Density Variant Glycan Microarray for Evaluating Crosslinking of Mucin-like Glycoconjugates by Lectins.

Kamil Godula[†,∫,|] and Carolyn R. Bertozzi[*,†,‡,§,∫,|]

[†]Department of Chemistry, [‡]Department of Molecular and Cell Biology, and [§]The Howard Hughes Medical Institute, University of California, Berkeley, California 94720. [∫]Materials Sciences Division and [|]The Molecular Foundry, Lawrence Berkeley National Laboratory, Berkeley, California 94720

Supporting Information

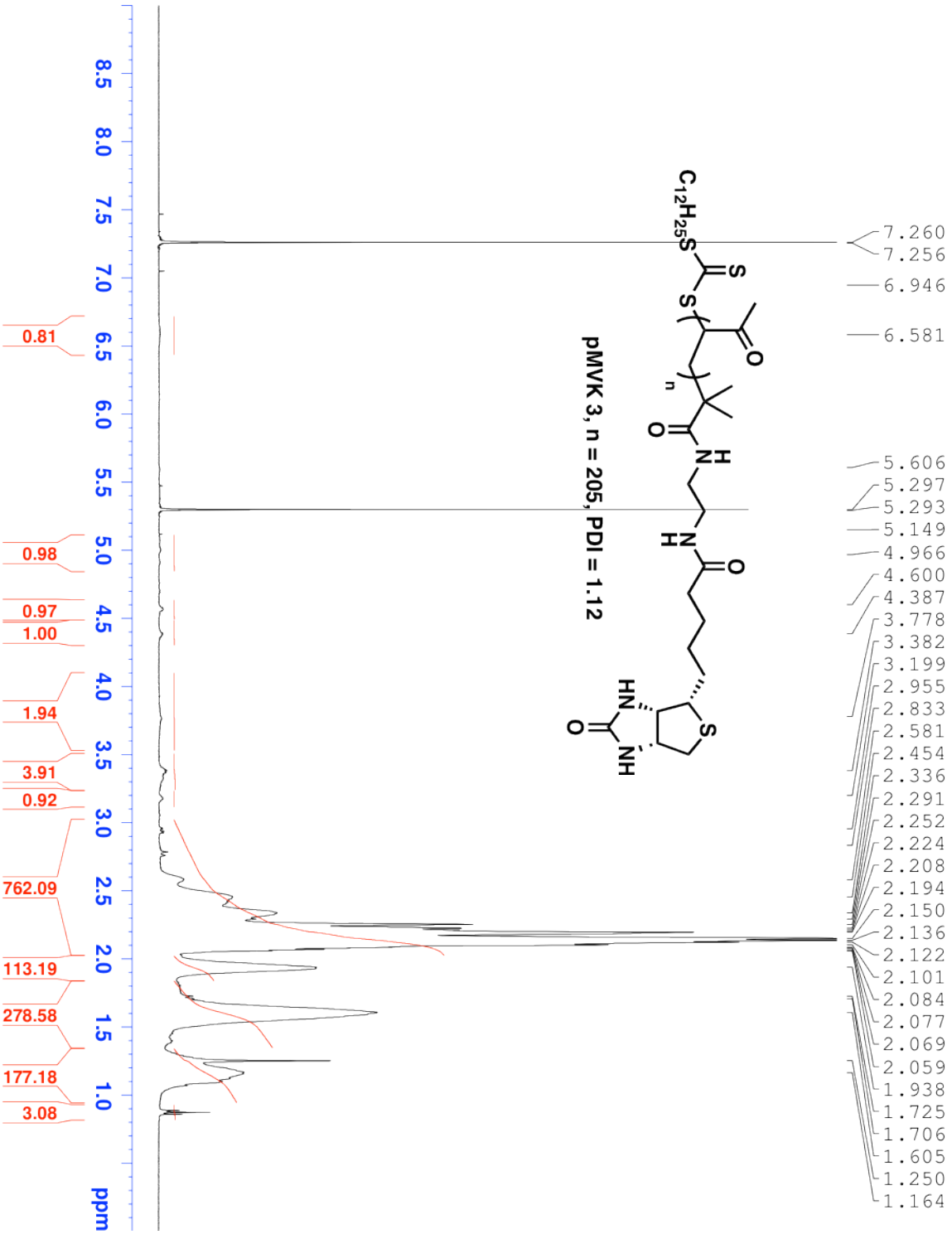
Content:

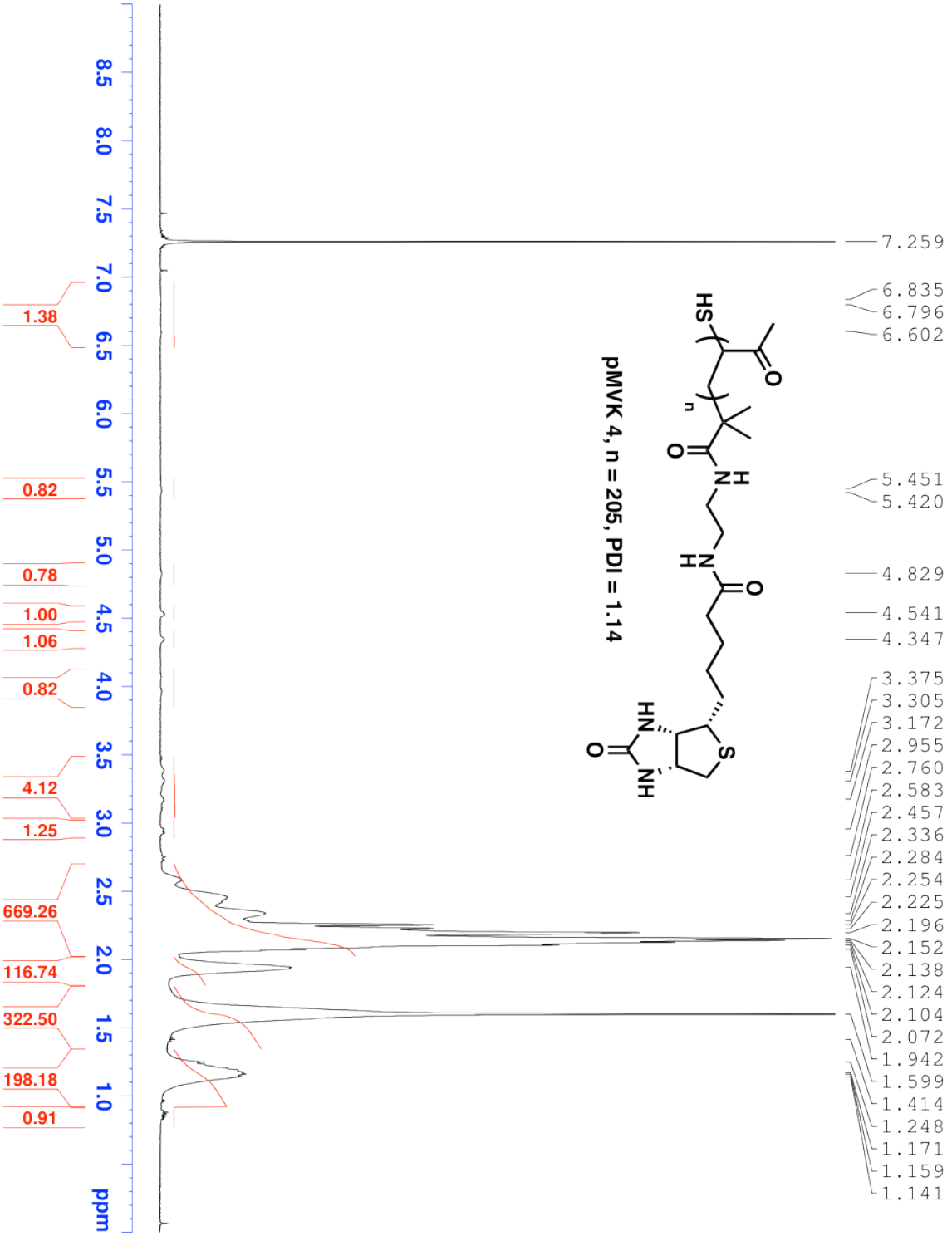
1. ^{1}H NMR spectra of pMVK precursors **3**, **4**, **7**, and **8**, and glycopolymers **6** and **9**.
2. SEC traces of pMVK polymers **3**, **4**, **7**, and **8**.
3. Determination of Mw of lectin monomers by SDS-PAGE.
4. Determination of apparent Mw of all lectins by FPLC.
5. Mucin mimetic array characterization.
   A) Images of a low-density array with polymers **6a-e**.
   B) Printing parameters.
   C) Derivation of equation 2 for the determination of average polymer spacing.
6. Images of a density variant array of polymer **9** and a plot of average polymer spacing.
7. Plot of average GalNAc density as a function of glycopolymer spacing.
8. Generation of a reduced, non-agglutinating form of WFL, RWFL.
9. Tables and charts:

   Table S1. Stoichiometry and efficiency of aminooxy-GalNAc (**5**) ligation to keto groups in polymers **4** and **8**.

   Table S2. Buffers, extinction coefficients at $\lambda = 280$, and extent of lectin labeling with AF647-NHS.

1. ^{1}H NMR spectra of pMVK precursors 3, 4, 7, and 8, and glycopolymers 6 and 9.

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6.796
6.602
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4.347
3.375
3.305
3.172
2.955
2.760
2.583
2.457
2.336
2.284
2.254
2.225
2.196
2.152
2.138
2.124
2.104
2.072
1.942
1.599
1.414
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1.159
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0.82
0.78
1.00
1.06
0.82
4.12
1.25
669.26
116.74
322.50
198.18
0.91
8.5
8.0
7.5
7.0
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6.0
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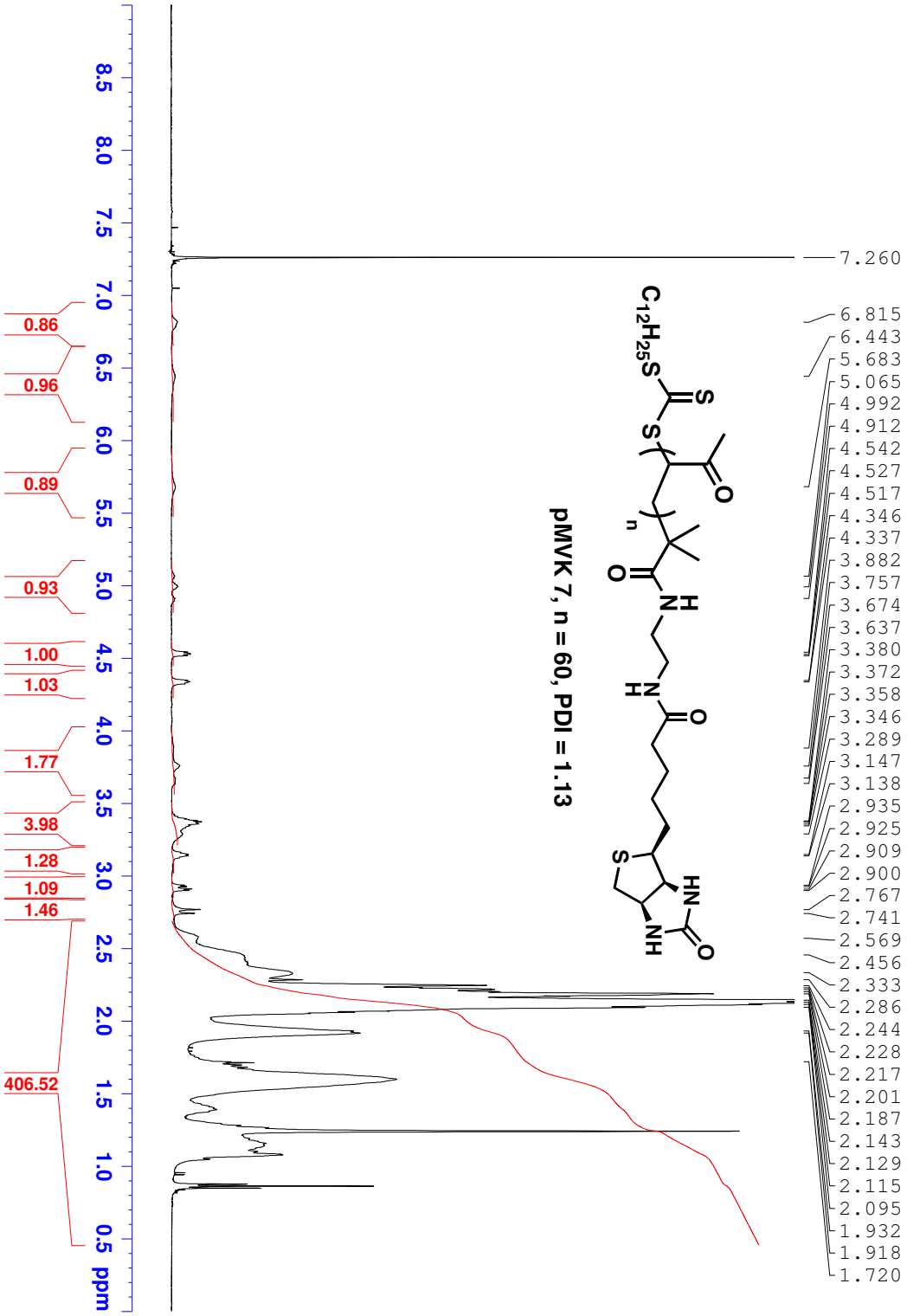
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6.815
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4.527
4.517
4.346
4.337
3.882
3.757
3.674
3.637
3.380
3.372
3.358
3.346
3.289
3.147
3.138
2.935
2.925
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2.900
2.767
2.741
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2.244
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2.217
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2.187
2.143
2.129
2.115
2.095
1.932
1.918
1.720
0.86
0.96
0.89
0.93
1.00
1.03
1.77
3.98
1.28
1.09
1.46
406.52
8.5
8.0
7.5
7.0
6.5
6.0
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3.5
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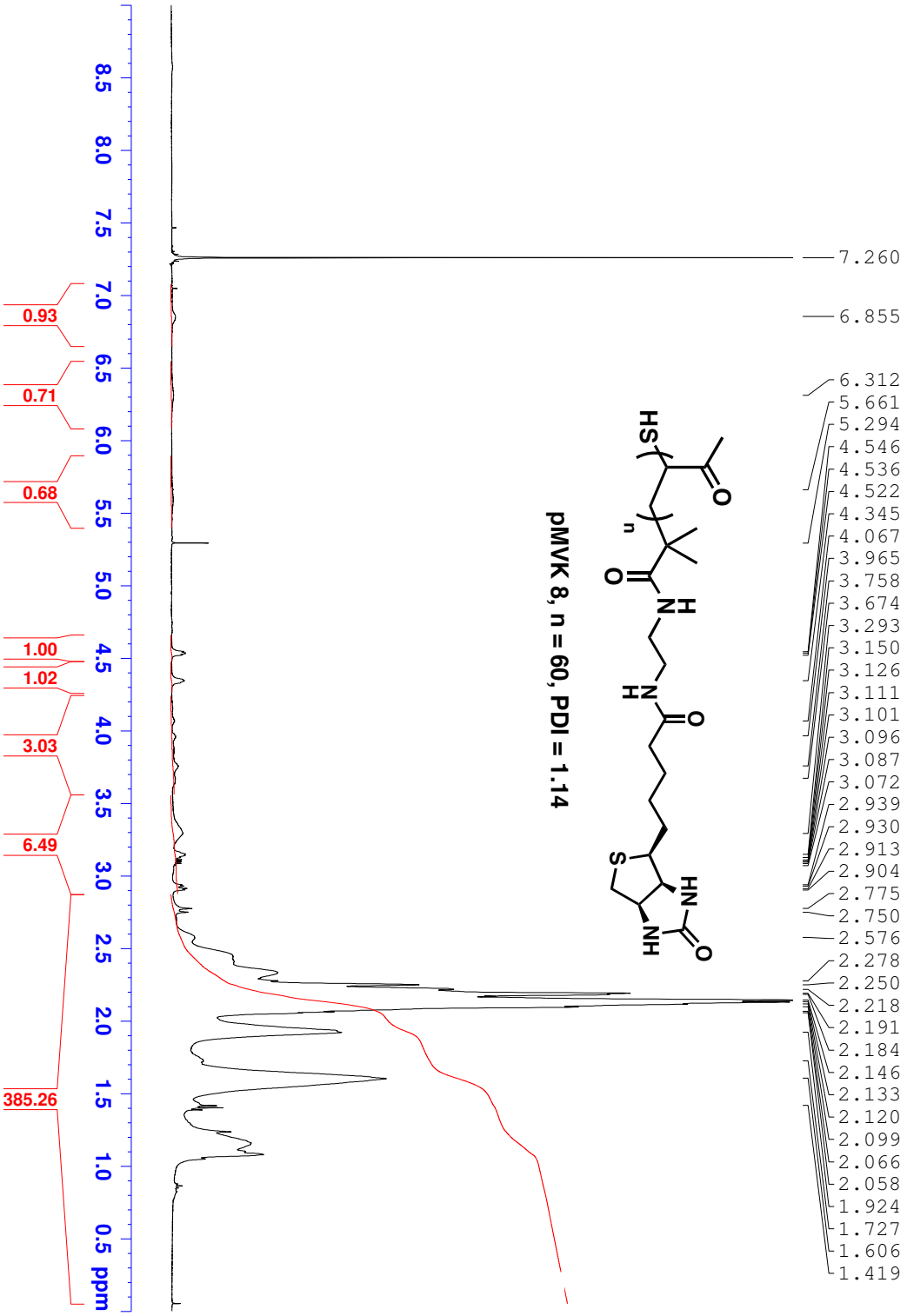
1.0
0.5
ppm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4.067
3.965
3.758
3.674
3.293
3.150
3.126
3.111
3.101
3.096
3.087
3.072
2.939
2.930
2.913
2.904
2.775
2.750
2.576
2.278
2.250
2.218
2.191
2.184
2.146
2.133
2.120
2.099
2.066
2.058
1.924
1.727
1.606
1.419
0.93
0.71
0.68
1.00
1.02
3.03
6.49
385.26
8.5
8.0
7.5
7.0
6.5
6.0
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1.0
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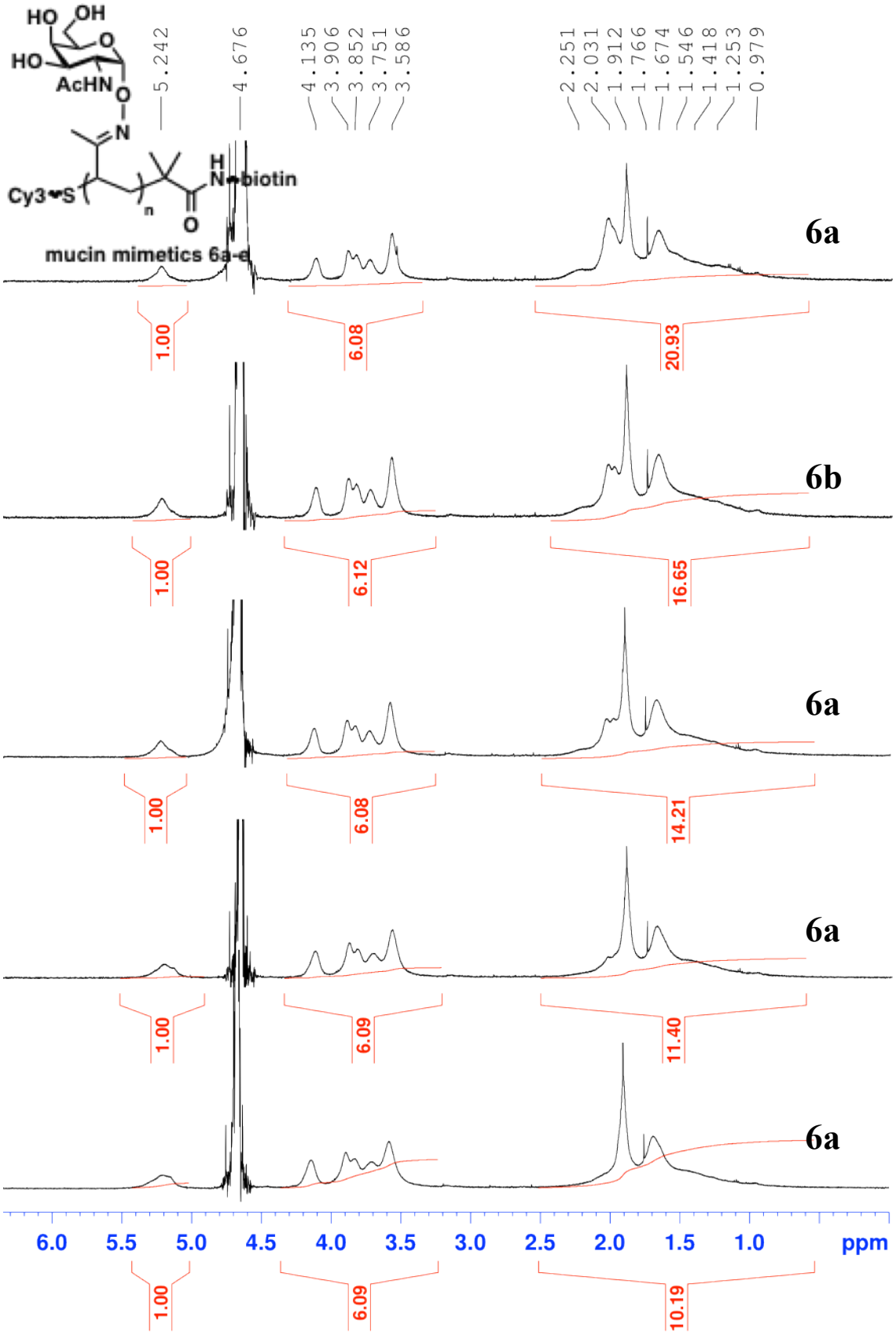
ppm


HO
OH
HO
O
AcHN
O
N
Cy3
S
n
H
N
biotin
O
mucin mimetics 6a-e
5.242
4.676
4.135
3.906
3.852
3.751
3.586
2.251
2.031
1.912
1.766
1.674
1.546
1.418
1.253
0.979
6a
1.00
6.08
20.93
6b
1.00
6.12
16.65
6a
1.00
6.08
14.21
6a
1.00
6.09
11.40
6a
1.00
6.09
10.19
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1.0
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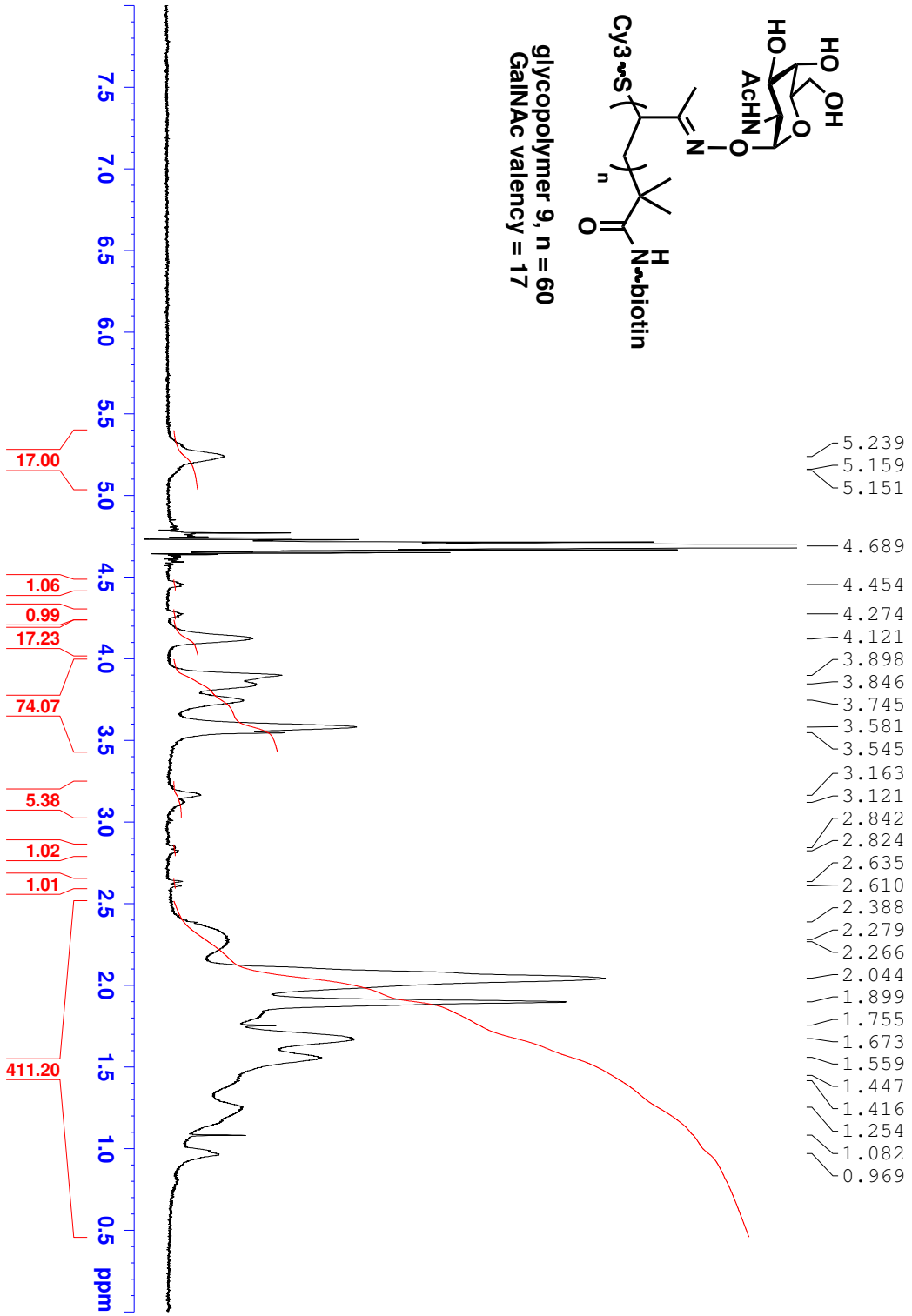
glycopolymer 9, n = 60
GalNAc valency = 17
Cy3
S
n
O
H
N
biotin
HO
OH
HO
AcHN
O
N
O
5.239
5.159
5.151
4.689
4.454
4.274
4.121
3.898
3.846
3.745
3.581
3.545
3.163
3.121
2.842
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2.635
2.610
2.388
2.279
2.266
2.044
1.899
1.755
1.673
1.559
1.447
1.416
1.254
1.082
0.969
17.00
1.06
0.99
17.23
74.07
5.38
1.02
1.01
411.20
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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2.5
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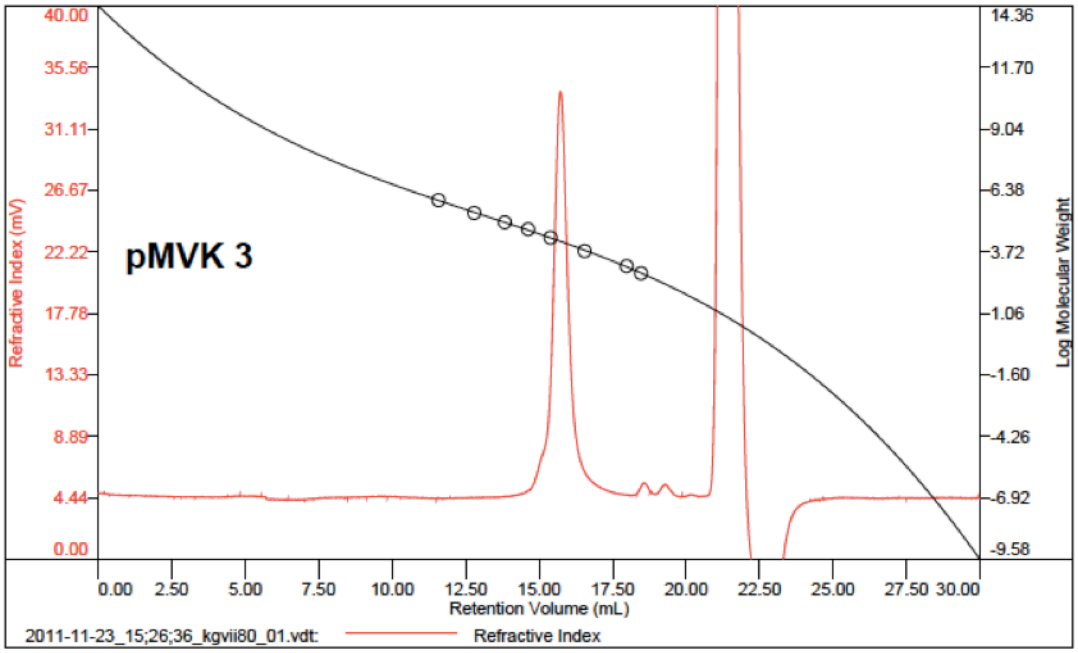
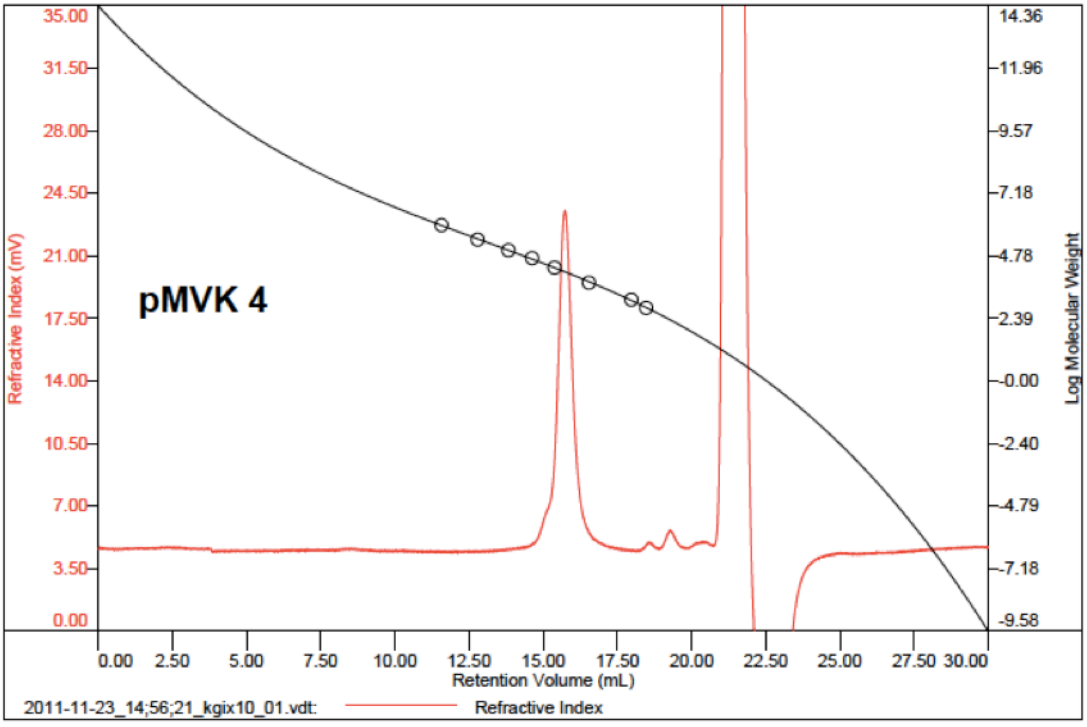
1.5
1.0
0.5
ppm

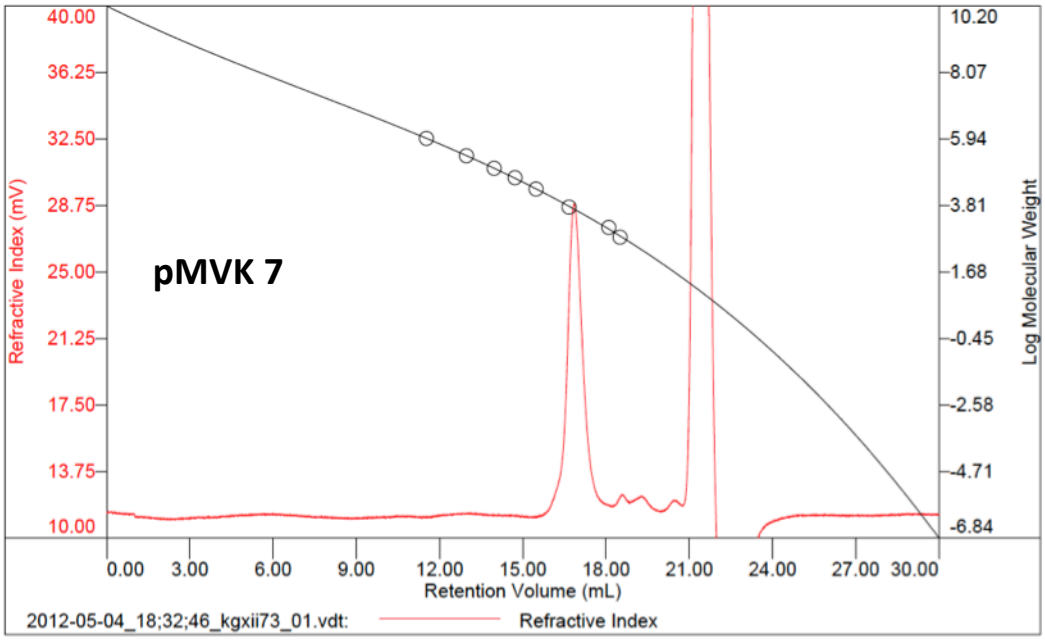
2. SEC traces of pMVK polymers 3, 4, 7, and 8.



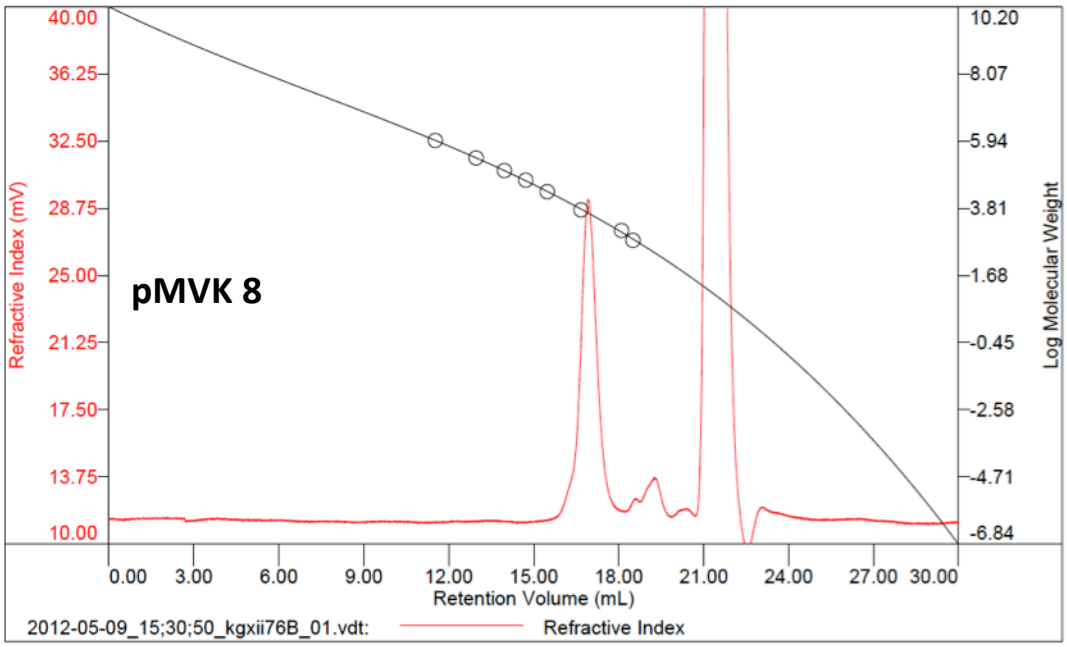
Sample	Mn	Mw	Mz	Mw/Mn
pMVK 3	13,183	15,080	17,049	1.144



Sample	Mn	Mw	Mz	Mw/Mn
pMVK4	13,426	15,255	17,233	1.136

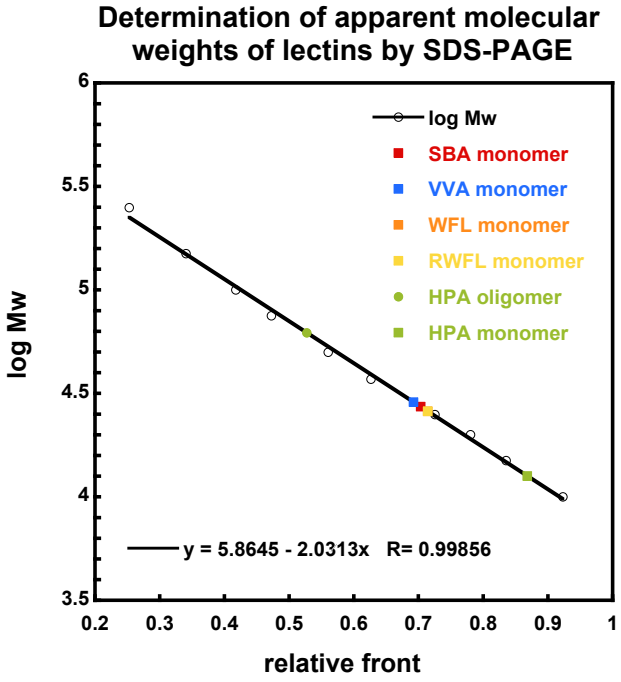


Sample	Mn	Mw	Mz	Mw/Mn
pMVK 7	4,345	4,905	5,490	1.129



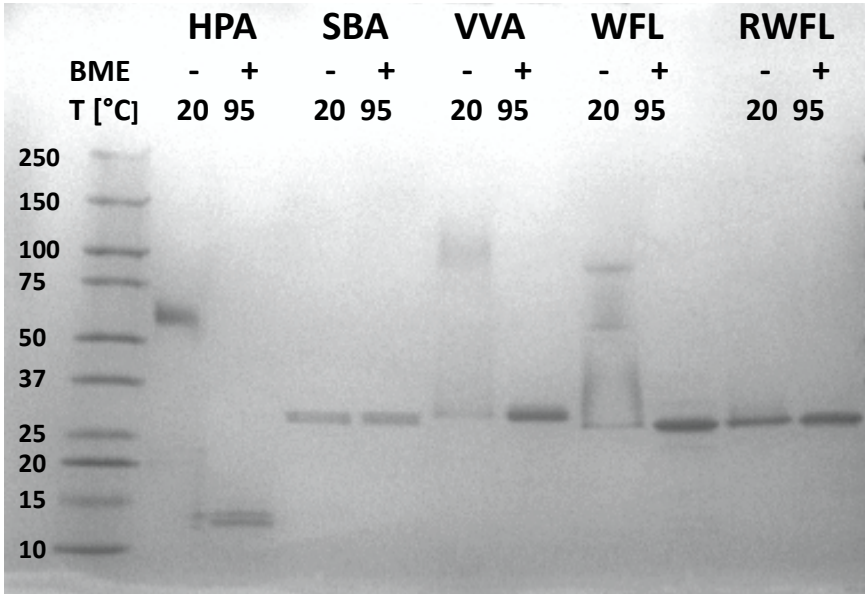
Sample	Mn	Mw	Mz	Mw/Mn
pMVK 8	4,011	4,582	5,256	1.142

3. Determination of Mw of lectin monomers by SDS-PAGE.



Lectin	relative front	Mw [Da]
SBA monomer	0.70	27,283
VVA monomer	0.69	28,722
WFL monomer	0.71	25,916
RWFL monomer	0.71	25,916
HPA oligomer	0.53	62,093*
HPA monomer	0.87	12,620

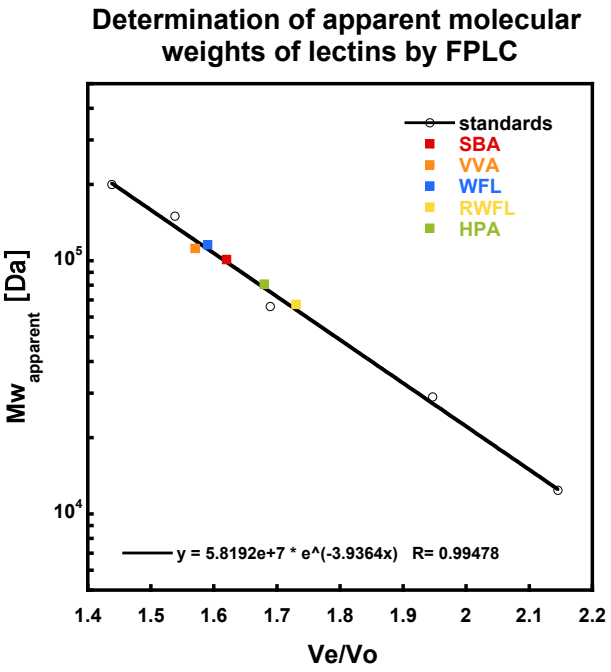
* the somewhat lower apparent Mw for HPA hexamer is consistent with previously reported observations (*ref*. Renwrantz L. *et al. J. Mulluscan Studies* **2009**, *75*, 41-49).



All lectins were dissociated into monomers by heating at 95 °C for 10 min in a loading buffer containing 5% β-mercaptoethanol (BME). Gel electrophoresis was performed on 4-20% PA gel in 0.1 % SDS in 1 x TRIS/glycine buffer, pH = 8.3.

* only HPA oligomer showed stability under non-reducing SDS-PAGE conditions (lectin samples prepared in Leammli loading buffer at 20 °C.

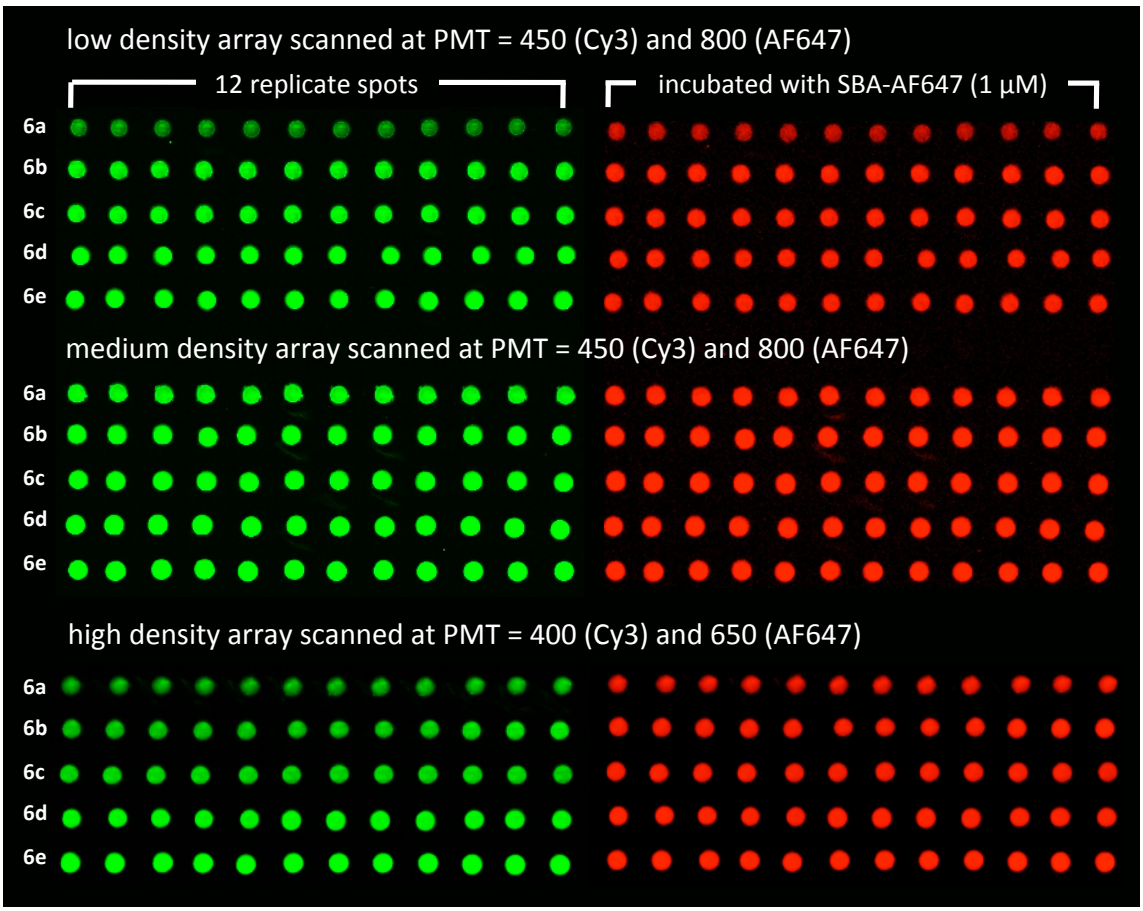
4. Determination of apparent Mw of lectins by FPLC.



Protein	Mw [Da]	Ve [mL]	Ve/Vo
Cytochrome c	12,400	17.96	2.15
Carbonic anhydrase	29,000	16.30	1.95
Bovine serum albumin	66,000	14.14	1.69
Alcohol dehydrogenase	150,000	12.88	1.54
β-Amylase	200,000	12.04	1.44
SBA	101,139	13.58	1.62
VVA	111,843	13.37	1.60
WFL	105,709	13.49	1.61
RWFL	67,317	14.45	1.73
HPA	80,862	14.06	1.68

5. Mucin mimetic array characterization

A) Images of a low-density array with polymers 6a-e.



B) Printing parameters.

wash cycles: 3**,** wash time: 3,000 msec**,** dry time: 8,000 msec
load time: 500 msec
slow pickup speed 2000
x-axis acceleration 6,000,000
y-axis acceleration 6,000,000
z-axis acceleration/deacceleration 3,500,000/350,000 (soft tap)

C) Derivation of equation 2 for the determination of average polymer spacing.

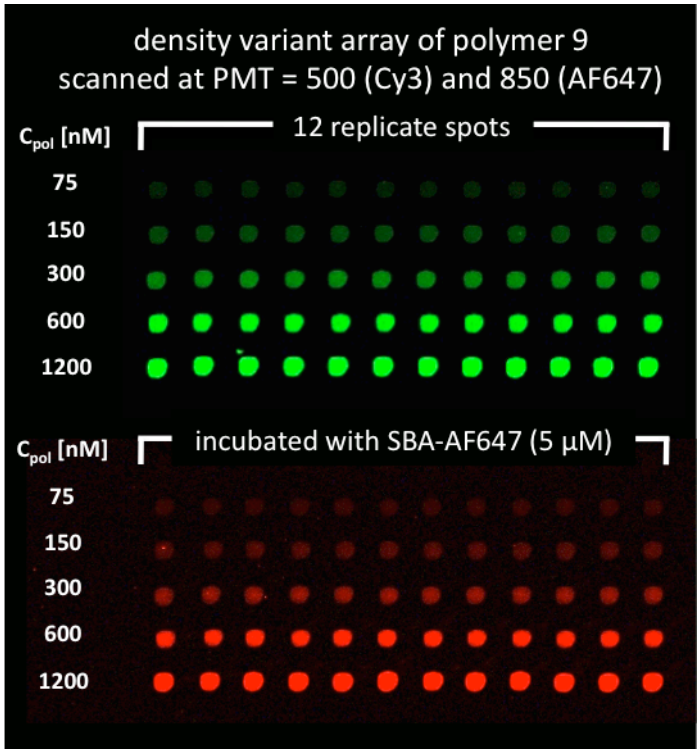
$$\rho = \frac{n_2 \cdot N_A}{\pi (r_{spot})^2},$$

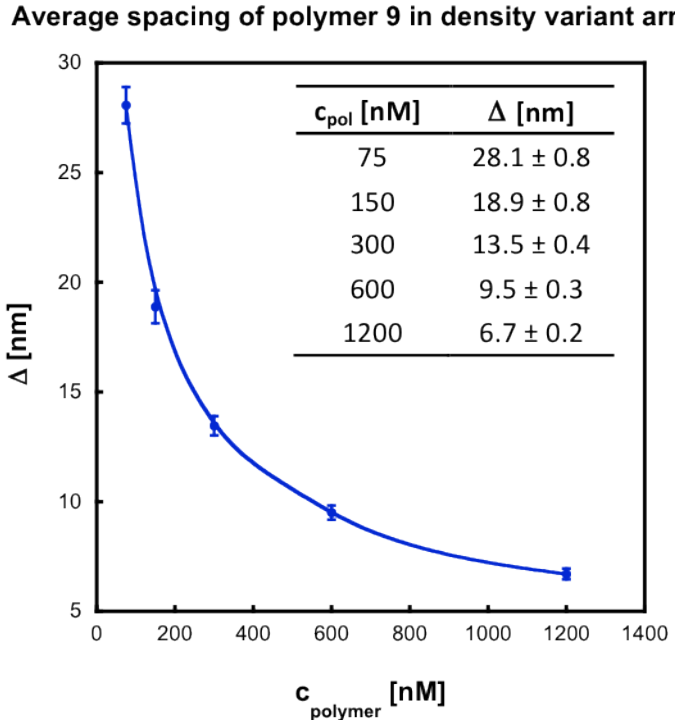
ρ ..... polymer surface density [nm^{-2}]

$$\Delta = \frac{1}{\sqrt{\rho}} = \frac{1}{\sqrt{\frac{n_2 \cdot N_A}{\pi (r_{spot})^2}}} = \frac{r_{spot} \cdot \sqrt{\pi}}{\sqrt{n_2 \cdot N_A}},$$

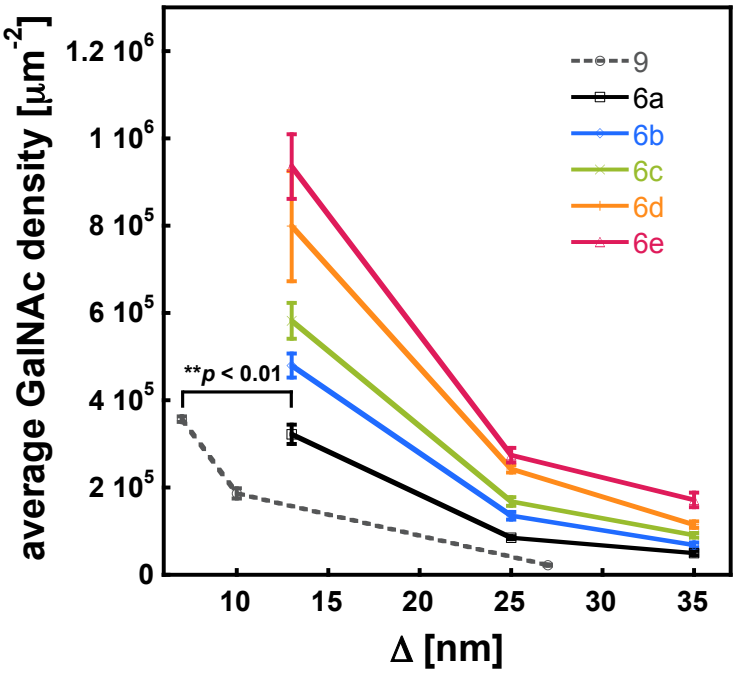
Δ ..... average spacing [nm]

6. Images of a density variant array of polymer 9 and a plot of average polymer spacing.





7. Plot of average GalNAc density as a function of glycopolymer spacing.



8. Generation of a reduced, non-agglutinating form of WFL, RWFL.

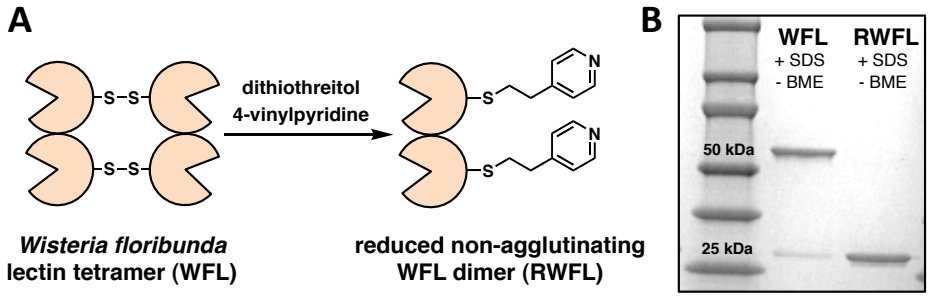


Figure S1. A) The disulfide bridged WFL tetramer was cleaved under reductive conditions followed by capping of the resulting free sulfhydryl groups with 4-vinylpyridine. B) SDS-PAGE showed complete reduction of WFL to RWFL (SDS = sodium dodecyl sulfate, BME = β-mercaptoethanol).

9. Tables and Charts.

Table S1. Stoichiometry and efficiency of aminooxy-GalNAc (**5**) ligation to keto groups in polymers **4** and **8**.

entry	equiv. of 5	product	GalNAc valency (%n)
1	0.3	**6a**	68 (33)
2	0.5	**6b**	92 (45)
3	0.6	**6c**	111 (54)
4	0.8	**6d**	146 (71)
5	1.0	**6e**	170 (83)
6	0.3	**9**	17 (28)

Table S2. Buffers, extinction coefficients at $\lambda = 280$, and extent of lectin labeling with AF647-NHS.

lectin-AF647	buffer	ε_{280} [$M^{-1} \cdot cm^{-1}$]	AF647/lectin
SBA	10 mM Na_2HPO_4, 150 mM NaCl, pH = 7.3	108,400 (tetramer)*	1.01
WFL	10 mM TRIS, 150 mM NaCl, 0.5 mM $CaCl_2$, pH = 8.5	155,200 (tetramer)*	1.40
RWFL	10 mM TRIS, 150 mM NaCl, 0.5 mM $CaCl_2$, pH = 8.5	77,600 (dimer)*	1.00
VVA	10 mM Na_2HPO_4, 150 mM NaCl, pH = 7.3	82,800 (tetramer)*	1.97
HPA	10 mM TRIS, 150 mM NaCl, pH = 8.0	116,800 (hexamer)*	1.14

*Oligomerization state of lectins was confirmed experimentally by FPLC (Section 3).

Table S3. Lectin dilutions and AF647/lectin ratios used in array assays.

lectin-AF647	dilution series	AF647/lectin
SBA	5 → 2.5 μM → 5 x dilution → 160 pM	0.10/tetramer
	10 → 5 μM → 5 x dilution → 320 pM (pol. **9**)	
WFL	5 → 2.5 μM → 5 x dilution → 160 pM	0.14/tetrmer
RWFL	10 → 5 μM → 5 x dilution → 320 pM	0.11/dimer
VVA	5 → 2.5 μM → 5 x dilution → 160 pM	0.09/tetramer
	10 → 5 μM → 5 x dilution → 320 pM (pol. **9**)	
HPA	2.5 μM → 5 x dilution → 32 pM	0.10/hexamer

Table S4. Apparent K_d values for soybean agglutinin from *Glycine max* (SBA).

pol.	apparent K_d [nM]						
	13 nm	**p value[1]**	**25 nm**	**p value[1]**	**35 nm**	**p value[1]**	**p value[2]**
6a	58 ± 6		106 ± 4		123 ± 27		0.003
6b	51 ± 6	0.127	80 ± 4	0.005	87 ± 18	0.068	0.009
6c	50 ± 4	0.091	72 ± 5	<0.001	80 ± 7	0.022	<0.001
6d	47 ± 4	0.327	54 ± 4	0.002	59 ± 10	0.011	0.071
6e	44 ± 6	0.016	53 ± 5	<0.001	51 ± 2	0.002	0.060

[1]p values refer to a comparison of K_d's between **6a** and each of the other glycopolymers (t-test, two-tailed distribution, equal variance). [2]p values refer to a comparison of K_d's for the same polymer at the lowest and highest surface densities (t-test, two-tailed distribution, equal variance).

Table S5. Apparent K_d values for Wisteria floribunda lectin (WFL).

pol.	apparent K_d [nM]						
	13 nm	**p value[1]**	**25 nm**	**p value[1]**	**35 nm**	**p value[1]**	**p value[2]**
6a	79 ± 17		85 ± 4		86 ± 8		0.483
6b	59 ± 9	0.076	62 ± 2	<0.001	72 ± 10	0.070	0.089
6c	49 ± 7	0.017	49 ± 3	<0.001	57 ± 3	0.001	0.067
6d	39 ± 6	0.087	39 ± 1	0.001	46 ± 4	0.004	0.150
6e	39 ± 7	0.005	40 ± 2	<0.001	44 ± 2	<0.001	0.263

[1]p values refer to a comparison of K_d's between **6a** and each of the other glycopolymers (t-test, two-tailed distribution, equal variance). [2]p values refer to a comparison of K_d's for the same polymer at the lowest and highest surface densities (t-test, two-tailed distribution, equal variance).

Table S6. Apparent K_d values for reduced form of *Wistaria floribunda* lectin (RWFL).

pol.	apparent K_d [nM]						
	13 nm	**p value[1]**	**21 nm**	**p value[1]**	**31 nm**	**p value[1]**	**p value[2]**
6a	378 ± 49		404 ± 62		398 ± 52		0.602
6b	313 ± 10	0.041	336 ± 31	0.096	363 ± 52	0.387	0.109
6c	252 ± 17	0.003	292 ± 20	0.014	322 ± 31	0.045	0.008
6d	196 ± 11	0.002	255 ± 19	0.034	268 ± 29	0.045	0.004
6e	188 ± 10	<0.001	247 ± 23	0.003	267 ± 21	0.003	<0.001

[1]p values refer to a comparison of K_d's between **6a** and each of the other glycopolymers (t-test, two-tailed distribution, equal variance). [2]p values refer to a comparison of K_d's for the same polymer at the lowest and highest surface densities (t-test, two-tailed distribution, equal variance).

Table S7. Apparent K_d values for *Vicia villosa-B_4* agglutinin (VVA).

pol.	apparent K_d [nM]						
	13 nm	p value[1]	25 nm	p value[1]	35 nm	p value[1]	p value[2]
6a	76 ± 4		60 ± 10		57 ± 7		0.003
6b	61 ± 5	0.003	45 ± 9	0.058	46 ± 7	0.071	0.015
6c	45 ± 4	<0.001	40 ± 5	0.012	36 ± 3	0.001	0.011
6d	32 ± 3	0.003	31 ± 3	0.020	28 ± 1	0.002	0.045
6e	29 ± 2	<0.001	27 ± 4	0.001	25 ± 3	<0.001	0.104

[1]p values refer to a comparison of K_d's between **6a** and each of the other glycopolymers (t-test, two-tailed distribution, equal variance). [2]p values refer to a comparison of K_d's for the same polymer at the lowest and highest surface densities (t-test, two-tailed distribution, equal variance).

Table S8. Apparent K_d values for *Helix pomatia* agglutinin (HPA).

pol.	apparent K_d [nM]						
	13 nm	p value[1]	25 nm	p value[1]	35 nm	p value[1]	p value[2]
6a	2.6 ± 0.2		1.3 ± 0.5		1.2 ± 0.5		0.002
6b	2.6 ± 0.3	0.962	1.3 ± 0.5	0.675	1.2 ± 0.5	0.952	0.002
6c	2.5 ± 0.3	0.643	1.3 ± 0.5	0.352	1.2 ± 0.5	0.891	0.003
6d	2.6 ± 0.3	0.567	1.2 ± 0.4	0.101	1.2 ± 0.3	0.992	<0.001
6e	2.7 ± 0.3	0.652	1.2 ± 0.4	0.012	1.2 ± 0.2	0.861	<0.001

[1]p values refer to a comparison of K_d's between **6a** and each of the other glycopolymers (t-test, two-tailed distribution, equal variance). [2]p values refer to a comparison of K_d's for the same polymer at the lowest and highest surface densities (t-test, two-tailed distribution, equal variance).

Chart S1. Apparent K_d's for lectins obtained from the lowest surface density array plotted against GalNAc valency in polymers **6.**

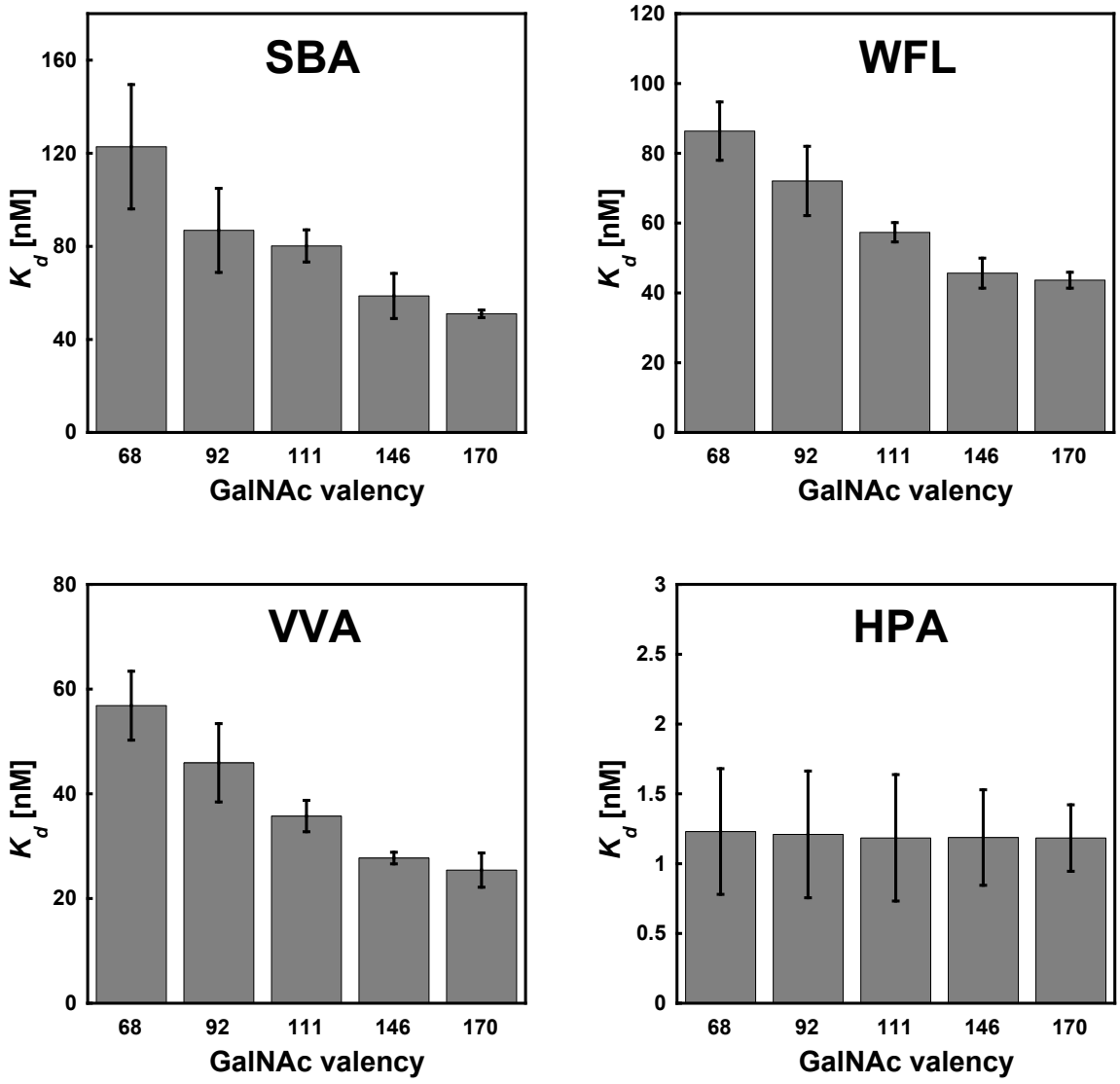


Table S9. Apparent K_d values for lectins toward polymer **9** in a density variant array.

lectin	apparent K_d [nM]				affinity enhancement K_{rel}[3]
	7 nm	15nm	28 nm	p value[1]	
SBA	361 ± 148	1245 ± 292	–*	0.009[2]	3.5[4]
WFL	866 ± 109	1284 ± 303	1587 ± 152	0.003	1.8
RWFL	748 ± 290	811 ± 262	629 ± 166	0.572	N/A
VVA	387 ± 134	514 ± 180	654 ± 191	0.011	1.7
HPA	3.4 ± 1.1	3.9 ± 1.1	5.0 ± 0.7	0.049	1.5

*apparent K_d too large to measure. [1]p values refer to a comparison of K_d's at the lowest and highest surface densities (t-test, two-tailed distribution, equal variance). [2]p value refers to a comparison of K_d's measured for average ligand spacing of 15 nm and 7 nm, respectively (t-test, two-tailed distribution, equal variance). [3] K_{rel} is calculated as the ratio between apparent dissociation constants measured at the lowest and the highest surface densities (28 and 7 nm average ligand spacing, respectively). [4]K_{rel} for SBA was calculated as the ratio between apparent dissociation constants measured for average ligand spacing of 15 nm and 7 nm, respectively.

Table S10. SBA and HPA precipitation by soluble glycopolymers **6b**, **6e**, and **9**.

pol.	valency	$P_{1/2}$ [μM]	p value[1]	p value[2]	lectin/ polymer[3]	GalNAc/ lectin	p value[4]
Soybean agglutinin (SBA):							
6e	170	2.9 ± 0.2	0.009		5.1 ± 0.3	33 ± 2	0.007
6b	92	3.1 ± 0.7	0.015		5.1 ± 1.4	19 ± 4	
9	17	6.6 ± 1.3			2.3 ± 0.4	7 ± 2	
Helix pomatia agglutinin (HPA):							
6e	170	4.4 ± 0.8	0.001	0.034	3.5 ± 0.6	50 ± 9	0.088
6b	92	5.7 ± 1.2	0.008	0.035	2.7 ± 0.6	35 ± 8	
9	17	9.5 ± 0.4		0.024	1.6 ± 0.1	11 ± 1	

[1]p values refer to a comparison of $P_{1/2}$ values for glycopolymers **6** and **9** (t-test, two-tailed distribution, equal variance). [2]p values refer to a comparison of $P_{1/2}$ values for the precipitation of both lectins by the same glycopolymer (t-test, two-tailed distribution, equal variance). [3]lectin to polymer ratio in the precipitate at $P_{1/2}$. [4]p values refer to a comparison of GalNAc/lectin ratios for polymers **6e** and **6b**. Lectin stoichiometries and GalNAc per lectin ratios were determined as described in reference 33.

Chart S2. SBA and HPA precipitation by soluble glycopolymers **6b**, **6e**, and **9**.