Photocrosslinking sugars to trap and identify host cell proteins that bind *H. pylori* glycans
Maren Cooper '27

Antibiotic resistance is an increasingly large public health threat. The misuse of current antibiotics has rendered many broad-spectrum antibiotics ineffective, and the resulting antibiotic crisis highlights the importance of developing new, narrow spectrum antibiotics[1]. One promising target for a bacterium-specific approach are bacterial glycans, as they are linked to pathogenesis, and they contain rare monosaccharides not found in human cells which allow them to be targeted without affecting the host cells[2]. These glycans are complex carbohydrate structures found on the surface of bacteria that, among other functions, are used to bind to human host cell surface receptors[3]. One specific bacterium of interest in the Dube lab is *Helicobacter pylori*, a gram-negative pathogen responsible for ulcers and gastric cancer.

Studying the binding interactions between *H. pylori* glycans and host cell proteins is an important step in targeting *H. pylori* infections, but identifying these binding interactions remains challenging. Often, protein-glycan interactions have low binding affinities, dissociate easily, and are therefore difficult to preserve[4]. Photocrosslinking offers a method to preserve protein-glycan interactions by trapping these interactions into stable, covalent complexes that can then be purified and identified. In this method, a reactive photocrosslinker is incorporated into glycans and, when it is irradiated with UV light, forms a highly reactive carbene that covalently bonds the protein-glycan complex[5].

In this research, I am using a diazirine (Daz) photocrosslinker incorporated into the monosaccharide GlcNAc. The resulting sugar probe, GlcNDaz, was generously provided by Dr. Jen Kohler at UT Southwestern[4]. In the first step of this research, this sugar probe is incorporated into *H. pylori* glycans. In the second step, the bacteria bearing the diazirine sugar are incubated with human adenocarcinoma (AGS) cells and irradiated, causing the diazirine on the bacteria to react and form a covalent bond with the human cell proteins to which the bacterial glycans are bound (Figure 1).

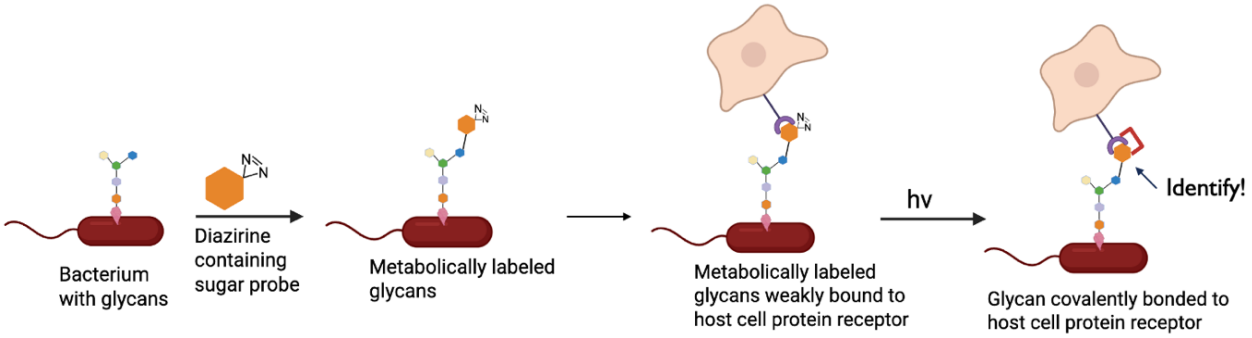

Figure 1. First, bacteria are metabolically labeled with a diazirine containing sugar probe. Next, these bacteria are incubated with AGS cells and irradiated, forming a covalent complex that allows the AGS cell receptors to be trapped and identified.

So far, my research has been focused on the first step—confirming metabolic incorporation of the diazirine sugar probe. I first tried an immunoprecipitation based approach, where I enriched specific glycoproteins that were expected to contain the diazirine sugar. This proved unsuccessful, so I cocultured the diazirine-treated bacteria with AGS cells and used western blotting to analyze proteins in the samples, with the expectation that if the sugar was incorporated, it would crosslink to the host cell receptors and a crosslinked protein would be seen on a blot. This approach failed to provide concrete evidence of incorporation due to the large number of proteins in the sample, making visualizing crosslinked proteins akin to finding a "needle in a haystack." Next, I will be turning to mass spectrometry as the most rigorous test for incorporation before I will move on to the big picture goal of identifying the host cell receptors to which *H. pylori* glycans bind.

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[1].Melander, R.J., D.V. Zurawski, and C. Melander, *Narrow-spectrum antibacterial agents.* MedChemComm, 2018. 9(1): p. 12-21. **[2]**. Champasa, K., et al., *Targeted Identification of Glycosylated Proteins in the Gastric Pathogen Helicobacter pylori (Hp).* Molecular & Cellular Proteomics, 2013. 12(9): p. 2568-2586. **[3]**.Barrett, K. and D.H. Dube, *Chemical tools to study bacterial glycans: a tale from discovery of glycoproteins to disruption of their function.* Isr J Chem, 2023. 63(1-2). **[4]**. Wu, H., et al., *A photo-cross-linking GlcNAc analog enables covalent capture of N-linked glycoprotein-binding partners on the cell surface.* Cell Chemical Biology, 2022. 29(1): p. 84-97.e8. **[5]**. Tanaka, Y. and J.J. Kohler, *Photoactivatable crosslinking sugars for capturing glycoprotein interactions.* J Am Chem Soc, 2008. 130(11): p. 3278-9.