Small molecule inhibition of *Helicobacter pylori* glycosylation
Daniel Williams, 2019

Helicobacter pylori (*H. pylori*) is a bacterial pathogen in the human digestive tract that produces chronic stomach inflammation. It has been associated with both stomach ulcers and stomach cancer, which is the second most common cause of death from cancer in humans worldwide [1,2]. Currently, the recommended treatment of *H. pylori* infection is a combination of antibiotics called "triple therapy" [3]. However, the efficacy of this treatment method has been found to be suboptimal in eradicating the bacteria due to the development of antimicrobial resistance [4] and deleterious effects on the host microbiome [5]. Therefore, new innovative alternative treatment methods are needed.

Previous work has identified certain *H. pylori* cell surface sugar-modified proteins, termed glycoproteins, as therapeutic targets due in part to the fact that they differ markedly from those found in human cells and certain of these glycoproteins have been found to be unique to *H. pylori*. The Dube lab has utilized metabolic oligosaccharide engineering (MOE) [6], a technique that uses the bacterial cell's natural biosynthetic pathways, to incorporate unnatural sugars into *H. pylori* cellular glycoproteins, while not affecting normal human host cells [7]. By using MOE, the lab has successfully labelled *H. pylori's* cell surface with unnatural sugars bearing chemical handles called azides, which can be used as binding sites for various therapeutics.

In the lab, several therapeutics were developed to treat *H. pylori* through this MOE-based approach: a phosphine-based immune-modulating therapeutic; a cyclooctyne-based photosensitizing therapeutic; and an alkyne-based immune-modulating therapeutic [7,8,9]. However, these therapeutics each suffered from drawbacks such as slow kinetics for the phosphine probe, non-specific delivery of the cyclooctyne probe to azide-free *H. pylori*, and potential cytotoxicity of the copper catalyst used to enhance delivery time of the alkyne probe [8,9,10].

Because of these limitations, alternative highly selective therapeutic targets need to be explored. Bacterial glycoproteins contain rare, exclusively bacterial monosaccharides that are frequently linked to virulence and essentially absent from human cells [11,12]. These surface glycoproteins can interfere with the protective mechanisms of host cells, promoting bacterial adherence and protective biofilm formation, enhance bacterial motility, and enable colonization [13]. Therefore, targeting these highly specific monosaccharides could provide a novel and highly effective method to disrupt bacterial pathogenicity, while protecting host cells and the gut microbiome.

Previous work has successfully developed several rare bacterial azide-containing monosaccharide analogs of *N*-acetylglucosamine (GlcNAc), bacillosamine (Bac), and 2,4-diacetamido-2,4,6-trideoxygalactose (DATDG) as unique alternatives for incorporation into *H. pylori* glycoproteins [14]. When *H. pylori* was grown in the presence of these analogs, there was substantial incorporation into its glycoproteins [14]. Incorporation of these unnatural sugars sets the stage for development of small molecule glycosylation substrate analog inhibitors based on GlcNAc, Bac, and DATDG. These inhibitors should be processed by *H. pylori's* glycoprotein biosynthetic enzymes and ultimately lead to the biosynthesis of truncated glycoproteins with resultant loss of function. To test this hypothesis, two classes of substrate-based analog inhibitors have been synthesized: chain-terminators, inspired by classic and modern examples of chain-terminating substrates [15-18], and substrate decoys, inspired by Esko's work with metabolic substrate decoys [19-21] (Figure 1). The ability of these molecules to inhibit normal glycoprotein biosynthesis in *H. pylori* was what I sought to assess this summer.

Toward this end, I was able to identify the capability of several of the substrate decoys to elicit *H. pylori* glycoprotein biosynthesis defects. Their ability to inhibit glycosylation was confirmed via western blot analysis. Future work will look to identify if any of the proposed chain terminators can also elicit such defects. Once successful inhibitors have been identified, further experimentation to characterize the glycan truncation products will be conducted. Additionally, the effects of these inhibitors on *H. pylori* virulence factors and viability will be assayed. Ultimately, this work will serve to confirm the importance of *H. pylori's* glycoproteins to the organism's virulence and their suitability as a highly selective therapeutic target.

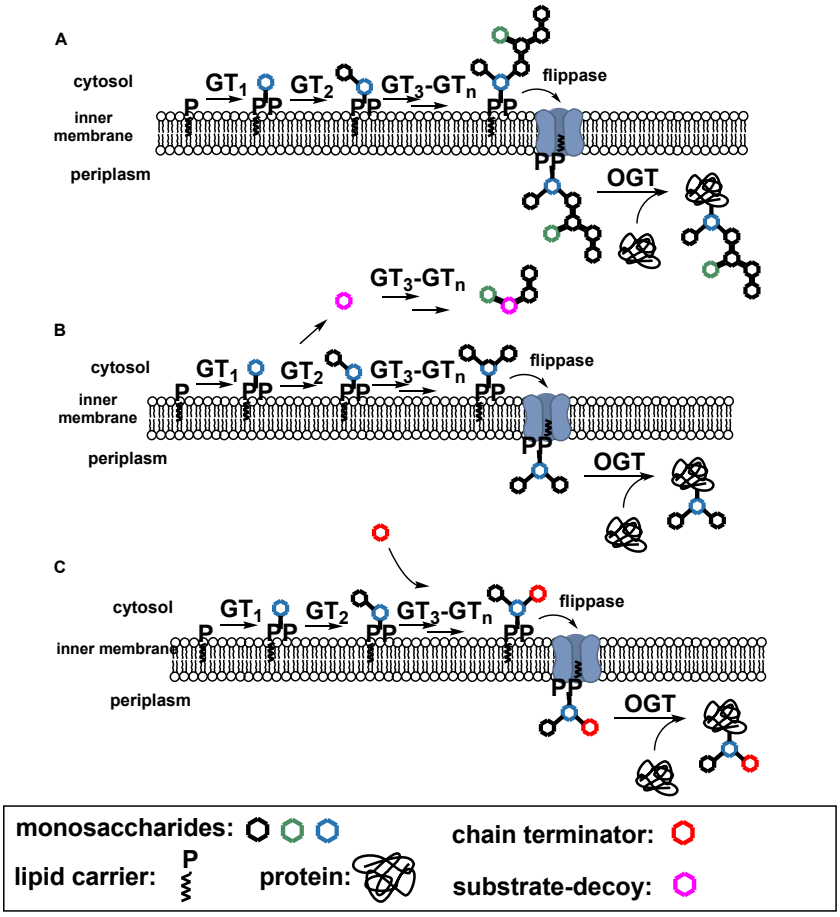

Figure 1. Effects of small molecule inhibitors on *Helicobacter pylori* glycosylation assembly. A) Normal lipid carrier-mediated glycosylation assembly pathway. Glycosyltransferases (GTs) sequentially transfer nucleotide-activated monosaccharides one sugar at a time onto a lipid carrier to construct lipid-linked oligosaccharides in the cytoplasm. The lipid-linked oligosaccharides are then flipped across the inner membrane into the periplasm, where an oligosaccharyltransferase (OGT) transfers the glycans onto protein substrates**. B)** Assembly inhibited by substrate decoy**.** Here the substrate decoy provides competition for the GTs as they are recognized as acceptor substrates. This will divert glycan synthesis onto these decoys leading to truncated glycoproteins. **C)** Assembly inhibited by a chain-terminator. Instead of transferring a sugar onto the lipid carrier, the GT transfers the chain terminator, which contains a functional group that lacks sites for additional extension, ultimately producing a truncated glycoprotein. Image adapted from Dube [22].

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