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

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**Chart S2.** SBA and HPA precipitation by soluble glycopolymers 6b, 6e, and 9.
1. $^1$H NMR spectra of pMVK precursors 3, 4, 7, and 8, and glycopolymers 6 and 9.
$p_{MWK} \ Z' \ u = 60', \ PDI = 1.13$
PMVK 8, n = 60, PDI = 1.14
GalNAc valency = 17
Glycopolymer \( n = 60 \)

\[
\text{GalNAc} \text{ Valency} = 17
\]

\[
\text{Glycopolymer} \quad n = 60
\]
2. SEC traces of pMVK polymers 3, 4, 7, and 8.

**Graph for pMVK 3:**
- Retention Volume (mL) vs. Refractive Index
- Mn = 13,183, Mw = 15,080, Mz = 17,049, Mw/Mn = 1.144

**Graph for pMVK 4:**
- Retention Volume (mL) vs. Refractive Index
- Mn = 13,426, Mw = 15,255, Mz = 17,233, Mw/Mn = 1.136
3. Determination of Mw of lectin monomers by SDS-PAGE.

![Graph showing determination of apparent molecular weights of lectins by SDS-PAGE](image)

$$y = 5.8645 - 2.0313x \quad R = 0.99856$$

| 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. Renwartz L. et al. J. Mulluscan Studies 2009, 75, 41-49).

| BME | HPA | SBA | VVA | WFL | RWFL |
|-----|-----|-----|-----|-----|------|
| T [°C] | 20  | 95  | 20  | 95  | 20  | 95  | 20  | 95  |

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.

**Determination of apparent molecular weights of lectins by FPLC**

![Graph showing apparent Mw vs Ve/Vo for lectins](image)

| 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.

\[ \rho = \frac{n_z \cdot N_A}{\pi (r_{spot})^2}, \]

\[ \rho \ldots ..\text{polymer surface density [nm}^2]\]

\[ \Delta = \frac{1}{\sqrt{\rho}} = \frac{1}{\sqrt{\frac{n_z \cdot N_A}{\pi (r_{spot})^2}}} = \frac{r_{spot} \cdot \sqrt{\pi}}{\sqrt{n_z \cdot N_A}}, \]

\[ \Delta \ldots ..\text{average spacing [nm]}\]
6. Images of a density variant array of polymer 9 and a plot of average polymer spacing.

![Density variant array of polymer 9 and average spacing plot](image)

**Average spacing of polymer 9 in density variant array**

| $c_{pol}$ [nM] | $\Delta$ [nm] |
|----------------|---------------|
| 75             | 28.1 ± 0.8    |
| 150            | 18.9 ± 0.8    |
| 300            | 13.5 ± 0.4    |
| 600            | 9.5 ± 0.3     |
| 1200           | 6.7 ± 0.2     |
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 (%) |
|-------|-------------|---------|--------------------|
| 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 | \( \varepsilon_{280} \) [M\(^{-1}\)·cm\(^{-1}\)] | AF647/lectin |
|--------------|--------|---------------------------------|--------------|
| SBA          | 10 mM Na\(_2\)HPO\(_4\), 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\(_2\)HPO\(_4\), 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/tetramer |
| 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] | p value\(^1\) | 13 nm | 25 nm | 35 nm | p value\(^1\) | 13 nm | 25 nm | 35 nm | p value\(^2\) |
|------|----------------------|----------------|-------|-------|-------|----------------|-------|-------|-------|----------------|
| 6a   | 58 ± 6               | 0.127          | 51 ± 6          | 50 ± 4          | 47 ± 4          | 44 ± 6          | 0.016          | 0.001          | 0.011          | 0.060          |
| 6b   | 106 ± 4              | 0.005          | 80 ± 4          | 72 ± 5          | 54 ± 4          | 53 ± 5          | <0.001         | <0.001         | <0.001         | <0.001         |
| 6c   | 123 ± 27             | 0.068          | 87 ± 18         | 80 ± 7          | 59 ± 10         | 51 ± 2          | 0.002          | 0.002          | 0.002          | 0.071          |
| 6d   | 123 ± 27             | 0.003          | 87 ± 18         | 80 ± 7          | 59 ± 10         | 51 ± 2          | 0.002          | 0.002          | 0.002          | 0.071          |
| 6e   |                      | 0.009          | 80 ± 7          | 80 ± 7          | 59 ± 10         | 51 ± 2          | 0.002          | 0.002          | 0.002          | 0.071          |

\(^1\) 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. | 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 *Wisteria floribunda* lectin (RWFL).

| pol. | 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 $K_{rel}$ |
|--------|----------------------|---------------------------------|
|        | 7 nm                 | 15 nm                           | 28 nm | p value$^1$ |                                    |
| SBA    | 361 ± 148            | 1245 ± 292                      | −*    | 0.009$^2$   |                                    |
| WFL    | 866 ± 109            | 1284 ± 303                      | 1587 ± 152 | 0.003           |                                    |
| RWFL   | 748 ± 290            | 811 ± 262                       | 629 ± 166 | 0.572         |                                    |
| VVA    | 387 ± 134            | 514 ± 180                       | 654 ± 191 | 0.011         |                                    |
| HPA    | 3.4 ± 1.1            | 3.9 ± 1.1                       | 5.0 ± 0.7 | 0.049         |                                    |

*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).  
$^3K_{rel}$ 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).  
$^4K_{rel}$ 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       | 3.5 ± 0.6           | 50 ± 9        | 0.088       |
| 6b           | 92             | 5.7 ± 1.2   | 0.008       | 2.7 ± 0.6           | 35 ± 8        |             |
| 9            | 17             | 9.5 ± 0.4   | 0.024       | 1.6 ± 0.1           | 11 ± 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.