SUPPORTING INFORMATION

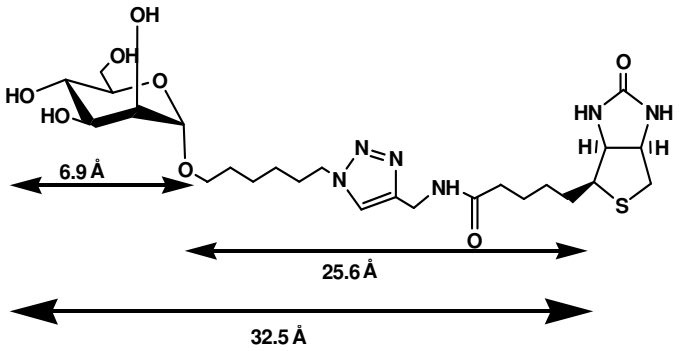
Supplementary Figure S1. Chemical structures of the glycoconjugates used in this study. Structures of the biotinylated mannose (A), PEG 3400 (B), and Pk analogue (C) glycoconjugates used in this study. The length of each molecule is noted.

Supplementary Figure S2. SPR sensograms for Stx B-subunit binding to Pk analogues from one representative experiment. A, Binding of Stx1B to Pk. B, Binding of Stx2B to NHAc-Pk. Dashed lines indicate the point in the binding curves (200 sec) from which binding values were taken.

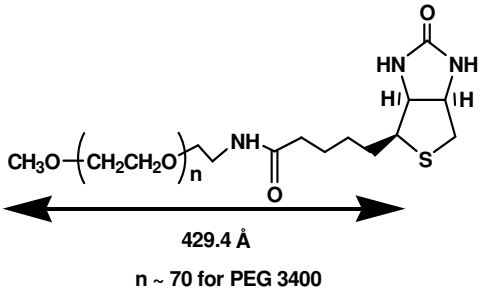
Supplementary Figure S3. Strong binding interactions between Stxs and Pk analogues occur very rapidly in ELISA platform. Analysis of the effect of Stx-glycan contact time on binding in the ELISA platform was tested by incubating with Stxs at 1.43 μM and 0.14 μM for 0.5, 1, 2, 3, 4, 5, 10, 15, 30 and 60 min. Binding is expressed as % of the value obtained for the highest toxin concentration tested for each plate.

Supplementary Figure S4. Nonlinear fits of Stx ELISA binding curves. ELISA data for Stx interactions with Pk analogues were fitted to a one site specific binding model (GraphPad 5 Software, La Jolla, CA) to give global affinities. A, Stx1 holotoxin binding to Pk, high binding-capacity ELISA. B, Stx2 holotoxin binding to NHAc-Pk, high binding-capacity ELISA. C, Stx1 B-subunit binding to Pk, high binding-capacity ELISA. D, Stx1 holotoxin binding to Pk, low binding-capacity ELISA. E, Stx2 holotoxin binding to NHAc-Pk, low binding-capacity ELISA. *Saturated binding was not obtained for the Stx1 concentrations tested in the low binding-capacity ELISA, therefore the K_d value is denoted as greater than or equal to (≥) the value obtained from the nonlinear fit.

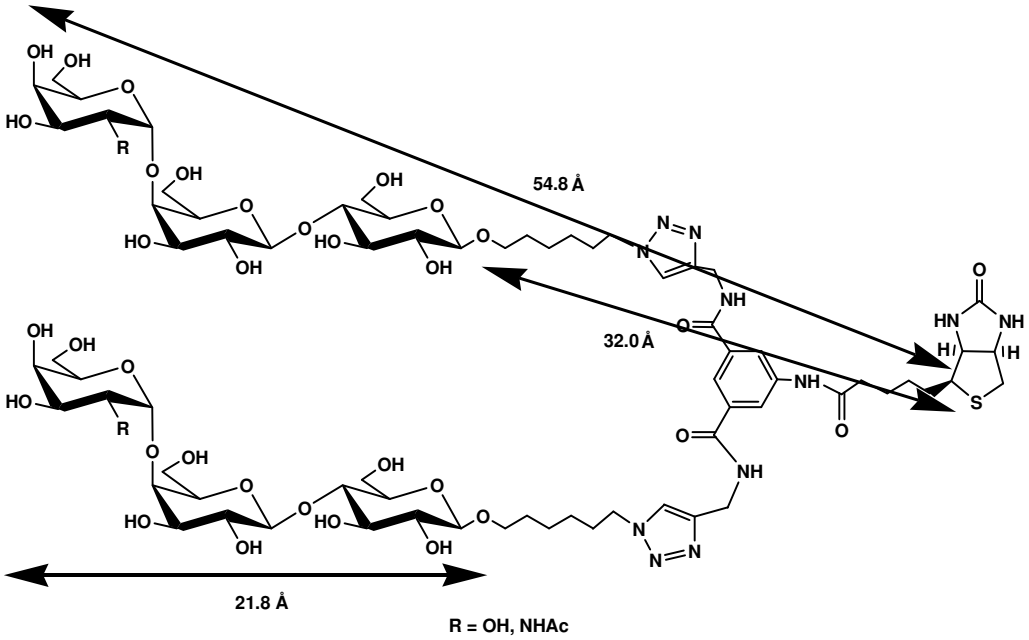
SUPPLEMENTARY FIGURES



A. Mannose



B. PEG 3400



C. Pk Analogues (Pk and NHAc-Pk)

Figure S1

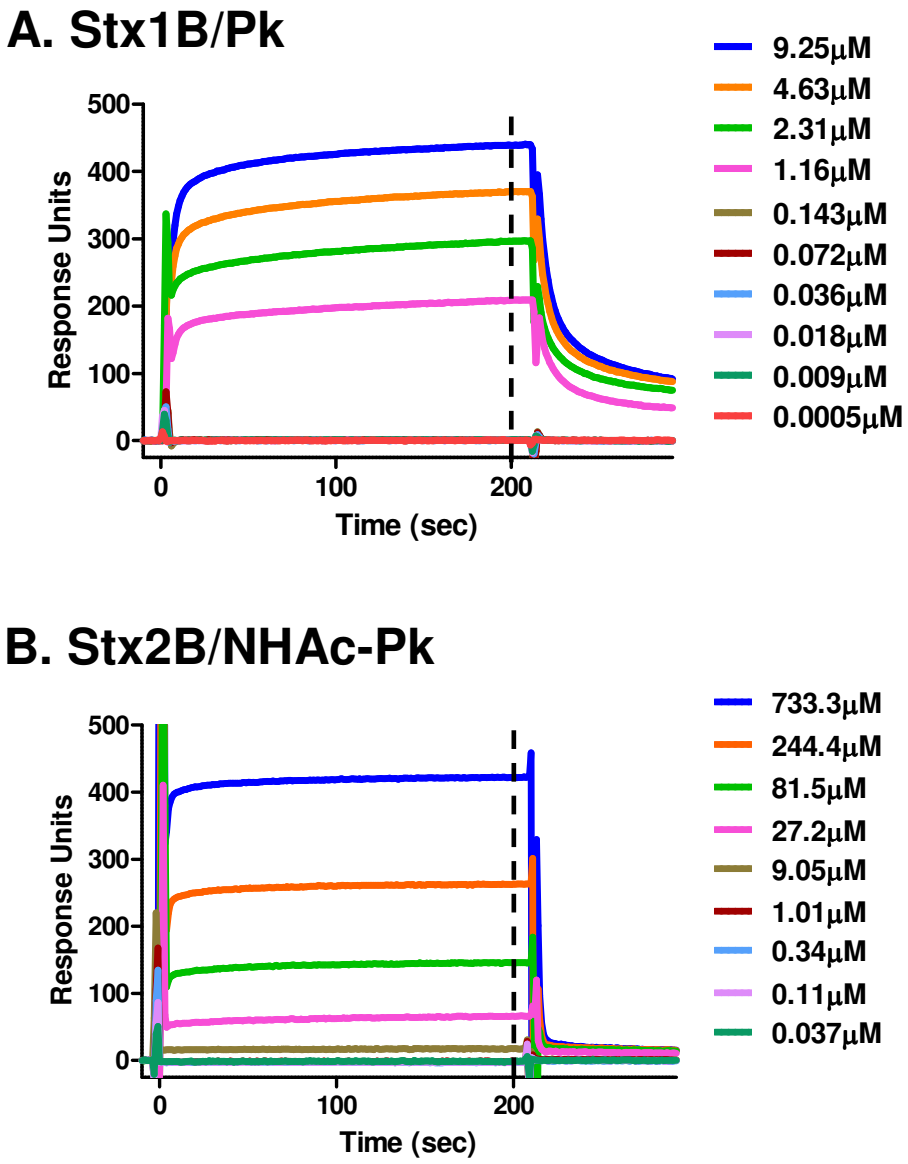

A. Stx1B/Pk
Response Units
500
400
300
200
100
0
0
100
200
Time (sec)
9.25µM
4.63µM
2.31µM
1.16µM
0.143µM
0.072µM
0.036µM
0.018µM
0.009µM
0.0005µM
B. Stx2B/NHAc-Pk
Response Units
500
400
300
200
100
0
0
100
200
Time (sec)
733.3µM
244.4µM
81.5µM
27.2µM
9.05µM
1.01µM
0.34µM
0.11µM
0.037µM

Figure S2

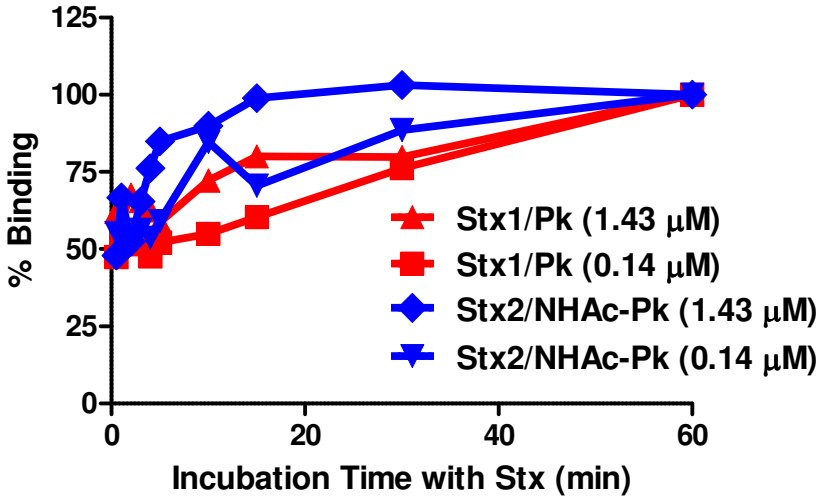

125
100
75
50
25
0
% Binding
0
20
40
60
Incubation Time with Stx (min)
Stx1/Pk (1.43 μM)
Stx1/Pk (0.14 μM)
Stx2/NHAc-Pk (1.43 μM)
Stx2/NHAc-Pk (0.14 μM)

Figure S3

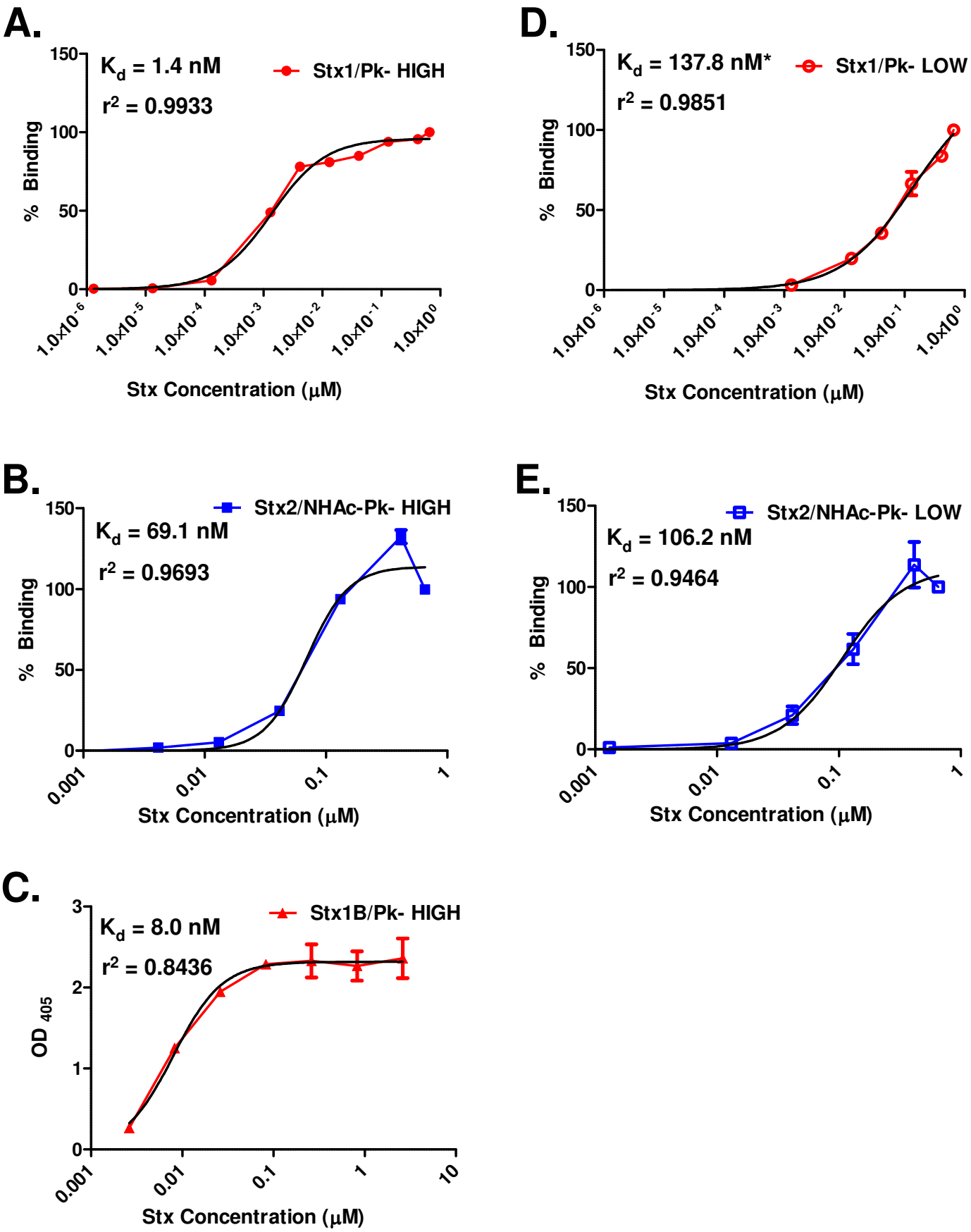

A.
K_d = 1.4 nM
r^2 = 0.9933
Stx1/Pk- HIGH
% Binding
Stx Concentration (μM)
D.
K_d = 137.8 nM*
r^2 = 0.9851
Stx1/Pk- LOW
% Binding
Stx Concentration (μM)
B.
K_d = 69.1 nM
r^2 = 0.9693
Stx2/NHAc-Pk- HIGH
% Binding
Stx Concentration (μM)
E.
K_d = 106.2 nM
r^2 = 0.9464
Stx2/NHAc-Pk- LOW
% Binding
Stx Concentration (μM)
C.
K_d = 8.0 nM
r^2 = 0.8436
Stx1B/Pk- HIGH
OD 405
Stx Concentration (μM)

Figure S4

Calculation of the distance between streptavidin (SA) molecules on SPR sensor chip
(assuming homogeneous distribution of the SA molecules in three-dimensional space):

SA sensor chip:
height = 50 nm = 5×10^{-5} mm (Reference 36)
flow cell area = 1.2 mm^2 (Reference 37, and manufacturer correspondence)
volume = (1.2 mm^2) × (5×10^{-5} mm) = 6×10^{-5} mm^3
SA capacity = 4000 to 6000 RU = 400 to 600 ng/cm^2 = 4 to 6 ng/mm^2 (Reference 36)
Amount of SA coupled to flow cell (assuming 6 ng SA/mm^2) =
(6 ng/mm^2) × (1.2 mm^2) = 7.2 ng

Molecular Weight of SA: 52,800 Da

Calculations:

52,800 Da SA = 52,800 g SA/mol = 5.28×10^{13} ng SA/mol

(1 mol SA/5.28×10^{13} ng) × (7.2 ng) = 1.36×10^{-13} mol SA

(1.36×10^{-13} mol SA) × (6.02×10^{23} molecules/mol) =
8.19×10^{10} molecules SA/flow cell

8.19×10^{10} molecules SA/flow cell = 8.19×10^{10} molecules SA/6×10^{-5} mm^3 =
1.37×10^{15} molecules SA/mm^3

cube root of 1.37×10^{15} molecules SA/mm^3 = 111,064 molecules SA/mm

(1 ÷ 111,064 molecules/mm) = 9.0×10^{-6} mm between SA molecules =
9.0 nm between SA molecules

(9.0 nm between SA molecules) × (10 Å/nm) = **90 Å between SA molecules***

*Assuming an SA capacity of 4000 RU, the distance between SA molecules is calculated to be 103 Å