Predicting and Controlling Translation Rate in Bacteria

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Short Abstract — Microbial engineering often requires fine control over protein expression; for example, to connect genetic circuits or control flux through a metabolic pathway. We have developed a predictive design method that generates a synthetic ribosome binding site sequence to achieve a user-selected translation rate on a proportional scale. The design method combines a predictive thermodynamic model of bacterial translation initiation with a Monte Carlo optimization algorithm. We experimentally test over 100 predictions in *Escherichia coli*.

***Keywords* — synthetic biology, ribosome binding sites, thermodynamics, optimization**

I. Introduction

Translation is a fundamental step in gene expression, where proteins are produced according to their mRNA sequence. In bacteria, the translation rate is controlled by the ribosome binding site – a relatively short mRNA sequence upstream of a start codon. Both intra-molecular interactions within the mRNA transcript and inter-molecular ones with the ribosome govern the translation rate.

II. A Statistical Thermodynamic Model

We have developed a thermodynamic model of bacterial translation initiation. Given an arbitrary mRNA sequence, the model will predict the translation rate from each of its start codons. The model contains five Gibbs free energy terms that quantify the strengths of the participating molecular interactions. We then use statistical thermodynamics to relate the sum of these free energies ΔG_{total} to the relative translation rate.

III. The RBS Calculator

By combining the thermodynamic model with a Monte Carlo optimization algorithm, we have developed a predictive design method for ribosome binding site sequences, named the RBS Calculator [1]. Given a protein coding sequence, the method optimizes the sequence of a synthetic ribosome binding site to yield a user-selected translation initiation rate, which proportionally affects the overall protein production rate (Figure 1). We also find that ribosome binding sites can be highly context dependent – small changes to the protein coding sequence can result in large changes to its translation rate. The method correctly accounts for these context effects.

IV. Summary

The RBS Calculator is a new predictive design method for ribosome binding sites. It provides the capability to rationally control the protein production rate in bacteria over a range of at least four orders of magnitude. It is currently available at http://voigtlab.ucsf.edu/software.

References

[1] H. Salis, E.A. Mirsky, C.A. Voigt, "Automated Design of Synthetic Ribosome Binding Sites to Precisely Control Protein Expression", (submitted)

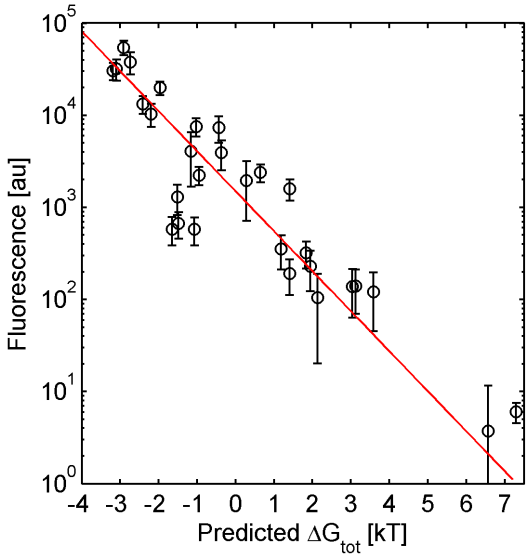

Figure 1: A comparison between the model predictions and experimental results. Using the RBS Calculator, 29 dissimilar synthetic ribosome binding site sequences are generated with increasing target translation rates. These sequences are inserted into a fluorescent protein measurement system and their relative translation rates are measured. The thermodynamic model is capable of predicting the translation rate to within a factor of about two and with an R^2 of 0.84.

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