Comparison of covalent delivery methods and their effects for immune-mediated killing of *Helicobacter pylori*

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Helicobacter pylori is a gram-negative bacteria infecting almost 50% of the world's population.[1] It is a pathogenic agent that is responsible for causing duodenal ulcers, gastritis, and even gastric cancer.[2] Current treatment of *H. pylori* infection involves triple therapy, which includes a combination of two antibiotics, clarithromycin and either amoxicillin or metronidazole, and a proton pump inhibitor omeprazole.[2] However, due to the rise in antibiotic resistant *H. pylori* strains, this treatment is not effective in some individuals.[3] In addition, even if the triple therapy is effective against *H. pylori*, there is convincing evidence that one week of triple therapy causes a shift in microbiota composition in the human gastrointestinal system for up to four years without further antibiotic treatment.[4] The ineffectiveness of antibiotics as well as non-selective killing of pathogenic bacterial cells suggests that a new therapeutic methodology that would more effectively and selectively kill *H. pylori* needs to be developed.

H. pylori's glycans, essentially carbohydrates expressed on bacterial cells, can be used to selectively target these pathogenic bacteria without harming the microbiota in the human gastrointestinal system, as different, unique bacterial glycans are expressed across species.[5] The presence of glycans is linked to the pathogenesis of *H. pylori*, and additionally, their glycans contain distinctive structures that human cells do not have, serving as a unique molecular target for therapeutics.[6] The therapeutic approach that the Dube Lab employs involves metabolic oligosaccharide engineering (MOE). MOE is a chemical tool that allows incorporation of a bioorthogonal chemical functional group into surface glycans through endogenous metabolic pathways.[7] Through MOE, surface glycans of *H. pylori* are labeled with the unnatural, azide-containing sugar *N*-azidoacetylglucosamine ($Ac_4GlcNAz$), an analog of the common metabolic precursor, *N*-acetylglucosamine (GlcNAc). The azide is a bioinert functional group that is normally not found in biological systems, meaning that it is perfect for the MOE strategy in terms of selectivity and safety.[8] Once azides are on the surface of *H. pylori*, a bioorthogonal reaction partner with the immune-stimulant 2,4-dinitrophenyl (DNP)[9] can then be covalently delivered to damage cells selectively through an immune-mediated process (Figure 1).

In the Dube Lab, three different methodologies have been explored to target azide-covered *H. pylori*: Staudinger ligation with a phosphine-based reactive partner (Phos-DNP)[11], strain promoted azide-alkyne cycloaddition (SPAAC) with a dibenzoazacyclooctyne (DIBAC)-based reactive partner (DIBAC-DNP)[10], and copper catalyzed azide-alkyne cycloaddition (CuAAC) with an alkyne-based reactive partner (Alkyne-DNP)[12]. In essence, my project is to compare the three therapeutic approaches in terms of delivery and immune-mediated killing of *H. pylori*.

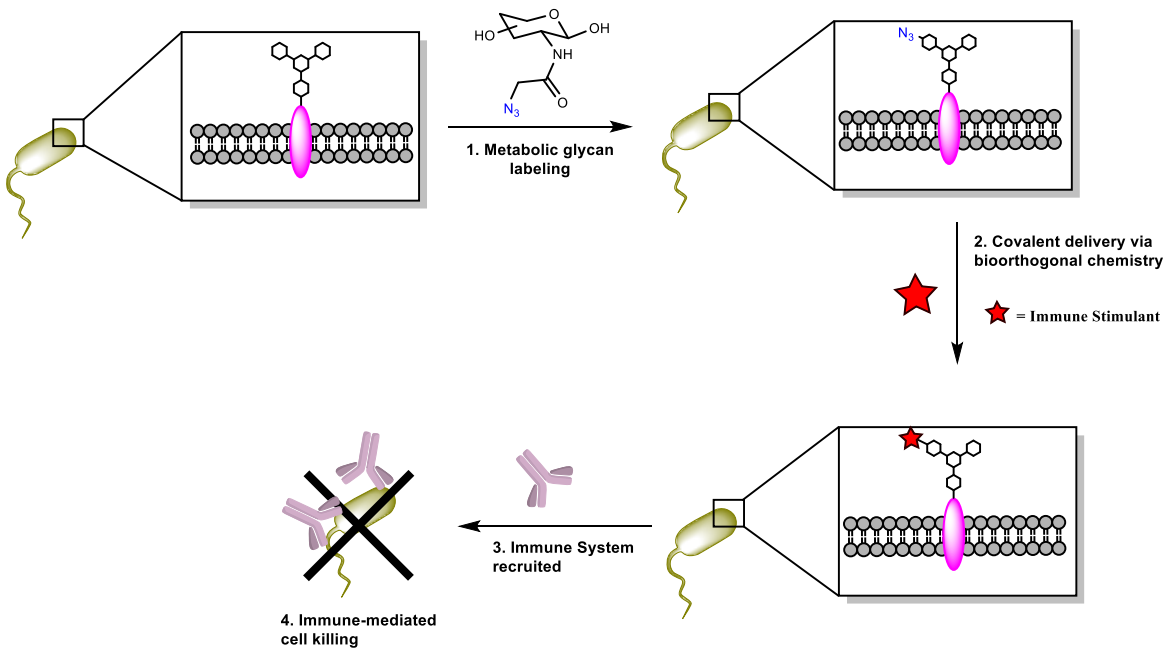

Figure 1. Use of MOE as a therapeutic strategy to azide-label and kill bacterial pathogens through an immune-mediated process. Glycans of *H. pylori* are labeled with azide-containing sugar. Azide-covered bacteria then react with a bioorthogonal reaction partner with the immune-stimulant 2,4-dinitrophenyl (DNP). The immune-system is finally triggered to kill the cells.[10]

The goal of this summer was to synthesize the three therapeutics, the phosphine-based, DIBAC-based and alkyne-based reactive partners, and purify these compounds. I was able to successfully synthesize these compounds, purify them via High-Performance Liquid Chromatography (HPLC), and begin to characterize them using Liquid Chromatography-Mass Spectrometry (LC-MS) and NMR (Nuclear Magnetic Resonance) data. Moving forward, I intend to conduct *in vitro* kinetic studies of the bioorthogonal chemistry, and begin immune-mediated killing experiments on *H. pylori*, comparing the killing effects of different therapeutics.

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