SELECTED TALK:

Dr. Kristin Low

A "terminal" case of glycan catabolism: Structural and enzymatic characterization of the sialidases of *Clostridium perfringens*

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Sialic acids are commonly found on the terminal ends of biologically important carbohydrates, including intestinal mucin *O*-linked glycans, and can be catabolized by commensal bacteria such as *Escherichia coli* and *Ruminococcus gnavus* contributing to inflammation during disease. Significantly, pathogens such as *Clostridium perfringens*, the causative agent of necrotic enteritis (NE) in poultry and humans, have the ability to degrade host mucins and colonize the mucus layer. In this process, the removal of terminal carbohydrates from intestinal mucins is postulated to be a rate-limiting step in the total dismantling of the protective glycans. Evidence suggests the removal of sialic acid by the carbohydrate-active enzymes (CAZymes) of *C. perfringens* is critical for virulence and invasion of host tissues. Characterizing the process for the hydrolysis of sialic acids from mucin glycans is critical to understanding the metabolism of sialic acid by *C. perfringens*, and thus may inform future efforts to mitigate the disease.

Here we present the structural and biochemical characterization of the three sialidases found in most *C. perfringens* strains, and probe the substrate specificity of these enzymes. We have solved the X-ray crystallographic structures of the GH33 domains from sialidases *Cp*NanH and *Cp*NanJ, and report co-crystal structures of catalytically inactive mutants of *Cp*NanH$_{GH33}$ and *Cp*NanJ$_{GH33}$ as well as wild-type *Cp*NanI$_{GH33}$ in complex with commercially available sialyllated substrates and/or different forms of sialic acids. Additionally, we have enzymatically characterized all three enzymes in the presence of commercially available neuraminidase inhibitors 2,3-dehydro-2-deoxy-N-acetylneuraminic acid (DANA) and siastatin B, and present the structural basis for their inhibition potency. Using high performance anion exchange chromatography with pulsed amperometric detection (HPAEC-PAD), liquid chromatography-mass spectrometry (LC-MS), and capillary electrophoresis with fluorescence detection (CE-FLD), we have also characterized activity against sialyllated substrates and determined linkage specificity for each of the three GH33s, and have probed enzymatic activity against a mixture of *O*-glycans purified from chicken intestinal mucins. The knowledge gained in these studies can be applied to *in vivo* models for *C. perfringens* growth and metabolism of mucin *O*-glycans probing the inhibition of sialidases via small molecules and substrate mimetics, with a view towards future mitigation of bacterial colonization and infection of intestinal tissues.