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

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

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Topic: Improved techniques for single-molecule mRNA detection and tracking in living *E. coli* cells

Background

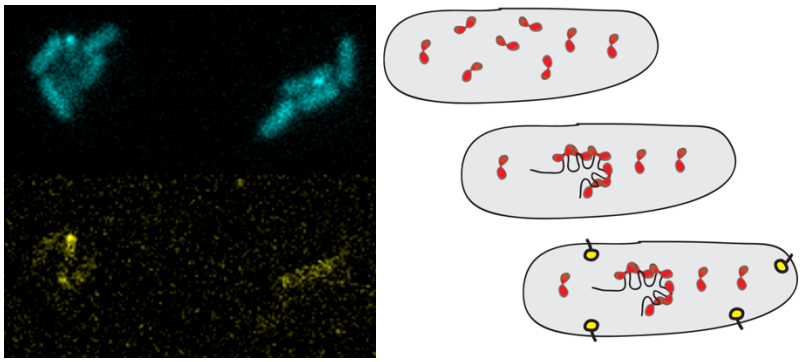
It would be possible to address many questions in microbiology if it were simple to observe single mRNA molecules in living cells. For example, in *E. coli* experiments have shown that even at high expression levels there is large cell-to-cell variation in protein expression levels; being able to observe mRNA and protein expression events at the same time in living cells would distinguish cell-to-cell variation at the transcription and translation levels. Improved single-molecule mRNA visualization will also help answer questions related to mRNA degradation and localization.

The most successful tools for single-molecule mRNA imaging in living cells today are based on fusion proteins containing an mRNA-binding sequence and a fluorescent protein. These proteins bind mRNA hairpin repeats, producing fluorescent spots corresponding to single mRNA molecules. However, this method has rarely been successful in prokaryotic organisms because of problems arising from the relatively high concentration of mRNA-binding proteins in small cells (high fluorescent background and protein/mRNA aggregation); the short lifetime of prokaryotic mRNAs is another difficulty. Preliminary results in our laboratory show that these problems can be overcome, and we are improving this type of method to observe single mRNA and protein molecules simultaneously in live *E. coli* cells.

Objectives

- Reproduce preliminary data; determine quality of current system based on bacteriophage PP7 by comparison to single-molecule mRNA FISH
- Compare PP7 system to mRNA detection based on components from bacteriophages MS2 and λ
- Explore regulatory DNA sequences and growth conditions to maximize the number of proteins produced per mRNA to simplify the observation of protein "bursts" from single mRNAs
- Apply improved methods to acquire mRNA expression time series and/or track mRNA diffusion

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: Single mRNA molecules (cyan spots) and protein produced from those mRNAs (yellow).

Right: Detecting mRNA and protein using red mRNA-binding proteins and yellow membrane-localized proteins.