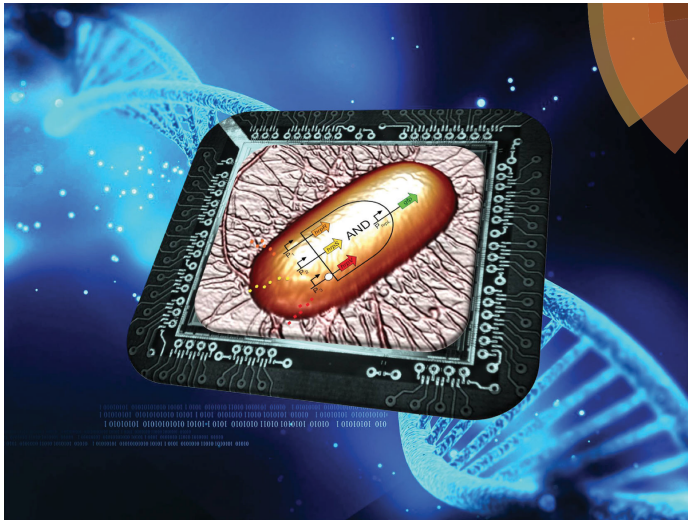


Rapid engineering of versatile molecular logic gates using heterologous genetic transcriptional modules

Baojun Wang and Martin Buck

Chemical Communications (2014 July) **50**: 11642-11644

Presented by Heejo Choi and Tushar Kamath

for 20.385

February 18th, 2015

Logic gates

Single-input buffer Gate:
- amplifier for input signal
- Does not change the logicality of gate

Input $NaAsO_2$	Output Buffer gate
0	0
1	1

Double-input AND gate:
- Requires two inputs
  - First input needs to ON, second one needs to be OFF

Inverting Non-inverting

Input 2 $HgCl_2$	Input 1 $NaAsO_2$	Output AND gate
0	0	0
0	1	1
1	0	0
1	1	0

Triple-input AND gate:
- Three inputs
  - First **and** second need to be ON, third needs to be OFF

Inverting Non-inverting

Input 3 $HgCl_2$	Input 2 $NaAsO_2$	Input 1 $3OC_6HSL$	Output AND gate
0	0	0	0
0	0	1	0
0	1	0	0
0	1	1	1
1	0	0	0
1	0	1	0
1	1	0	0
1	1	1	0

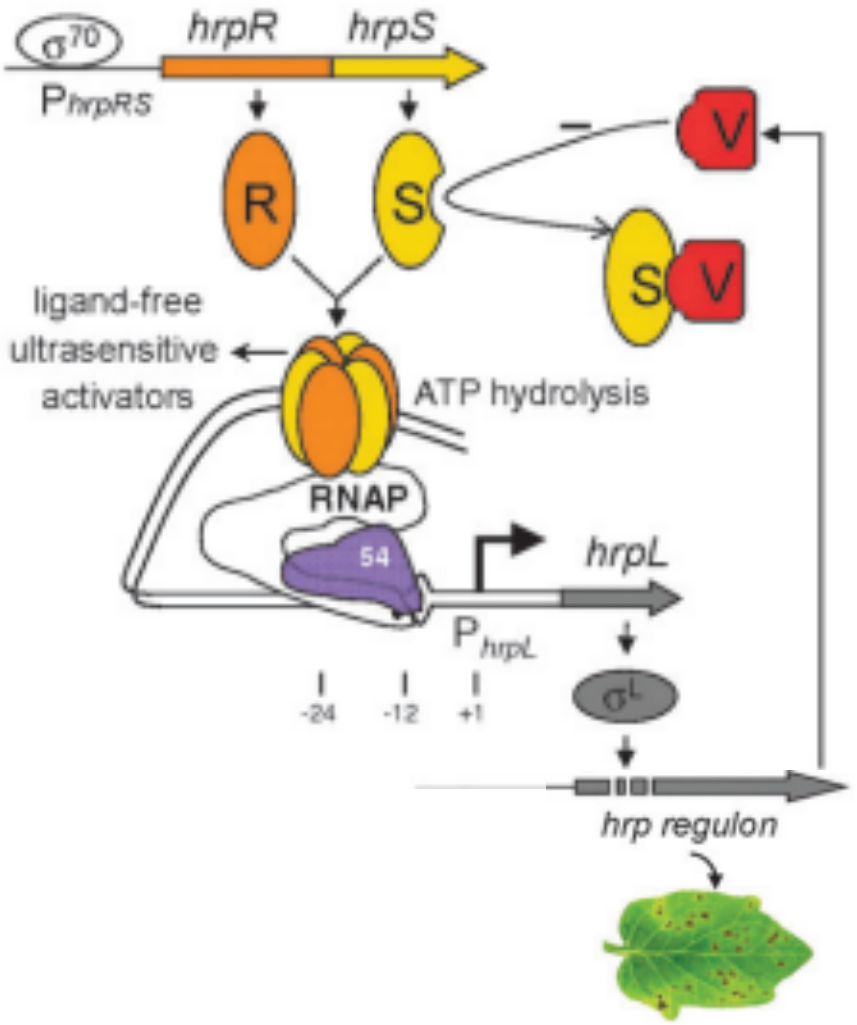
Hrp regulatory system - type III secretion and multiple input

Type III secretion

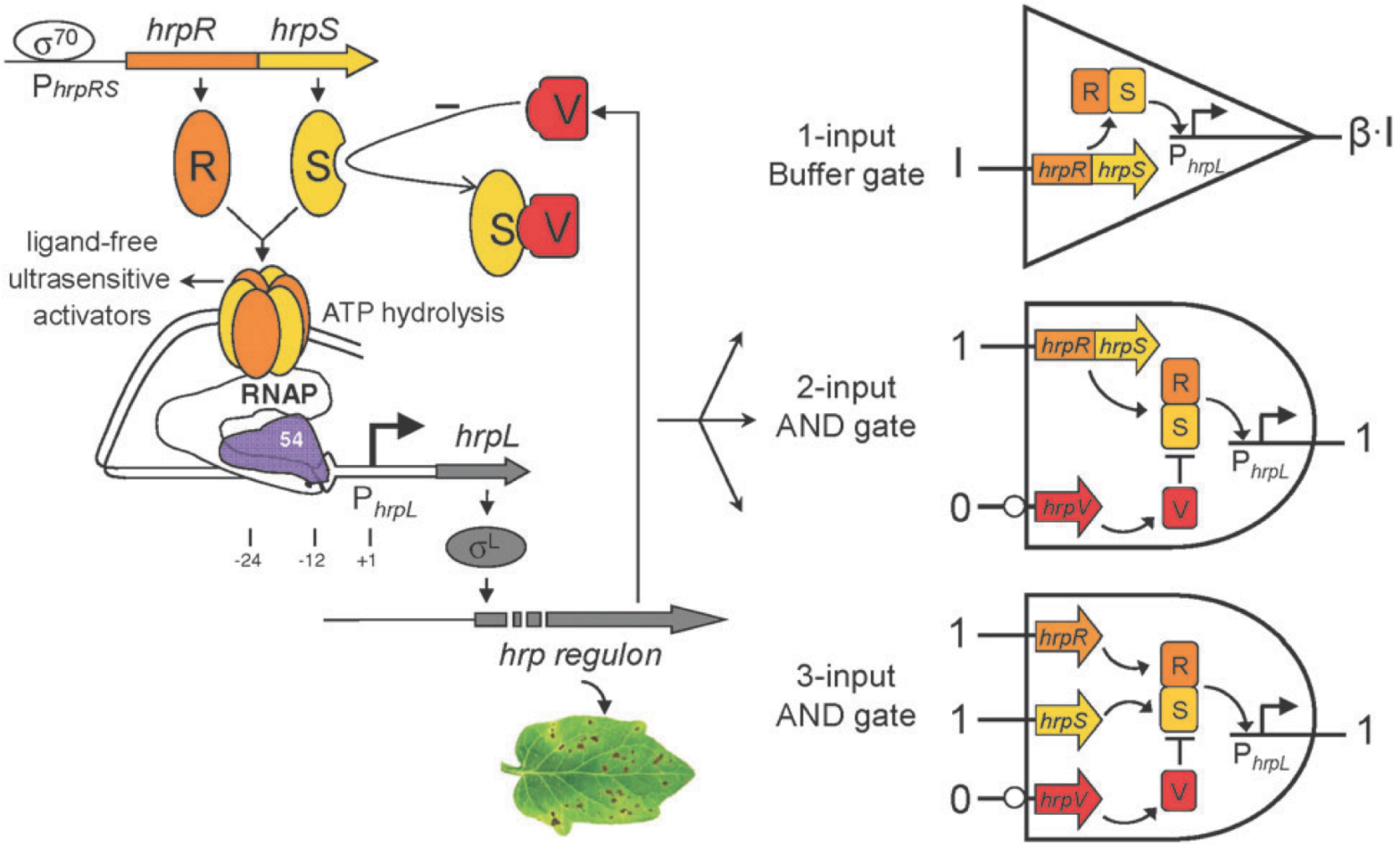
- Underlying basis for pathogenicity of *Pseudomonas syringae*

Multiple input

- hrpR/hrpS: both required to form high-order co-complex
- Co-complex activates by opening sigma-dependent *hrpL* promoter → activates *hrp* regulon
- hrpV: negative regulation directly interacts with hrpS



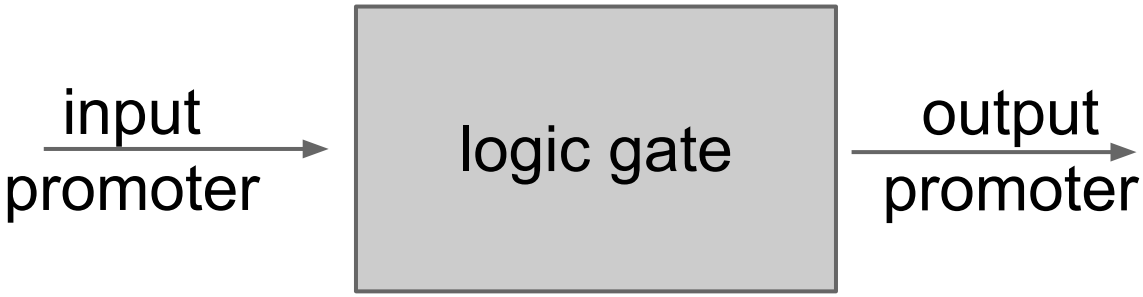
Constructing a set of modular genetic logic gates with versatile digital logic functions



What are the advantages?

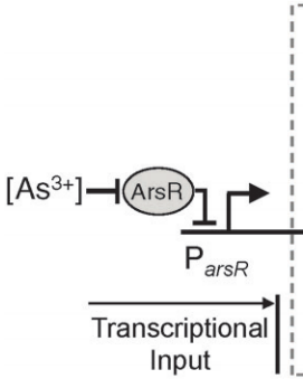
- Modularity (reusability)
  - inputs can be connected to different environment-responsive promoters
  - output may be wired to drive various useful genes

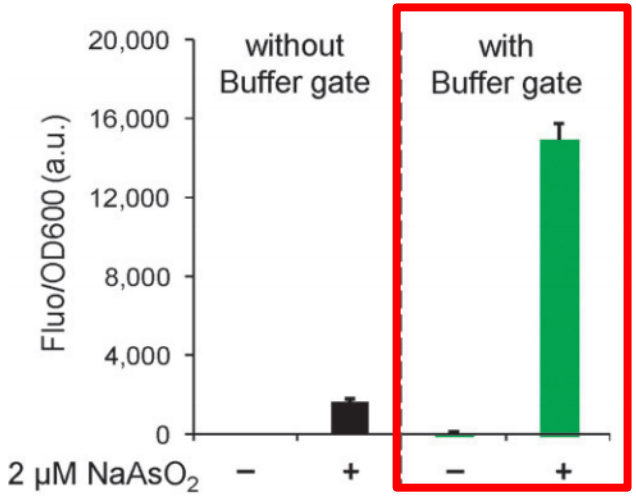
- Genetic components used are heterologous to *E. coli*
  - less likely to interfere with the host endogenous genetic programs
  - enable these logic devices to behave more robustly



Design and characterization of the hrp system as a single-input buffer gate

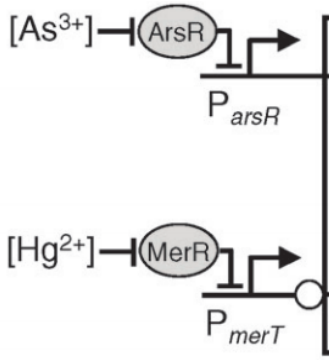
- Input connected to an arsenic-responsive promoter
- hrpR/hrpS act synergistically to produce hrpL promoter
- hrpL promoter activates gfp protein, creating fluorescent output
- Output is significantly amplified with buffer gate

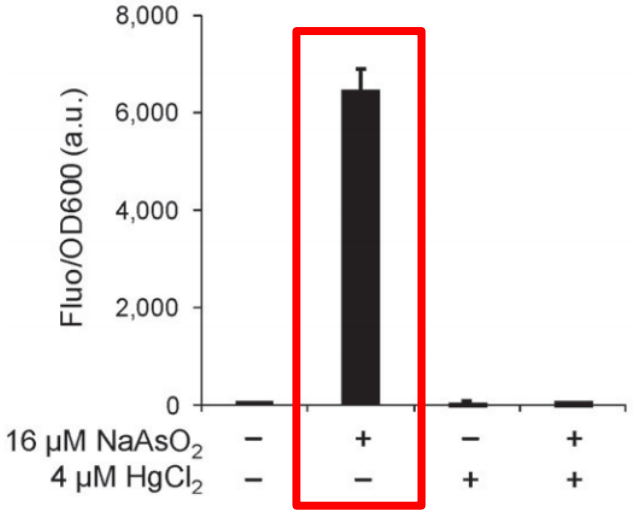




The *hrp* regulatory system, in response to mercury and arsenic, functions as a logic AND gate with one inverting input

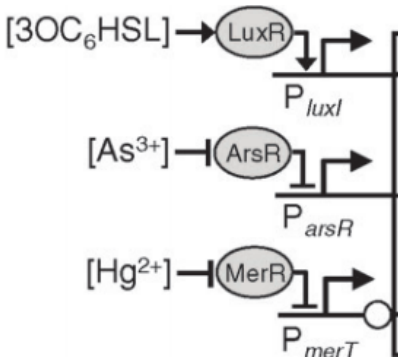
- One inverted input and one non-inverted input
- HrpR/HrpS expressed with a non-inverted input and HrpV with an inverted input
- weak RBS coupled with HrpR/HrpS and strong RBS with HrpV
- Arsenic present/mercury absent produces strongest output

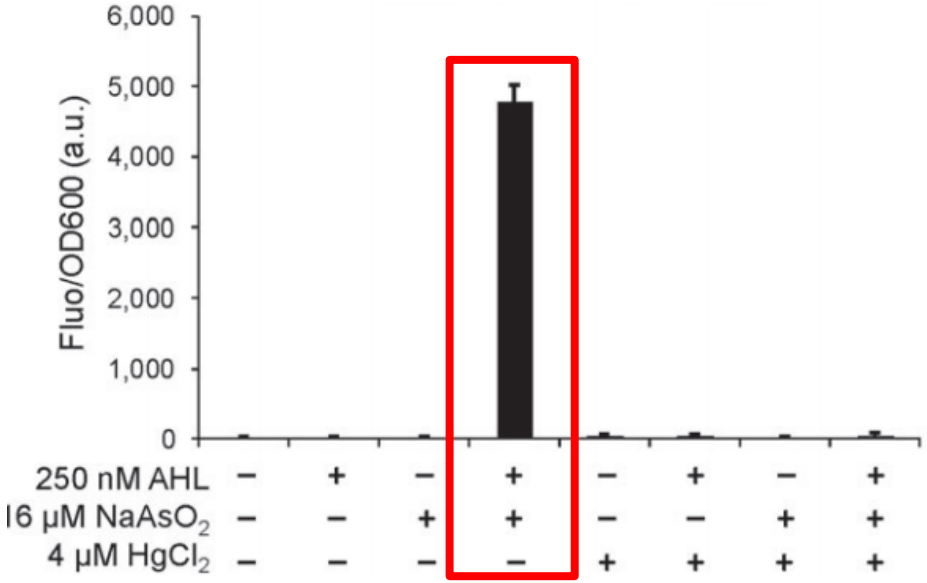




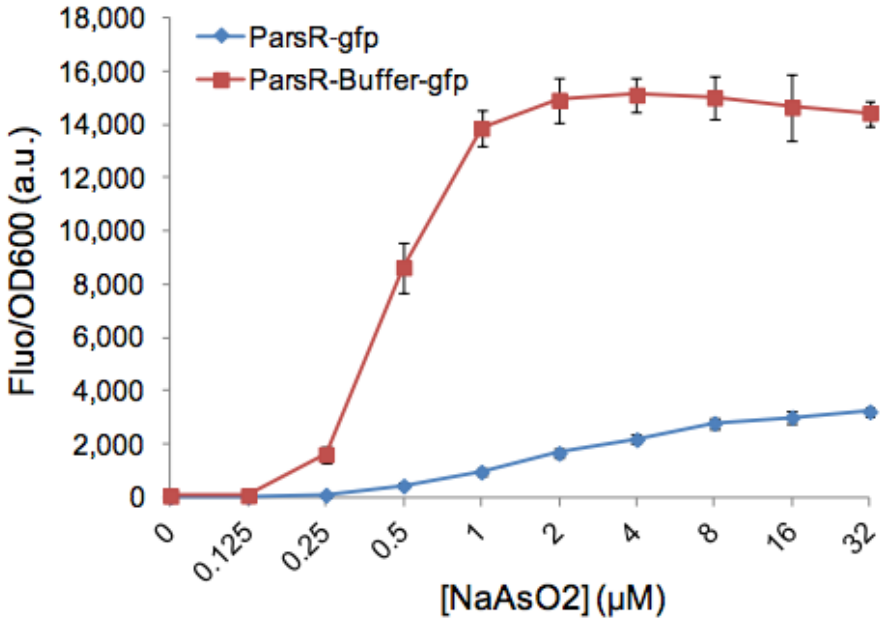
The hrp system functions as a triple-input AND logic gate with a single non-inverting input

- *HrpR* and *hrpS* control two non-inverted inputs independently
- *HrpV* acts as a third inverting input with a mercury promoter
- *HrpL* activated to produce GFP output





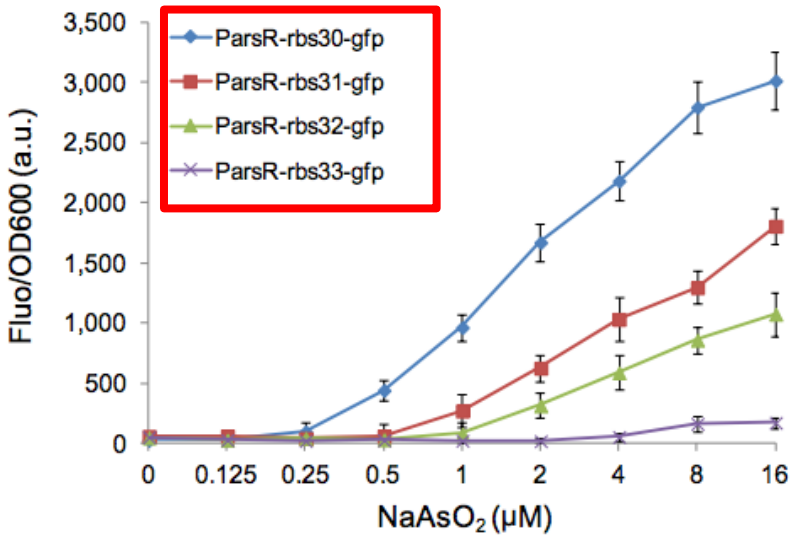
Quantifying the effects of a buffer logic gate on GFP output

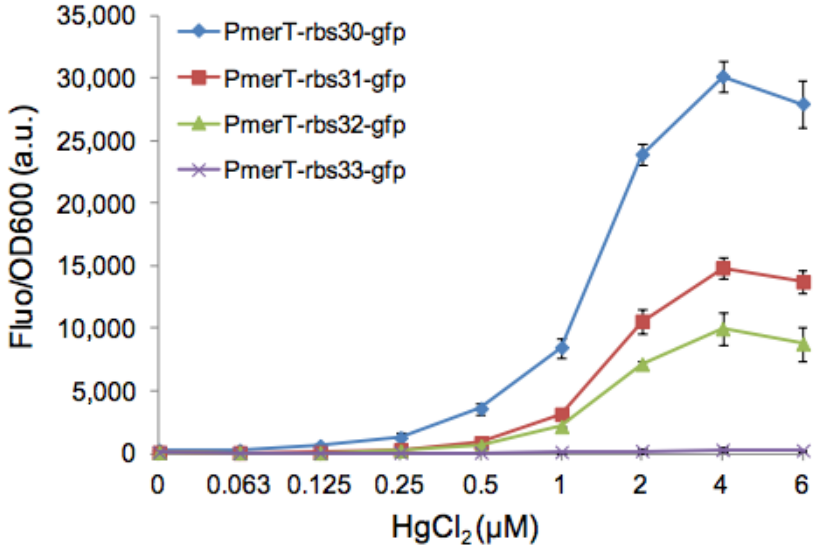


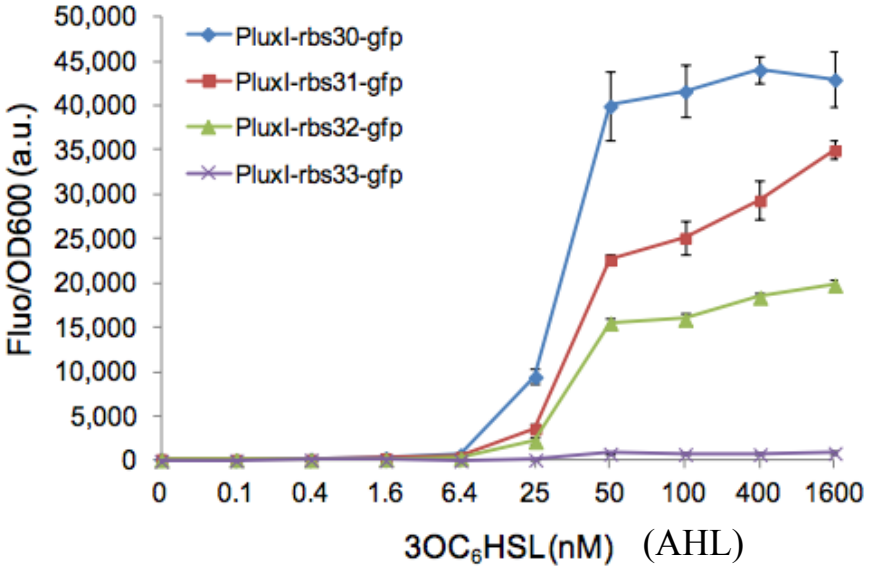
Identifier[a]	Type	DNA sequence (5'– 3')	Reported strength[1]
rbs30	RBS[b]	TCTAGAGATTAAAGAGGAGAAATACTAG**ATG**	Very strong
rbs31	RBS[b]	TCTAGAGTCACACAGGAAACCTACTAG**ATG**	Middle strong
rbs32	RBS[b]	TCTAGAGTCACACAGGAAAGTACTAG**ATG**	Weak
rbs33	RBS[b]	TCTAGAGTCACACAGGACTACTAG**ATG**	Very weak
rbs34	RBS[b]	TCTAGAGAAAGAGGAGAAATACTAG**ATG**	Very strong
J115	Promoter	TTTATAGCTAGCTCAGCCCTTGGTACAATGCTAGC	– (constitutive)
J101	Promoter	TTTACAGCTAGCTCAGTCCTAGGTATTATGCTAGC	– (constitutive)

[a]The regulatory sequences are from the Registry of Standard Biological Parts (http://partsregistry.org).
[b]Sequence of RBS (ribosome binding site) is underlined and start codon is in bold.

Quantifying fluorescent effects by ribosomal binding site changes with various promoters







(AHL)

Rapid engineering of versatile molecular logic gates using heterologous genetic transcriptional modules

Key assumption
- genetic heterology protects robustness

As a reviewer
- Explain existing hrp regulation system
  - Clarify how hrpR and hrpS co-complex causes a synergistic amplification
- Justify input substrate concentration (ie. At which substrate concentrations does the system lose robustness?)
- Assays to show robustness (What level of Fluo/OD600 is considered “1”?)

Significance
- building blocks in kit for gene circuit engineering
- modular (reusable) ie. easy to incorporate into other cellular circuits by changing inputs and outputs
- versatile (adaptable) ie. easy to make various logic gates from the same existing regulatory system
- heterologous to *E. coli* → less likely to interfere with with host endogenous genetic programs

Genetic logic device incorporated into kit for gene circuit engineering

Designer cell signal processing circuits for biotechnology

Robert W. Bradley[1,2] and Baojun Wang[2]

January 2015

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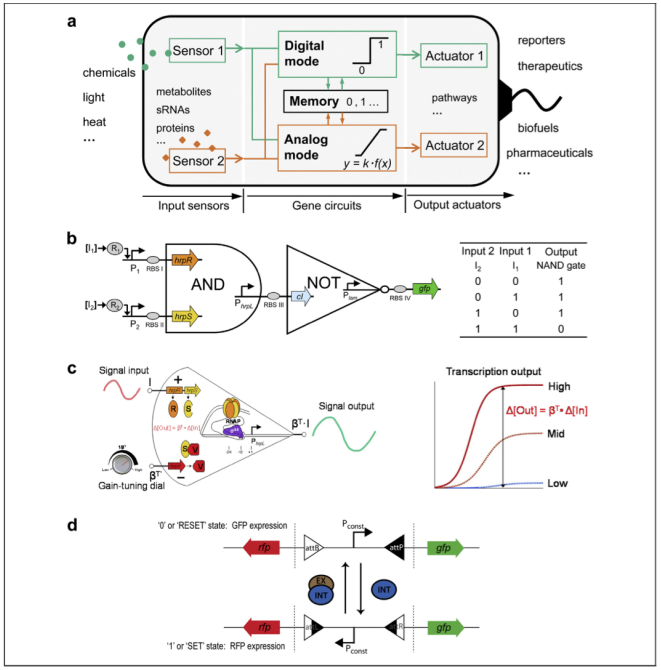
[2] Centre for Synthetic and Systems Biology, School of Biological Sciences, University of Edinburgh, Edinburgh EH9 3JR, UK

Figure 1



Follow-up

- Expanding range of available orthogonal genetic components
- Insufficient orthogonal elements in the current toolkit to design large synthetic biological systems
- Robustness & safety
- Demonstrate repeated efficacy to improve and maintain public perception in synthetic biology