

Stochastic Switching in a Cell-Free Positive-Feedback Circuit

Background

Positive-feedback gene circuits can generate nonlinear responses, bistability, and switch-like behaviour. In previous experiments in our lab, an AraC positive-feedback circuit in *E. coli* cell-free transcription–translation (TXTL) showed stochastic switching: nominally identical reactions could remain OFF or transition to a strongly expressing ON state.

A recent project rebuilt the circuit as separate transcriptional units after problems were identified in the original plasmids. Most required constructs are now cloned and sequence verified, providing a cleaner starting point for systematic characterization.

Project goal

The main goal is to **reproduce and characterize the stochastic switching observed previously using the rebuilt modular circuit.**

Main tasks

The project will begin by validating the individual circuit components in TXTL, including reporter expression, AraC expression, the pLac/ara and pBAD regulatory modules, and their responses to arabinose and IPTG.

The validated components will then be combined to reconstruct the AraC positive-feedback circuit and test whether heterogeneous OFF/ON behaviour can be reproduced.

Key parameters—including AraC input, DNA concentrations, arabinose, IPTG, and TXTL reaction conditions—will be varied systematically to identify the switching regime. Automated liquid handling may be used for parameter screening.

If stochastic switching is recovered, the system will be characterized by measuring switching probability, switching time, expression level, replicate-to-replicate variability, and dependence on experimental conditions.

For a Master's thesis, the project can include more extensive parameter screening and comparison of the experimental results with a simple deterministic or stochastic model of the circuit.

Expected outcome

The project will determine whether the previously observed switching phenotype can be reproduced with the rebuilt circuit and identify the conditions controlling transitions between OFF, intermediate, and ON states.

Even if the original phenotype is not fully recovered, systematic characterization will define the operating regime of the circuit and provide a basis for future modelling and circuit design.

Methods

The student will gain experience with *E. coli* cell-free transcription–translation, synthetic gene circuits, plasmid preparation, PCR and DNA purification, fluorescence plate-reader measurements, automated liquid handling, experimental design, parameter screening, and time-series data analysis. Quantitative modelling can be included depending on the thesis scope.