Supplementary Glycan Microarray Document

Based on MIRAGE Guidelines (doi:10.3762/mirage.3)

Peng W, de Vries RP, Grant OC, Thompson AJ, McBride R, Tsogtbaatar B, Lee PS, Razi N, Wilson IA, Woods RJ, Paulson JC. Recent H3N2 Viruses Have Evolved Specificity for Extended, Branched Human-type Receptors, Conferring Potential for Increased Avidity. *Cell Host Microbe*. 21:23-34 (2017). DOI:10.1016/j.chom.2016.11.004. PubMed

Classification	Guidelines
1. Sample: Glycan Binding Sample	
Description of Sample	Recombinant hemagluttinin (HA) proteins originating from Influenza TypeA viruses. HA: Fig2C A/HK/1/68 A/Victoria/3/75 A/Beijing/353/89 A/Wyoming/3/03 A/Perth/16/09 A/Victoria/361/11 HA: Fig4A A/VN/1203/0 Fig4B A/Duck/UKR/63 Fig4C A/HK/6934/10 Fig4D A/Cal/04/09 (Cal/04/09) Codon-optimized HA-encoding cDNA (GenScript) was cloned into the pCD5 expression vector as described previously. The resulting expression vector encodes an HA protein containing a heterologous signal peptide, a C-terminal trimerization domain (GCN4), and a streptavidin tag (Streptag-II) and lacking the transmembrane and cytoplasmic domains. All mutant H1 proteins were created by site-directed mutagenesis with a QuikChange site-directed mutagenesis kit (Stratagene, CA). The HA proteins were expressed in HEK293S GnT1(−) cells, purified from the cell culture supernatants using streptavidin beads, and quantified as described previously. de Vries et al., JVI (2013) PMID: 24109242 Whole H3N2 Influenza A viruses Fig3A A/Florida/2/06 Fig3B A/Georgia/4/06 Fig3C A/Honduras/3112/06 Fig3D A/New Hampshire/3/06

	Fig3E A/New Jersey/2/06 Fig3F A/New York/2/06 Fig3G A/New York/3/06 Fig3H A/Pennsylvania/4/07 Fig3I Vic/11 Influenza A/H3N2 clinical samples with Ct values less than 33.22 were included in this study and cultured using the MDCK.2 (ATCC; CRL-2936) cell-line with a single passage history. Trypsin-treated MDCK viral culturing was performed with reference to CLSI Viral Culture; Approved Guideline. Briefly, the shell vial monolayer of the culture was first rinsed with 0.5 mL minimum essential media with 0.8% trypsin (TMM) to remove traces of growth media. The clinical samples were aspirated into a 5-mL sterile syringe and filtered through a Minisart syringe filter of 0.45 μm pore size (Sartorius, Goettingen, DE) directly into the MDCK monolayer shell vials, followed by centrifugation of 760 relative centrifugal force for 30 minutes at 28 °C. The filtrates were decanted. The cell vial cultures were maintained with 1 mL of TMM and incubated at 33 °C in 5% CO2 for up to 7 days, until the characteristic influenza virus cytopathic effect was observed. For the 20 culture replicates, clinical sample influenza A/Singapore/H2011.704/2011(H3N2), which contained a high viral load, was diluted 50 with universal transport medium (Copan Diagnostics Inc., Corona, CA) prior to culture. Stevens et al., JVI (2010) PMID: 20519409
Sample modifications	Not relevant.
Assay protocol	Please see method section in the main text.
2. Glycan Library	
Glycan description for defined glycans	In-house sialoside array, consisting of 135 defined glycans (Supplementary Table 1). The synthesis of the contained glycans are described in Supplemental Experimental Procedures 5.Experimental Section. Peng et al., Glycobiology (2012) PMID: 22786570
Glycan description for undefined glycans	No glycans are undefined.
Glycan modifications	No modifications after initial synthesis were made.
3. Printing Surface; e.g., Microarray Slide	
Description of surface	NHS-ester functionalized hydro-polymer
Manufacturer	Schott SlideH (Applied Microarrays 1070936)
Custom preparation of surface	None

Non-covalent Immobilization	All glycans are terminated with primary amine linker (either natural amino acid or chemical linker) Xu et al. JVI (2012) PMID: 22072785
4. Arrayer (Printer)	
Description of Arrayer	MicroGrid II (Digilab)
Dispensing mechanism	Contact microarray pins (SMP3, ArrayIt)
Glycan deposition	Manufacturer estimation is 0.7nL per spot. However, actual delivery volume of each printed spot is not determined. Each glycan was "pre-spotted" 3 times on Poly-L-Lysine derivatized slides (made in-house) before being spotted on SlideH slides. Each array contains 6 replicate spots of each individual glycan.
Printing conditions	Glycans were diluted to 100uM in 150mM NaPO4 buffer, pH 8.4 + 0.005% Tween-20. 10uL of each glycan was transferred to a 384-well microtiter plate and printed at ambient temperature and relative humidity of 50-65%.
5. Glycan Microarray with "Map"	
Array layout	Each slide contains 3 replicate arrays, consisting of a 4x4 (16) subarray pattern with each subarray containing 12x18 features (not all features contain a printed sample). Array Layout file = "SialosideArrayv1.GAL"
Glycan identification and quality control	In-house sialoside array, consisting of 135 defined glycans (Supplementary Table 1). Quality control was assessed by incubation with plant lectins, AAL, ECA and SNA, to monitor fucosylations, de-sialylation and NeuAc-a(2-6) terminated glycans, respectively. Supplemental Experimental Procedures 2.Array Quality Control by Plant Lectins. Supplemental Figure S3
6. Detector and Data Processing	
Scanning hardware	Innoscan 1100AL (Innopsys)
Scanner settings	Scanning resolution: 10 μm / pixel Laser channel: 635 PMT Voltages: Adjusted for each sample to achieve maximum signal without saturation of any single spot. Scan power: Adjusted for each sample to achieve maximum signal without saturation of any single spot.
Image analysis software	Mapix (Innopsys)

Data processing	Output .txt files containing calculated data were processed in MS Excel to determine the mean signal value of 6 replicate spots with highest and lowest signals removed (e.g. average of 4 spots).
7. Glycan Microarray Data Presentation	
Data presentation	The microarray binding results are in **Figures 2, 3 and 4** and **Supplementary Figure S7-B**. Binding results are presented as 2D bar graphs with bars representing averaged mean signal of each glycan and error bars representing standard deviation.
8. Interpretation and Conclusion from Microarray Data	
Data interpretation	No software or algorithms were used to interpret processed data.
Conclusions	Recent H3N2 viruses prefer extended, branched glycan receptors and have evolved over recent history to be selective to those glycans versus "shorter" sialosides.

References

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