Research Project Proposal for Master's Degree March 31, 2017

Workplace: Single Molecule Microbiology laboratory, ITQB NOVA, Oeiras, Portugal

Supervisor: Dr. Zach Hensel / zach.hensel@itqb.unl.pt

Topic: Engineering low-noise gene expression systems for single-molecule experiments

Background

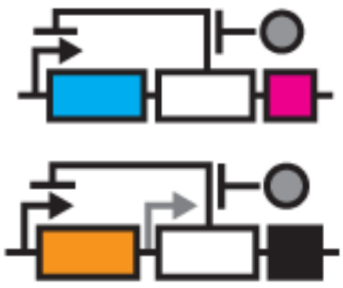
In *E. coli*, experiments have shown that even at high expression levels there is large cell-to-cell variation in protein expression levels; this is especially true at low expression levels. Sources of noise include the intrinsic randomness of molecular diffusion and biochemical reactions, gene copy number variation, and cell cycle periodicity. Many single-molecule experiments call for low cell-to-cell variability at low gene expression levels, but the most commonly used vectors for recombinant gene expression are poorly suited for this task.

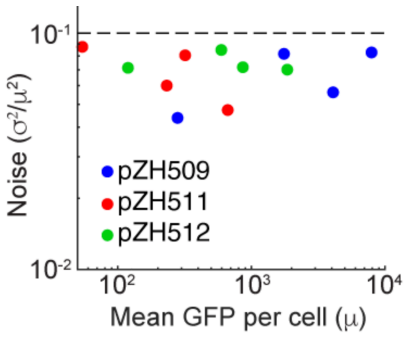
Our lab recently developed and characterized an *E. coli* gene expression system that uses the principles of negative autoregulation and bicistronic gene expression to reduce gene expression noise below that seen for endogenous genes. We are looking for students interested in (**1**) using this principle to produce orthogonal expression systems (e.g. compatible plasmid origins of replication and antibiotic selection; different inducible transcription repressors) and (**2**) developing improved expression systems with a wider range of expression levels.

Objectives

- Develop methods for characterizing gene expression noise by flow cytometry and single-molecule fluorescence microscopy
- Engineer and characterize orthogonal expression system based on principles of negative autoregulation and bicistronic expression
- Engineer and characterize a hybrid expression system with a larger range of expression levels
- Apply the improved system to a problem of interest in the lab requiring simultaneous, low-noise, inducible expression of two proteins

An individualized work plan will be developed based upon two or more of these objectives based upon the interests, skills, and career goals of the student.





Left: Expression of transgene (blue) controlled by bicistronic, autoregulated expression of repressor (white). Bottom: hybrid expression to expand range of expression levels

Right: Achieving expression noise below the *E. coli* noise limit (~10^{-1})