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# Single Pass Design of Genetic Circuits Using Absolute Binding Free Energy Measurements and Dimensionless Analysis

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**Abstract :** Engineered genetic circuits reprogram cellular behavior to act as living computers with applications in detecting cancer, creating self-controlling artificial tissues, and dynamically regulating metabolic pathways. Phenemenological models are often used to simulate and design genetic circuit behavior towards a desired behavior. While such models assume that each circuit component's function is modular and independent, even small changes in a circuit (e.g. a new promoter, a change in transcription factor expression level, or even a new media) can have significant effects on the circuit's function. Here, we use statistical thermodynamics to account for the several factors that control transcriptional regulation in bacteria, and experimentally demonstrate the model's accuracy across 825 measurements in several genetic contexts and hosts. We then employ our first principles model to design, experimentally construct, and characterize a family of signal amplifying genetic circuits (genetic OpAmps) that expand the dynamic range of cell sensors. To develop these models, we needed a new approach to measuring the in vivo binding free energies of transcription factors (TFs), a key ingredient of statistical thermodynamic models of gene regulation. We developed a new high-throughput assay to measure RNA polymerase and TF binding free energies, requiring the construction and characterization of only a few constructs and data analysis (Figure 1A). We experimentally verified the assay on 6 TetR-homolog repressors and a CRISPR/dCas9 guide RNA. We found that our binding free energy measurements quantitatively explains why changing TF expression levels alters circuit function. Altogether, by combining these measurements with our biophysical model of translation (the RBS Calculator) as well as other measurements (Figure 1B), our model can account for changes in TF binding sites, TF expression levels, circuit copy number, host genome size, and host growth rate (Figure 1C). Model predictions correctly accounted for how these 8 factors control a promoter's transcription rate (Figure 1D). Using the model, we developed a design framework for engineering multi-promoter genetic circuits that greatly reduces the number of degrees of freedom (8 factors per promoter) to a single dimensionless unit. We propose the Ptashne (Pt) number to encapsulate the 8 co-dependent factors that control transcriptional regulation into a single number. Therefore, a single number controls a promoter's output rather than these 8 co-dependent factors, and designing a genetic circuit with N promoters requires specification of only N Pt numbers. We demonstrate how to design genetic circuits in Pt number space by constructing and characterizing 15 2-repressor OpAmp circuits that act as signal amplifiers when within an optimal Pt region. We experimentally show that OpAmp circuits using different TFs and TF expression levels will only amplify the dynamic range of input signals when their corresponding Pt numbers are within the optimal region. Thus, the use of the Pt number greatly simplifies the genetic circuit design, particularly important as circuits employ more TFs to perform increasingly complex functions.

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