# Glycosidase and Glycan

## Glycan profiles of bovine serum fetuin

GlycoStation® Technical Note **5**

GlycoTechnica

## Glycosidase

Glycosidase is a collective term for enzymes that catalyze the hydrolysis of glycosidic linkages. The cleavage sites of these enzymes are indicated by dotted lines in Figure 1.

In this technical note, we introduce the changes in glycan profiles for the glycoprotein generated by neuraminidase digestion. Neuraminidase, also called sialidase, cleaves terminal sialic-acid residues, and is relevant to biological processes; for example, influenza A viruses, which are known to cause flu pandemics, are divided into subtypes based on hemagglutinin (H) and “neuraminidase (N)”. These viruses utilize neuraminidase for cell-to-cell spread by cleaving sialic acids.

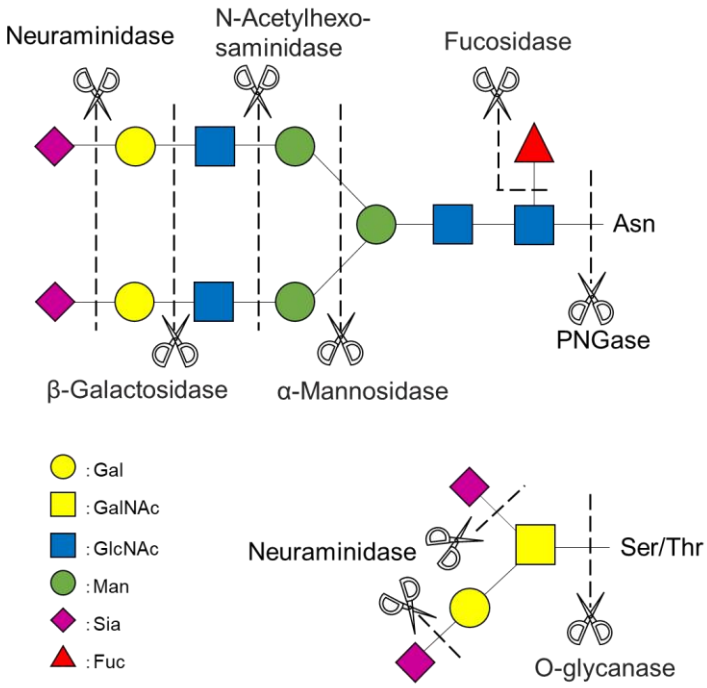
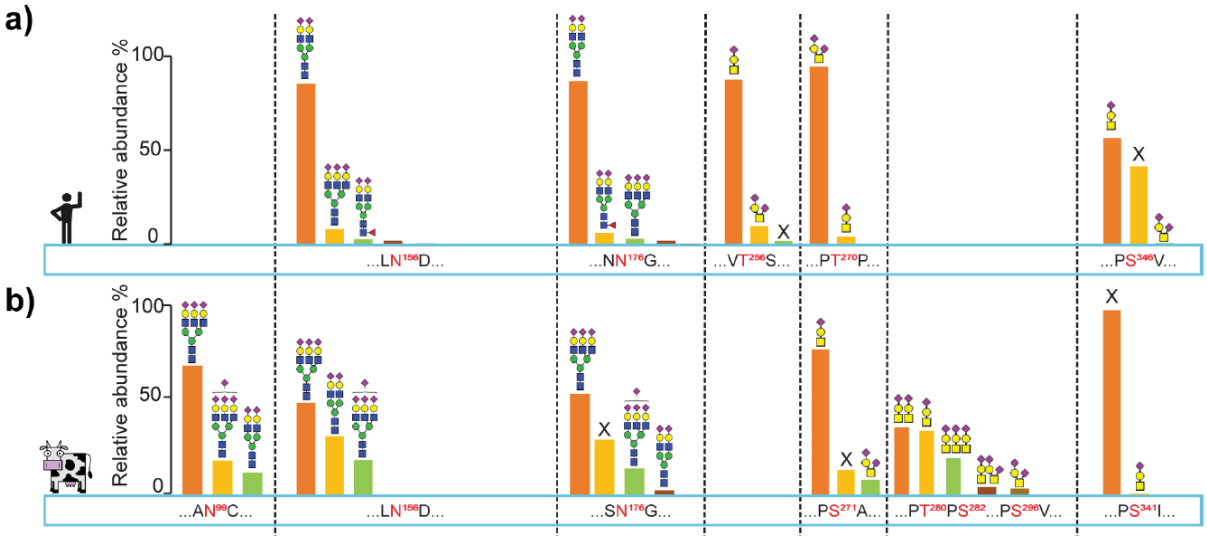



**Fig.1 The cleavage sites of glycosidases.**

## Fetuin

Here, we used bovine serum fetuin, which harbors three *N*-glycosylation and three *O*-glycosylation sites, as a glycoprotein sample. Bovine fetuin contains mainly sialylated tri-antennary complex *N*-glycans, whereas human fetuin predominantly contains bi-antennary glycan structures (Green et al. 1988; Lin et al. 2018). The dominant *O*-glycans in both fetuins are of the core 1 mucin-type, and harbor one or two sialic acids (Lin et al. 2018).




**Reference (Lin et al. 2018. J. Proteome Res., 17: 2861−2869, Fig.3a, b)**

## Analysis by Lectin microarray with 45 lectins

**Protocol:** Cy3-labeled samples with or without neuraminidase treatment were diluted with probing solution buffer, and then 100 µL of the samples (125 ng/mL) were applied to LecChip®. The LecChip® was directly scanned using the GlycoStation® Reader after overnight incubation at 20 ℃ (No need to wash LecChip®).

**Result:** We compared the lectin signals between neuraminidase (+) fetuin and neuraminidase (-) fetuin (Fig. 2). Removal of Sia residues using neuraminidase treatment resulted in decreased signal intensity of Sia-binding lectins, MAL and ACG (α2-3 Sia-binders), SNA, SSA, and TJA-1 (α2-6 Sia-binders), and WGA (a multivalent Sia-binder), and increased signal intensity of Gal-binding lectins (ECA, RCA120, DSA, BPL, and TJA-Ⅱ) and core1-binding lectins (ABA, Jacalin, and ACA). In contrast, GlcNAc oligomer binding lectins (LEL, STL, and UDA) showed little change.

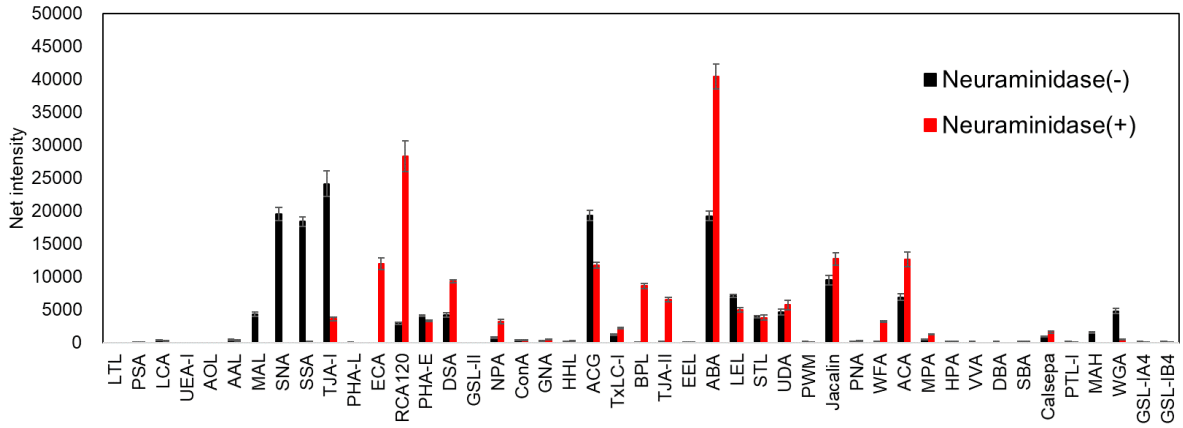



**Fig. 2 Glycan profiles of fetuin without (black bar) or with (red bar) neuraminidase treatment.**
Data are represented as mean signals of three spots ± standard deviations.

The signal shift from Sia-binders (MAL, ACG, SNA, SSA, and TJA-Ⅰ) to Gal-binders (ECA, RCA120, DSA, BPL, and TJA-Ⅱ) suggested that a change from highly sialylated complex N-glycans to galactosylated complex *N*-glycans (Fig. 3). No signal changes at LEL, STL, and UDA, suggested that these lectins interacted with the *N, N*'-diacetylchitobiose core of *N*-glycans. ABA, ACA, and Jacalin, which strongly bind to T-antigen, were increased in contrast to a decreasing signal of ACG that recognizes α2-3 sialylated T-antigen, suggesting a change in the O-glycan structure from the α2-3 sialylated T-antigen (*ST*) to T-antigen. Decreasing of MAH signal, a *DiST* binder, by neuraminidase treatment also suggests the existence of a low amount of *DiST* in intact fetuin and its change to T-antigen.

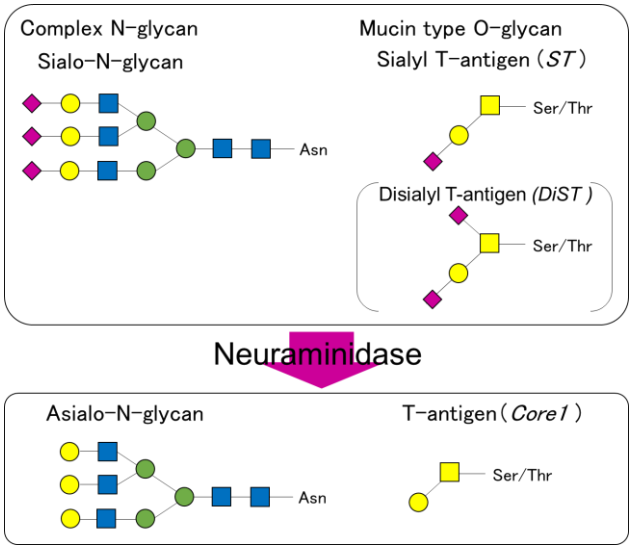



**Fig. 3 Glycan changes of fetuin by neuraminidase treatment**

Lectin microarray is a technology for rapid and sensitive profiling of glycan expression patterns, which can analyze both *N*-glycan and *O*-glycan at a time with a small amount of various glycoproteins. Alteration of glycan profiles with glycosidase treatment provides additional data about glycan structures, such as different degrees of sialylation or fucosylation among samples.

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