Are multiple feedbacks in biological systems redundant or overkill?

Multiple feedback systems are observed in genetic circuits (such as the trp regulation in E. coli) and signalling networks (such as in MAPK pathway and insulin signalling pathway). To characterize the relevance of multiple feedback loops we make use of synthetic genetic network (strinsic) comprising of multiple negative feedback loops. Experimental and modelling studies on the synthetic network demonstrate reduction in noise in expression and further that the noise propagates to the phenotypic level.

Design of the synthetic genetic constructs

4 different plasmids were generated developing a modified lac operon using three promoters, trc, plac, pLB and pIPT. The synthetic network has the capability of self replicating through (pLB) or plac/CP to alter the plasmid copy number and also express the LacI - CFP fusion protein and YFP. YFP characterizes the plasmid copy number. Strain 1 represents the open loop circuit with no level of control, while strain 3 represents a multiple input output (MIMO) circuit with regulation on both lac expression and plasmid replication. Strain 2 and strain 3 represent single input single output (SISO) circuits with regulation on lac expression and plasmid replication respectively.

Regulation of Protein Expression

Our aim was to demonstrate the increased level of regulation and the decreased variance for protein expression on the MIMO strain as compared to the SISO and the open loop strains. This protein expression was studied when the cells were grown on varying amounts of IPTG (Isopropyl- β-D-thiogalactoside) which acts as an inducer for the system.

Experimentation

YFP expression was measured using a Fluorescence-Activated Cell Sorting system (FACS) for various values of IPTG in the system, for all the four strains. The values of Cpf however could not be measured due to technical problems.

Key Results

- YFP expression characterized by FACS indicates MIMO has minimum variance in protein expression as compared to other 3 constructs, indicating lower noise.
- Comparative expression of β-galactosidase to the lac concentration, by MIMO demonstrates, multiple feedback loops yield optimum behavior.
- Variance in growth rate was lowest for MIMO indicating that the multiple feedback loop yields robust protein expression which transfers to stable growth rates, validated by Growth curve and Agar Plate Experiments.
- Significant reduction in inherent system noise was observed for MIMO in competition to the Open Loop, as per the characterisation by Stochastic modelling.
- Deterministic model was successful in capturing the experimental results for specific growth rates on LacI.
- Control analysis was successful in demonstrating that the cell could respond rapidly to internal and external changes and is robust to uncertainties.

Future work

- Experimental analysis of CFP: Due to unavailability of cyan laser for FACS we were unable to measure CFP expressions. By December 2009, we aim to generate CFP expression profiles for the four systems.
- More experimentation for different values of lactose and IPTG, to get more data for β-gal expression and growth rates for all 4 strains.
- 3 detailed Model: Accurately finding the kinetic constants from literature and fitting them to accurately correlate with the experimental results.
- Control analysis: We have done the analysis for only one kind of feedback system. We could use different feedback systems characterized by different H11 coefficients to try to do further analysis on the same lines as the analysis done here.

References


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