High-Throughput Strategies for the Scalable Generation of RNA Component Functions

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ABSTRACT

Engineered biological systems hold great promise in providing solutions to many global challenges, including environmental remediation, sustainability, scalable manufacturing, and health and medicine. Synthetic biology is an emerging research field with a primary goal of making the engineering of biology more streamlined and reliable. Recent advances in synthetic RNA biology have led to design of RNA-based gene-regulatory devices from assembly of functional RNA components that encode more basic functions, including sensing, information transmission, and actuation functions. These synthetic RNA control devices allow access and control information on cellular state, thereby advancing our ability to interact with and program biology.

A modular ribozyme device platform was recently described to link an aptamer (sensor) to a hammerhead ribozyme (actuator) through a distinct sequence (information transmitter) capable of a strand-displacement event. The utilization of ribozyme as the actuator in the platform, whose mechanism of action is independent of cell-specific machinery, allows transport of the resultant devices to *in vitro* or different cellular environments. The broad implementation of these devices requires enabling technologies to support efficient generation of new functional RNA components and quantitative tailoring of device regulatory performance for specific cellular applications. Current component generation and device tailoring strategies are limited in their throughputs and efficiencies, and thus have hampered our ability to generate new ribozyme devices for cellular engineering applications.

To support scalable generation and tailoring of ribozyme devices, we have described high-throughput *in vitro* selection and *in vivo* screening strategies based on the

modular ribozyme device platform. We proposed a high-throughput solution-based *in vitro* selection strategy to generate new sensing functions within the device platform. A high-throughput and quantitative two-color FACS-based screening strategy was developed to complement the *in vitro* selection strategy by allowing efficient tailoring of device regulatory activities in the cellular environments. We further developed quantitative assays based on the surface plasmon resonance (SPR) technology to allow rapid measurements of the device and component activities. Together, these enabling strategies will offer a scalable and integrated process for the construction and programming of RNA control devices for broad cellular engineering applications, thus laying an important foundation for engineering more complex biological systems.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES