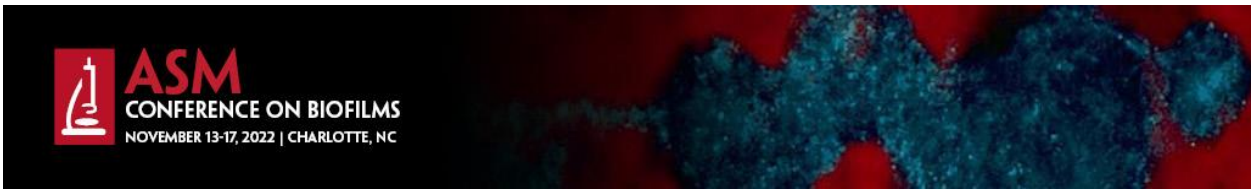

Tools for the Detection of Polysaccharide Precursors and Surface Polymers

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Complex capsular and secreted polysaccharides make up the major component of bacterial biofilms. My group has focused extensively on developing new methods for the reconstruction of glycan biosynthesis pathways *in vitro* making use of fluorescent polyisoprenoids as easily detectable anchors for key phosphoglycosyl and glycosyl transferases. More recently we have begun to reconstruct foreign glycans in *E. coli*, using this organism as an amenable polysaccharide assembly factory that could be used to monitor the impact of glycans of different structures on biofilm formation. This talk will describe the methods used to identify proteins required for the production of the immunomodulatory *Bacteroides fragilis* Capsular Polysaccharide A (CPSA). LC-MS analysis of isoprenoid-linked glycan intermediates is used to help reconstruct the production of the foreign polysaccharide in *E. coli.* We have found that the *B. fragilis* CPSA biosynthesis gene locus alone is not enough for CPSA production and added additional sugar-modifying enzyme encoding genes from *Campylobacter jejuni* and *Vibrio vulnificus* to complete the development of an effective CPSA assembly system. In addition, several additional changes were made in the gene locus to promote ribosome binding of each gene transcript and enhance the production of the glycan. The impact of CPSA production on *E. coli* growth has been surprising, and our work is currently focused on redesigning *E. coli* for effective glycan production. I will describe some of our efforts in this area including the removal of other glycan assembly systems and a directed evolution approach to improving the growth of *E. coli* without disrupting its ability to produce a foreign glycan.