Sialic acids are a large group of substituted derivatives of neuraminic acid, a monosaccharide with a nine-carbon backbone. Most influenza virologists use sialic acid to refer to 5-*N*-acetylneuraminic acid (Neu5Ac), which is the most common sialic acid together with 5-*N*-glycolyl (Neu5Gc). In biological relevant glycans, sialic acid is always linked to a LacNAc (Galactose linked to an N-acetylglucosamine). This LacNAc is in turn often modified by sulfates and fucose structures that are species and organ specific.

As there is hardly any data on the specificity towards these structures, nor on the display of these in tissues. A key objective of the proposed research is to elucidate influenza A virus specificity to N-linked glycans terminating in various modified sialic acid and LacNAc structures.

Specific Aim 1. A chemoenzymatic approach will be employed to synthesize a representative set of complex glycans found on upper airway epithelial cells from different influenza virus host species. These will then be employed for the printing of glycan arrays on which we will screen a large library of influenza A virus hemagglutinins.

Specific Aim 2. We will create tissue arrays of species that are hosts to a variety of influenza A viruses. It will allow us to probe different parts of the respiratory and gastrointestinal tract for the display of these highly varied N-glycans in different animal species.

Specific Aim 3. Use different enzymatic and non-enzymatic treatments on tissues to demonstrate the necessity of LacNAc modifications on binding biological relevant epithelial cell surfaces.

Results The results are described in two papers given below, in which we used a chemoenzymatic approach to synthesize a library of complex N-glycan presenting sialyl lewis X epitopes, screened for these epitopes in tissues of various species and used enzymatic treatment to remove these epitopes. We mainly focused on H5 and H7 subtypes because of their zoonotic abilities.

- Spruit CM, Palme DI, Li T, Ríos Carrasco M, Gabarroca García A, Sweet I, Kuryshko M, Maliepaard JC, Reiding KR, Scheibner D, Boons GJ, Abdelwhab EM, **de Vries RP**[#] *Journal of Virology (2024)* Complex N-glycans are important for interspecies transmission of H7 influenza A viruses

- Wu Y, Bosman GP, Chapla D, Huang C, Moremen KW, **de Vries RP**, Boons GJ[#] *Journal of the American Chemical Society (2024)* A biomimetic synthetic Strategy can provide keratan sulfate I and II oligosaccharides with diverse fucosylation and sulfation patterns.