Supporting Information

Metabolic Oligosaccharide Engineering with Alkyne Sialic Acids Confers Neuraminidase Resistance and Inhibits Influenza Reproduction

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Supporting Materials and Methods

Reagents

Ac_5SiaNAz and Ac_5SiaNPoc were synthesized as described before.[9] The biotinylated lectins MALII, SNA-I, PNA and carbo-free blocking solution were obtained from Vector Laboratories, Inc. (Burlingame, CA). Streptavidin-phycoerythrin (PE) was purchased from BD Pharmingen (Franklin Lakes, NJ), NeutrAvidin conjugated with Texas Red and DAPI nucleic acid stain from Molecular Probes, Inc. (Eugene, OR) and Mowiol 4-88 mounting medium from Merck (Frankfurt, Germany). *Clostridium perfringens* neuraminidase, azide-PEG3-biotin, alkyne-PEG4-biotin, sodium L-ascorbate, L-histidine and copper(II) sulfate pentahydrate were purchased from Sigma-Aldrich (St. Louis, MO).

Cell culture

Human monocytic THP-1 cells (TIB-202, ATCC) and A549 (CCL-185, ATCC) cells were cultured in RRMI-1640 medium (Life Technologies, Burlington, Ontario, Canada) supplemented with 10 % FBS (Greiner Bio-one, Frickenhausen, Germany), 2 mM glutamine (Lonza, Walkersville, MD) and 1 x antibiotic-antimycotic solution (Life Technologies) in a humidified CO_2 incubator at 37 °C.

Confocal microscopy

To incorporate and detect poc and azide sialic acids on the cell surface, THP-1 cells were cultured for 3 days with 100 µM Ac_5SiaNAz or 100 µM Ac_5SiaNPoc. PBS treated cells were used as control. The cells were washed with 1 x PBS and fixed for 10 minutes with 2 % paraformaldehyde on ice. Next, the cells were extensively washed with 1 x PBS and incubated for 20 minutes at 37 °C in 1 x PBS containing 250 µM $CuSO_4$, 200 µM L-histidine, 500 µM sodium ascorbate and 50 µM alkyne-PEG4-biotin conjugate or azide-PEG3-biotin conjugate, respectively. After washing the cells in 1 x PBS containing 1 % bovine serum albumin (BSA), they were stained for 20 minutes at 4 °C with 5 µg/ml NeutrAvidin-Texas Red followed by staining of the nuclei with DAPI for 10 minutes at room temperature. Samples were washed, mounted with Mowiol and images were acquired using an Olympus FV1000 confocal laser scanning microscope (Olympus, Tokyo, Japan). Images were processed using ImageJ (NIH, Bethesda, MD).

Sialidase treatment, lectin staining and flow cytometry

THP-1 cells or A549 were incubated for 3 days with 1 x PBS, 100 µM Ac_5SiaNAz or 100 µM Ac_5SiaNPoc. To detect azide and poc groups at the cell surface, the cells were reacted for 20 minutes at 37 °C with click buffer (1 x PBS, 250 µM CuSO4, 200 µM L-histidine, 500 µM sodium ascorbate) containing 50 µM alkyne-PEG4-biotin conjugate or azide-PEG3-biotin conjugate, respectively, and stained with streptavidin-PE for 10 minutes at 4 °C. The cells were resuspended in 1 x PBS containing 1 % BSA and fluorescence was assessed using a CyAn ADP flow cytometer (BD Biosciences, San Jose, CA) followed by analysis with FlowJo software (Tree Star Inc., Ashland, OR). To remove surface sialic acids, cells incubated with 1 x PBS, 100 µM Ac_5SiaNAz or 100 µM Ac_5SiaNPoc were treated for 1 hour with 250 mU/ml neuraminidase from *Clostridium perfringens, Vibrio cholera or Arthrobacter ureafaciens* at 37 °C in unsupplemented RRMI-1640 medium. Next, cell surface azide and poc sialic acids were clicked to alkyne-biotin or azide-biotin, respectively, stained with streptavidin-PE as described above and analyzed by flow cytometry. For the lectin staining, THP-1 cells incubated for 3 days with 1 x PBS, 100 µM Ac_5SiaNAz or 100 µM Ac_5SiaNPoc treated with or without sialidase were stained for 1 hour with biotinylated MALII (5 µg/ml), SNA-I (1 µg/ml) or PNA (5 µg/ml) in carbo-free blocking solution containing 1 mM $CaCl^{2+}$ and 1 mM $MgCl^{2+}$ at 4 °C. These lectins recognize α2,3-linked sialic acids (MALII), α2,6-linked sialic acids (SNA-I) and terminal β-galactose (PNA). The cells were washed in 1 x PBS containing 1% BSA and cell-bound biotinylated

lectins were conjugated for 10 minutes at 4 °C with 2 µg/ml streptavidin-PE and analyzed by flow cytometry. Untreated cells stained only with streptavidin-PE were used as background control and the percentage lectin binding was calculated by normalizing the mean fluorescence intensity values from the bound lectins to the respective control.

Influenza infection and flow cytometry

A549 cells (1.10E5 per well) were seeded in a 24-wells plate cells and grown to confluence in 3 days with DMSO control, 100 µM Ac_5SiaNAz or 100 µM Ac_5SiaNPoc. A549 cells were washed twice with PBS and infected with influenza virus strain A/PR8-GFP/8/34 in 100 µL Optimem + 4 % BSA. After 1 hour, cells were washed with PBS and 500 µL Optimem + 4 % BSA was added. After 6 or 24 hours, A549 cells were washed 2 x with PBS and were released from the 24-wells plate by incubation with Trypsin-EDTA. Cells were fixed with 2 % PFA for 30 minutes and GFP expression was determined by flow cytometry using a FACS LSR II (BD Biosciences). Data were analyzed using FlowJo v10.1.

Statistical Analysis

Statistical significance between two groups was determined using an unpaired t-test and P-values < 0.05 were considered significant ($p<0.05$ *, $p<0.01$ **, $p<0.001$ ***).

Supporting Figures

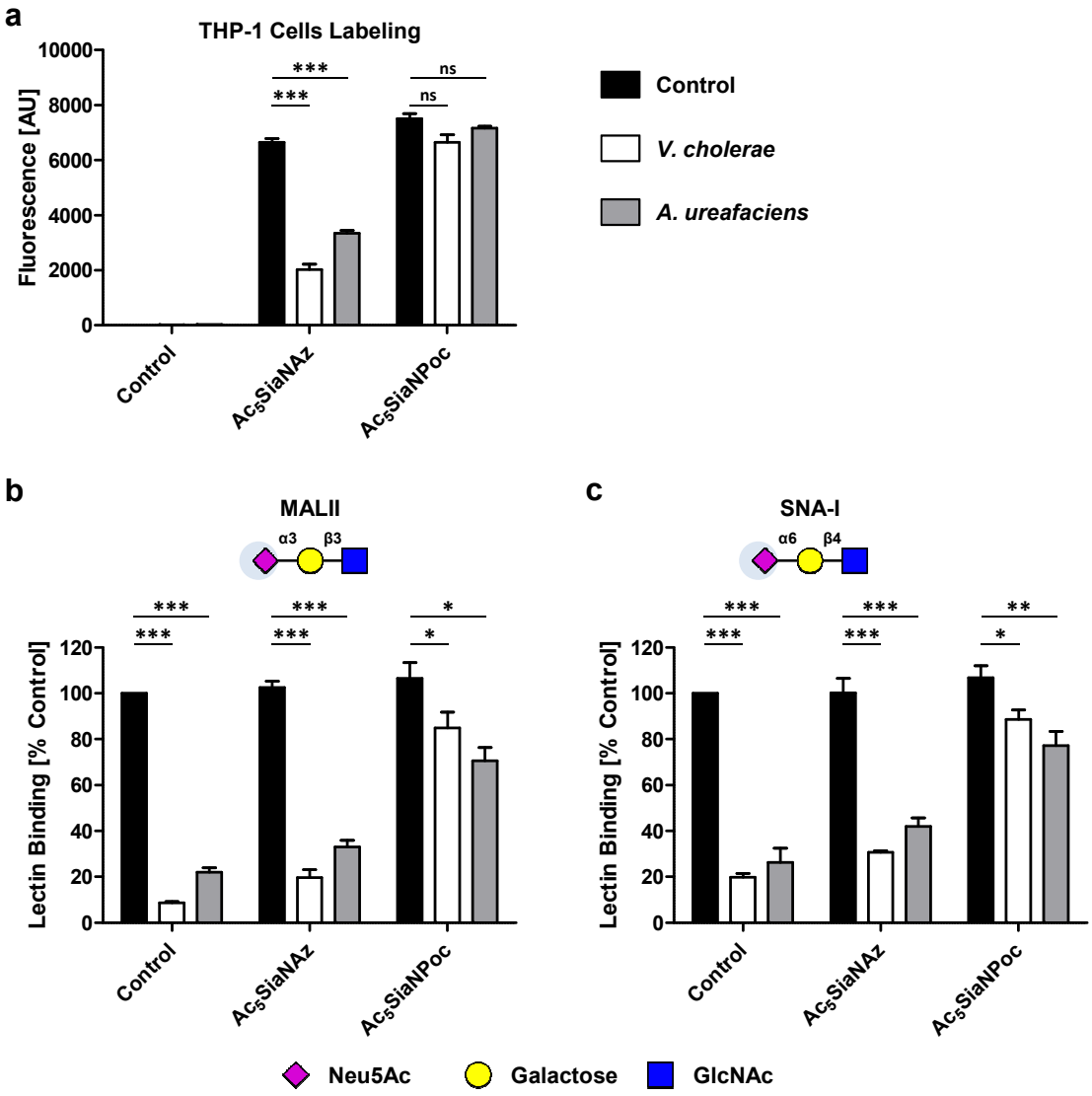

Figure S1 Removal of Az and Poc sialic acids from the cell surface by bacterial sialidases. THP-1 cells incubated for 3 days with PBS, 100 µM Ac_5SiaNAz or 100 µM Ac_5SiaNPoc were treated for 1 hour with 250mU/ml neuraminidase from *V. cholera* or *A. ureafaciens*. Az and Poc sialoglycans were reacted to fluorescent biotin using CuAAC (**a**) and α2,3-sialoglycans were detected with MALII lectin (**b**) and α2,6-sialoglycans with SNA-I lectin (**c**), respectively. Bar diagrams show mean fluorescence intensity or mean lectin binding normalized to control ± SEM.

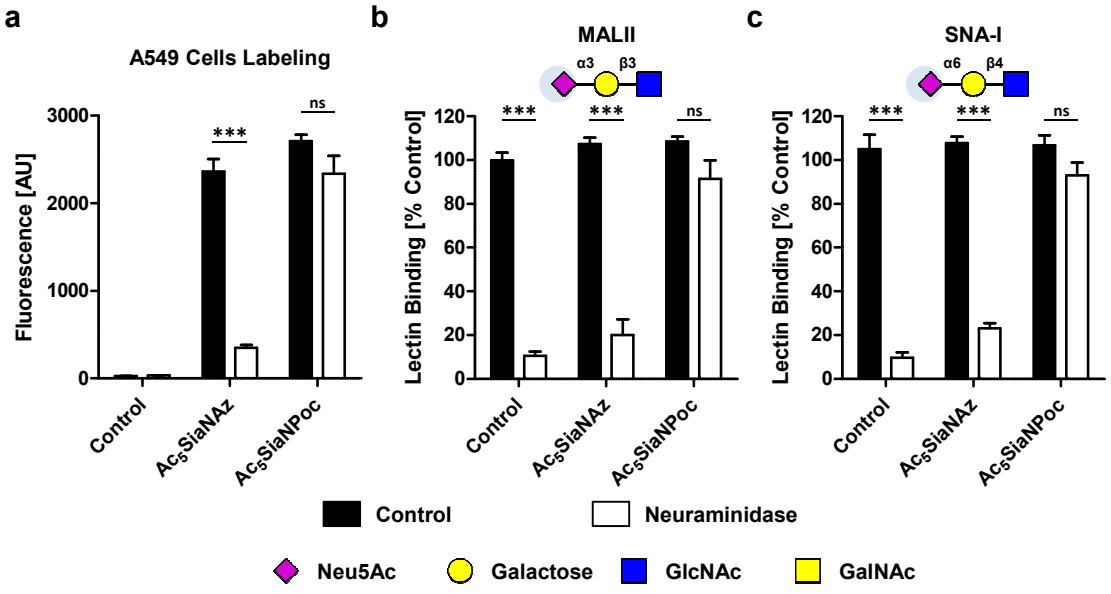

Figure S2 Poc sialic acids are sialidase resistant in A549 cells. A549 cells incubated for 3 days with PBS, 100 µM Ac_5Sia*N*Az or 100 µM Ac_5Sia*N*Poc were treated for 1 hour with 250mU/ml neuraminidase from *C. perfringens*. Az and Poc sialoglycans were reacted to fluorescent biotin using CuAAC (**a**), α2,3-sialoglycans were detected with MALII lectin (**b**) and α2,6-sialoglycans with SNA-I lectin (**c**). Bar diagrams show mean fluorescence intensity or mean lectin binding normalized to control ± SEM (n = 3).

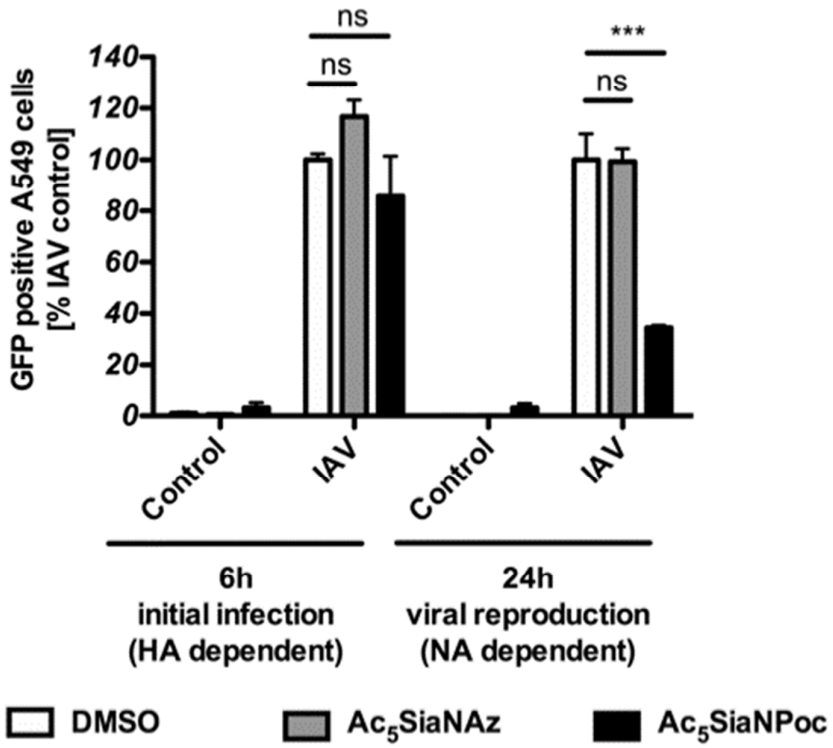

Figure S3 Effect of Az and Poc sialic acid on influenza reproduction (1000x dilution). A549 cells cultured with either Ac_5SiaNAz or Ac_5SiaNPoc were treated with influenza virus strain A/PR8-GFP/8/34 and the GFP expression was measured as readout for successful viral infection after 6 hours and reinfection after 24 hours.