβ-3Gaiβ-4Gic-DH NeuAcα-3/6Galβ-3GlcNAcβ-3Galβ-4Glc-DH		-
82	SA(3/6)LNFP-I	Fuca-2	-	55
62	SA(2) INED II	NeuAcα-3Galβ-3GlcNAcβ-3Galβ-4Glc-DH		77
65	JA(J)-LINFP-II	Fucα-4 NeuAcα-6Galβ-4GlcNAcβ-3Galβ-4Glc-DH		3/
84	SA(6')-LNFP-VI	Fuca-3	-	92

85 SA(3)-INIP-III Image: Balance Statute For Control - Cont			NeuAca-3Galβ-4GlcNAcβ-3Galβ-4Glc-DH		
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87 pLNnH contractions of point of the second of the secon	86	pLNH	Call-3GloNAcA-3GalR-4GloNAcA-3GalA-4Glo-DH	-	156
9 Partner Part	87	nINnH	Salp Solewap Solar Aslawa 2010 401 Di	171	168
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100 pLNFH-IV FUCa-3 - 131 101 DFpLNH-II Galp-3GlcNAcp-3Galp-4Glc-DH - 25 102 TFpLNH-II FUCa-3 FUCa-3 - 38 102 TFpLNH-I FUCa-4 FUCa-3 - 38 103 MFLNH-III Galp-4Glc-DH - 38 103 MFLNH-III Galp-4Glc-DH - 38			Galβ-3GlcNAcβ-3Galβ-4GlcNAcβ-3Galβ-4Glc-DH		
101 DFpLNH-II Fucor-4 Fucor-3 Galp-4Glc-DH - 25 102 TFpLNH-I Fucor-4 Fucor-3 Galp-4Glc-DH - 38 102 TFpLNH-I Galp-4GlcNAcp-3Galp-4Glc-DH - 38 103 MFLNH-III Galp-4GlcNacp-3 91 217	100	pLNFH-IV	Fuca-3		131
IOI DrpLintiti Fuca-4 Fuca-4 Fuca-3 Calp-3Galp-4GlcNAcp-3Galp-4Glc-DH - 25 102 TFpLNH-I Fuca-4 Fuca-3 Galp-4GlcNAcp-3Galp-4Glc-DH - 38 103 MFLNH-III Galp-4GlcNAcp-3 Galp-4GlcDH - 38 103 MFLNH-III Galp-4GlcNAcp-3 91 217	101		Gaip-SGICNACP-SGaib-4GICNACB-SGaib-4GIC-DH		25
102 TFpLNH-I Fuca-3 Fuca-3 - 38 103 MFLNH-III Galβ-4Glc-DH 91 217	101		Fucα-4 Fucα-3 Fucα-2Galβ-3GlcNAcβ-3Galβ-4GlcNAcβ-3Galβ-4Glc-DH	-	25
Image: Constraint of the	102	TFpLNH-I	Fuce-4 Fuce-3	-	38
103 MFLNH-III Fucor-3 Galß-4Glc-DH 91 217		10	Galβ-4GlcNAcβ-6		
103 MFLNH-III Gal\$-3G1cNAc\$-3 91 217			Fuco-3 Galβ-4Glc-DH		
	103	MFLNH-III	GalB-3GlcNAcB-3	91	217

1		Galβ-4GlcNAcβ-6		
		Fuca-3 Galβ-4Glc-DH		
		Co18-20100808-2		
104		Parb Parcinch 2		2
104	DENTI(D)	Fucα-4 Galβ-4GlcNAcβ-6	-	5
		Gal8-4Glc-DH		
105		Fuca-2GalB-3GICNAcB-3	120	70
105	DFLINH(C)	Fuca-4 GalR-4GlcNBcR-6	139	79
106		Puca-5 Gaip-4Gic-DH		
100	DFLINH(a)	Fuca=2GalB=3GlcNAcB=3 GalB=4GlcNAcB=6	-	-
		Ruco-3 Cal8-4Clo-DH		
107	TELNUL	Fuca-zGalp-3GICNACB-3		24
107	TELINH	Fucα-4 Gal8-3GlcNAc8-3Gal8-4GlcNAc8-6	-	24
100		rucu-s Galp-4610-Dh	260	00
100	INIFILINO-IV	GalB-3GlcNAcB-3GalB-4GlcNAcB-6	200	00
		Fuge 4 Fuge 2 Calls (Clarby		
		a la pal va a la		
100	TELNO	Galβ-3GlcNAcβ-3	274	C 2
109	THILNU	Fuca-4	3/1	68
		Fuca=3 Galb=4GlcNAcb=6		
		Galβ-3GlcNAcβ-3 Galβ-4Glc-DH		
110	MFLND	Galβ-3GlcNAcβ-3	-	119
		Galp-4GICNACB-6		
		Fucα-3 Galβ-4Glc-DH		
111	MFLNnH(a)	Galβ-4GlcNAcβ-3	-	-
		Galp=4GlCNACP=6		
		Fuca-3 Galβ-4Glc-DH		
		Galβ-4GlcNAcβ-3		
112	DFLNnH	Fuca=3	-	167
		Galα-3Galβ-4GlcNAcβ-6		
		Fucα-2 Galβ-4GlcNAcβ-3Galβ-4Glcβ-Cer		
		Gala-3Galβ-4GlcNAcβ-3		
113	B-III dodecaosylceramide	Fuca-2	-	372
		Gala-3Galβ-4GlcNAcβ-6		
		Fucα-2 Galβ-4GlcNAcβ-3Galβ-4Glcβ-Cer		
	B-IV	Galα-3Galβ-4GlcNAcβ-3Galβ-4GlcNAcβ-3		
114	tetradecaosylceramide	Fuca-2	666	184
		NeuAcα-6Galβ-4GlcNAcβ-6		
		Galβ=4Glc=DH		
115	MSLNH	Galβ-3GlcNAcβ-3	-	-
		Galβ-4GlcNAcβ-6		
		Galβ-4Glc-DH		
116	MSLNnH-I	NeuAca-6Galβ-3GlcNAcβ-3	-	-
		NeuAca-6Galβ-4GlcNAcβ-6		
		Galβ-4Glc-DH		
117	DSLNnH	NeuAca-6Galβ-4GlcNAcβ-3	222	58
		Galβ-4GlcNAcβ-6		
		Fuca-3 Galβ-4Glc-DH		
118	MSMFLNH	NeuAcα-3Galβ-3GlcNAcβ-3	-	-
		Gaip-4GicNACB-6		
		Fuca-3 Galβ-4Glc-DH		
119	MFMSLNnH	NeuAcα-6Galβ-3GlcNAcβ-3	-	-
120	CALL	NeuAca-3Gaip-4GiCNAcβ-3Gaiβ-3GiCNAc-DH		
120	C4U	SU-6 SU-6 SU-6 Fuca-3	66	-
		NewSer 20-10 401-ND-0 20-10 201-ND- 20		
101	FucCALL	Menaca-searp-aercNACb-3991b-301CNAC-DH		
122	Full40	SU-6 SU-6 SU-6	-	-
122	ivian2(α2)	Mana-2Man-DH	-	41

123	Man2(α3)	Mano-3Man-DH	366	32
		Manq-6Man-DH		
124	Man3(α3,α6)	Mana-3 Mana-3	267	669
		Mana-6Mana-DH		
125	Man5(α3,α6)	Manor-3	-	357
126	Man1GN1	Manβ-4GlcNAc-DH	-	-
127	Man2GN1	Mana-3Manβ-4GlcNAc-DH	1,761	5,615
128	Man2aGN2	Mana-6Manβ-4GlcNAcβ-4GlcNAc-DH	1,570	1,416
		Manor-6		
129	Man3GN2	Manp-4GlcNAcp-4GlcNAc-DH	2 602	8 1 2 2
125	IVIAII3GIV2	Mana - 3 Mana - 6	2,002	0,122
		Xylb-2Manb-4GlcNAcb-4GlcNAc-DH		
130	Man3XylGN2	Manor=3	837	546
		Manor-6 Fucor-6		
121	Man2ECN2	Manβ-4GlcNAcβ-4GlcNAc-DH	C 001	20 447
151	Manardinz	Manα-3 Manα-6	6,091	20,447
		Xy1β-2Manα-4GlcNAcβ-4GlcNAc-DH		
132	Man3FXylGN2	 Μαηα-3 Ευςα-3	1,156	1,213
		Manor-3Manor-6		
		Manβ-4GlcNAcβ-4GlcNAc-DH		
133	Man4aGN2	Manα-3 Μαηα-6	3,485	10,141
		Mano-3Mano-6		
134	Man4bGN2	Mang-4G1 cNAcg-4G1 cNAc-DH	541	882
		Mana=6		
		Mana-3Mana-6		
		Manß-4GlcNAcß-4GlcNAc-DH		
135	Man5GN2	Mano-3	1,792	4,992
136	Man6GN2	Mano-2Mano-2	1.358	1.247
		Mana 2Mana 3 Mana 6	_,	_,
		Manα-3Manα-6		
		Manβ-4GlcNAcβ-4GlcNAc-DH		
137	Man7(D1)GN2	Mano-2Mano-2Mano-3	321	1,244
		Mano-JMano-6		
138	Man7(D1)GN2-AO	Manp-4GICNACP-4GICNAC-AU	1 202	676
130		mana-zmana-zmana-3 Mana-2Mana-6	1,202	0,0
		Mana-3Mana-6		
		Manβ-4GlcNAcβ-4GlcNAc-DH		
139	Man7(D3)GN2	Mano-2Mano-3	382	1,090
		Manor-2Manor-6		
		manα-JManα-6		
140	Man8(D1D3)GN2	Manβ-4GlcNAcβ-4GlcNAc-DH	105	368
140	אוטנטעדטאוטע	Manα-2Manα-2 Manα-2Manα-6	473	308
		Mano-2Mano-6		
		Manβ-4GlcNAcβ-4GlcNAc-DH		
141	Man9GN2	Mano-2Mano-2Mano-3	253	241
		Mana-2Mana-6		
		Manα-2Manα-3Manα-6		
140		Manβ-4GlcNAcβ-4GlcNAc-AO		110
142	IVIAN9GN2-AO	Mana-2Mana-2Mana-3		112

	F			
		Mana-2Mana-6		
		Mano-6		
		Manα-2Manα-3 Manβ-4GlcNAcβ-4GlcNAc-DH		
143	Glc1Man9GN2	Glco-3Mano-2Mano-2Mano-3	609	286
		Mana-2Mana-6		
		Mano-6		
144		Mana-2Mana-3 Manp-4GICNACP-4GICNAC-AD	200	24
144	GICINIan9GN2-AU	Glca-3Mana-2Mana-3	399	31
		Hand-0		
		Mana-3Mana-6		
		Manß-4GlcNAc-AO		
145	Glc2Man7(D1)GN1-AO	Glca-3Glca-3Mana-2Mana-3	641	899
		Manor-6		
		Manα-3Manα-6		
		Man6-4G1 cNAc-AQ		
1/16	Glc3Man7(D1)GN1-AO		869	808
140	Glesinan (Bi)GNI AO	Glca-2Glca-3Glca-3Mana-2Mana-2Mana-3 Galβ-4GlcNAcβ-2Mana-6 Fuca-6	005	000
1 4 7	NI	Manp-4GICNACp-4GICNAC-DH	F 767	14 200
147	NI	Manor-3	5,767	14,300
		ANGLINE O		
		Manß-4GlcNAcβ-4GlcNAc-DH		
148	N2	Galβ-4GlcNAcβ-2Manα-3	4,024	11,294
		Galβ-4GlcNAcβ-2Manor-6		
		Manβ-4GlcNAcβ-4GlcNAc-DH		
149	N4	Mana-3	2,429	7,224
		GlcNAcβ-2Manα-6 Fucα-6		
		Galβ-4 Manβ-4GlcNAcβ-4GlcNAc-DH		
150	N3	GlcNAcβ-2Manα-3	2,825	5,689
		GlcNAcβ-2Manα-6		
		Manβ-4GlcNAcβ-4GlcNAc-DH		
151	NGA2	G1cNAc6-2Mano-3	1,225	1,499
		GlcNAcβ-2Mana-6 Fuca-6		
		Manß-4GlcNAcβ-4GlcNAc-DH		
152	NGA2F	CleNbcG-2Mapper 3	5.772	8.604
		GlcNAcβ-2Manα-6	-,	-,
		GlcNAcB-4ManB-4GlcNAcB-4GlcNAc-DH		
153	NGA2B		1 390	3 5 2 3
133	NGALD	GLCNACB-2Mano-3 GLCNACB-2Mano-6	1,550	3,323
		oremep manp receive m		
		GlcNAcβ-4Manα-3		
154	NGA3B	GlcNAcβ-2	184	112
		GTCMMCD-0		
		GlcNAcB-2Mano-6		
		Manβ-4GlcNAcβ-4GlcNAc-DH		
		GlcNAcβ-2Manα-3		
155	NGA4	GleNAc6-4	173	332
		GlcNAcβ-2	1,5	552
		GI CNAC6-4Mano-6		
		GTCWWCD-0		
		GlcNAcβ-4Manβ-4GlcNAcβ-4GlcNAc-DH		
		GlcNAcb-4Mana-3		
156	NGA5B	GlcNAcβ-2	32	27
		Manor-6		
		Mana-3Mana-6		
		GlcNAcB-4ManB-4GlcNAcB-4GlcNAc-DH		
157	GNMan5BGN2		072	909
1.57	Grandisbonz	GICNACP=ZMANG=3 Galβ=4GlcNAcβ=2Mang=6	510	505
		M = R = A C (1 = N) A R = A C (1 = N) A = D H		
150	NAC	Manp-agrowed-agrowedby	2 0 2 2	4 742
128	INAZ	Galβ-4GlcNAcβ-2Manα-3	2,032	4,742

	1			1 1	
		Galβ-4GlcNAcβ-2Manα-6	Fuca-6		1
		Manβ-4G	LCNAcβ-4GlcNAc-DH		1
159	NA2F	Galβ-4GlcNAcβ-2Manα-3	Galβ-4GlcNAcβ-2Manα-3		
		Galβ-4GlcNAcβ-2Manα-6	Galβ-4GlcNAcβ-2Manα-6 Fucα-6		
		Manβ-4G	Manß-4GlcNAcβ-4GlcNAc-AO		
160	NA2F-AO	Gal8-4GlcNAc8-2Manα-3	2,356	3,594	
		Galβ-4GlcNAcβ-2Manα-6	Galβ-4GlcNAcβ-2Manα-6 Fucα-6		
		GlcNAcβ-4Manβ-4G	GlcNAcB-4Man6-4GlcNAc6-4GlcNAc-DH		
161	NA2FB	Cold (Clowbod Shops 3		841	652
		Galβ-4GlcNAcβ-2Manα-6	Galβ-4GlcNAcβ-2Manα-6		
		Man6-4G	CNAC6-4G] CNAC-DH		1
					1
1.60		Galβ-4GlcNAcβ-4Manα-3			
162	NA3	Gal6-4GlcNAc6-2		-	27
		Galp-4GlCMACp-2Manu-6			1
		Manß-4G	Manß-4GlcNAcβ-4GlcNAc-DH		
		Galβ-4GlcNAcβ-4Manα-3			1
163	NA3-Lex	Galβ-4GlcNAcβ-2		-	143
		Galβ-4GlcNAcβ-6			
		Galβ-4GlcNAcβ-2Manα-6			1
		Man6-4G	CNAC6-4GICNAC-DH		1
		Patip-4G.	consequences and and		1
		Galβ-4GlcNAcβ-4Manα-3			
164	NA4	Galβ-4GlcNAcβ-2		149	9
		NeuAcα=3Ga1β=4G1cNAcβ=2Manα=	-6 Fuca-6		1
			Manß-4GlcNAcB-4GlcNAc-DH		1
165	A2F(2-3)	NeuAcα-3Galβ-4GlcNAcβ-2Manα	-3	3,163	2,063
		NeuAcα-6Galβ-4GlcNAcβ-2Manα-	-6		
			Manβ-4GlcNAcβ-4GlcNAc-DH		1
166	A2(2-6)	NeuAca-6Gal8-4GlcNAc8-2Mana-	-3	813	1,510
		NeuAcα-6Galβ-4GlcNAcβ-2Manα	-6		i
			Manβ-4GlcNAcβ-4GlcNAc-DH		1
167	AGP-Bi-Ac2	Neules (Cold (CleNted Mana		927	1.411
107		NeuGcα-6Galβ-4GlcNAcβ-2Manα	-6	527	
			Man8-4GlcNAc8-4GlcNAc-DH		1
160	ACD BI Co2			2 5 2 5	2 2 7 1
100	AGF-BI-GC2	NeuGcα-6Galβ-4GlcNAcβ-2Manα NeuGcα-6Galβ-4GlcNAcβ-2Manα	-3	2,323	2,271
			I		1
1.00			Manβ-4GlcNAcβ-4GlcNAc-DH	1.1.10	4 4 7 0
169	AGP-BI-ACGC	NeuAcα-6Galβ-4GlcNAcβ-2Manα-	-3	1,146	1,179
		Neukcu Starp Harenkep Zhanu	Ĩ		1
			Manß-4GlcNAcB-4GlcNAc-DH		1
		NeuAcα-3Galβ-4GlcNAcβ-4Manα	-3		1
170	A3	NeuAca-6Gal8-4GlcNAc8-2		94	33
171	Fuc-GlcNAc	Fuca-6GlcNAc-DH		27	-
172	GM4	N A AG 10 -			-
172	SW12	NeuAcα=3Ga1β=Cer		++	114
1/3		SU-3Galβ-4Glcβ-Cer			114
174	Haematoside	NeuAca-3Galβ-4Glcβ-Cer		-	-
175	GM3	NeuAca-3Galβ-4Glcβ-Cer		-	61
176	GM3(Gc)	NeuGca-3Galβ-4Glc-Cer		250	107
177	Asialo-GM2	GalNAc6-4Gal6-4Glc6-Cer		202	-
		GalNAcβ-4Galβ-4Glcβ-Cer		1 1	
178	SM2	su-3		130	
		SU-3GalNAcβ-4Galβ-4Glcβ-Cer		1	
179	SB2	su-3		-	
		GalNAcB-4GalB-4GlcB-Cer			
180	GM2	NeuAca-3		131	-
181	Asialo-GM1	GalB-3GalNAcB-4GalB-4GlcB-Ce		-	88
182	Asialo-GM1-Tetra	Call-3CalNice Acale Aci- pr		_	
101		Galp-3GalNAcp-4Galp-4Glcp-Ce	er	+ +	
183	SM1a	err_9		_	
		SU-3Galβ-3GalNAcβ-4Galβ-4Glo	2β-Cer	+ +	
184	SB1a	R = 112		_	
185	GM1h	Newber 20-10 00 197 0 17 17	401-0.0	-	-
100	0.0110	weuAcα=3Ga1β=3Ga1NAcβ=4Ga1β	-wordb_net	-	-
		Galβ-3GalNAcβ-4Galβ-4Glcβ-Ce	er		

		C-10 2C-103-0 4C-10 4C1- DU		
187	GM1-penta	Galp-JGalp-4Galp-4GIC-DH NeuAco-3	-	-
100	GM1(Gc)	Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer		
100	001(00)	NeuGcor-3 Galβ-3GalNAcβ-4Galβ-4Glc-DH		
189	GM1(Gc)-penta	NeuGoo-3	193	-
190	GD1a	Mennor-Searp-Searmerp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-dearp-de	-	-
		NeuAca-3GalB-3GalNAcB-4GalB-4Glc-DH		
191	GD1a-hexa	NeuAca-3	-	-
		MauGon 3 Mauhon 3		
		GalNack-4Galk-3GalNack-4Galk-4Glck-Cer		
192	GalNAc-GD1a(Ac.Gc)		-	-
193	GD3	Neulou 5 Neulou 5 Neulou 5 Neulou 5	-	-
194	GD3-tetra	NeuAca-8NeuAca-3Galβ-4Glc-DH	-	-
195	GD3-tetra-AO	NeuAca-8NeuAca-3Galβ-4Glc-AO	-	-
106	CD3	GalNAcβ-4Galβ-4Glcβ-Cer	272	
196	GDZ	NeuAcα-8NeuAcα-3 Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer	273	-
197	GD1b	NeuAca-8NeuAca-3	338	-
100	CT1-	NeuAca-8NeuAca-3Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer	100	
198	6110	NeuAca-3 NeuAca-3Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer	123	-
199	GT1b	NeuAca-8NeuAca-3	-	-
200	C011	NeuAca-8NeuAca-3Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer		
200	GQID ColNAc Sor	NeuAca-8NeuAca-3	-	-
201	GalNAc-Ser	GalNAc-Ser	514	-
202	GalR-3GalNAc	GalNAc-Thr		62
203	Galß-6GalNAc	GalB-GGalMAC-DH	263	46
201		GalNAcB-4GalB-OX	200	
205	B12/3	NeuGoa-3	-	-
206	DST	Neukca-Scarb-Scarbe-DH	-	-
207	Man-Ser	Mano-Ser	-	10
208	Man-Ser-Succ	Man-Ser-Succ	-	63
209	Man-Thr	Man-Thr	45	28
210	Man-Thr-Succ	Man-Thr-Succ	-	-
211	Notch-1	Fuca-Thr	-	-
212	Notch-2	GlcNAcβ-3Fuca-Thr	-	-
213	Notch-3	Galβ-4GlcNAcβ-3Fucα-Thr	-	-
214	SA2(α8)	NeuAca-8NeuAc-DH	72	-
215	SA3(α8)	NeuAca-8NeuAca-BH	- 125	-
210	SA5(α8)	NeuAca-8NeuAca-8NeuAca-DH	125	-
218	SA6(q8)	Neulaca-sneulaca-sneulaca-sneulac-DH	127	-
219	SA7(α8)	Neulog-Shenlog-Shenlog-Shenlog-Shenlog-Shenlog-Shenlog-DH	-	-
220	SA8(α8)	NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAc-8NeuAc-8NeuAca-8NeuAc-DH	-	-
221	SA9(α8)	NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAc-8NeuAca-8NeuAca-8NeuAca-DH	-	-
222	SA10(α8)	NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca- 8NeuAc-DH	-	-
223	SA11(α8)	NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca-8NeuAca	-	-
		AUA-4G1cNS-DH		
224	Hep-Di IS	SU-2 SU-6	-	216
		AUA-4G1CNS-AO		
225	Hep-Di-IS-AO	SU-2 SU-6	-	-
226	Lam-2	Glcβ-3Glc-DH	223	27
227	Glc2(α2)	Glca-2Glc-DH	-	13
228	Glc2(a3)	Glca-3Glc-DH	118	-
229	Glc2(α2)-AO	Glca-2Glc-AO	-	-
230	Glc2(α3)-AO	Glca-3Glc-AO	-	-

-				
231	Gal	Gal-DH	-	150
232	Gal-AO	Gal-AO	-	-
233	GalNAc	GalNAc-DH	-	228
234	GalNAc-AO	GalNac-AO	-	-
235	Glc	Glc-DH	-	9
236	Glc-AO	Glc-AO	20	-
237	GN	GlcNAc-DH	-	-
238	GN-AO	GlcNAc-AO	-	-
239	Man	Man-DH	-	49
240	Man-AO	Man-AO	-	-
241	Fuc	Fuc-DH	26	262
242	Fuc-AO	Fuc-AO	-	-
243	NeuAc	NeuAc-DH	-	-
244	NeuAc-AO	NeuAc-AO	-	-
245	NeuGc	NeuGo-DH	117	-
246	NeuGc-AO	NeuGc-A0	-	-
247	Rha	Rha-DH	367	-
248	Rha-AO	Rha-AO	-	-
249	Galα-6Glc-AO	Gala-6Glc-AO	-	-
250	(6P)-Man	P-6Man-DH	-	145
251	(6P)-Man-AO	P-6Man-AO	-	-
252	(6P)-Man5	P-6Manα-3Manα-3Manα-2Man-DH	-	-
253	SU-Tyr	SU-Tyr	-	-
254	SU-Cholesterol	SU-Cholesterol	-	-
255	Glc(α6,α4,α4)	Glca-6Glca-4Glca-4Glc-DH	631	76

^a Pos, Probe position in the screening microarray.

^bThe oligosaccharide probes are all lipid-linked, neoglycolipids (NGLs) or glycosylceramides and are from the collection assembled in the course of research in Glycosciences Laboratory. Unless otherwise specified the NGLs are prepared from reducing oligosaccharides by reductive amination with the amino lipid, 1,2-dihexadecyl-*sn*-glycero-3-phosphoethanolamine (DHPE); AO, NGLs prepared from reducing oligosaccharides by oxime ligation with an aminooxy (AO) functionalized DHPE (Liu et al., Chem. Biol. 14, 847–859, 2007); Cer, natural glycolipids with various ceramide moieties; CerA and CerB denote different natural ceramides; Cer36 and Cer42, synthetic glycolipids with ceramide having a total of 32 and 42 carbon atoms, respectively; C30, a synthetic lipid [2-(tetradecyl)hexadecanol] with 30 carbon atoms. UA, 4,5-unsaturated hexuronic acid; aMan, 2,5-anhydromannose; aGal, 3,6-anhydro-galactose.

-, signal less than 1.

coupled to AKTA Purifier (GE Healthcare, Bio-Science Inc. Germany), previously equilibrated with phosphate-buffered saline (PBS) containing 0.5 M NaCl. After washing with equilibrating buffer, the adsorbed material was eluted with 0.1 M D-mannose in equilibrating buffer. The preparations obtained were ultradiafiltered against PBS using a YM10 membrane (Amicon Division, W.R. Grace, Beverly, MA) and biotinylated using sulfo-NHS-LC-biotin (Sigma-Aldrich, St. Louis, USA) according to the manufacturer instructions.

2.2. Glycan microarray analyses

Microarray analyses were performed using the neoglycolipid (NGL)-based system [4], with lipidlinked glycan probes, including NGLs and glycolipids, and comprising a total of 255 oligosaccharides (in-house designation 'Array Sets 18–22bis'; list of probes are in Table 1). These were robotically printed on nitrocellulose-coated glass slides, at 2 and 7 fmol per spot, using a non-contact arrayer (Piezorray; PerkinElmer LAS, Beaconsfield, UK). The microarray binding assays were performed as described [1]. In brief, microarray slides were blocked at ambient temperature with 1% w/v bovine serum albumin (BSA; Sigma-Aldrich) in casein blocker solution (Pierce Chemical Co, USA) for 1 h. The biotinylated lectin samples were overlaid at 50 µg/mL, and binding was detected using Alexa Fluor 647-labeled streptavidin (Molecular Probes-Life Technologies, CA, USA) at 1 μ g/mL in blocker solution. Glycoarray data analysis was performed with dedicated software [5]. The binding signals were probe-dose dependent. The results of glycan probes at 7 fmol per spot are shown in Fig. 1 and Table 1.

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