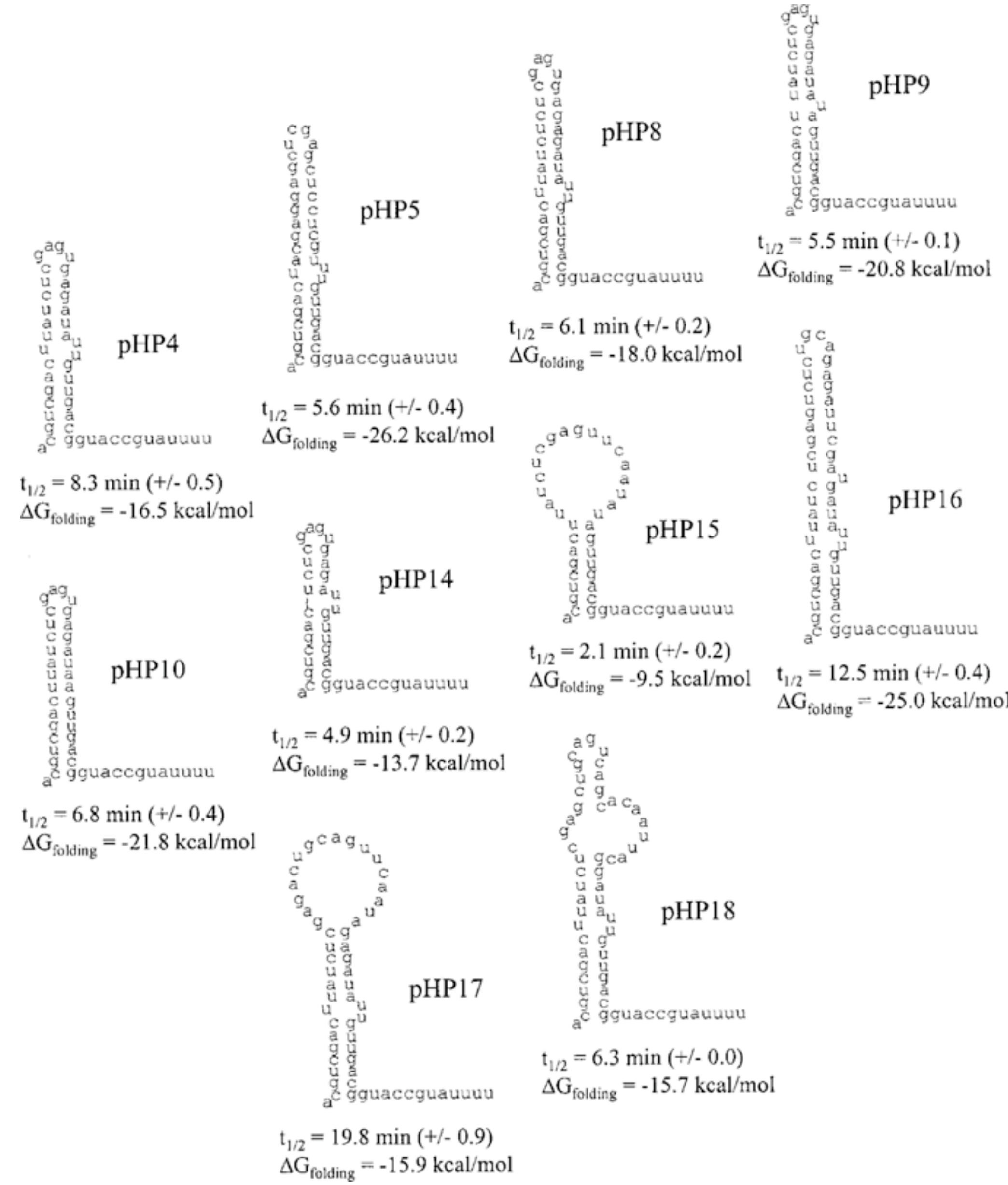
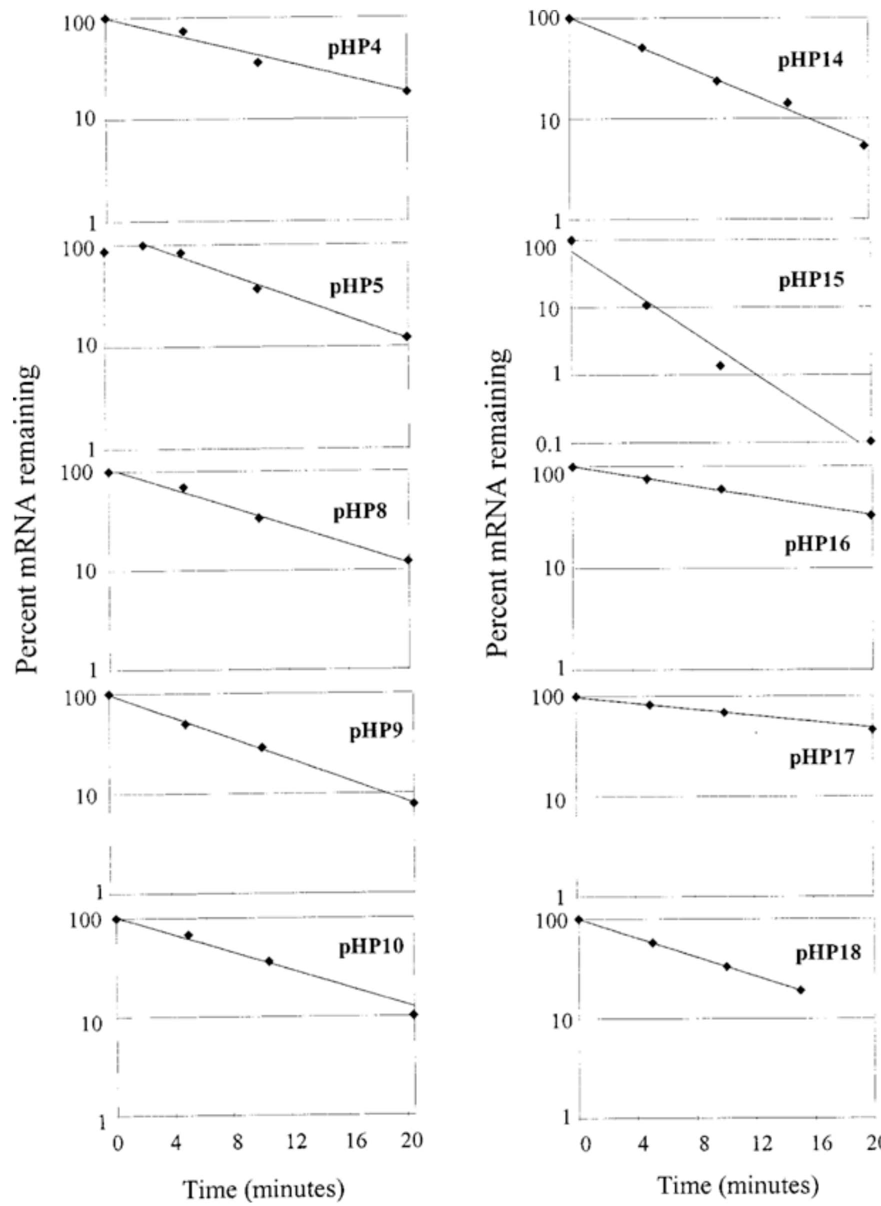


Library of synthetic 5' secondary structures to manipulate mRNA stability in Escherichia coli.
Carrier TA, Keasling JD. Biotechnol Prog. 1999 Jan-Feb;15(1):58-64.



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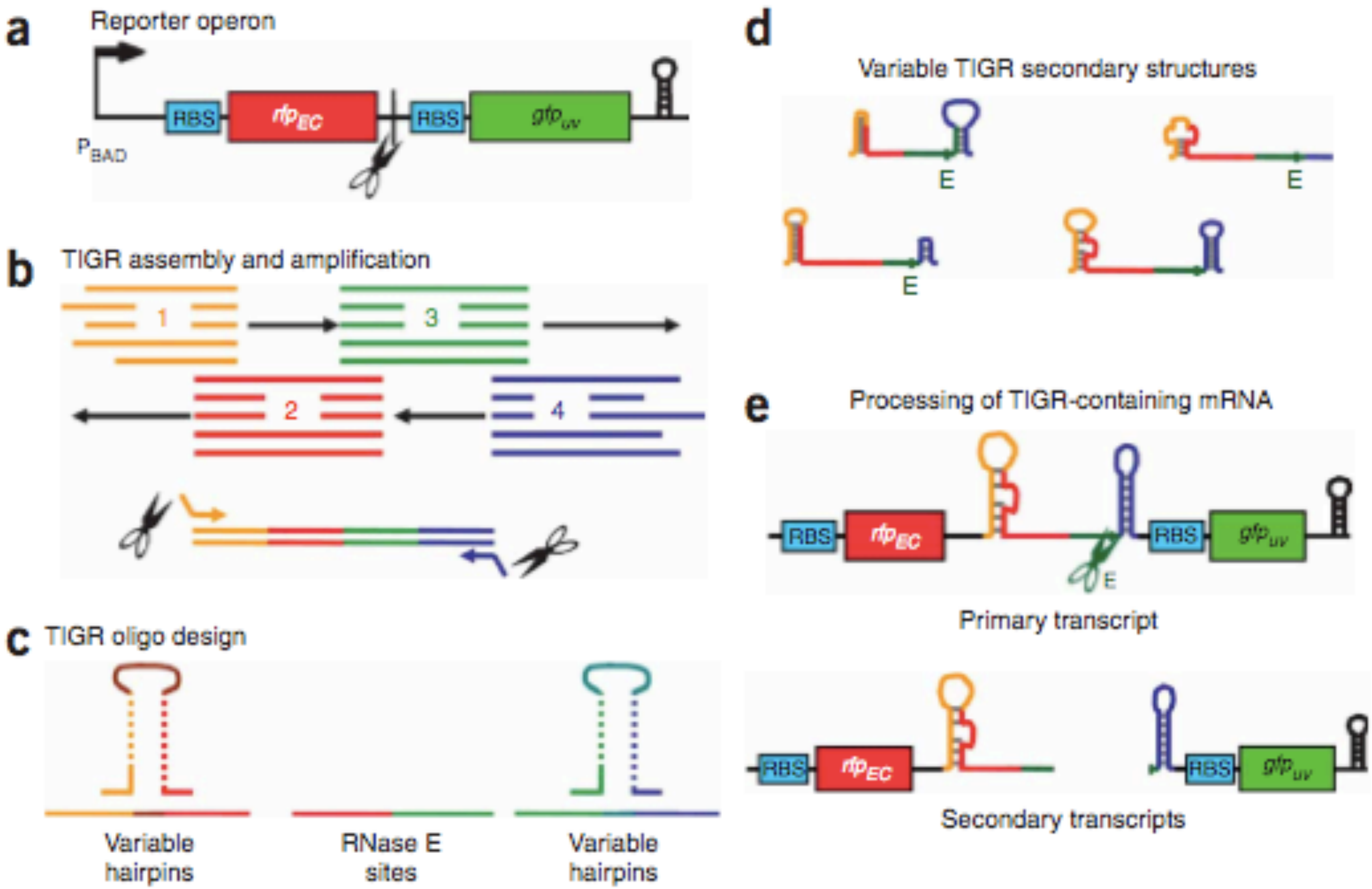
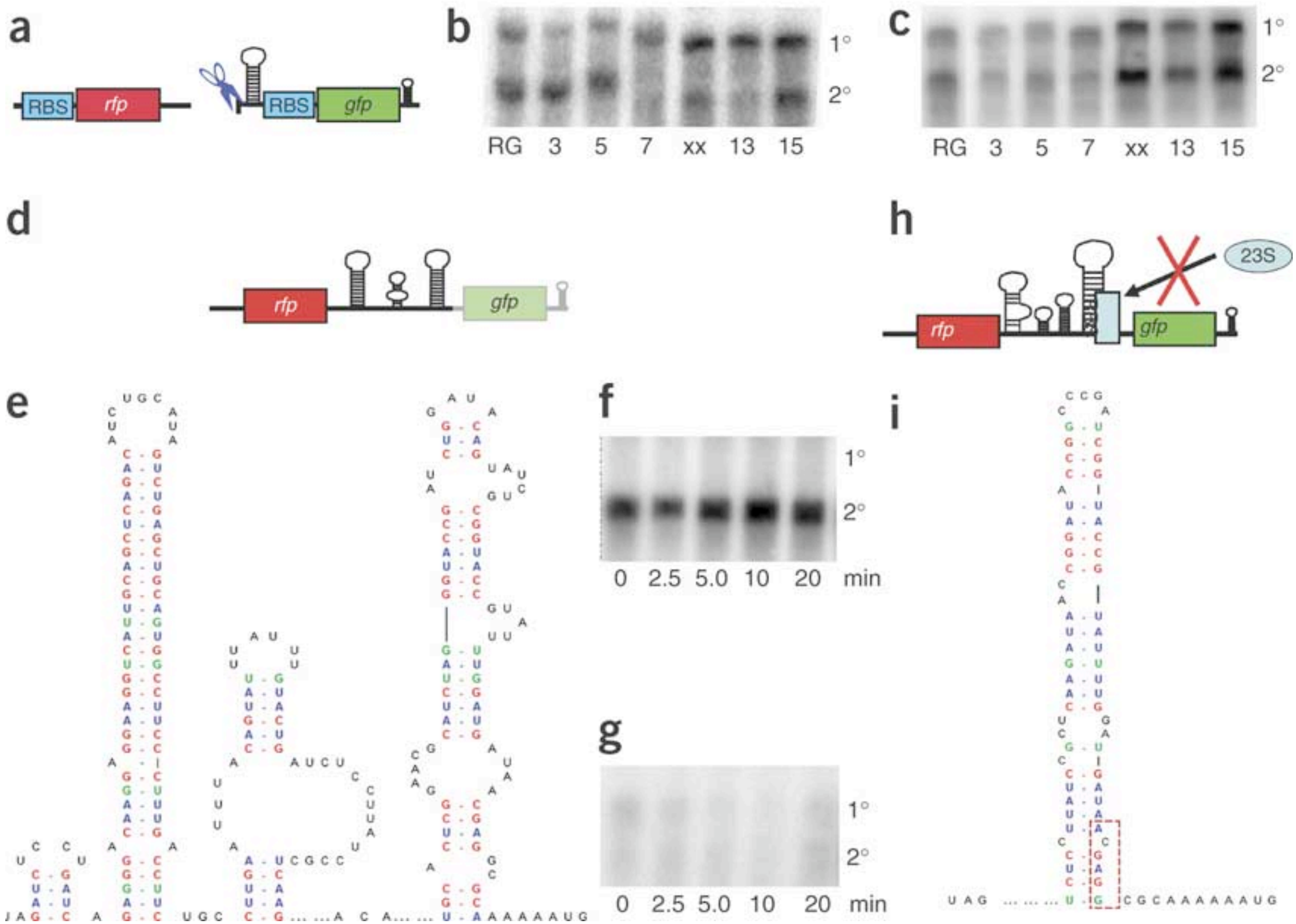


Combinatorial engineering of intergenic regions in operons tunes expression of multiple genes.
Pfleger BF, Pitera DJ, Smolke CD, Keasling JD. Nat Biotechnol. 2006 Aug;24(8):1027-32. Epub 2006 Jul 16.



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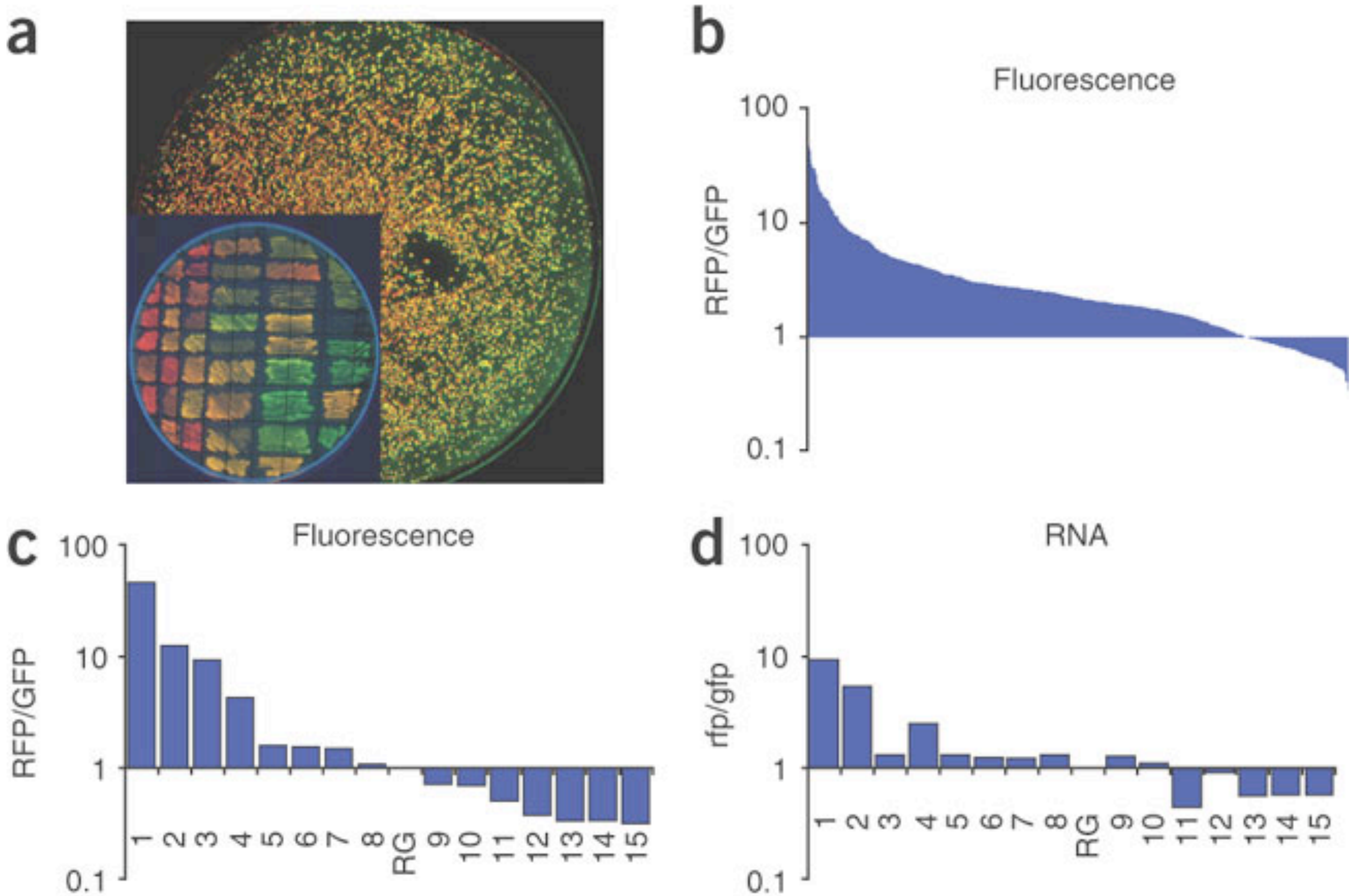

Combinatorial engineering of intergenic regions in operons tunes expression of multiple genes.
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A modular and extensible RNA-based gene-regulatory platform for engineering cellular function

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Edited by Arthur D. Riggs, Beckman Research Institute, City of Hope, Duarte, CA, and approved July 12, 2007 (received for review May 1, 2007)

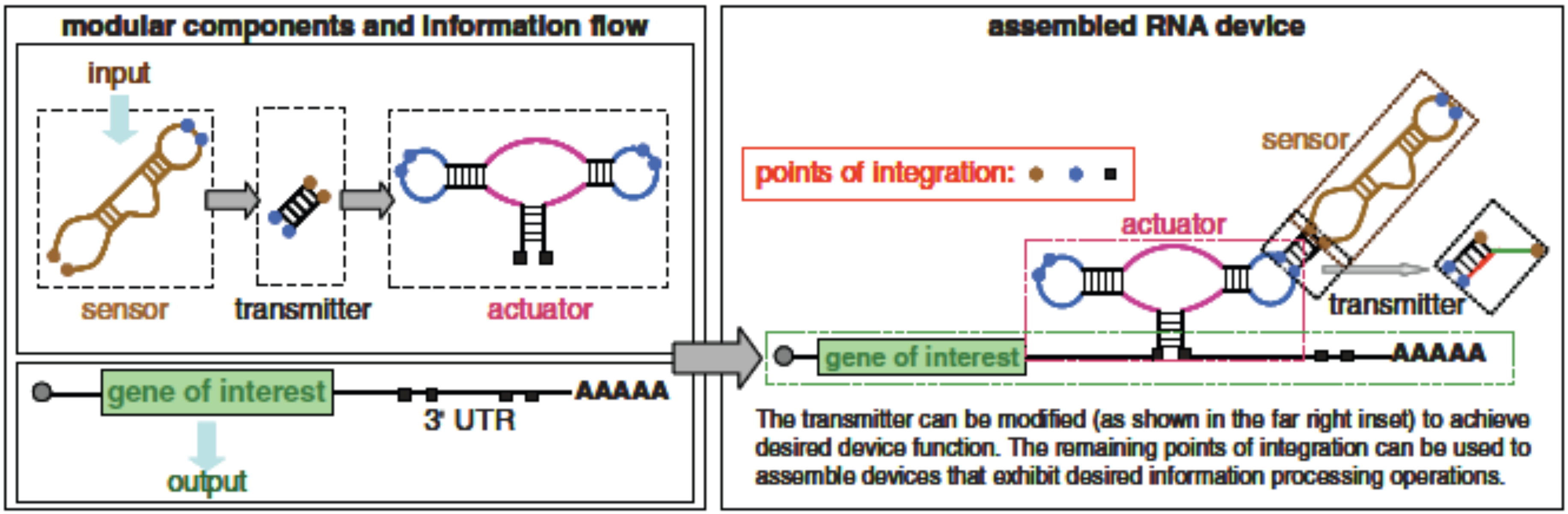
Engineered biological systems hold promise in addressing pressing human needs in chemical processing, energy production, materials construction, and maintenance and enhancement of human health and the environment. However, significant advancements in our ability to engineer biological systems have been limited by the foundational tools available for reporting on, responding to, and controlling intracellular components in living systems. Portable and scalable platforms are needed for the reliable construction of such communication and control systems across diverse organisms. We report an extensible RNA-based framework for engineering ligand-controlled gene-regulatory systems, called ribozyme switches, that exhibits tunable regulation, design modularity, and target specificity. These switch platforms contain a sensor domain, comprised of an aptamer sequence, and an actuator domain, comprised of a hammerhead ribozyme sequence. We examined two modes of standardized information transmission between these domains and demonstrate a mechanism that allows for the reliable and modular assembly of functioning synthetic RNA switches and regulation of ribozyme activity in response to various effectors. In addition to demonstrating examples of small

lack portability across organisms and systems, and their designs and construction do not support modularity and component reuse.

We set out to develop a universal and extensible RNA-based platform that will provide a framework for the reliable design and construction of gene-regulatory systems that can control the expression of specific target genes in response to various effector molecules.† We implemented five engineering design principles (DPs) in addressing this challenge: DP1, scalability (a sensing platform enabling *de novo* generation of ligand-binding elements for implementation within the sensor domain); DP2, portability (a regulatory element that is independent of cell-specific machinery or regulatory mechanisms for implementation within the actuator domain); DP3, utility (a mechanism through which to modularly couple the control system to functional level components); DP4, composability (a mechanism by which to modularly couple the actuator and sensor domains without disrupting the activities of these individual elements); and DP5, reliability (a mechanism through which to standardize the transmission of information from the sensor domain to the actuator domain).

Results

A Functional composition of an RNA device



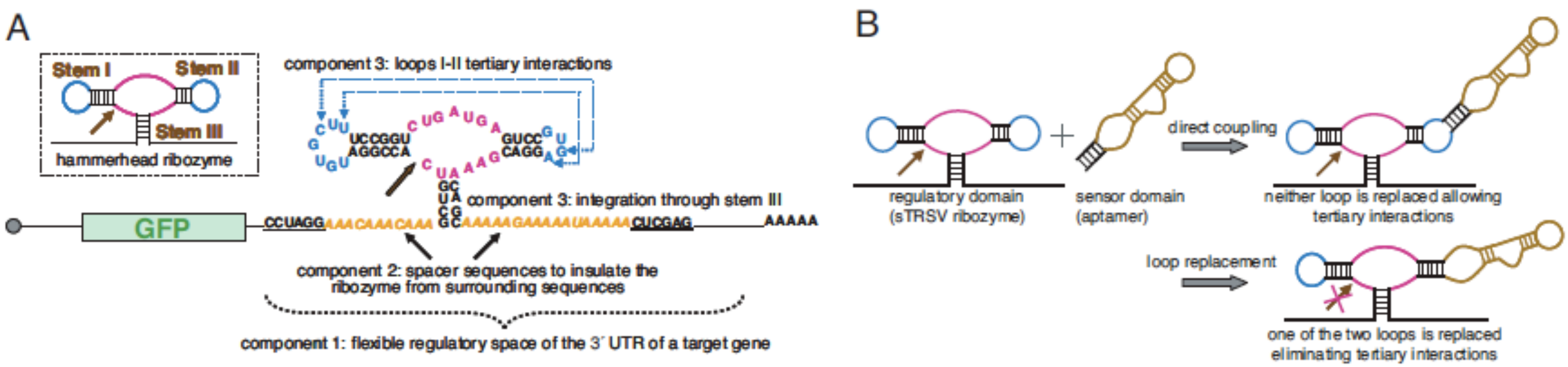


Fig. 1. General design strategy for engineering ribozyme switches. The color scheme is as follows: catalytic core, purple; aptamer sequences, brown; loop sequences, blue; spacer sequences, yellow; brown arrow, cleavage site. (*A*) General compositional framework and design strategy for engineering cis-acting hammerhead ribozyme-based regulatory systems. Restriction enzyme sites are underlined. (*B*) Modular coupling strategies of the sensor and regulatory domains to maintain *in vivo* activity of the individual domains.

See http://en.wikipedia.org/wiki/Hammerhead_ribozyme for much more information and references

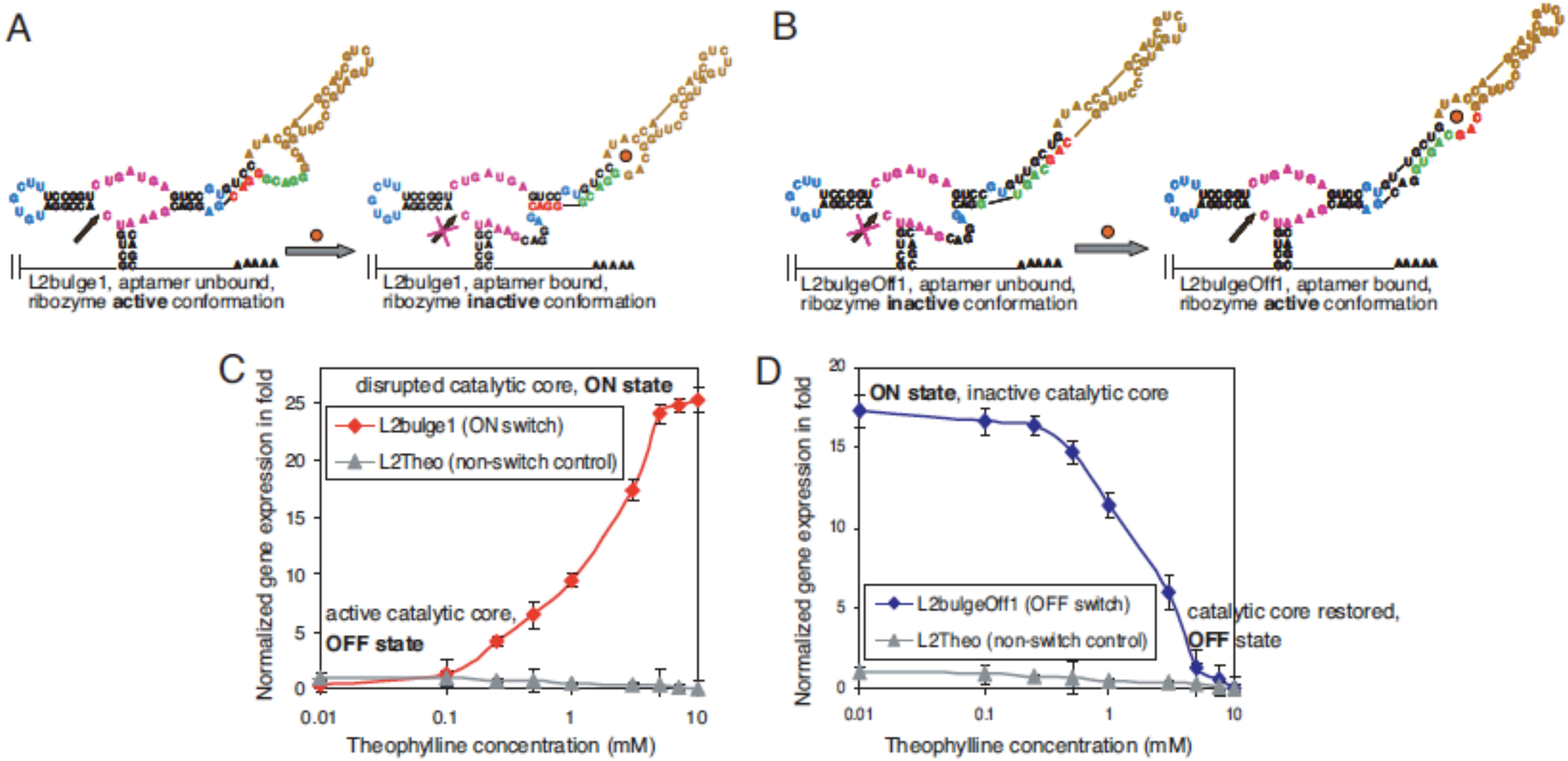
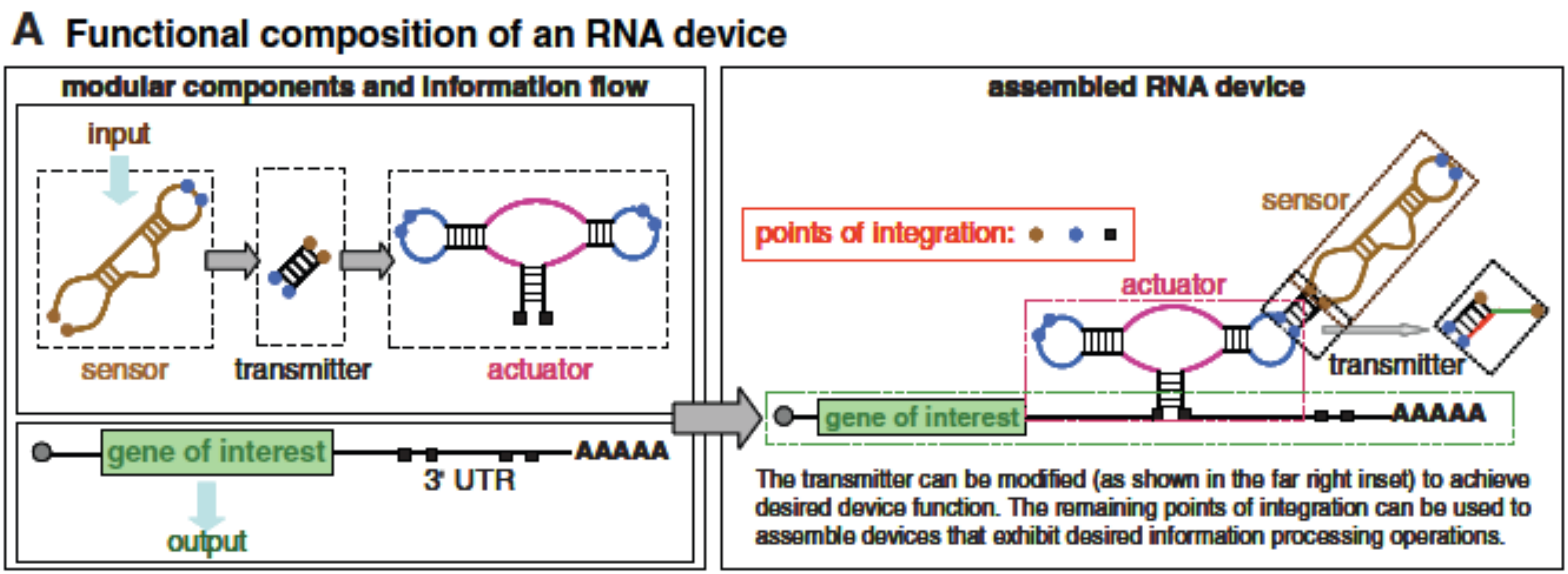


Fig. 2. Regulatory properties of the strand-displacement information transmission mechanism. The color scheme corresponds to that used in Fig. 1 with the following exceptions: switching strand, red; competing strand, green. (*A*) Gene expression ON ribozyme switch platform, L2bulge1. (*B*) Gene expression OFF ribozyme switch platform, L2bulgeOff1. (*C* and *D*) The theophylline-dependent gene-regulatory behavior of L2bulge1 (ON switch) (*C*), L2bulgeOff1 (OFF switch) (*D*), and L2Theo (nonswitch control). Gene-expression levels are reported in fold as defined in *SI Text* and were normalized to the expression levels in the absence of effector.

Higher-Order Cellular Information Processing with Synthetic RNA Devices

Maung Nyan Win and Christina D. Smolke*

The engineering of biological systems is anticipated to provide effective solutions to challenges that include energy and food production, environmental quality, and health and medicine. Our ability to transmit information to and from living systems, and to process and act on information inside cells, is critical to advancing the scale and complexity at which we can engineer, manipulate, and probe biological systems. We developed a general approach for assembling RNA devices that can execute higher-order cellular information processing operations from standard components. The engineered devices can function as logic gates (AND, NOR, NAND, or OR gates) and signal filters, and exhibit cooperativity. RNA devices process and transmit molecular inputs to targeted protein outputs, linking computation to gene expression and thus the potential to control cellular function.


A Functional composition of an RNA device
modular components and information flow
input
sensor
transmitter
actuator
gene of interest
AAAAA
3' UTR
output
assembled RNA device
points of integration:
sensor
actuator
transmitter
gene of interest
AAAAA
The transmitter can be modified (as shown in the far right inset) to achieve desired device function. The remaining points of integration can be used to assemble devices that exhibit desired information processing operations.

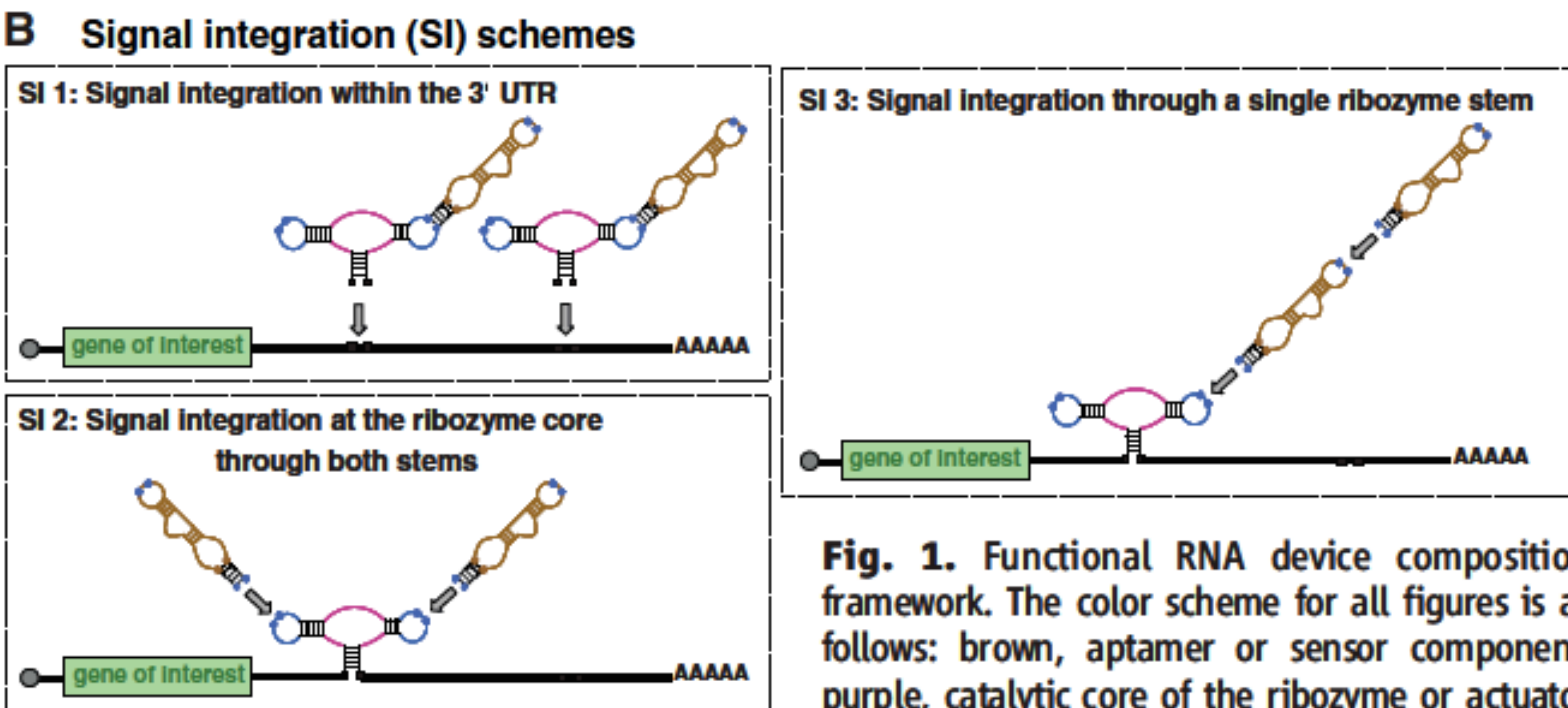
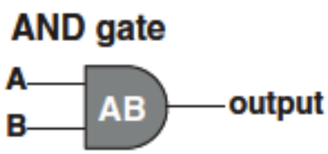
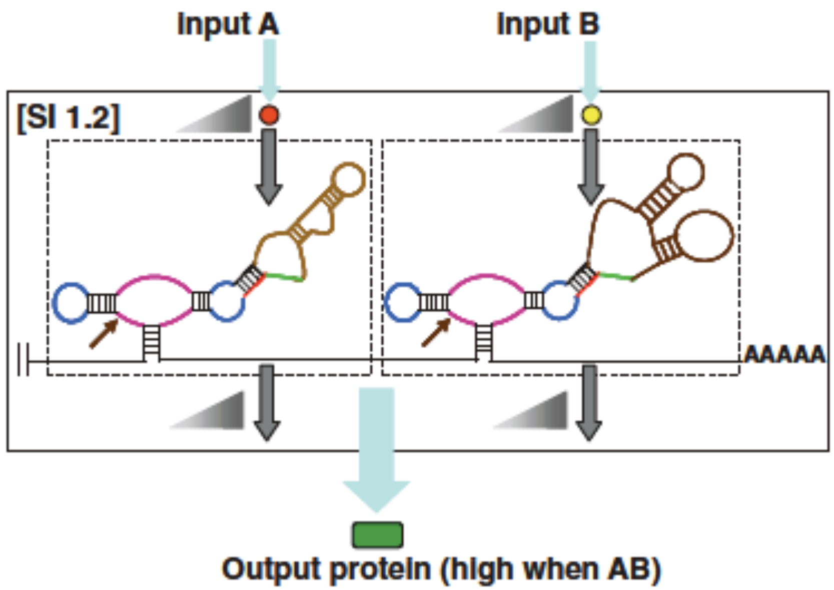


Fig. 1. Functional RNA device composition framework. The color scheme for all figures is as follows: brown, aptamer or sensor component; purple, catalytic core of the ribozyme or actuator component; blue, loop regions of the actuator

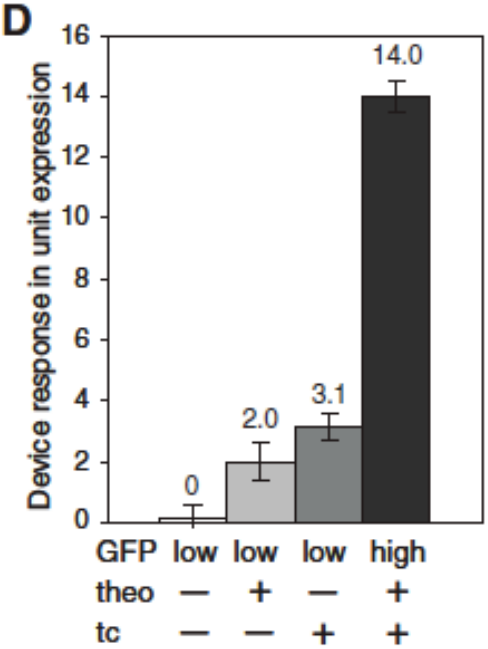
C



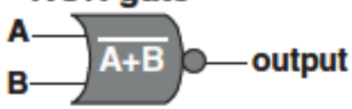
A	B	output
theo	tc	GFP
0	0	0
0	1	0
1	0	0
1	1	1



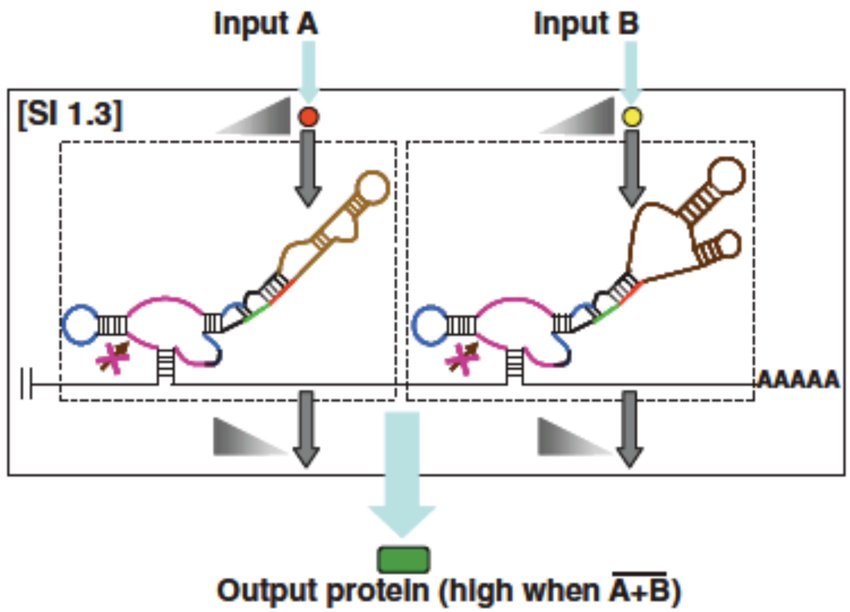
D



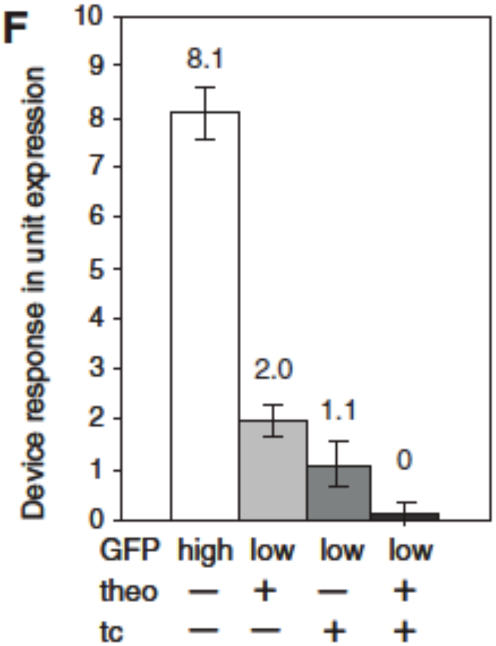
E NOR gate



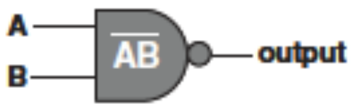
A	B	output
theo	tc	GFP
0	0	1
0	1	0
1	0	0
1	1	0



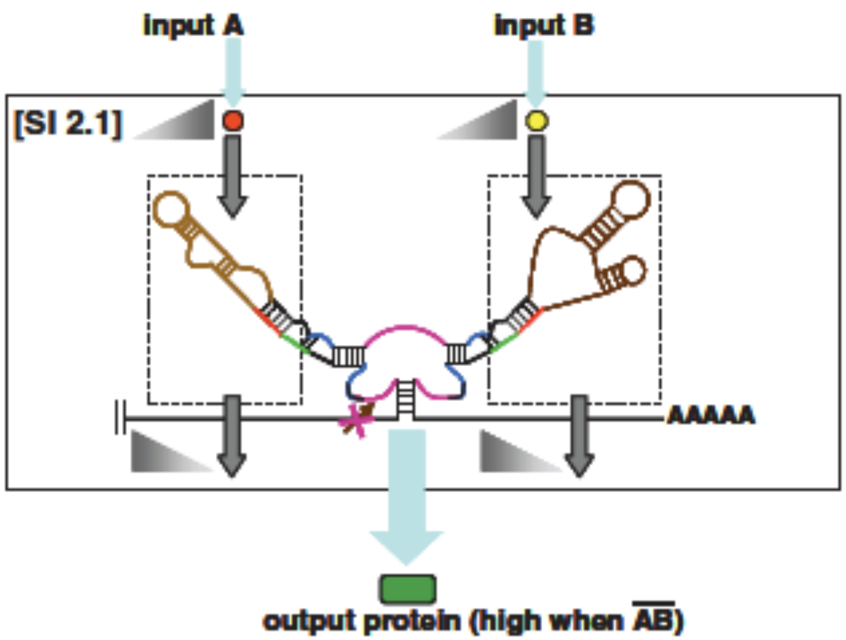
F



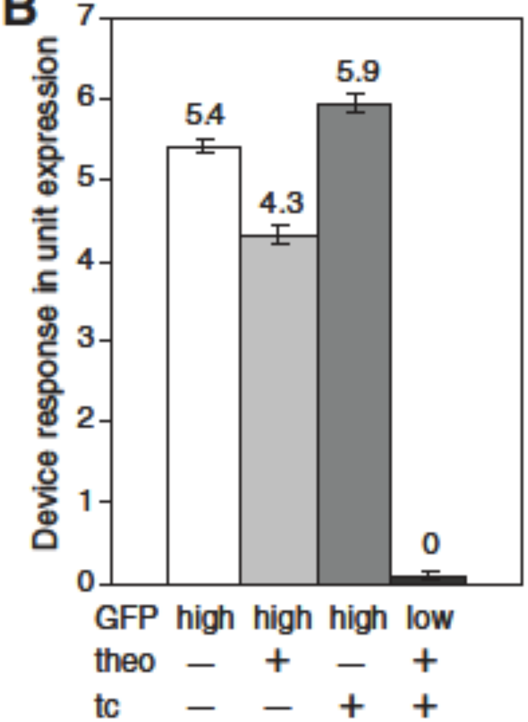
A NAND gate

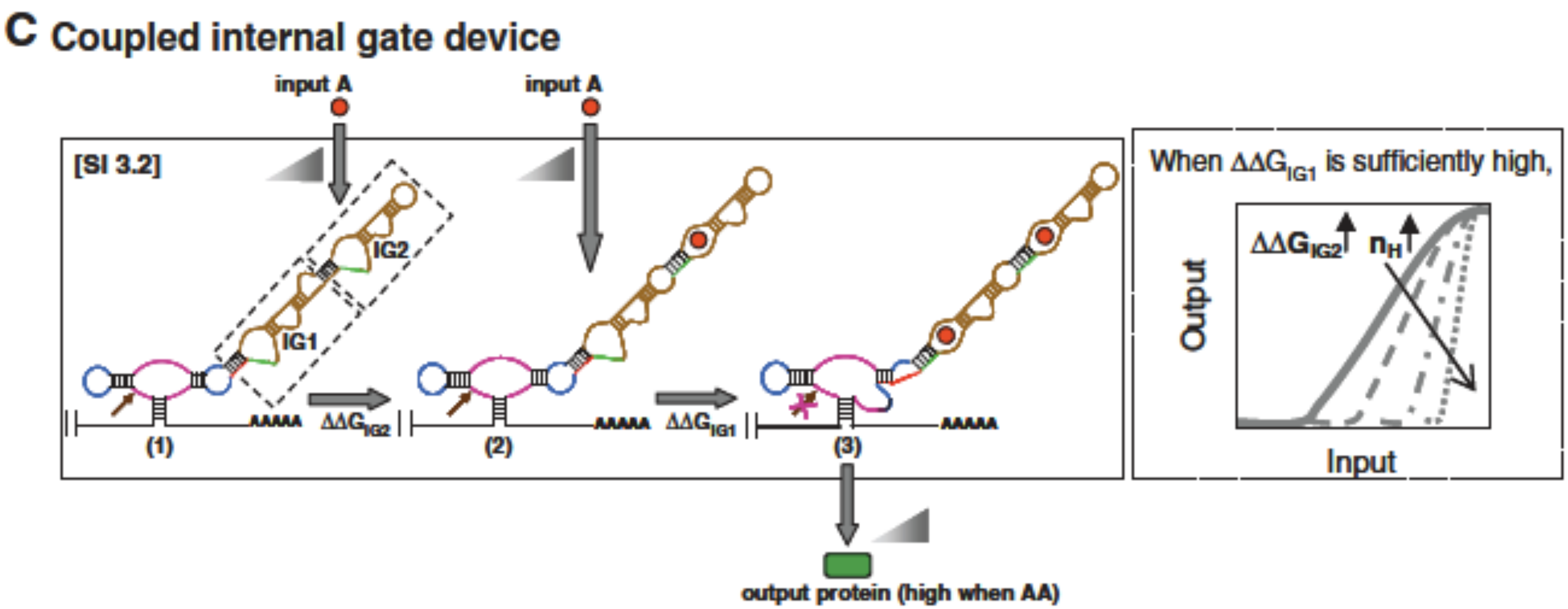


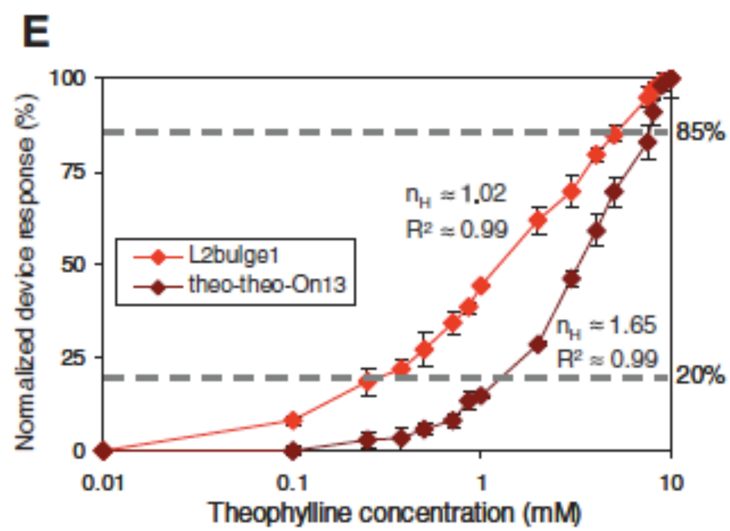
A theo	B tc	output GFP
0	0	1
0	1	1
1	0	1
1	1	0



B




C Coupled internal gate device
input A
input A
[SI 3.2]
IG2
IG1
ΔΔG_IG2
ΔΔG_IG1
AAAAA
AAAAA
AAAAA
(1)
(2)
(3)
When ΔΔG_IG1 is sufficiently high,
ΔΔG_IG2
n_H
Output
Input
output protein (high when AA)


E
Normalized device response (%)
100
75
50
25
0
0.01
0.1
1
10
Theophylline concentration (mM)
85%
20%
n_H ≈ 1.02
R² ≈ 0.99
n_H ≈ 1.65
R² ≈ 0.99
L2bulge1
theo-theo-On13

Q. But, where do the "sensors" come from?!