Supporting Information for

Glycomics characterization of an influenza H5N7 hemagglutinin series with engineered glycosylation patterns: Implications for structure-function relationships

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Table of Contents

Table S1: Glycans found in each H5N7 sample by MALDI-TOF MS analysis Page S-2

Table S2. Comparison of glycoforms detected by MALDI-ToF MS of permethylated glycans and Q-ToF MSE glycopeptides. Page S-3

Figure S1: Comparison of glycan abundances of RNAseB from released, permethylated glycans measured by MALDI-TOF MS and unlabeled glycopeptides measured by LC/MS. Page S-4

Figure S2: Percent abundance of LC/MS measured glycopeptides at each site from previously studied goose/Qinghai/14/2008 H5N1 HA. Page S-5

Table S1. Glycans found in each sample by MALDI-TOF MS analysis. Percent abundances ± the standard deviation between three sample spots are shown for each glycan found.

Glycan	Calculated $[M+Na]^+$	Sample			
		293T	293T-VCNA	293S	S2
Hex2HexNAc2	967.48			0.19 ± 0.07	6.08 ± 1.25
dHex1Hex2HexNAc2	1141.57				7.03 ± 0.55
Hex3HexNAc2	1171.58			0.16 ± 0.03	39.47 ± 2.18
dHex1Hex3HexNAc2	1345.67				35.59 ± 1.24
Hex3HexNAc3	1416.71				3.06 ± 0.51
dHex1Hex3HexNAc3	1590.80			0.51 ± 0.05	
Hex3HexNAc4	1661.84	0.39 ± 0.07	0.33 ± 0.14		
dHex1Hex3HexNAc5	2081.05	0.55 ± 0.01	0.78 ± 0.27		
NeuAc1Hex3HexNAc5	2268.14	2.03 ± 0.46	4.62 ± 1.47		
Hex3HexNAc6	2152.09	0.11 ± 0.05			
Hex4HexNAc2	1375.68			2.90 ± 0.16	2.60 ± 0.39
dHex1Hex4HexNAc2	1549.77			0.27 ± 0.09	
Hex4HexNAc4	1865.94	0.27 ± 0.03	0.31 ± 0.18		
dHex1Hex4HexNAc4	2040.03	0.36 ± 0.04	0.55 ± 0.17		
Hex4HexNAc5	2111.06	0.71 ± 0.05	0.92 ± 0.26		
NeuAc1Hex4HexNAc5	2472.24	2.37 ± 0.54	5.62 ± 2.00		
dHex1Hex4HexNAc5	2285.15	0.27 ± 0.09	0.33 ± 1.4		
NeuAc1dHex1Hex4HexNAc5	2646.33	0.18 ± 0.04	0.30 0.10		
Hex5HexNAc2	1579.78	4.21 0.54	4.72 ± 1.58	95.98 ± 0.21	6.18 ± 0.48
Hex5HexNAc3	1824.91	0.37 ± 0.05	0.31 ± 0.25		
NeuAc1Hex5HexNAc3	2186.08	0.39 ± 0.2	0.28 ± 0.20		
dHex1Hex5HexNAc3	1999.00	0.39 ± 0.02	0.28 ± 0.20		
NeuAc1dHex1Hex5HexNAc3	2360.17	0.67 ± 0.09	0.63 ± 0.20		
Hex5HexNAc4	2070.04	0.74 ± 0.15	1.16 ± 0.29		
NeuAc1Hex5HexNAc4	2431.21	0.44 ± 0.04	0.45 ± 0.10		
dHex1Hex5HexNAc4	2244.12	0.58 ± 0.03	1.17 ± 0.44		
NeuAc1dHex1Hex5HexNAc4	2605.30	2.14 0.15	2.01 ± 0.57		
NeuAc2dHex1Hex5HexNAc4	2966.47	1.55 ± 0.22	1.76 ± 0.08		
Hex5HexNAc5	2315.16	0.37 ± 0.04	0.57 ± 0.16		
NeuAc1Hex5HexNAc5	2676.34	0.37 ± 0.09	0.29 ± 0.06		
dHex1Hex5HexNAc5	2486.25	0.20 ± 0.06			
Hex6HexNAc2	1783.88	0.50 ± 0.12	0.55 ± 0.21		
Hex6HexNAc3	2029.01	0.25 ± 0.07	0.42 ± 0.22		
Hex6HexNAc5	2519.26	1.85 ± 0.05	3.37 ± 0.65		
NeuAc1Hex6HexNAc5	2880.44	0.83 ± 1.19	1.22 ± 0.38		

NeuAc3Hex6HexNAc5	3602.78	0.12 ± 0.0			
dHex1Hex6HexNAc5	2693.35	2.04 ± 0.53	3.33 ± 1.25		
NeuAc1dHex1Hex6HexNAc5	3054.52	12.00 ± 1.74	10.48 ± 2.82		
NeuAc2dHex1Hex6HexNAc5	3415.70	0.86 ± 0.07			
Hex7HexNAc2	1987.98	0.57 ± 0.05	0.46 ± 0.29		
dHex1Hex7HexNAc2	2162.07				
Hex7HexNAc5	2723.36	0.32 ± 0.03	0.24 ± 0.10		
NeuAc1Hex7HexNAc5	3084.54	0.79 ± 0.00	1.50 ± 0.09		
NeuAc2Hex7HexNAc5	3445.71	0.64 ± 0.24			
NeuAc1Hex7HexNAc6	3329.66	0.86 ± 0.50	4.56 ± 1.77		
NeuAc2Hex7HexNAc6	3690.84	0.97 ± 0.33			
NeuAc3Hex7HexNAc6	4052.01	0.30 ± 0.05			
dHex1Hex7HexNAc6	3142.58	0.72 ± 0.07	1.82 ± 0.16		
NeuAc1dHex1Hex7HexNAc6	3503.75	19.23 ± 2.07	38.06 ± 0.46		
NeuAc2dHex1Hex7HexNAc6	3864.92	31.71 ± 1.16	5.70 ± 1.69		
NeuAc3dHex1Hex7HexNAc6	4226.10	4.54 ± 0.76			

Table S2. Comparison of glycoforms detected by MALDI-ToF MS of permethylated glycans and Q-ToF MS^E glycopeptides.

	MALDI				**Q-ToF**			
Glycan	293T	293T-VCNA	293S	S2	293T	293T-VCNA	293S	S2
Hex2HexNAc2			+	+			+	+
dHex1Hex2HexNAc2				+				+
Hex3HexNAc2			+	+			+	+
dHex1Hex3HexNAc2				+				+
Hex3HexNAc3				+				+
dHex1Hex3HexNAc3			+				+	
Hex3HexNAc4	+	+						
dHex1Hex3HexNAc5	+	+			+	+		
NeuAc1Hex3HexNAc5	+	+						
Hex3HexNAc6	+				+	+		
Hex4HexNAc2			+	+			+	+
dHex1Hex4HexNAc2			+				+	
Hex4HexNAc4	+	+						
dHex1Hex4HexNAc4	+	+						
Hex4HexNAc5	+	+			+	+		
NeuAc1Hex4HexNAc5	+	+			+	+		
dHex1Hex4HexNAc5	+	+			+	+		
NeuAc1dHex1Hex4HexNAc5	+	+			+	+		
Hex5HexNAc2	+	+	+	+	+	+	+	+

Hex5HexNAc3	+	+			+	+		
NeuAc1Hex5HexNAc3	+	+			+	+		
dHex1Hex5HexNAc3	+	+			+	+		
NeuAc1dHex1Hex5HexNAc3	+	+			+	+		
Hex5HexNAc4	+	+			+	+		
NeuAc1Hex5HexNAc4	+	+			+	+		
dHex1Hex5HexNAc4	+	+			+	+		
NeuAc1dHex1Hex5HexNAc4	+	+			+	+		
NeuAc2dHex1Hex5HexNAc4	+	+			+	+		
Hex5HexNAc5	+	+			+	+		
NeuAc1Hex5HexNAc5	+	+						
dHex1Hex5HexNAc5	+				+	+		
Hex6HexNAc2	+	+			+	+		
Hex6HexNAc3	+	+						
Hex6HexNAc5	+	+			+	+		
NeuAc1Hex6HexNAc5	+	+			+	+		

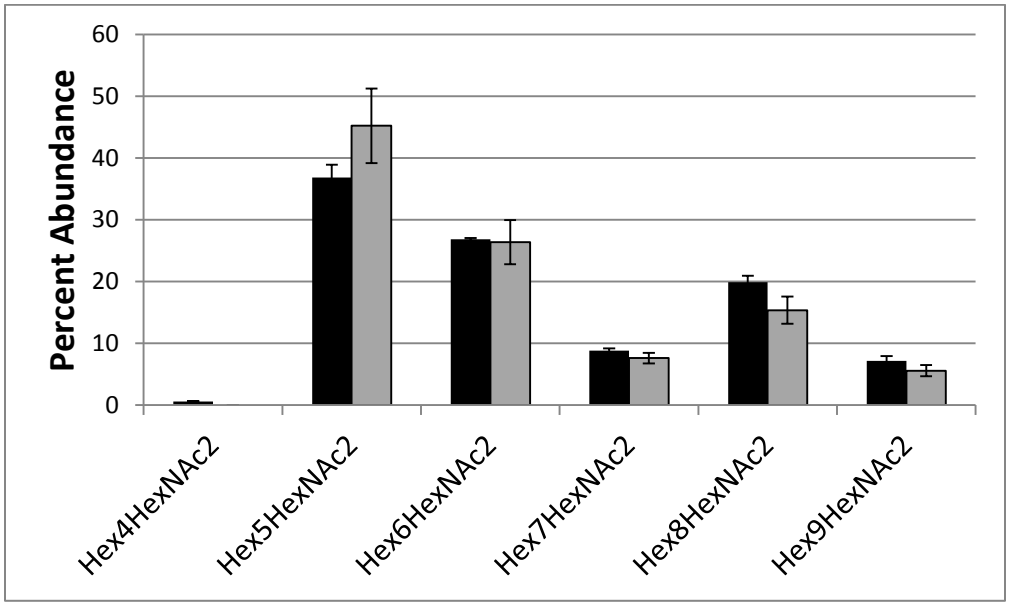

Figure S1. Comparison of glycan abundances from RNAseB assigned from released, permethylated glycans via MALDI-TOF MS (black bars) and unlabeled glycopeptides via LC/MS (gray bars). Bars are average of three sets of data. Error bars represent one standard deviation.

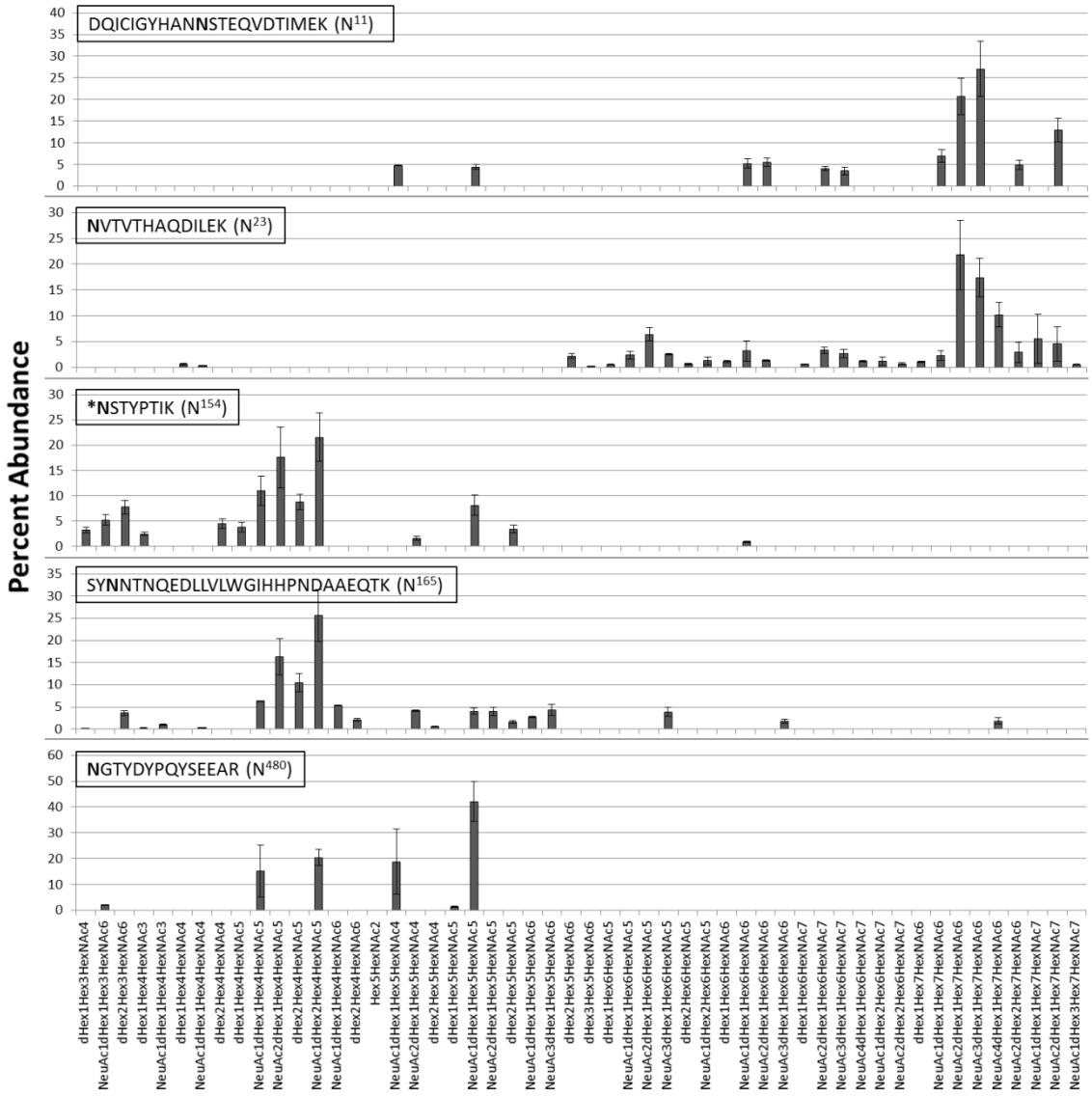

Figure S2. Percent abundance of LC/MS data for each site in a previous study of goose/Qinghai/14/2008 H5N1 HA grown in HEK293T cells [1]. The peptide sequence is shown at the top of each chart. The corresponding glycosylation site in the current study is shown in parenthesis. Bars are average of three sets of data. Error bars represent one standard deviation. *Site N^{154} is not glycosylated in the current study due to a mutation in the sequon from NST to NNA.

1. An, Y.; Rininger, J. A.; Jarvis, D. L.; Jing, X.; Ye, Z.; Aumiller, J. J.; Eichelberger, M.; Cipollo, J. F., Comparative glycomics analysis of influenza Hemagglutinin (H5N1) produced in vaccine relevant cell platforms. *Journal of Proteome Research* **2013,** *12* (8), 3707-20.