A composable cell-free gene regulatory architecture using nucleic acid transcription factors and semi-synthetic RNA polymerase

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Living cells use information encoded in biochemical circuits to make complex decisions and perform sophisticated tasks. Inspired by their rich functionality, synthetic gene circuits are currently being developed to model biology and engineer organisms for various applications. Recently, there have also been increasing interests in developing in vitro gene circuits that operate using reconstituted molecular components.[1] Compared to cellular devices, these cell-free devices have the advantages of being more portable and accessible. These features are being explored for a number of applications ranging from point-of-care diagnostics, reconfigurable materials, artificial cells, to education. However, as with cellular devices, a principle challenge in scaling up the complexity of gene circuits is the lack of a sufficiently large gene regulatory toolset for "wiring up" genetic elements without introducing cross-talk. In living cells, the specificity of circuit wiring is achieved via the interactions between distinct protein regulatory assemblies called transcription factors (TFs) with *cis*-regulatory elements distributed throughout the genome. The molecular properties of TFs enable sophisticated self-assembly-mediated regulatory behaviors, including specific promoter recognition, combinatorial binding, and signal integration via multicomponent assembly. Engineering these properties has been a rate-limiting step in gene circuit fabrication.[2]

Here we use concepts from DNA nanotechnology to synthetically recapitulate features of TF-mediated gene regulation in cell-free gene networks actuated by the relatively primitive T7 phage RNA polymerase (RNAP). Our architecture controls gene expression via programmable nucleic acid hybridization interactions between an oligonucleotide-tethered T7 RNAP with genetic templates displaying single-stranded DNA (ssDNA) regulatory domains, and auxiliary nucleic acid assemblies serving as "artificial TFs" (Fig. 1A). By relying on nucleic acid hybridization, we demonstrate the ability to computationally design de novo regulatory assemblies that emulate features of protein-based TFs (Fig. 1B) while offering unique advantages such as programmability, chemical stability, and scalability. We show how synthetic, nucleic-acid based TFs can be used to implement transcriptional logic, cascade, feedback, and multiplexing (Fig. 1C). Finally, we also demonstrate the integration of this regulatory mechanism with engineered DNA nanostructures to spatially localize gene expression. The composability of this gene regulatory architecture lends itself to design abstraction, standardization, and scaling. We therefore imagine this new regulatory architecture to enable rapid prototyping of increasingly complex in vitro genetic devices for various applications.

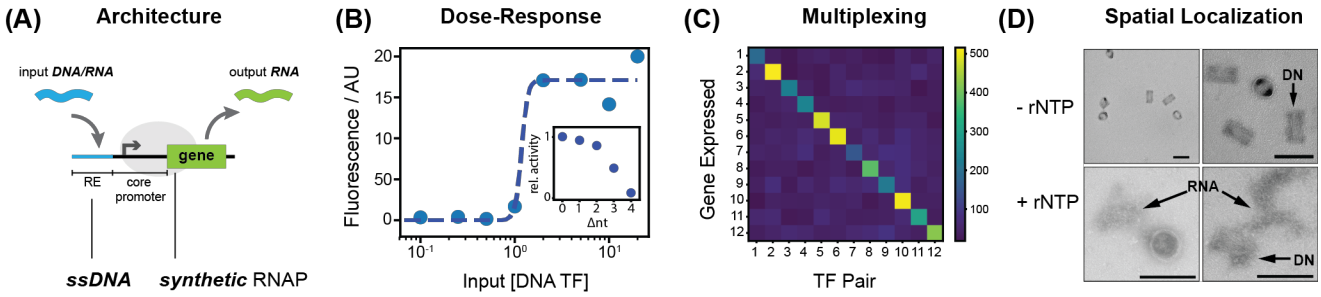

Figure 1: (A) Schematic of gene regulatory architecture. RE = regulatory element (B) Dose-response of an inducible gene system as a function of nucleic acid TF input. Inset shows how the response changes with deletion mutations on the nucleic acid TF. (C) Example of transcriptional multiplexing, showing independent control over the expression of twelve genes in a pooled format. (D) Transmission electron micrographs showing rolling-circle transcription from within the lumen of a 3D DNA cylinder. DN = DNA nanostructure.

References

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