Design Rules for Bacterial Enhancers

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The most commonly occurring gene regulatory elements in biology, ubiquitous in all organisms, are called enhancers. Enhancers occupy vast genomic regions, which can span from tens (in bacteria) to thousands (in metazoans) of base-pairs. Despite their central role in biological regulation, our current understanding of the their structure-function relationship is highly limited. Here, we present a new synthetic-biology based strategy to decipher the enhancer regulatory code. Our goal is to develop a "Rosetta-Stone type algorithm" or design rules based on an analysis of synthetic enhancers, systematically constructed from the ground up via a combined theoretical and experimental approach. We show the potential of this approach by demonstrating our ability to construct synthetic bacterial enhancers, which allow us to qualitatively predict the output for naturally occurring bacterial enhancers.

I. Purpose

One of the premises of synthetic biology is to develop novel biological functioning modules from existing biological "parts". One biological class of components that has not been utilized by the synthetic biology community are **enhancers**, which correspond to the most commonly occurring gene regulatory element found in biology. An enhancer typically comprises of multiple binding sites for transcription factors, and functions as a type of molecular integrator that determines when, where, and how much a particular gene gets expressed. Due to the large variability in their genomic size, enhancers are notoriously difficult to dissect, thus making them a part, which is under-characterized for synthetic biological purposes.

In order to make enhancers useful for synthetic biology applications, we would like to develop a biological Rosetta Stone for the decipherment of the regulatory code encoded within natural enhancers. The premise of our approach is based on two pillars: first, enhancers are modular objects that can be divided into three or more irreducible modules, and second, it is possible to construct functional synthetic enhancers from the ground up by mixing and matching modules from naturally occurring cis regulatory elements. As a result, we can systematically dissect several synthetic enhancers that differ by some control parameter, which in turn allows us to map a large space of possible regulatory behavior. The plethora of data obtainable with this method is used as input to thermodynamic models, which then allows us to formulate predictive algorithms for the regulatory output of natural enhancers with similar sequence and binding-site architectures to the synthetic ones.

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II. Results

Recently[1-3] we demonstrated the feasibility of this approach using synthetic bacterial enhancers. Bacterial enhancers have three modules: a driver module which is absolutely essential for transcription initiation, an expression modulation region which either up or down regulates expression, and a poised promoter which integrates all the inputs. In the bacterial examples the driver module loops and contacts the poised promoter to initiate expression, while the expression modulation region either promotes or inhibits the formation of the loop by altering the ability of DNA to bend.

Using our synthetic enhancers, we showed that it is possible to generate new classes of non-monotonic regulatory response transfer functions based on the binding site content of the expression modulation region. The functions obtained with our synthetic enhancers exhibited analog to digital conversion, band pass filtering capabilities, sharp sigmoidal behavior and were for the most part dependent on **four control parameters or design rules that can be read-off directly from the sequence**. This, in turn, allowed us to formulate a set of qualitative thermodynamics models or a Rosetta Stone algorithm that can predict the regulatory output of unrelated naturally occurring bacterial enhancers.

III. Conclusion

In conclusion, we developed an experimental and theoretical methodology that allows us to engineer enhancers with designed specialized transfer functions based on simple design rules that can in turn be utilized in gene circuits to execute higher-level computational operations than is currently possible with conventional regulatory elements. In addition, our analysis has shed light on important regulatory effects that emerge from cooperative and anti-cooperative interactions between TFs bound to adjacent binding sites that may play a crucial role in various naturally occurring regulatory phenomenon.

References

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