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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	Biology
More specific subject area	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, retrieving 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 Manα1-3(Manα1-6) 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 β1-2-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.

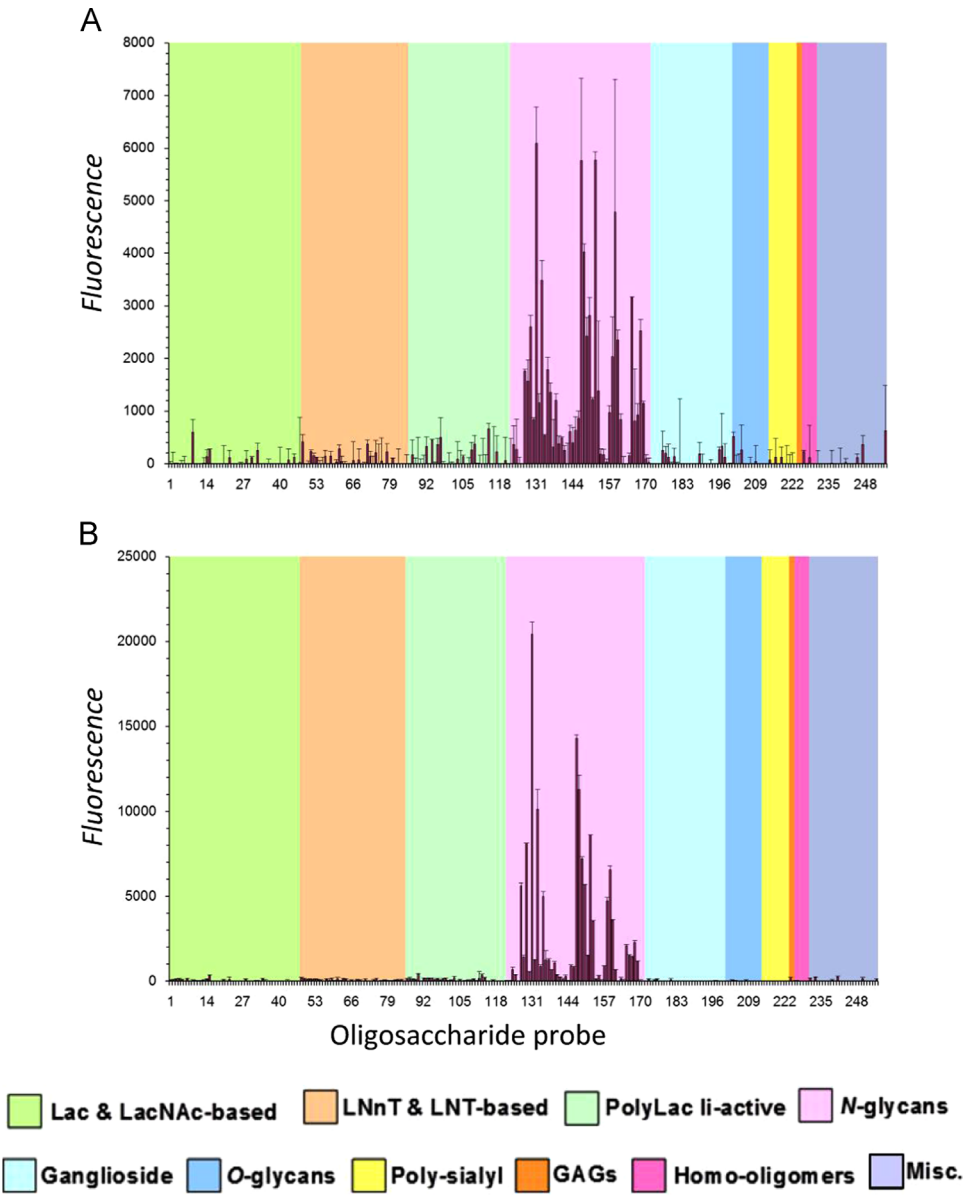


Fig. 1. Carbohydrate microarray analyses of n-ArtinM (*A*) and y-ArtinM (*B*). Numerical scores of the binding signals are means of duplicate spots at 7 fmol/spot (with *error bars*). The complete list of probes and their sequences and binding scores are in Table 1.

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.

Pos.[a]	Probe[b]	Structure	Fluorescence signals	
			n-ArtinM	y-ArtinM
1	Galactocerebrosides	Galß-Cer	-	74
2	H-Di	Fucα-2Gal-DH	-	76
3	A-Tri	GalNAcα-3Gal-DH | Fucα-2	-	83
4	B-Tri	Galα-3Gal-DH | Fucα-2	-	133
5	Sulfatide	SU-3Galß-Cer	6	89
6	GSF-1	SU-3Galß-C30	-	-
7	Glucocerebrosides	Glcß-Cer	-	128
8	GSF-19	SU-6Glcß-C30	-	-
9	Lactocerebrosides	Galß-4Glcß-Cer	598	61
10	Lac	Galß-4Glc-DH	-	44
11	Lac-AO	Galß-4Glc-AO	-	-
12	GalNAcα-3Galß-4Glc	GalNAcα-3Galß-4Glc-DH	-	5
13	Ceramide trihexoside	Galα-4Galß-4Glcß-Cer	20	79
14	Globoside (P-antigen)	GalNAcß-3Galα-4Galß-4Glcß-Cer	136	106
15	Forssmann glycolipid	GalNAcα-3GalNAcß-3Galα-4Galß-4Glcß-Cer	258	321
16	NeuAcα-(3')Lac	NeuAcα-3Galß-4Glc-DH	-	25
17	NeuAcα-(3')Lac-AO	NeuAcα-3Galß-4Glc-AO	-	-
18	Neu4,5Ac-(3')Lac	(4-OAc)NeuAcα-3Galß-4Glc-DH	-	-
19	Neu4,5Ac-(3')Lac-AO	(4-OAc)NeuAcα-3Galß-4Glc-AO	-	-
20	Neuα-(3')Lac	Neuα-3Galß-4Glc-DH	-	110
21	Neuα-(3')Lac-AO	Neuα-3Galß-4Glc-AO	-	-
22	NeuAcα-(6')Lac	NeuAcα-6Galß-4Glc-DH	118	108
23	NeuAcα-(6')Lac-AO	NeuAcα-6Galß-4Glc-AO	-	-
24	Neuα-(6')Lac	Neuα-6Galß-4Glc-DH	-	-
25	Neuα-(6')Lac-AO	Neuα-6Galß-4Glc-AO	-	-
26	NeuAcß-(3')Lac	NeuAcß-3Galß-4Glc-DH	-	-
27	NeuAcß-(3')Lac-AO	NeuAcß-3Galß-4Glc-AO	-	-
28	NeuAcß-(6')Lac	NeuAcß-6Galß-4Glc-DH	88	96
29	NeuAcß-(6')Lac-AO	NeuAcß-6Galß-4Glc-AO	-	-
30	LacNAc(1-3)	Galß-3GlcNAc-DH	113	-
31	LacNAc(1-3)-AO	Galß-3GlcNAc-AO	-	-
32	LacNAc	Galß-4GlcNAc-DH	252	-
33	LacNAc-AO	Galß-4GlcNAc-AO	-	-
34	Galα-4Galß-4GlcNAc	Galα-4Galß-4GlcNAc-DH	-	131
35	SU(3')-LN	SU-3Galß-4GlcNAc-DH	-	5
36	Lea-Tri	Galß-3GlcNAc-DH | Fucα-4	-	-
37	Lea-Tri-AO	Galß-3GlcNAc-AO | Fucα-4	-	-
38	Lex-Tri	Galß-4GlcNAc-DH | Fucα-3	-	-
39	Lex-Tri-AO	Galß-4GlcNAc-AO | Fucα-3	-	-
40	Lex-Tri-(Me)AO	Galß-4GlcNAc-(Me)AO | Fucα-3	-	-
41	SU(3')-Lea-Tri	SU-3Galß-3GlcNAc-DH | Fucα-4	-	-
42	SU(3')-Lex-Tri	SU-3Galß-4GlcNAc-DH | Fucα-3	-	-
43	NeuAcα-(3')LN	NeuAcα-3Galß-4GlcNAc-DH	72	-
44	NeuAcα-(3')LN-AO	NeuAcα-3Galß-4GlcNAc-AO	-	-
45	NeuAcα-(6')LN	NeuAcα-6Galß-4GlcNAc-DH	123	-
46	Neu5,9Ac-(6')LN	(9-OAc)NeuAcα-6Galß-4GlcNAc-DH	-	-

Table 1 (*continued*)

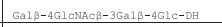
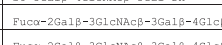
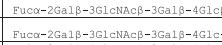
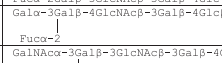
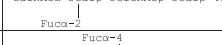
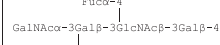
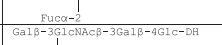
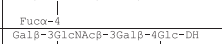
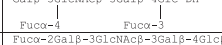
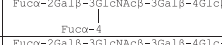
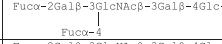
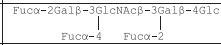
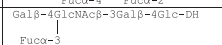
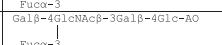
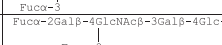
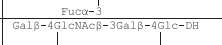
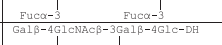
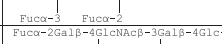
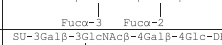
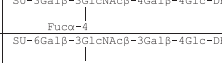
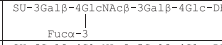
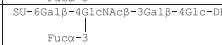
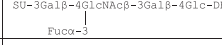
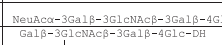
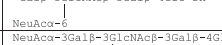
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49	LNT		-	124
50	Paragloboside		-	75
51	LNnT		236	107
52	B-like pentaosylceramide		150	95
53	Klaus glycolipid		114	75
54	SU(3')-Tri		-	72
55	Led-II pentaosylceramide		-	84
56	Led-I pentaosylceramide		146	-
57	LNFP-I		-	111
58	B-hexaosylceramide		145	60
59	A-Hexa		-	139
60	A-Hepta		32	44
61	LNFP-II		284	129
62	LNDFH-II		40	54
63	Leb-hexaosylceramide		-	139
64	LNDFH-I		-	103
65	LNTFH-I		-	-
66	LNFP-III		63	44
67	LNFP-III-AO		-	-
68	LNnDFH-I		73	70
69	LNnDFH-II		-	79
70	LNnDFH-V		-	4
71	LNnTFH-I		374	69
72	SU(3')-LNFP-II		141	-
73	SU(6')-LNFP-II		-	-
74	SU(3')-LNFP-III		209	51
75	SU(6')-LNFP-III		-	132
76	SU(3',6)-LNFP-III		52	-
77	LSTa		-	30
78	LSTb		230	30
79	DSLNT		-	43
80	Sialylparagloboside		96	18
81	LSTc		-	-
82	SA(3/6)LNFP-I		-	55
83	SA(3')-LNFP-II		-	37
84	SA(6')-LNFP-VI		-	92

Table 1 (*continued*)

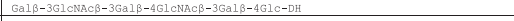
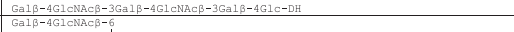
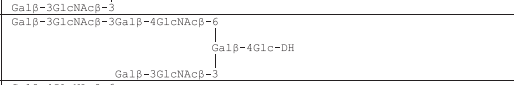
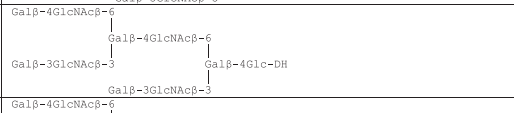
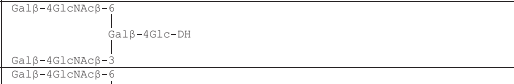
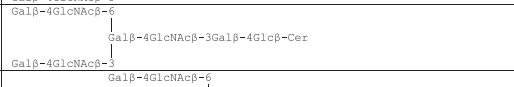
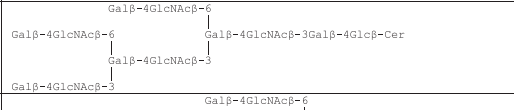
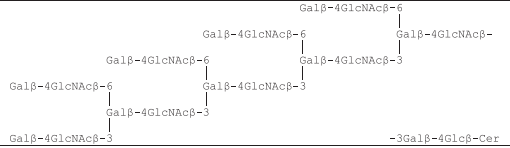
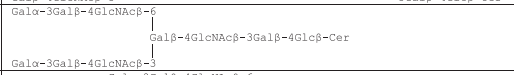
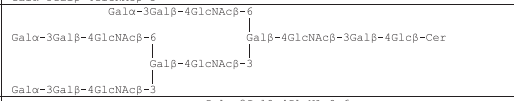
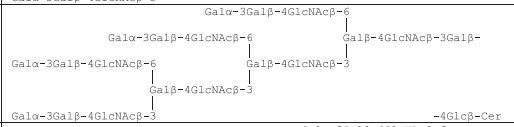
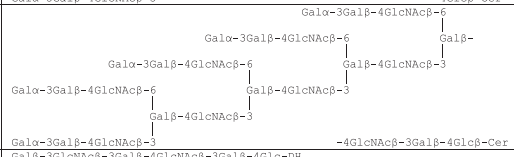
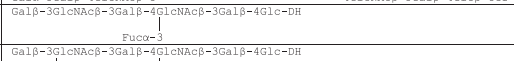
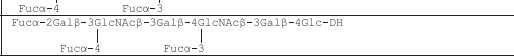
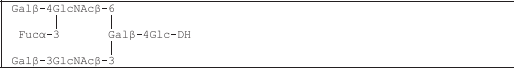
85	SA(3')-LNFP-III		-	-
86	pLNH		-	156
87	pLNnH		171	168
88	LNH		10	110
89	iLNO		-	92
90	LND		-	421
91	LNnH		-	-
92	I-octaosylceramide		326	146
93	I-dodecaosylceramide		-	163
94	I-hexadecaosylceramide		458	158
95	I-eicosaosylceramide		-	180
96	B-like decaosylceramide		366	111
97	B-like pentadecaosylceramide		501	126
98	B-like eicosaosylceramide		-	54
99	B-like pentaeicosaosylceramide		-	148
100	pLNFH-IV		-	131
101	DFpLNH-II		-	25
102	TFpLNH-I		-	38
103	MFLNH-III		91	217

Table 1 (*continued*)

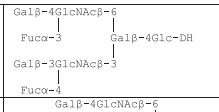
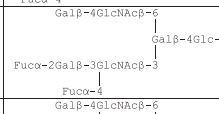
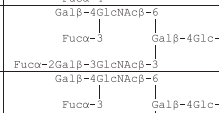
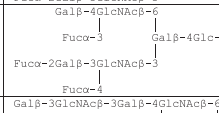
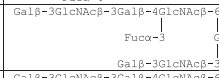
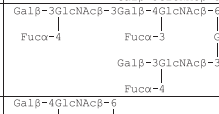
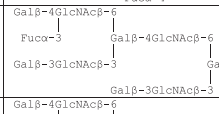
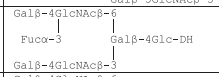
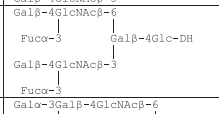
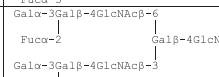
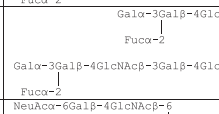
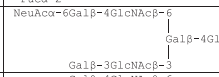
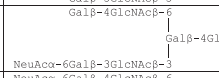
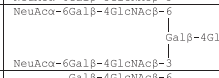
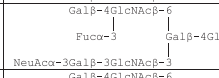
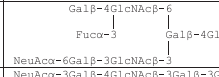
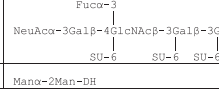
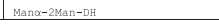
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105	DFLNH(c)		139	79
106	DFLNH(a)		-	-
107	TFLNH		-	24
108	MFiLNO-IV		260	88
109	TFiLNO		371	68
110	MFLND		-	119
111	MFLNnH(a)		-	-
112	DFLNnH		-	167
113	B-III dodecaosylceramide		-	372
114	B-IV tetradecaosylceramide		666	184
115	MSLNH		-	-
116	MSLNnH-I		-	-
117	DSLNnH		222	58
118	MSMFLNH		-	-
119	MFMSLNnH		-	-
120	C4U		66	-
121	FucC4U		-	-
122	Man2(α2)		-	41

Table 1 (*continued*)

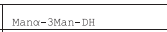
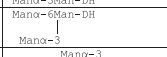
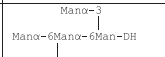
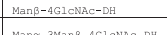
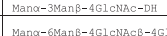
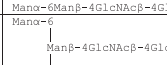
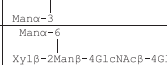
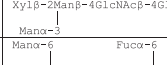
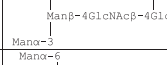
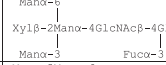
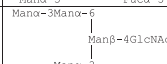
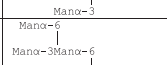
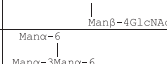
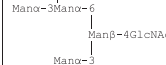
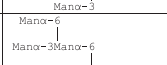
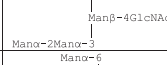
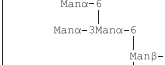
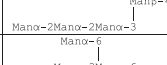
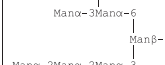
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124	Man3(α3,α6)		267	669
125	Man5(α3,α6)		-	357
126	Man1GN1		-	-
127	Man2GN1		1,761	5,615
128	Man2aGN2		1,570	1,416
129	Man3GN2		2,602	8,122
130	Man3XylGN2		837	546
131	Man3FGN2		6,091	20,447
132	Man3FXylGN2		1,156	1,213
133	Man4aGN2		3,485	10,141
134	Man4bGN2		541	882
135	Man5GN2		1,792	4,992
136	Man6GN2		1,358	1,247
137	Man7(D1)GN2		321	1,244
138	Man7(D1)GN2-AO		1,202	676
139	Man7(D3)GN2		382	1,090
140	Man8(D1D3)GN2		495	368
141	Man9GN2		253	241
142	Man9GN2-AO		-	112

Table 1 (*continued*)

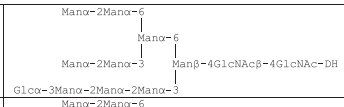
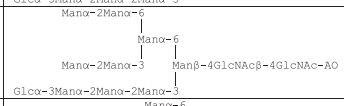
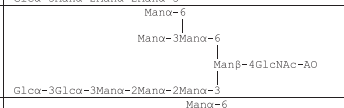
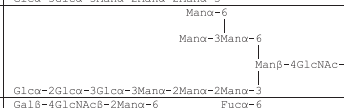
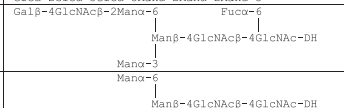
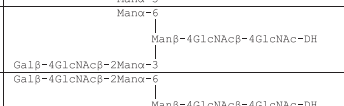
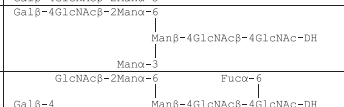
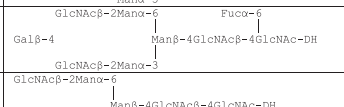
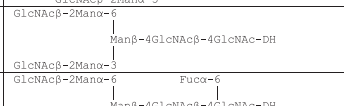
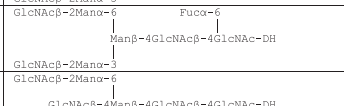
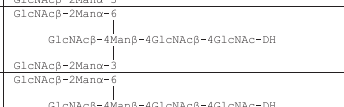
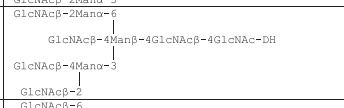
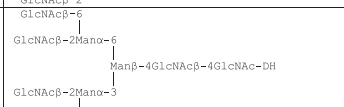
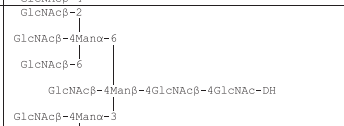
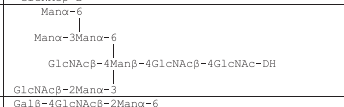
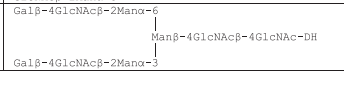
143	Glc1Man9GN2		609	286
144	Glc1Man9GN2-AO		399	31
145	Glc2Man7(D1)GN1-AO		641	899
146	Glc3Man7(D1)GN1-AO		869	808
147	N1		5,767	14,300
148	N2		4,024	11,294
149	N4		2,429	7,224
150	N3		2,825	5,689
151	NGA2		1,225	1,499
152	NGA2F		5,772	8,604
153	NGA2B		1,390	3,523
154	NGA3B		184	112
155	NGA4		173	332
156	NGA5B		32	27
157	GNMan5BGN2		978	909
158	NA2		2,032	4,742

Table 1 (*continued*)

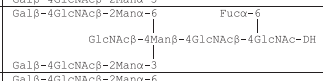
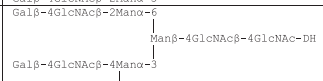
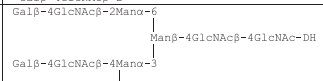
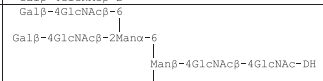
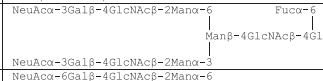
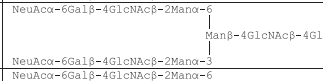
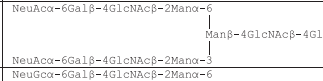
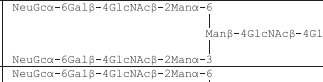
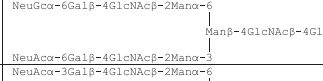
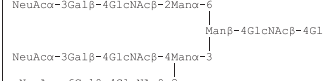
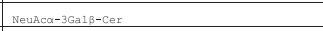
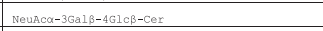
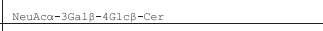
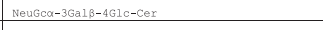
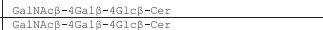
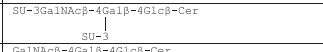
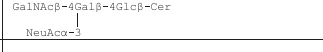
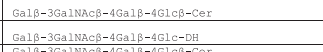
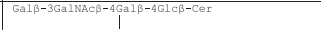
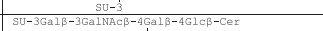
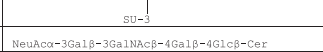
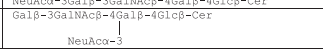
159	NA2F		4,782	6,557
160	NA2F-AO		2,356	3,594
161	NA2FB		841	652
162	NA3		-	27
163	NA3-Lex		-	143
164	NA4		149	9
165	A2F(2-3)		3,163	2,063
166	A2(2-6)		813	1,510
167	AGP-Bi-Ac2		927	1,411
168	AGP-Bi-Gc2		2,525	2,271
169	AGP-Bi-AcGc		1,146	1,179
170	A3		94	33
171	Fuc-GlcNAc		27	-
172	GM4		-	-
173	SM3		-	114
174	Haematoside		-	-
175	GM3		-	61
176	GM3(Gc)		250	107
177	Asialo-GM2		202	-
178	SM2		130	-
179	SB2		-	-
180	GM2		131	-
181	Asialo-GM1		-	88
182	Asialo-GM1-Tetra		-	-
183	SM1a		-	-
184	SB1a		-	-
185	GM1b		-	-
186	GM1		-	-

Table 1 (*continued*)

187	GM1-penta	Galβ-3GalNAcβ-4Galβ-4Glc-DH NeuAcα-3	-	-
188	GM1(Gc)	Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer NeuGcα-3	-	-
189	GM1(Gc)-penta	Galβ-3GalNAcβ-4Galβ-4Glc-DH NeuGcα-3	193	-
190	GD1a	NeuAcα-3Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer NeuAcα-3	-	-
191	GD1a-hexa	NeuAcα-3Galβ-3GalNAcβ-4Galβ-4Glc-DH NeuAcα-3	-	-
192	GalNAc-GD1a(Ac,Gc)	GalNAcβ-4Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer NeuGcα-3 NeuAcα-3 GalNAcβ-4Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer NeuAcα-3 NeuGcα-3	-	-
193	GD3	NeuAcα-8NeuAcα-3Galβ-4Glcβ-Cer	-	-
194	GD3-tetra	NeuAcα-8NeuAcα-3Galβ-4Glc-DH	-	-
195	GD3-tetra-AO	NeuAcα-8NeuAcα-3Galβ-4Glc-AO	-	-
196	GD2	GalNAcβ-4Galβ-4Glcβ-Cer NeuAcα-8NeuAcα-3	273	-
197	GD1b	Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer NeuAcα-8NeuAcα-3	338	-
198	GT1a	NeuAcα-8NeuAcα-3Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer NeuAcα-3	123	-
199	GT1b	NeuAcα-3Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer NeuAcα-8NeuAcα-3	-	-
200	GQ1b	NeuAcα-8NeuAcα-3Galβ-3GalNAcβ-4Galβ-4Glcβ-Cer NeuAcα-8NeuAcα-3	-	-
201	GalNAc-Ser	GalNAc-Ser	514	-
202	GalNAc-Thr	GalNAc-Thr	-	-
203	Galß-3GalNAc	Galβ-3GalNAc-DH	-	62
204	Galß-6GalNAc	Galβ-6GalNAc-DH	263	46
205	B12/3	GalNAcβ-4Galβ-OX NeuGcα-3	-	-
206	DST	NeuAcα-3Galβ-3GalNAc-DH NeuAcα-6	-	-
207	Man-Ser	Manα-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	Fucα-Thr	-	-
212	Notch-2	GlcNAcβ-3Fucα-Thr	-	-
213	Notch-3	Galβ-4GlcNAcβ-3Fucα-Thr	-	-
214	SA2(α8)	NeuAcα-8NeuAc-DH	72	-
215	SA3(α8)	NeuAcα-8NeuAcα-8NeuAc-DH	-	-
216	SA4(α8)	NeuAcα-8NeuAcα-8NeuAcα-8NeuAc-DH	125	-
217	SA5(α8)	NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAc-DH	-	-
218	SA6(α8)	NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAc-DH	127	-
219	SA7(α8)	NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAc-DH	-	-
220	SA8(α8)	NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAc-8NeuAcα-8NeuAc-DH	-	-
221	SA9(α8)	NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAc-8NeuAcα-8NeuAc-8NeuAcα-DH	-	-
222	SA10(α8)	NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAc-DH	-	-
223	SA11(α8)	NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAcα-8NeuAc-DH	-	-
224	Hep-Di IS	ΔUA-4GlcNS-DH SU-2 SU-6	-	216
225	Hep-Di-IS-AO	ΔUA-4GlcNS-AO SU-2 SU-6	-	-
226	Lam-2	Glcβ-3Glc-DH	223	27
227	Glc2(α2)	Glcα-2Glc-DH	-	13
228	Glc2(α3)	Glcα-3Glc-DH	118	-
229	Glc2(α2)-AO	Glcα-2Glc-AO	-	-
230	Glc2(α3)-AO	Glcα-3Glc-AO	-	-

Table 1 (*continued*)

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	NeuGc-DH	117	-
246	NeuGc-AO	NeuGc-AO	-	-
247	Rha	Rha-DH	367	-
248	Rha-AO	Rha-AO	-	-
249	Galα-6Glc-AO	Galα-6Glc-AO	-	-
250	(6P)-Man	P-6Man-DH	-	145
251	(6P)-Man-AO	P-6Man-AO	-	-
252	(6P)-Man5	P-6Manα-3Manα-3Manα-3Manα-2Man-DH	-	-
253	SU-Tyr	SU-Tyr	-	-
254	SU-Cholesterol	SU-Cholesterol	-	-
255	Glc(α6,α4,α4)	Glcα-6Glcα-4Glcα-4Glc-DH	631	76

[a] Pos, Probe position in the screening microarray.

[b] The 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-anhydro-mannose; 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 lipid-linked 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 μg/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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