Doctoral Thesis Defense

Engineering Probiotic Microbes for *In Vivo* Applications

Isaak Müller

May 4th, 2020 at 9:00 am (EST)

Zoom ID: 913-7372-8515
Password: Sboulardii

Our overall goal is to engineer probiotic microbes as localized diagnostic and therapeutic tool for diseases in the gastrointestinal tract, like inflammatory bowel disease. Current treatments often rely on systemic supplementation of immunomodulatory drugs, potentially leading to severe side effects. The probiotic yeast *Saccharomyces boulardii* has shown promising results for the use as probiotic supplement for the amelioration of disease-related symptoms in the GI tract. However, its genetic engineering has been limited to date. This work focuses on the development of fundamental engineering tools for *S. boulardii*, including recombinant protein secretion, a new vector integration system and inducible promoters. Another major obstacle to move synthetic biology technologies from the bench to a patient's bedside is the need for gene circuits to function in a complex environment where unexpected crosstalk can occur. We show that synthetic gene networks can be engineered to compensate for crosstalk by integrating pathway signals, rather than by pathway insulation. We demonstrate this principle using reactive oxygen species (ROS)-responsive gene circuits in *Escherichia coli* that exhibit concentration-dependent crosstalk with the non-cognate ROS. By designing gene circuits that introduce compensatory crosstalk at the gene network level, the resulting gene network exhibits reduced crosstalk in the sensing of the two different ROS. The development of both fundamental genetic parts in a probiotic chassis as well as more complex genetic networks will contribute to the future implementation of living cell therapeutics in the clinic.

Thesis advisor
Timothy K. Lu, Associated Professor, Research Laboratory of Electronics and Biological Engineering, MIT

Thesis Committee
Kristala L. Jones Prather, Arthur D. Little Professor of Chemical Engineering, MIT

James J. Collins, Termeer Professor of Medical Engineering & Science Professor, MIT

Ahmad Khalil, Dorf-Ebner Distinguished Associated Professor, BU