On-Virus Construction of Polyvalent Glycan Ligands for Cell-Surface Receptors

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Supporting Information

A. General

1. Production and Isolation of Virus Particles

CPMV particles were produced in cowpea plants and isolated using previously published procedures.[1] Briefly, CPMV was isolated from infected leaves of black-eye cowpea plants. Primary leaves from 10-day old cowpea plants were first dusted with carborundum and inoculated with homogenized infected leaves in phosphate buffer. Symptoms of infection appear within a week and a systemic infection is observed after three weeks. Leaves were collected, weighed and frozen for future purification of CPMV. Blended leaf tissue was separated from virus as previously described.[1] Expression of the Qβ coat protein from a recombinant plasmid has been previously reported;[2] we created our own vector to allow for more convenient genomic manipulation, as will be described in detail elsewhere.[3] A 133-amino acid version of the Qβ coat protein gene was cloned into the vector pQE-60 and expressed under IPTG control in M15MA cells in SOB media. After expression, collected cells were lysed by sonication and lysozyme treatment and then centrifuged to remove insoluble cell components. Assembled particles were precipitated from the resulting supernatant using 8% PEG 8000. Following further centrifugation, the isolated pellet was resuspended in 0.1M potassium phosphate pH 7.0. The virus-like particles then underwent a final purification by ultracentrifugation through 10-40% sucrose gradients followed by ultrapelleting and resuspension in 0.1M potassium phosphate pH 7.0.

Final purification of all viruses was performed by ultracentrifugation through 10-40% sucrose gradients; we find that this is more reliable than size-exclusion "spin columns" previously employed (and still used for preliminary cleanup in some cases). It may be possible to improve upon the maximum recovery of 70-80% from sucrose gradients with the use of molecular weight cutoff filtration (resin or membranes), but this was not attempted in the studies described here.

CPMV concentrations were determined by absorbance at 260 nm (0.1 mg/mL virus sample gives an absorbance of 0.8). Qβ concentrations were determined using the modified Lowry protein assay.[4] Unless otherwise indicated, all virus samples were handled in 0.1 M potassium phosphate buffer (pH 7.0).

A reviewer asked for an estimate of the average distances between attached glycans in the various particle conjugates. Based on previous characterizations of the reactivities of various amine nucleophiles in the structure,[5] CPMV (average loading = 190 per particle), 120 of the 190 attachments are made at the K38 and K99 positions of the small and large subunit, respectively.

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[1] Siler, D. J.; Babcock, J.; Bruening, G. *Virology* **1976**, *71*, 560-567.

[2] Kozlovska, T. M.; Cielens, I.; Dreilinna, D.; Dislers, A.; Baumanis, V.; Ose, V.; Pumpens, P. *Gene* **1993**, *137*, 133-137.

[3] Strable, E., Prasuhn, D., Udit, A., Brown, S., and Finn, M.G. "Virus-Like Particles Incorporating Unnatural Amino Acids;" manuscript in preparation.

[4] Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. *J. Biol. Chem.* **1951**, *193*, 265-275.

[5] (a) Wang, Q.; Kaltgrad, E.; Lin, T.; Johnson, J. E.; Finn, M. G. *Chem. Biol.* **2002**, *9*, 805-811.
(b) Chatterji, A.; Ochoa, W.F.; Paine, M.; Ratna, B.R.; Johnson, J.E.; Lin, T. *Chem. Biol.* **2004**, *11*, 855-863.

The remaining 70 are likely to be scattered over the K34 and K199 residues (both in the large subunit), since the ring of lysines at the K82 position is passivated by a 5-fold salt bridge interaction with neighboring aspartate (D81) residue. For Qβ, the wild-type particle was decorated with approximately 470 glycans, which will cover the majority of the sites shown in Figure S1B. In both cases, due to the dense array of attachment points, there will be no clear set of distances between attached glycans. Instead, neighboring glycans will be found at distances of 20-70 Å, over the entire surface of both particles, due to the polyvalent nature of icosahedral symmetry. In order to get discrete sets of distances, the loading must be reduced as in the Qβ (K16HPG) particle. In this species, approximately half of the K16 positions will be occupied by glycan, and the glycan is attached nowhere else. There will therefore be pairs of glycans separated by approximately 28, 40, and 35 Å (the last distance is the K16-K16 separation around the pseudo 6-fold axis, not shown), ignoring the lengths and uncertainties associated with the triazole tether.

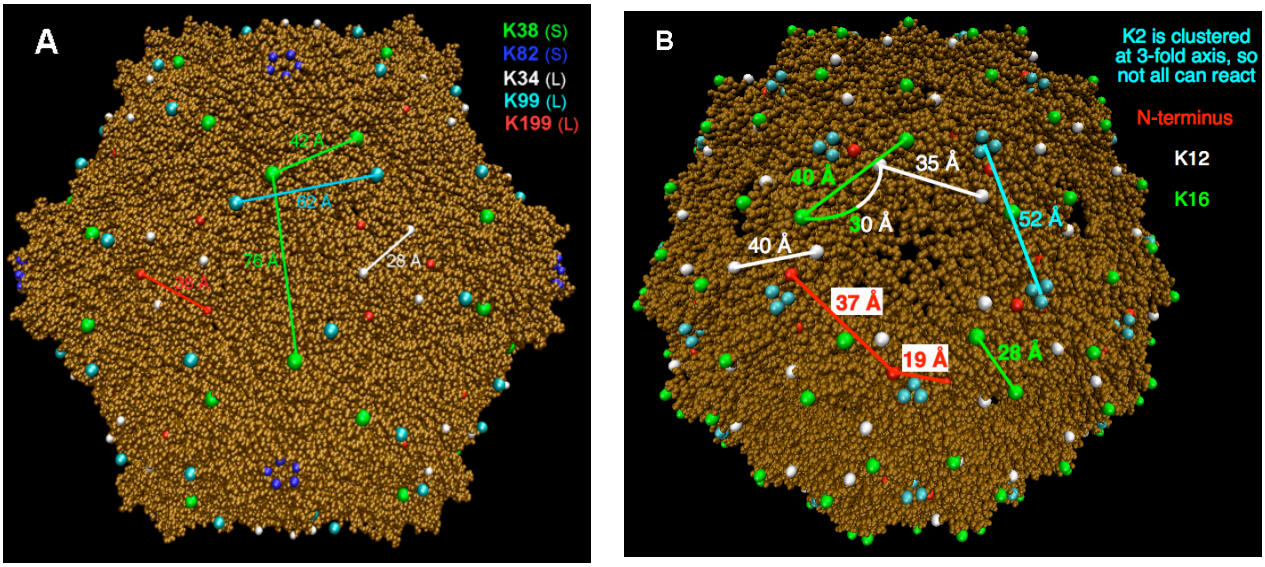


Figure S1. Placement of reactive amino groups on the surfaces of (A) CPMV and (B) bacteriophage Qβ virus-like particles, according to their respective x-ray crystal structures. These figures were made using coordinates provided by the VIPER database (http://viperdb.scripps.edu/) using the program VMD. The spheres in (A) are sized in approximate correspondence to the relative reactivities of the respective lysine residues, with K38 being the most reactive, followed by K99. Such measurements of relative reactivity have not been done on the Qβ capsid, but preliminary indications show no great differences between the reactivities of its lysine residues in different sites.

2. Cell lines and antibodies

B cell lymphoma cell lines Raji and Daudi were maintained in RPMI 1640/10% FBS. For Raji cells, 50 μM 2-mercaptoethanol was included. CHO cells were maintained in 1:1 F12:DMEM containing 10% FBS and either zeocin for wild-type cells or hygromycin as a selection marker for CHO cells stably transfected with a plasmid encoding full-length hCD22.[6] Anti-CPMV was raised in rabbits and was kindly provided by Prof. Marianne Manchester of The Scripps Research Institute. Anti-Qβ was raised in chickens as described below. FITC-goat anti-

[6] Tateno, H.; Li, H.; Schur, M.J.; Bovin, N.; Crocker, P.R.; Wakarchuk, W.; Paulson, J.C. *Mol. Cell. Biol.* **2007**, *27,* 5699-5710.

rabbit was purchased from Jackson Immunoresearch. FITC-rabbit anti-chicken was purchased from GenWay Biotech, Inc.

B. Glycan Synthesis

Compounds **1** and **2** were prepared as previously described.[7] Compound **7** was synthesized as previously described[8] and was used as an unnatural substrate for sialyltransferase reactions.

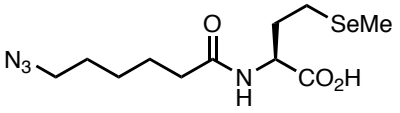
2-azidoethyl α-5-acetamido-9-(biphenylcarbonylamino)-3,5,9-trideoxy-D-glycero-2-nonulosonic acid–pyranosyl-(2→3)-β-D-galactopyranosyl-(1→3)-2-acetamido-2-deoxy-glucopyranoside (**3**).

Compound **1** (10 mg, 0.034 mmol), UDP-glucose (33.5 mg, 0.055 mmol, 1.6 equiv.), compound **7** (34 mg, 0.068 mmol), Cytidine 5'-triphosphate disocium salt (CTP, 40.8 mg, 0.068 mmol) were mixed in sodium cacodylate buffer (100 mM) containing $MgCl_2$ (20 mM) and $MnCl_2$ (20 mM). To this solution β1-4galactotransferase-galactoepimerase fusion enzyme (0.15 U), CMPNeu5Ac synthetase (100 U), and Galβ1-4GlcNAc α2-6-sialyltransferase (0.15 U) were added. The reaction mixture was slowly stirred at room temperature for 2 days. The reaction was monitored by TLC (Etheyl Acetate : Methanol : Acetic Acid : H_2O = 10 : 3 : 3 : 2). When the reaction is completed, the mixture was centrifuged to remove precipitated enzymes. The solution was then lyophilized and loaded onto a size exclusion column (Sephadex G15, 1x120 cm). Fractions were pooled and lyophilized to give compound **3** as a white powder (25 mg, 78%). ^{1}H-NMR (600 MHz) 7.79, 7.78 (2d, J = 1.8 Hz, 2 x 2H), 7.66 (dd, J = 1.2, 8.4 Hz, 2H), 7.45 (t, J = 6.6, 7.8 Hz, 2H), 7.37 (m, 1H), 4.44 (d, J = 7.8 Hz, 1H), 4.31 (d, 7.8 Hz, 1H) 3.96 (m, 1H), 3.89 (m, 2H), 3.84 (m, 1H), 3.81 (m, 1H), 3.74 – 3.55 (m, 8H), 3.50 – 3.40 (m, 5H), 3.31 (m, 1H), 3.26 (m, 1H), 2.54 (dd, J = 4.8, 12.6 Hz, 1H), 1.93 (s, 3H), 1.89 (s, 3H), 1.64 (t, J = 12.6, 12.6 Hz, 1H); ^{13}C-NMR (175 MHz) 144.8, 140.5, 133.0, 130.0, 128.7, 128.0, 128.0, 104.3, 101.6, 99.8, 81.5, 75.3, 74.4, 73.4, 73.4, 73.3, 71.5, 71.0, 70.6, 69.5, 69.2, 68.7, 64.3, 61.1, 55.5, 52.7, 51.1, 43.9, 40.6, 23.2, 22.8. HRMS by ESI-MS: calcd for $C_{40}H_{54}N_6O_{19}$ ($M+H^+$) 923.3516; found, 923.3515. The NMR spectra are reproduced at the end of this file.

C. Synthesis and Characterization of Virus Conjugates

1. Quantitation of glycan attachments.

The number of triazole-tethered molecules attached to the particle surface was estimated by parallel reactions using the selenomethionine azide derivative **8**, which more closely mimics the hydrophilic character of carbohydrates than do dyes such as fluorescein that we have previously used for this purpose.[9] Selenium, not present in detectable levels as background, can be measured at sub-μM concentrations using inductively coupled plasma optical emission spectroscopy (ICP-OES). Reproducible measurements of 190±20 selenium atoms per **CPMV-SeMet**, 470±50 per WT-derived **Qβ-SeMet** and 85 ± 9 selenium atoms per K16HPG-derived particle **K16HPG-SeMet**, were made.



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[7] Blixt, O.; Brown, J.; Schur, M. J.; Wakarchuk, W.; Paulson, J. C. *J. Org. Chem.* **2001**, *66*, 2442-2448.

[8] Han, S.; Collins, B.E.; Paulson, J.C. "Synthesis of 9-Substituted Sialic Acids As Probes for CD22-Ligand Interactions on B cells." A.V. Demchenko, Ed., *In* Frontiers in Modern Carbohydrate Chemistry. ACS Symposium Series **2007**, *960*, 2-14.

[9] (a) Raja, K. S.; Wang, Q.; Finn, M. G. *ChemBioChem* **2003**, *4*, 1348-1351. (b) Wang, Q.; Kaltgrad, E.; Lin, T.; Johnson, J. E.; Finn, M. G. *Chem. Biol.* **2002**, *9*, 805-811.

2. Representative procedures for on-virus glycosyltransferase reactions.

(a) **CPMV-GlcNAc** to **CPMV-LacNAc**. The following materials were mixed in a 1 mL vial in a total reaction volume of 277 uL: **CPMV-GlcNAc** (2.9 mg/mL; *ca.* 103 μM in GlcNAc), UDP-Glucose (6.4 mg/mL, 11.6 mM), β-(1,4)-galactotransferase-galactose epimerase fusion enzyme[6] (0.1 U), sodium cacodylate buffer (36 mM, pH 7.5) containing $MgCl_2$ (7.2 mM) and $MnCl_2$ (7.2 mM). The reaction was stirred at room temperature for 24 h with addition of NaOH solution (1 M) to keep pH at 7.5.

(b) **CPMV-LacNAc** to **CPMV-BPCsial**. The following materials were mixed in a 1 mL vial in a total reaction volume of 497 uL: **CPMV-LacNAc** (1.58 mg/mL; *ca.* 56 μM in LacNAc), compound **7** (2.75 mg/mL, 5.6 mM), CTP (3.4 mg/mL, 11.2 mM, approx. 2 equiv. with respect to **7**), CMP-synthetase (approx. 2 U), α-(2,6)-sialyl transferase (0.1U), 35 mM Na cacodylate (pH 7.5) containing $MgCl_2$ and $MnCl_2$ (7 mM each). The reaction was stirred at room temperature for 24 h with addition of NaOH solution (1 M) to keep pH at 7.5.

(c) One-pot transformation of **CPMV-GlcNAc** to **CPMV-BPCsial**. [The success of this protocol is made possible by the fact that the sialyltransferase used to attach the BPC substrate does not react until UDP-galactose has been added to the tethered GlcNAc moiety of the starting virus conjugate.] The following materials were mixed in a 1 mL vial in a total reaction volume of 327 uL: **CPMV-GlcNAc** (2.4 mg/mL, *ca.* 78 μM in GlcNAc), UDP-Glucose (5.4 mg/mL, 9.3 mM), compound **7** (2.6 mg/mL, 5.3 mM), CTP (3 mg/mL, 10 mM, approx. 2 equiv. with respect to **7**), β-(1,4)-Galactotransferase-Galactose epimerase fusion enzyme[6] (0.1 U), CMP-synthetase (approx. 1.3 U), α-(2,6)-sialyl transferase (0.06 U), 30 mM Na cacodylate (pH 7.5) containing $MgCl_2$ and $MnCl_2$ (6 mM each). The reaction was stirred at room temperature for 24 h with addition of NaOH solution (1 M) to keep pH at 7.5.

3. Generation and affinity purification of chicken anti-Qβ polyclonal antibody for flow cytometry.

Two white Leghorn hens (3-6 months old) were immunized with 200 μg of wild-type Qβ virus-like particles; Freund's complete adjuvant (CFA) was co-injected with the initial dose. Hens were boosted with an additional 100 μg of antigen and incomplete Freund's adjuvant (IFA) on days 14, 28, and 49. Eggs were collected and cataloged from days 24 to 70. The final 12 eggs from each bird were pooled; isolation of total IgY by Genway Biotech (San Diego, CA) provided approximately one gram of polyclonal antibody per chicken.

In order to increase the specificity and potency of the polyclonal chicken anti-Qβ IgY antibodies, a column was constructed for affinity purification. Wild type Qβ particles were ultracentrifuged and dissolved in pH 7.0 MES coupling buffer. 20 mg of a 5 mg/ml Qβ solution was added to 4 mL of amine terminated CarbpoxyLink Gel (Pierce Biotechnology). 60 mg of EDC was added and immediately mixed for 1 hour to avoid excess cross-linking of Qβ coat proteins. 10 column volumes of 1 M NaCl were flowed through the column to remove any non-specifically bound Qβ and the column was equilibrated with PBS buffer.

50 mg of anti-Qβ IgY antibody was run through the column and the flowthrough was collected. The column was then washed with 100 mL of buffer, followed by elution with 5 mL glycine•HCl (pH 2.0) and immediate neutralization to pH 7.0 with 1M Tris-HCl buffer. The column was reequilibrated with 10 mL PBS and most of the flowthrough fraction was added to the column, followed by collection of a second flowthrough fraction. Washing and elution was repeated as described above.

The different collected fractions were analyzed by ELISA during the affinity purification

process, as follows. Qβ (1 μg in 5 μL buffer) was deposited in each well of a 96-well plate and allowed to stand overnight, followed by blocking with 5% dry milk for 1 hour at room temperature. 100 μL of 50 μg/mL solutions of each antibody fraction was used to probe against the immobilized Qβ for 1 hour at room temperature, followed by washing with PBS-T 3 times for 5 minutes. The microtiter plate was then probed with HRP-conjugated goat anti-IgY secondary antibody (GenWay Biotech, Inc.) followed by another 3 washes and development with TMB substrate. 1 M HCl was added to stop the peroxidase reaction and a 96-well plate reader was used to record absorbance intensities at 450 nm. As shown in Figure S2, strong signals were observed for bulk and elution fractions and background levels of signal were obtained for both flowthrough fractions, indicating that almost all anti-Qβ antibodies bound the affinity column and were successfully eluted. The enriched (eluted) material was substantially more active in flow cytometry assays.

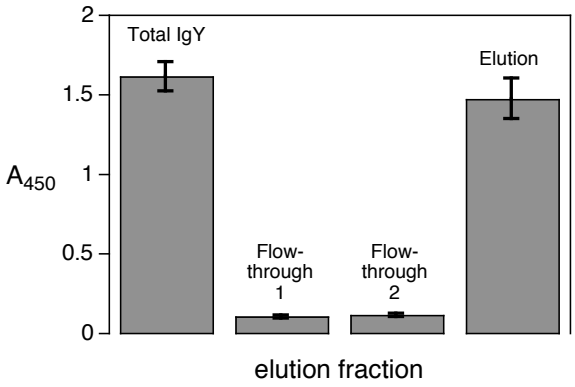

Figure S2. ELISA results establishing the affinity purification of anti-Qβ IgY.

4. Characterization of virus-glycan conjugates.

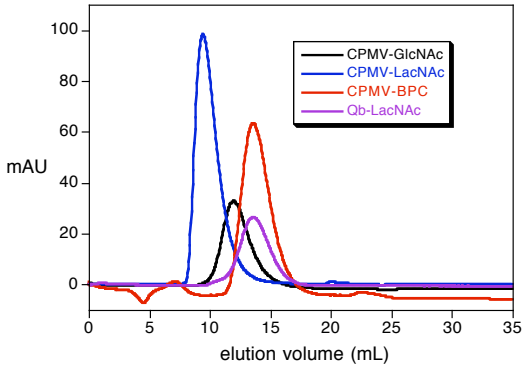

Figure S3. FPLC (Superose-6) of virus glycoconjugates. In each case, the elution profile is the same as the underivatized intact virus; the elution volume varies because the samples were analyzed on different columns with slightly different properties.

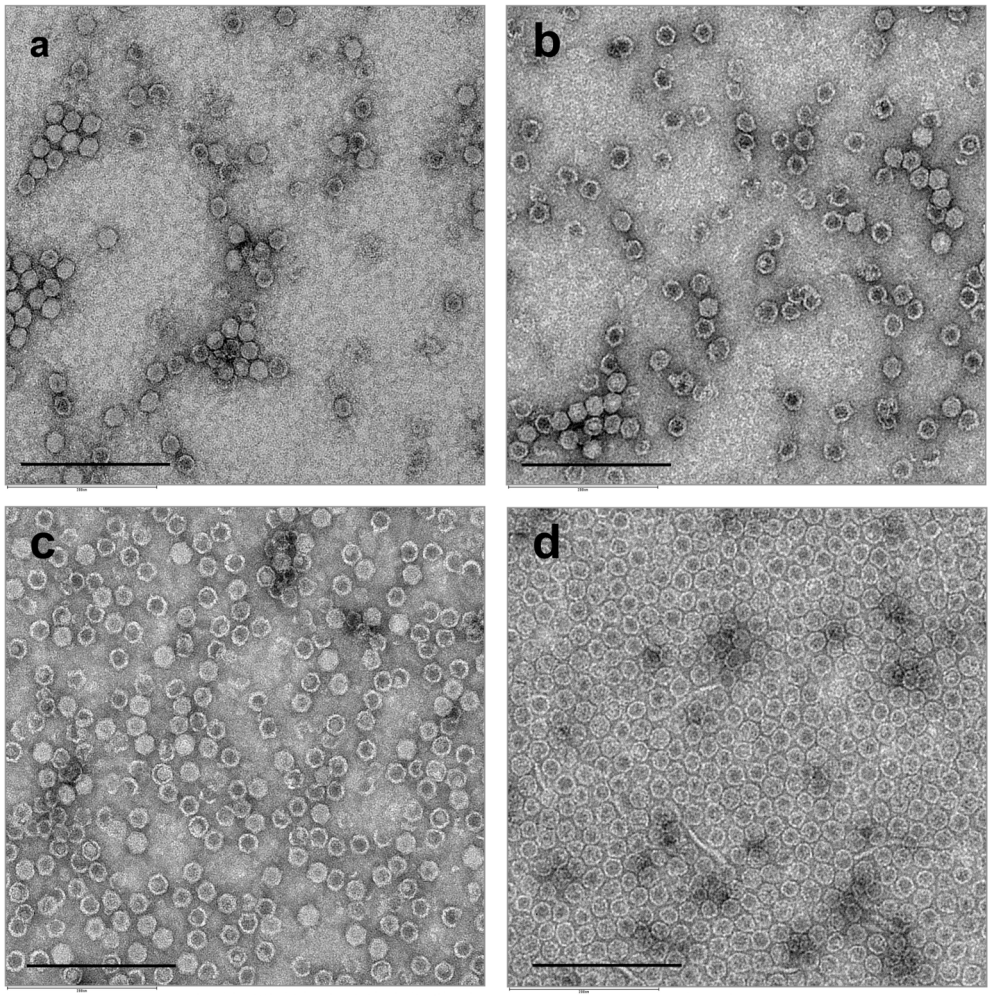

Figure S4. Visualization of glycan-labeled particles by transmission electron microscopy. (a) **CPMV-GlcNAc**, (b) **CPMV-LacNAc**, (c) **CPMV-BPCsial**, (d) **Qβ-LacNAc**. Scale bars are 200 nm.

D. Biding Assays

1. Viral capsid binding to beads

ECA beads were prepared by immobilizing biotinylated ECA (Vector laboratories) onto Streptavidin-conjugated Dynabeads (Invitrogen). hCD22-Fc beads were prepared by immobilizing hCD22-Fc chimaera, produced by transient transfection of COS cells with a plasmid encoding hCD22-Fc, a gift of Ajit Varki (UCSD) (Brinkman-Van der Linden et al, *J. Biol. Chem.* **2000**, *275*, 8633-8640.), onto Protein A Dynabeads (Invitrogen). After protein immobilization, beads were stored as a slurry in Hank's Buffered Saline Solution containing 5 mg/mL BSA (HBSS/B) at one-tenth the original density, and 2 µL/experiment were used.

Viruses (2-3 µg) were incubated with beads in HBSS/B (100-300 µL total) at room temperature for one hour, followed by magnetic isolation of beads and removal of virus solution. Beads were washed with HBSS/B, then incubated in HBSS/B with either anti-CPMV (1:5,000) or anti-Qβ (1:1,000, or 1:5,000 for affinity-purified antibody), then washed and followed by FITC anti-rabbit (1:300) or FITC-anti-chicken (1:10,000). After washing, bead fluorescence was measured by flow cytometry (FACSCaliber, Becton Dickinson), and data was analyzed using CellQuest software.

2. Viral capsid binding to cells

Viruses (2-3 µg) were incubated with 2-3 x 10^5 cells in HBSS/B (1-2 x 10^6 cells/mL) for 1 hour at 4 °C. Capsid binding was detected as described for bead binding, except that all steps were done at 4 °C and cells were isolated by centrifugation in washing steps.

E. Binding of Qβ-glycan to CD22 beads and cells

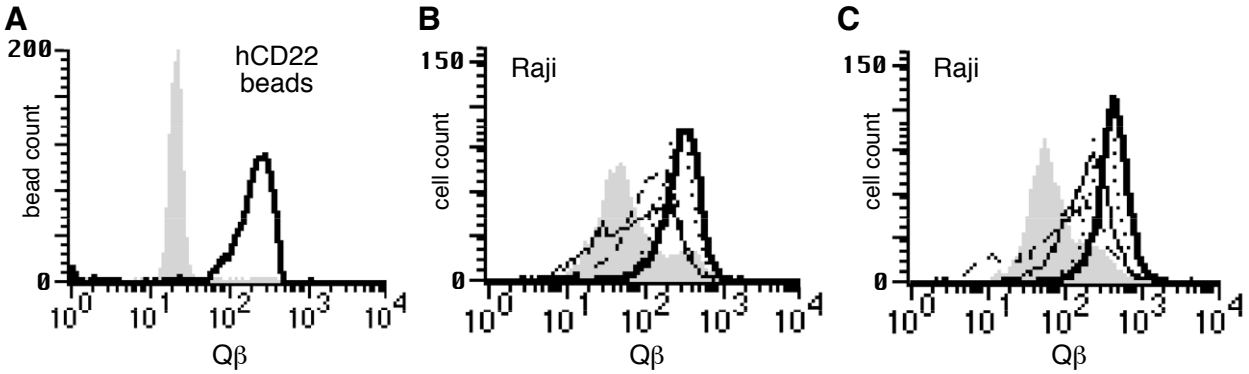

Figure S5. Flow cytometry analyses of the binding of Qβ-glycans with beads and cells in a CD22- and sialic acid-dependent manner. (A) **Qβ-LacNAc** (grey filled) or **Qβ-BPCsial** incubated with hCD22 beads. (B) Competition of compound **7** with wild-type **Qβ-BPCsial** for binding to Raji cells. (C) Competition of compound **7** with K16HPG **Qβ-BPCsial** for binding to Raji cells. Shown in (B) and (C) are **Qβ-LacNAc** (grey filled), **Qβ-BPCsial** (thick solid black), and **Qβ-BPCsial** including 0.01 (dotted), 0.1 (thin solid), or 1.0 mM (dashed) concentrations of compound **7**.

E. Optimization of glycosyltransferase and sialyltransferase reactions on virus substrates

For both the conversion of **CPMV-GlcNAc** to **CPMV-LacNAc** and **CPMV-LacNAc** to **CPMV-BPCsial**, maximal binding was obtained from the use of approximately 0.1 U enzyme (β-1,4-GalT and α-2,6-sialyltransferase ST6G1, respectively). This was determined as follows.

The reactions of **CPMV-GlcNAc** with GalT and UDP-glucose, and **CPMV-LacNAc** with **7** and the α-2,6-sialyltransferase ST6G1, were performed with the addition of increasing amounts of transferase enzymes, with the concentrations of other reagents constant except for an approximate 75% overall dilution in the reactions going from 0.0002 units enzyme to 0.2 units. In each case, the reactions were allowed to proceed for 24 hours at room temperature. Following purification, the resulting capsids were analyzed by flow cytometry in the standard manner, with the results shown in Figure S6. Virus recoveries in all cases were good (>60%).

An increase in affinity toward ECA lectin was observed with the use of higher concentrations of GalT, peaking at 0.1U (Figure 6A). Figure 6B,C shows substantial loss of ECA affinity and gain of association to CD22-coated beads upon the use of the lowest concentration of enzyme (0.001 U), showing that the assay in this case cannot discriminate between different loadings of glycosylated product. The gain of binding to the sialic acid-binding SNA lectin was more gradual, with maximum affinity reached at 0.1 U. It appears that the ECA and CD22 beads bind too strongly to discriminate between lightly loaded (low concentrations of enzyme used) and heavily loaded (higher concentrations of enzyme) particles, because binding is saturated when only a few of the proper glycans are on the particles. Since ECA recognizes sialic acid, and not the biphenylcarbonyl derivative, it presumably binds less tightly to **CPMV-BPCsial**, and this

weaker sensitivity allows us to see the dose-response behavior that indicates when the greatest loading is achieved.

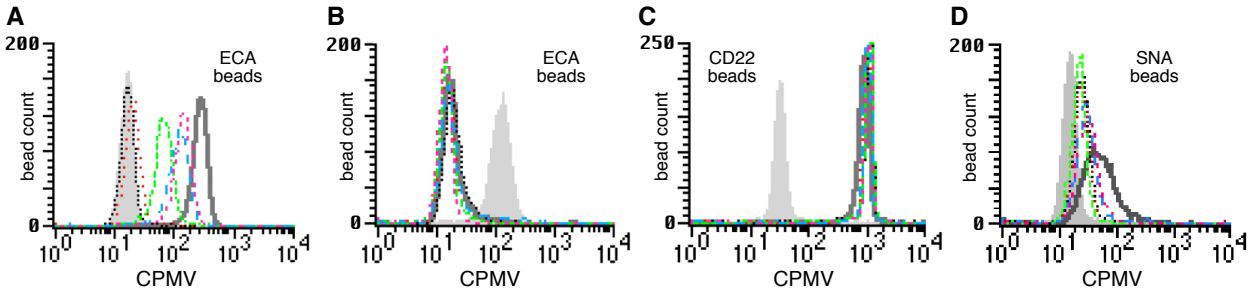

Figure S6. Reaction optimization. (A) Galactosyltransferase (GalT) reaction monitored using ECA beads to detect the addition of Gal. Shown are **CPMV-GlcNAc** (filled grey), **CPMV-LacNAc** (solid dark grey), and the products of reactions catalyzed by 0.0002 U (black dotted), 0.002 U (red), 0.02 U (green), 0.1 U (magenta), or 0.2 U (blue) of GalT. (B) Sialyltransferase (ST6Gal1) reaction monitored using ECA beads to detect the disappearance of the terminal Gal. (C) Sialyltransferase (ST6Gal1) reaction monitored using CD22 beads to detect the addition of the BPCsial. (D) Sialyltransferase (ST6Gal1) reaction monitored using SNA beads to detect the addition of the sialic acid. Shown in B–D are **CPMV-LacNAc** (filled light grey), **CPMV-BPCsial** (dark grey solid), and the products of reactions catalyzed by 0.001 U (black dotted), 0.002 U (red), 0.02 U (green), 0.1 U (magenta), or 0.2 U (blue) of ST6Gal1.

Discussion of expected rates of reactions relevant to Figure S6.

The reactions analyzed in Figure S6A were performed with the following amounts of reagents:

Enz total [a]	[Enz] (U/mL)	[CPMV-GlcNAc] (μM) [b]	[UDP-Glc] (mg/mL) [c]	[UDP-Glc] (mM)	Vol. (μL)	k_{rel} [d]
0.2 U	0.56	80.8	5.0	9.1	354	2.30
0.1 U	0.36	103	6.4	11.6	277	2.42
0.02 U	0.099	133	8.2	14.9	215	1
0.002 U	0.0099	142	8.7	15.8	202	0.13
0.0002 U	0.0010	143	8.8	16.0	200	0.013

a) Total amount of enzyme in the reaction. b) Stock solution of 8.0 mg/mL contained 286 μM of attached GlcNAc. 100 μL of this stock solution was used in each reaction. c) 1.76 mg (100 equiv. with respect to GlcNAc) diluted in the indicated reaction volume. The β(1,4)-galactotransferase enzyme is fused with galactoepimerase which very rapidly converts UDP-Glc to UDP-Gal. d) k_{rel} = expected relative rate = [Enz]•[CPMV-GlcNAc]•[UDP-Gal], with the value for the third entry set to a relative value of 1.0, assuming that Michaelis-Menten kinetics do not apply.

The reactions analyzed in Figure S6B,C were performed with the following amounts of reagents:

Enz stock [a]	[enzyme] (U/mL)	[CPMV-LacNAc] (μM) [b]	[BPCNeuAc] (mg/mL) [c]	CTP (mg/mL) [d]	Volume (μL)	k_{rel} [e]
0.2 U	0.28	38.9	1.9	2.3	719	0.87
0.1 U	0.20	56.3	2.8	3.4	497	1.32
0.02 U	0.063	87.8	4.3	5.3	319	1
0.002 U	0.0072	100	4.9	6.0	279.4	0.15
0.001 U	0.0036	101	4.9	6.1	277.2	0.076

a) Total amount of enzyme in the reaction. b) Stock solution of 7.9 mg/mL contained approx. 280 μM of attached LacNAc. 100 μL of this stock solution was used in each reaction. c) 1.37 mg (100 equiv. with respect to GlcNAc) diluted in the indicated reaction volume. d) 1.68 mg (100 equiv. with respect to GlcNAc) diluted in the indicated reaction volume. e) k_{rel} = expected relative rate = [enz]•[CPMV-LacNAc]•[BPCNeuAc], with the value for the third entry set to a relative value of 1.0, assuming that Michaelis-Menten kinetics do not apply. The variable concentration of CTP does not enter into the calculation because the CMP synthetase reaction is not rate-limiting.

Calculations of expected relative rates are complicated by the polyvalent nature of the acceptor and the difference in concentration of the particles *vs.* the glycan displayed on the surface. The concentrations of donor are far in excess of K_m (approx. 4.4 μM)[10] and so the enzyme-donor interaction is assumed to be saturated. The total concentrations of virus-displayed acceptor are much lower than K_m (approx. 0.6 mM), and so this portion of the reaction is decidedly not saturated. However, it is not clear if we should regard the acceptor concentration as that of the tethered glycans or the virus particles, nor is it certain that all acceptor molecules tethered to the virus remain equivalent throughout the reaction. Thus, it is difficult to generate predictive values of the expected rates in these reactions in which one can have confidence. (K_m values for the sialyltransferase step are similar: for LacNAc, recombinant ST6 from rat liver, K_m = 2.4 mM;[11] bovine enzyme, K_m = 1.6 mM;[12] the bovine K_m for CMP-NeuAc = 0.169 mM [8] (of course the BPC molecule may be different).

In each case, it was observed that the extent of reaction reached a peak and then declined with the use of the greatest amount of transferase enzyme tested. It seems likely that this result would be contrary to expectations if Michaelis-Menten style kinetics applied. Interestingly, if one invokes simple a third-order model (rate = k_{obs}[enzyme][donor][acceptor]), which does not account for reversibility, the dilution of the reaction mixtures is sufficient to explain the peak in observed rate, as shown in the above tables. While we are far from advocating such a proposal, the high *local* concentration of glycan acceptor on the virus surface may give rise to unusual, heterogeneous-like, kinetics.

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[11] Williams, M. A.; Kitagawa, H.; Datta, A. K.; Paulson, J. C.; Jamieson, J. C. *Glycoconjugate J.* **1995**, *12*, 755-761

[12] Paulson, J. C.; Rearick, J. I.; Hill, R. L. *J. Biol. Chem.* **1977**, *252*, 2363-2371

F. Further demonstration of the functional similarity of polyvalent virus-glycans obtained by conjugation of pre-formed di- and trisaccharides *vs.* the products of on-virus synthesis

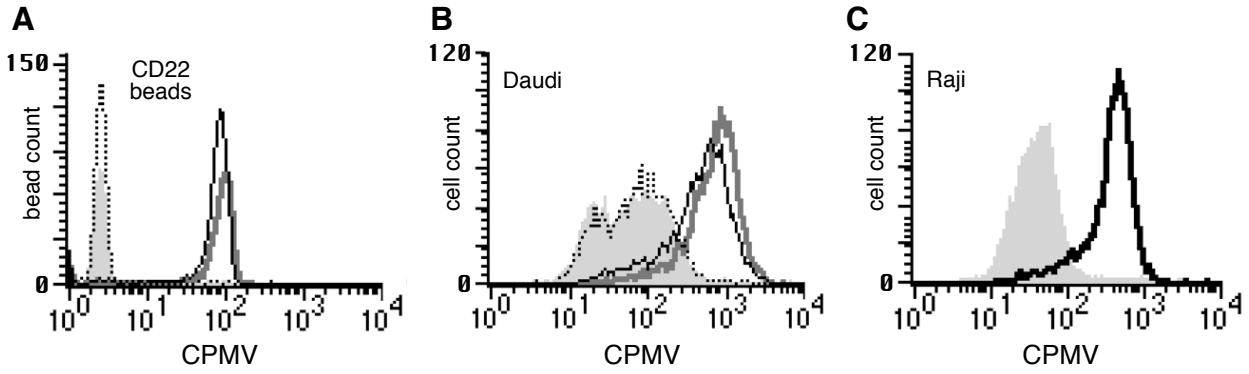

Figure S7. Comparisons of on-virus reactions to particles obtained by conjugation of pre-formed glycans. (A) and (B) report on the ST6Gal1 reaction converting **CPMV-LacNAc** to **CPMV-BPCsial**, using CD22 beads or Daudi B cells as indicated. Shown in (A) and (B) are **CPMV-LacNAc** prepared by coupling **2** to the capsid (grey filled), **CPMV-LacNAc** prepared by on-virus conversion of **CPMV-GlcNAc** (black dotted), **CPMV-BPCsial** prepared by coupling **3** to the capsid (thick grey solid), and **CPMV-BPCsial** prepared by on-virus conversion of **CPMV-LacNAc** (thin black solid). (C) Binding of **CPMV-LacNAc** prepared by on-virus reaction (filled grey) and **CPMV-BPCsial** prepared by on-virus reaction (black) to Raji cells.

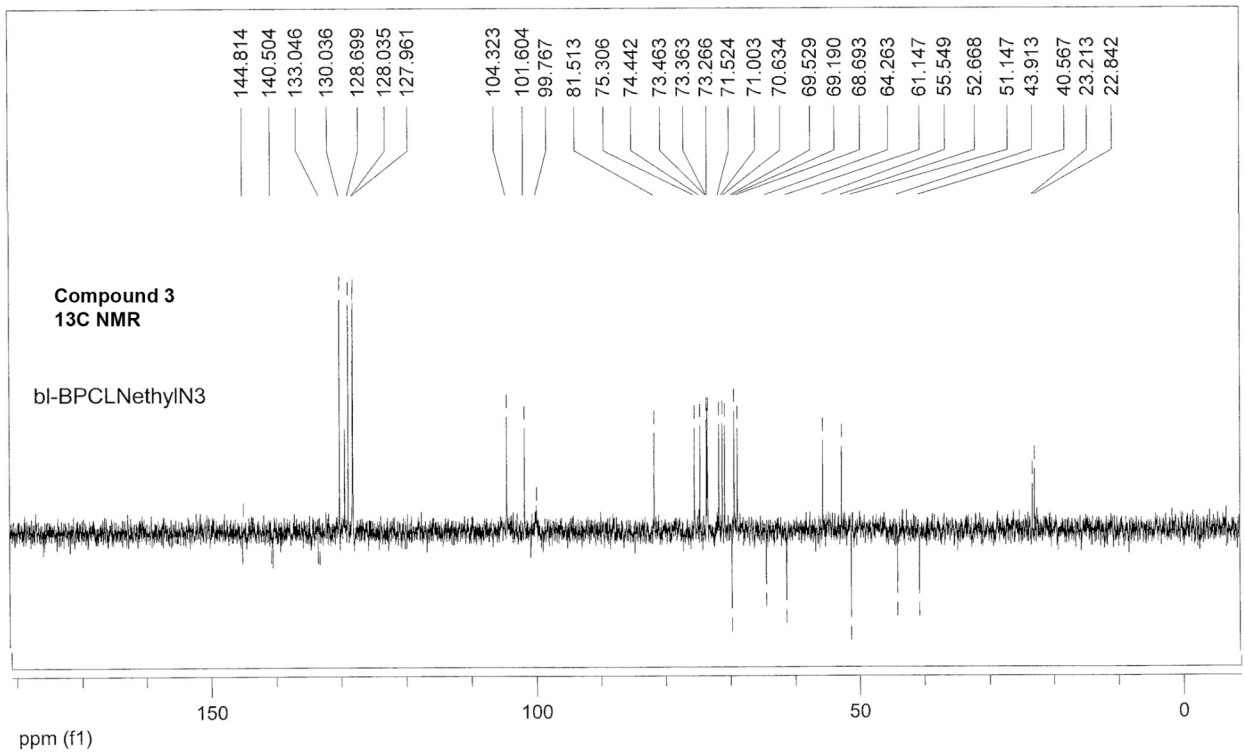

Figure S8. ^{13}C NMR of compound **3** (see section B).

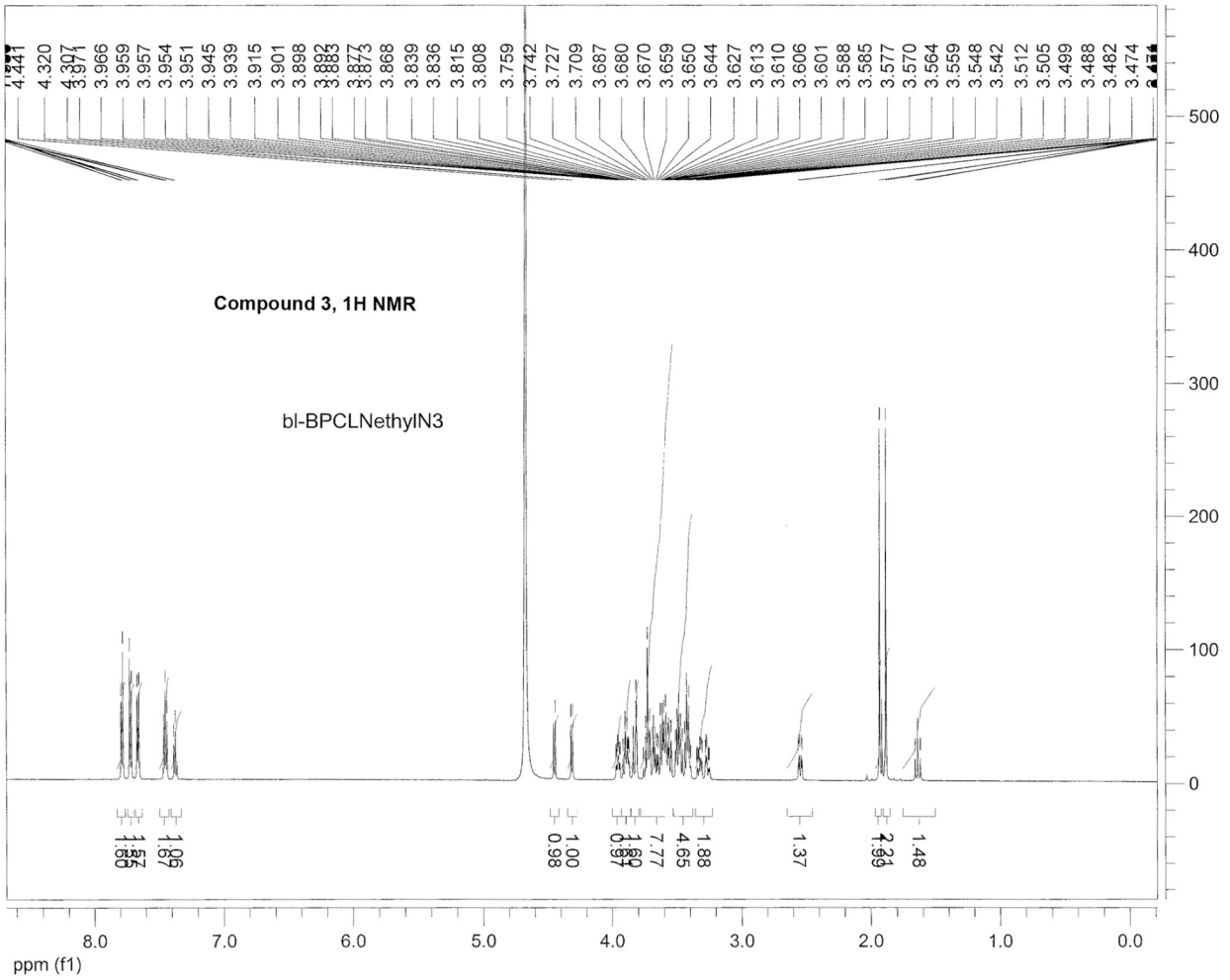

Figure S9. ^{1}H NMR of compound **3** (see section B).