TITLE

A general microarray technique for immobilization and screening of natural glycans

AUTHORS

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ABSTRACT

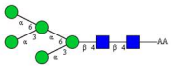
We here present a printed covalent glycan microarray for protein binding studies, using low femtomol quantities of glycans. Glycans, either natural glycans which were released from glycoproteins and glycolipids from natural sources, or synthetic glycans, were labeled with common fluorescent labels (e.g., 2-aminobenzamide or 2-aminobenzoic acid) by reductive amination and purified by HPLC. The purified glycoconjugates were covalently immobilized on commercial epoxide-activated glass slides via the secondary amine group that links the glycan moiety with the fluorescent tag. This immobilization procedure is generally applicable to reductively aminated glycans with different established fluorescent labels and allows the spatial arrangement of oligosaccharides.

The microarray comprised a variety of natural glycans from various biological sources and synthetic glycans and provided informative binding fingerprints for the lectin concanavalin A as well as fourteen monoclonal antibodies. Recognized glycans were characterized by tandem mass spectrometry revealing binding motifs.

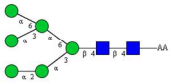
This natural glycan array allowed the characterization of the specificity of carbohydrate-binding proteins for oligosaccharide ligands from sparse biological sources. Moreover, it was applied for the characterization of the microarray glycans by using known carbohydrate-binding proteins.

SUPPORTING FIGURES AND LEGENDS

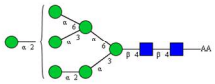
1. 1 μM
2. 10 μM



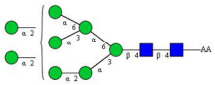
3. 0.01 μM
4. 0.1 μM
5. 0.5 μM
6. 3 μM
7-13. 10 μM
14. 100 μM



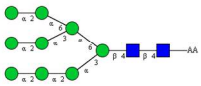
15. 1 μM
16. 10 μM



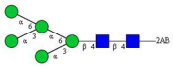
17. 1 μM
18. 10 μM



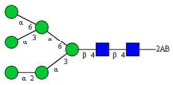
19. 1 μM
20. 10 μM



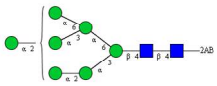
21. 1 μM
22. 10 μM



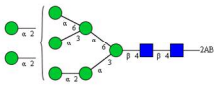
23. 0.01 μM
24. 0.1 μM
25. 0.5 μM
26. 3 μM
27. 10 μM
28. 100 μM



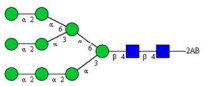
29. 1 μM
30. 10 μM



31. 1 μM
32. 10 μM



33. 1 μM
34. 10 μM



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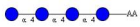

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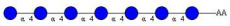
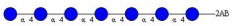
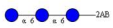


37. 10 μM



38. 10 μM

39. 10 μM



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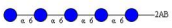
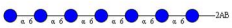
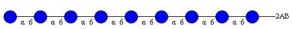


41. 10 μM



42. 10 μM



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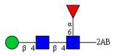

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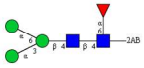

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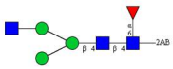
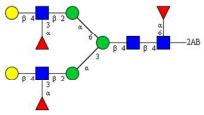


46. 10 μM



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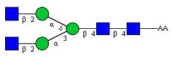

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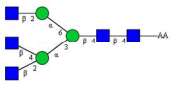

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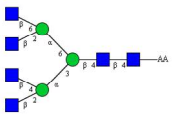
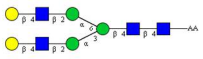


50. 10 μM

51. 10 μM



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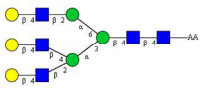

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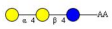

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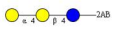

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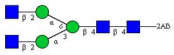

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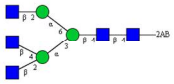

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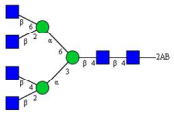
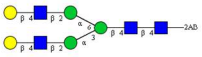


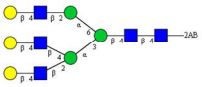
58. 10 μM

59. 10 μM H3N1F1

60. 10 μM



61. 10 μM



62. 10 μM
63. 100 μM

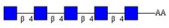
64. 10 μM
65. 100 μM

66. 10 μM
67. 100 μM

68. 10 μM

69. 10 μM

70. 10 μM



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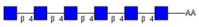
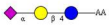


72. 10 μM

73. 10 μM



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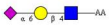
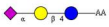


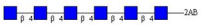
75. 10 μM



76. 10 μM

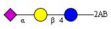
77. 10 μM

78. 10 μM

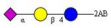


79. 10 μM

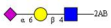
80. 10 μM



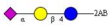
81. 10 μM



82. 10 μM

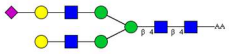


83. 10 μM

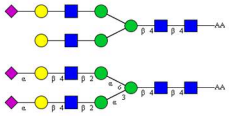


84-85. blank

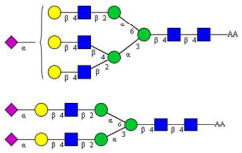
86-87.



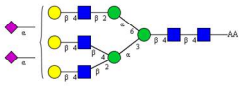
88.



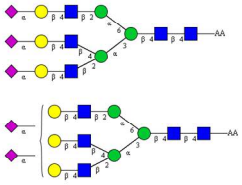
89-90.



91-92.



93-94.

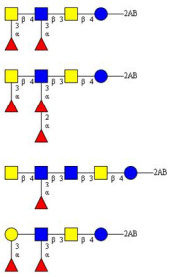


95-96. -

97-100. blank

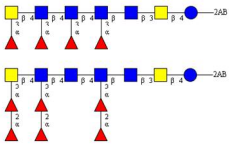
101. *S.mansoni* eggs fr. 8.1

102. *S.mansoni* eggs fr. 8.2

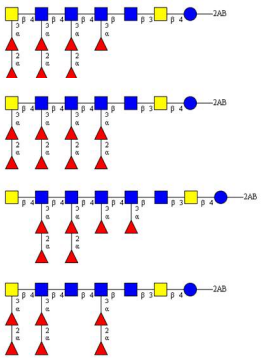


103-104. *S.mansoni* eggs fr. 8.3-8.4

105. *S.mansoni* eggs fr. 15.1

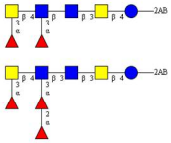


106. *S.mansoni* eggs fr. 15.2



107-110. *S.mansoni* eggs fr. 15.3-15.7

111. *S.mansoni* eggs fr. 10.1



112-117. *S.mansoni* eggs fr. 10.3-10.7

118-120. blank

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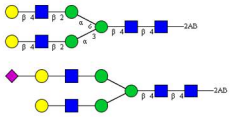

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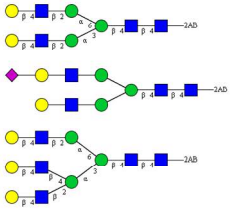
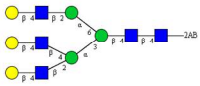


123-124



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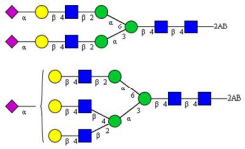

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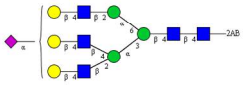
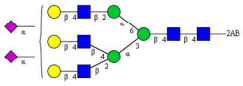


127-128.



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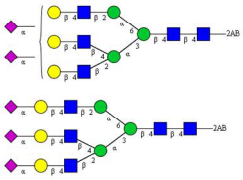

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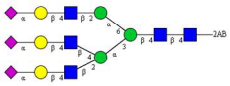

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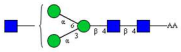

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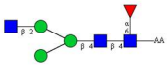

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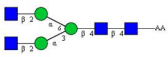

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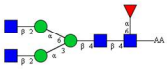

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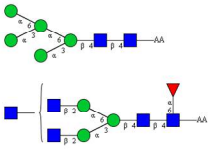

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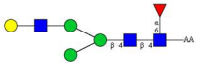

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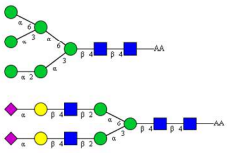

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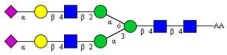

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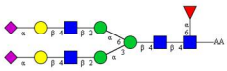
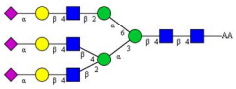
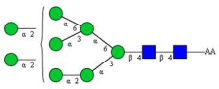
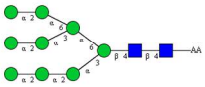
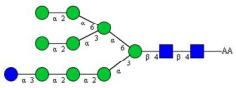


141. 

142. 

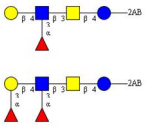
143. 

144. 

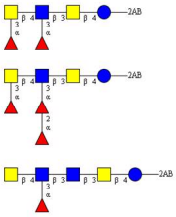
145-146. -

147-152. *S.mansoni* eggs fr. 1-6

153. *S.mansoni* eggs fr. 7

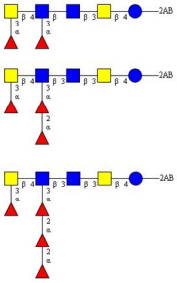


154. *S.mansoni* eggs fr. 8



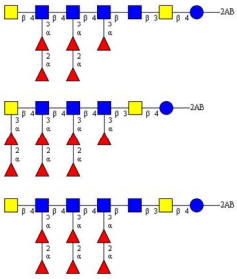
155. *S.mansoni* eggs fr. 9

156. *S.mansoni* eggs fr. 10

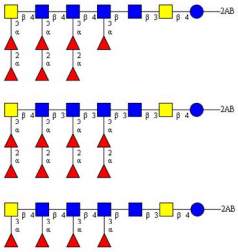


157-159. *S.mansoni* eggs fr. 11-13

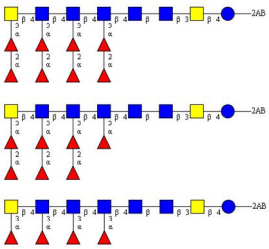
160. *S.mansoni* eggs fr. 14



161. *S.mansoni* eggs fr. 15



162. *S.mansoni* eggs fr. 16



163-164. *S.mansoni* eggs fr. 17-18

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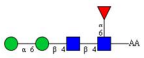

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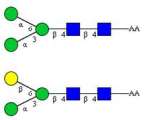

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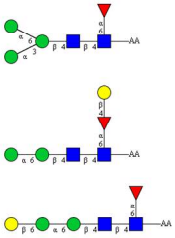

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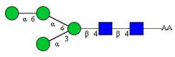

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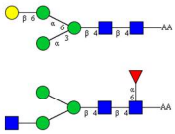

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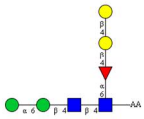

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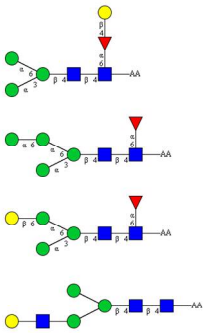

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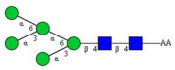

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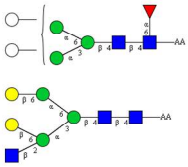

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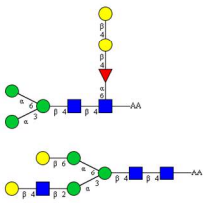

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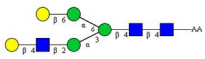

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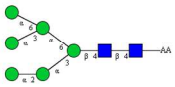

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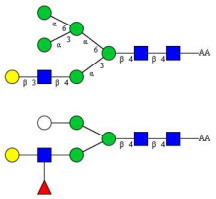

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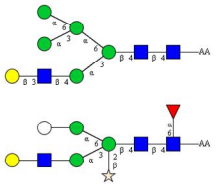

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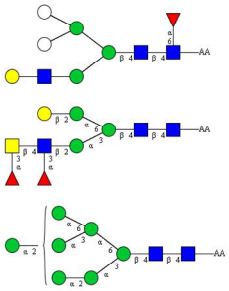

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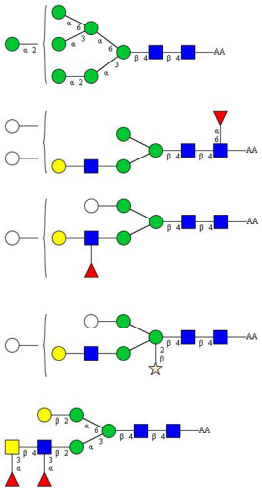

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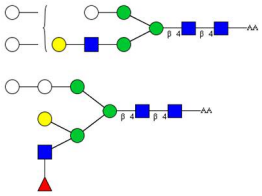

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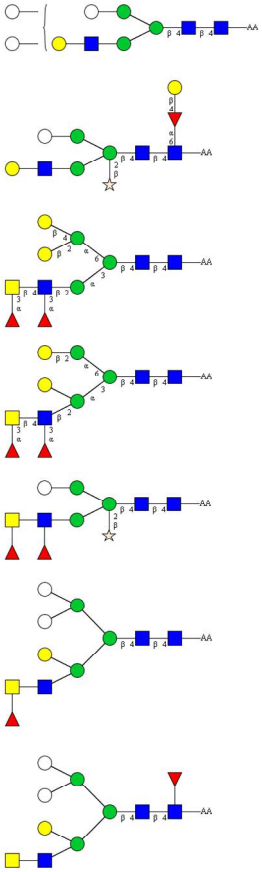

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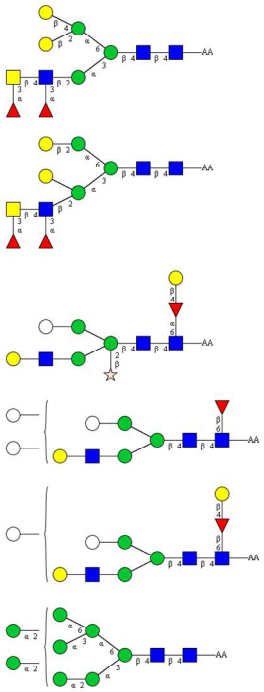

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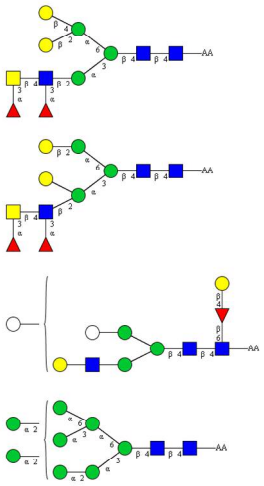

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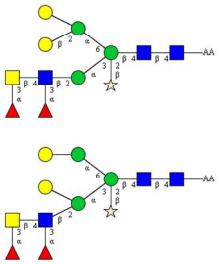

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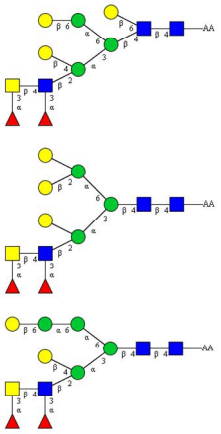

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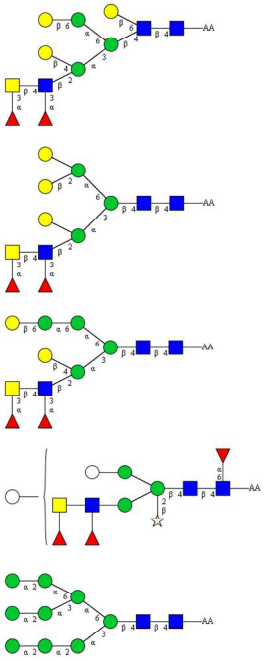


189. -

190-192. blank

193. 20 µg/mL
194. 200 µg/mL

195. 20 µg/mL
196. 200 µg/mL

197. 20 µg/mL
198. 200 µg/mL

199. 20 µg/mL
200. 200 µg/mL

201-202. blank

203. 20 µg/mL RNase A
204. 200 µg/mL

205. 20 µg/mL RNase B
206. 200 µg/mL

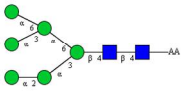
207. 20 µg/mL fetuin
208. 200 µg/mL

209. 20 µg/mL asialofetuin
210. 200 µg/mL

211. 20 µg/mL BSA
212. 200 µg/mL
213. 500 µg/mL

214-217. blank

218-230. 10 µM



231. blank

232. 0.01 µg/mL
233. 0.1 µg/mL
234. 0.5 µg/mL
235. 1 µg/mL
236. 5 µg/mL
237. 10 µg/mL
238. 20 µg/mL
239. 200 µg/mL

240. blank

- galactose (Gal)
- glucose (Glc)
- mannose (Man)
- hexose, unspecified (Hex)
- *N*-acetylgalactosamine (GalNAc)
- *N*-acetylglucosamine (GlcNAc)
- fucose (Fuc)
- xylose (Xyl)
- sialic acid (Sia)

<u>Natural glycans:</u>

1-34, 218-230.	RNase B
43-46.	*S. mansoni* adult worms
51-52, 60-61.	asialofetuin
86-96, 121-130.	fetuin ~10 µM (average value)
101-117.	*S. mansoni* eggs subfractions ~5 µM (average value)
131-146.	human serum ~10 µM (average value)
147-164.	*S. mansoni* eggs fractions ~5 µM (average value)
166-189.	KLH ~10 µM (average value)

Figure S-1. Full list of glycan structures that were spotted on the microarray.

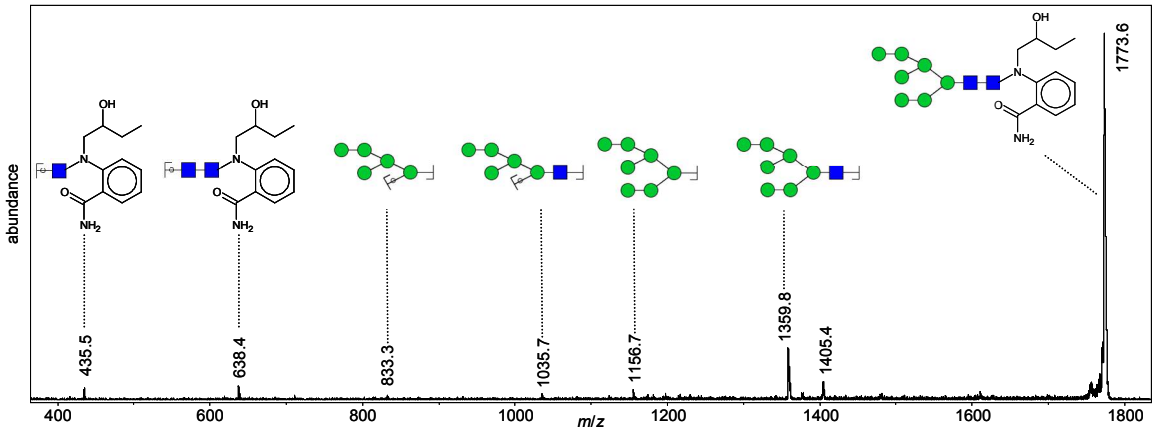


Figure S-2. MALDI-TOF/TOF-MS/MS spectrum of $Man_7GlcNAc_2$-2AB incubated with an excess of 1,2-epoxybutane in 100 mmol/L triethylamine, pH 8.4, for 60 h at room temperature. *M/z* 1773.6 ($[M+Na]^+$) corresponds to $Man_7GlcNAc_2$-2AB-1,2-epoxybutane, *m/z* 435.5 and *m/z* 638.4 correspond to $GlcNAc_1$-2AB-1,2-epoxybutane and $GlcNAc_2$-2AB-1,2-epoxybutane, respectively. Spectrum was acquired in the positive ion mode.

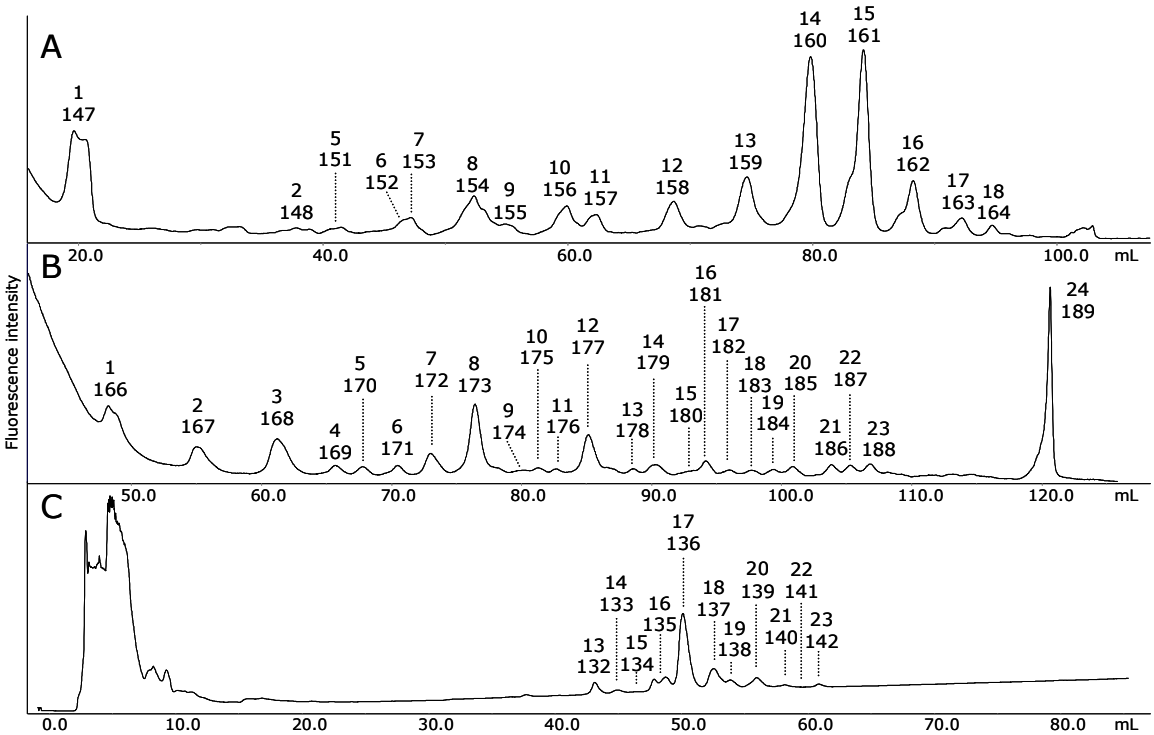


Figure S-3. HILIC fractionation of (A) O-glycans from *S. mansoni* eggs, (B) N-glycans from KLH, and (C) N-glycans from human serum. Peaks are labeled with fraction number (top) and microarray sample number (bottom).

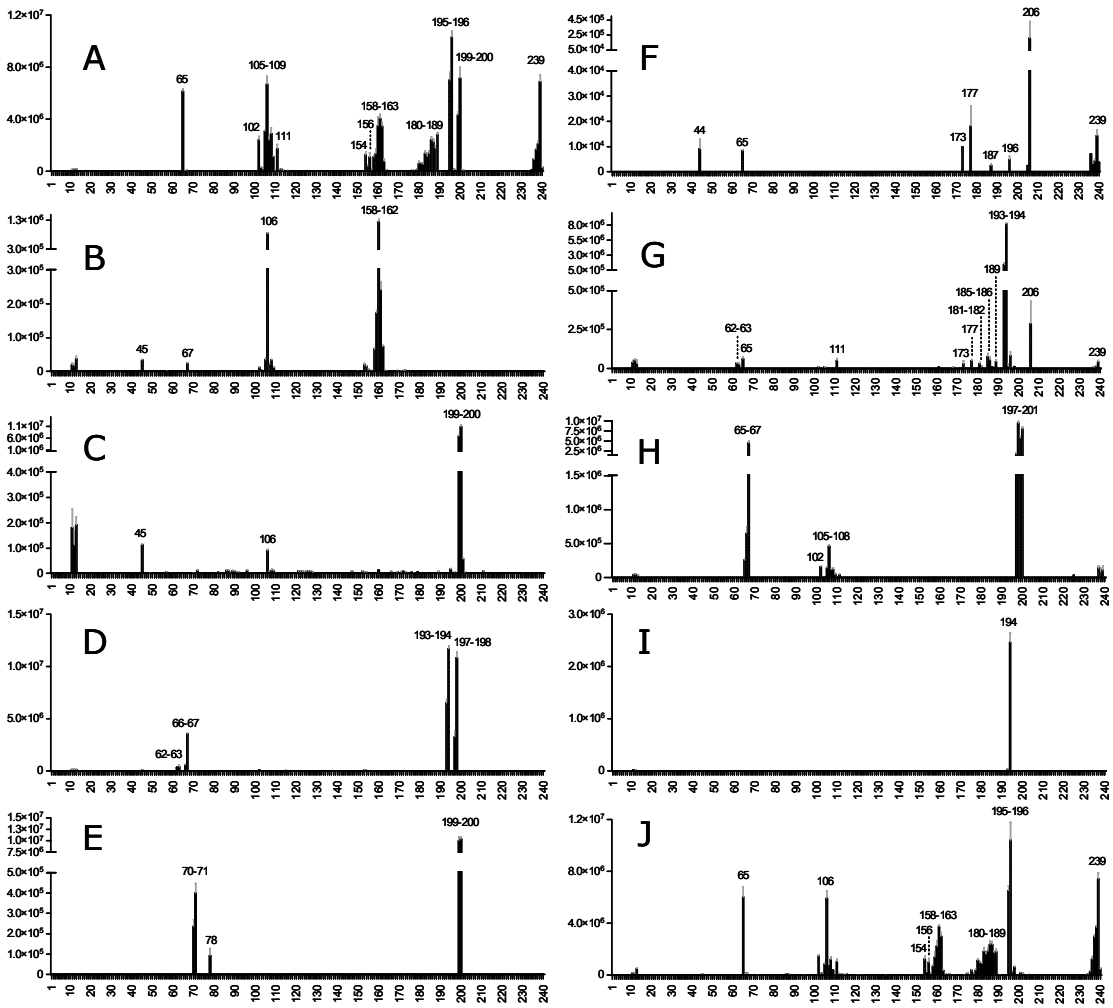


Figure S-4. Additional microarray read-outs of tested mAbs that were not shown in Figure 3. (A) 258-3E3, (B) 114-5B1-A, (C) 259-2A1, (D) 290-2E6, (E) 114-3A5, (F) 114-4E8-A, (G) 128-4F9-A, (H) 273-3F2-A, (I) 99-1G3 and (J) 291-5D5.

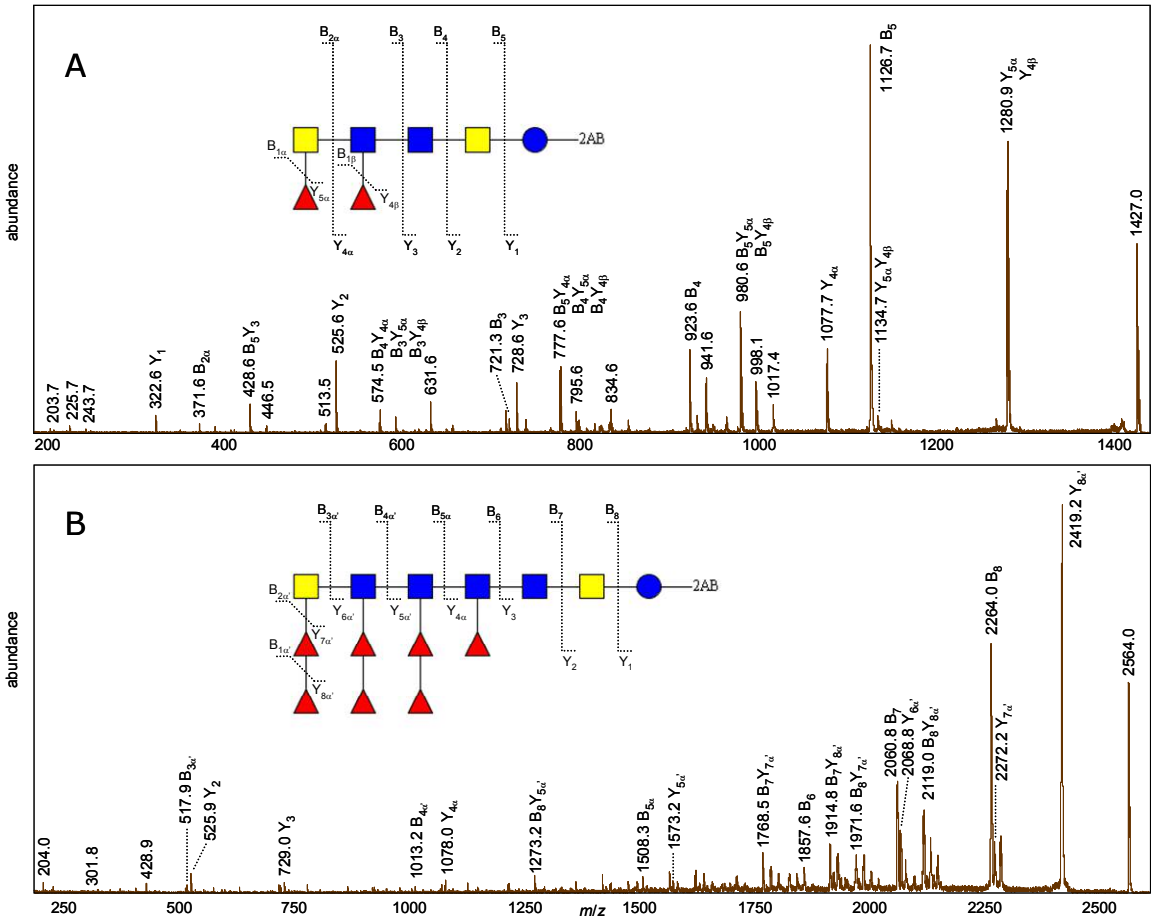


Figure S-5. (A) MALDI-TOF/TOF-MS/MS data of the FLDNF-epitope containing structure in sample *#111* with *m/z* 1427.0 ([M+Na]$^+$) (B) MALDI-TOF/TOF-MS/MS of sample *#106* with *m/z* 2564.0 ([M+Na]$^+$). Spectra were acquired in the positive ion mode.