Principal Investigator: Nichollas E Scott
Grant Title: Understanding the substrate targeting of the pglL oligosaccharyltransferase family

Abstract:

Within members of the *Neisseria* genus the oligosaccharyltransferase PglL is responsible for mediating O-linked glycosylation. Recently we observed that *Neisseria* PglL enzymes possessed discrete targeting ranges which may play previously unrecognized roles in modulating the *N. gonorrhoeae* proteome. Using a panel of chimeric PglL enzymes generated from domain swaps of $pglL_{cinerea}$ and $pglL_{meningitidis}$ expressed within *N. gonorrhoeae* we explore the glycoproteome and proteomic impacts of PglL enzymes with differing targeting ranges using glycoproteomic analysis with high-field asymmetric waveform ion mobility spectrometry (FAIMS) as well as Data-Independent Acquisition (DIA) to track proteome alterations. We demonstrate FAIMS based glycopeptide enrichment allows robust analysis of the *N. gonorrhoeae* glycoproteome and reveals differences in the glycoproteome of chimeric PglL enzymes leading to the identification of 44 unique glycoproteins. To understand how changes in glycosylation impacts the proteome we undertook DIA analysis revealing widespread changes in response to different PglL enzymes with > 30% of the proteome (481 proteins) impacted by the expression of different PglL chimeras. Surprisingly only a single glycoprotein appears impacted across this panel of PglL chimeras suggesting despite changes in glycosylation the abundance of known glycoproteins are unaffected. These findings suggest that beyond its roles in antigenic variation and pathogenesis O-linked glycosylation plays additional functions supporting a central role for glycosylation in fine tuning the *N. gonorrhoeae* proteome. Beyond the implications of these findings on understanding the function of glycosylation, this work supports that discrete targeting specificities may be commonplace even within evolutionarily closely related PglL enzymes. Combined this work expands our understanding of the *N. gonorrhoeae* glycoproteome and the distinct targeting activities of PglL enzymes.