SUPPLEMENTAL MATERIAL

A systematic analysis of acceptor specificity and reaction kinetics of five human α(2,3)sialyltransferases: Product inhibition studies illustrates reaction mechanism for ST3Gal-I

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KINETICS ANALYSIS

The following sections present rate expressions for the ordered sequential bi-bi, random sequential bi-bi and ping-pong reaction mechanisms [1]:

ORDERED SEQUENTIAL BI-BI MECHANISM

A. Overall expression for product formation

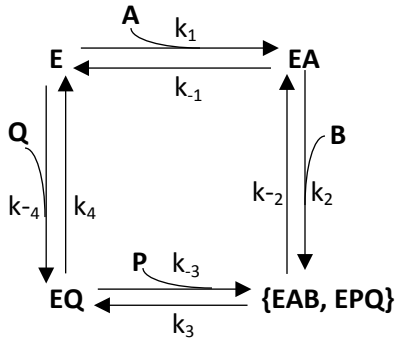
$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA$$

$$EA + B \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} EAB$$

$$EAB \underset{k_{-p}}{\overset{k_p}{\rightleftharpoons}} EPQ$$

$$EPQ \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} EQ + P$$

$$EQ \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + Q$$



Upon solving the above set of equations algebraically using the King-Altman method, a rate expression for product formation can be derived:

$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B} - \frac{V_r[P][Q]}{K_i^Q K_M^P}}{1 + \frac{[A]}{K_i^A} + \frac{K_M^A[B]}{K_i^A K_M^B} + \frac{K_M^Q[P]}{K_i^Q K_M^P} + \frac{[Q]}{K_i^Q} + \frac{[A][B]}{K_i^A K_M^B} + \frac{K_M^Q[A][P]}{K_i^A K_M^P K_i^Q} + \frac{K_M^A[B][Q]}{K_i^A K_M^B K_i^Q} + \frac{[P][Q]}{K_i^Q K_M^P} + \frac{[A][B][P]}{K_i^A K_M^B K_i^P} + \frac{[B][P][Q]}{K_i^B K_M^P K_i^Q}}$$

[Eq. 1]

where

$$V_f = \frac{k_3 k_4 E_0}{k_3 + k_4};\ V_r = \frac{k_{-1} k_{-2} E_0}{k_{-1} + k_{-2}};\ K_M^A = \frac{k_3 k_4}{k_1(k_3 + k_4)};\ K_M^B = \frac{k_4(k_{-2} + k_3)}{k_2(k_3 + k_4)};$$

$$K_M^P = \frac{k_{-1}(k_{-2} + k_3)}{k_{-3}(k_{-1} + k_{-2})};\ K_M^Q = \frac{k_{-1} k_{-2}}{k_{-4}(k_{-1} + k_{-2})};\ K_i^A = \frac{k_{-1}}{k_1}$$

$$K_i^B = \frac{(k_{-1} + k_{-2})}{k_2};\ K_i^P = \frac{(k_3 + k_4)}{k_{-3}};\ K_i^Q = \frac{k_4}{k_{-4}}$$

B. Initial velocity rate expression

The initial reaction velocity then follows upon setting [*P*]= [*Q*]= 0 in the above equation:

$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B}}{1 + \frac{[A]}{K_i^A} + \frac{K_M^A[B]}{K_i^A K_M^B} + \frac{[A][B]}{K_i^A K_M^B}} = \frac{V_f[A][B]}{[A][B] + K_M^B[A] + K_M^A[B] + K_i^A K_M^B}$$

i.e. when [A] is varied, the above expression in double-reciprocal form is:

$$\frac{1}{v} = \frac{K_M^A}{V_f}\left(1 + \frac{K_i^A K_M^B}{K_M^A[B]}\right)\frac{1}{[A]} + \frac{1}{V_f}\left(1 + \frac{K_M^B}{[B]}\right)$$

[Eq. 2a]

When [B] is varied, the above expression becomes:

$$\frac{1}{v} = \frac{K_M^B}{V_f}\left(1 + \frac{K_i^A}{[A]}\right)\frac{1}{[B]} + \frac{1}{V_f}\left(1 + \frac{K_M^A}{[A]}\right)$$

[Eq. 2b]

This expression is similar to the standard equation used for the Lineweaver Burk Plot:

$$\frac{1}{v} = \frac{K_{M,app}}{V_{app}}\frac{1}{[A]} + \frac{1}{V_{app}}$$

In both the above equations, both the slope and intercept change upon varying [A] or [B]. The intercept lies above, at or below the *x*-axis depends on the relative values of K_i^Aversus K_M^A.

C. Product inhibition

In the case of product inhibition, [A] is set to be the variable and [B] is fixed. Here, if [P] is finite and [Q]=0, it follows that:

$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B}}{1 + \frac{[A]}{K_i^A} + \frac{K_M^A[B]}{K_i^A K_M^B} + \frac{K_M^Q[P]}{K_i^Q K_M^P} + \frac{[A][B]}{K_i^A K_M^B} + \frac{K_M^Q[A][P]}{K_i^A K_M^P K_i^Q} + \frac{[A][B][P]}{K_i^A K_M^B K_i^P}}$$

$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B}}{\left[1 + \frac{K_M^A[B]}{K_i^A K_M^B} + \frac{K_M^Q[P]}{K_i^Q K_M^P}\right] + \frac{[A]}{K_i^A}\left[1 + \frac{[B]}{K_M^B} + \frac{K_M^Q[P]}{K_M^P K_i^Q} + \frac{[B][P]}{K_M^B K_i^P}\right]}$$

$$\frac{1}{v} = \frac{K_M^A}{V_f}\left[1 + \frac{K_i^A K_M^B}{[B]K_M^A}\left(1 + \frac{K_M^Q[P]}{K_i^Q K_M^P}\right)\right]\frac{1}{[A]} + \frac{1}{V_f}\left[1 + \frac{K_M^B}{[B]}\left(1 + \frac{K_M^Q[P]}{K_M^P K_i^Q}\right) + \frac{[P]}{K_i^P}\right]$$

[Eq. 3a]

Here, both the apparent K_M and V_{max} are functions of [P]. Similar to above, in the case of product inhibition when [A] is set to be variable and [B] is fixed, [P]=0 and [Q] is finite, it follows that:

$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B}}{1 + \frac{[A]}{K_i^A} + \frac{K_M^A[B]}{K_i^A K_M^B} + \frac{[Q]}{K_i^Q} + \frac{[A][B]}{K_i^A K_M^B} + \frac{K_M^A[B][Q]}{K_i^A K_M^B K_i^Q}}$$

or

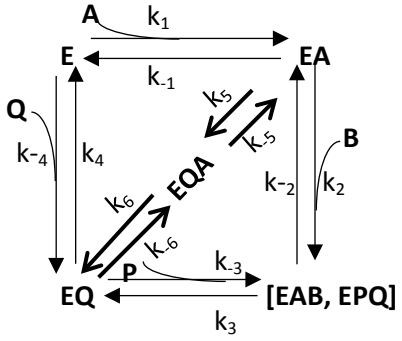
$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B}}{\left[1 + \frac{K_M^A[B]}{K_i^A K_M^B} + \frac{[Q]}{K_i^Q} + \frac{K_M^A[B][Q]}{K_i^A K_M^B K_i^Q}\right] + \frac{[A]}{K_i^A}\left[1 + \frac{[B]}{K_M^B}\right]}$$

$$\frac{1}{v} = \frac{K_M^A}{V_f}\left[\left(1 + \frac{[Q]}{K_i^Q}\right)\left(1 + \frac{K_i^A K_M^B}{[B]K_M^A}\right)\right]\frac{1}{[A]} + \frac{1}{V_f}\left[1 + \frac{K_M^B}{[B]}\right]$$

[Eq. 3b]

Here, only the apparent K_M is a function of [*Q*] and thus this is a type of competitive inhibition. In addition, if the product inhibition type is mixed when [A] is set to be variable and [B] is fixed, [P]=0 and [Q] is finite, the schematic stated below describes enzymatic mechanism.

The set of enzymatic equations solved algebraically using King-Altman method results in the rate expression with both apparent K_M and V_{max} as the function of [*Q*].



$$\frac{1}{v} = \frac{K_M^A}{V_f}\left(1 + \frac{K_i^A K_M^B}{[B]K_M^A}\right)\left[\left(1 + \frac{[Q]}{K_i^Q}\right)\right]\frac{1}{[A]} + \frac{1}{V_f}\left(K_i^{PQ} + \frac{K_M^B}{[B]}\right)\left[1 + \frac{[Q]}{K_i^{QA}\left[1 + \frac{[B]}{K_i^{PQ}K_M^B}\right]}\right]$$

[Eq. 3c]

where

$$V_f = \frac{k_3 E_0}{}; K_M^A = \frac{k_3}{k_1}; K_M^B = \frac{(k_{-2} + k_3)}{k_2};\ K_i^A = \frac{k_{-1}}{k_1}$$

$$K_i^{QA} = \frac{k_{-5}}{k_5};\ K_i^{PQ} = \frac{k_4}{(k_3 + k_4)};\ K_i^{Q} = \frac{k_4}{k_{-4}}$$

RANDOM SEQUENTIAL BI-BI MECHANISM

A. Overall expression for product formation

The rate expression here is similar that of the ordered sequential bi-bi reaction, only simpler since many of the steps are in rapid equilibrium:

$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B} - \frac{V_r[P][Q]}{K_i^Q K_M^P}}{1 + \frac{[A]}{K_i^A} + \frac{[B]}{K_i^B} + \frac{[P]}{K_i^P} + \frac{[Q]}{K_i^Q} + \frac{[A][B]}{K_i^A K_M^B} + \frac{[P][Q]}{K_i^Q K_M^P}}$$

[Eq.4]

B. Initial velocity rate expression

The initial reaction velocity follows by setting [P]=[Q]=0:

$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B}}{1 + \frac{[A]}{K_i^A} + \frac{[B]}{K_i^B} + \frac{[A][B]}{K_i^A K_M^B}} = \frac{V_f[A][B]}{[A][B] + K_M^B[A] + \frac{K_i^A K_M^B[B]}{K_i^B} + K_i^A K_M^B}$$

When [A] is varied, the above expression in double-reciprocal form is:

$$\frac{1}{v} = \frac{1}{V_f}\left(\frac{K_i^A K_M^B}{K_i^B} + \frac{K_i^A K_M^B}{[B]}\right)\frac{1}{[A]} + \frac{1}{V_f}\left(1 + \frac{K_M^B}{[B]}\right)$$

[Eq.5]

In the above equations, both the slope and intercept change upon varying [B].

C. Product inhibition

For product inhibition [P]=0 and [Q] is finite. In this case, when [A] is set to be the variable and [B] is fixed:

$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B}}{1 + \frac{[A]}{K_i^A} + \frac{[B]}{K_i^B} + \frac{[Q]}{K_i^Q} + \frac{[A][B]}{K_i^A K_M^B}}$$

$$\frac{1}{v} = \frac{K_i^A K_M^B}{V_f[A][B]}\left(1 + \frac{[B]}{K_i^B} + \frac{[Q]}{K_i^Q}\right) + \frac{K_M^B}{V_f[B]}\left(1 + \frac{[B]}{K_M^B}\right)$$

[Eq.6]

PING-PONG REACTION

A. Overall expression for product formation

$$E + A \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} EA$$

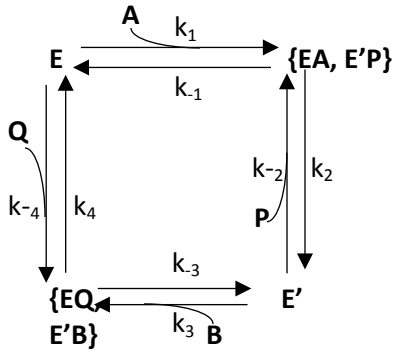
$$EA \rightleftharpoons E'P$$

$$E'P \underset{k_{-2}}{\overset{k_2}{\rightleftharpoons}} E' + P$$

$$E' + B \underset{k_{-3}}{\overset{k_3}{\rightleftharpoons}} E'B$$

$$E'B \rightleftharpoons EQ$$

$$EQ \underset{k_{-4}}{\overset{k_4}{\rightleftharpoons}} E + Q$$



Upon solving the above set of equations algebraically, a rate expression for product formation can be derived:

$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B} - \frac{V_r[P][Q]}{K_i^P K_M^Q}}{\frac{[A]}{K_i^A} + \frac{K_M^A[B]}{K_i^A K_M^B} + \frac{[P]}{K_i^P} + \frac{K_M^P[Q]}{K_i^P K_M^Q} + \frac{[A][B]}{K_i^A K_M^B} + \frac{[A][P]}{K_i^A K_M^B} + \frac{K_M^A[B][Q]}{K_i^A K_M^B K_i^Q} + \frac{[P][Q]}{K_i^P K_M^Q}}$$

[Eq. 7]

where

$$V_f = \frac{k_2 k_4 E_0}{k_2 + k_4};\ V_r = \frac{k_{-1} k_{-3} E_0}{k_{-1} + k_{-3}};\ K_M^A = \frac{(k_{-1} + k_2) k_4}{k_1 (k_2 + k_4)};\ K_M^B = \frac{k_2 (k_{-3} + k_4)}{k_3 (k_2 + k_4)};$$

$$K_M^P = \frac{k_{-3}(k_{-1} + k_2)}{k_{-2}(k_{-1} + k_{-3})};\ K_M^Q = \frac{k_{-1}(k_{-3} + k_4)}{k_{-4}(k_{-1} + k_{-3})};\ K_i^A = \frac{k_{-1}}{k_1}$$

$$K_i^B = \frac{k_{-3}}{k_3};\ K_i^P = \frac{k_2}{k_{-2}};\ K_i^Q = \frac{k_4}{k_{-4}}$$

B. Initial velocity rate expression

At the initial time point, [*P*]=[*Q*]=0 and thus when [*A*] is varied:

$$v = \frac{\frac{V_f[A][B]}{K_i^A K_M^B}}{\frac{[A]}{K_i^A} + \frac{K_M^A[B]}{K_i^A K_M^B} + \frac{[A][B]}{K_i^A K_M^B}}$$

$$\frac{1}{v} = \frac{1}{[A]}\frac{K_M^A}{V_f} + \frac{1}{V_f}\left(\frac{K_M^B}{[B]} + 1\right)$$

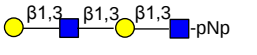
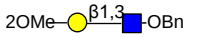
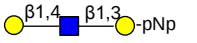
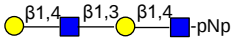
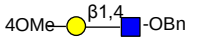
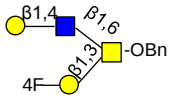
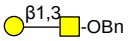
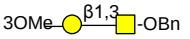
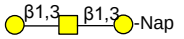
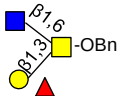
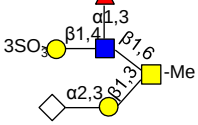
[Eq. 8]

Upon changing [*B*], Lineweaver Burk plot results in a series of lines with identical slope.

Reference:

1. Segel, I., *Enzyme kinetics*. 1975, John Wiley & Sons, Inc.: United States of America. p. 560-621.

Table S1: Substrates used for enzymology

Structures	Glycan Type	*
	***Type-I*** Galβ1,3GlcNAcβ1,3lac-pNp	**[1]**
	2-O-MeGalβ1,3GlcNAc-OBn	**[1a]**
	Type-II	
	Galβ1,4GlcNAcβ1,3Gal-pNp	**[2]**
	Galβ1,4GlcNAcβ1,3Galβ1,4 GlcNAc-pNp	**[2a]**
	4-O-MeGalβ1,4GlcNAc-OBn	**[2b]**
	Galβ1,4GlcNAcβ1,6(4FGalβ1, 3)GalNAc-OBn	**[2c]**
	Type-III	
	Galβ1,3GalNAc-OBn	**[3]**
	3-O-MeGalβ1,3GalNAc-OBn	**[3a]**
	Galβ1,3GalNAcβ1,3Gal-Nap	**[3b]**
	Galβ1,3(GlcNAc β1,6) GalNAc-OBn	**[4]**
	NeuAcα2,3Galβ1,3(3OSulfo) Galβ1,4(Fucα1,3)GlcNAcβ1,6) GalNAc-Me	**[Sia4a]**

* The corresponding Neu5Ac terminated glycans are referred to as **[Sia1]**, **[Sia2]**, **[Sia3]**, **[Sia4]** etc.

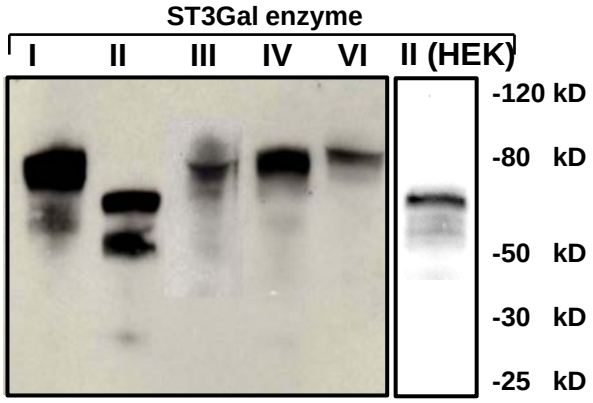


Figure S1. Sialyltransferase expression – Western blot detected the ST3Gal fusion proteins using HRP conjugated anti-human Fc Ab. All enzymes were purified from CHO cell culture media using nickel-chelate column, except for ST3Gal-II which was also obtained from HEK cells.

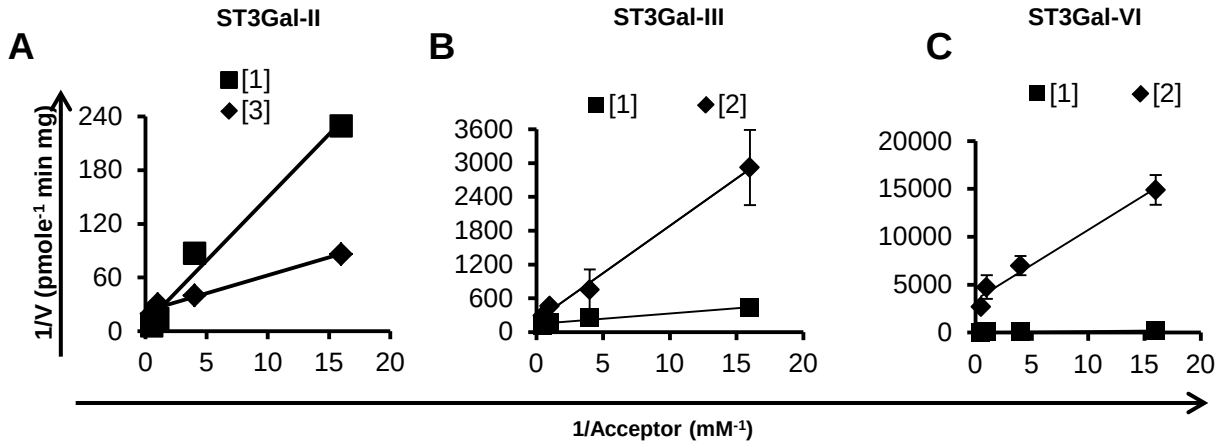


Figure S2. Kinetic analysis for sialylTs – A – C. Lineweaver-Burk plot for 0.24mU/mL ST3Gal-II, 0.05mU/mL ST3Gal-III and 0.063mU/mL ST3Gal-VI, respectively. In all cases, reactions contained 0.0625-2.0mM of either acceptor **[1]** or **[2]** or **[3]** as indicated, along with 0.5mM CMP-Neu5Ac.

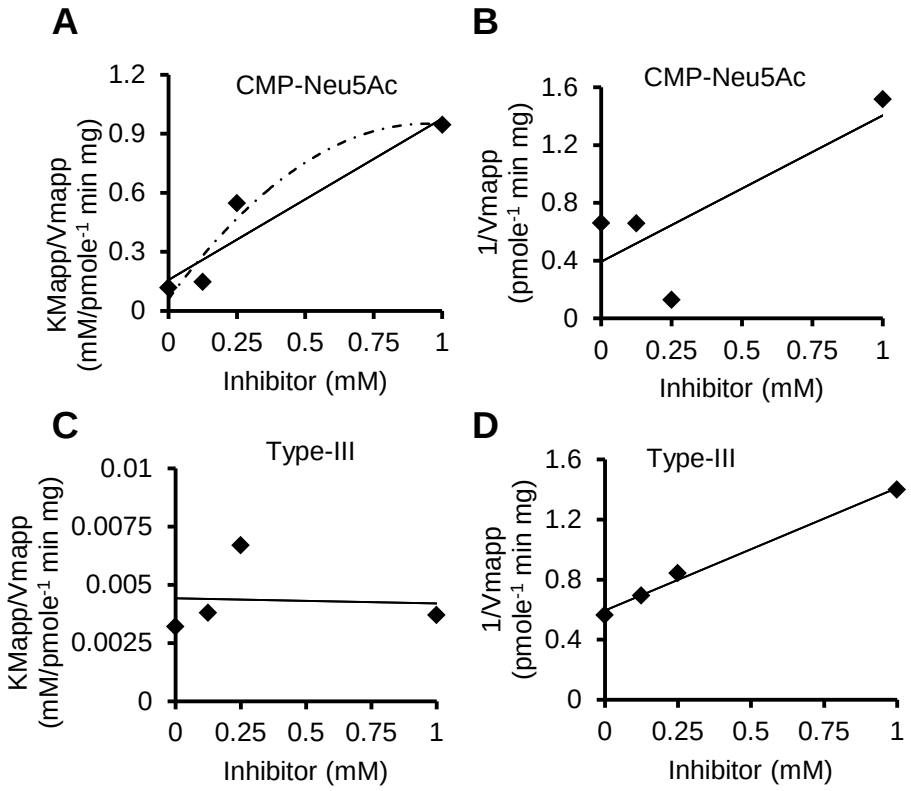


Figure S3. Secondary plots for inhibition reactions – A. Secondary plots obtained from the Lineweaver-Burk plots generated by varying CMP-Neu5Ac in the presence of 5'CMP. Hyperbolic profile indicates partial effect of inhibitor. **B.** $1/V_{Mapp}$ vs inhibitor 5'-CMP together with the response in **A** suggests partially mixed or competitive inhibition. **C.** and **D.** Secondary plots obtained by varying Galβ1,3GalNAc suggests uncompetitive inhibition with 5'CMP. Equation 5 described in methods illustrates these plots.

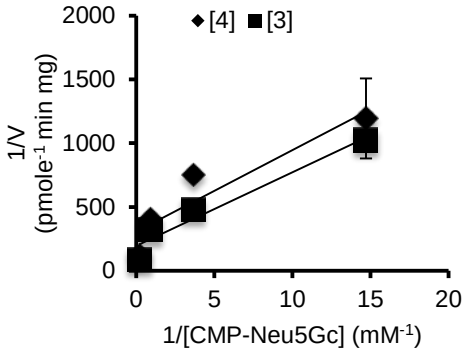


Figure S4. Kinetic analysis based on CMP-Neu5Gc – Lineweaver-Burk plot upon varying CMP-Neu5Gc at fixed concentrations of 1mM **[3]** and **[4]** in the presence of 0.1 mU/mL ST3Gal-I.