BCHM 421/422 – 2020/2021

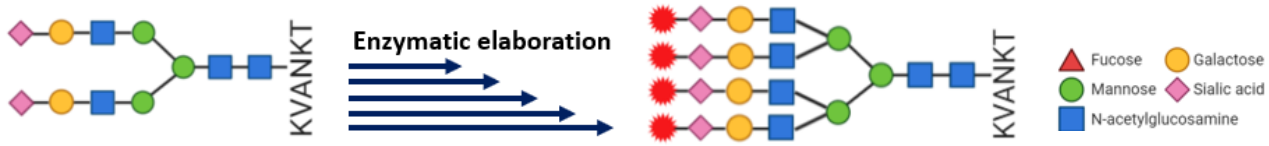
Project Title: Developing Antibody-drug Conjugate through Site-specific Glycoengineering

Supervisor: Chantelle Capicciotti

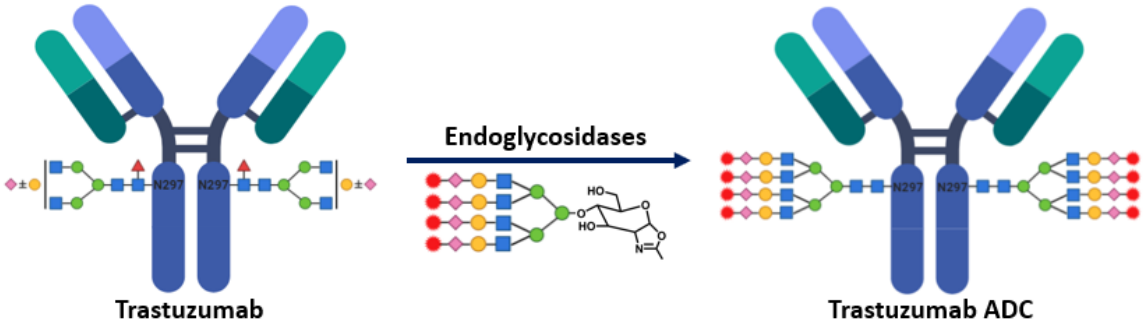
Project Outline: Antibody-drug conjugates (ADCs), are complex therapeutic molecules that have been developed as a target-specific drug delivery system for various diseases, especially cancers. They are composed of monoclonal antibodies that are directed toward specific antigens, and highly potent drugs conjugated to the antibodies through chemical linkers. Previously, drugs have been conjugated by chemical reactions through natural amino acids such as cysteine or lysine distributed throughout antibodies. These methods can disrupt the integrity of antibodies and result in heterogeneous products with compromised biological activities due to their different drug-to-antibody ratios (DARs) and drug load distributions. There have been attempts to produce homogeneous ADCs by using genetically introduced unnatural amino acids to conjugate drugs with specificity. Although these methods can result in homogeneous products, the process is more complicated, and the resulting ADCs have low DARs. Therefore, there is a need for a better and simpler strategy to produce homogeneous ADCs with higher DARs.

Glycosylation of proteins is a common post-translational modification. Immunoglobulin G (IgG) has a single N-glycosylation site on Asn297 residues in each heavy chain. We hypothesize that using N-glycans on IgG is a promising strategy to develop homogeneous ADCs because the pre-existing site-specific glycan can ensure homogeneous products with minimal disruption to antibodies. We aim to develop homogeneous ADCs with a high DAR (eight drugs per antibody), by installing defined modified tetra antennary glycans. This project will involve enzymatically preparing a tetra-antennary glycan structure with drug/imaging agents from a bi-antennary N-glycan precursor and remodeling antibody glycans by trans-glycosylation reactions catalyzed by endoglycosidases using a model IgG1, trastuzumab, which is a clinical grade monoclonal antibody for HER2 positive breast cancers.

1) Preparing a **tetra-antennary glycan structure with cargo** from bi-antennary precursor



2) Remodeling antibody glycans by **trans-glycosylation**, trastuzumab as a model antibody



Keywords:

1. **Glycoengineering**
2. **Antibody-drug conjugate**
3. **Antibody glycan remodeling**
4. **Trans-glycosylation**

Project Goals:

1. Enzymatically prepare defined complex N-glycan substrates with drug/imaging agents from a N-glycan precursor.
2. Express and characterize model IgG1 antibody, trastuzumab, in mammalian protein expression platform.
3. Examine substrate promiscuity of various endoglycosidases for different glycan substrates (e.g. bi-, tri-, tetra-antennary complex glycans)
4. Remodel antibody glycans with the prepared glycan substrates through a trans-glycosylation reaction catalyzed by endoglycosidases.

Experimental Approaches:

- Extraction of N-glycan precursor, sialyl glycopeptide from egg yolk powder
- Mammalian and bacterial antibody and enzyme expression & purification
- Chemoenzymatic synthesis of nucleotide sugars
- Enzymatic synthesis of complex N-glycan substrates
- Enzymatic reactions with glycosyltransferases, glycosidases, and endoglycosidases
- Biochemical assays, western blotting, immunoprecipitation, flow cytometry, microscopy, structure determination by NMR and mass spectroscopy.

References:

1. Liu, L., Prudden, A. R., Capicciotti, C. J., Bosman G. P., Yang, J., Chapla, D. G., Mremen K. W., and Boons, G. Streamlining the chemoenzymatic synthesis of complex N-glycans by a stop and go strategy. *Nature Chemistry*, **2019**, 11, 161-169
2. Tang, F., Wang, L-X, and Huang, W. Chemoenzymatic synthesis of glycoengineered IgG antibodies and glycosite-specific antibody-drug conjugates, *Nature Protocol*, **2017**, 12(8), 1702-1721