Supplemental Data for Levan, *et al*.

"*Vibrio cholerae* cytolysin recognizes the heptasaccharide core of complex N-glycans with nanomolar affinity."

Supplemental Data Figure Captions

Figure S1. Isothermal Titration Calorimetry data. ITC data for the titration of the VCC β-prism domain with six monosaccharides. In each panel, the raw data is shown above and the fit binding isotherm is shown below. No binding was reported in cases where the isotherm exhibited zero slope. Fit values are listed in Table 1 of the main manuscript.

Figure S2. ITC data for six additional carbohydrates.

Figure S3. ITC data for wild-type VCC binding to Me-α-mannose. The binding affinity for Me-α-mannose binding to full-length VCC was determined to be 1.4 ± 0.1 mM, which is consistent with the measured value of 3.24 ± 0.1 mM obtained for Me-α-mannose binding to the isolated VCC β-prism domain.

Figure S4. Crystallographic data statistics for the structure of the VCC β-prism domain with Me-α-mannose bound.

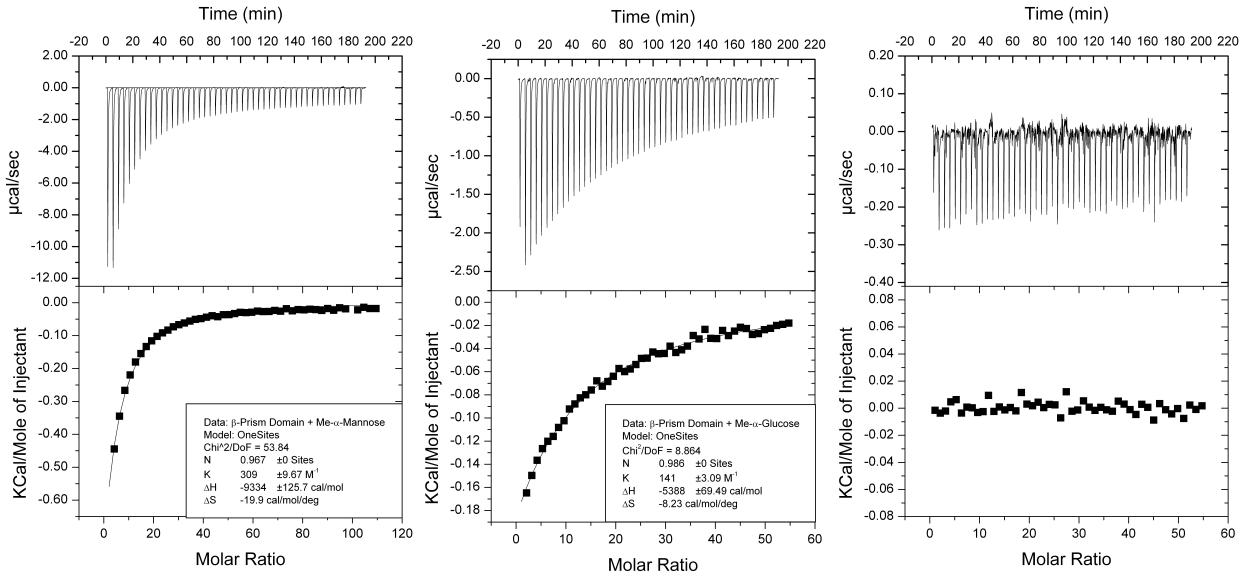
Figure S5. Glycan array data for the 25 glycans with the strongest fluorescence signals. Data is shown for Alexa fluor 488-labeled wild-type VCC incubated with the array at 1 and 10 μg/mL concentrations. The table contains the identification number of the glycan on the array, a schematic using CFG standardized nomenclature, the chemical structure of the glycan, and the magnitude of the relative fluorescence unit signal normalized to the highest value on the chip (#328). The top 25 binders were identified by averaging the normalized

signal measured for each glycan with 1, 10, and 180 μg/mL VCC. A key for the CFG nomenclature and the chemical substrate that the glycan is attached to are listed.

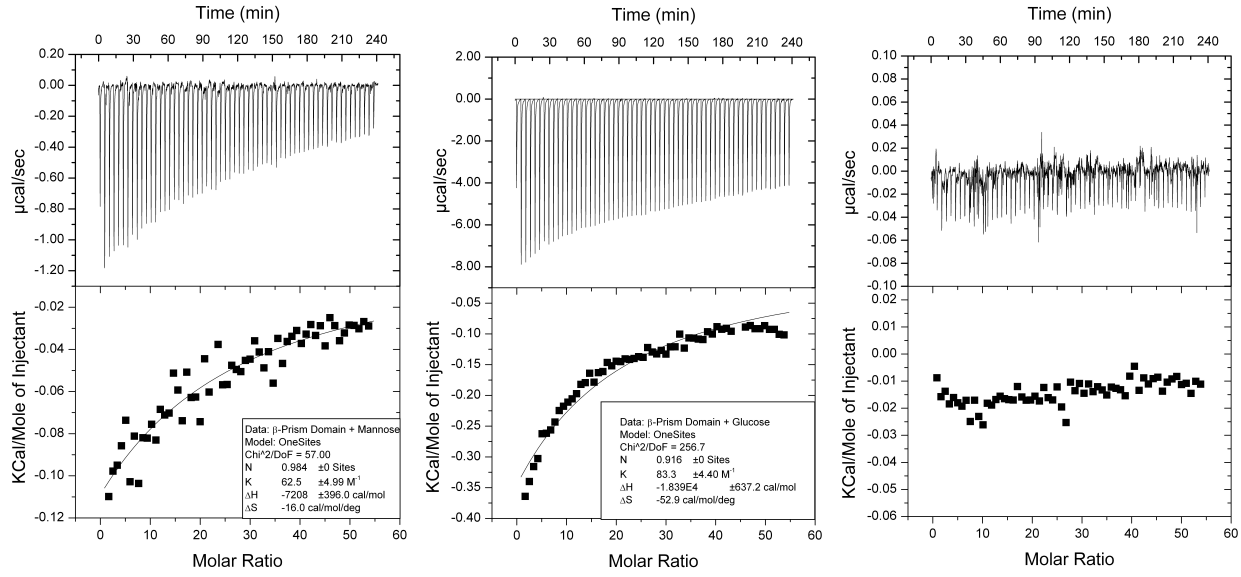
Figure S6. Glycan array data for additional glycans of interest. VCC exhibits relatively little binding to high-mannose type glycans as well as fragments of the top binders in Figure S5.

Figure S7. Fluorescence anisotropy for $VCC_{\beta\text{-prism}}$ D617A mutant binding to 2AA-labeled NGA2. Fluorescence anisotropy of a solution of 100 nM of 2AA-labled NGA2 was monitored as purified β-prism domain (D617A) was titrated into the cuvette. A fit of the data from three replicates using the RandoA model in Origin 6.0 indicated a dissociation constant of 20 μM ± 6.1 μM.

Figure S8. Trp706 adopts two rotamer positions. Surface representation showing the different rotamer positions of Trp706 (red) in $VCC_{\beta\text{-prism}}$ chain B (left) and chain E (right). The closed position (left) is seen in five of six chains in the crystal asymmetric unit as well as in all 14 copies of the β-prism domain in the VCC heptamer structure (PDB 3O44). The open position (right) is seen in one chain of the current structure as well as the monomer structure bound to β-octyl glycoside (PDB 1XEZ). Movement of the side-chain into the open position creates a cleft (indicated by arrow) that could accommodate a polysaccharide glycan.

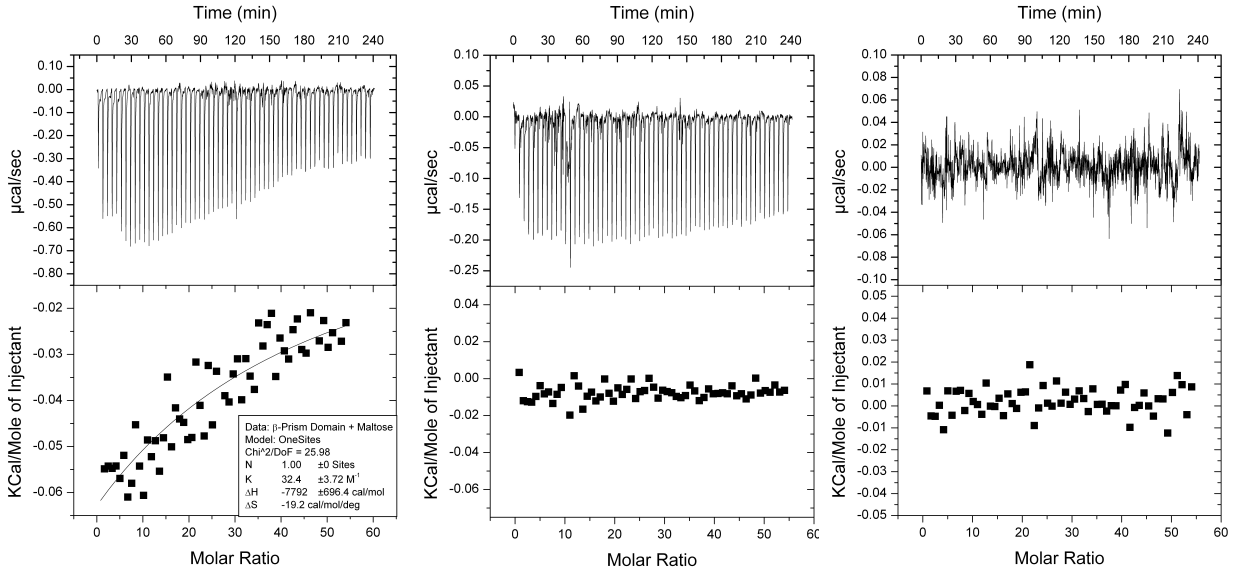


Me-α-Mannose **Me-α-Glucose** **Me-α-Galactose**



D-Mannose **D-Glucose** **D-Galactose**

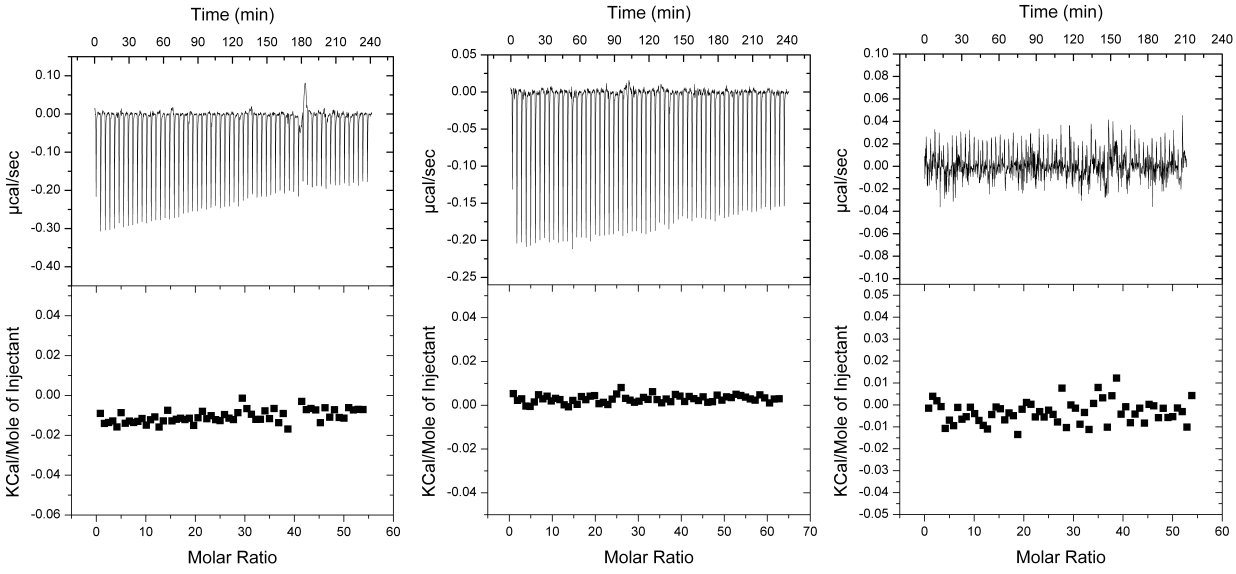
Figure S1



Maltose (Glcα1-4Glc)

Cellobiose (Glcβ1-4Glc)

N-Acetylglucosamine



Me-β-Glucose

Me-β-Galactose

L-Fucose

Figure S2

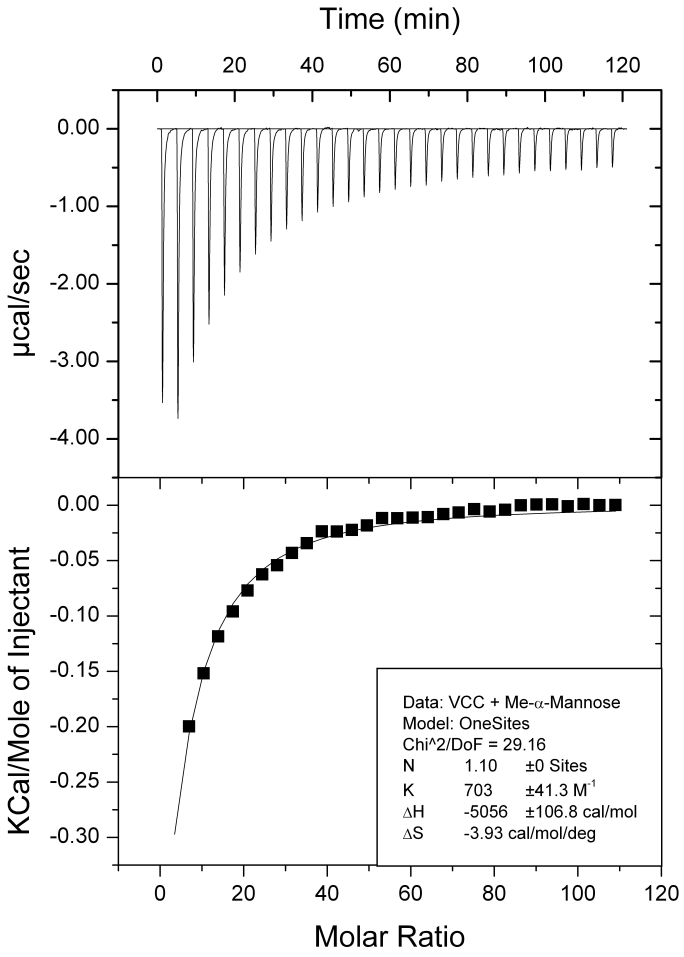
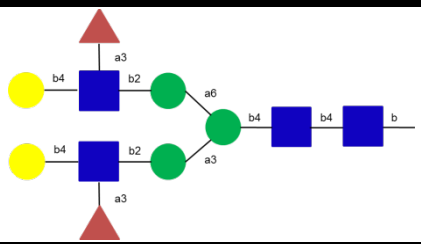
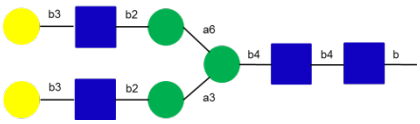
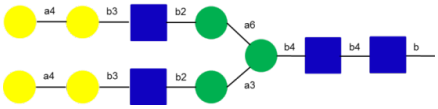
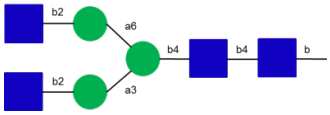
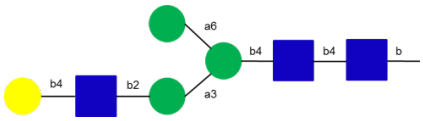
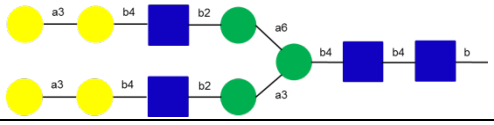
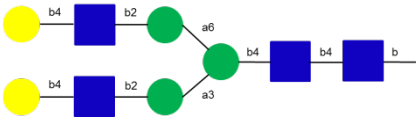

Time (min)
0
20
40
60
80
100
120
0.00
-1.00
-2.00
-3.00
-4.00
µcal/sec
0.00
-0.05
-0.10
-0.15
-0.20
-0.25
-0.30
KCal/Mole of Injectant
Data: VCC + Me-α-Mannose
Model: OneSites
Chi^2/DoF = 29.16
N 1.10 ±0 Sites
K 703 ±41.3 M-1
ΔH -5056 ±106.8 cal/mol
ΔS -3.93 cal/mol/deg
Molar Ratio

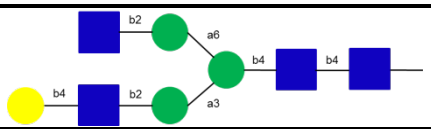
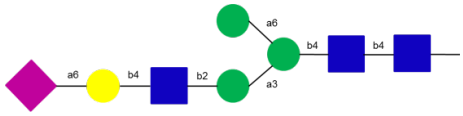
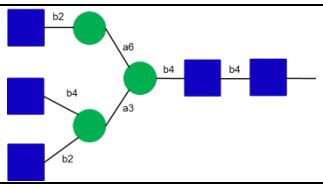
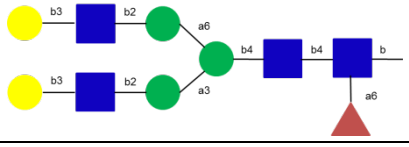
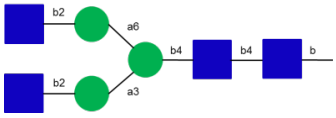
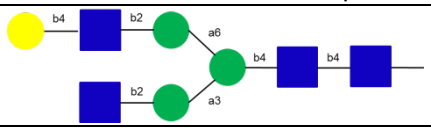
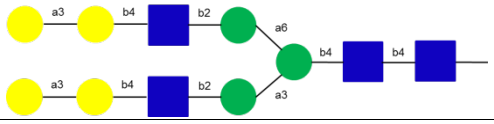
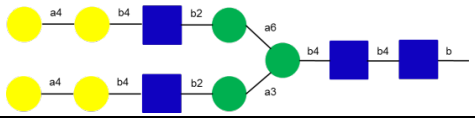
Figure S3

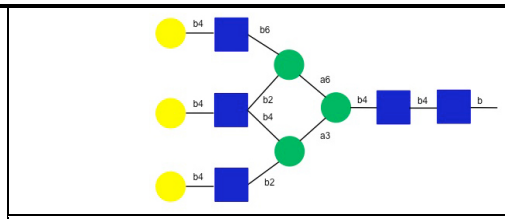
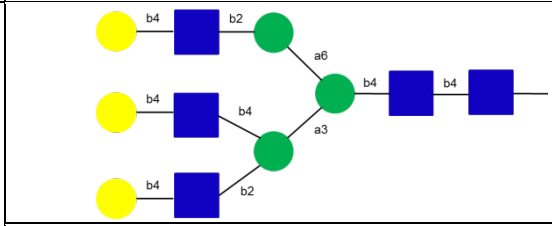
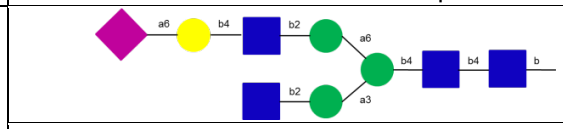
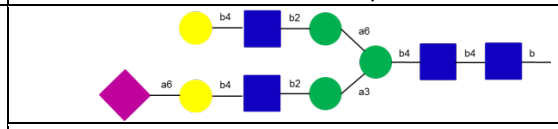
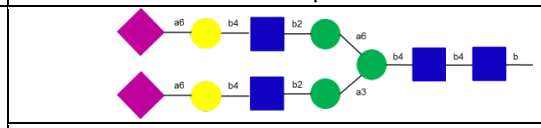
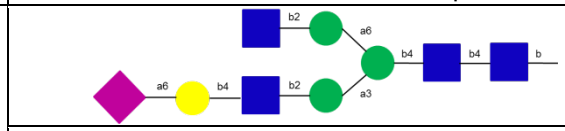
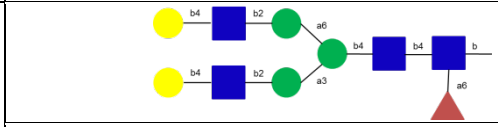
Data Statistics	
Resolution limits (Å)	86.4 - 2.7 (3.0-2.85)*
Space Group	$P3_12_1$
Cell dimensions *a*, *b*, *c* (Å); γ (°)	99.8, 99.8, 172.4; 120.0
Total Reflections (N)	116,049 (16,805)
Unique Reflections (N)	23,691 (3,397)
Redundancy (%)	4.9 (4.9)
Completeness (%)	99.8 (100.0)
R_{sym} (%)	21.1 (47.7)
R_{pim} (%)	10.6 (24.0)
I/σI (Mn(I/sd))	7.1 (3.2)
Refinement Statistics	
Atoms	5853
R-work (%)	19.5
R-free (%)	24.8
R.m.s. dev. bonds (Å)	0.005
R.m.s. dev angles (°)	1.045
Average B-value	24.7
Ramachandran statistics	
Most favored (%)	96.4
Disallowed (%)	0.0

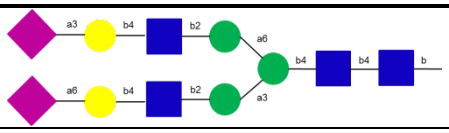
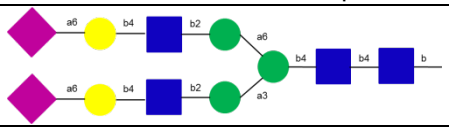
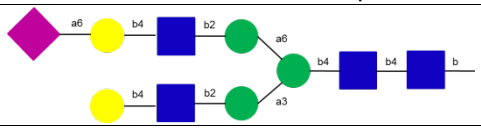
Figure S4

* Numbers in parentheses denote highest resolution shell.

Chart Number	Glycan Structure	Normalized Signal	RFU Measured	
			10 ug/mL VCC	1 ug/mL VCC
328	 Galb1-4(Fuca1-3)GlcNAcb1-2Mana1-6(Galb1-4(Fuca1-3)GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb-Sp20	1.00	21665	2089
325	 Galb1-3GlcNAcb1-2Mana1-6(Galb1-3GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb-Sp19	0.77	19702	1322
404	 Gala1-4Galb1-3GlcNAcb1-2Mana1-6(Gala1-4Galb1-3GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb-Sp19	0.66	14686	1325
53	 GlcNAcb1-2Mana1-6(GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb-Sp13	0.57	11490	1278
352	 Mana1-6(Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb-Sp12	0.49	6875	1394
364	 Gala1-3Galb1-4GlcNAcb1-2Mana1-6(Gala1-3Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb-Sp20	0.38	6848	942
54	 Galb1-3GlcNAcb1-2Mana1-6(Galb1-3GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4(Fuca1-6)GlcNAcb-Sp22	0.36	5026	1027

398	 GlcNAcb1-2Mana1-6(Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAc-Sp12	0.35	5230	971
347	 Mana1-6(Neu5Aca2-6Galb1-4GlcNAcb1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAc-Sp12	0.34	2745	1152
389	 GlcNAcb1-2Mana1-6 (GlcNAcb1-4(GlcNAcb1-2) Mana1-3)Manb1-4GlcNAcb1-4 GlcNAc-Sp21	0.33	4904	911
355	 Galb1-3GlcNAcb1-2Mana1-6 (Galb1-3GlcNAcb1-2Mana1-3) Manb1-4GlcNAcb1-4 (Fuca1-6)GlcNAcb-Sp22	0.30	5670	719
52	 GlcNAcb1-2Mana1-6 (GlcNAcb1-2Mana1-3) Manb1-4GlcNAcb1-4 GlcNAcb-Sp12	0.30	4237	862
399	 Galb1-4GlcNAcb1-2Mana1-6 (GlcNAcb1-2Mana1-3) Manb1-4GlcNAcb1-4 GlcNAc-Sp12	0.27	3154	844
561	 Gala1-3Galb1-4GlcNAcb1-2 Mana1-6(Gala1-3Galb1-4 GlcNAcb1-2Mana1-3)Manb1-4 GlcNAcb1-4GlcNAc-Sp24	0.25	3235	744
405	 Gala1-4Galb1-4GlcNAcb1-2 Mana1-6(Gala1-4Galb1-4 GlcNAcb1-2Mana1-3)Manb1-4 GlcNAcb1-4GlcNAcb-Sp24	0.24	2579	739

388	 Galb1-4GlcNAcb1-6(Galb1-4 GlcNAcb1-2)Mana1-6(Galb1-4 GlcNAcb1-4(Galb1-4 GlcNAcb1-2)Mana1-3) Manb1-4GlcNAcb1-4 GlcNAcb-Sp21	0.22	2244	713
370	 Galb1-4GlcNAcb1-2Mana1-6 (Galb1-4GlcNAcb1-4(Galb1-4 GlcNAcb1-2)Mana1-3) Manb1-4 GlcNAcb1-4 GlcNAc-Sp21	0.21	2340	663
309	 Neu5Aca2-6Galb1-4 GlcNAcb1-2Mana1-6 (GlcNAcb1-2Mana1-3) Manb1-4GlcNAcb1-4 GlcNAcb-Sp12	0.21	2216	650
320	 Galb1-4GlcNAcb1-2Mana1-6 (Neu5Aca2-6Galb1-4 GlcNAcb1-2Mana1-3)Manb1-4 GlcNAcb1-4GlcNAcb-Sp12	0.21	1743	692
57	 Neu5Aca2-6Galb1-4 GlcNAcb1-2Mana1-6 (Neu5Aca2-6Galb1-4 GlcNAcb1-2Man-a1-3) Manb1-4GlcNAcb1-4 GlcNAcb-Sp21	0.20	1846	657
321	 GlcNAcb1-2Mana1-6 (Neu5Aca2-6Galb1-4 GlcNAcb1-2Mana1-3)Manb1-4 GlcNAcb1-4GlcNAcb-Sp12	0.19	2504	572
354	 Galb1-4GlcNAcb1-2Mana1-6 (Galb1-4GlcNAcb1-2Mana1-3) Manb1-4GlcNAcb1-4 (Fuca1-6)GlcNAcb-Sp22	0.19	1732	632

327	 Neu5Aca2-3Galb1-4 GlcNAcb1-2Mana1-6 (Neu5Aca2-6Galb1-4 GlcNAcb1-2Mana1-3)Manb1-4 GlcNAcb1-4GlcNAcb-Sp12	0.18	2106	530
56	 Neu5Aca2-6Galb1-4 GlcNAcb1-2Mana1-6 (Neu5Aca2-6Galb1-4 GlcNAcb1-2Mana1-3)Manb1-4 GlcNAcb1-4GlcNAcb-Sp13	0.18	1918	585
302	 Neu5Aca2-6Galb1-4 GlcNAcb1-2Mana1-6(Galb1-4 GlcNAcb1-2Mana1-3)Manb1-4 GlcNAcb1-4GlcNAcb-Sp12	0.17	2861	443

Key:

Sugar	Symbol
Neuraminic Acid	
Galactose	
N-acetyl glucosamine	
Mannose	
Fucose	

Sp12 – Asparagine

Sp13 - Glycine

Sp19 – EN or NK

Sp20 - GENR

Sp21 - $-N(CH_3)-O-(CH_2)_2-NH_2$

Sp22 - NST

Sp23 – $(OCH_2CH_2)_6NH_2$

Sp24 - KVANKT

Figure S5

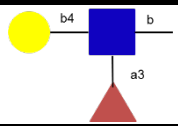
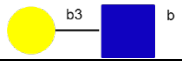
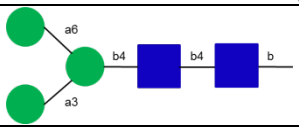
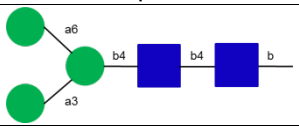
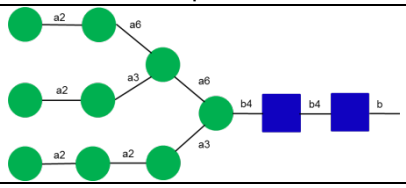
Chart Number	Glycan Structure	Normalized Signal	RFU Measured	
			10 ug/mL VCC	1 ug/mL VCC
152	 Galb1-4(Fuca1-3)GlcNAcb-Sp0	0.003	70	54
150	 Galb1-3GlcNAcb1-Sp0	0.003	70	87
116	 Gala1-3Galb1-4GlcNAcb-Sp8	0.004	97	52
51	 Mana1-6(Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb-Sp13	0.05	1153	679
50	 Mana1-6(Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb-Sp12	0.01	296	193
213	 Mana1-2Mana1-6(Mana1-2Mana1-3)Mana1-6(Mana1-2Mana1-2Mana1-3)Manb1-4GlcNAcb1-4GlcNAcb-Sp12	0.005	107	72

Figure S6

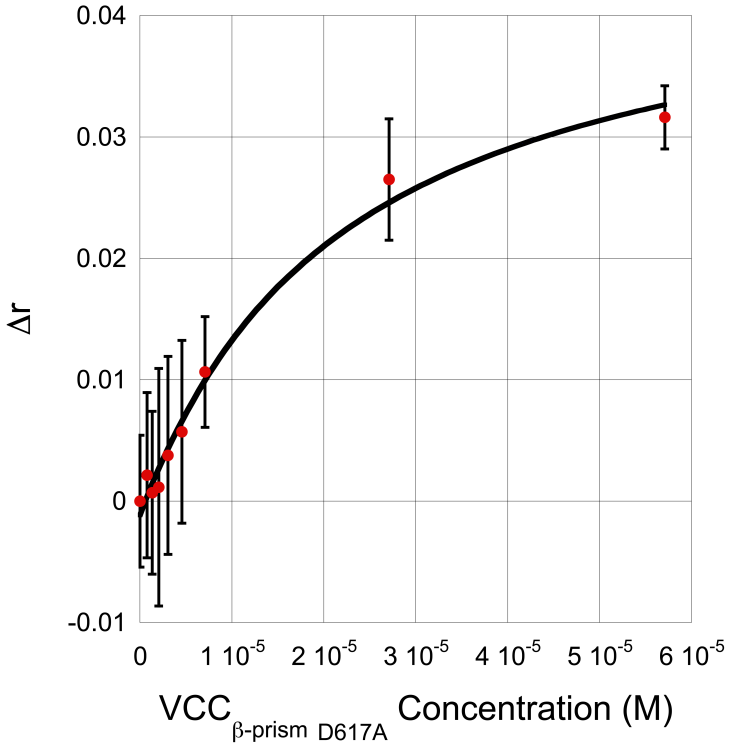

0.04
0.03
0.02
0.01
0
-0.01
Δr
0
1 10-5
2 10-5
3 10-5
4 10-5
5 10-5
6 10-5
VCCβ-prism D617A Concentration (M)

Figure S7

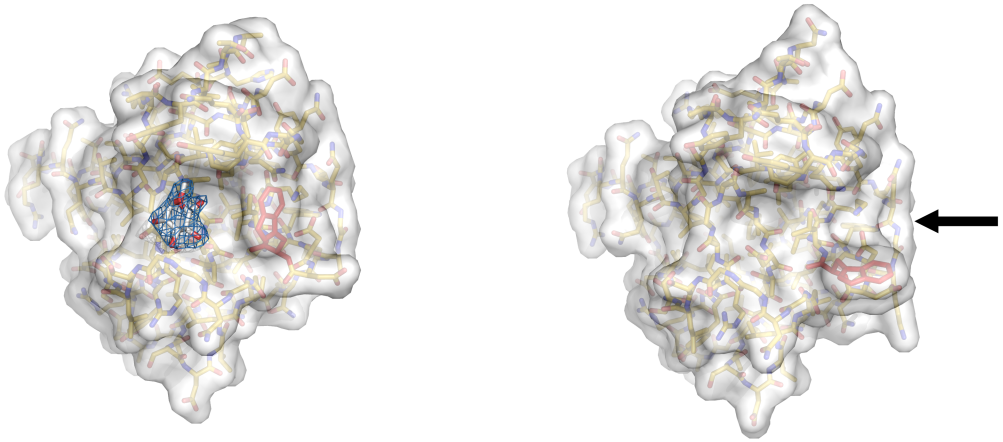


Figure S8