Abstract
Sialic acids, also called neuraminic acids, are negatively charged monosaccharides that are usually located on the non-reducing ends of glycans on secretory glycoproteins. Due to their unique positionings, sialic acids frequently act as receptors or ligands for cell-cell and cell-pathogen interactions.11 Accordingly, the functions expressed by mammalian cells and pathogens are known to bind cell surface sialic acids.4

Types of sialyltransferases
Sialic acids are introduced to glycans through specific sialyltransferases. The human genome has: six β-galactoside α-2,3-sialyltransferases (ST3Gal1-6), six β-galactoside α-2,6-sialyltransferases (ST6Gal1, 2), two β-galactoside α-2,6-sialyltransferases (ST6GalNAc1, 2), six N-acetyl-neuraminic α-2,6-sialyltransferases (ST6GalNAc6, 2).

Principle of the Method
The carrying of an azide group allows for the glycan to be functionalized with an azido-sugar and a clickable monosaccharide. The incorporation of clickable monosaccharides using ST3Gal1, 2, 6A4, 81, 84 exhibited self-labeling and are also indicated. Some of the glycans labeled by this method can be ascertained based on the specificities of the labeling enzymes. For example, enzyme 6A4 incorporated a hexasaccharide disialyl core-1 O-glycan (Siaα2-3Gal β1-3GalNAc) and a disialylated hexasaccharide on the protein include a disialylated core-1 O-glycan (Siaα2-3Gal β1-3GalNAc). For more information, see the references.

Results
Figure 1. Strategies for glycan detection using glycosiltransferases and clickable monosaccharides. A) Glycan detection. Glycans that can be terminated with sialic acids.5 The most common types are disialylated core-1 O-glycans that can be terminated with sialic acids.5 The most common types are disialylated core-1 O-glycans and a disialylated hexasaccharide (Siaα2-3Gal β1-3GalNAc) and a disialylated hexasaccharide (Siaα2-3Gal β1-3GalNAc). The glycans revealed by this method with great certainty are indicated. 1, 2, 4, 5, 6 refer to ST6GalNac1,2,4,5, and 61.

Figure 2. Probing fetuin samples with sialyltransferases. Upper panels, protein staining. Lower panels, strep-HRP detection. A) Probing untreated fetuin. B) Probing neuraminidase treated fetuin. Some of the glycans labeled by this method can be ascertained based on the specificities of the labeling enzymes. For example, enzyme 644 directly recognizes Siaα2-3Gal β1-3GalNAc. Enzyme 624, 63, 66, 64, 61 exhibits self-labeling and are also indicated.

Figure 3. Probing fetuin with Siaα2-3Gal β1-3GalNAc. A) Probing untreated fetuin and O-glycans with different GLcNAc transferases. B) Probing ST3Gal1, 2, 6A4, 81, 84. \( \text{N}-\text{glycans} \) are labeled with biotin. D-fetuin, decapsidated fetuin. 84ST6GalNAc2 reveals core-1 O-glycans (GalNAc-0/3, 5). 81ST6GalNAc1 reveals high mannose glycans (indicated with arrows).

Figure 4. Specifications of ST6GalNAc2 enzymes. A) Probing pre-treated fetuin samples with ST6GalNAc2 enzymes. B) Sialidase treated fetuin. Some of the glycans labeled by this method can be ascertained based on the specificities of the labeling enzymes. For example, enzyme 644 directly recognizes Siaα2-3Gal β1-3GalNAc. Enzyme 624, 63, 66, 64, 61 exhibits self-labeling and are also indicated.

Figure 5. Probing fetuin, D-fetuin and CS/DS with various sialyltransferases. Activity samples were also treated with PNGase F under native condition and run on SDS-PAGE with the untreated samples. The glycans revealed by this method with great certainty are indicated. CS/DS contains polysialic acid (PSA) attaching site that is recognized by ST8Sia4 (84).

Summary
Using sialic acid as a testing glycoprotein, we demonstrated the use of glycosyltransferases and clickable monosaccharides to probe glycan structure or epitope. We confirmed that the non-reducing end Gal residues on both N-glycans and O-glycans on the protein are fully sialylated, while the O-linked Gal/Gal residues are not, the protein contains abundant sialyl core-1 glycan. In addition, we are able to detect sialyl fucosyl epitope on the antigen, suggesting the high sensitivity and specificity of this method for glycan epitope detection.

Advantages of the Method
- **Great Specificity**: Labeling is achieved through enzymes that are known to be selective for substrate recognition.
- **Superior Sensitivity**: Labeling is through covalent bonding via click chemistry.
- **Direct**: No need for releasing glycans from conjugation.
- **Simple**: Only common laboratory equipment for Western blotting is needed.
- **Fast**: Results can be obtained in a day.
- **Applicable**: To glycan analysis on biotherapeutics.