**EMSL Research and Capability Development Proposals**

**Systematic characterization of protein glycosylation of bacteria cell surface proteins**

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Protein glycosylation of cell surface proteins exists in both bacteria and archaea. *N*-glycosylated and *O*-glycosylated proteins have been identified in both lineages, though the latter are less frequently observed in archaea. *N*-glycoproteins have been found in *Methanothermus fervidus*, an organism that grows optimally at 83°C, and it has been speculated that its extracellular saccharides may contribute to the stability of its cell envelope proteins. To date, there has been no systematic examination of the roles that extracellular glycoproteins play in the thermostabilization of cell envelope proteins. The overall goal of this proposal is to develop an integrated top-down and bottom-up approach for bacterial glycoprotein profiling assisted by accurate glycan structure identification, which will be applied to study bacterial glycosylations.
This platform will combine multiple, cutting-edge “omics” techniques (proteomics, glycomics, and bioinformatics) uniquely provided by EMSL. Using the proposed platform, we will test the hypothesis that the structure of the glycan moieties of thermophilic bacteria glycoproteins are different from those of bacteria that thrive at low temperatures.

Products and Output

This work will integrate various capabilities available at EMSL (mass spectroscopy, nuclear magnetic resonance, and computational capabilities) to develop a novel method for comprehensive, high-throughput, systems-level analysis of protein glycosylation. Successful development of this capability will notably enhance proteomics capacities at EMSL and increase the EMSL’s prestige as a unique user facility.