Supplementary Materials:

Multivalency and the Mode of Action of Bacterial Sialidases

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General Methods and Materials

Sialidases of *Clostridium perfringens*, *Vibrio cholerae,* and *Salmonella typhimurium* were purchased from Sigma and used without further purification. The synthesis of sialyl lactosamine will be reported elsewhere. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Varian Inova500 spectrometer and a Varian Inova600 spectrometer equipped with Sun workstations. Assignments were made by standard gCOSY and gHSQC. Negative ion matrix assisted laser desorption ionization time of flight (MALDI-TOF) mass spectra were recorded using a HP-MALDI 2030A instrument using gentisic acid as a matrix**.**

Synthesis of Monovalent 1

To a solution of 3-acetamidopropyl *O*-[sodium (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid) onate]-(2,3)-*O*-(β-D-galactopyranosyl)-(1,4)–2-acetamido-2-deoxy-β-D-glucopyranoside (5.5 mg, 6.1 μmol) in methanol (1 mL) was added acetic anhydride (0.5 mL). After stirring for 16 h at room temperature, the reaction mixture was concentrated *in vacuo* and the residue co-evaporated with toluene (3 x 5 mL). The residue was dissolved in water (1 mL) and 1M aqueous NaOH (0.2 mL). After stirring for 1 h, the reaction mixture was purified by Sephadex G-15 size-exclusion column chromatography using water as an eluent to furnish monovalent compound **1** as a white glass (5.5 mg, 96%). MALDI-TOF *m/z* = 819 $[M + Na]^+$; ^{1}H NMR (D_2O) 500 MHz: δ 4.58 (d, 1H, H-1', $J_{1',2'}$ = 7.8 Hz), 4.54 (d, 1H, H-1, $J_{1,2}$ = 8.3 Hz), 4.15 (dd, 1H, H-3', $J_{2',3'}$ = 9.8 Hz, $J_{3',4'}$ = 2.9 Hz), 4.03 (dd, 1H, H-6a, $J_{5,6a}$ =<1.0 Hz, $J_{6a,6b}$ = 10.7 Hz), 3.99 (d, 1H, H-4', $J_{4',5'}$ =<1.0 Hz), 3.97 – 3.86 (m, 5H, OCH_{2a}, H-5'', H-8'', H-9a'', H-6b), 3.80 – 3.70 (m, 7H, H-2, H-3, H-4, H-5', H-6a', H-6b', H-4''), 3.70 – 3.61 (m, 5H, OCH_{2b}, H-5, H-6'', H-7'', H-9b''), 3.60 (dd, 1H, H-2'), 3.31 – 3.25 (m, 1H, CH_{2a}-N), 3.22 – 3.16 (m, 1H, CH_{2b}-N), 2.79 (dd, 1H, H-3e'', $J_{3e'',3a''}$ = 12.7 Hz, $J_{3e'',4''}$ = 4.9 Hz), 2.06 (x2) (s, 6H, 2 x NH-CO-C<u>H</u>$_3$), 2.01 (s, 3H, NH-CO-C<u>H</u>$_3$), 1.83 (t, 1H, H-3a''), 1.82 – 1.77 (m, 2H, O-CH_2C<u>H</u>$_2$$CH_2$); ^{13}C NMR ($D_2O$): δ 175.2, 174.4, 174.2 (3 x <u>C</u>O-CH_3), 174.0 (C-1''), 102.4 (C-1'), 101.0 (C-1), 99.7 (C-2''), 78.5 (C-4), 75.6 (C-3'), 75.3 (C-5'), 74.9 (C-5), 73.0 (C-6''), 72.5 (C-3), 71.8 (C-8''), 69.5 (C-2'), 68.3 (C-4''), 68.1 (C-7''), 67.9 (O-CH_2), 67.7 (C-4'), 62.7 (C-9''), 61.1 (C-6), 60.3 (C-6'), 55.1 (C-2), 51.9 (C-5''), 39.6 (C-3''), 36.3 (CH_2-N), 28.3 (O-CH_2<u>C</u>$H_2$$CH_2$), 22.1 (2 x NH-CO-<u>C</u>H_3), 21.9 (NH-CO-<u>C</u>H_3).

Synthesis of Glycopolymer 2

To a solution of 3-acetamidopropyl *O*-[sodium (5-acetamido-3,5-dideoxy-D-*glycero*-α-D-*galacto*-non-2-ulopyranosid) onate]-(2,3)-*O*-(β-D-galactopyranosyl)-(1,4)–2-acetamido-2-deoxy-β-D-glucopyranoside (2.1 mg, 2.75μmol) and poly[*N*-(acryloxy)succinimide] (2.5 mg, 14.6 μmol of *N*-hydroxysuccinimide ester) in DMF (0.5 mL) was added diisopropylethylamine (1.4 μL, 8.3 μmol).[1] The solution was stirred at room temperature for 18 h, then heated at 65°C for 5 h, followed by cooling to room temperature. An aqueous solution of NH_4OH (20%, 2 mL) was then added and the mixture stirred at room temperature for 18 h. The resulting mixture was dialyzed against water for 3 days (1L of milli-Q water changed 4 times) and then lyophilized to afford glycopolymer **2** as a white

foam (2.8 mg, 95%). Selected ^{1}H NMR data (D_2O) 500 mHz: δ 4.56 (dd, 2H, H-1', H-1), 4.14 (d, 1H, H-3'), 3.98 (s, 1H, H-4'), 3.31 – 3.16 (m, 2H, CH_2-N), 2.79 (dd, 1H, H-3e'', $J_{3e'',3a''}$ = 12.7 Hz, $J_{3e'',4''}$ = 4.9 Hz), 2.06 (s, 6H, 2 x NHCO-CH_3).

General procedure for sialidase assay

Sialosides **1** and **2** (25 μL) at different concentrations in an appropriate buffer were incubated at 37 °C for 5 minutes. Aliquots of sialidase were added and after incubation of 5 minutes at 37 °C, the reactions were quenched by boiling (2 min) followed by cooling on ice. All transformations were performed in duplicate. *Vibrio cholerae* and *Salmonella typhimurium* sialidases were performed in sodium acetate buffer (pH 5.50, 50 mM sodium acetate, 10 mM calcium chloride, 50 mM sodium chloride in milli-Q water) and *Clostridium perfringens* assays were performed in potassium phosphate buffer (pH 5.16, 0.05 M potassium phosphate/sodium hydroxide in Milli-Q water). An appropriate amount of enzyme was employed to assure that consumption of substrate was less than 15% for initial rate conditions.

Analysis using DIONEX HPLC

Sialic acid standards and HPLC eluents were made using the Hardy and Townsend procedure.[2] Samples were concentrated to dryness using a Savant AES 1000 Environmental SpeedVac at the low drying rate. Next, milli-Q water (30 μL) was added and the samples were vortexed for 15 seconds and then sonicated for 5 minutes to assure a completely homogeneous solution. Samples were then transferred into screw capped autosampler vials with spring tipped inserts and loaded into a DIONEX AS 3500 autosampler. A DIONEX carbopac PA10 column was used in conjunction with a GP40 Gradient pump. The gradient program setup through DIONEX PeakNet software was t = 0 min, NaOH = 95%, NaOAc = 5%; t = 5 min, NaOH = 95%, NaOAc = 5%; t = 25 min, NaOH = 82%, NaOAc = 18%; t = 30 min, NaOH = 82%, NaOAc = 18%; t = 32 min, NaOH = 95%, NaOAc = 5% with a cycle set for 60 minutes. Free sialic acid detection was made by an ED40 Electrochemical Detector. The autosampler was used with a 10μL syringe. Data analysis, Michaelis curves, and Lineweaver-Burk plots were performed with GraphPad Prism version 3.0. PeakNet software was used for raw data conversion. Standard curves were run with each assay to account for slight changes in eluent batches. Data analysis was performed with GraphPad Prism version 3.0.

Determination of Polymer Loading

Total carbohydrate content was determined by phenol sulfuric acid assay.[3] In addition, every sialidase assay included an experiment in which all sialic acids were cleaved by prolonged treatment with excess sialidase. In each case, the amount of free sialic acid was within experimental error.

Table 1. Kinetic parameters for the three sialidases determined from Michaelis-Menten and Lineweaver-Burk plots. Lineweaver-Burk data excludes the lowest concentrations to assure more accurate values.[4] For the *Clostridium perfringens,* 2.5 mU of enzyme was used for compound **1** while only 0.2 mU was required for compound **2** to obtain initial rate conditions. For *Vibrio cholerae,* 2.5 mU of enzyme was used for compound **1** while 0.25 mU was required for compound **2** to obtain initial rate conditions.

		Michaelis-Menten			Lineweaver-Burk		
enzyme	**cmpd**	**Km (mmol)**	**Vmax (μmol/m)**	**Rel. Vmax**	**Km (mmol)**	**Vmax (μmol/m)**	**Rel. Vmax**
C. perfringens	**1**	2.2 ± 0.3	3.1 ± 0.6	1.0	2.7 ± 0.3	2.8 ± 0.1	1.0
	2	0.04 ± 0.02	0.21 ± 0.03	0.85	0.04 ± 0.01	0.18 ± 0.08	0.80
V. cholerae	**1**	5.7 ± 0.4	24.0 ± 1.6	1.0	5.7 ± 0.3	24.1 ± 0.7	1.0
	2	0.04 ± 0.01	0.56 ± 0.04	0.23	0.04 ± 0.01	0.6 ± 0.1	0.25
S. typhimurium	**1**	2.1 ± 0.5	0.9 ± 0.1	1.0	2.2 ± 0.1	0.89 ± 0.06	1.0
	2	2.9 ± 0.3	1.3 ± 0.1	1.4	2.6 ±0.5	1.2 ± 0.2	1.3

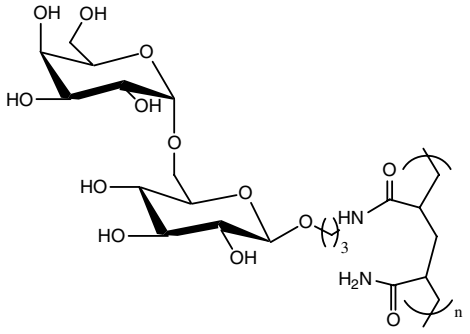


Figure 1. Structure of polyvalent melibiose (18% loading)

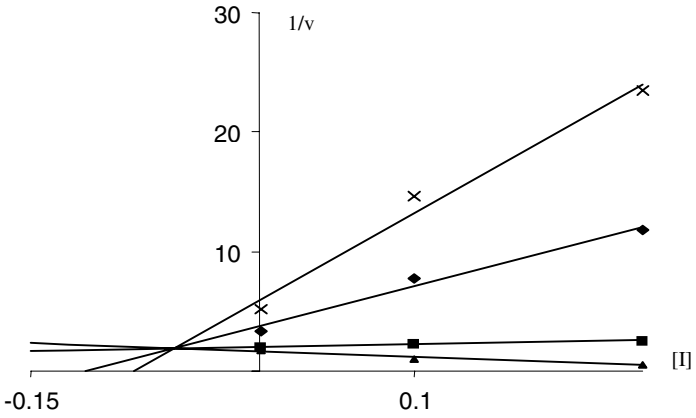
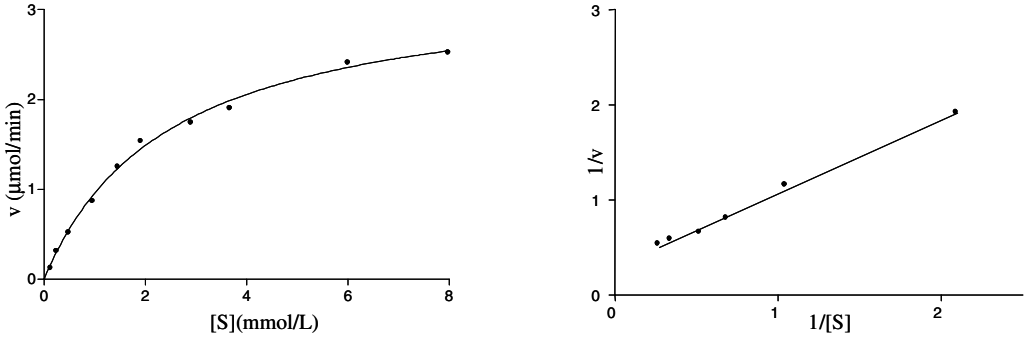
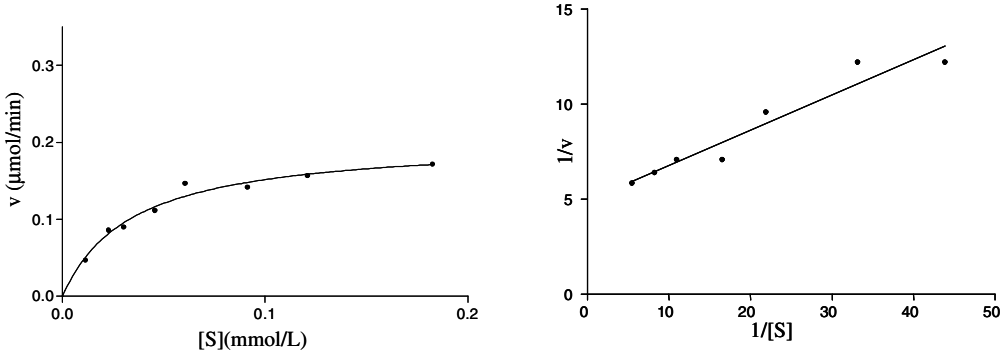


Figure 2. Dixon Plot of hydrolysis of sialoside **2** by sialidase of *Vibrio cholerae* in the presence of different concentrations of (I) valency corrected polymeric melibiose (18% loading). x = 0.025 mmol , ♦= 0.050 mmol, ■ = 0.250 mmol, ▲ = 2.00 mmol (valency corrected concentrations of substrate).

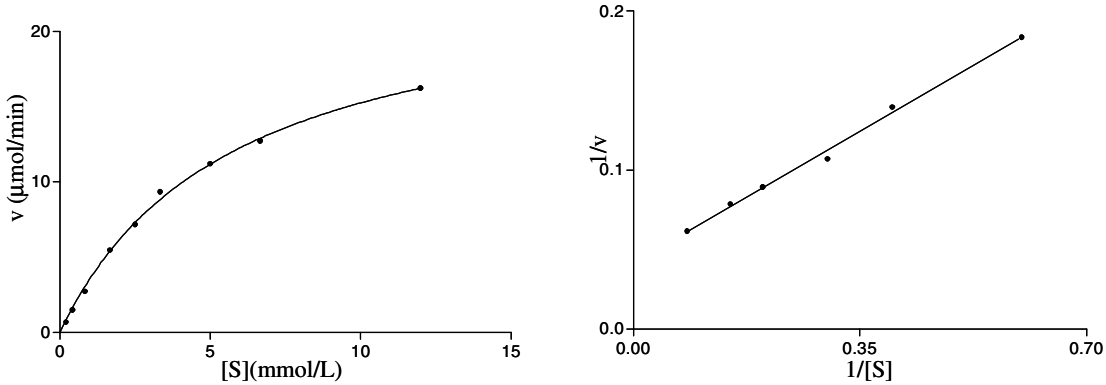
Compound **1** – *Clostridium perfringens*



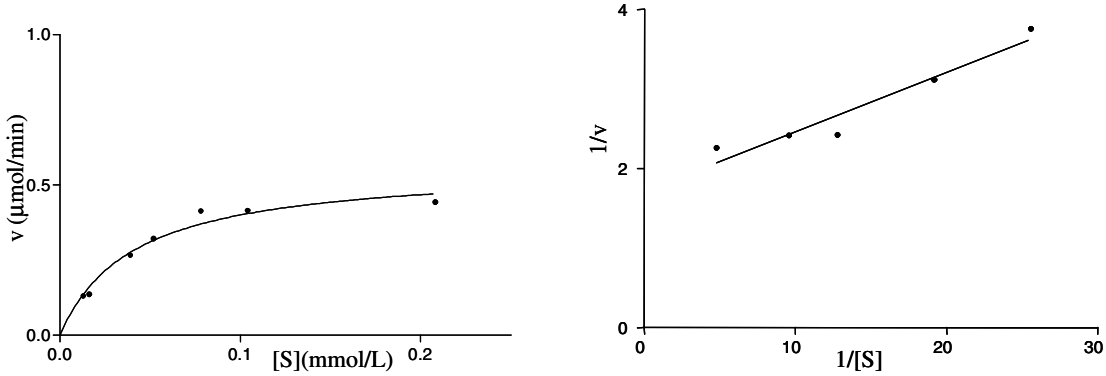
Compound **2** – *Clostridium perfringens*



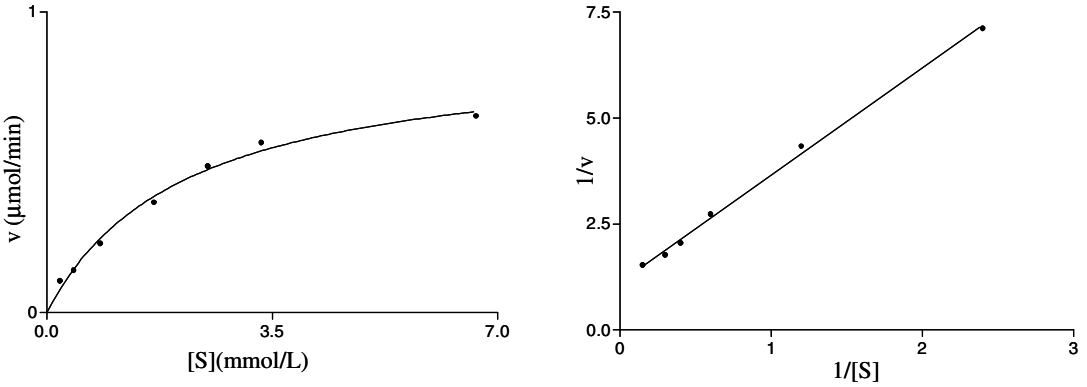
Compound **1** – *Vibrio cholerae*



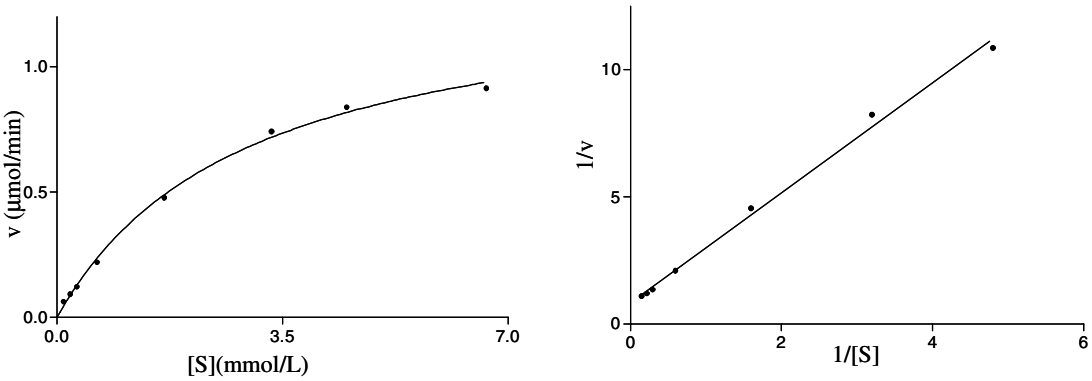
Compound **2** – *Vibrio cholerae*



Compound **1** – *Salmonella typhimurium*



Compound **2** – *Salmonella typhimurium*



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3. Dubois, M.; Gilles, K. A.; Hamilton, J. K.; Rebers, P. A.; Smith, F. *Nature* **1951**, 168,167; *Anal Chem.* **1956**, *28*, 350.
4. Copeland, R. Enzymes: A Practical Introduction to Structure, Mechanism, and Data Analysis. **2000**.