Programming cellular behavior with RNA controllers

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Stanford University

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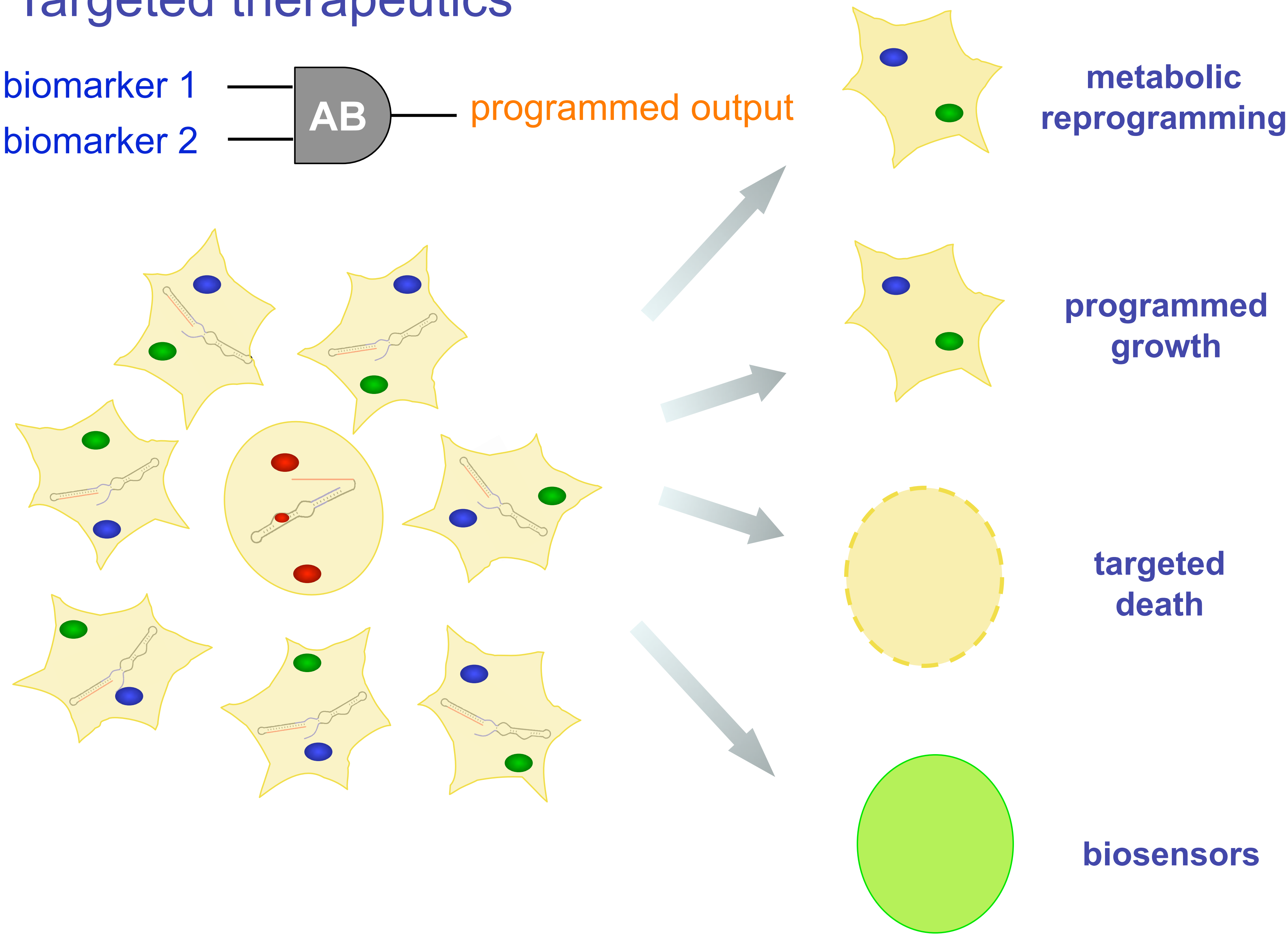
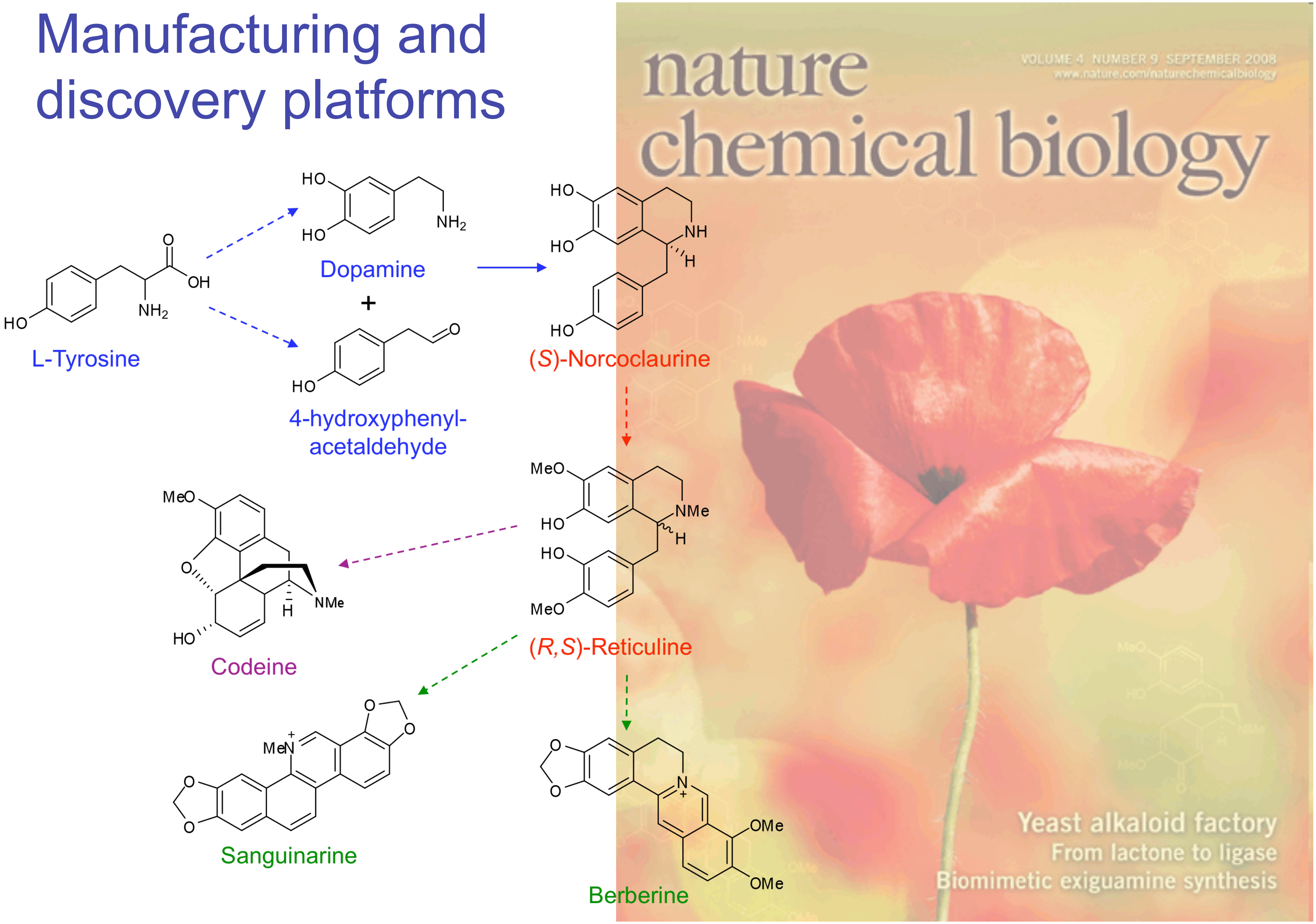
RoSBNet Synthetic Biology Workshop
St. Anne’s College, University of Oxford

Targeted therapeutics



Manufacturing and discovery platforms



Hawkins KM, Smolke CD. 2008. *Nat Chem Biol.* 4:564-73

Apps

Tools

c/o D. Endy (Stanford University)

7. Control & dyn. systems

6. Reverse engineering

5. Fab, CAD & EDA

4. Standards & abstraction

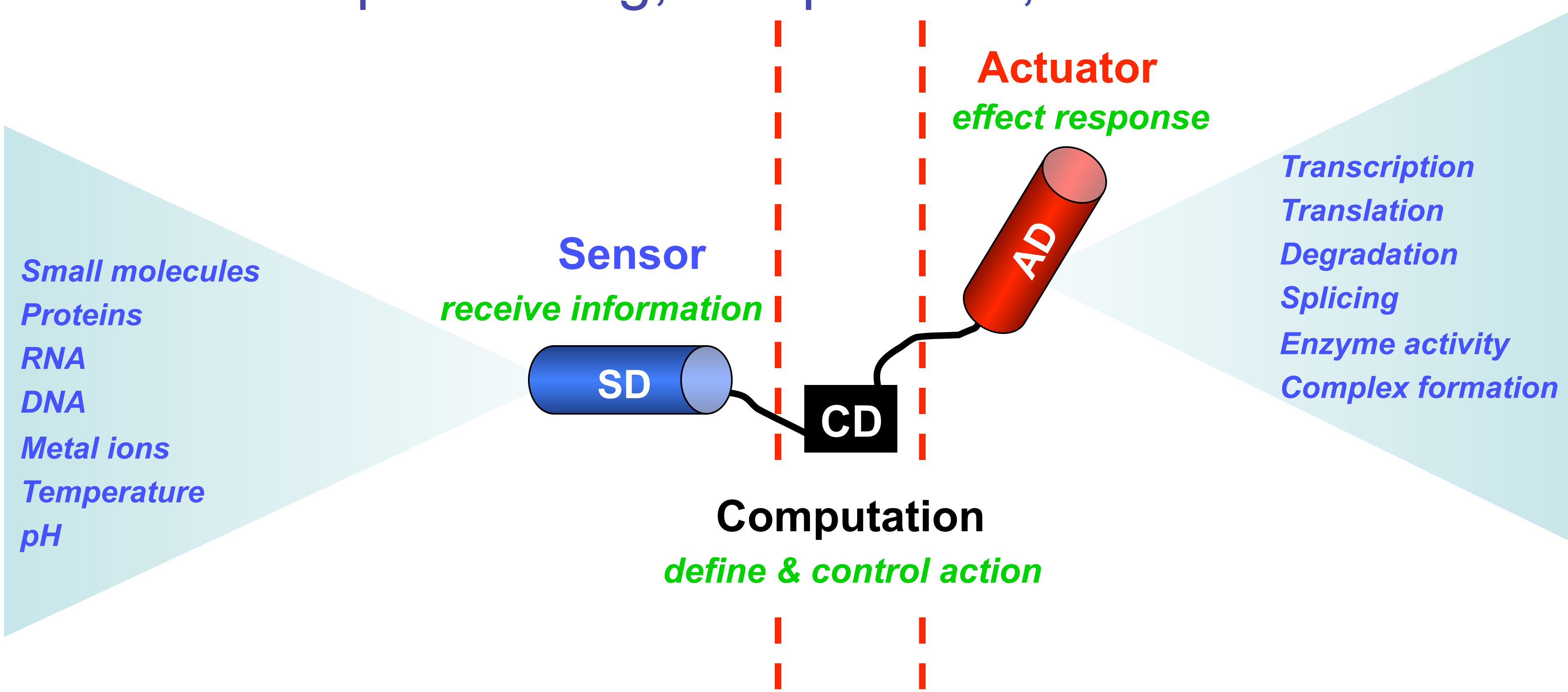
3. Languages & grammars

2. Device design

1. Info. theory & signal proc.

c/o D. Endy (Stanford University)

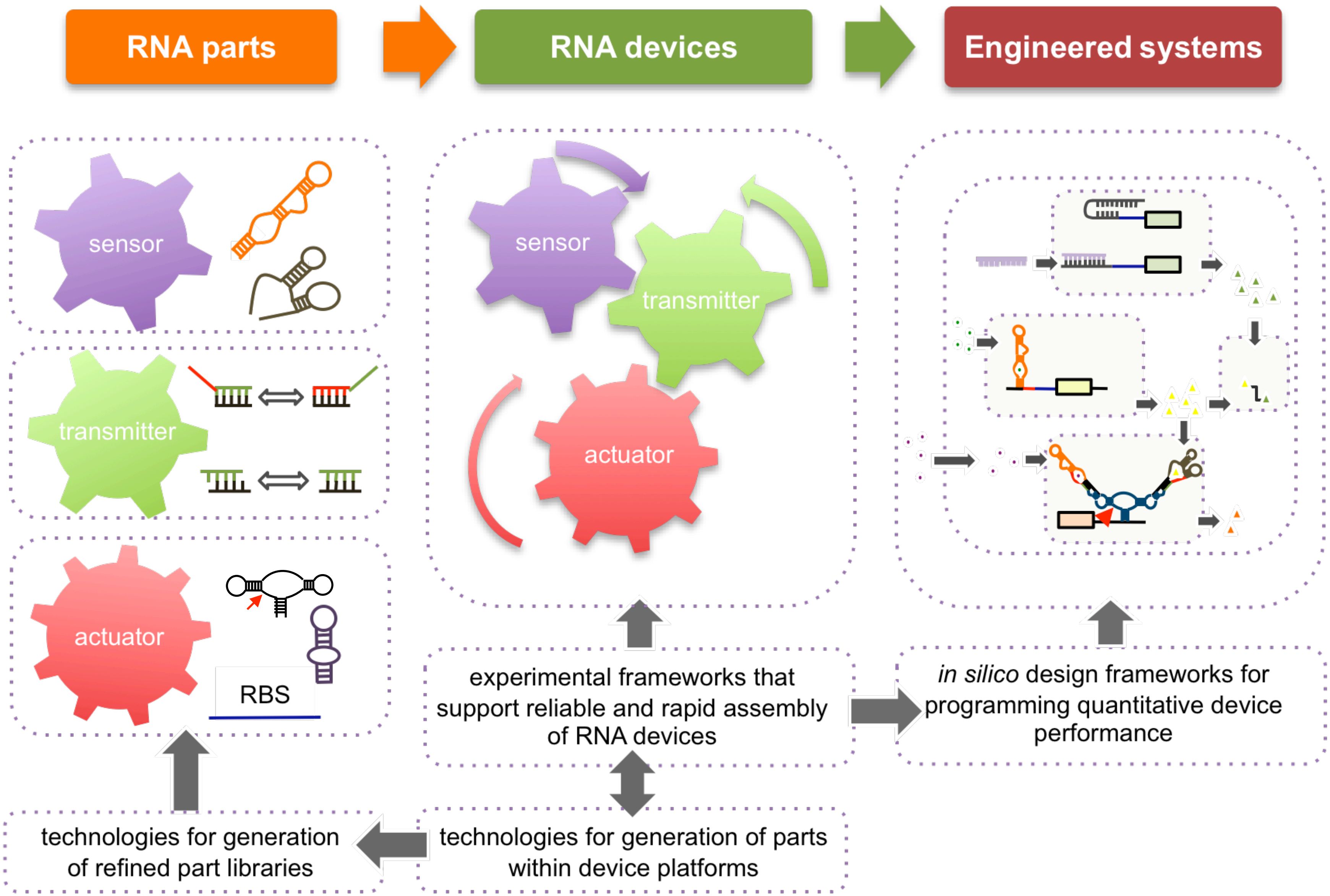
Information processing, computation, and control



Key Engineering Properties

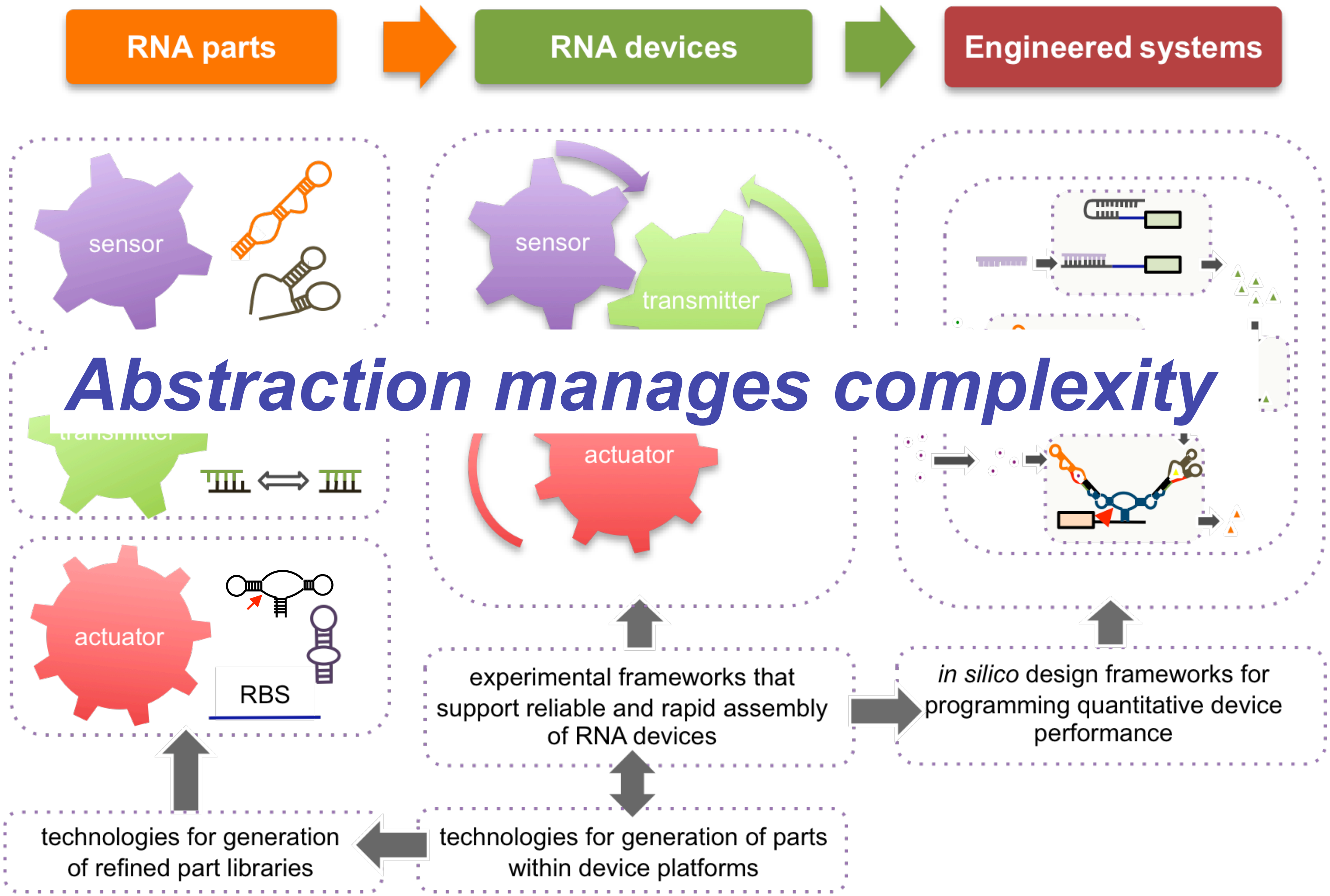
- ✓ scalability
- ✓ portability
- ✓ utility
- ✓ composability
- ✓ reliability

Plan for developing a programmable input/output tool



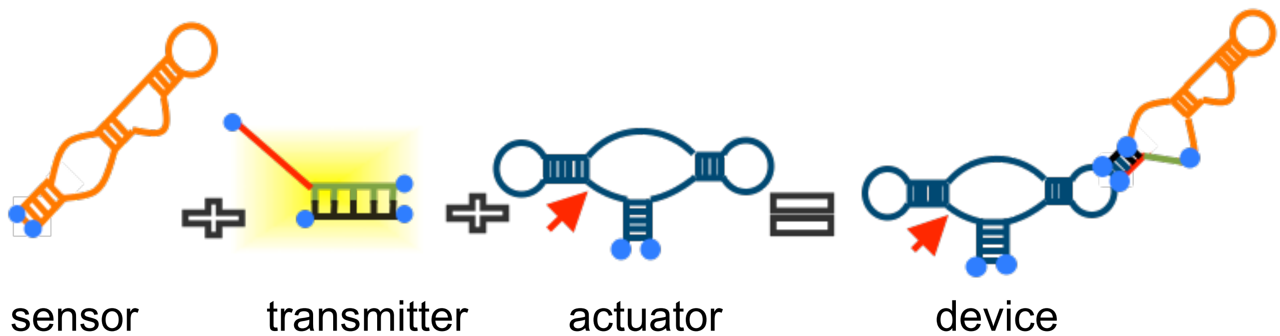
Win MN, Liang JC, Smolke CD. 2009. *Chem Biol.* 16: 298-310

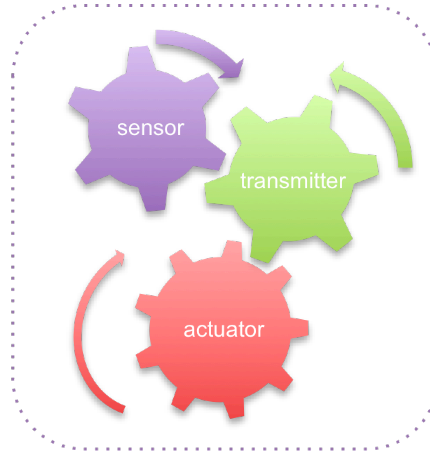
Plan for developing a programmable input/output tool



Win MN, Liang JC, Smolke CD. 2009. *Chem Biol.* 16: 298-310

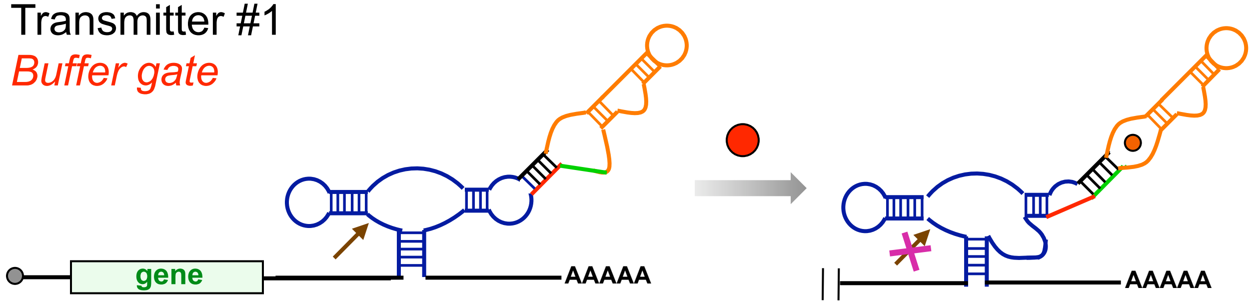
A platform for programming device function





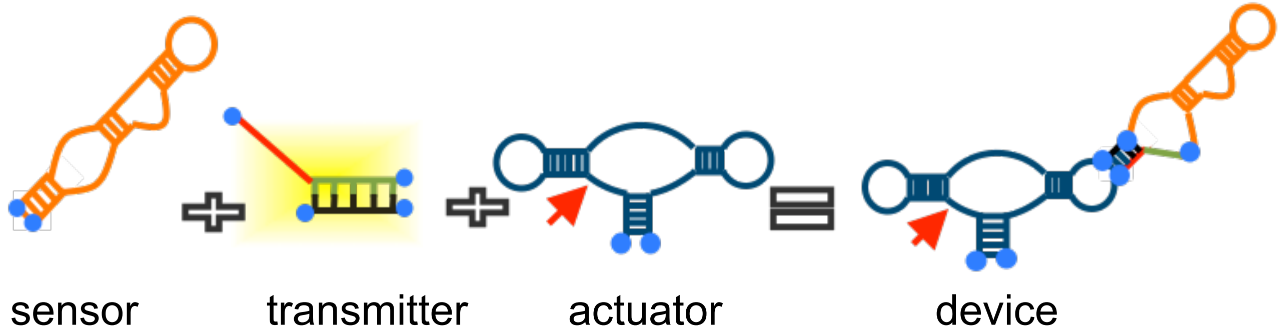
Transmitter #1

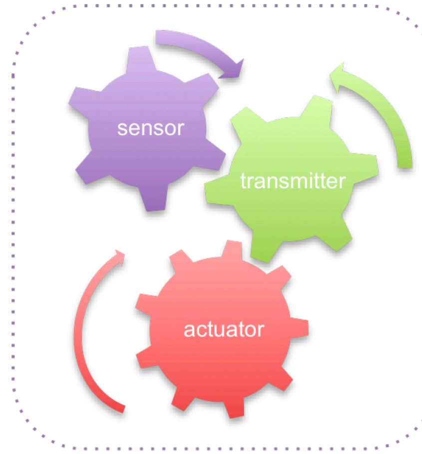
Buffer gate



Win MN, Smolke CD. 2007. *PNAS*. 104: 14283-8

A platform for programming device function





Transmitter #1

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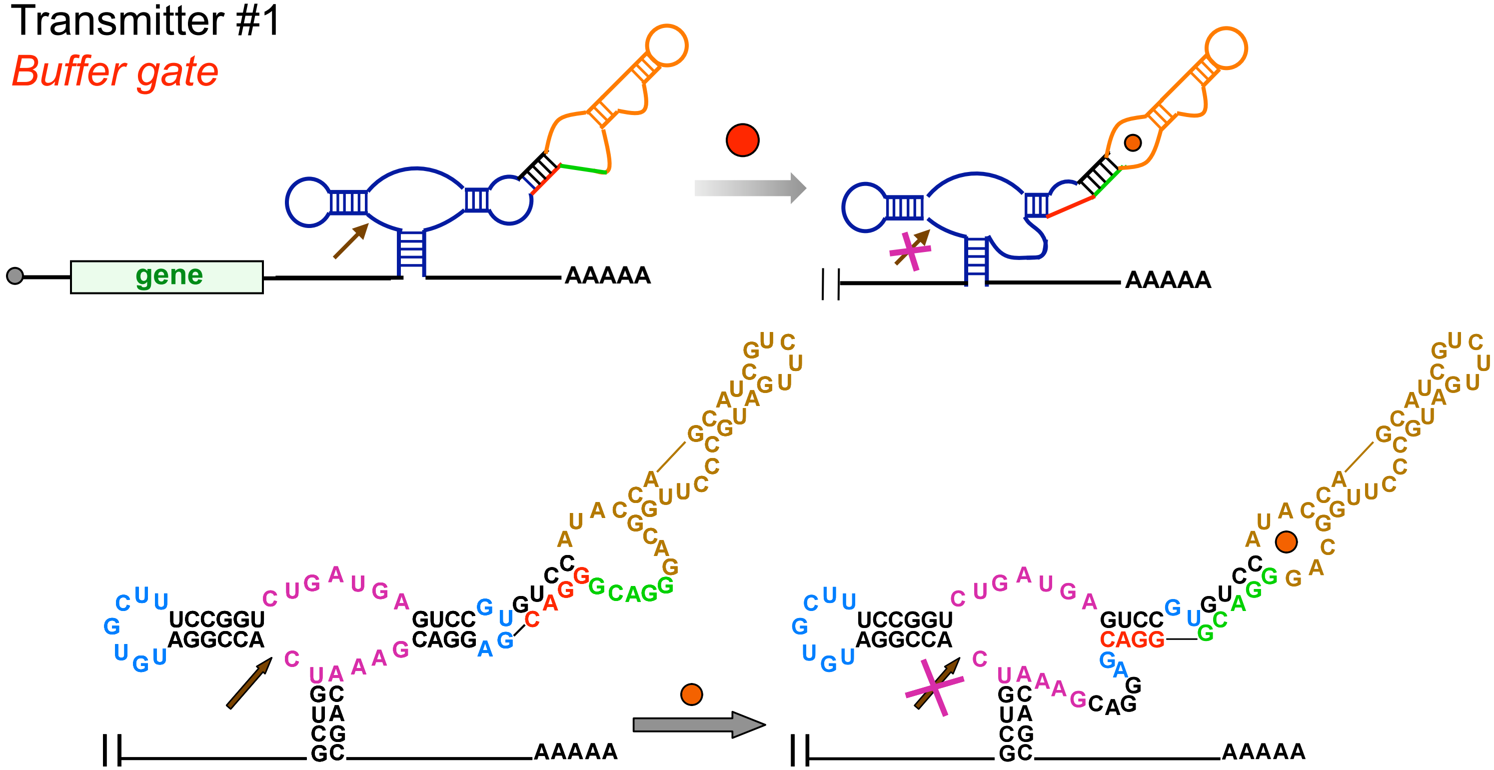

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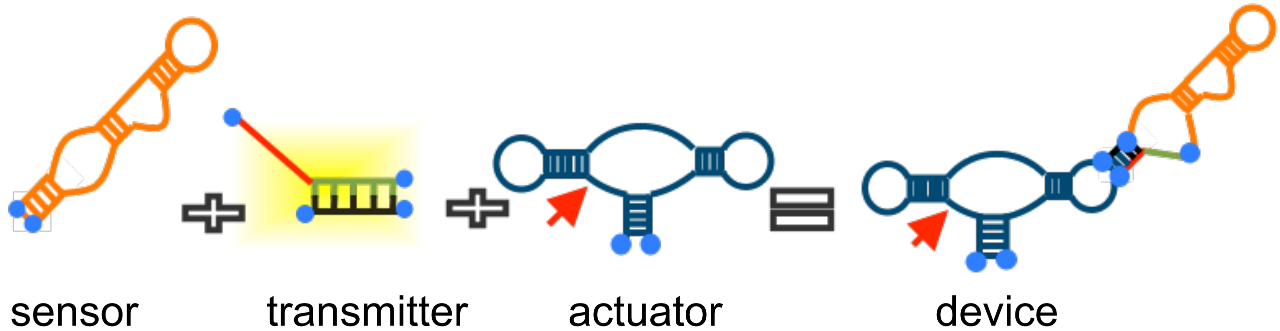
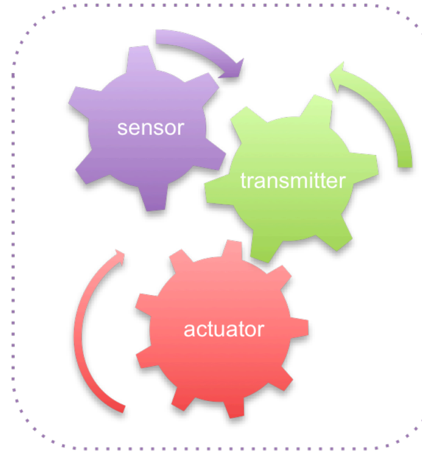
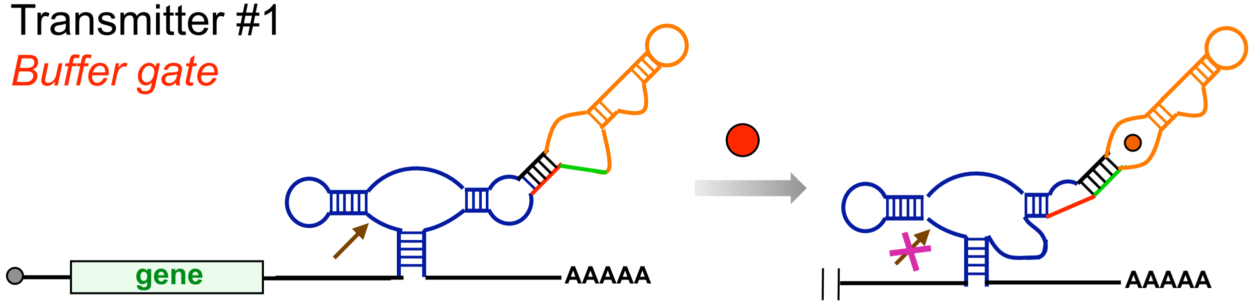
A platform for programming device function

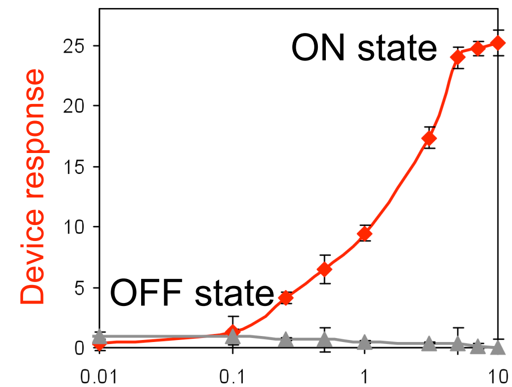


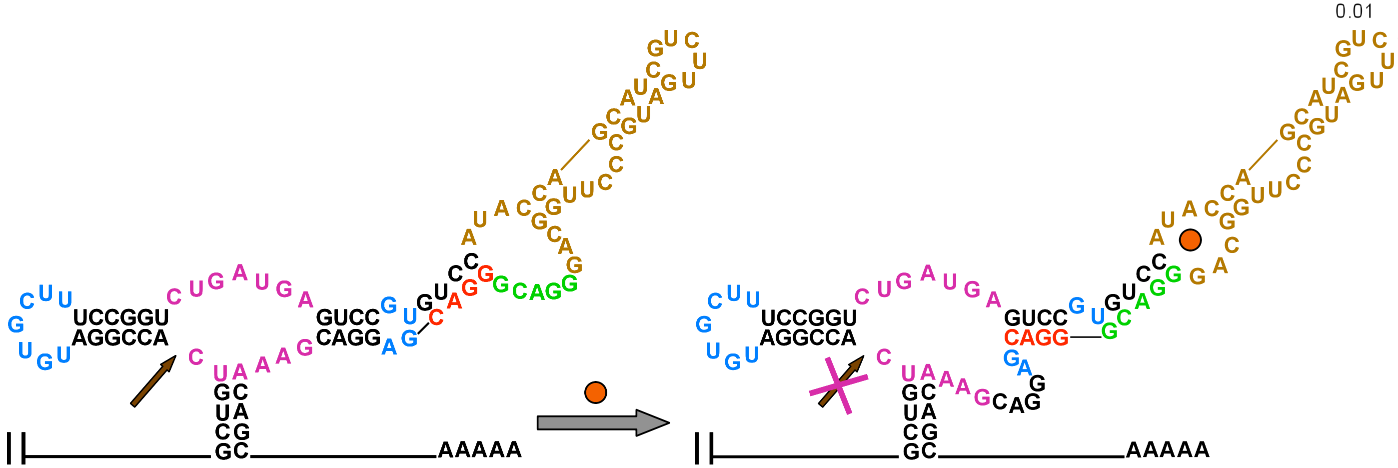


Transmitter #1

Buffer gate







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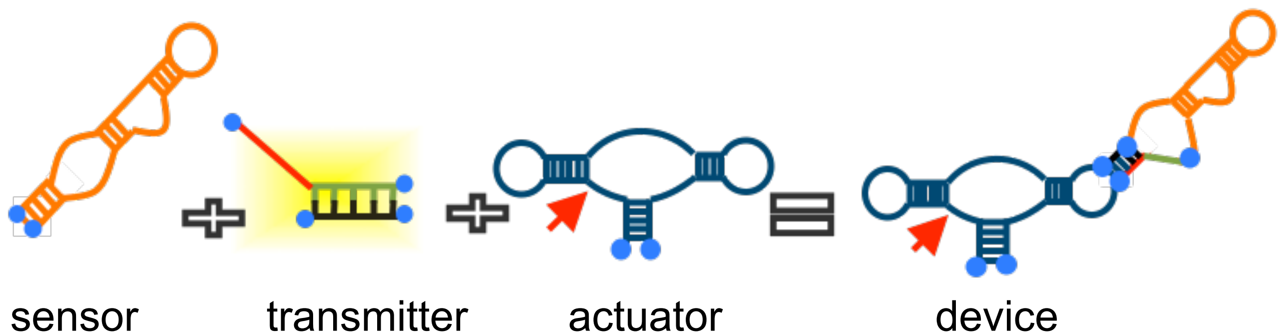
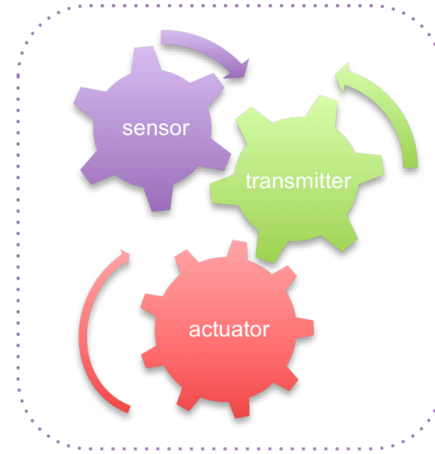
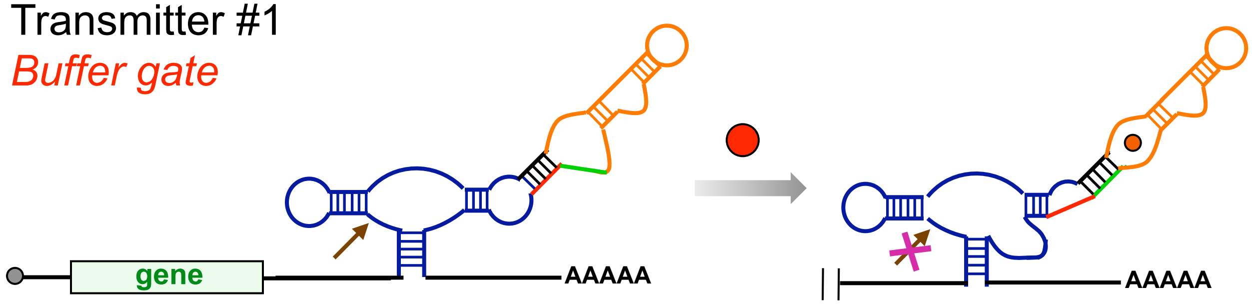
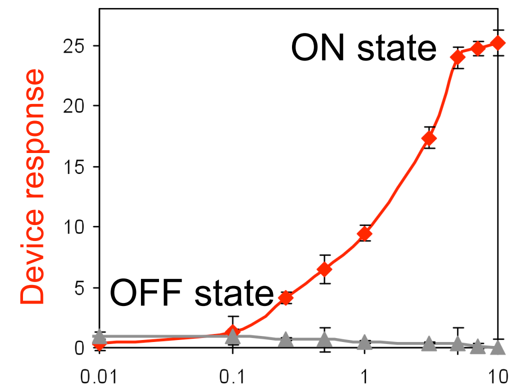
A platform for programming device function



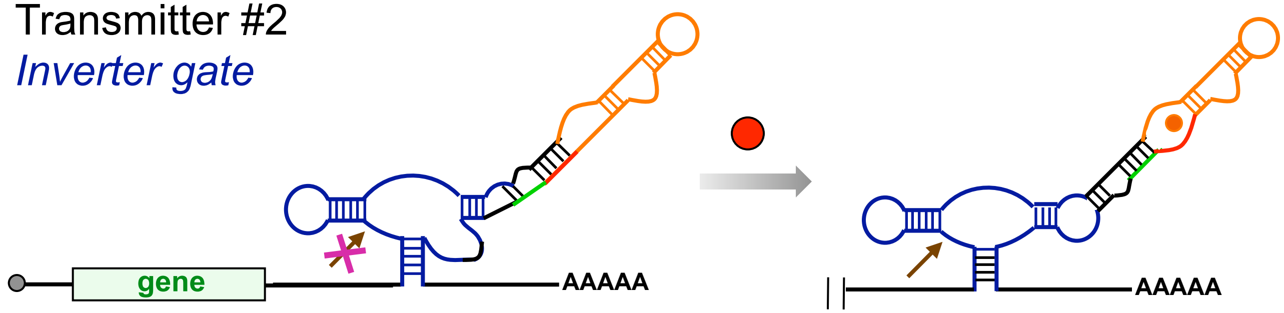


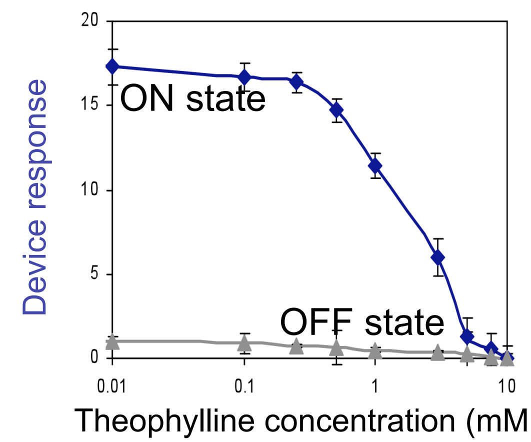
Transmitter #1
Buffer gate





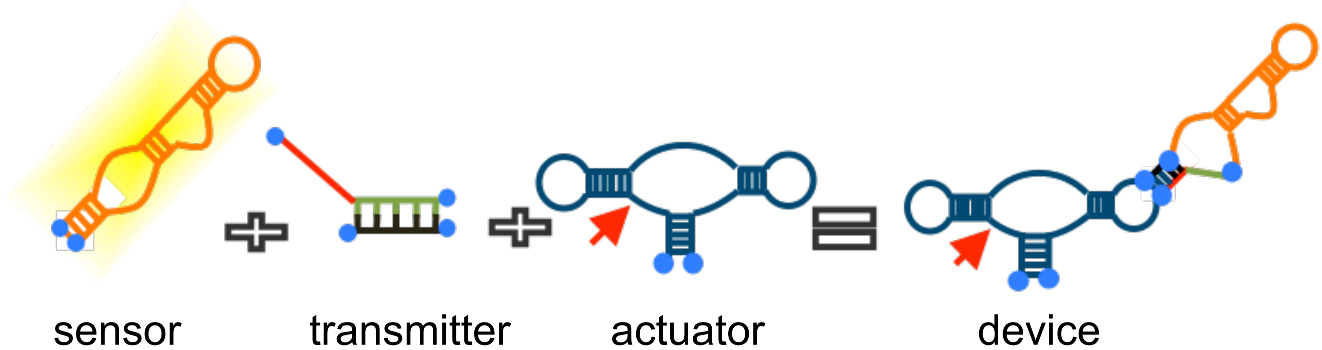
Transmitter #2
Inverter gate

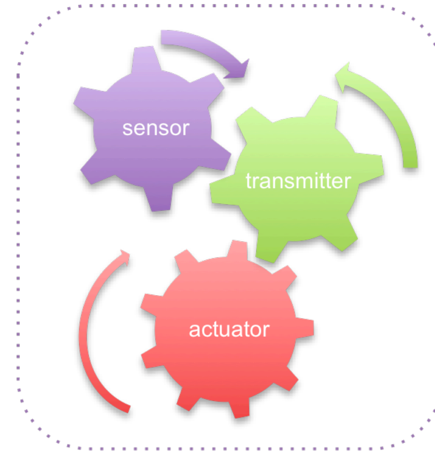




Win MN, Smolke CD. 2007. *PNAS*. 104: 14283-8

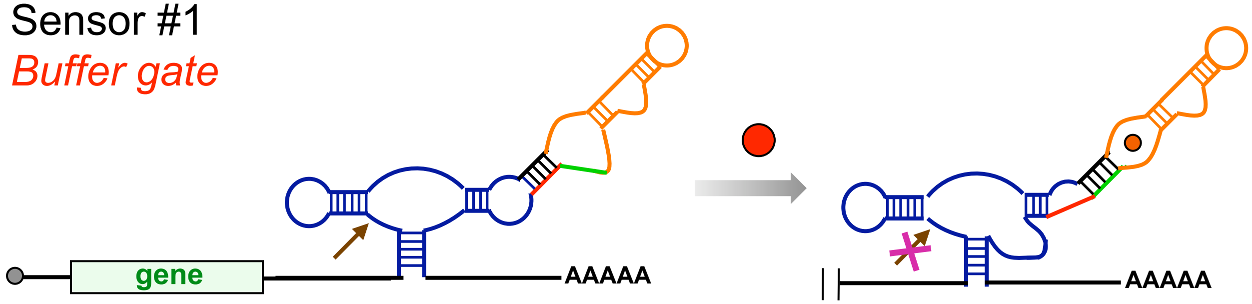
A platform for programming device inputs





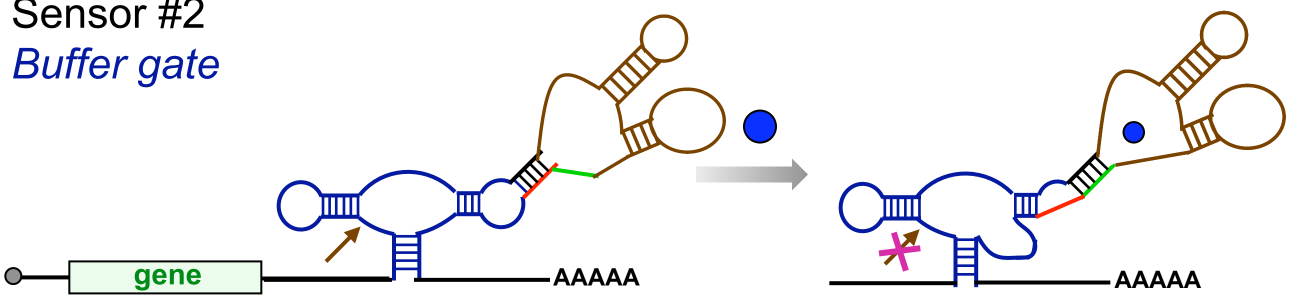
Sensor #1

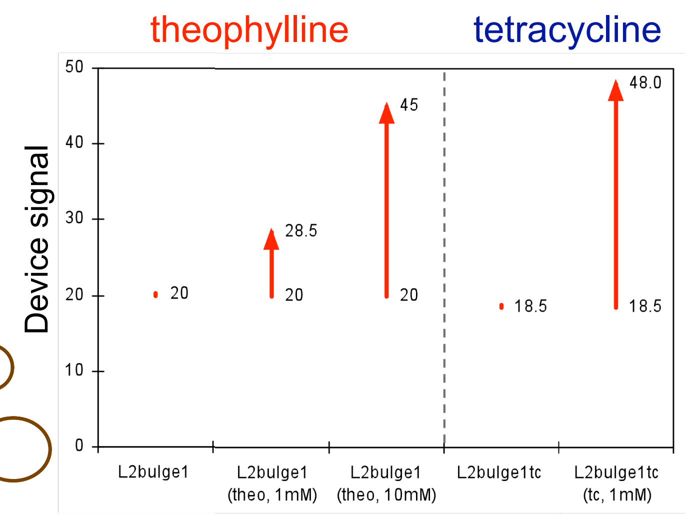
Buffer gate



Sensor #2

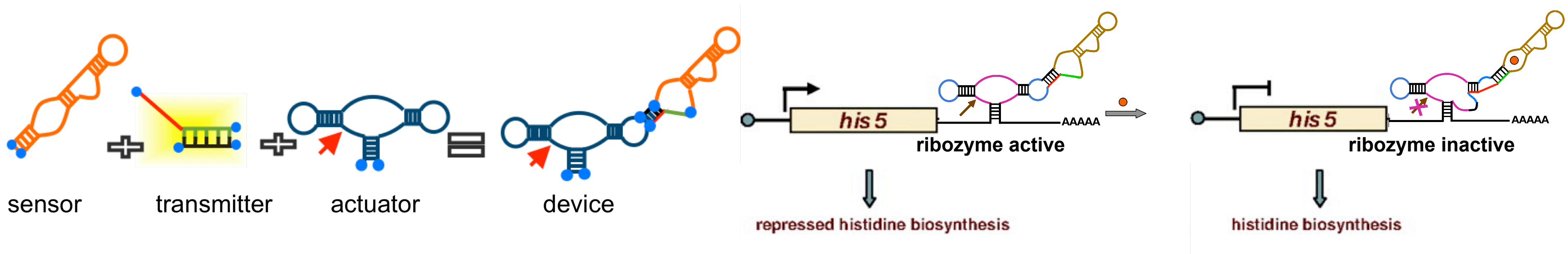
Buffer gate

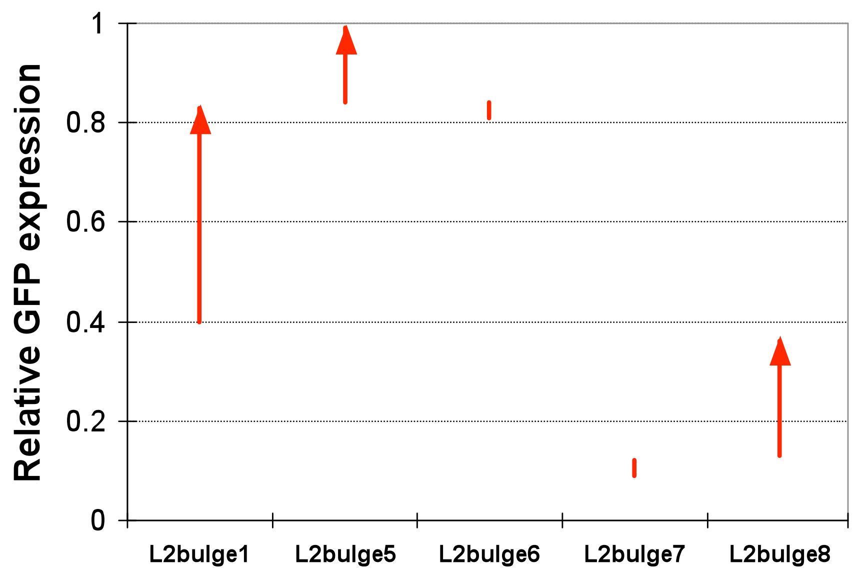


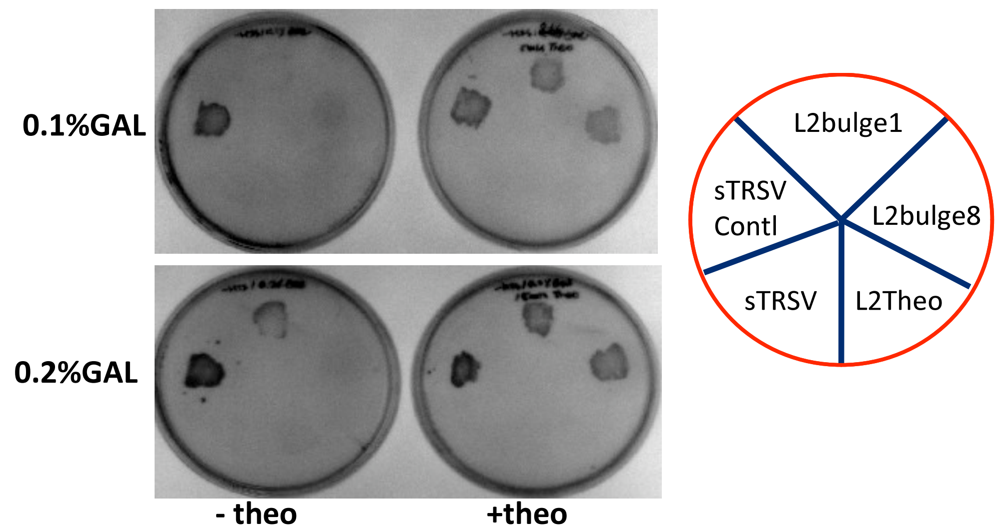


Win MN, Smolke CD. 2007. *PNAS*. 104: 14283-8

A platform for programming response properties





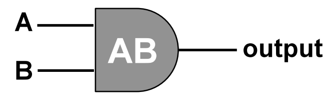


Win MN, Smolke CD. 2007. *PNAS*. 104: 14283-8

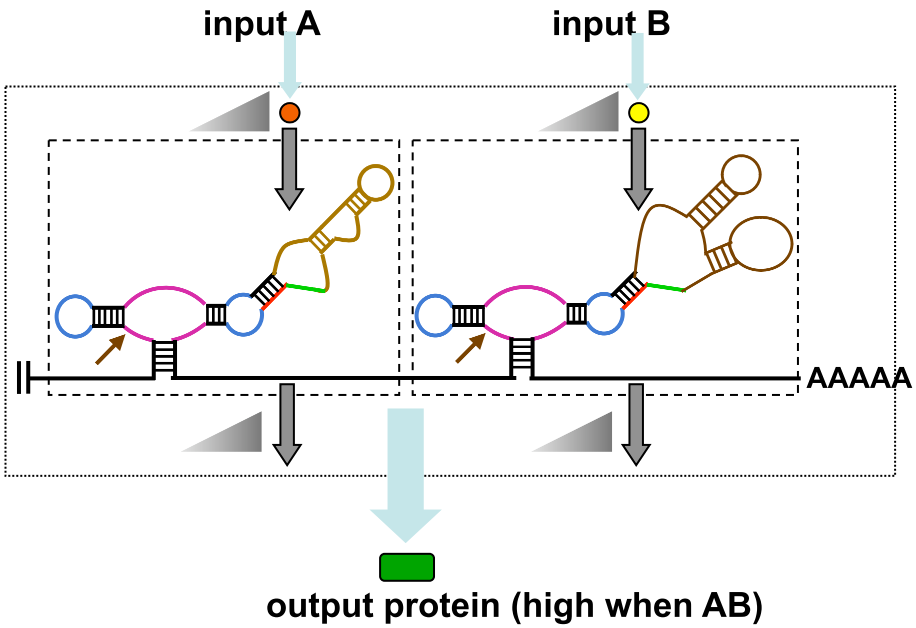
Win MN, Smolke CD. 2008. *Science*. 322: 456-60

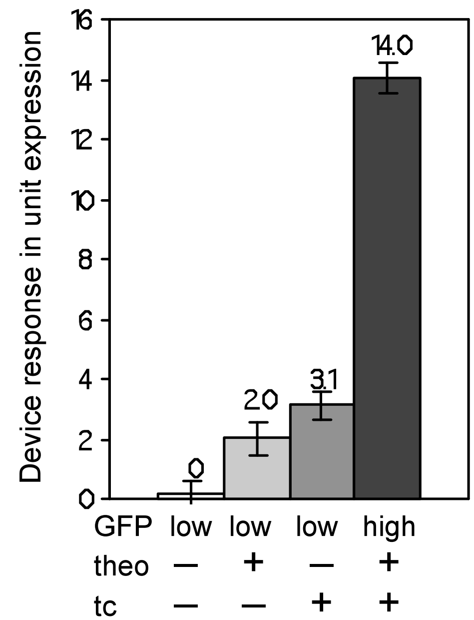
Logic gate devices

AND gate

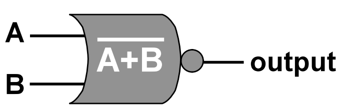


A theo	B tc	output GFP
0	0	0
0	1	0
1	0	0
1	1	1

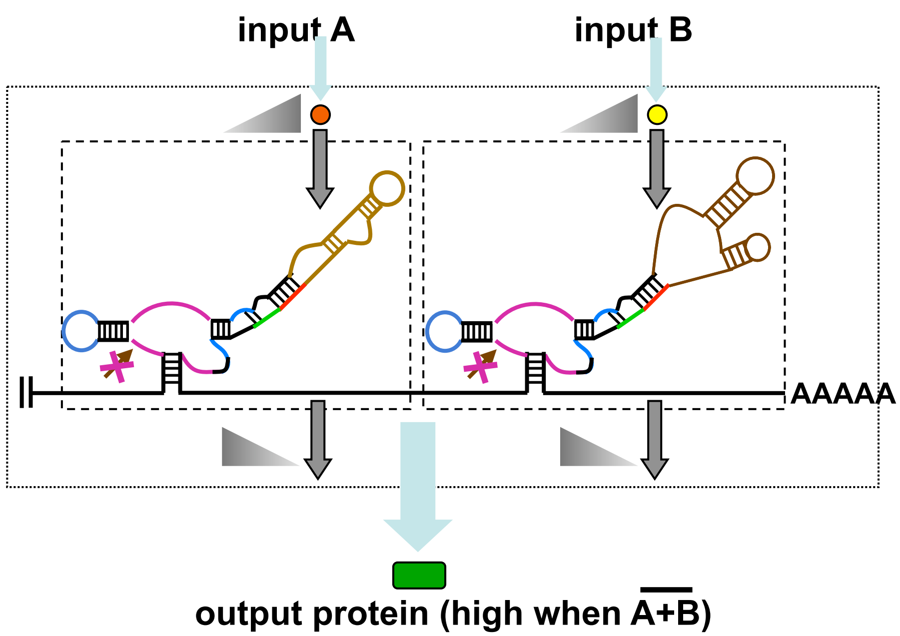


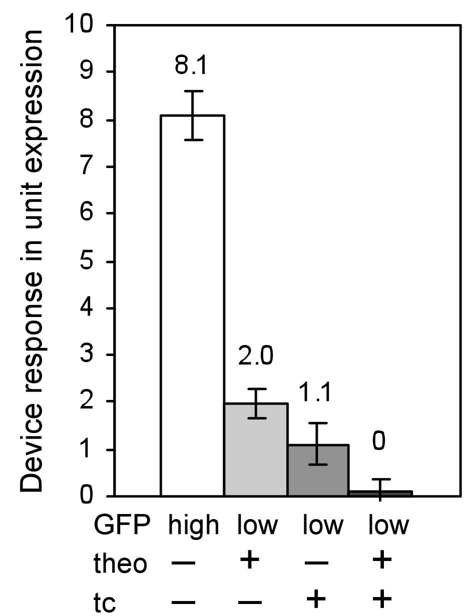


NOR gate

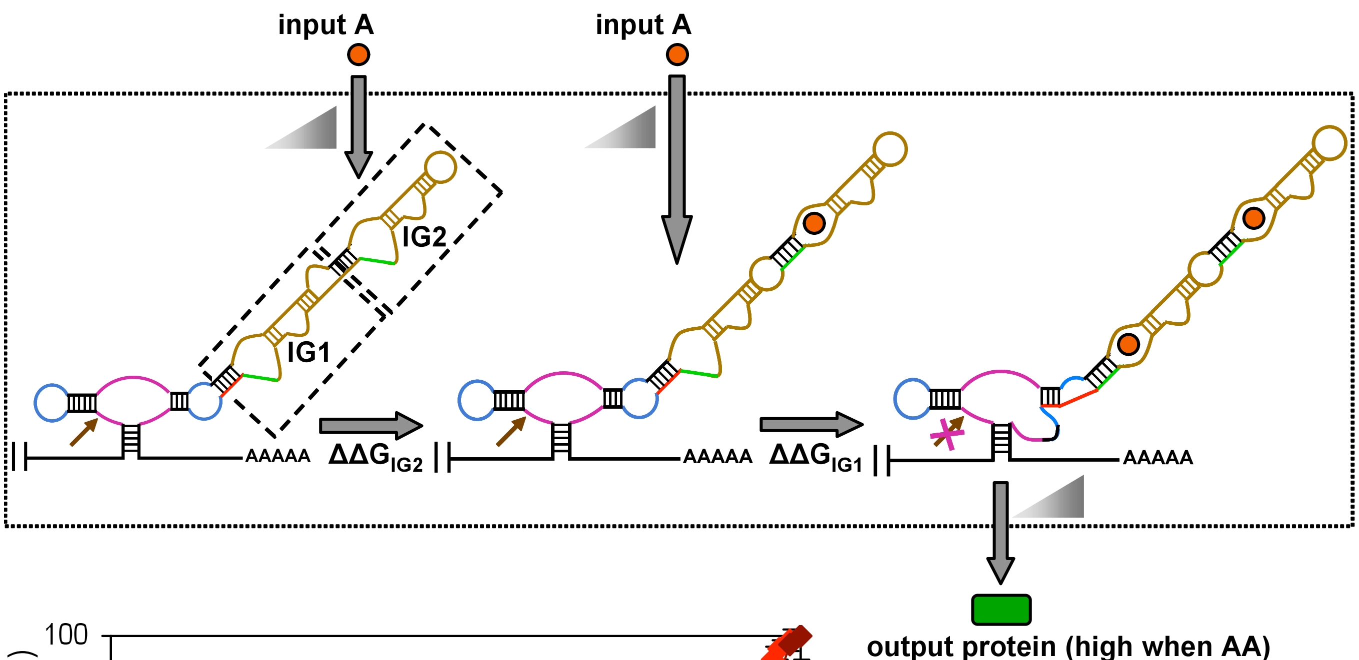


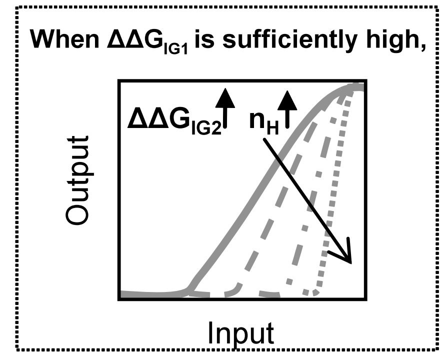
A theo	B tc	output GFP
0	0	1
0	1	0
1	0	0
1	1	0

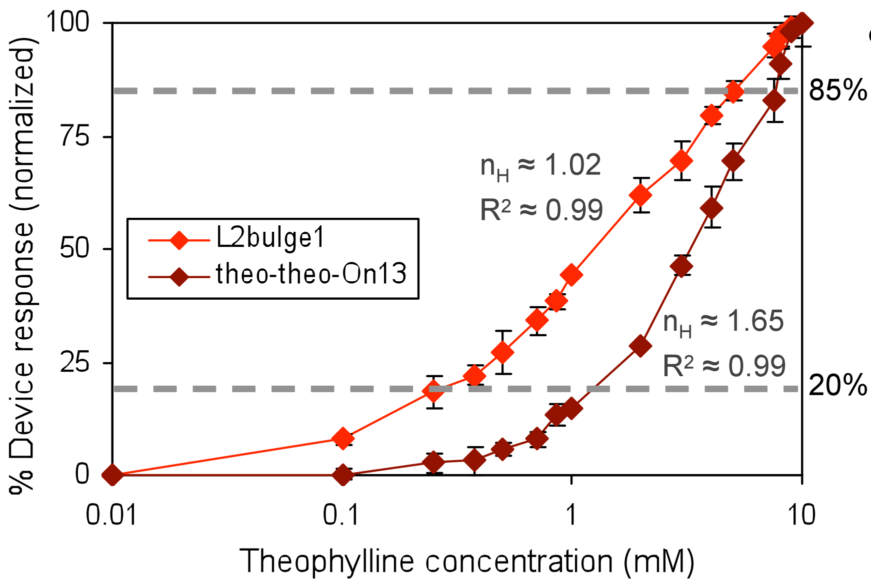




Single gain device – programmed cooperativity

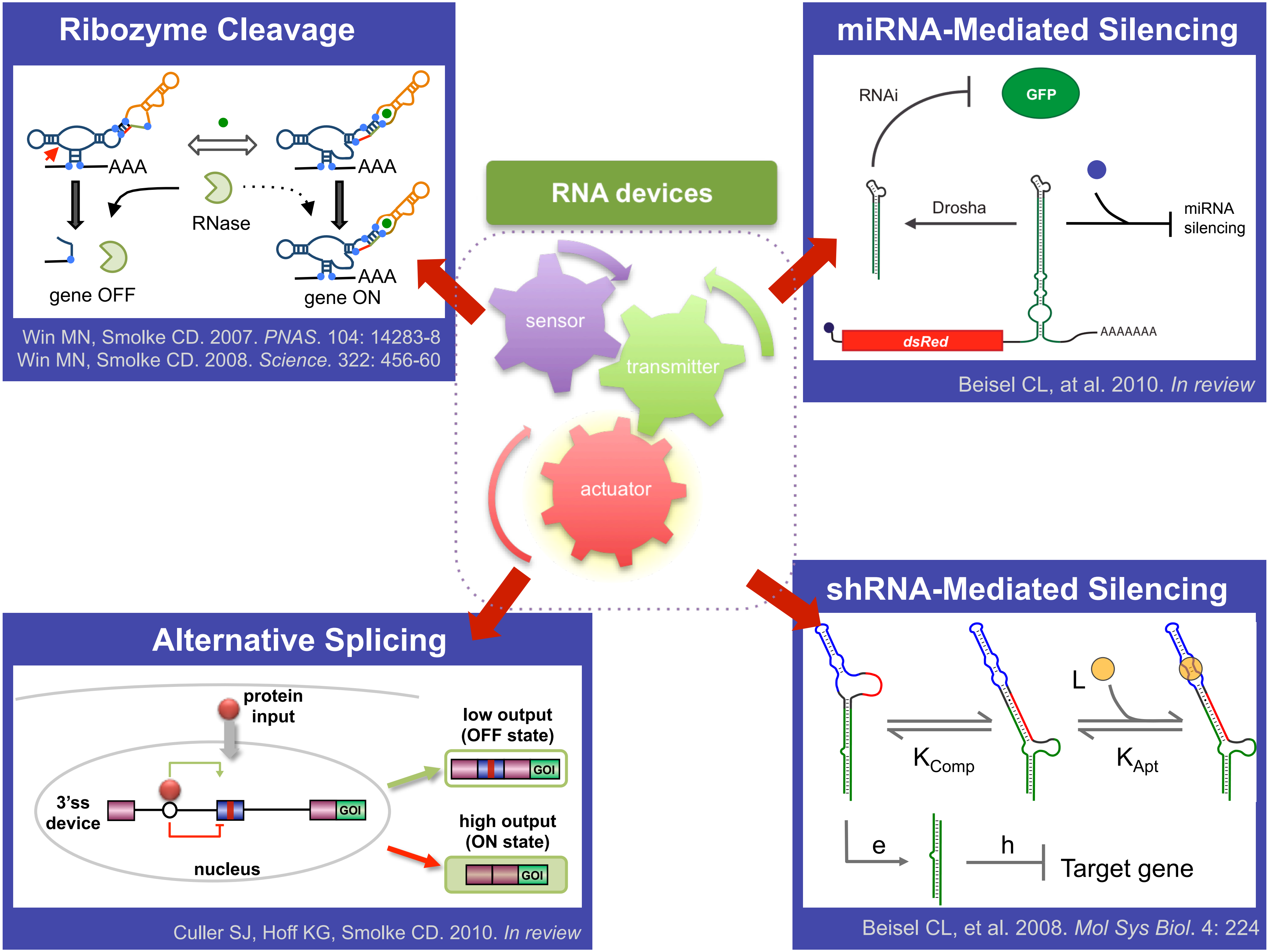


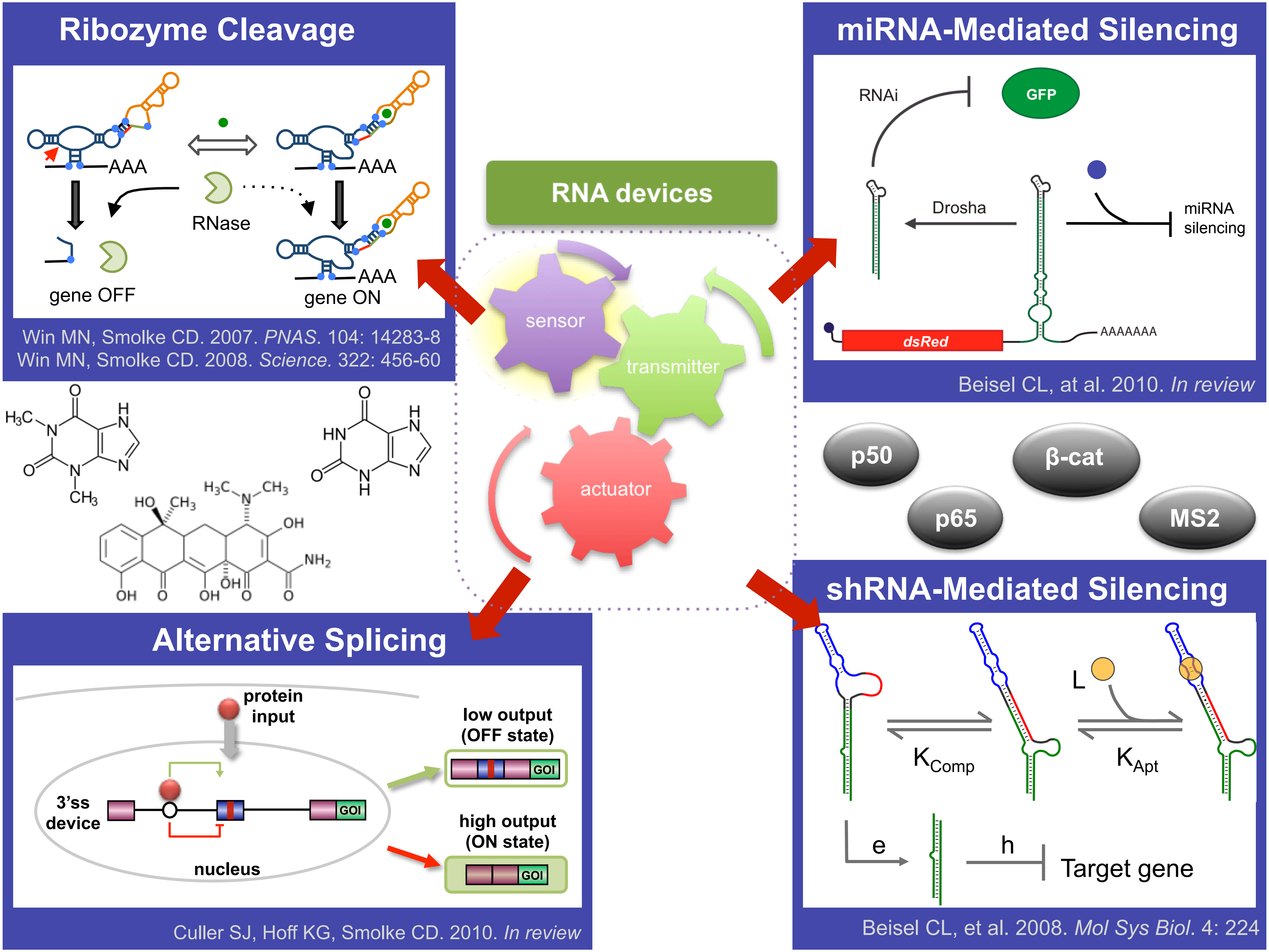




theo-theo-On13 Hill coefficient = 1.65

L2bulge1 Hill coefficient = 1.02


Ribozyme Cleavage
AAA
AAA
RNase
AAA
gene OFF
gene ON
Win MN, Smolke CD. 2007. PNAS. 104: 14283-8
Win MN, Smolke CD. 2008. Science. 322: 456-60
miRNA-Mediated Silencing
RNAi
GFP
Drosha
miRNA silencing
dsRed
AAAAAAA
Beisel CL, at al. 2010. In review
RNA devices
sensor
transmitter
actuator
Alternative Splicing
protein input
low output (OFF state)
GOI
3'ss device
GOI
high output (ON state)
GOI
nucleus
Culler SJ, Hoff KG, Smolke CD. 2010. In review
shRNA-Mediated Silencing
L
K Comp
K Apt
e
h
Target gene
Beisel CL, et al. 2008. Mol Sys Biol. 4: 224



Ribozyme Cleavage
AAA
RNase
AAA
AAA
gene OFF
gene ON
Win MN, Smolke CD. 2007. PNAS. 104: 14283-8
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RNA devices
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RNAi
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dsRed
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Beisel CL, at al. 2010. In review
p50
β-cat
p65
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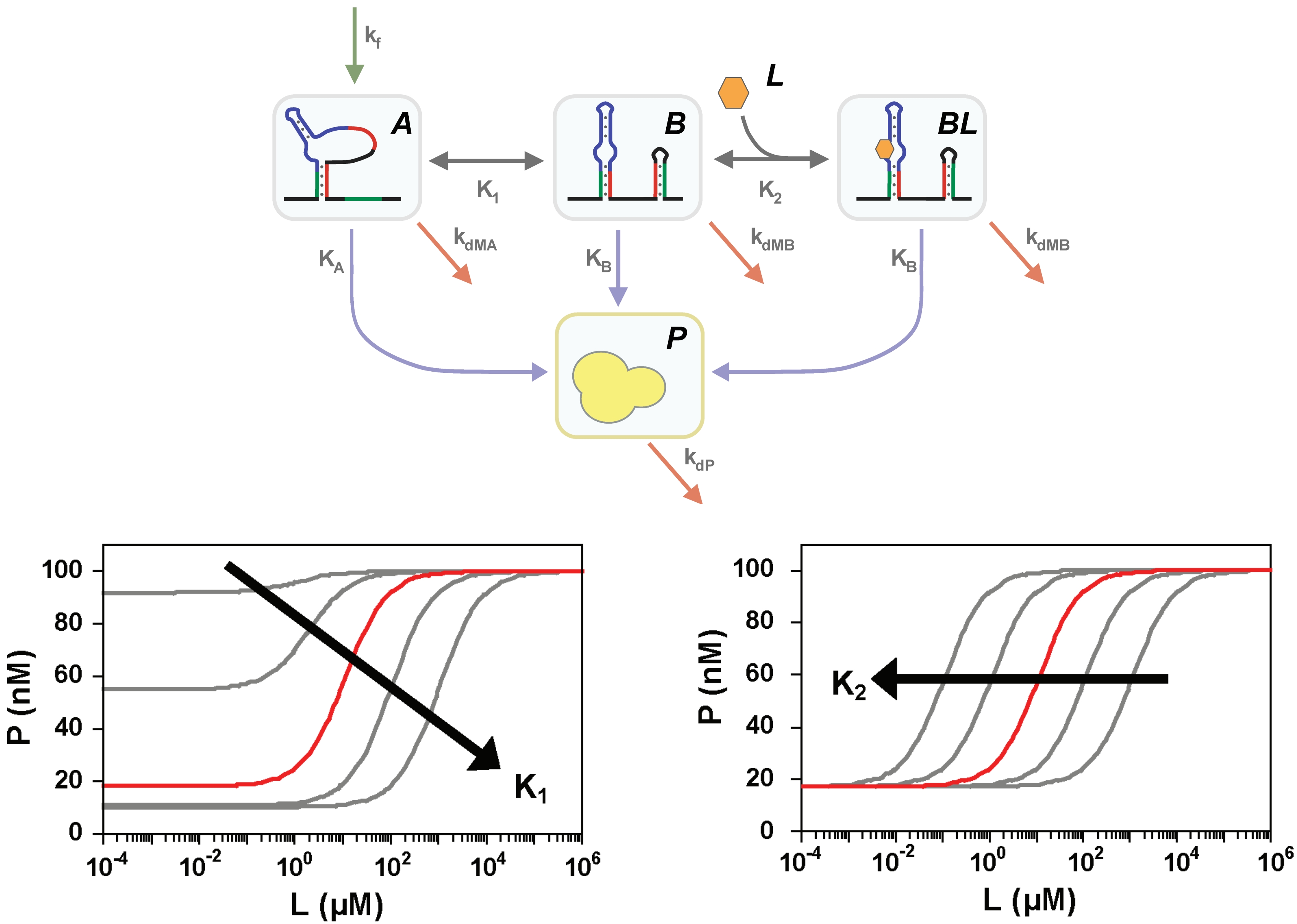
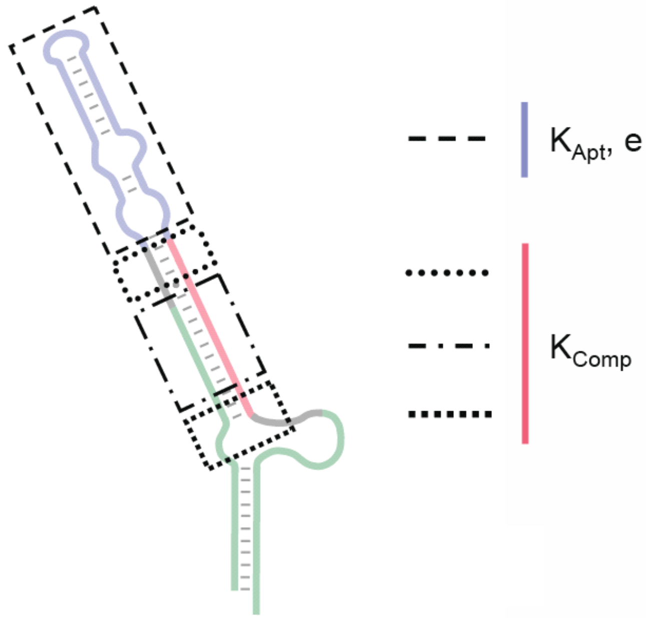
L
K_Comp
K_Apt
e
h
Target gene
Beisel CL, et al. 2008. Mol Sys Biol. 4: 224

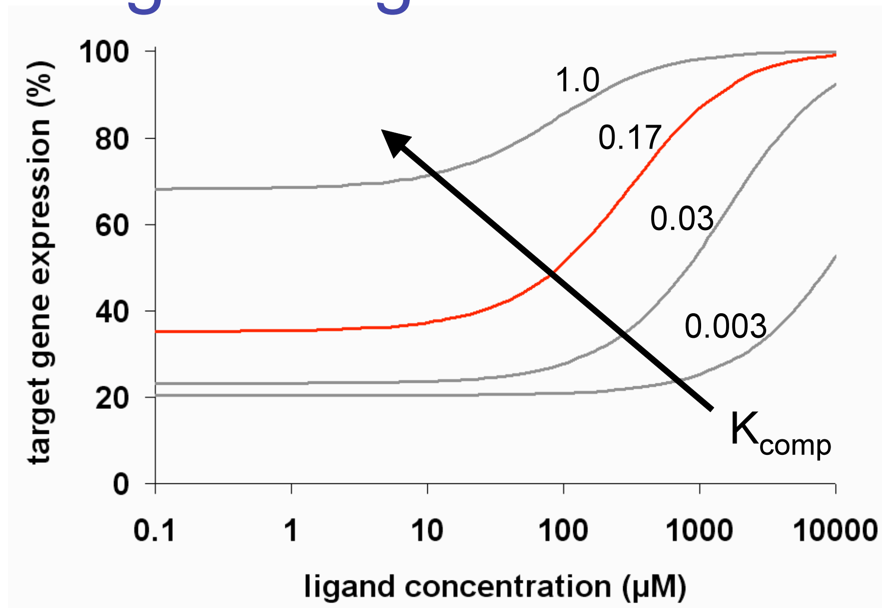
Computational models can predict parameter effects



Beisel CL, Smolke CD. 2009. *PLoS Comp Biol*. 5: e1000363

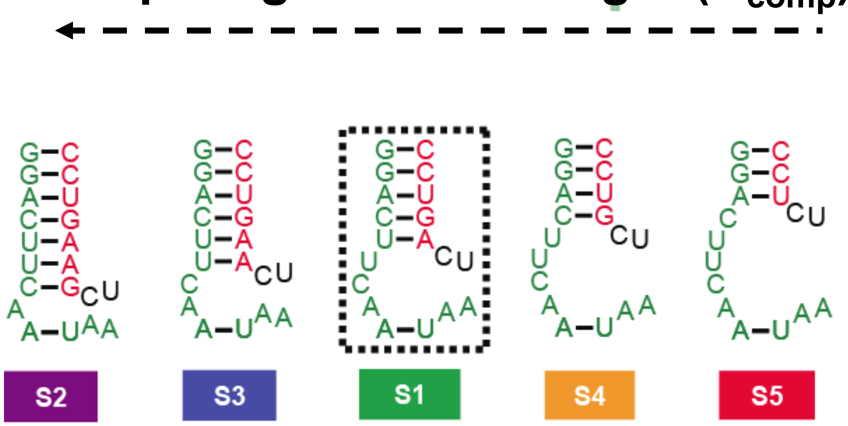
Models predict transmitter tuning strategies

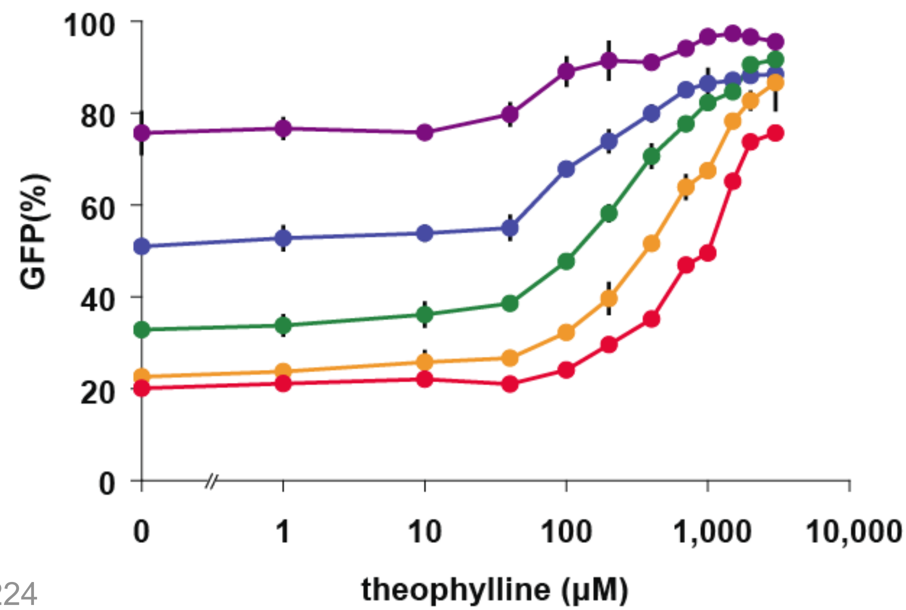




Construction and experimental validation

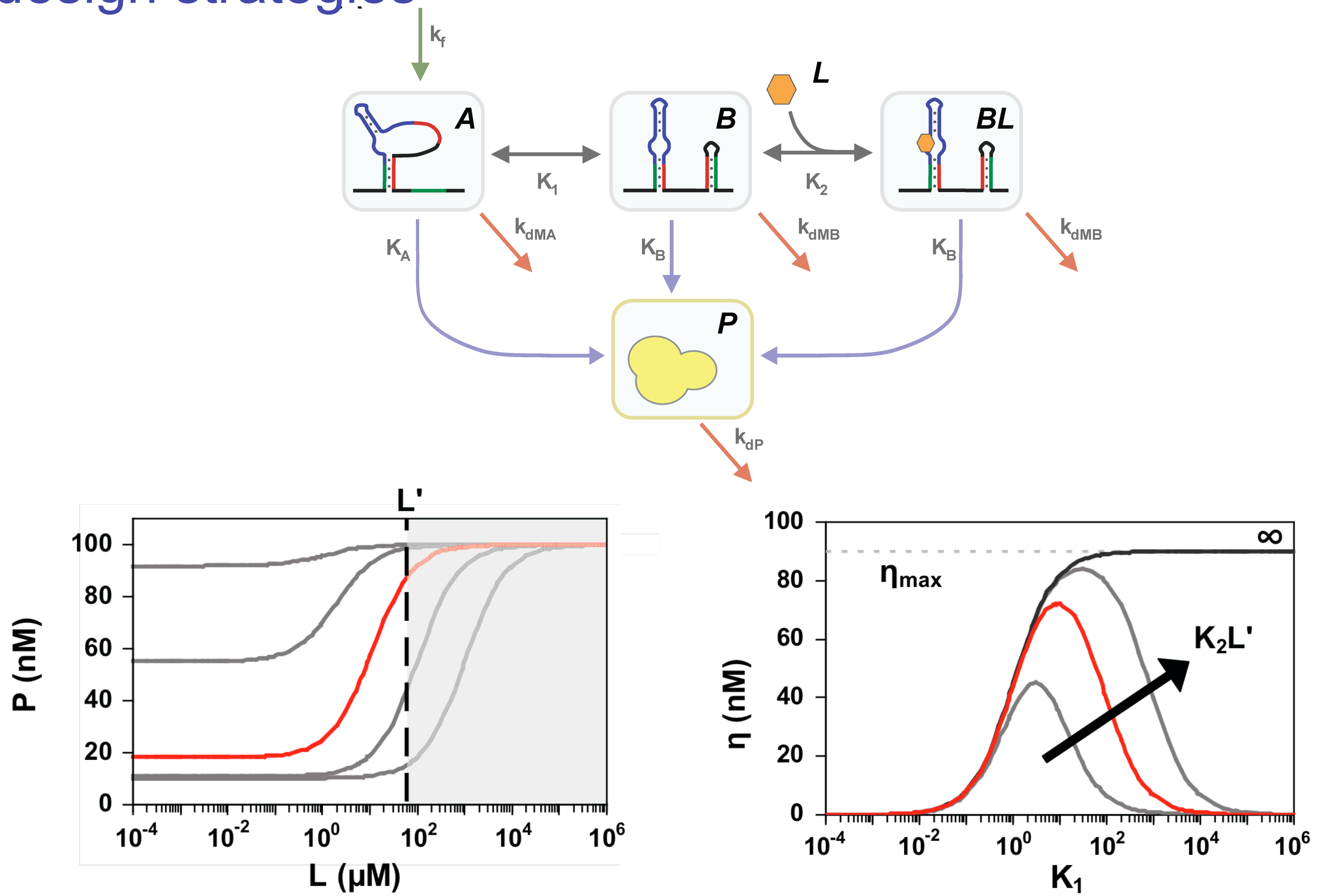
Competing strand 3' length (K_{comp})





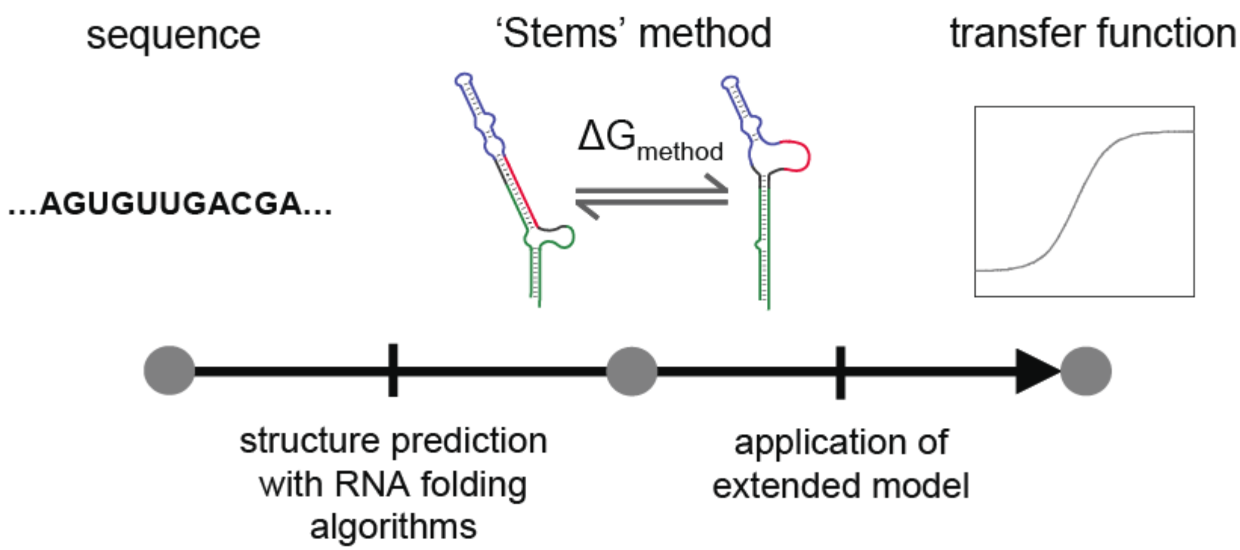
Beisel CL, Bayer TS, Hoff KH, Smolke CD. 2008. *Mol Sys Biol*. 4: 224

System restrictions lead to optimization in RNA device design strategies

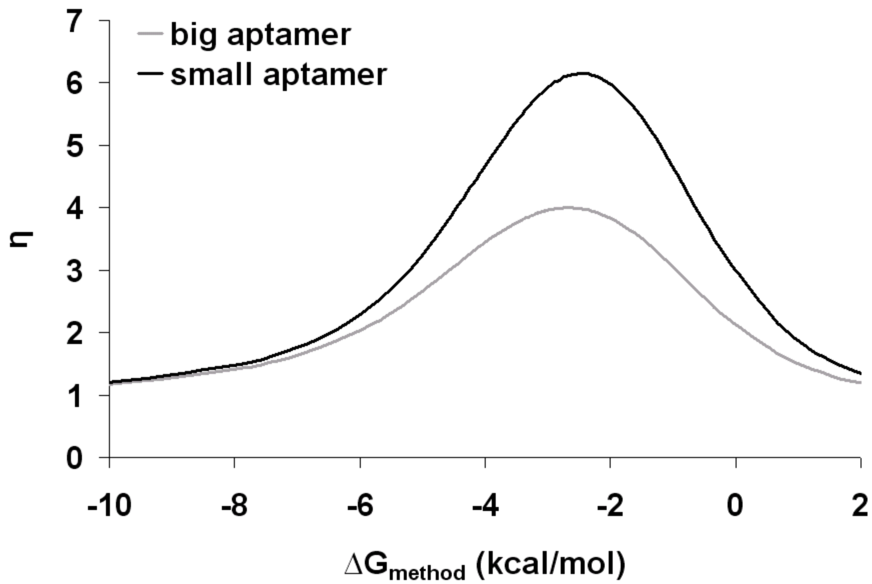


Beisel CL, Smolke CD. 2009. *PLoS Comp Biol*. 5: e1000363

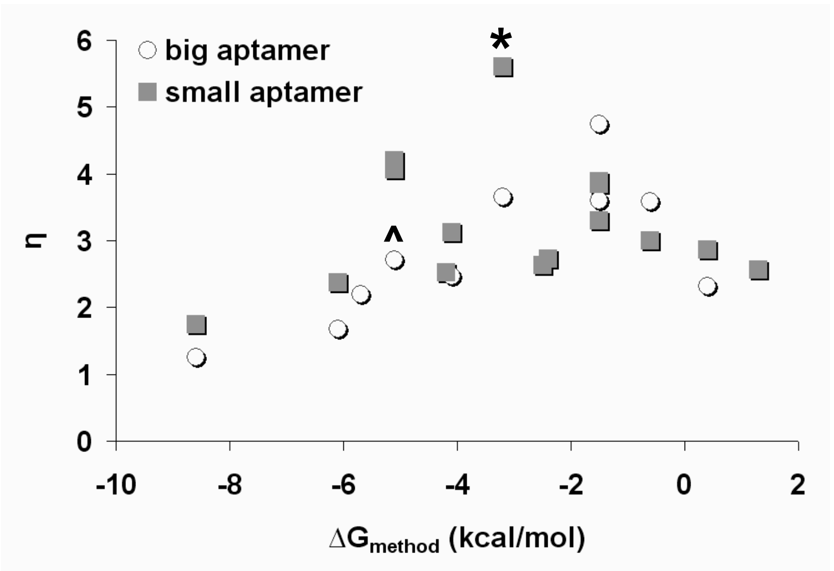
A framework for forward engineering device response



Model predictions

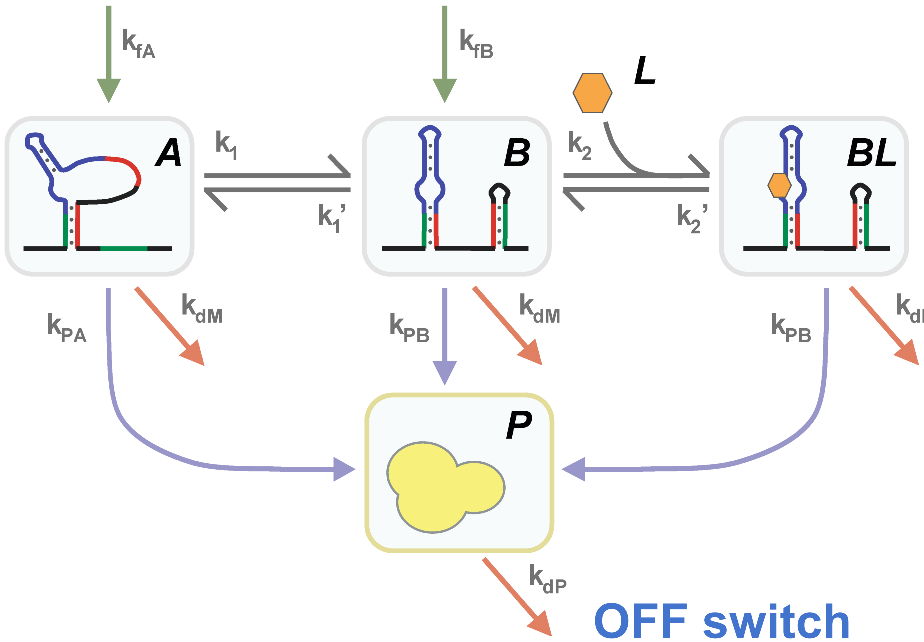


Experimental results

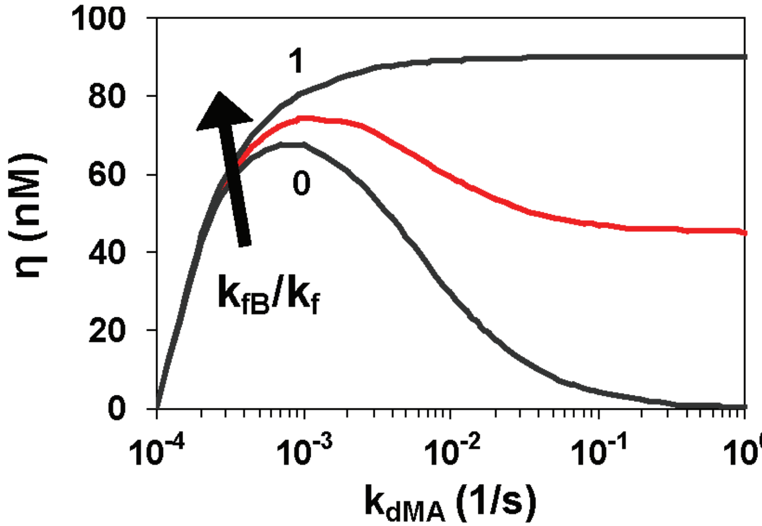


Beisel CL, Bayer TS, Hoff KH, Smolke CD. 2008. *Mol Sys Biol*. 4: 224

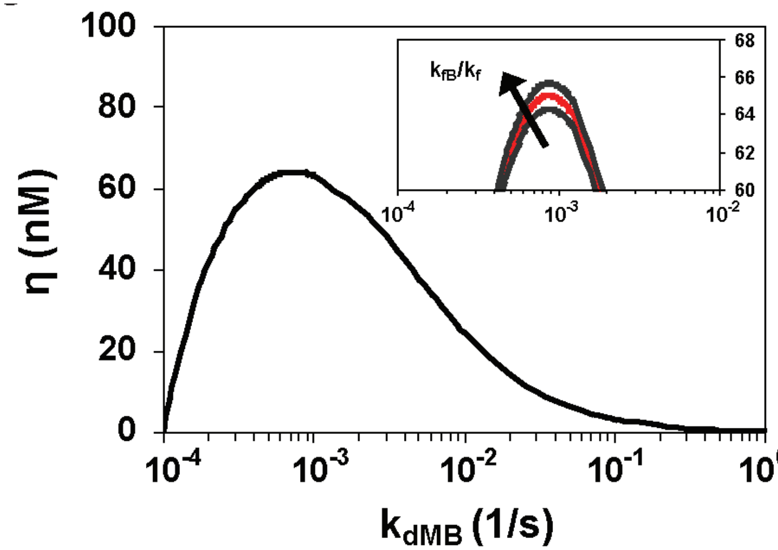
Irreversible rates can play an important role in RNA device performance



ON switch

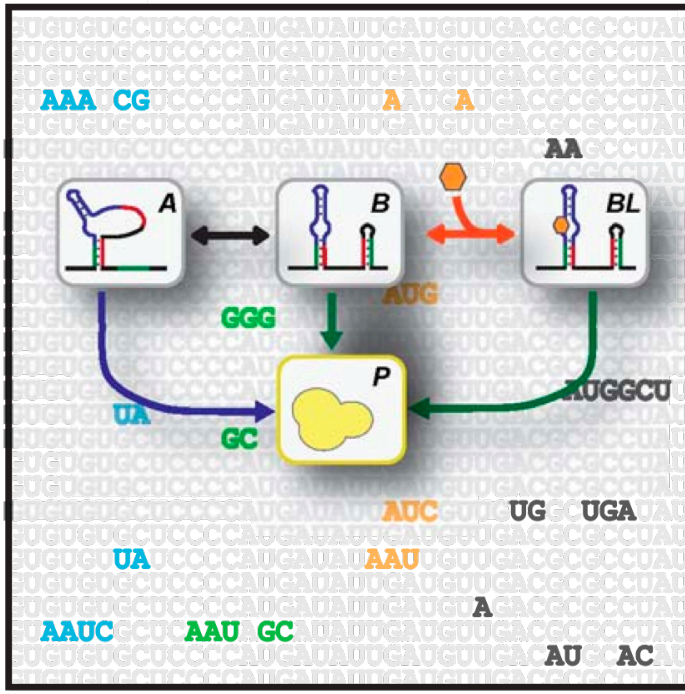


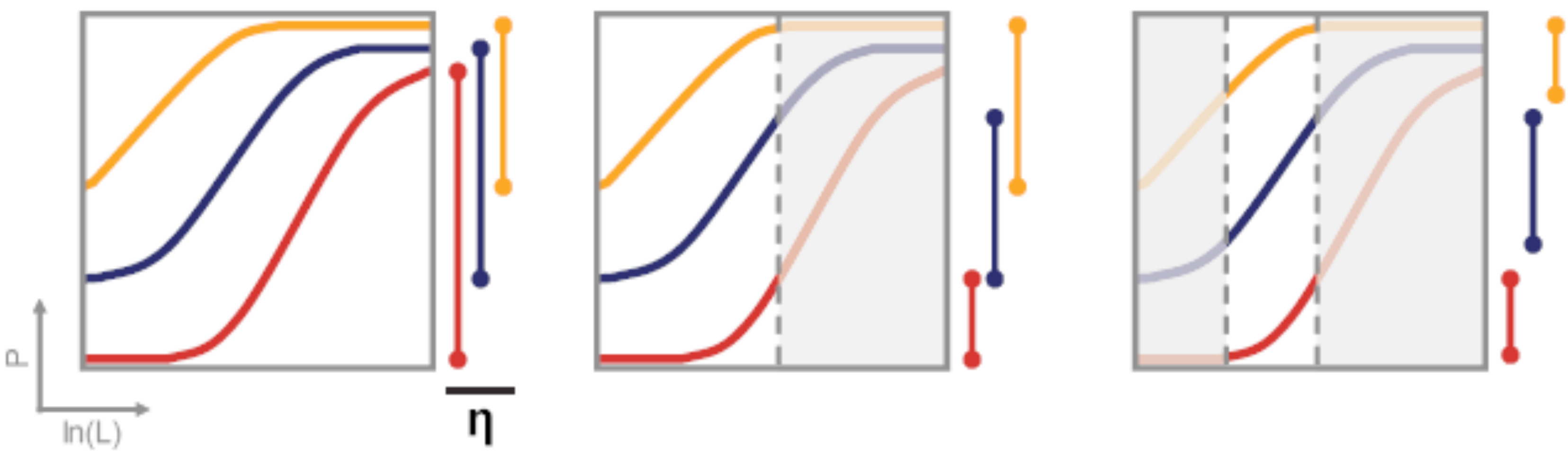
OFF switch



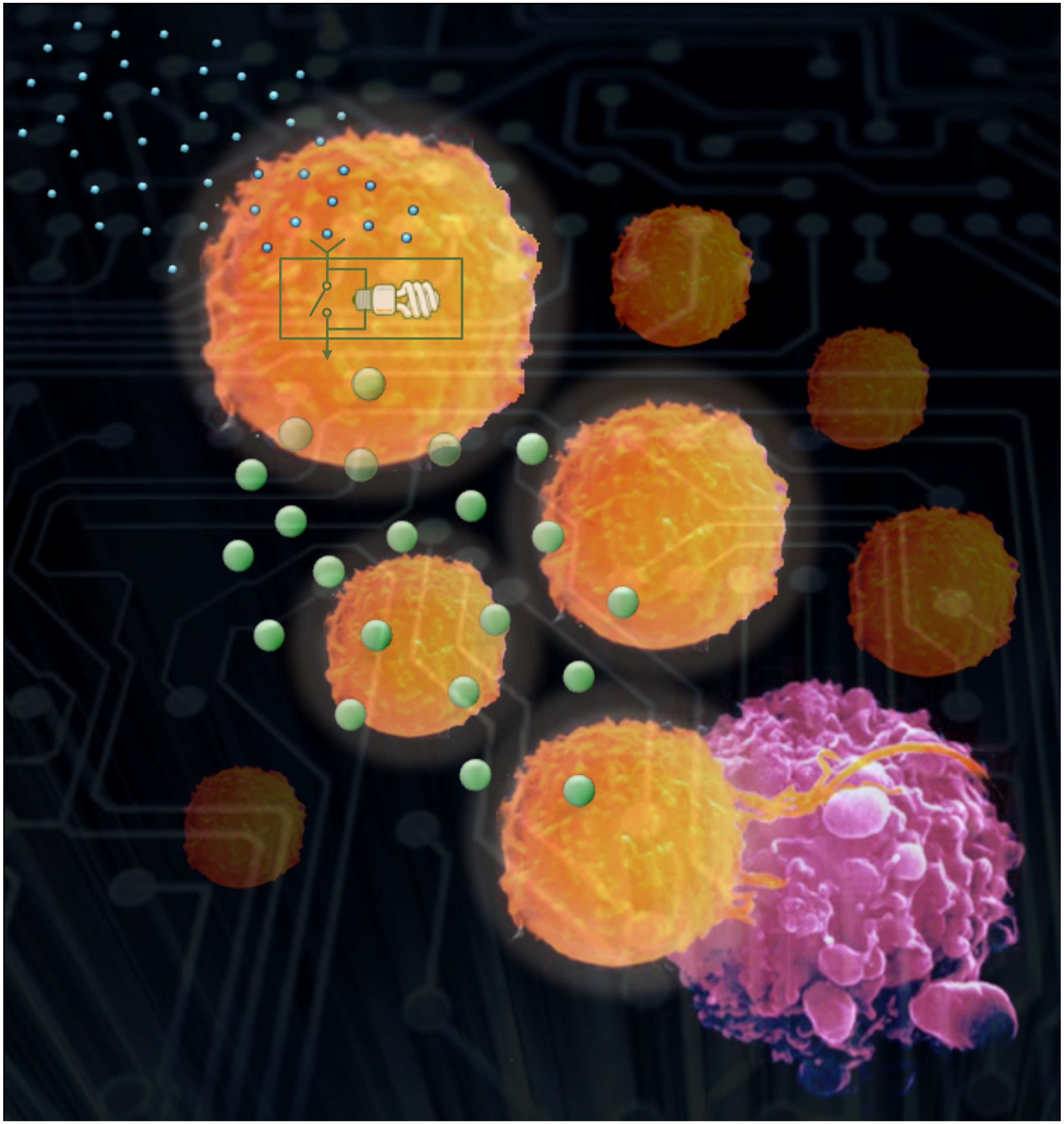
Beisel CL, Smolke CD. 2009. *PLoS Comp Biol*. 5: e1000363

Quantitative tuning of device response



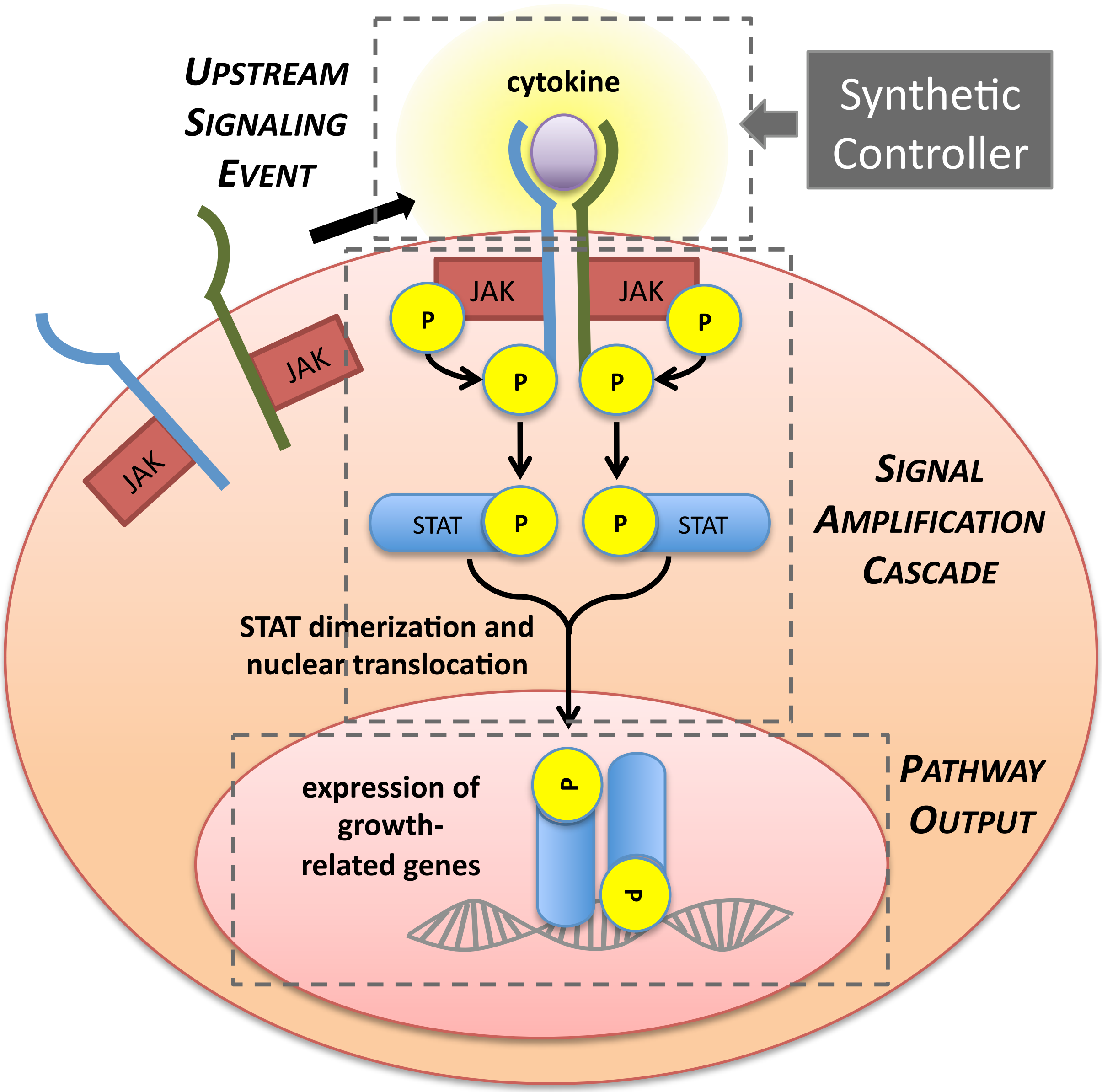


Advancing cell-based therapies

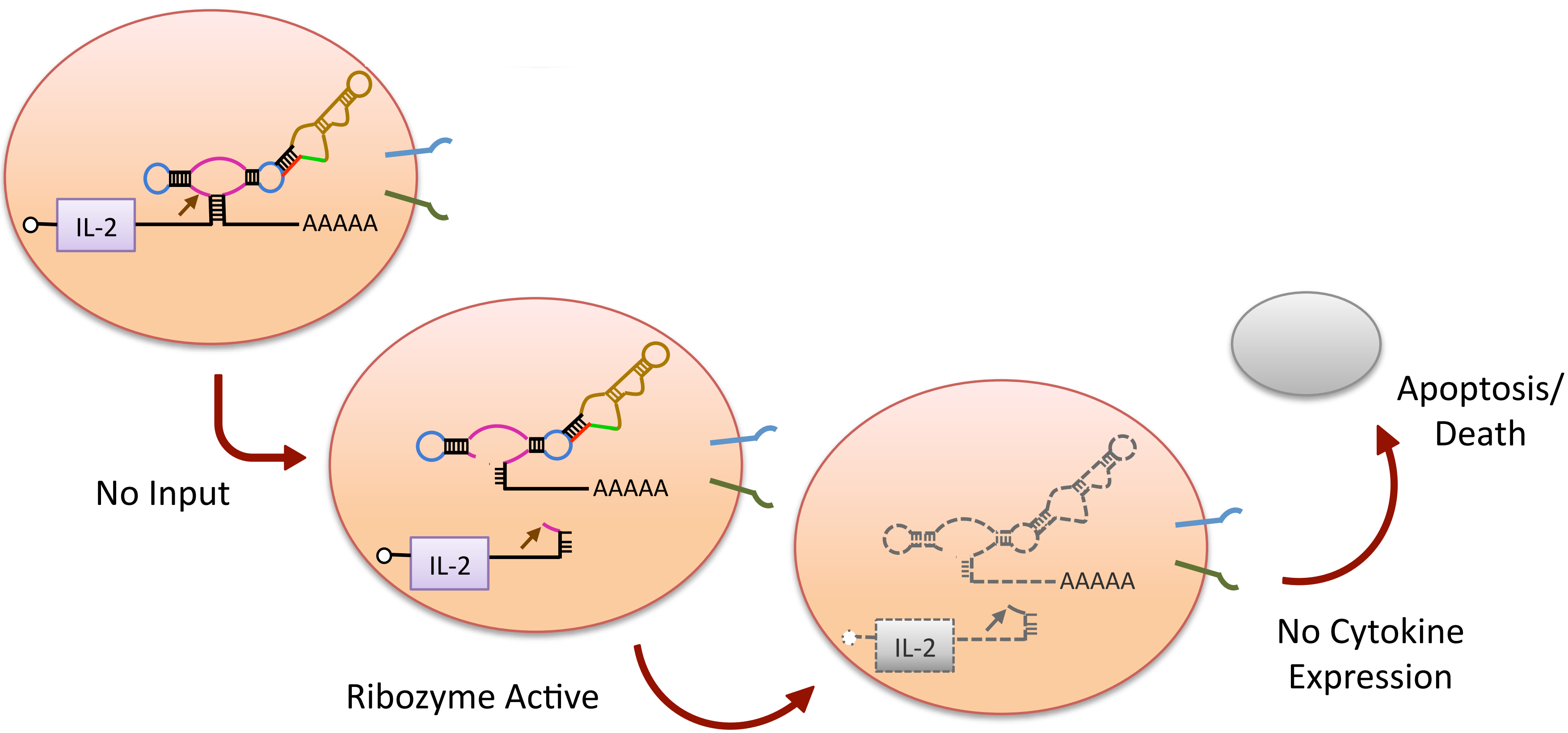


Chen YY, Jensen MC, Smolke CD. 2010. *PNAS.* 107: 8531-6

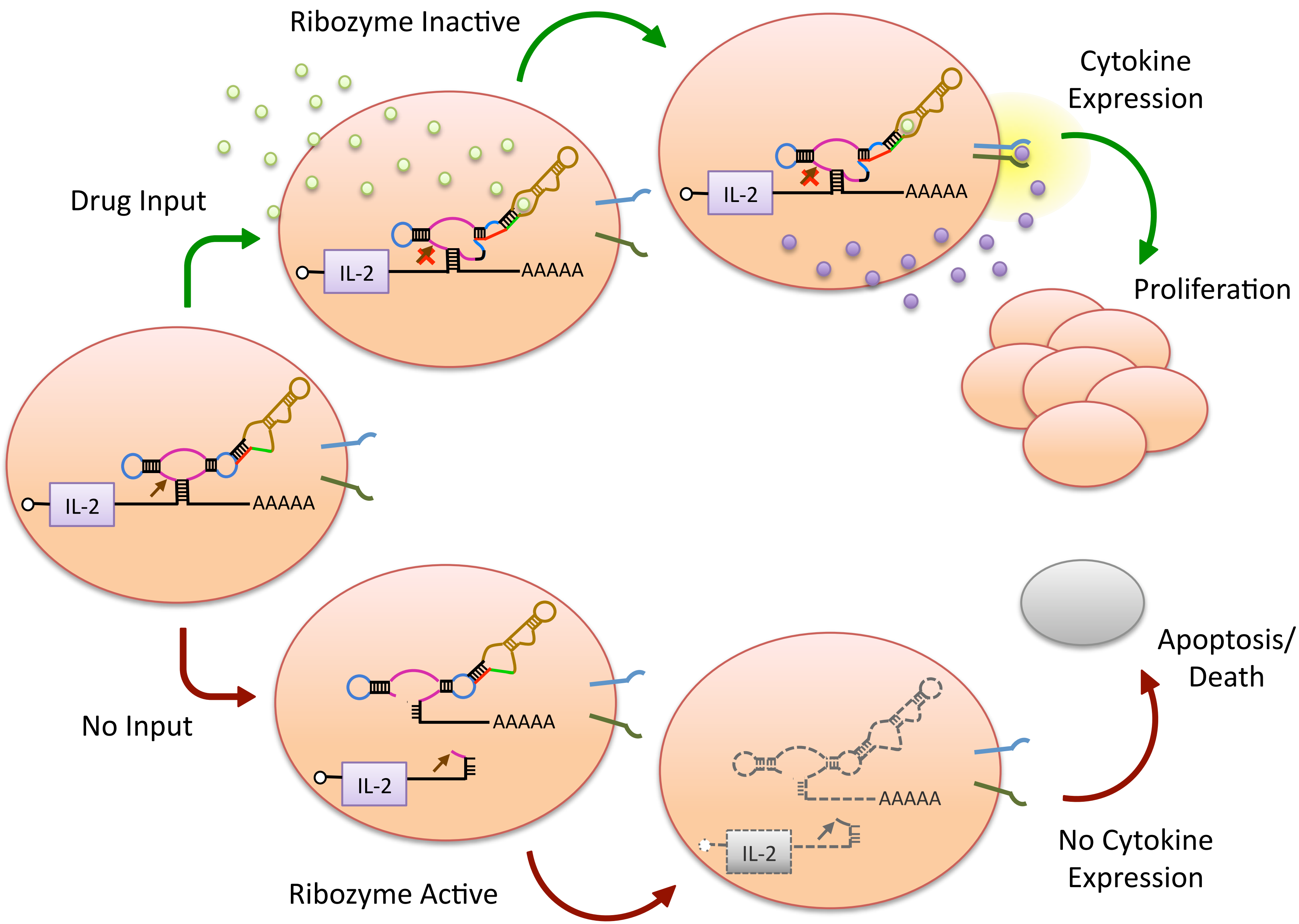
T-cell proliferation pathway



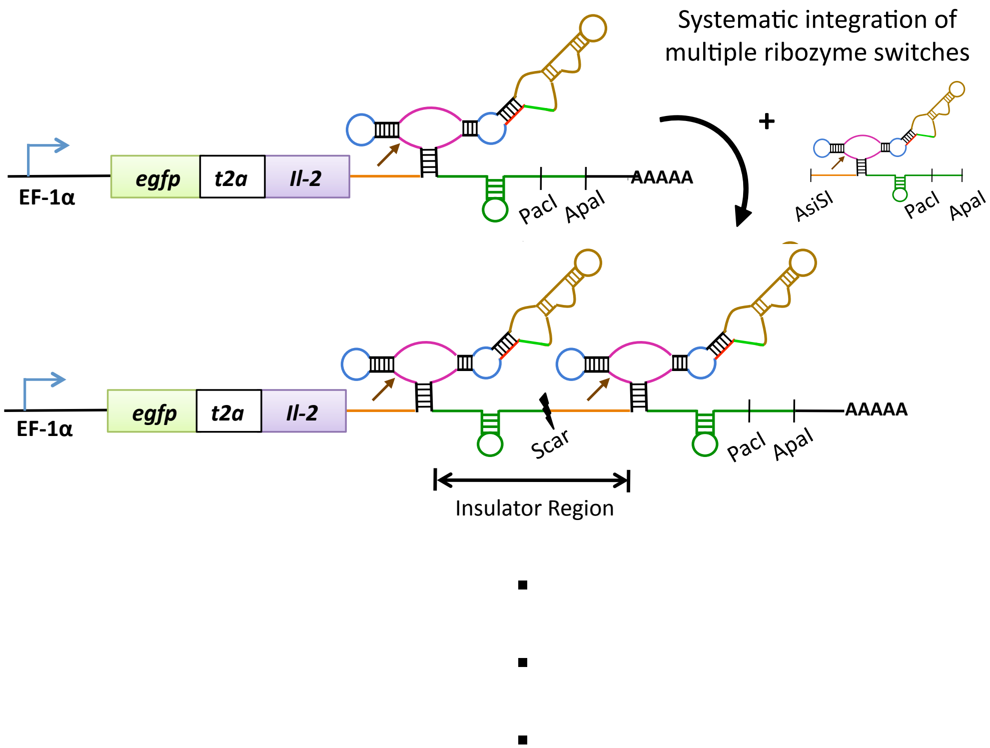
A synthetic T-cell regulatory network

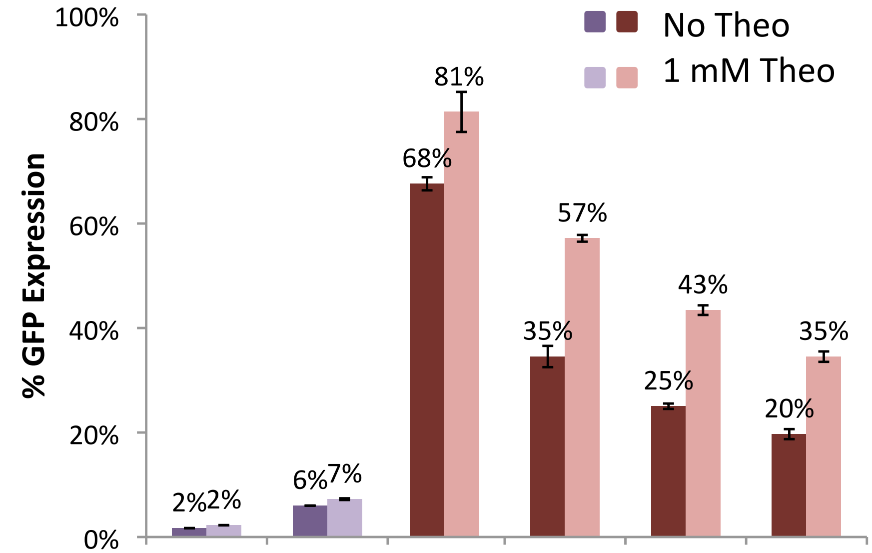


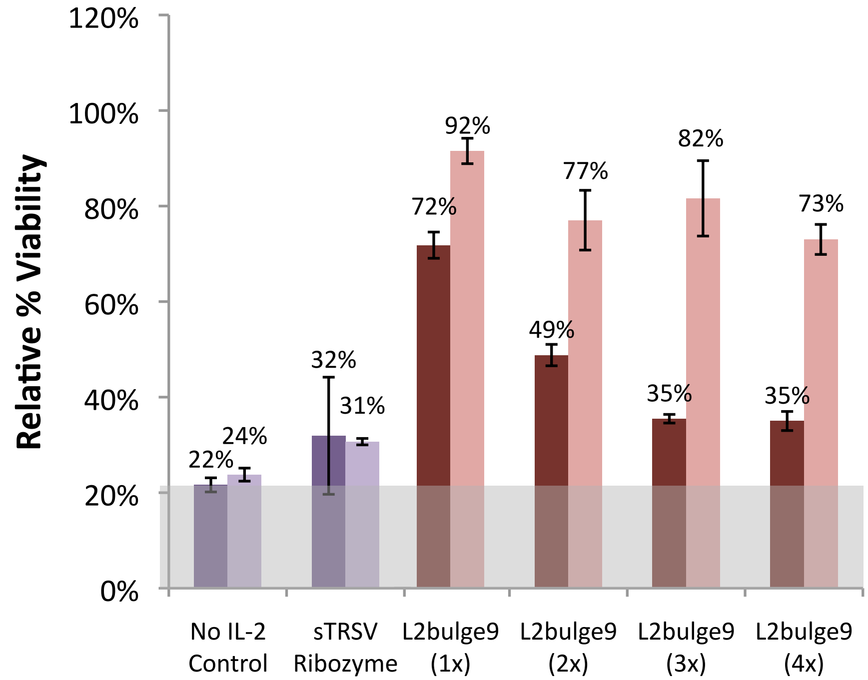
A synthetic T-cell regulatory network



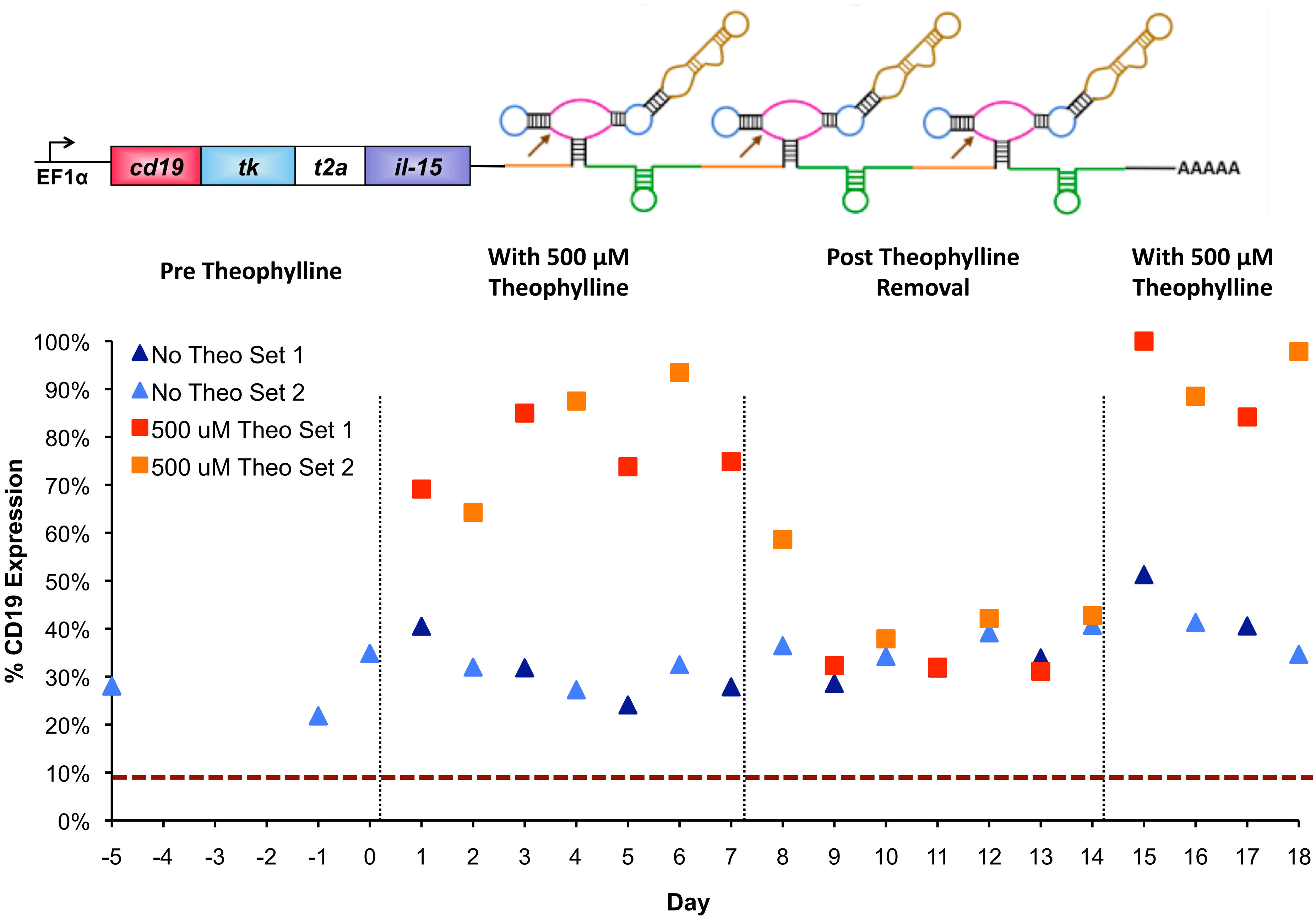
Rapid prototyping and optimization of a T-cell proliferation control system



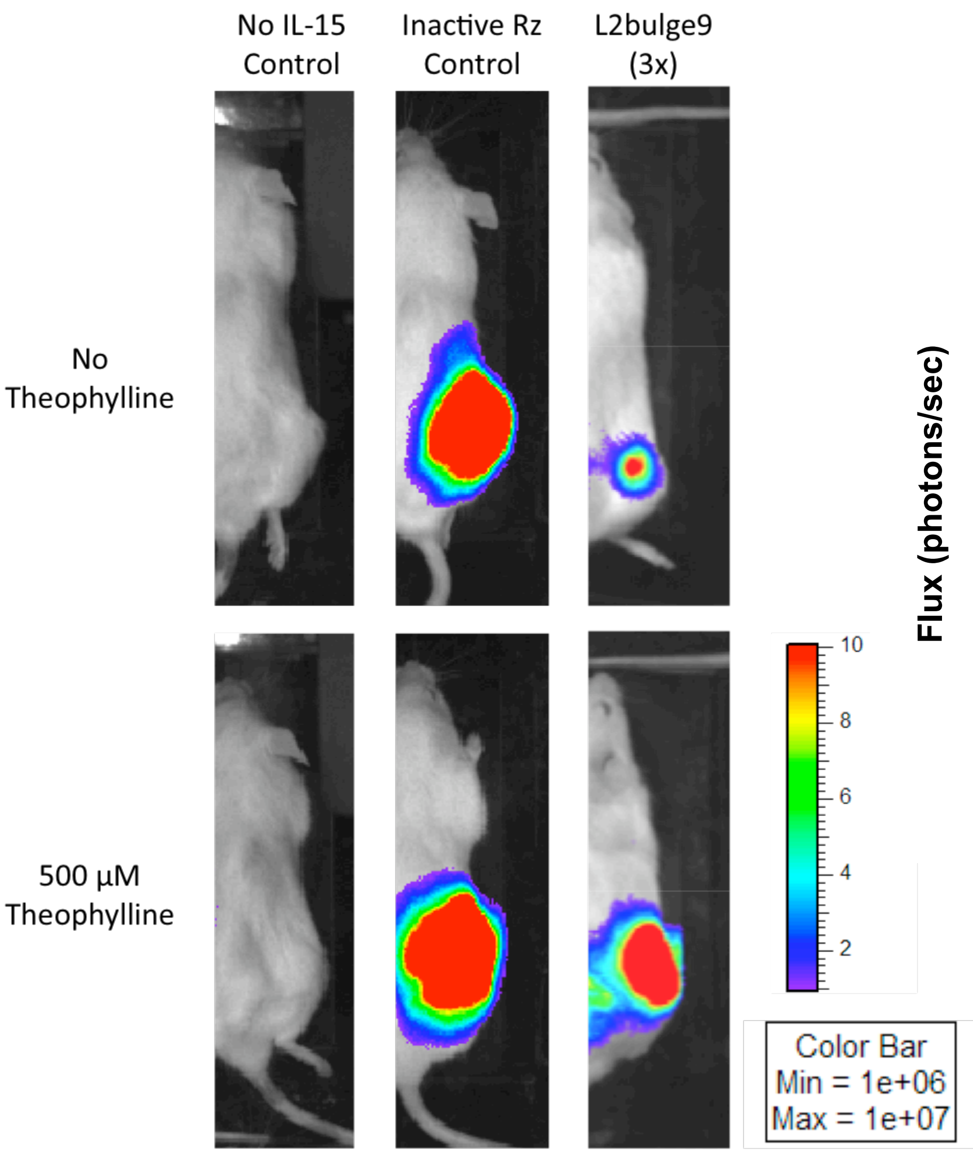


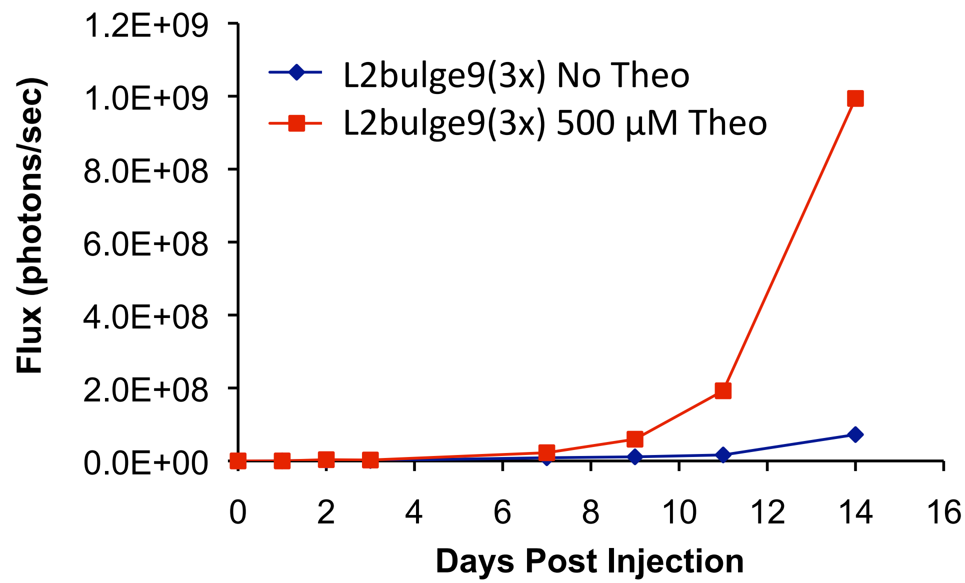


Stable cell lines show dynamic response to input

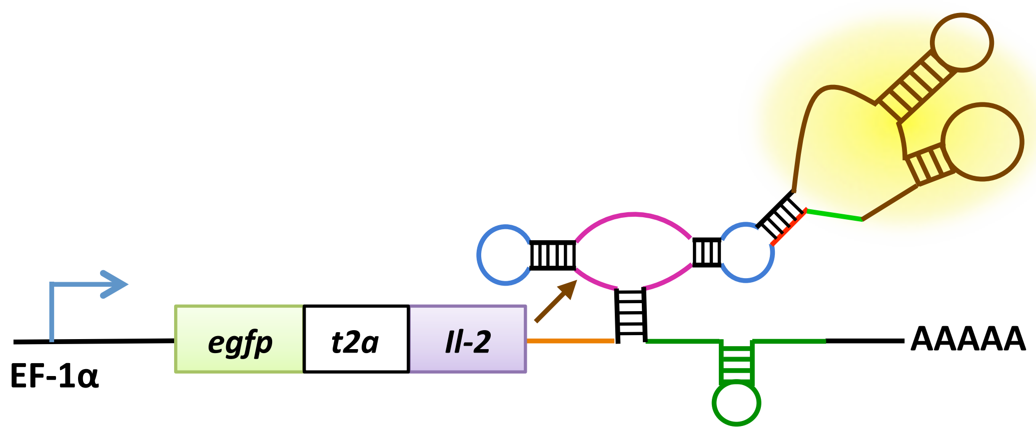


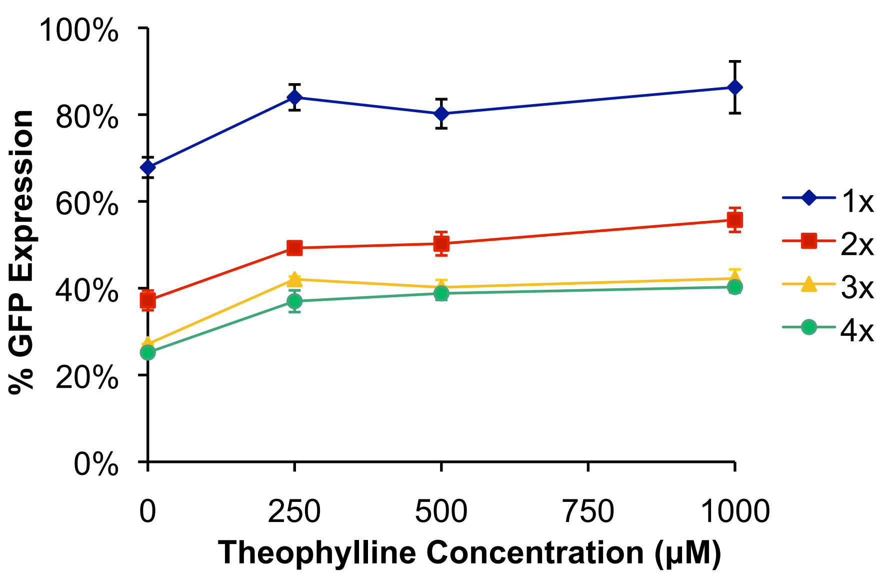
RNA devices regulate T-cell proliferation *in vivo*

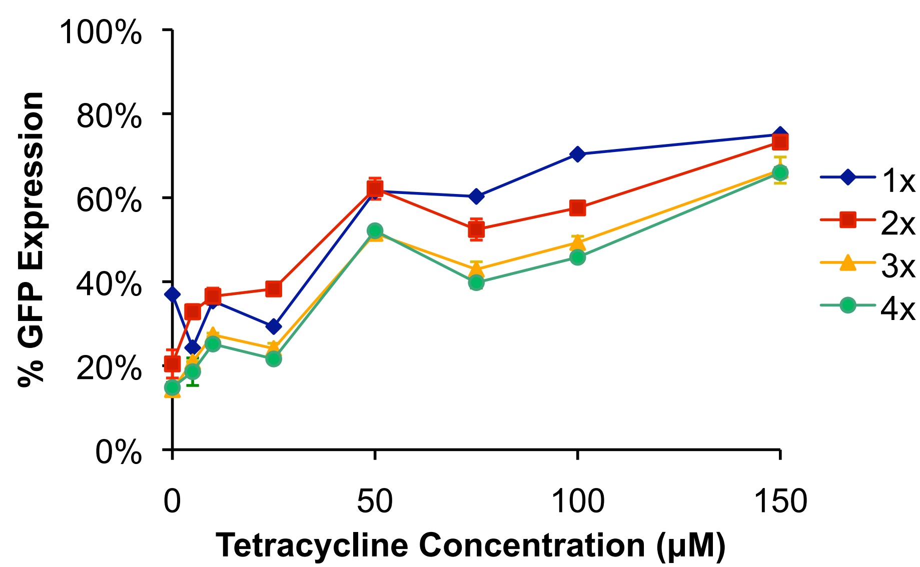




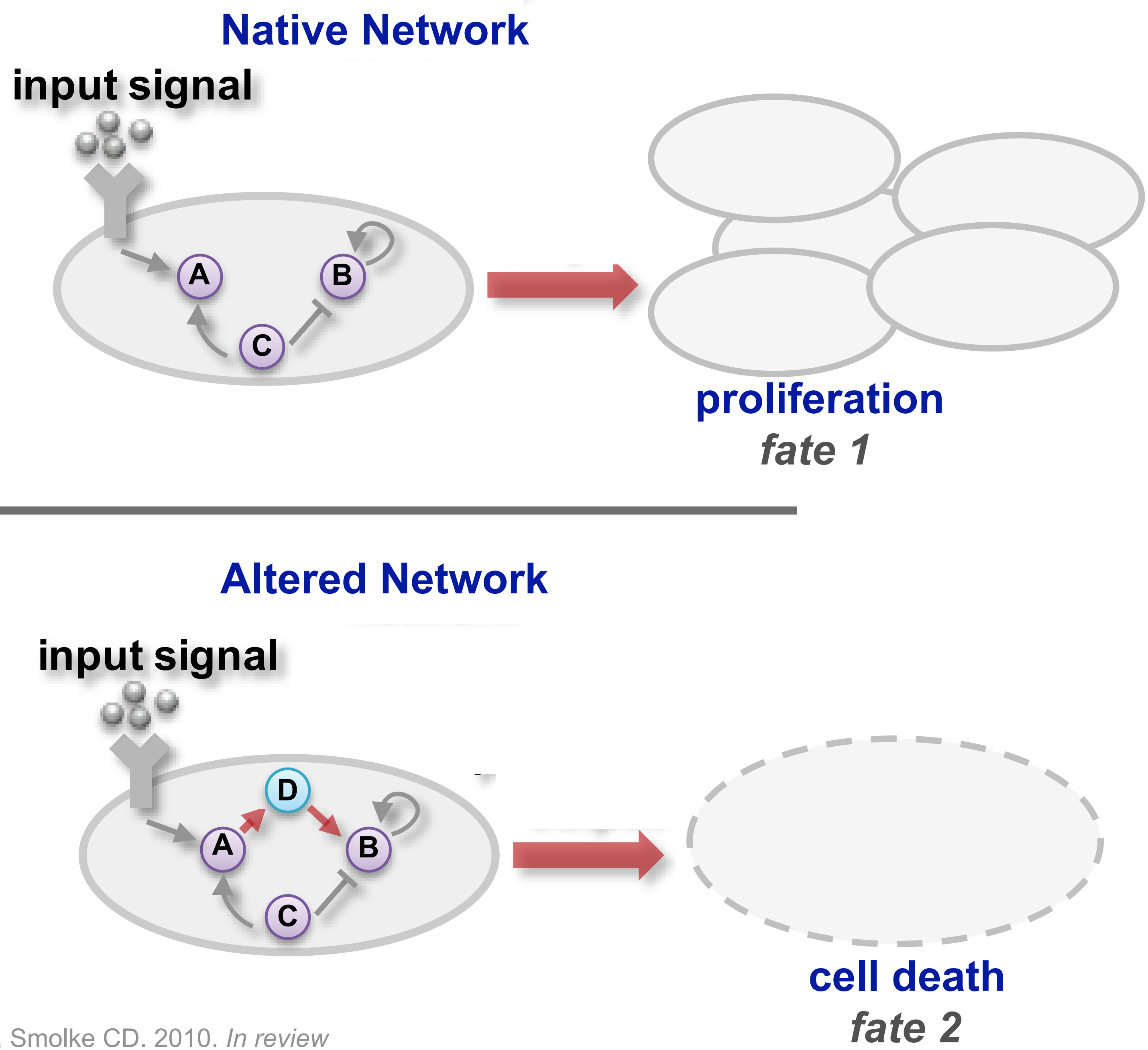
Choice of drug-sensor pair affects device performance





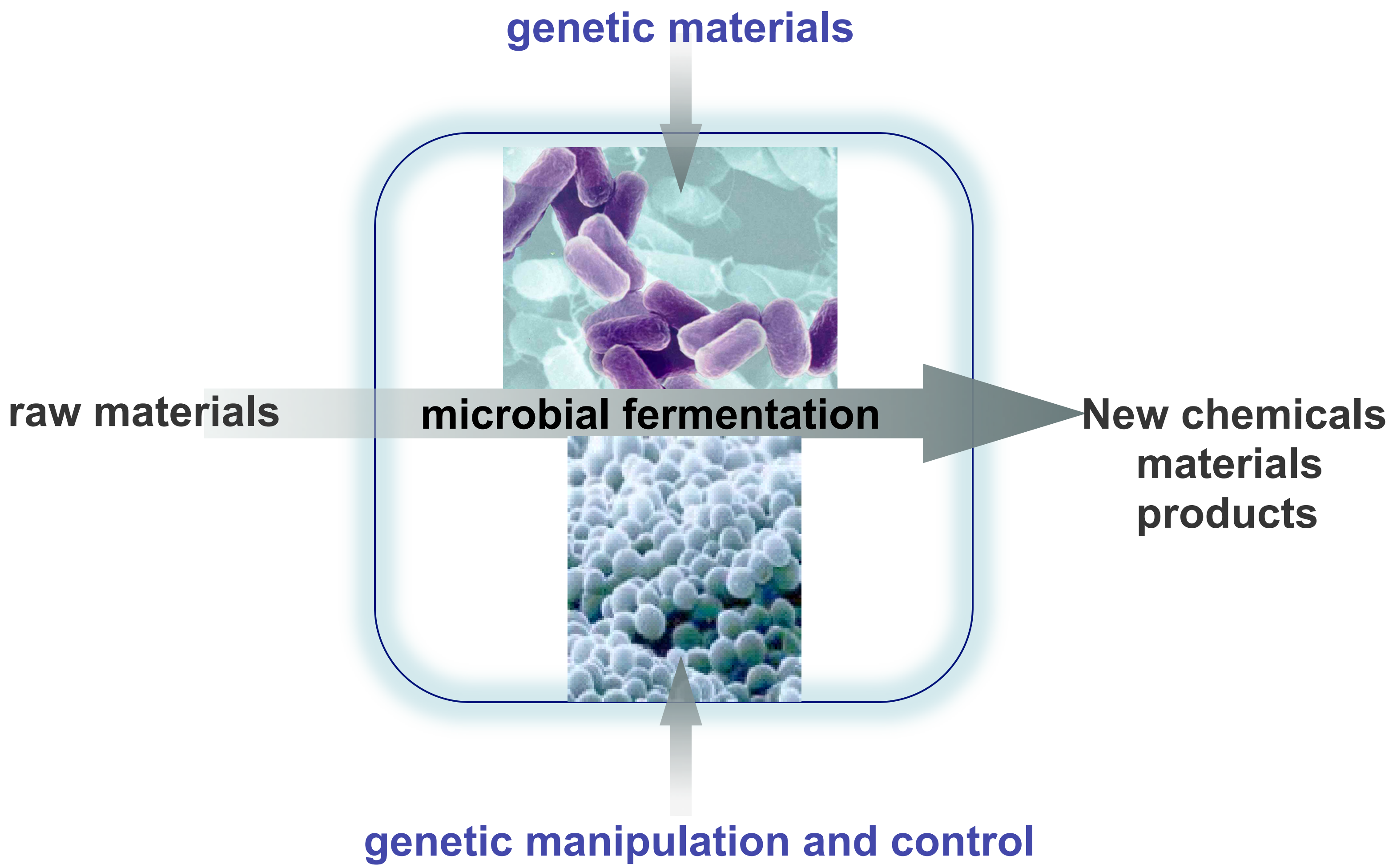


Moving between exogenous and embedded control



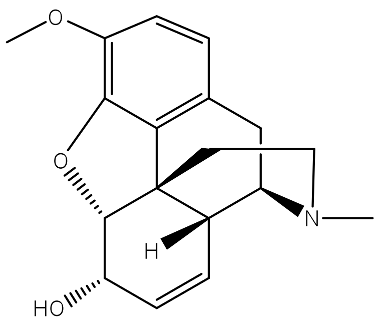
Culler SJ, Hoff KG, Smolke CD. 2010. *In review*

Microbial factories

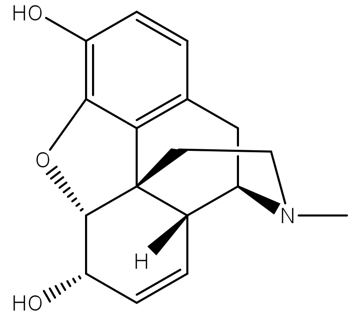


http://lifeboat.com/images/bacteria.purple.jpg

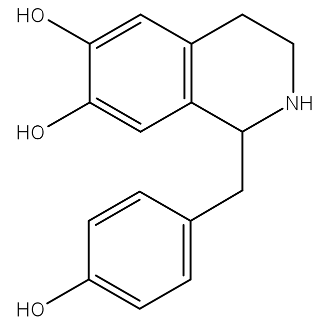
Benzylisoquinoline Alkaloids (BIAs) are an important class of natural products

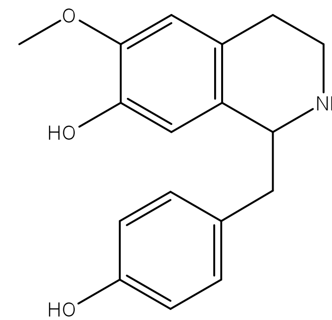


Codeine



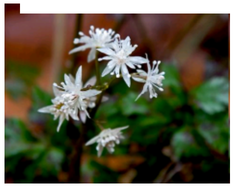
Morphine

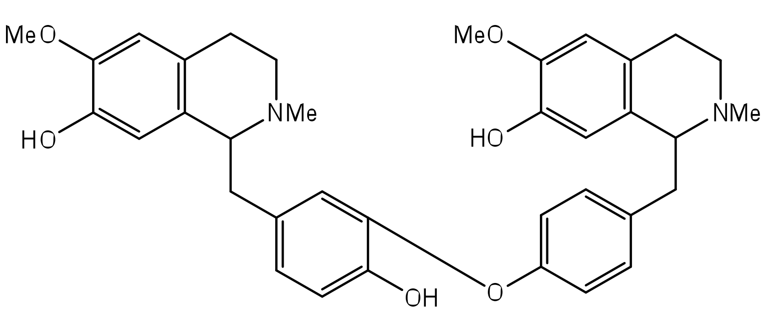




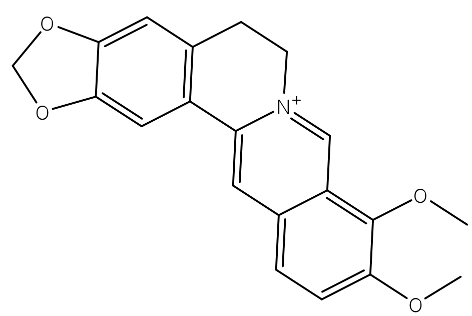
Norcoclaurine, Coclaurine

Over 2,500 known structures

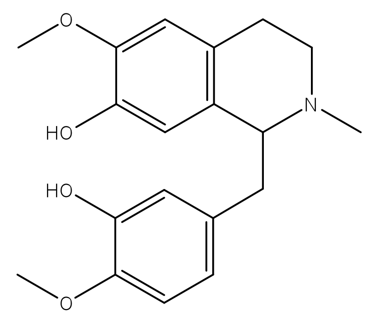




bis-BIAs (Berbamunine)
Antimicrobial, anticancer

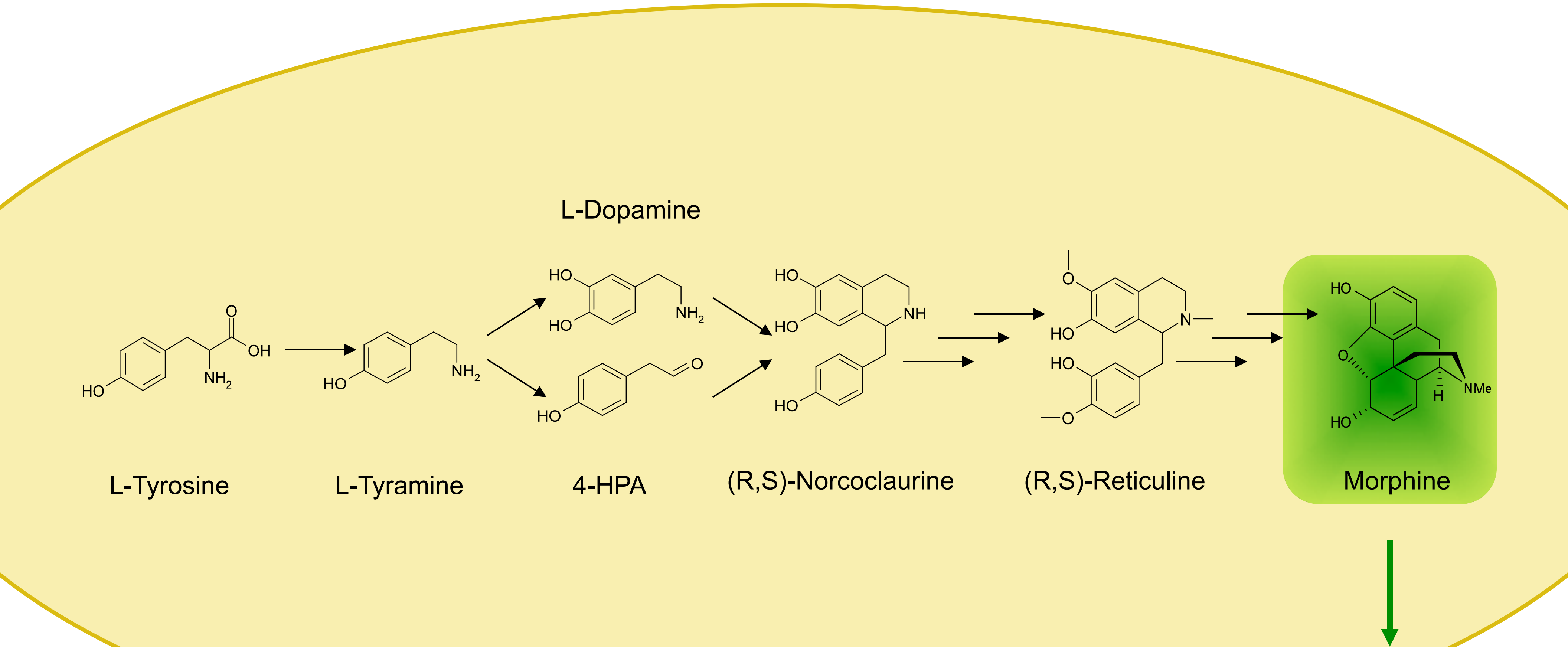


Berberine
Antimicrobial, antioxidant



Reticuline
stimulates hair growth

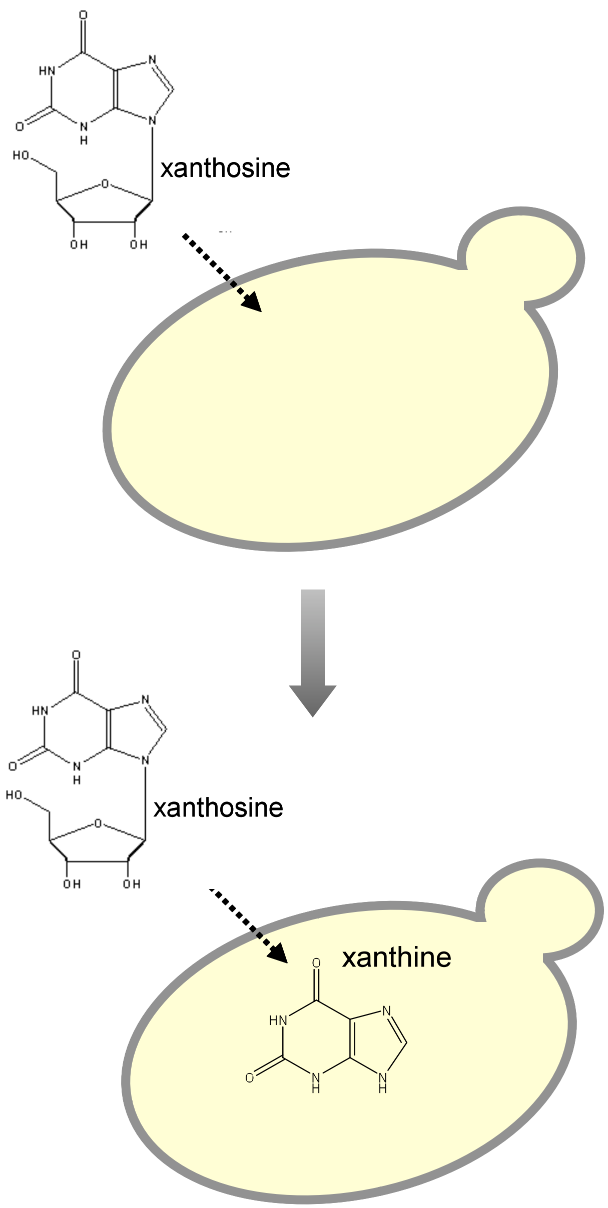
Molecular tools for optimizing metabolite production

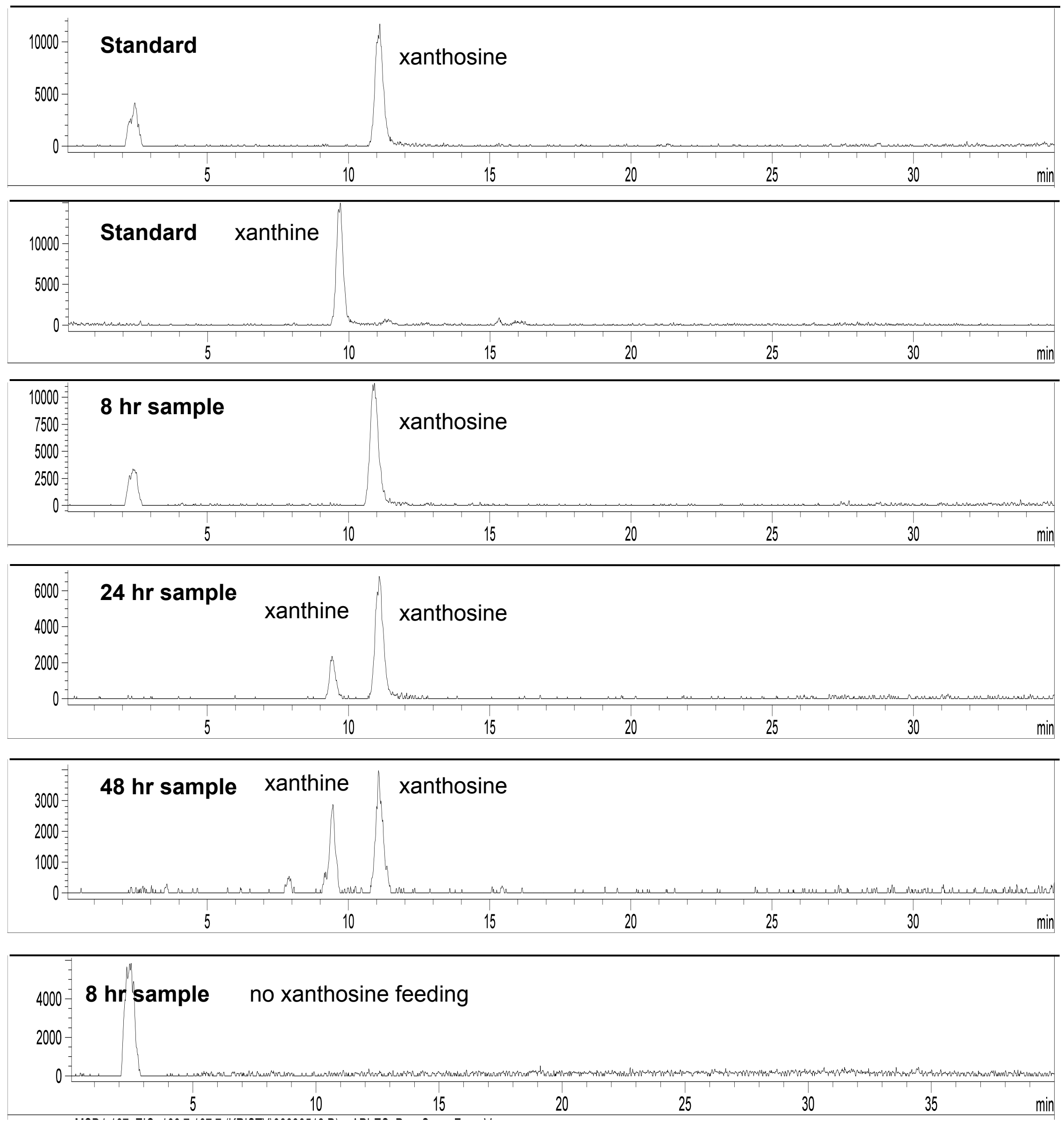


Often need to screen through large libraries of pathway/enzyme variants – invasive / analytical procedures are too time and resource intensive

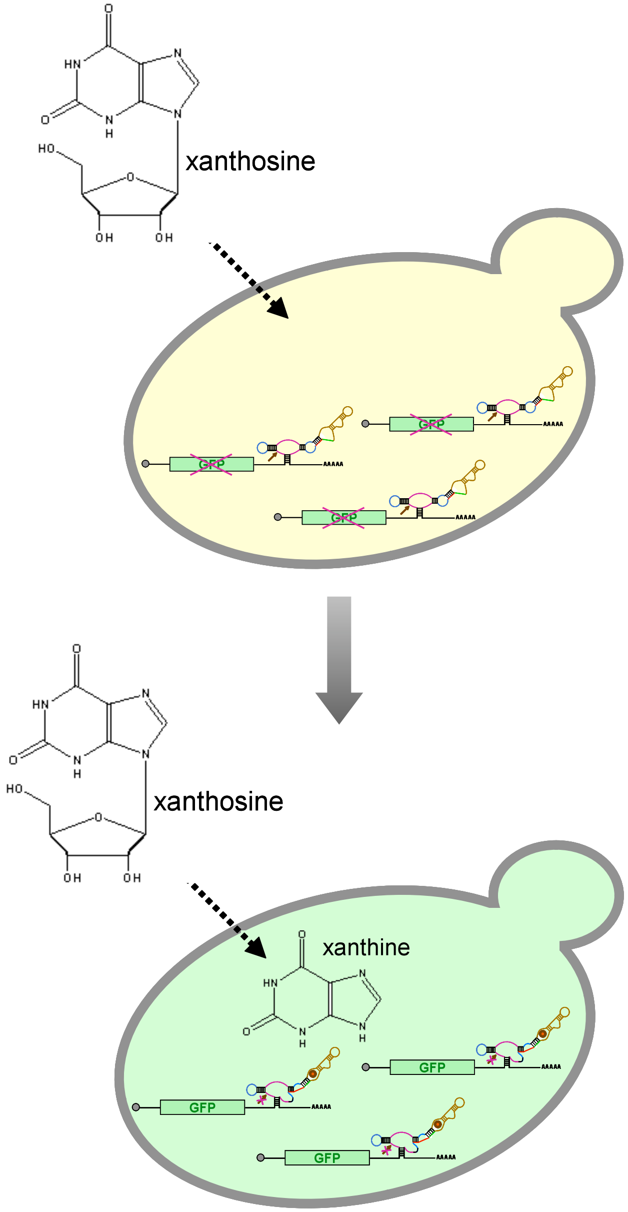
Engineer noninvasive sensors of metabolite concentrations in living cells

Integrating RNA devices as noninvasive sensors of metabolite concentration

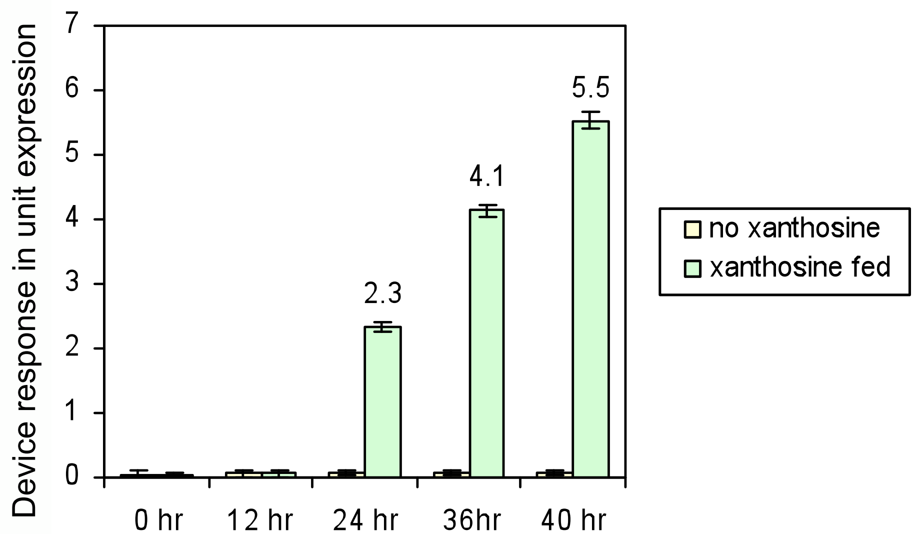




RNA devices enable noninvasive sensing of metabolite concentration

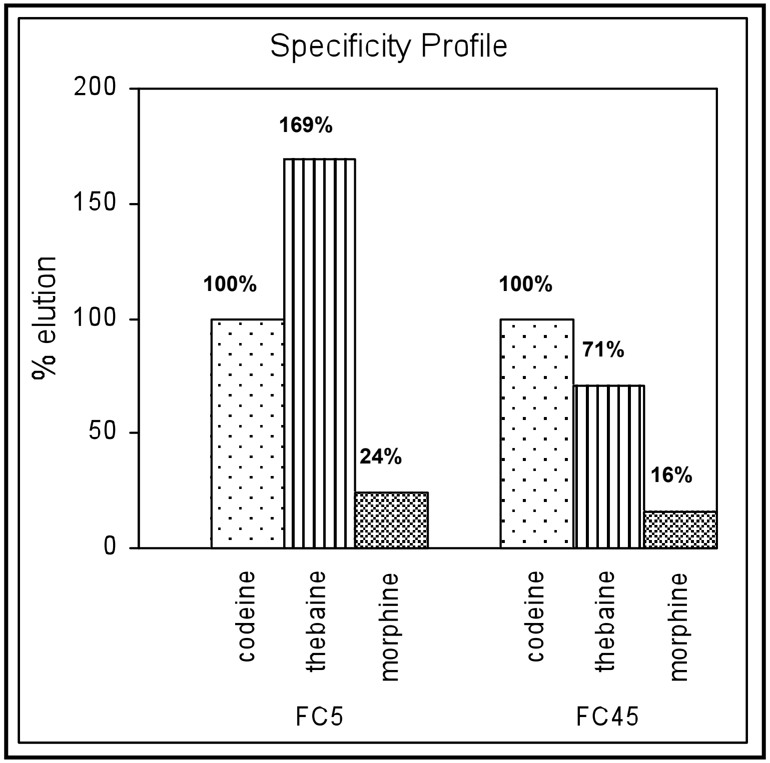


Implementation of Buffer device as a real-time noninvasive xanthine sensor

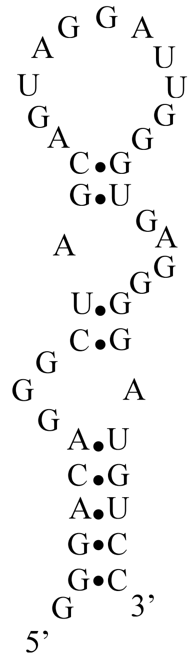


Win MN, Smolke CD. 2007. *PNAS*. 104: 14283-8

RNA sensors generated to key BIAs

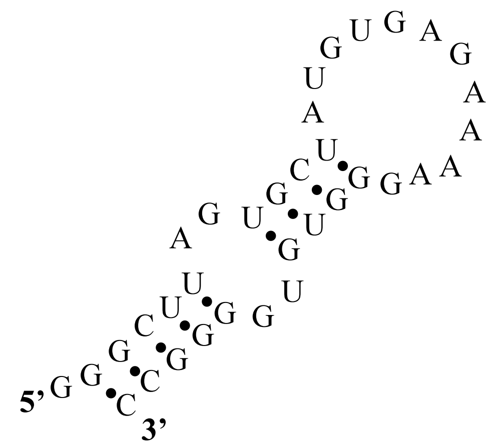


FC5

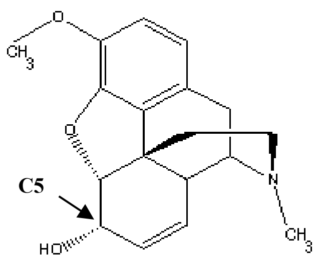


K_d **(FC5L) = 4.55±0.14 μM**

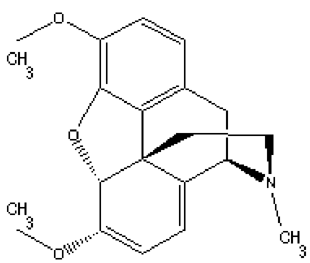
FC45



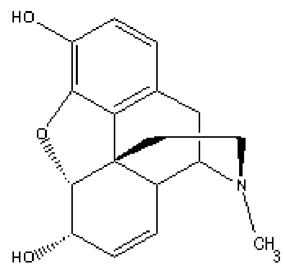
Kd (FC45L) = 2.59 ± 0.09 μM



codeine



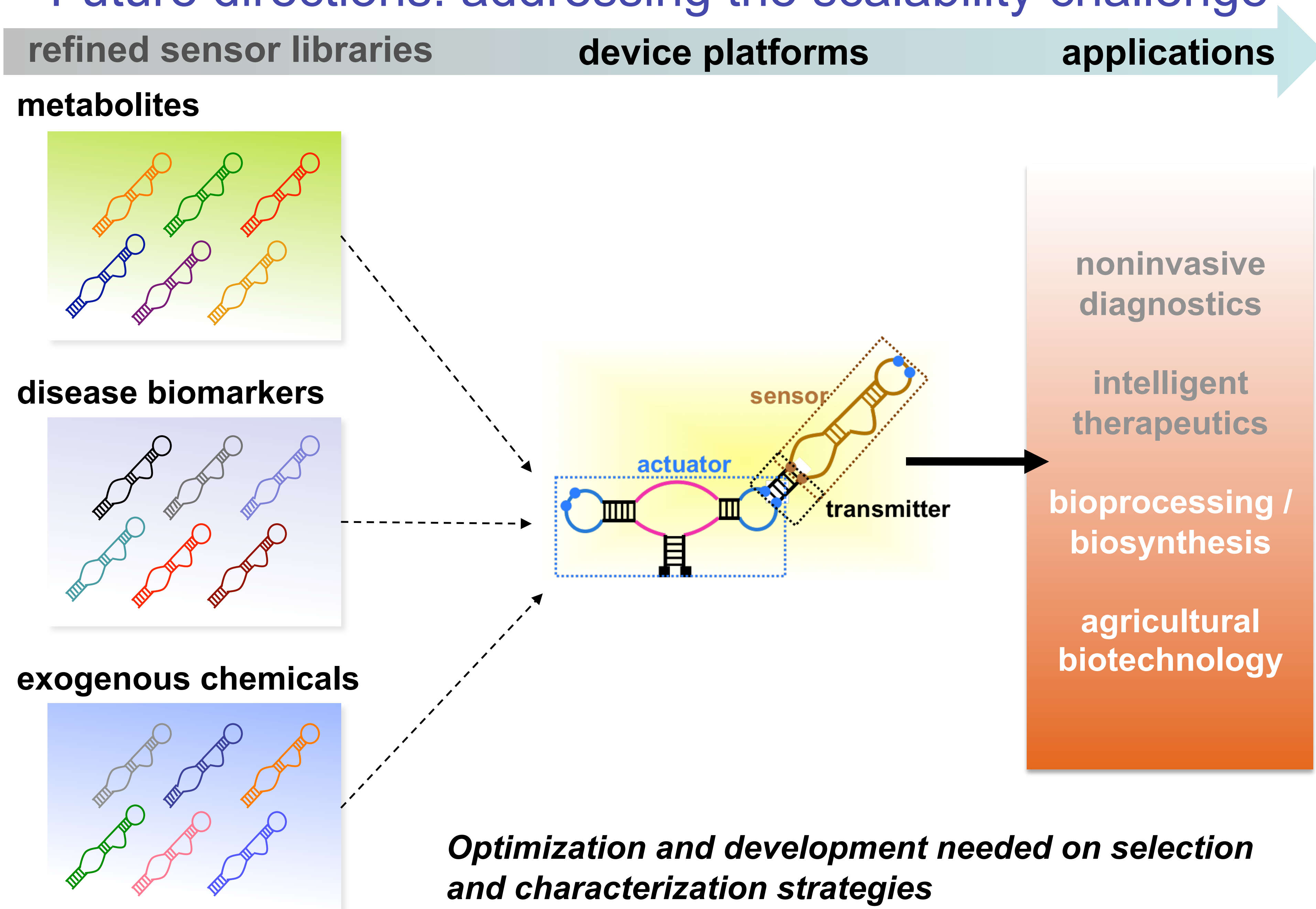
thebaine



morphine

Win MN, Klein JS, Smolke CD. 2006. *Nuc Acids Res.* 34: 5670-82.

Future directions: addressing the scalability challenge



Optimization and development needed on selection and characterization strategies

Acknowledgements

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Maung Nyan Win

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The Jensen Lab (City of Hope)

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