# Precision measurements of regulatory energetics in living cells

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**Transcription in all organisms is regulated by weak protein-DNA and protein-protein interactions, but measuring these interactions in living cells remains exceedingly difficult. Here we show how reporter assays can be used to measure the Gibbs free energy of critical regulatory interactions in vivo and with high precision (~0.1 kcal/mol). We further demonstrate how this approach can be used to distinguish which kinetic steps in the transcription initiation pathway are regulated. The prospects for a massively parallel implementation of this approach are promising, and we will discuss ongoing work in this direction.**

## I. Background

DESPITE an imposing arsenal of experimental techniques for studying transcriptional regulation genome-wide, knowledge of what regulatory proteins actually do when bound to individual regulatory sequences remains highly limited. Indeed, an understanding of how elemental protein-DNA and protein-protein interactions mechanistically control transcription has been established for only a small handful of intensively studied bacterial promoters. New experimental methods are needed if we are to understand the mechanistic basis for transcriptional regulation more broadly.

The weak nature of regulatory interactions presents a major experimental difficulty. Transcription is controlled by multi-protein-DNA complexes that continually form and break apart due to thermal fluctuations. The dynamic nature of these complexes, and thus their physiological function, is strictly governed by the quantitative strength of the interactions that hold them together. For example, a transcription factor (TF) can up-regulate transcription as much as 5-fold through a stabilizing interaction with RNA polymerase (RNAP) of as little as 1 kcal/mol (1.6 $k_BT$ at 37 C, ~1/4 of a hydrogen bond). Even a crude mechanistic understanding of such interactions therefore requires knowing their energies to a precision of substantially less than 1 kcal/mol. Standard biochemical methods, however, cannot measure such interactions in living cells to this precision.

## II. Results

Here we show how reporter assays can be used as a general method for measuring protein-DNA and protein-protein interactions in living cells. Each Gibbs free energy is measured by quantifying expression from a variety of synthetic regulatory sequences, the activities of which form a one-dimensional "expression manifold" embedded in a two-dimensional space. Quantitative energy values are then obtained by mathematically modeling this manifold. This modeling task is far simpler and more transparent than other model-based approaches to transcriptional biophysics (e.g., [1-4]). Indeed, accurate free energy values can often be discerned by eye from raw data.

We demonstrate this approach by measuring TF-DNA, RNAP-DNA, and TF-RNAP interactions in *Escherichia coli*. In doing so, we demonstrate the ability to robustly measure free energies to high precision (~0.1 kcal/mol). We find that the well-studied transcription factor CRP can activate transcription far more strongly than has long been thought [5,6]. This expression manifold strategy can further distinguish which kinetic steps in the transcript initiation pathway CRP activates when bound to DNA at varying positions upstream of RNAP.

## III. Outlook

Because this approach requires only mRNA expression measurements, it has the potential to be applied in high throughput using massively parallel reporter assays in a wide variety of systems, including human cells [7]. Progress toward this goal will be discussed, as will outstanding challenges. The potential utility of studying expression manifolds in higher dimensions will also be discussed.

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