**BCHM 421/422 – 2018/2019**

**Project #2 Outline:** Microbes have developed a diverse repertoire of enzyme systems to efficiently degrade biomass-based polysaccharides. One particularly elaborate and highly efficient cellulose-degrading assembly is the cellulosome, which is a multi-enzyme complex produced by several anaerobic cellulose-degrading bacteria. Within these complexes the various enzymes are assembled onto a central scaffold subunit via a high-affinity protein-protein interaction involving dockerin modules of the enzyme subunits and cohesin modules of the scaffold subunit. While there is no obvious binding specificity between this modules within a single bacterial species, there is a very high degree of binding specificity among species. This unique recognition has led to the engineering of designer cellulosomes in which chimeric scaffolds comprises cohesin modules from various cellulolytic bacteria produced to allow the directed additional of complementary cellulolytic enzymes containing the partnering dockerin modules.

We are interested in utilizing the designer cellulosome system to assemble unique multi-enzyme complexes and assess their synergistic modification of marine and terrestrial polysaccharides.

**Supervisor:** Steven Smith

**Project Title:** Engineering designer enzyme complexes for directed production of biomass-based high-value chemicals

**Keywords (3-5):**

1. Protein engineering
2. Carbohydrate modification
3. Multi-enzyme complexes
4. Protein biochemistry

**Project Goals:**

1. Clone, expression, and purify carbohydrate-active enzyme (CAZyme)-dockerin fusion protein constructs; expression and purify chimeric scaffold constructs comprising complementary cohesin modules.
2. Assembly the multi-enzyme complexes and assess their purity and stability.
3. Assess the enzymatic properties of these engineering complexes and compare those of the free enzyme mixtures.

**Experimental Approaches:** Towards pursuing our research goals, you will design and produce CAZyme-dockerin fusion proteins and cohesin scaffold chimeric proteins in *E. coli* and purify them using standard chromatographic methods. You assemble the multi-enzyme complexes and assess their activities on targeted polysaccharide substrates.

**References:**

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3. Carvalho, A.L., et al (2003) Cellulosome assembly revealed by the crystal structure of the cohesin-dockerin complex. *Proc Natl Acad Sci USA* 100: 13809-13814.
4. Stern, J., et al. (2017) Carbohydrate depolymerization by intricate cellulosomal systems. *Methods Mol Biol* 1588: 93-116.
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