Department of Molecular and Cellular Biology

Graduate Seminar MCB*7500

Friday, Feb. 2, 2018 in SSC 1511@ 12:45 p.m.

presented by:

Taylor Forrester

(Advisor: M. Kimber)

"A Structural & Functional Investigation into O-antigen Polysaccharide Regulatory Machinery: $WbbB^{Kdo}$ and WbbY"

O-antigen polysaccharides (OPS) are Gram-negative bacterial cell surface sugar polymers that differ greatly between strains. OPS variability is important for protection of the organism from phages and, in pathogens, avoiding host immune surveillance. OPS glycotype variability is generated by a diverse array of glycosyltransferases which vary greatly in their specificity, architecture and mechanism. My research will be an investigation into the structures and mechanisms of OPS glycosyltransferases $WbbB^{Kdo}$ and WbbY. $WbbB^{Kdo}$ from *R. terrigena* (ATCC 33257) is a β-Kdo transferase, which adds a single Kdo residue to OPS from CMP-Kdo, signalling the readiness of this OPS for export. We hypothesize $WbbB^{Kdo}$ operates through a novel mechanism which utilizes a covalent enzyme-substrate intermediate, two sequential SN2 reactions and two spatially distinct half-sites. We plan to elucidate the details of this mechanism by determining structures of $WbbB^{Kdo}$ in complex with CMP-Kdo, as a Kdo-adduct, with synthetic acceptor and with product. Mass spectrometry and kinetic analyses will supplement the structural data. WbbY from *K. pneumonia* O1 caps OPS repeat D-Galactan I with a polymer, D-Galactan II. Bioinformatic analyses suggest WbbY possesses dual glycosyltransferase modules, a coiled-coil at the C-terminus and amphipathic helixes. We hypothesize that the amphipathic helices bind the membrane and the coiled-coil region extends the glycosyltransferase domains into the cytosol, regulating the maximal length of the D-Galactan I domains. I will explore the enzymatic mechanism and function of the coiled-coil domain using crystallography and manipulations of coiled-coil length. This research may have applications in both glycoengineering and engineering novel therapeutics.

■ *MCB Brown Bag Seminar*