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Creating Synthetic Protein-Protein Interaction Networks and Implications for Endogenous Network Discovery

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Weiss Lab
Synthetic Biology Center @ MIT

May 17, 2018
ATCC Webinar

ebrc Engineering Biology Research Consortium

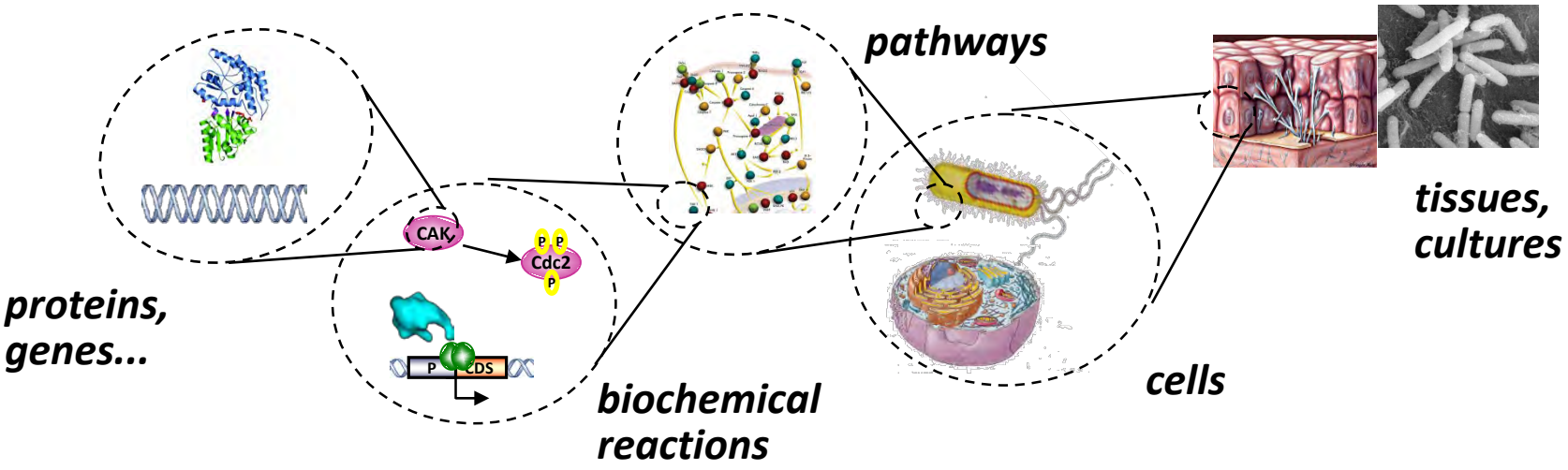
ICB

MIT CISB | Center for Integrative Synthetic Biology
An NIGMS-funded National Center for Systems Biology

synberc
Building the Future with Biology

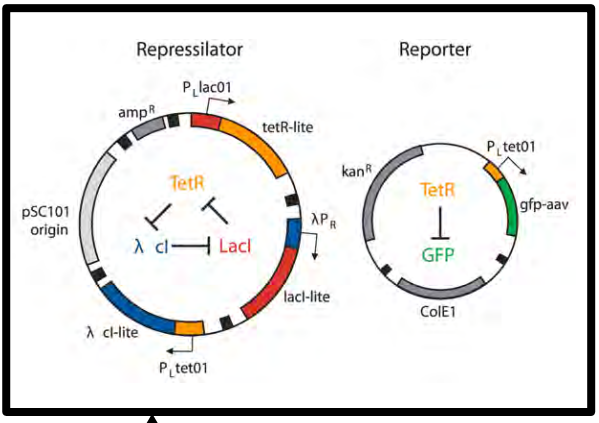
NSF

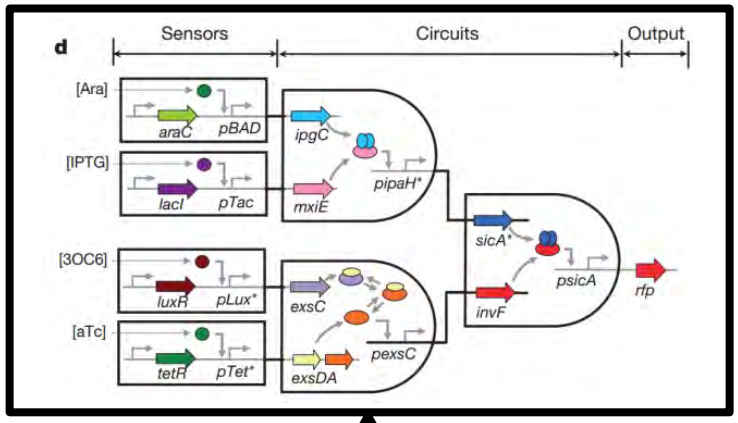
Developing Biological Systems with Novel Function



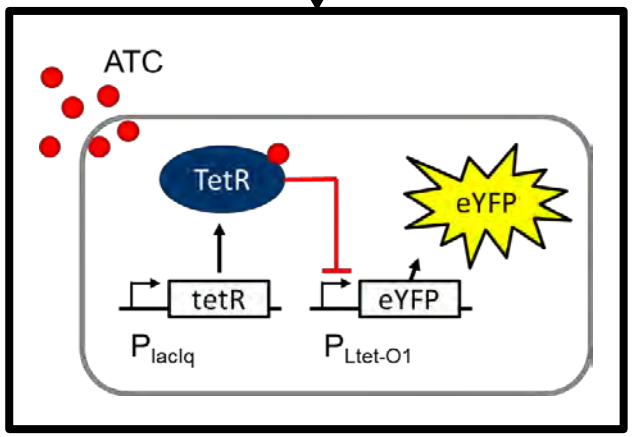
By engineering underlying elements and reactions, can we program the resulting behaviors of cells/tissues?

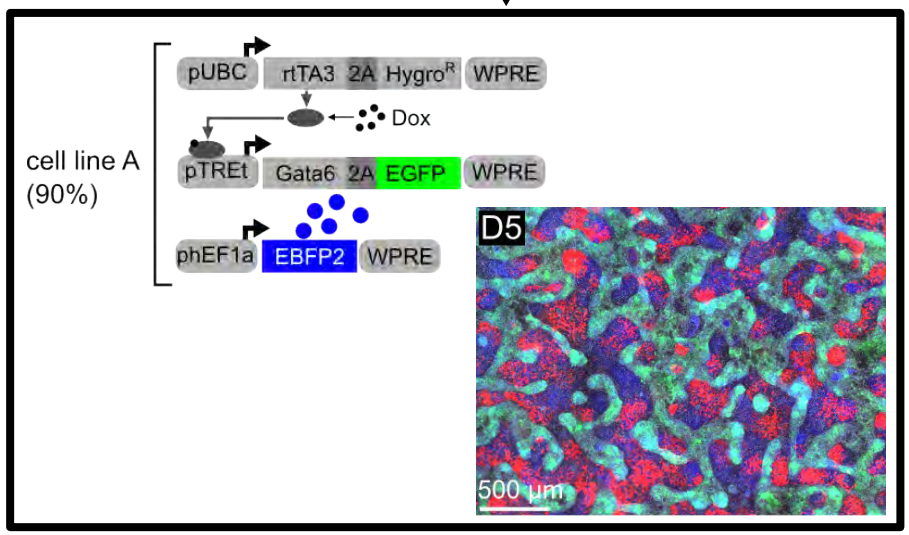
Brief History of Transcriptional Systems in Synthetic Biology





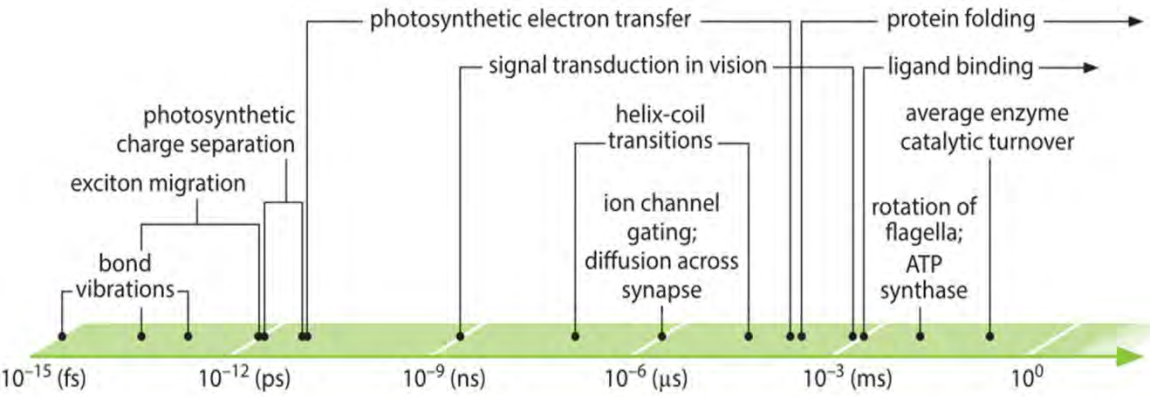
2000 2006 2012 2018

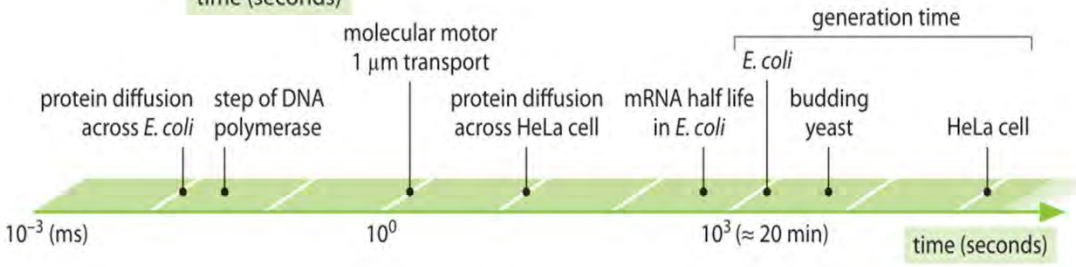




Elowitz *et al,* Nature (2000), Basu *et al,* Nature (2005); Moon *et al,* Nature (2012); Guye *et al,* NatCom(2015)

Life Is NOT Governed By Transcriptional Speeds



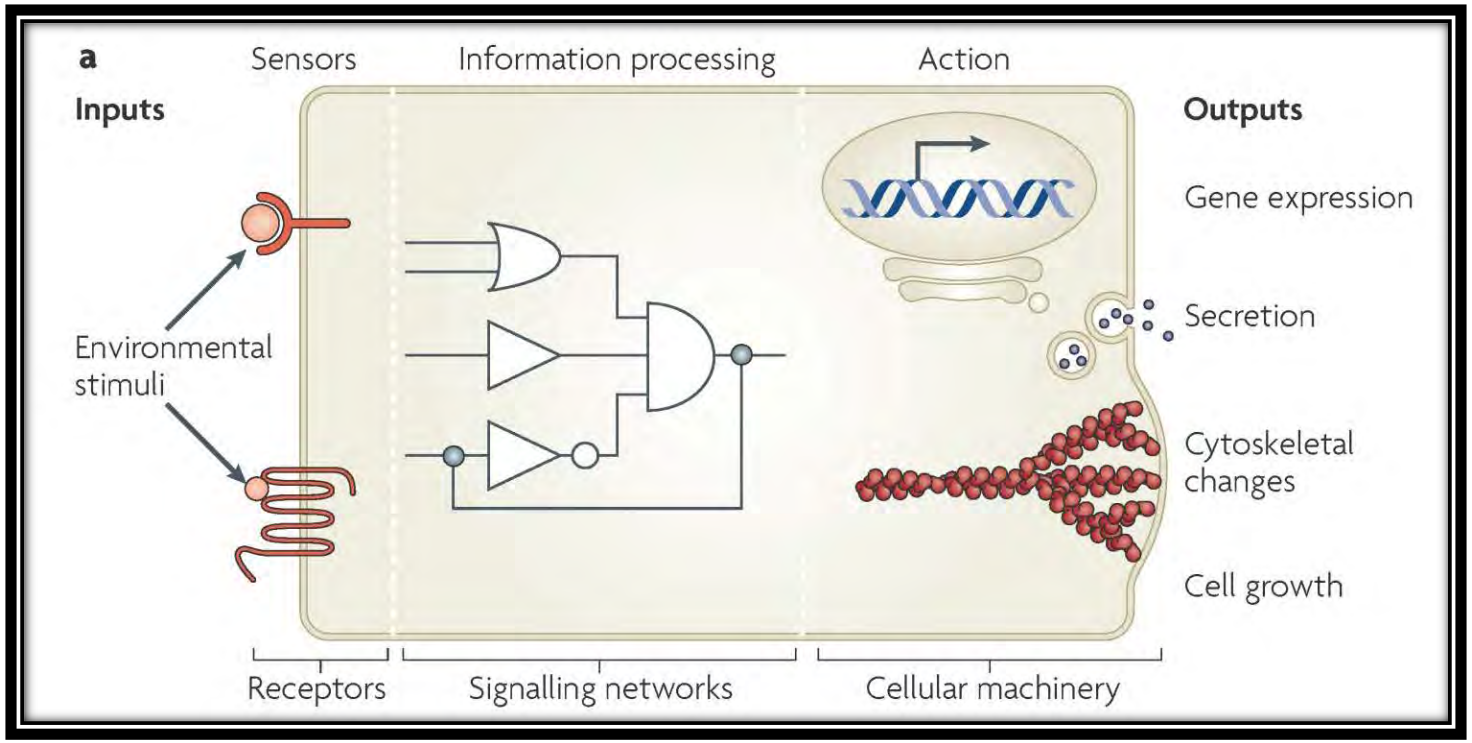


Milo *et al,* NAR (2010) BioNumbers

Protein-Protein Interactions Represent a Frontier in SynBio

Organism	Transcription Factors	Receptors, Kinases, and Phosphatases
S. cerevisiae	113 [1]	1850 [3]
H. sapiens	229 [2]	2211 [3]

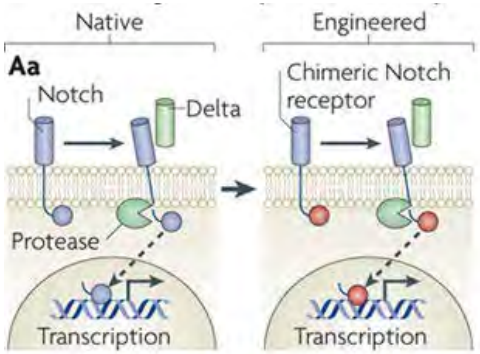
Similar order of magnitude naturally but NOT in synbio



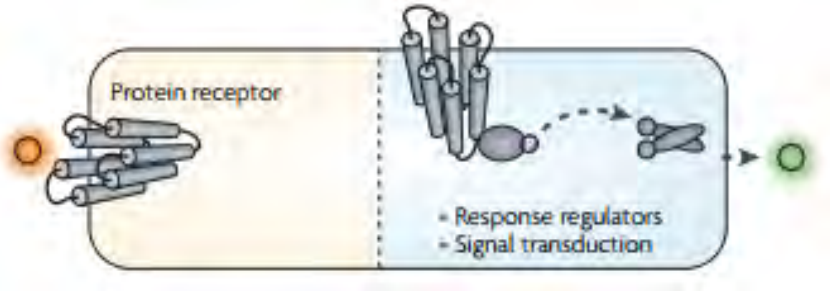
[1] Teixeira *et al, NAR,* 2014 [2] Breitkreutz *et al, Science,* 2010 [3] Papin *et al, Nat. Rev. MCB* 2005 [4] Lim *NatRevMCB 2010*

Why Build Protein-Protein Networks?

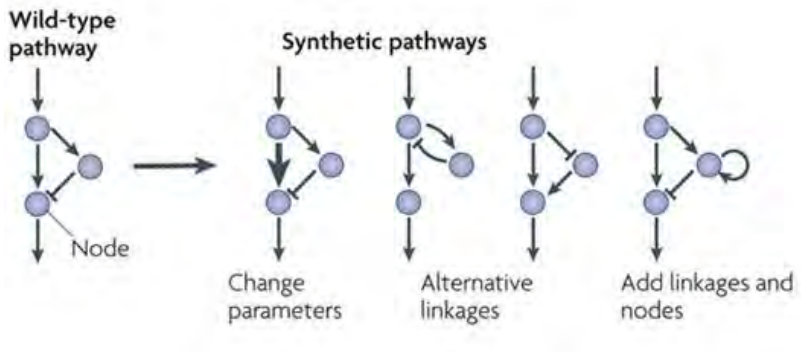
Modulate/modify existing pathways



Sophisticated cellular sensing directly coupled to actuation



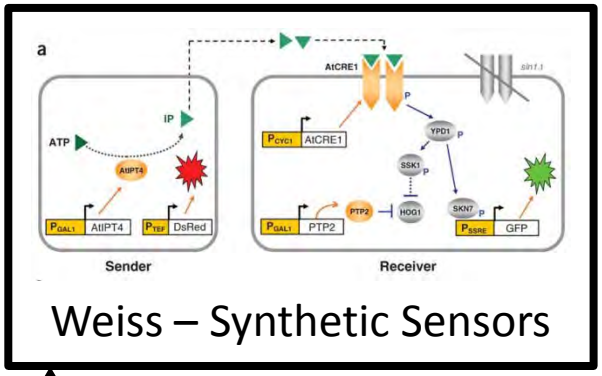
Build to understand motifs



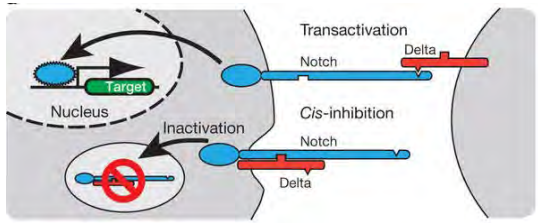
Fast networks with potentially low operating energy

Lim *NatRevMCB 2010;* Khalil & Collins *NatRevGen 2010*

Strategies for implementing protein interactions in SynBio



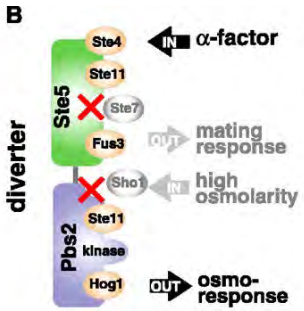
Weiss – Synthetic Sensors



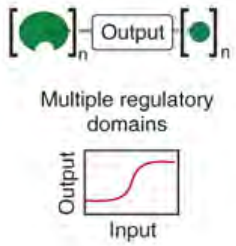
Elowitz – Natural Sensors

2003 2005 2007 2009 2011 2013

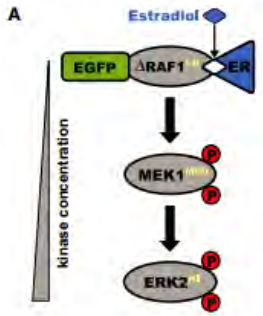
Lim – Hubs



Lim – Switches

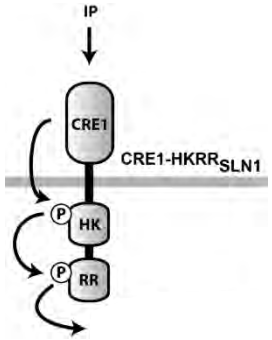


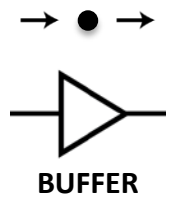
Sarkar – Response

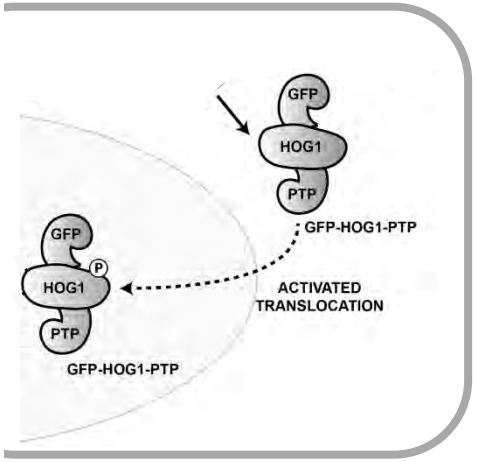


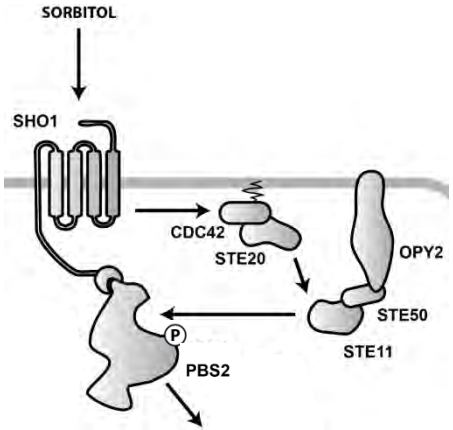
Park *et al,* Science (2003), Chen *et al,* NBT (2005); Dueber *et al,* NBT (2007); O'Shaughnessy *et al,* Cell (2011) Sprinzak *et al, Nature* (2010)

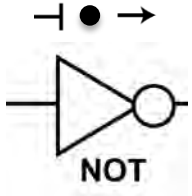
Protein networks requires rules to build protein devices

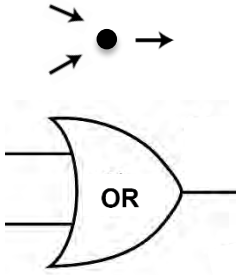


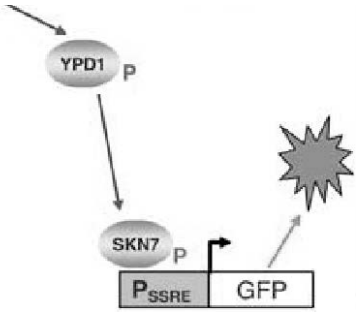






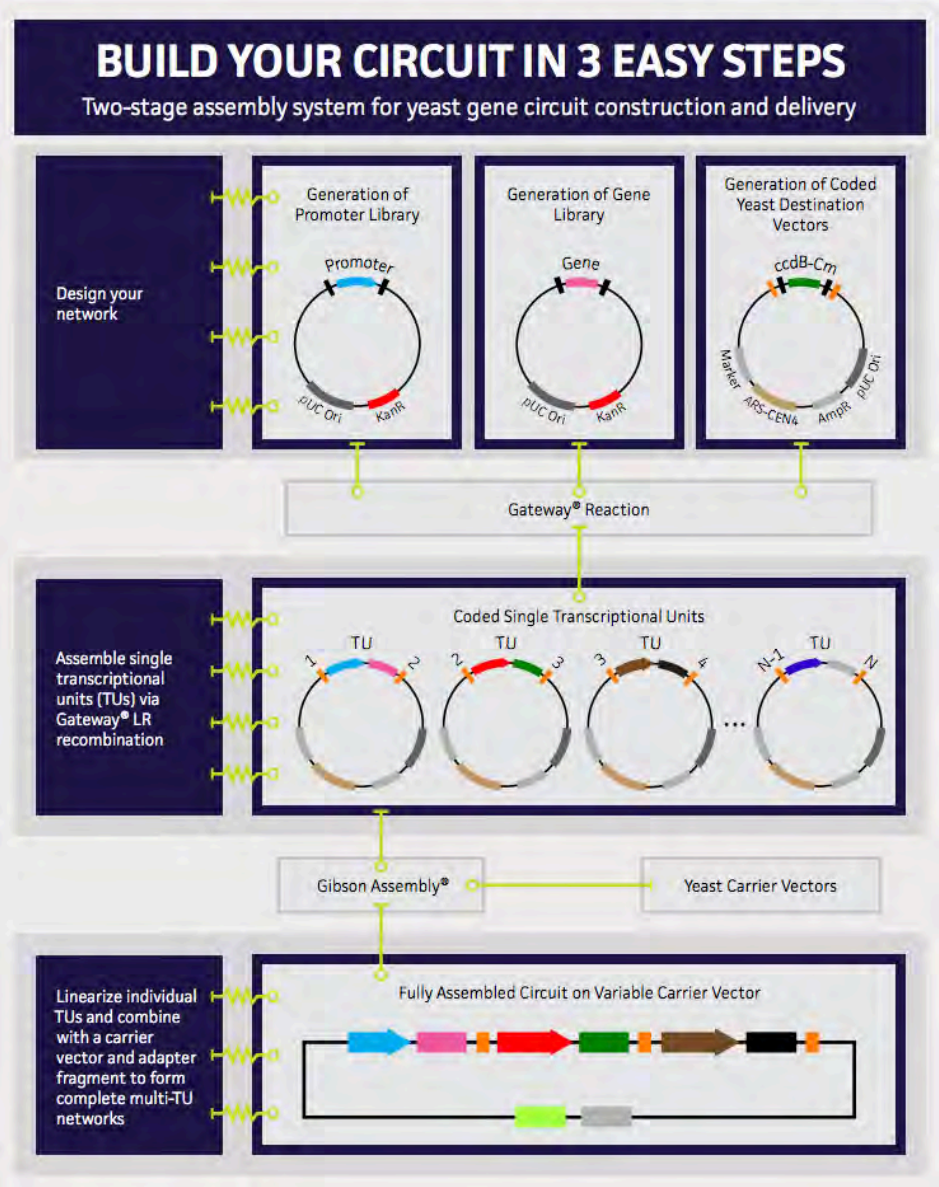






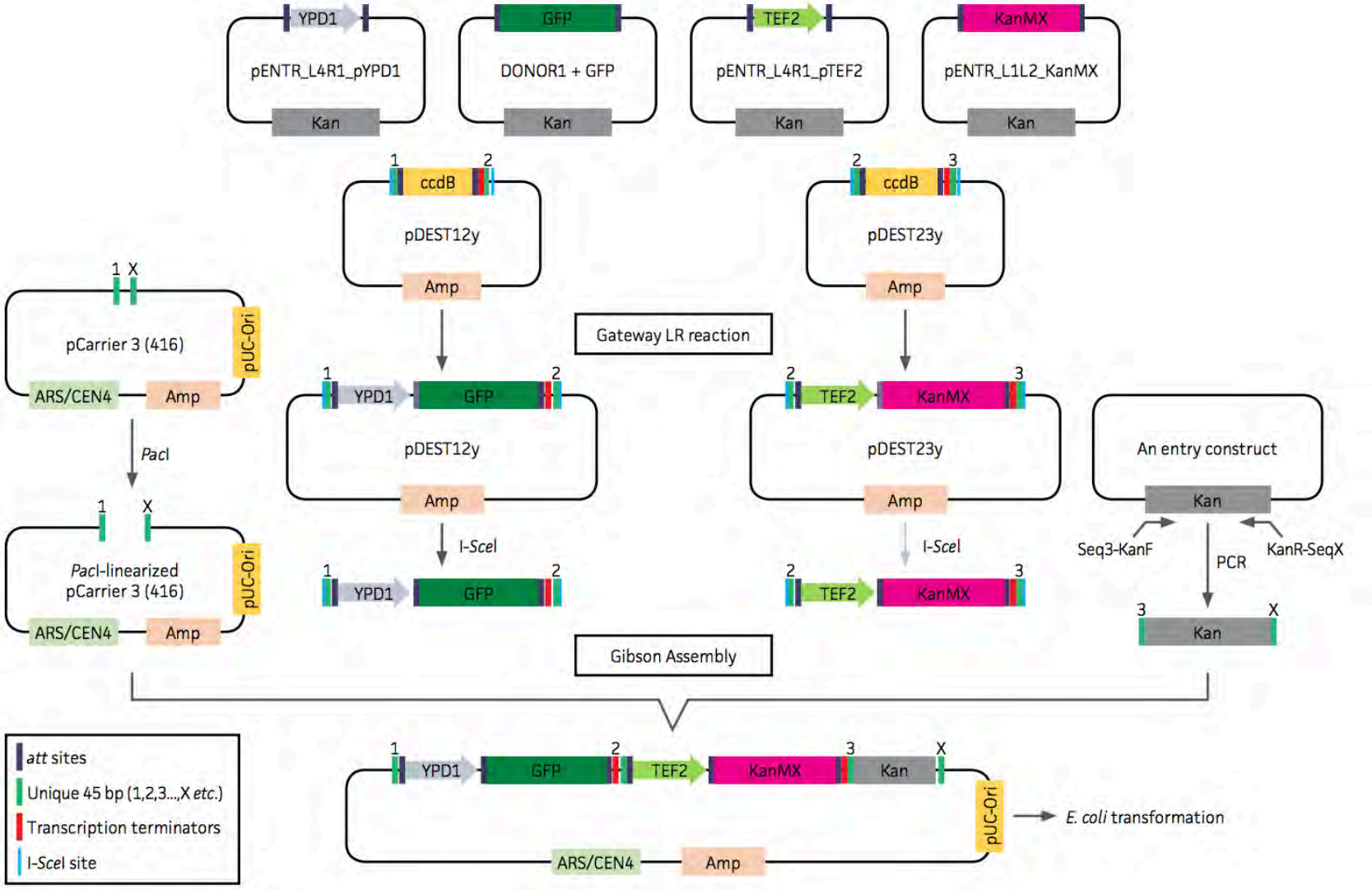
SENSE **COMPUTE** **ACTUATE**

Rapid Design and Assembly of Complex Networks



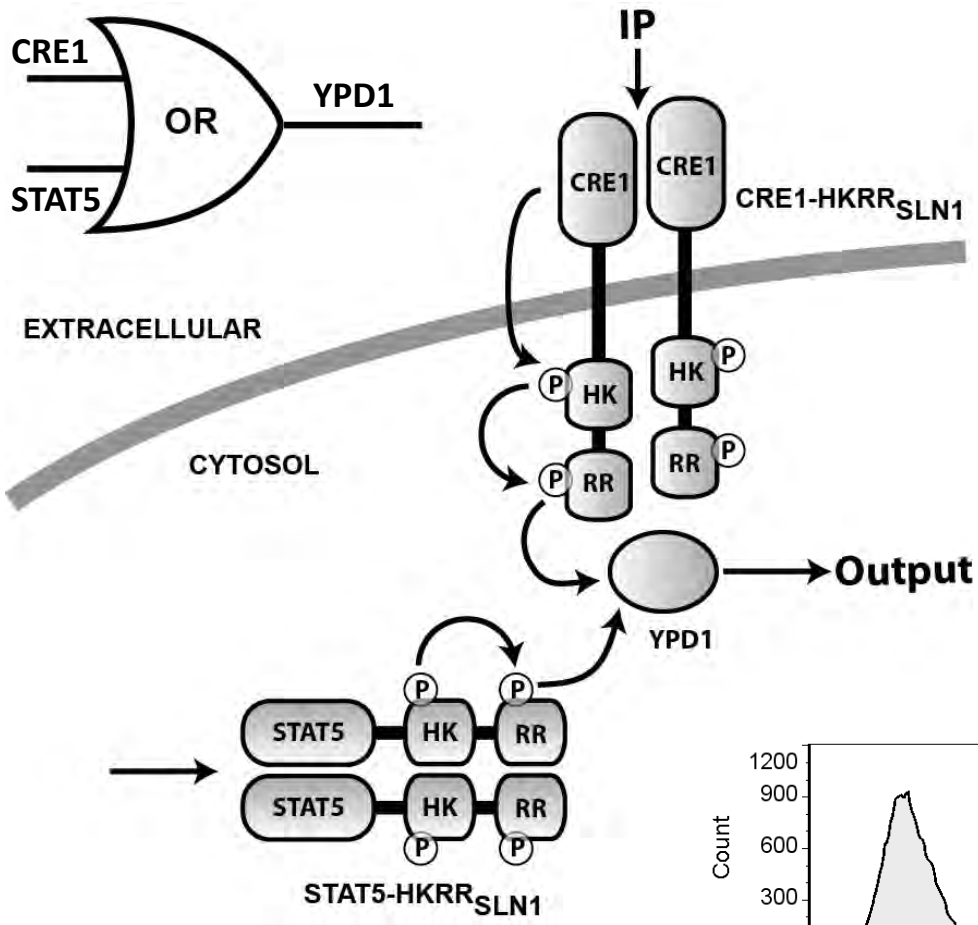
- Highly efficient (Recombinase cloning with selection, double-antibiotic Gibson assembly)
- No PCR Mutation Risk
- Uses L1L2 gene plasmids available from common NIH repositories (ASU/Harvard)
- Flexible Delivery
- Easy to make network variants
- Available as SB-2000 from ATCC!

DNA Assembly Example



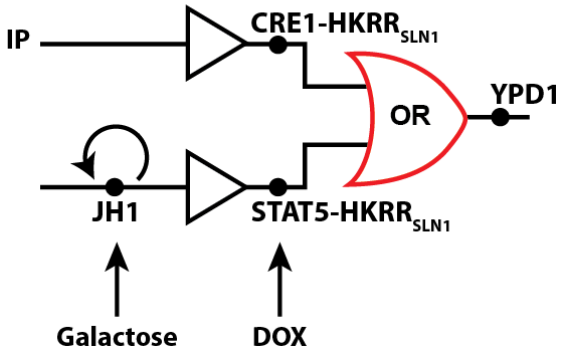
Engineering Pathway Convergence for Synthetic OR Gate

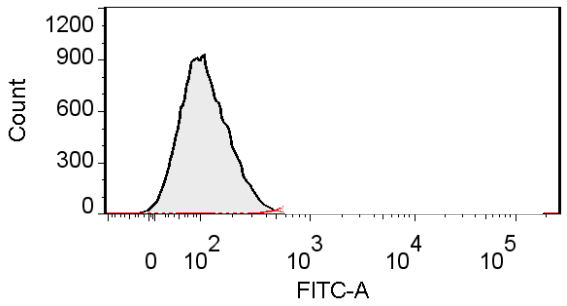
Objective: Bridge CRE1 and STAT5 to YPD1 to create OR logical gate

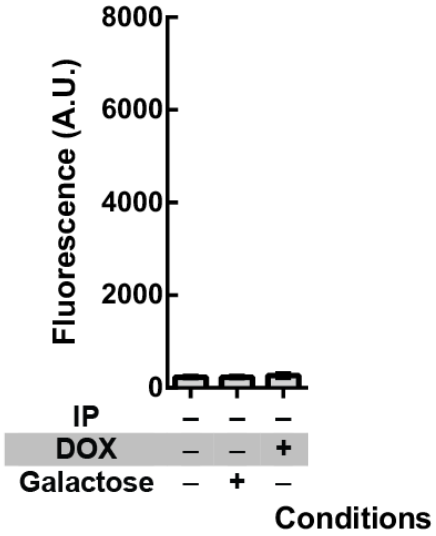


Testing OR Gate

IP OR (DOX AND Galactose)

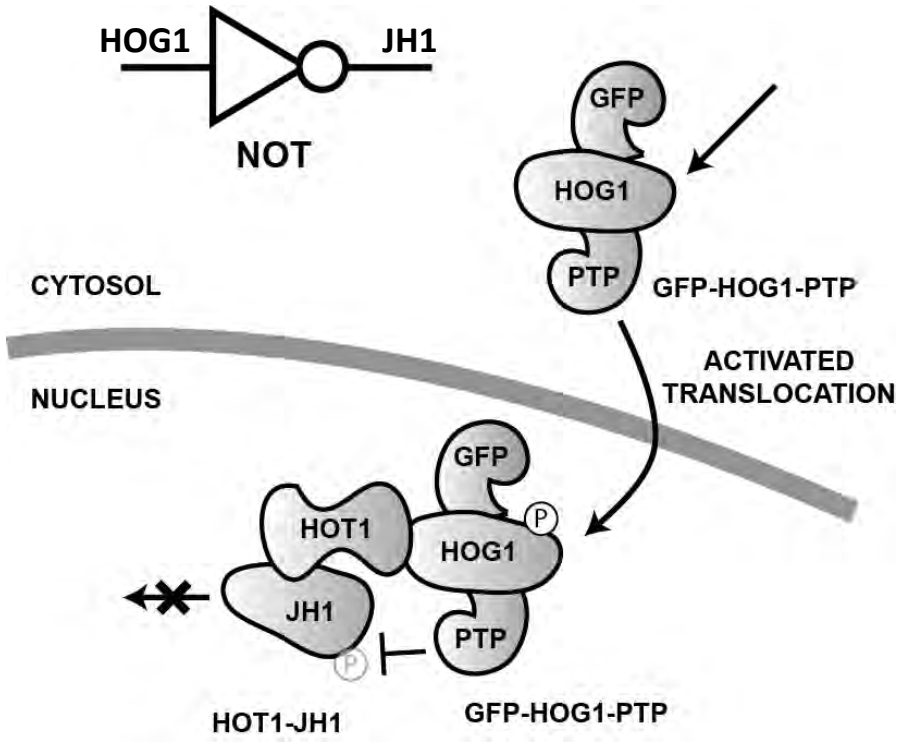






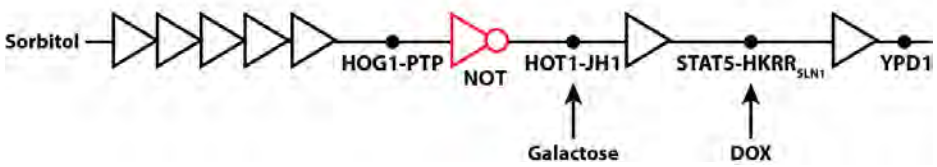
Phosphorylation Activated Localization (PAL) for Synthetic NOT Gate

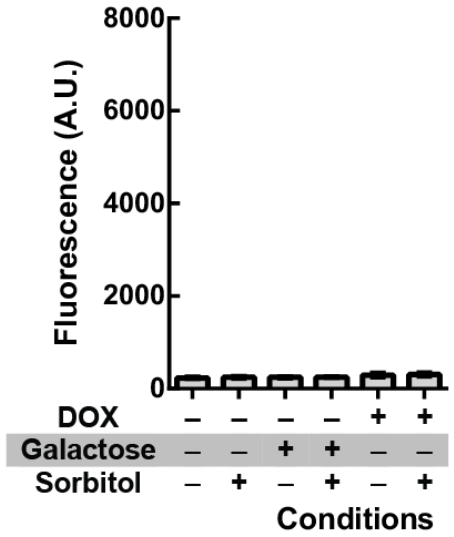
Objective: Bridge HOG1 and JH1 to create NOT logical gate

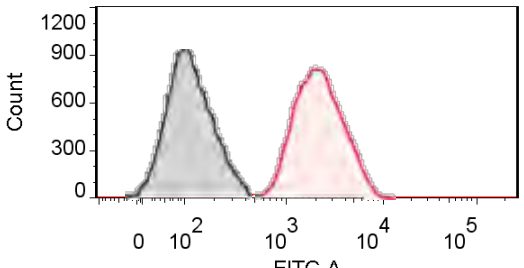


Testing NOT Gate

(DOX AND Galactose) AND NOT Sorbitol

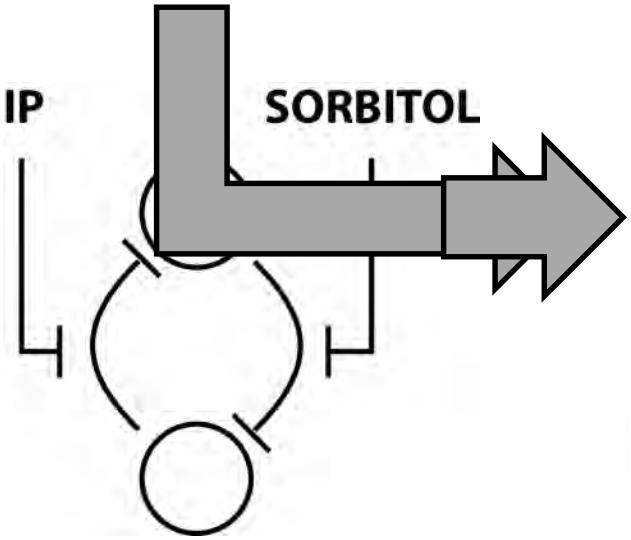


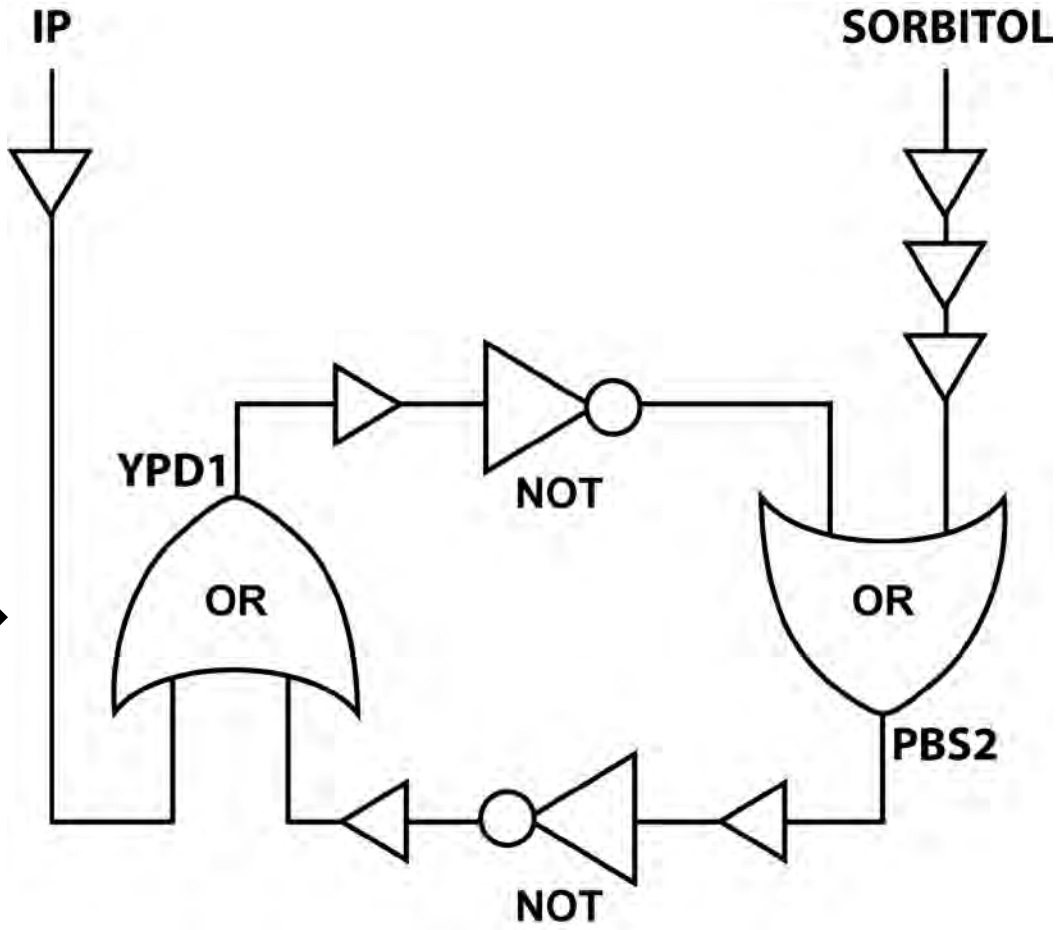




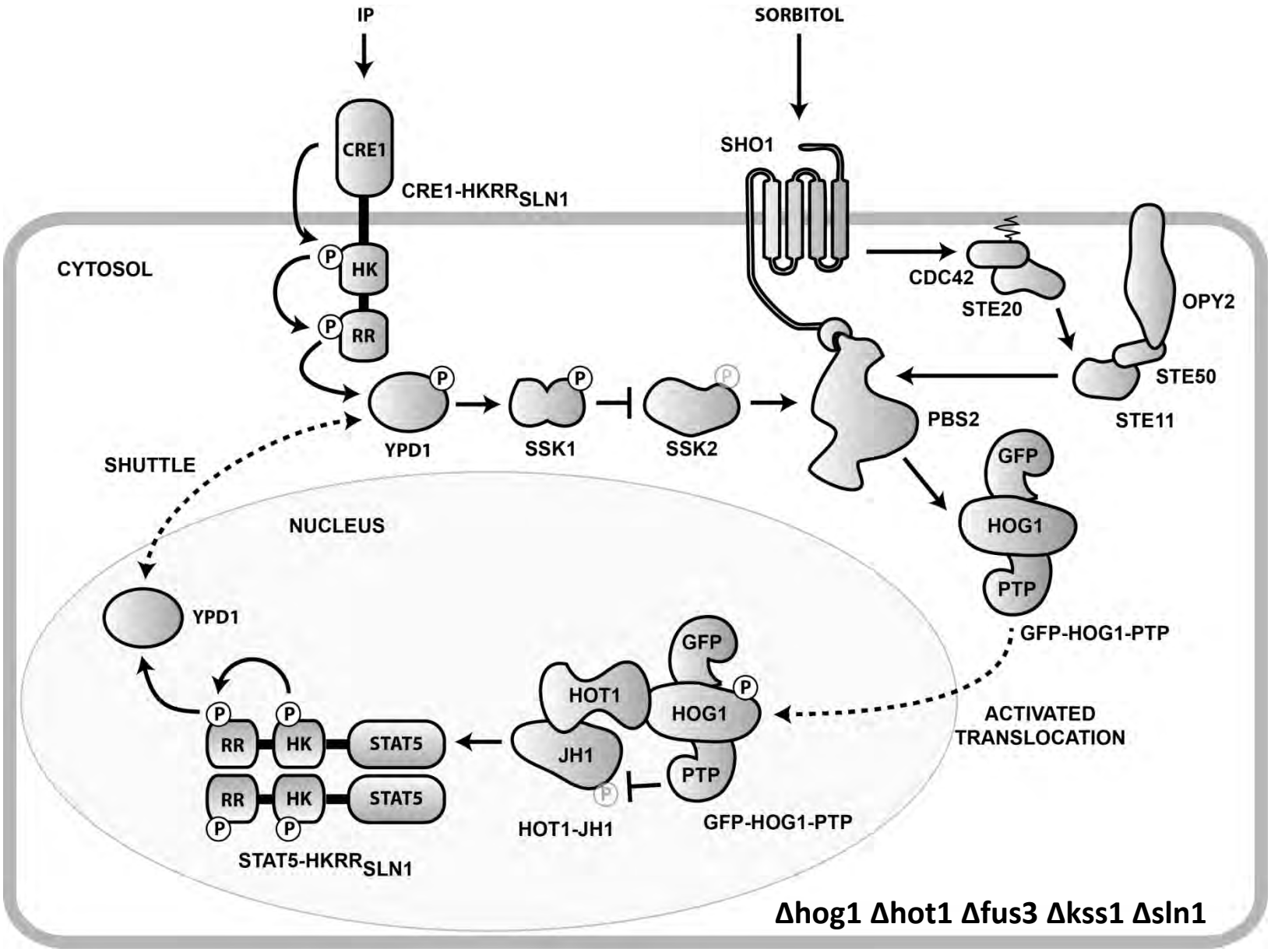
Forming a toggle network from protein-protein ORs and NOTs

If IP (transiently) administered:
set YPD1 branch to HIGH
set PBS2 branch to LOW
If Sorbitol (transiently) administered:
set YPD1 branch to LOW
set PBS2 branch to HIGH





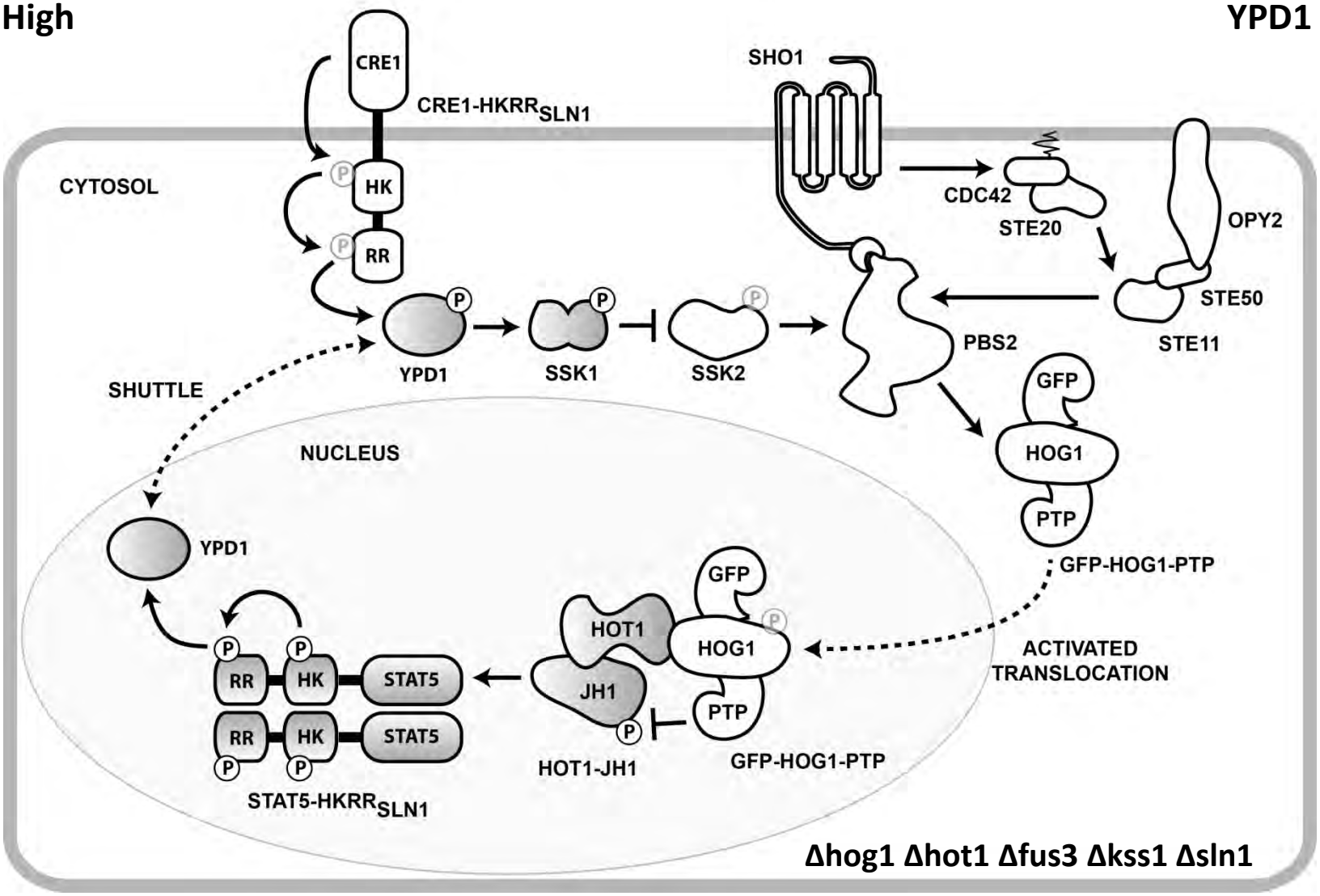
Mapping a Logic Network to a Protein Network



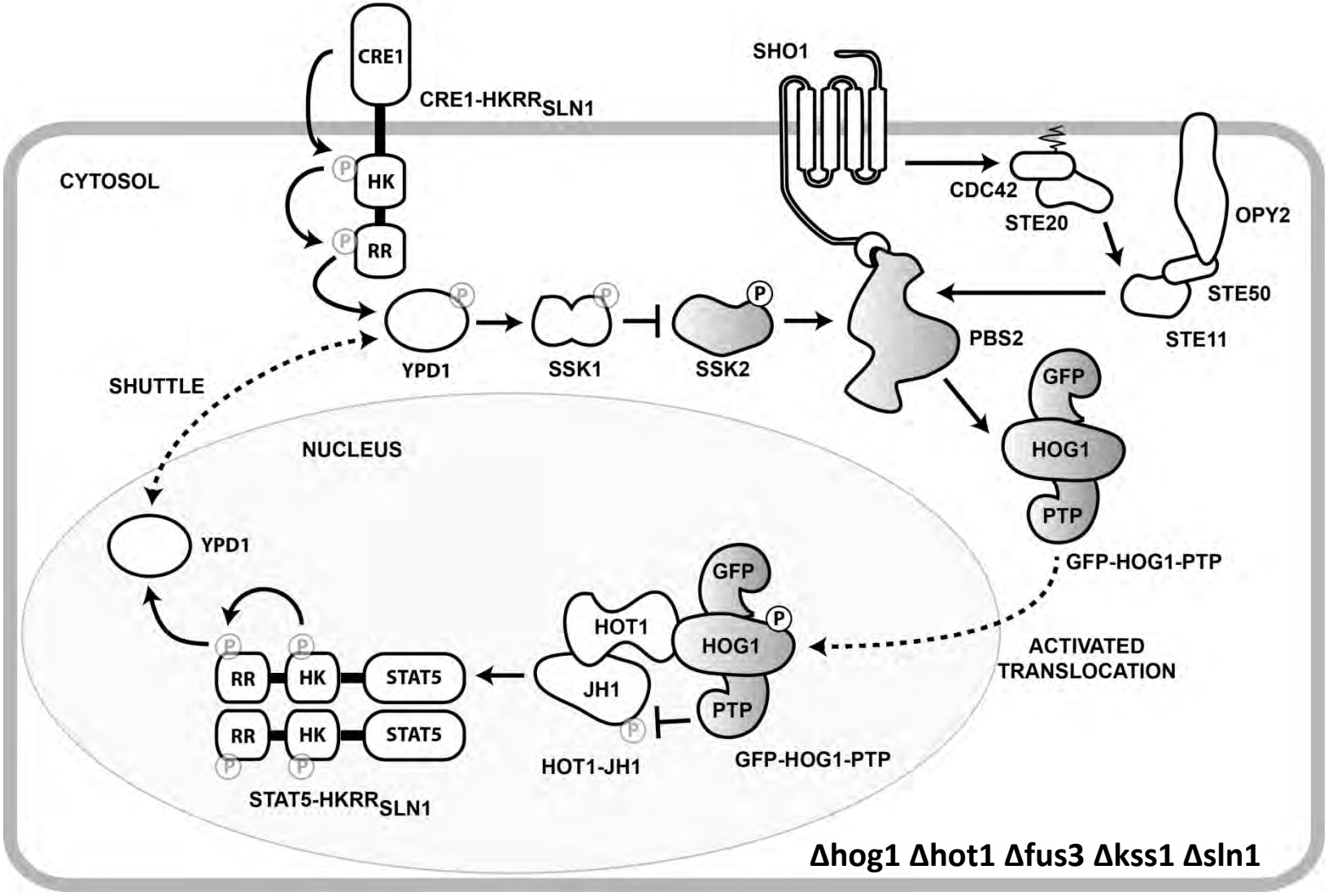
Protein Network Operation – Two Stable States

State One:
PBS2 High

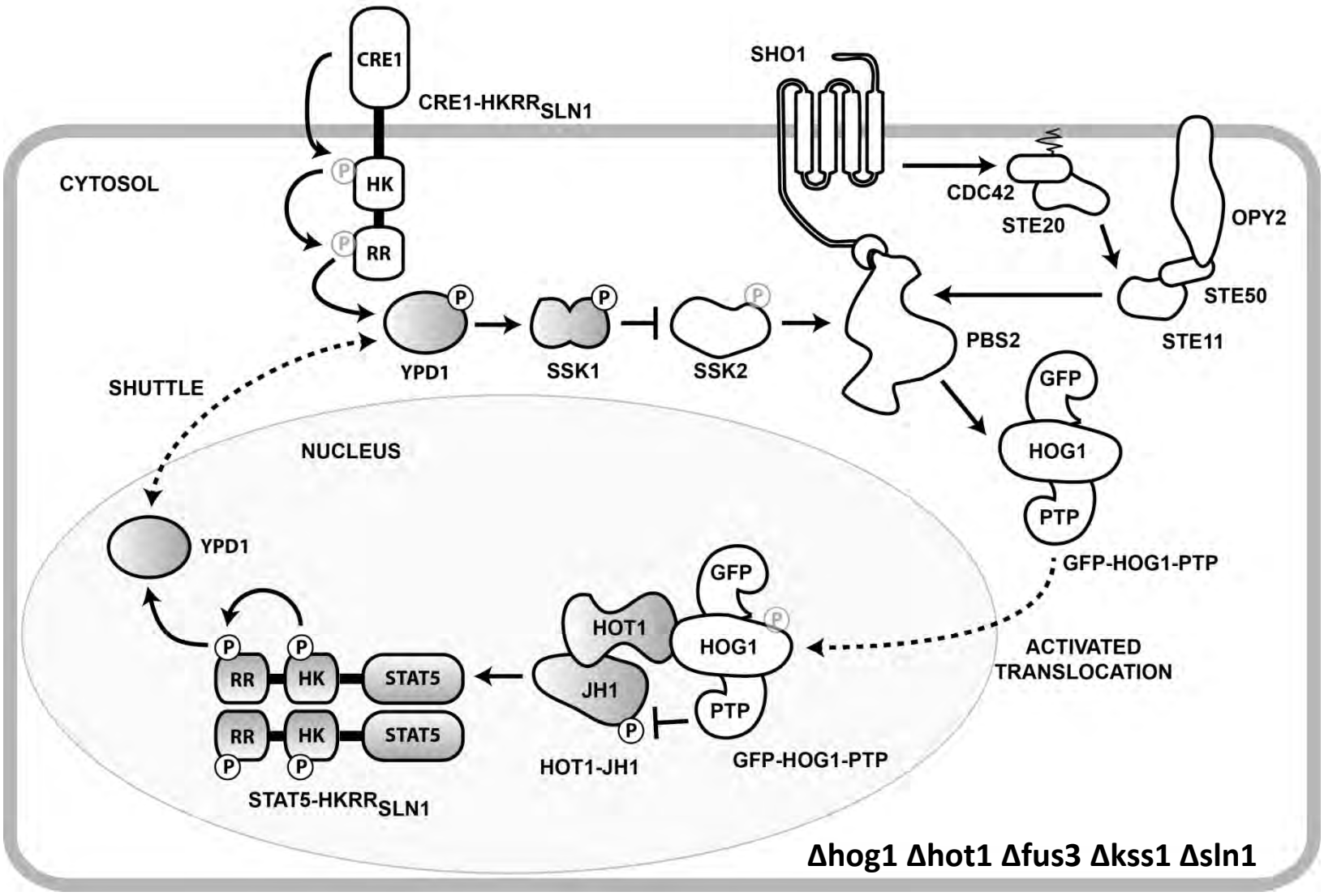
State Two:
YPD1 High



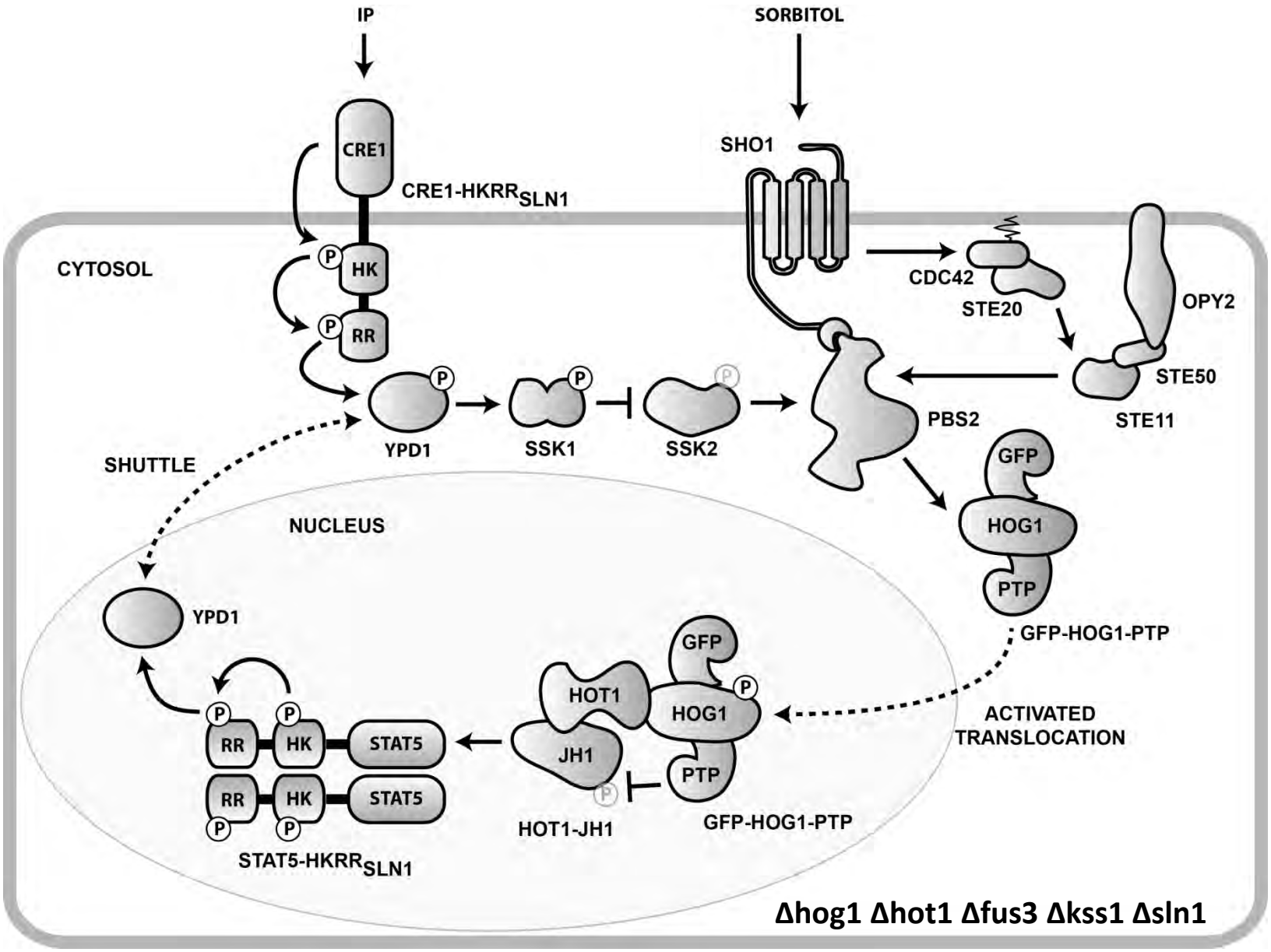
Switching to PBS2 High Stable State



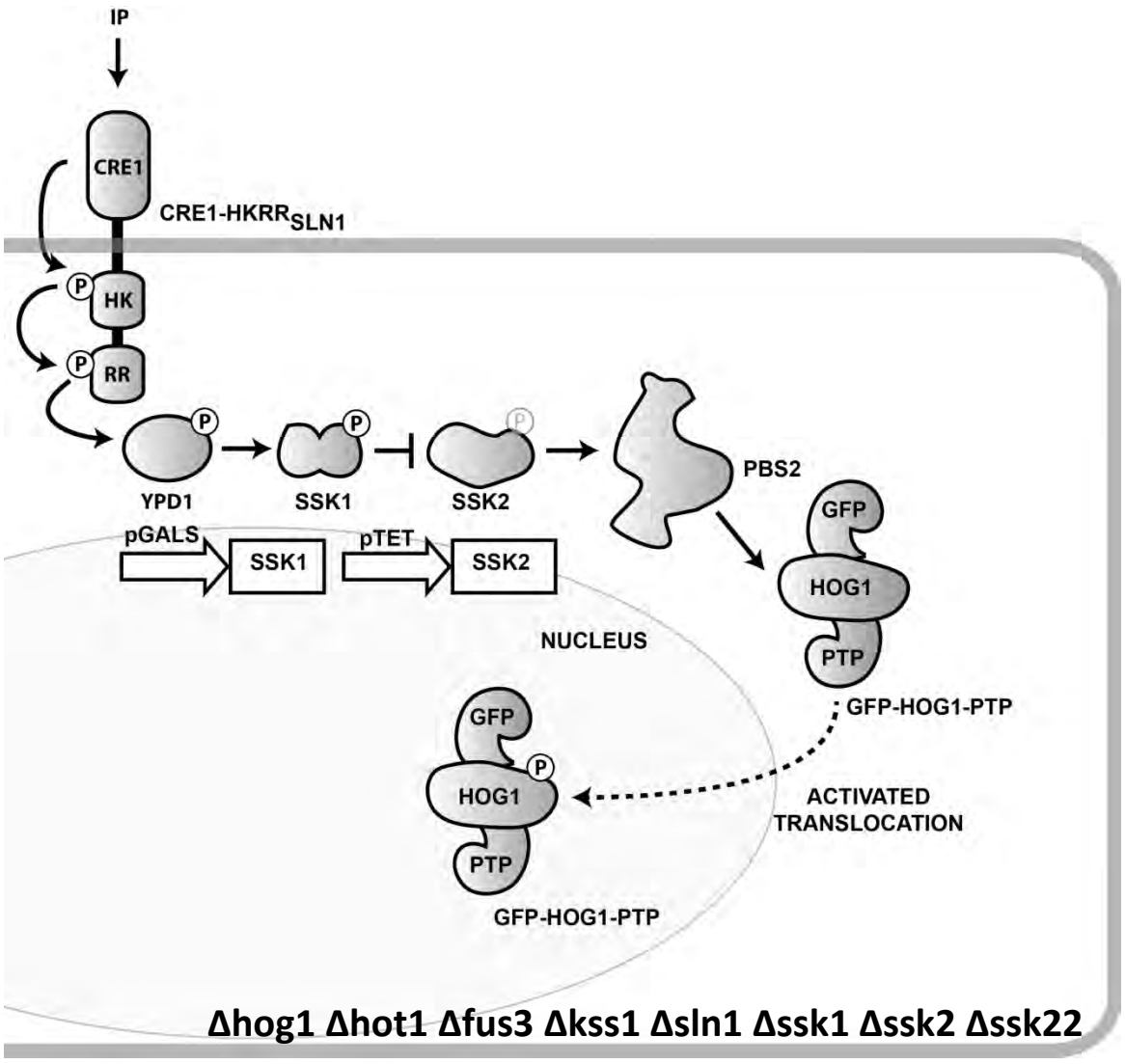
Switching to YPD1 High Stable State



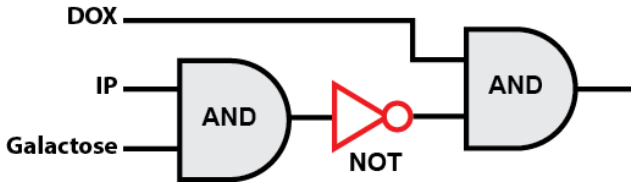
Characterizing the Toggle Network

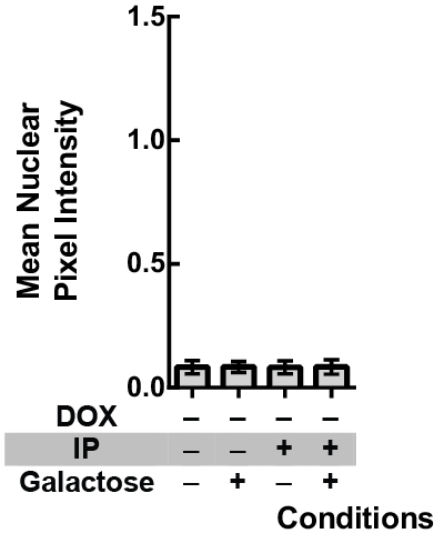


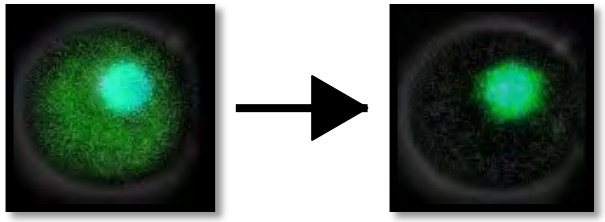
Characterizing Endogenous NOT gate



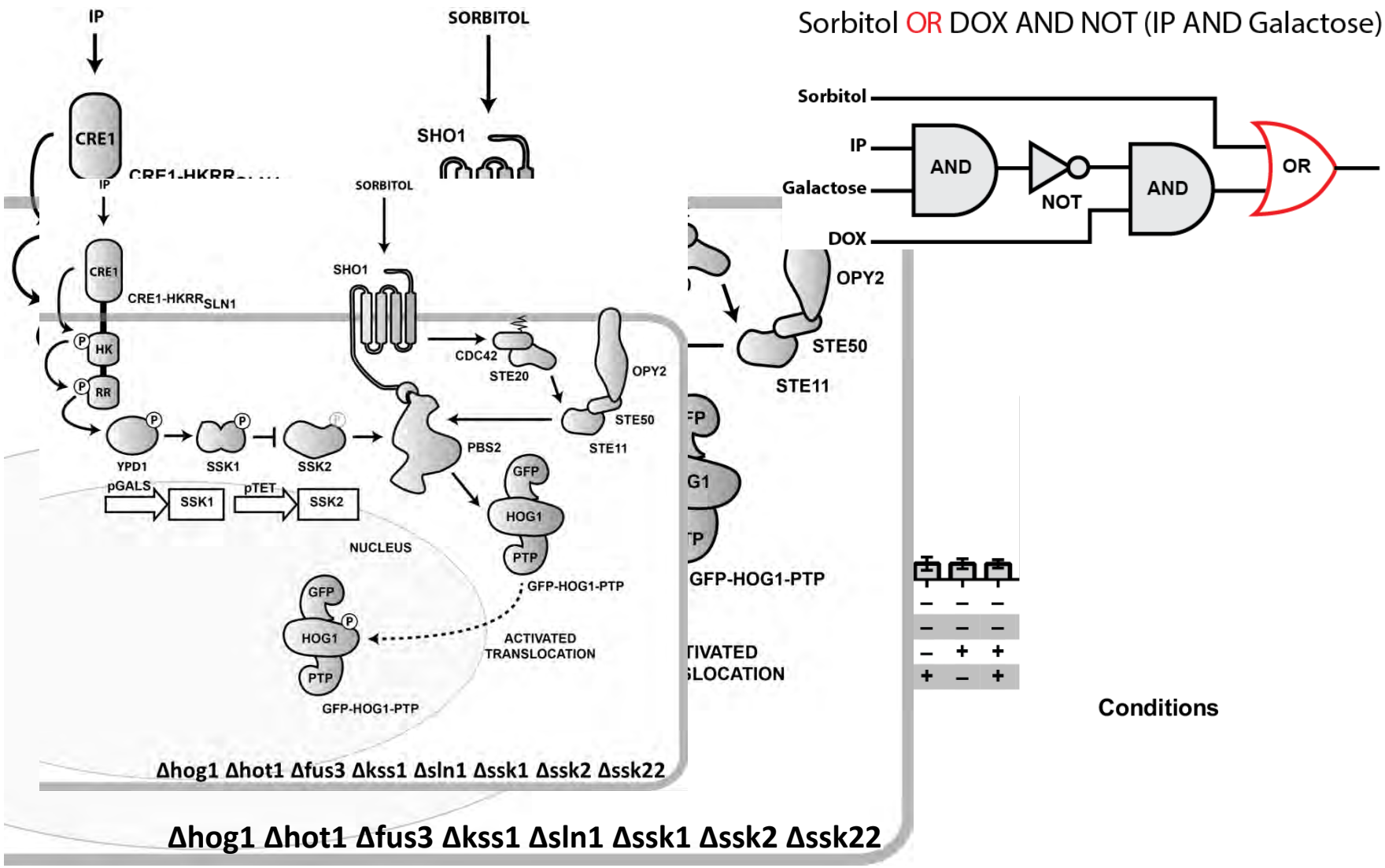
DOX AND NOT (IP AND Galactose)





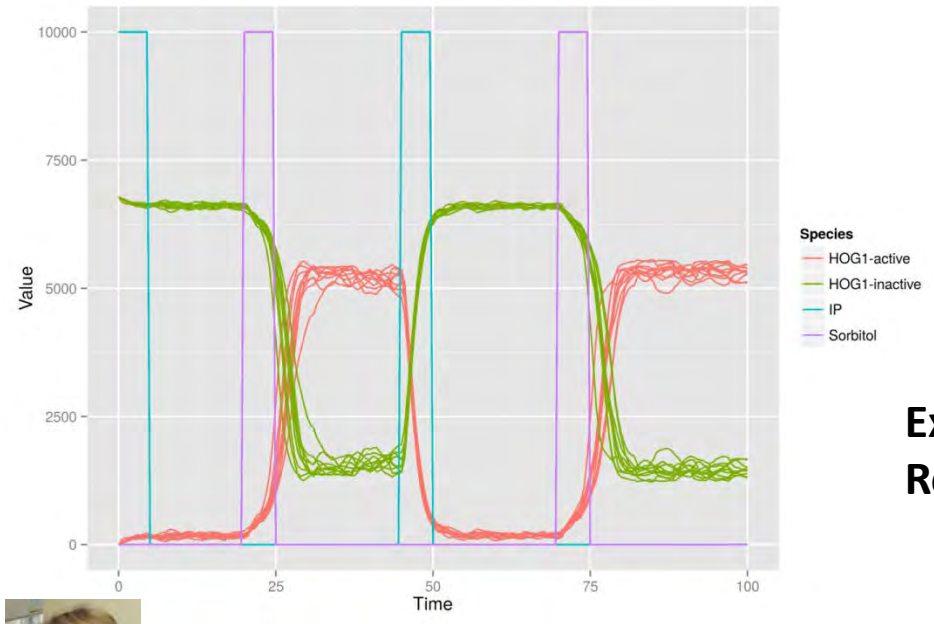


Characterizing OR gate from Engineered Signal Diversion



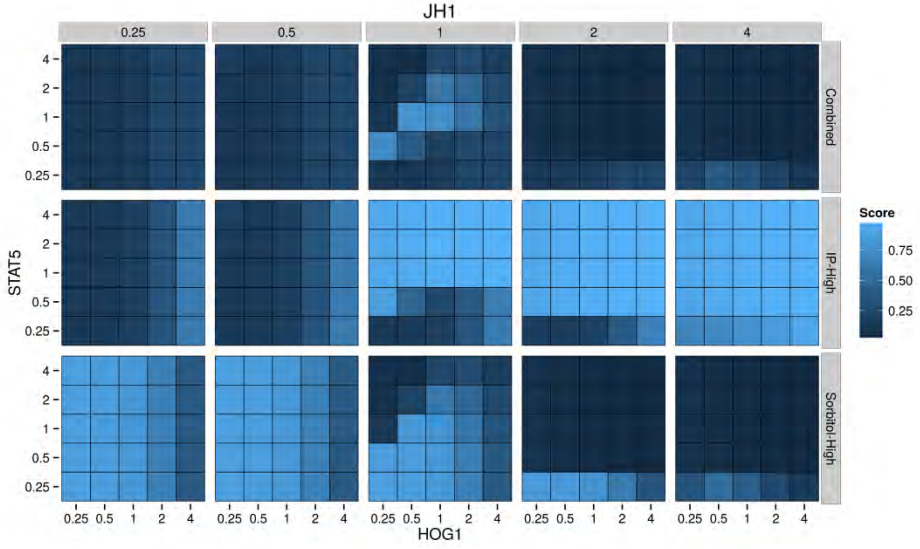
Computational model for toggle optimization

- **Created stochastic mathematical model (~2 dozen species)**
- **Parameter sweep across gene expression levels and reaction rates**

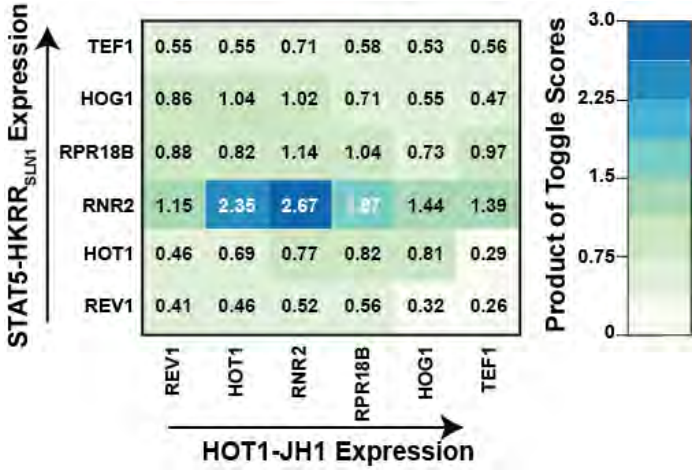


Tristan Bepler

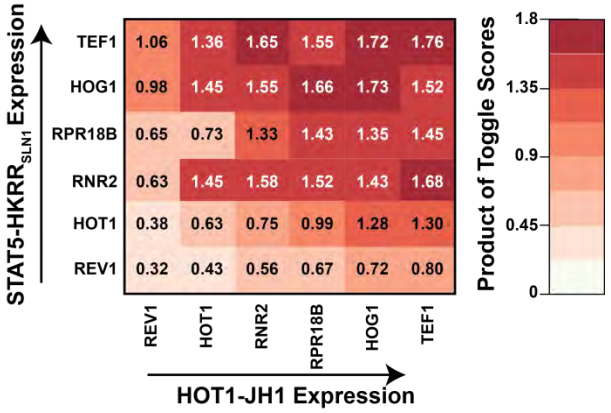
- **Identified expression amount and relative ratios of HOG1/HOT1/STAT5 as critical for bistable behavior**



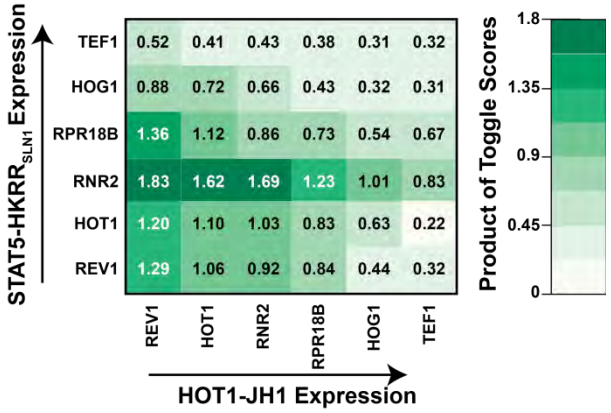
Experimental Results



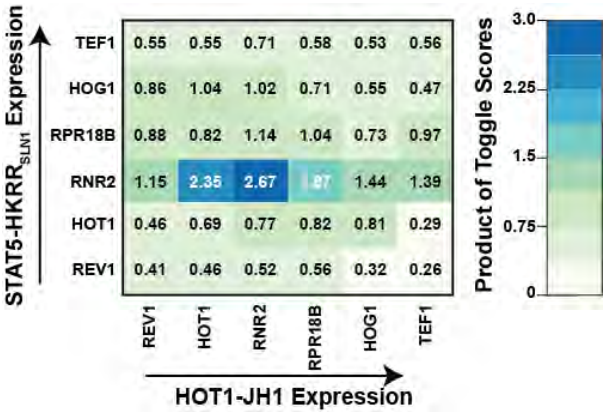
Toggle Optimization and Computational Prediction



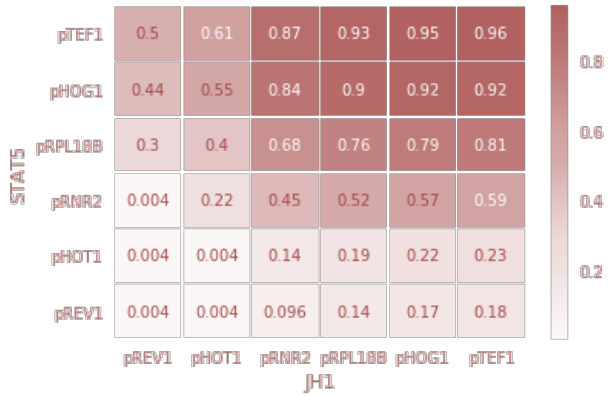
IRR/SRR

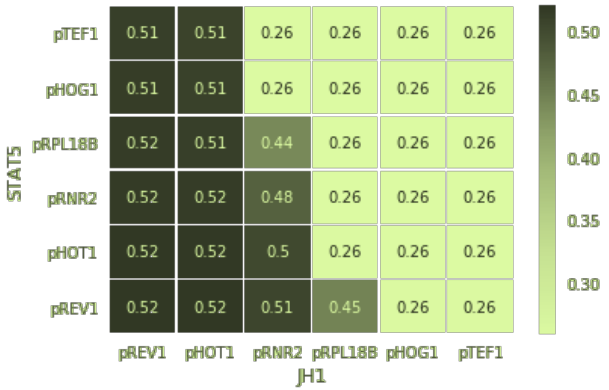


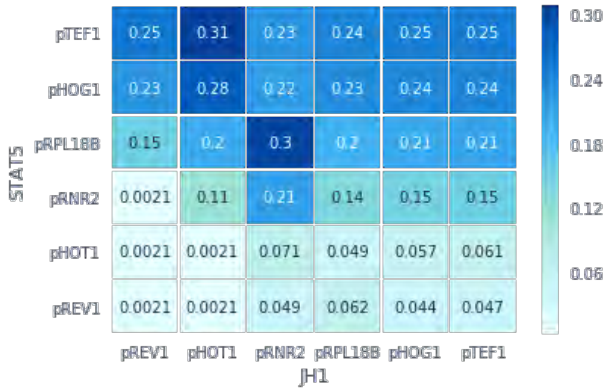
SRG/IRG



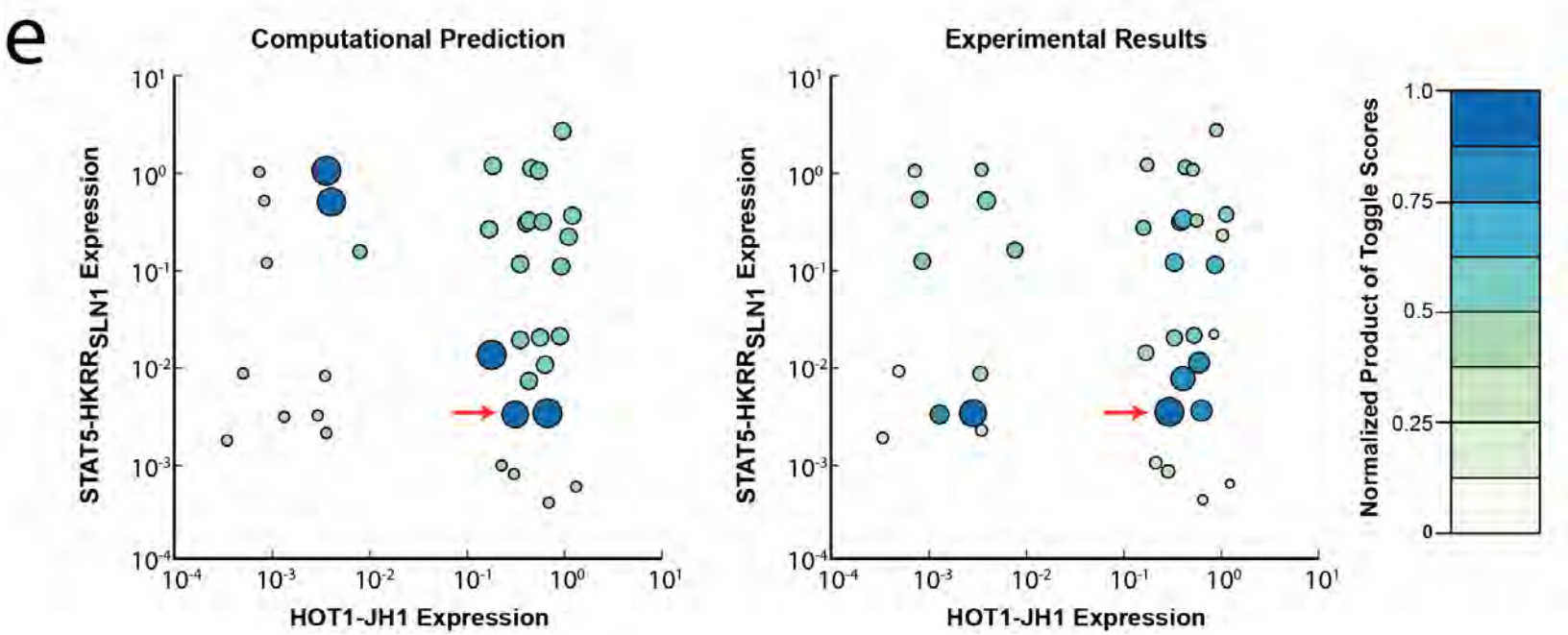
Product



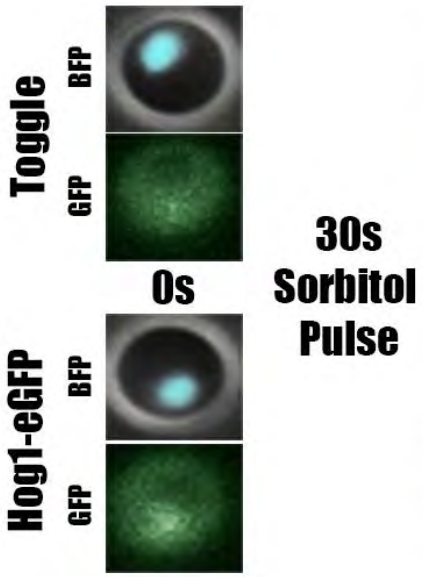


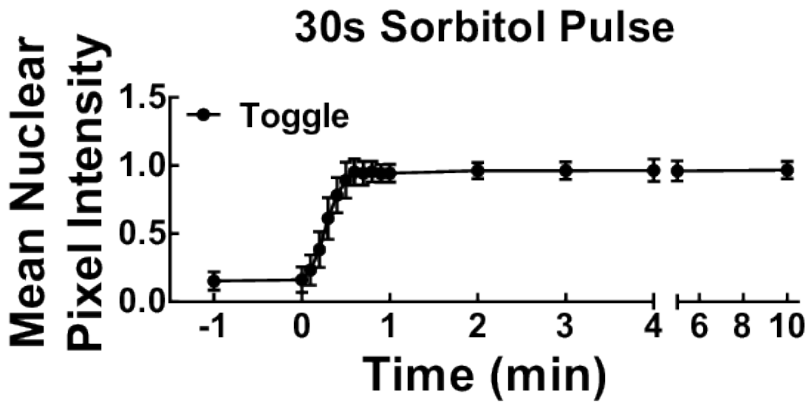


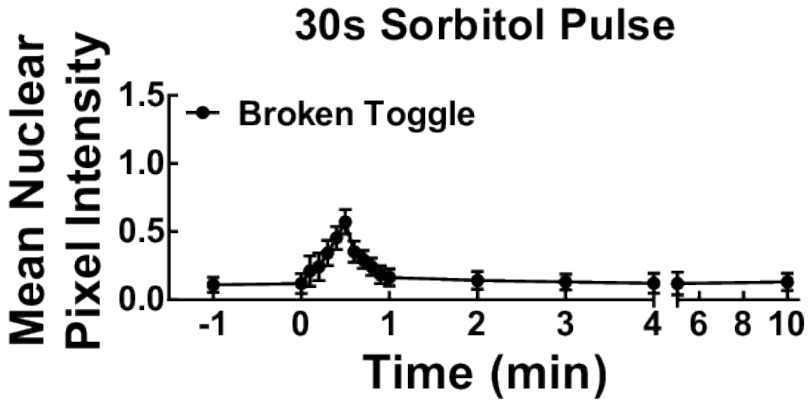
qPCR Validated Expression & Rearrangement of Data



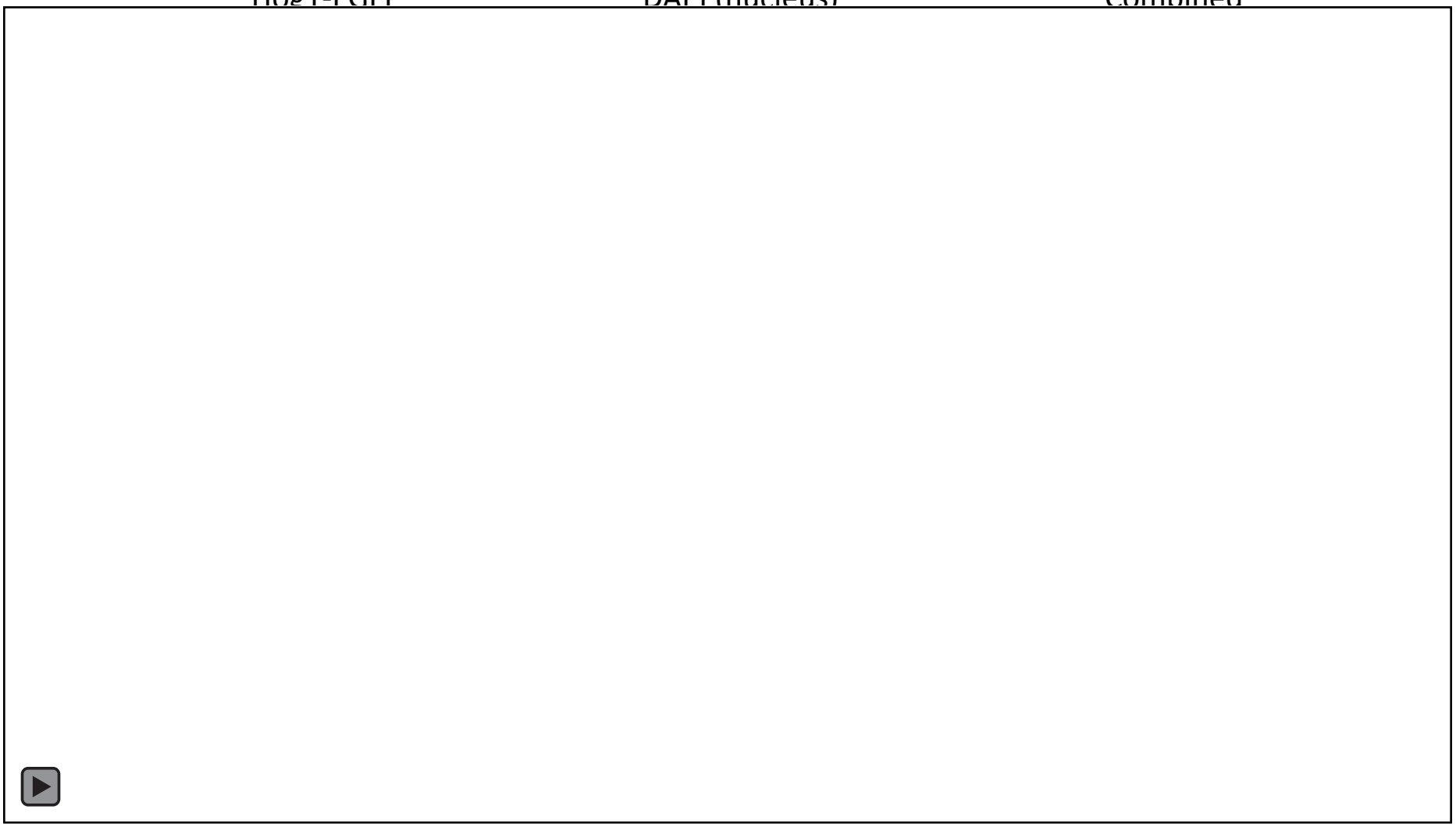
Toggle Network Dynamic Operation with Sorbitol



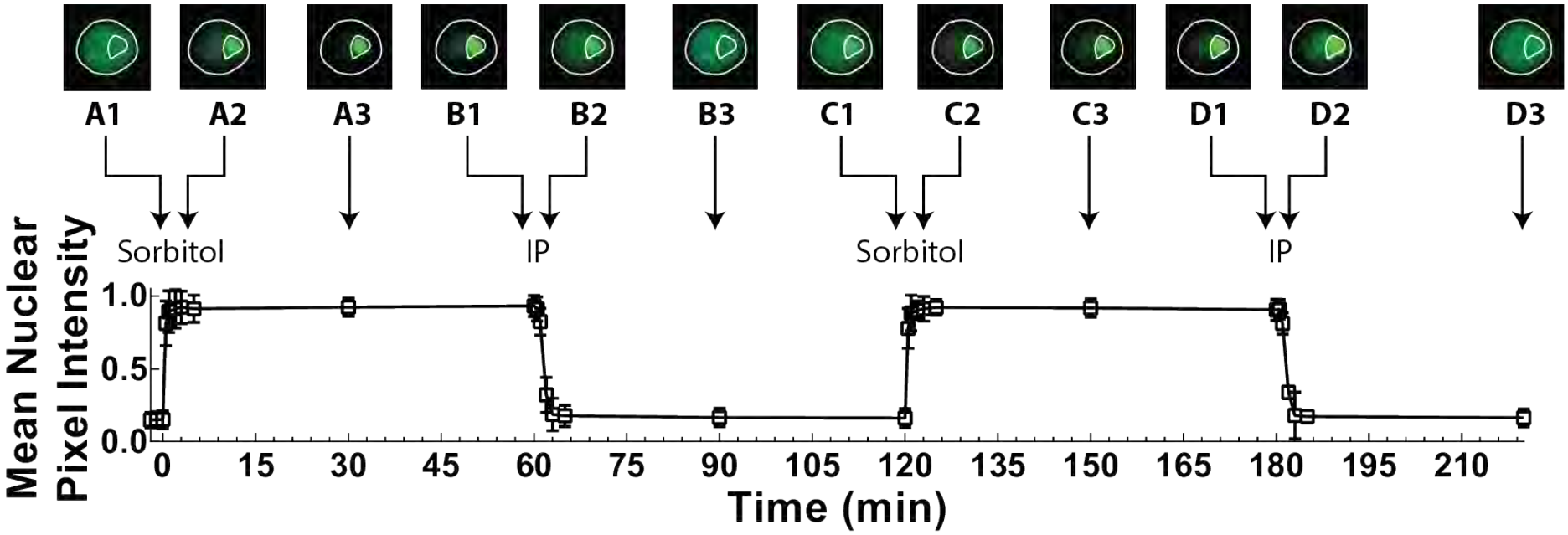




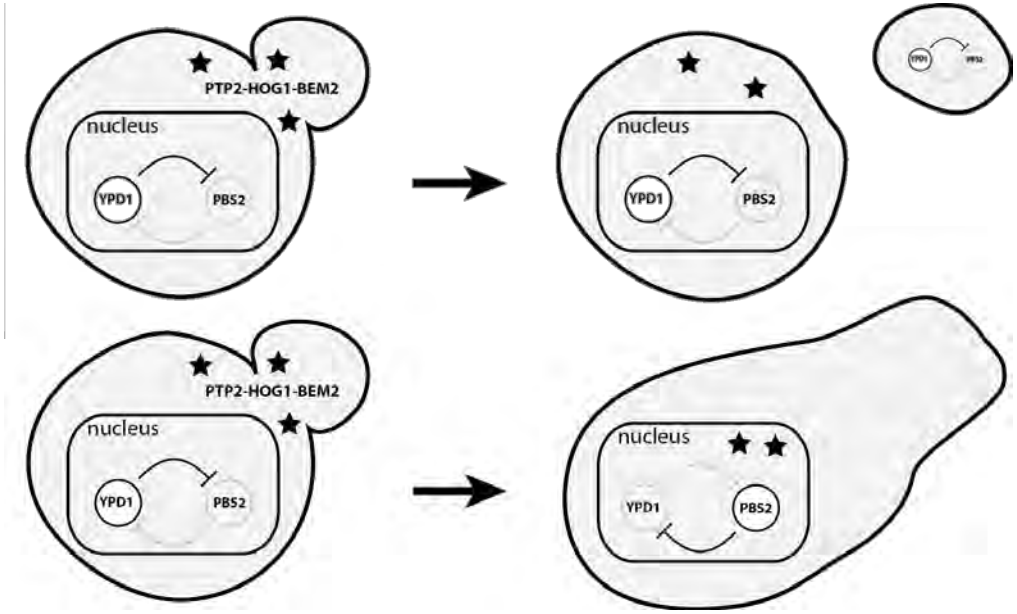
Demonstrating Toggle Network Bistability

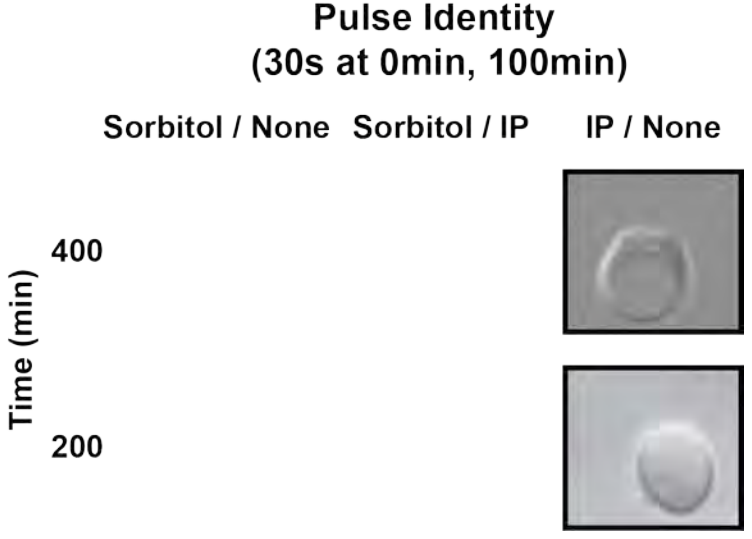


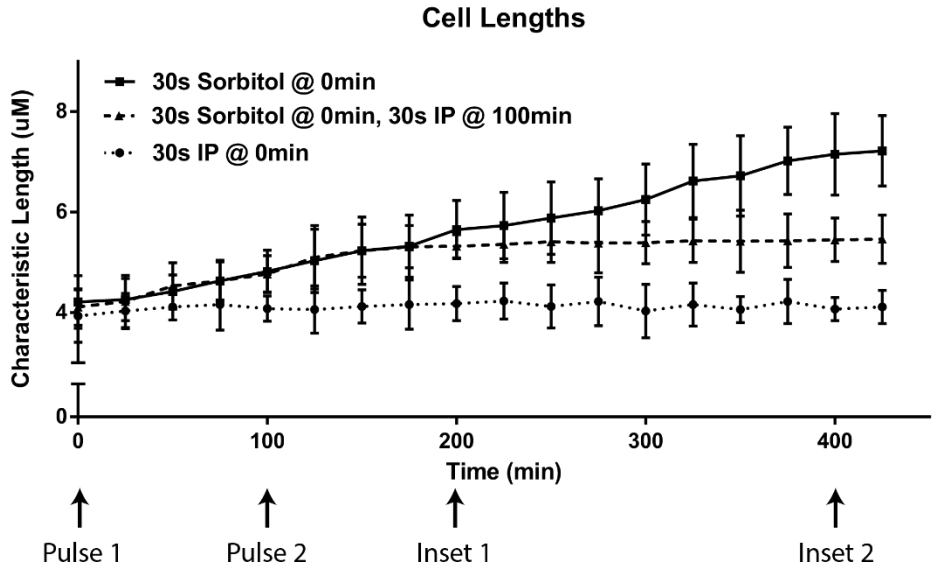
Demonstrating Toggle Network Bistability



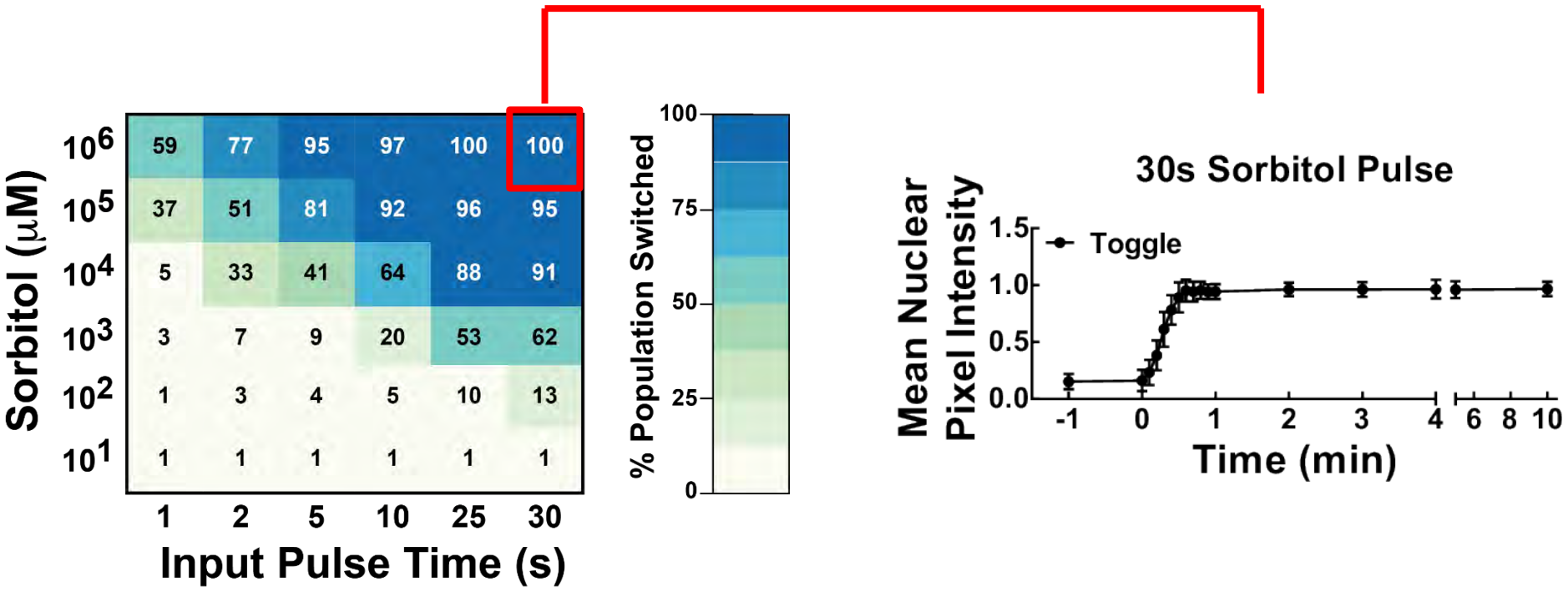
Controlling Cellular Phenotype



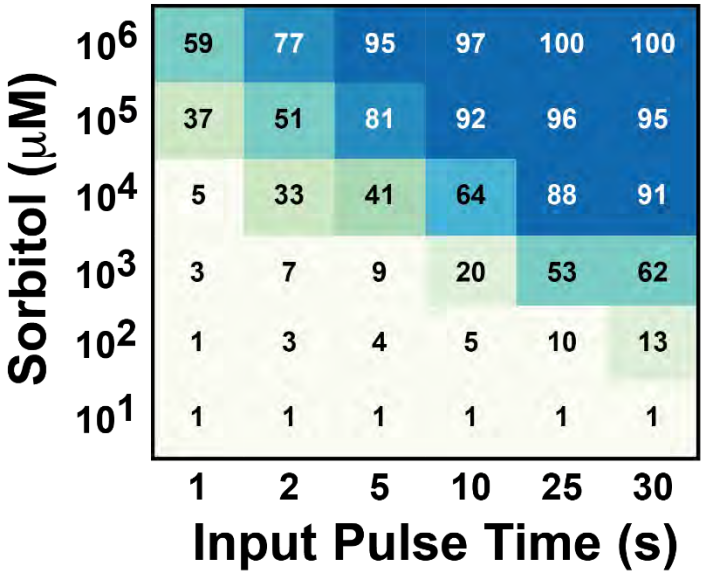


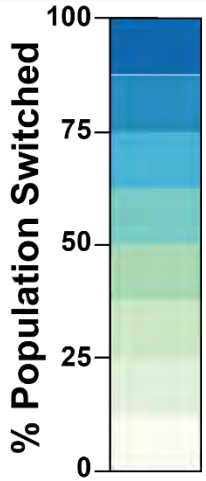


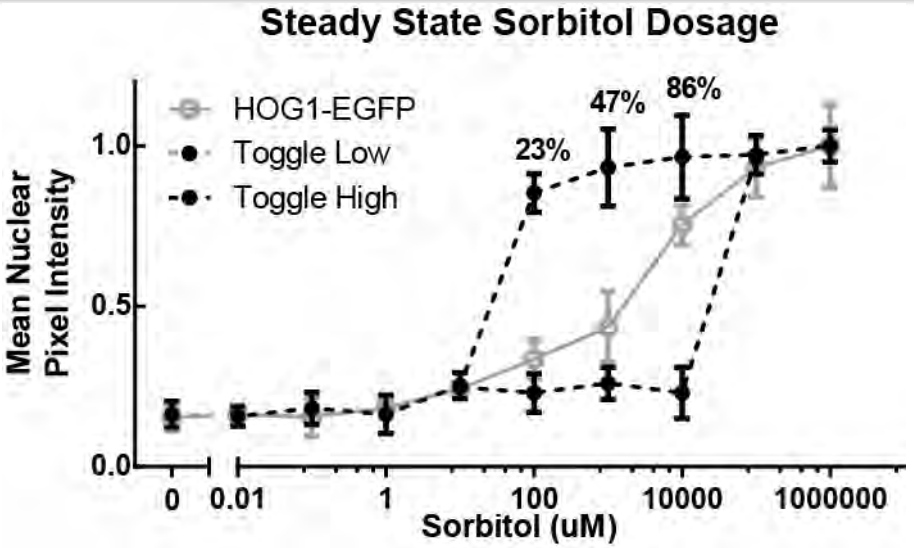
Toggle Network Dynamic Operation with Sorbitol

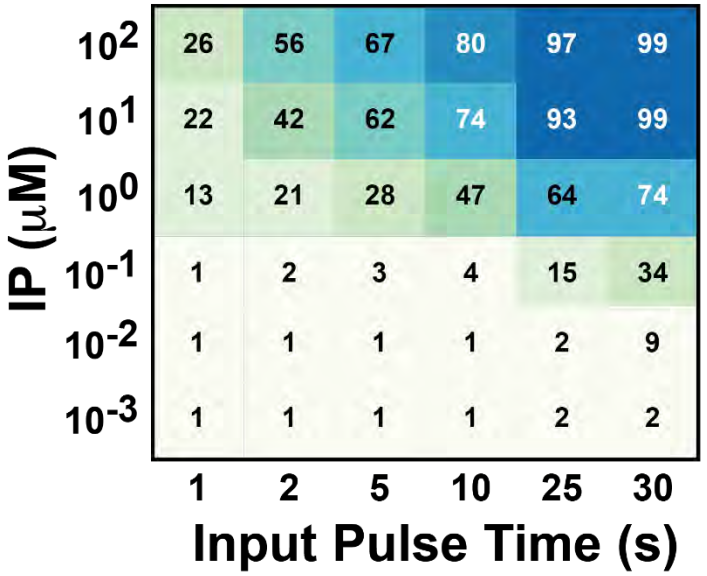


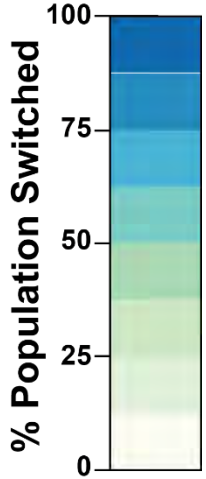
Toggle Network Operation

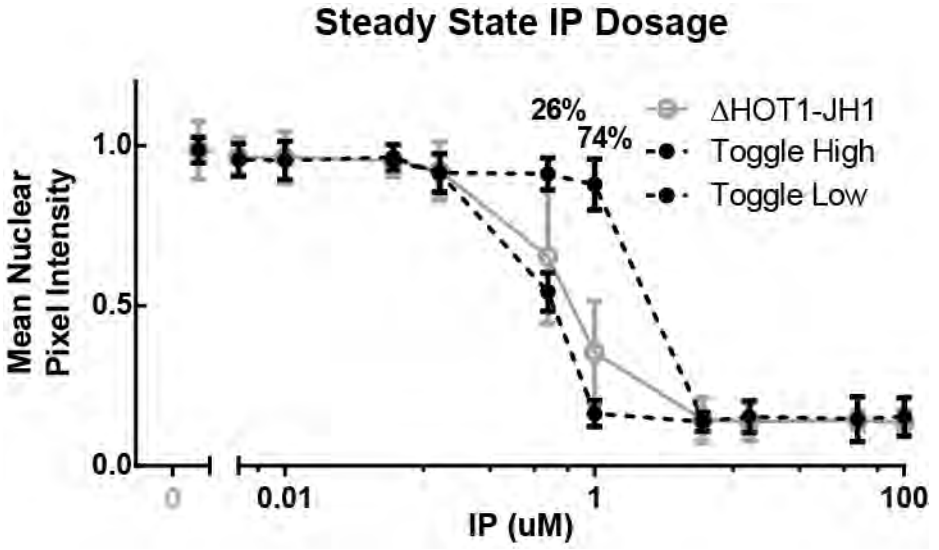




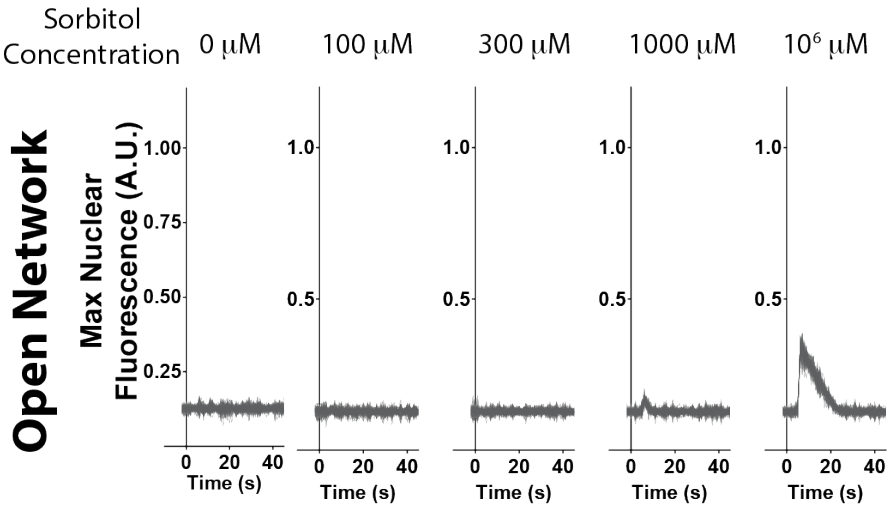


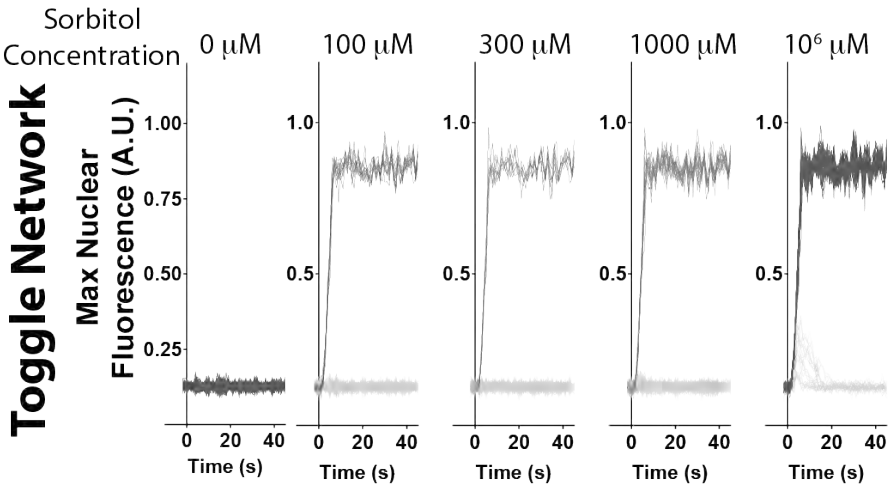


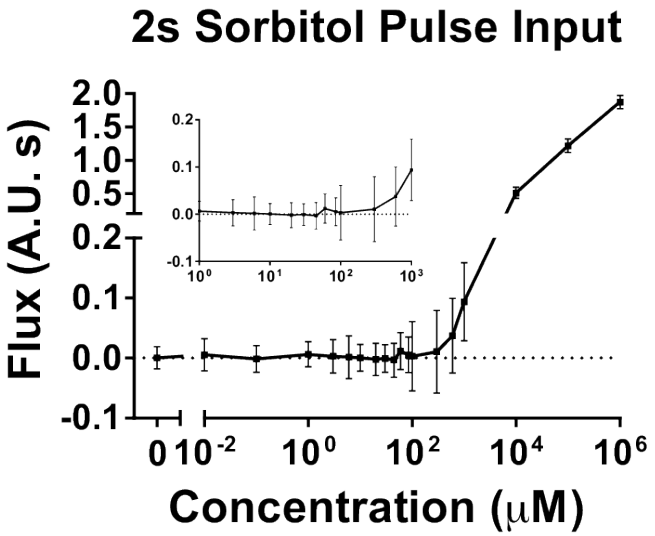


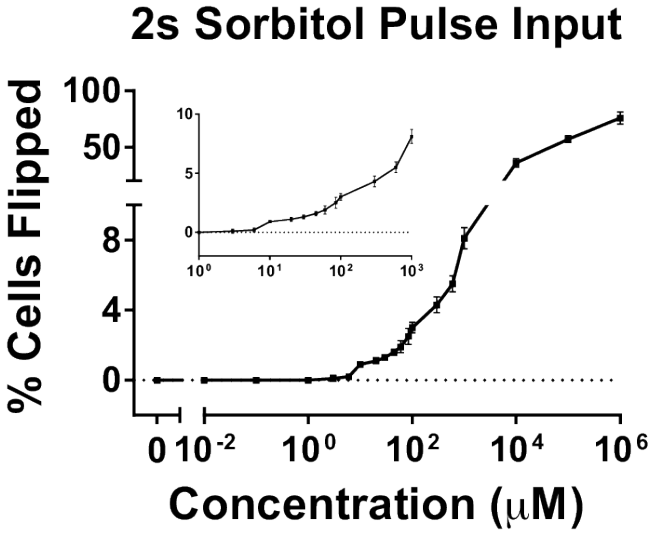


Stochastic Ultrasensitivity

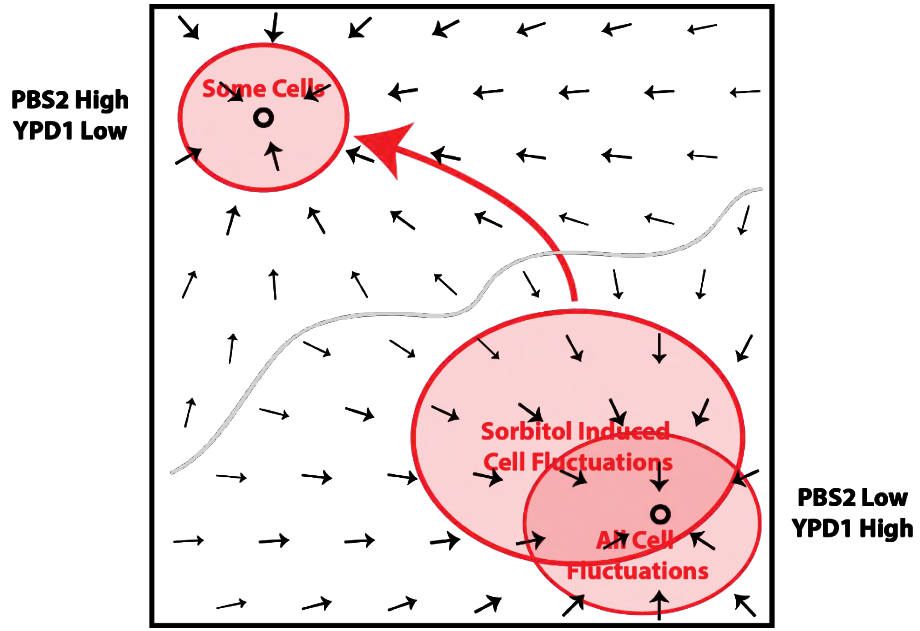


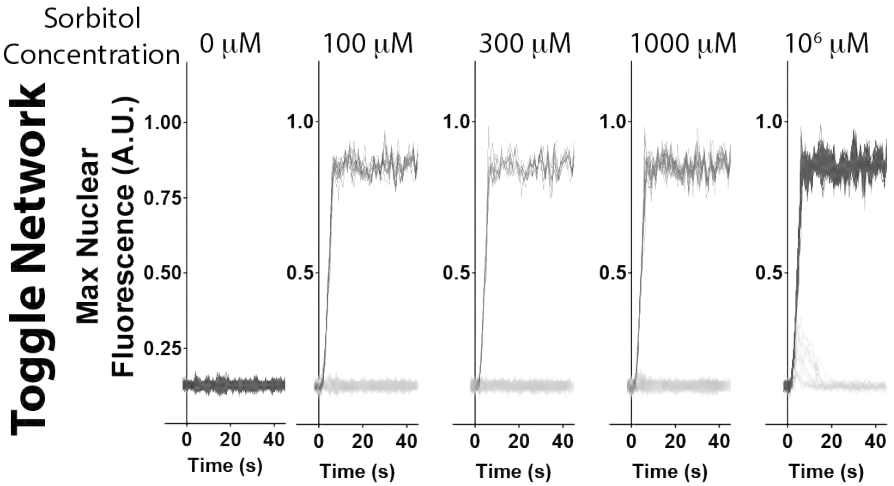




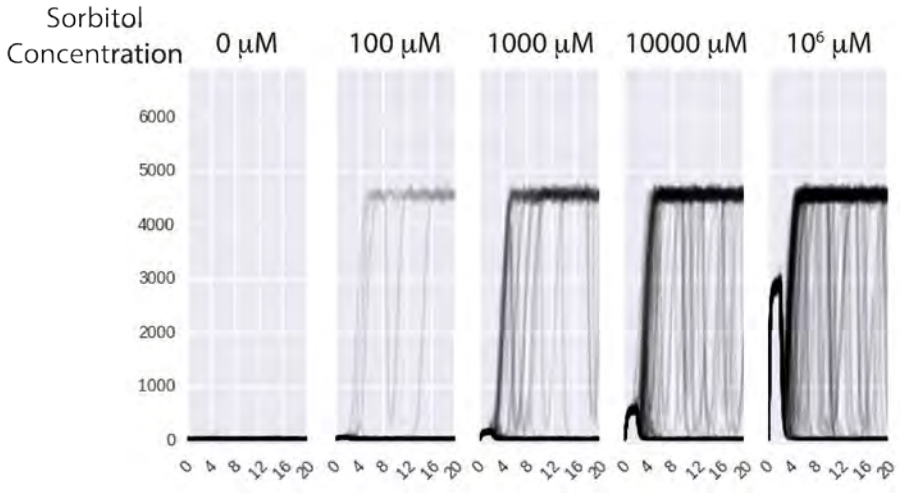


Stochastic Ultrasensitivity



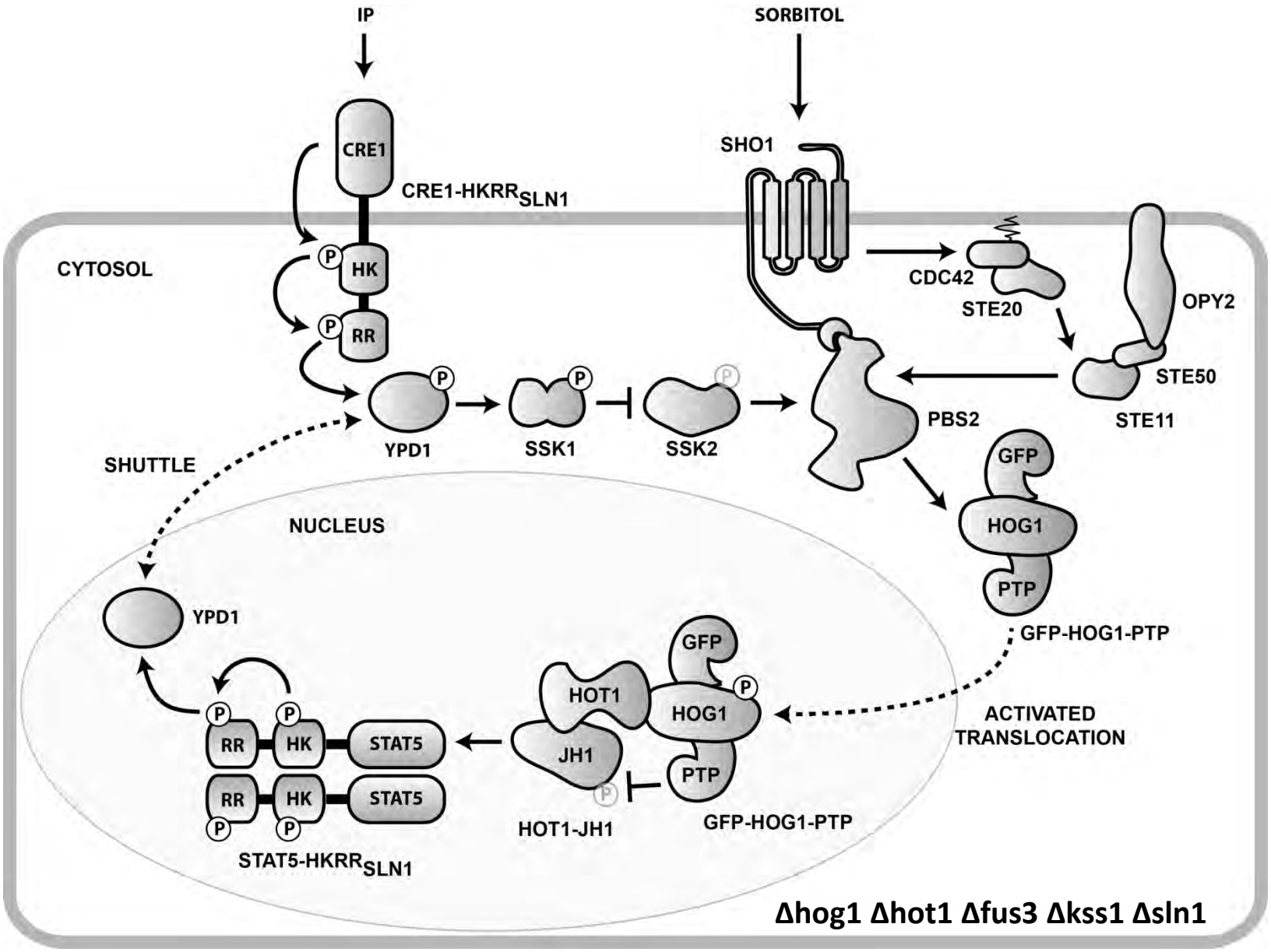


Experimental Results

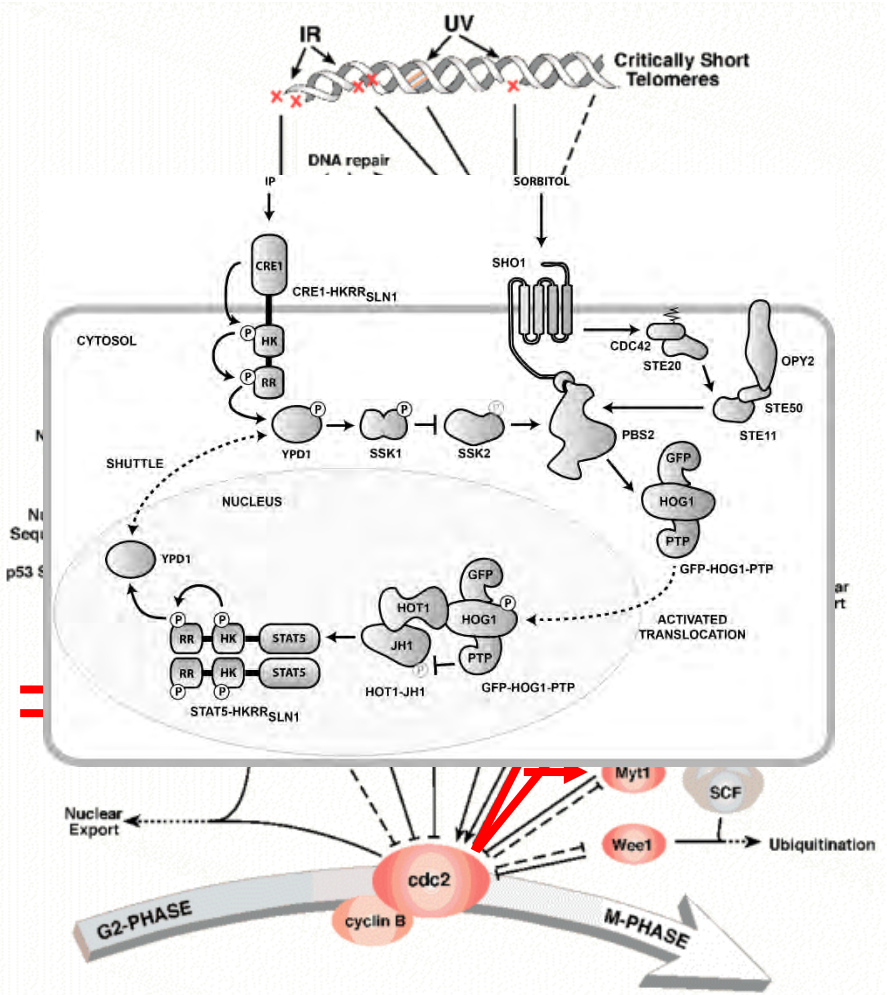


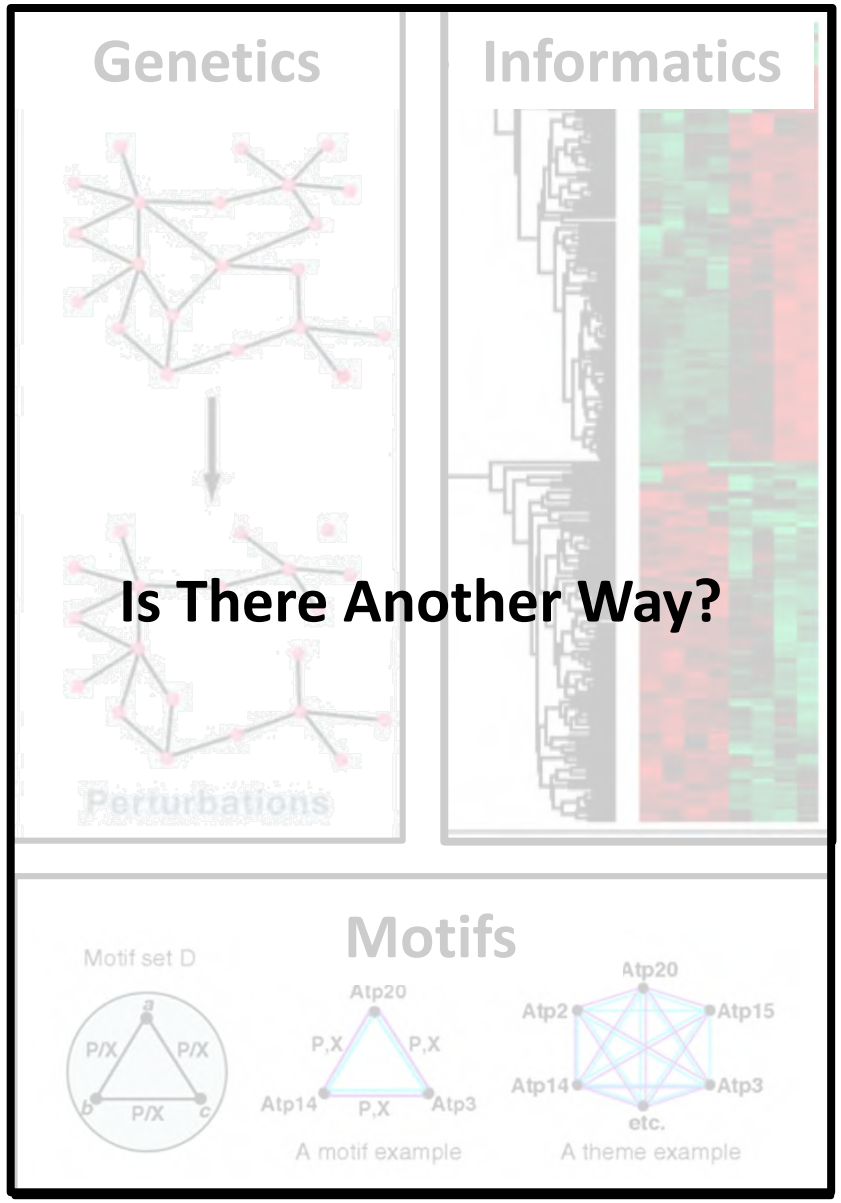
Mechanistic Simulations

Deciphering Protein Networks

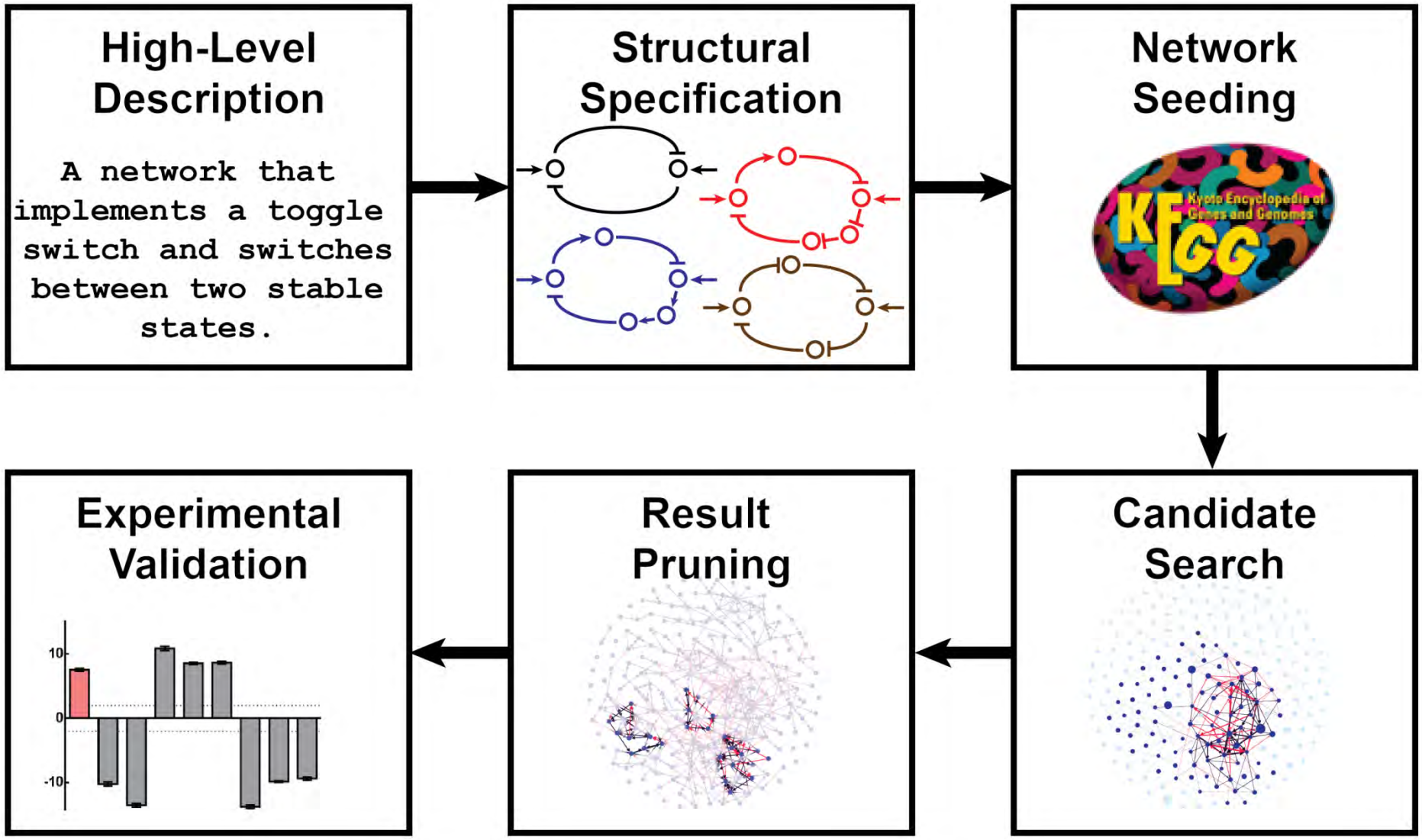


Deciphering Protein Networks





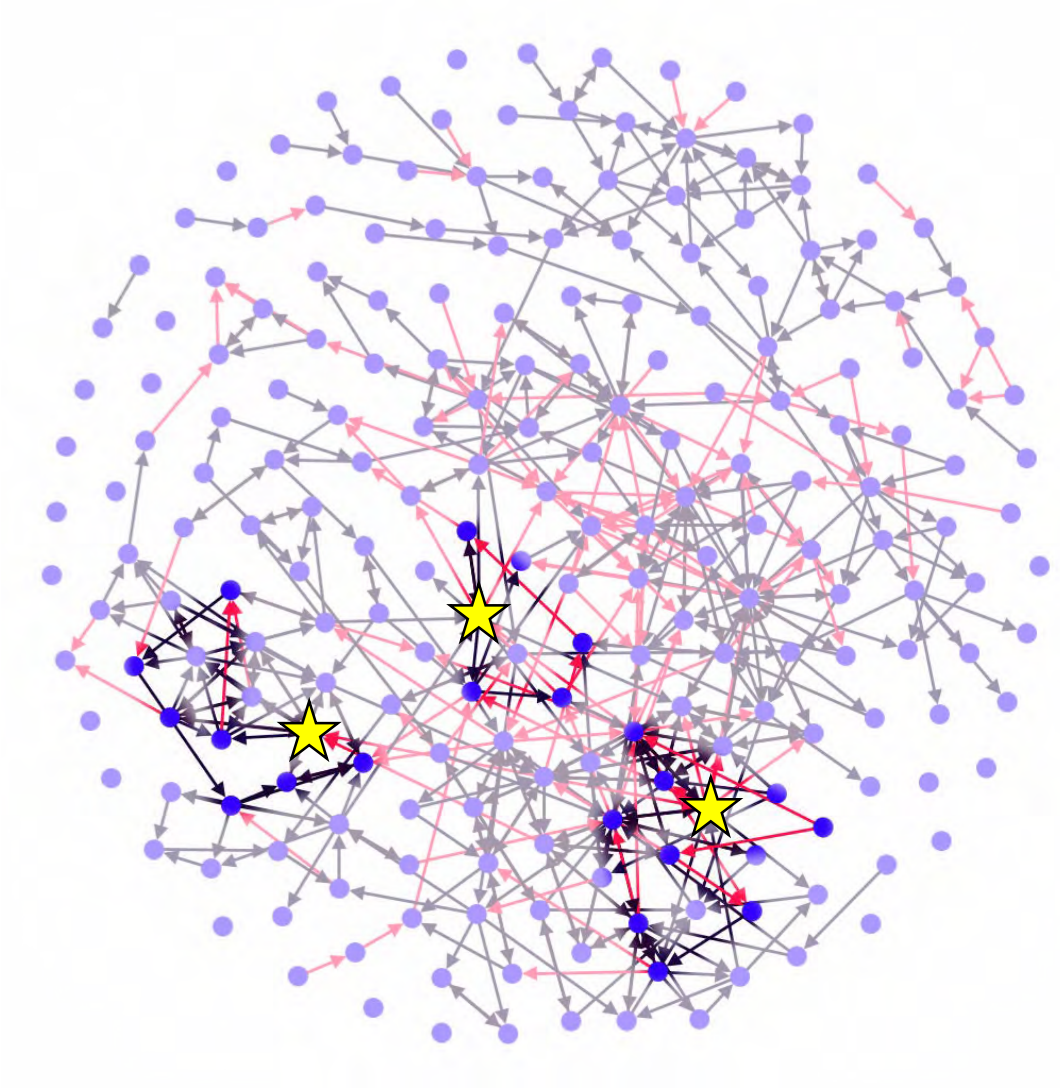
Biological Function ENcoded by Connected Edges (BioFENCE)



Biological Function ENcoded by Connected Edges (BioFENCE)

A toggle may be implemented with a cycle without repeating nodes with an even, non-zero number of inhibitory nodes.

1. Start with a single node
2. Trace a looped path that starts and ends with a single node that does not repeat any node AND has an even number of inhibitory connections.



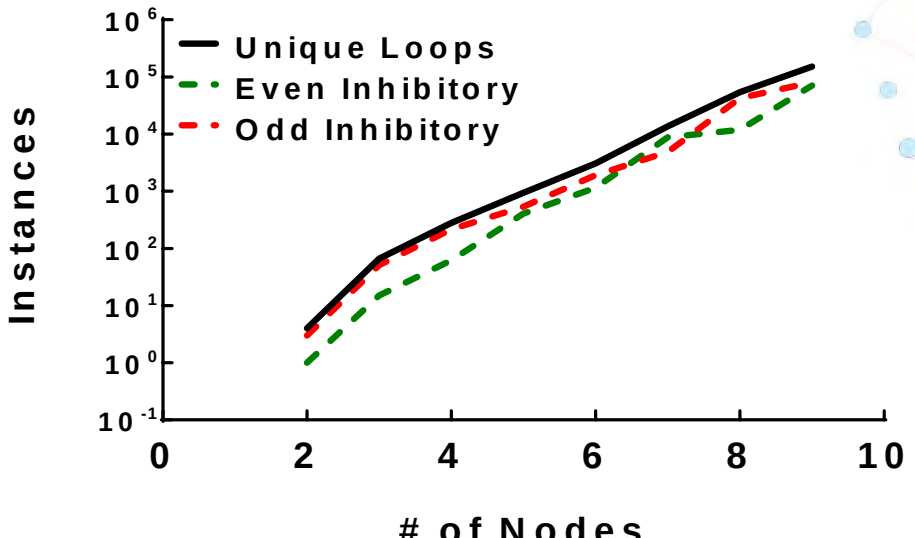
BioFENCE: Seeding and Searching

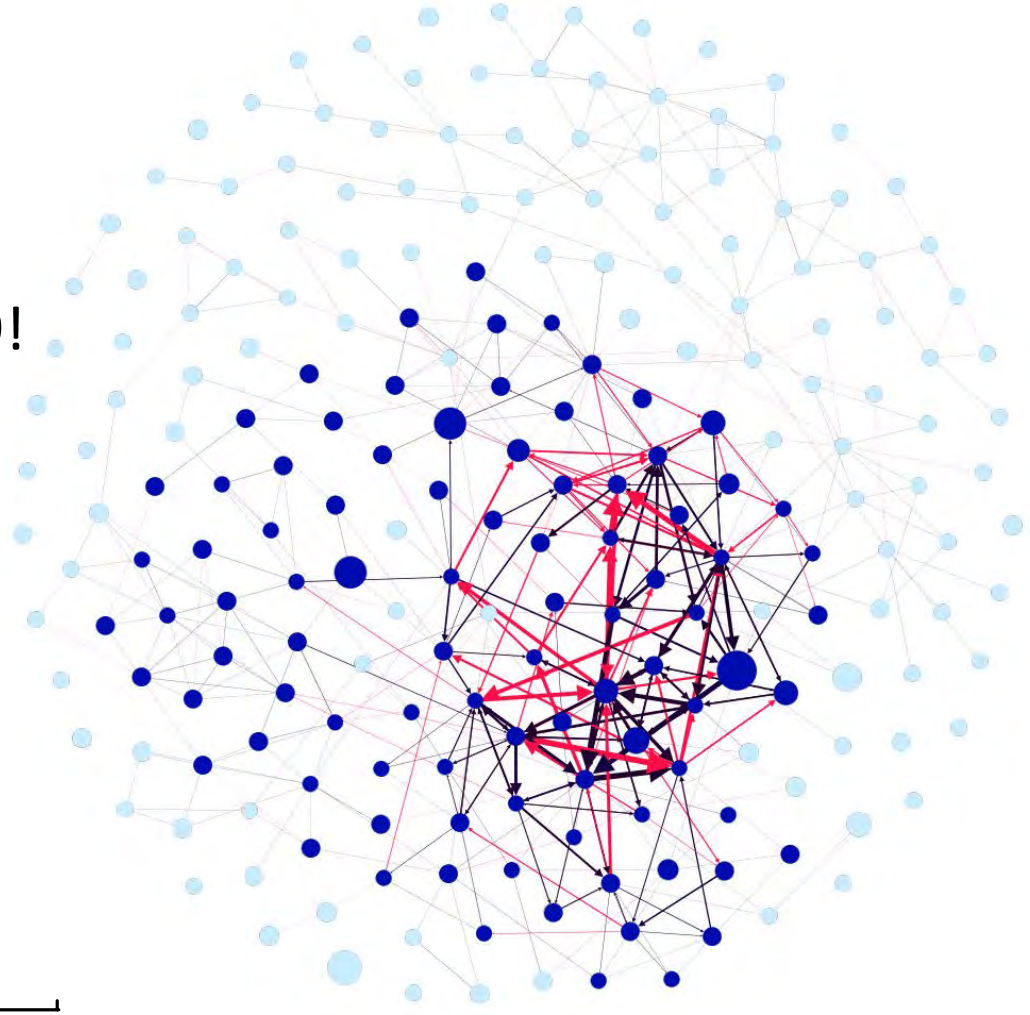
Seeded with *S. cerevisiae* KEGG

- 216 nodes
- 124 inhibitory edges
- 408 activation edges

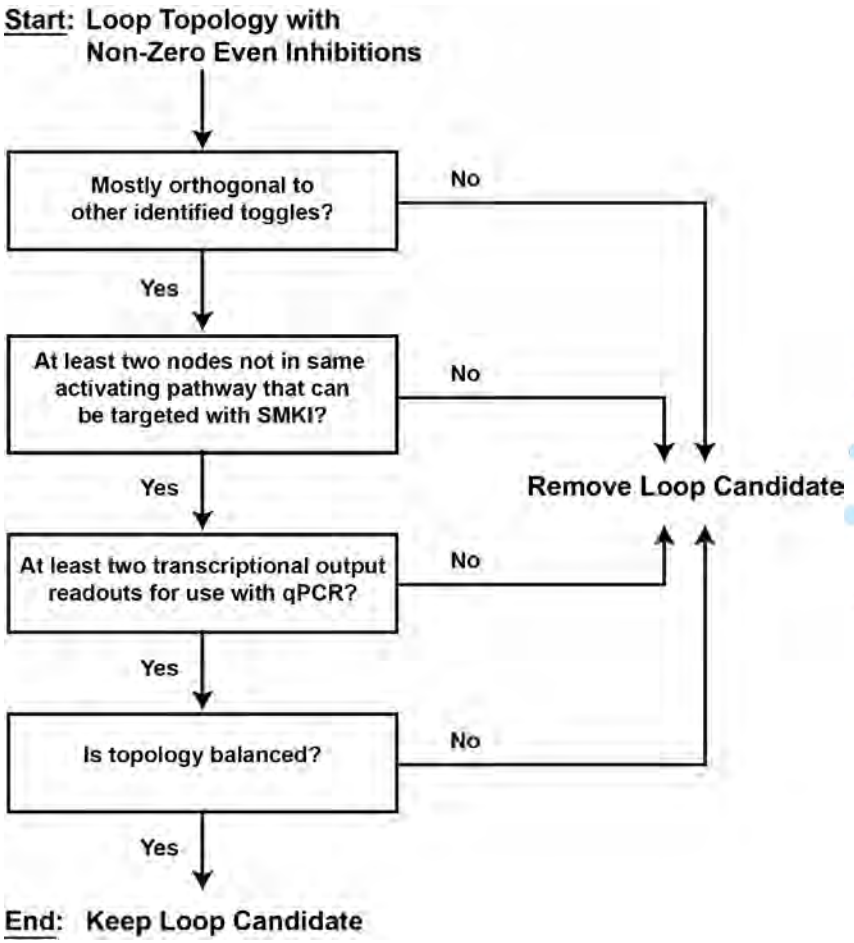
Yields 109401 networks to n=9!

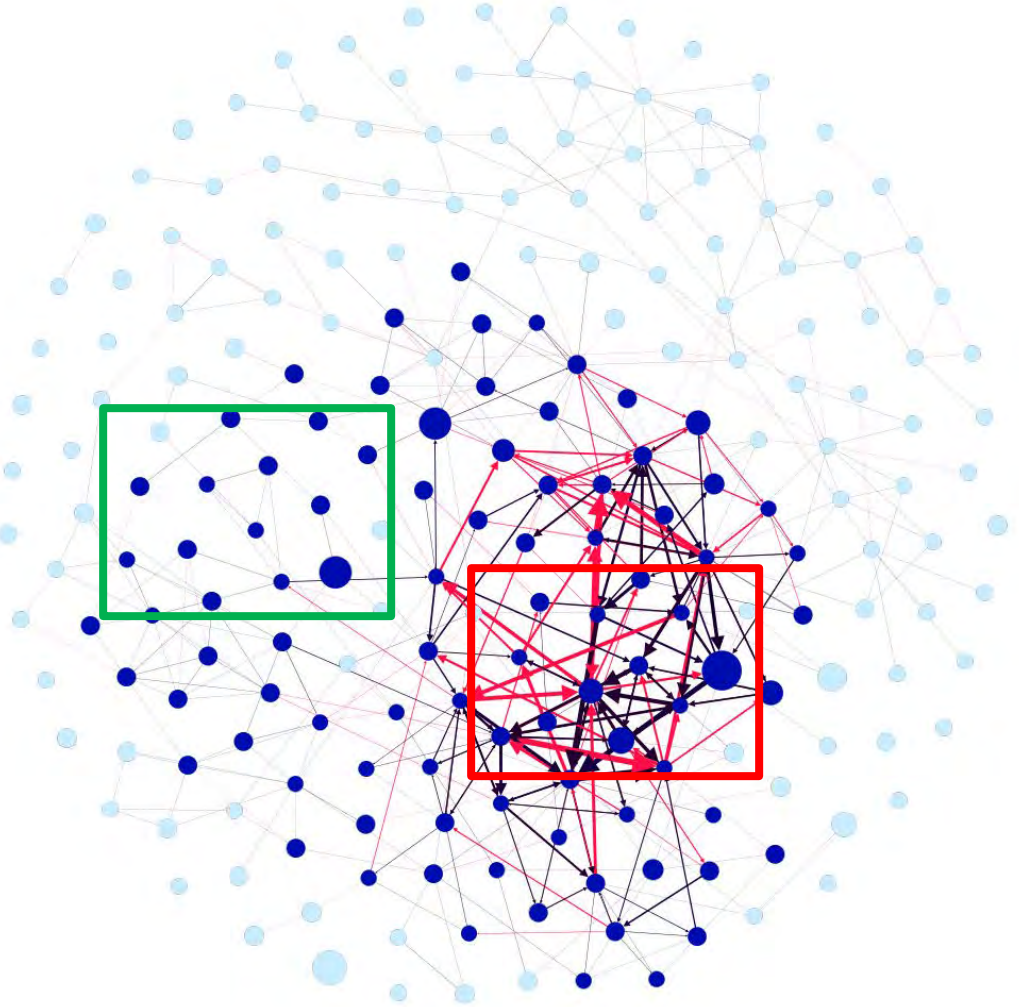
- 91/216 nodes
- 327/532 edges





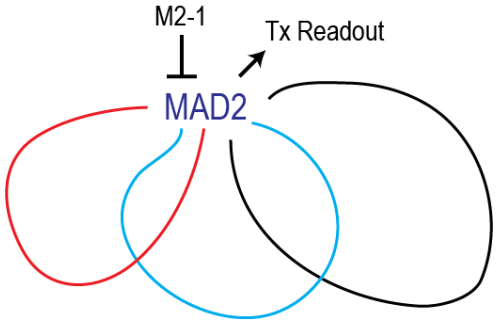
Choosing Good Candidates using BioFENCE To Validate



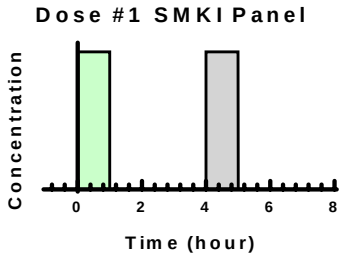


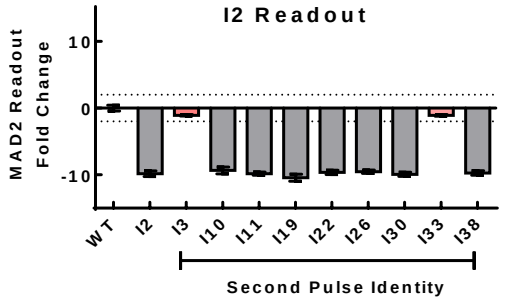
Experimental Validation of BioFENCE Candidates

ID nodes with several candidates AND input SMKI and TX readout

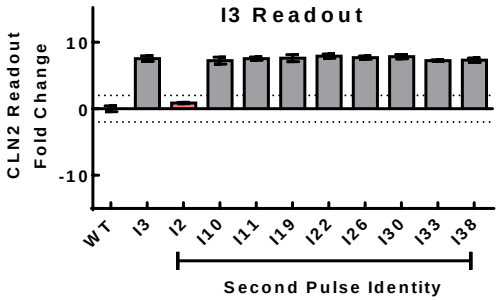


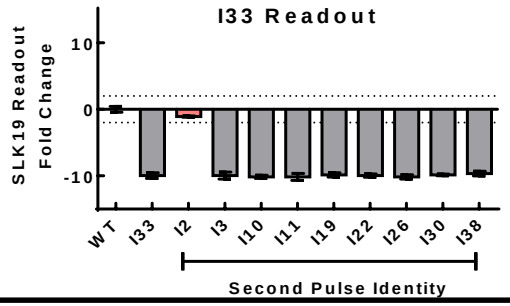
Screen with two sequential SMKI pulses and cognate Tx qPCR



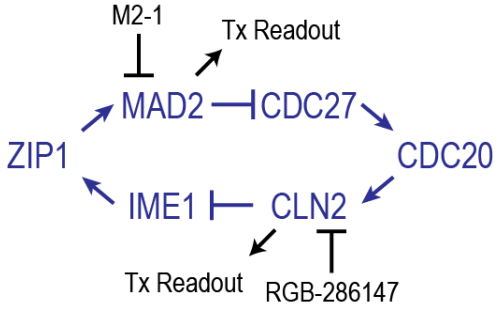


Validate 2-Input Network

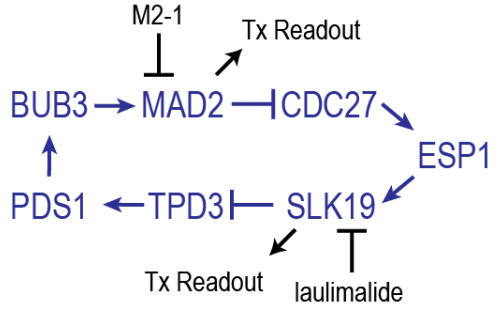




Identify target candidates

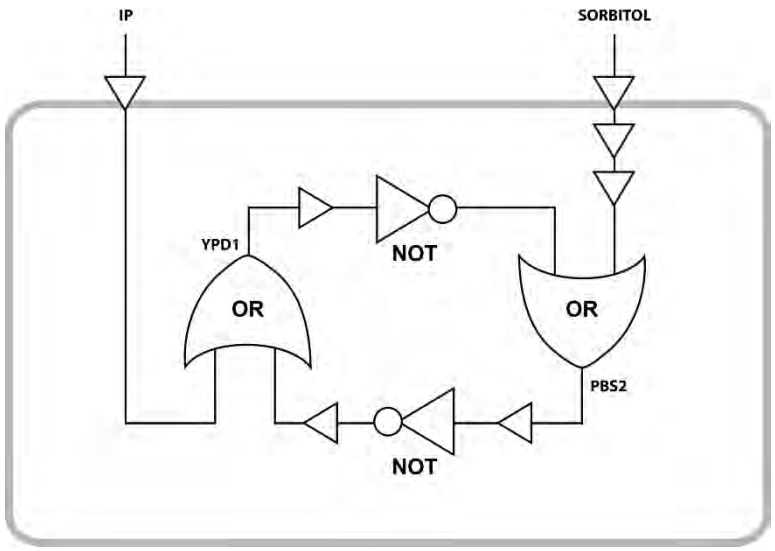


I2 inhibits MAD2
I3 inhibits CLN2

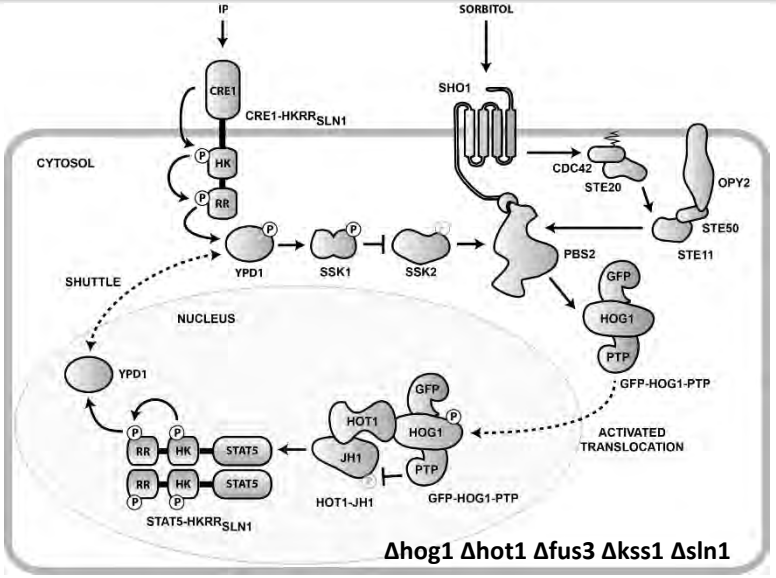


I2 inhibits MAD2
I33 inhibits SLK19

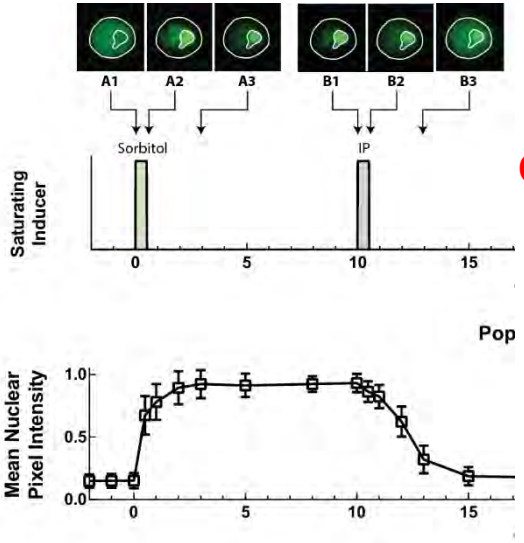
Synthetic and Endogenous Toggle Design Principles



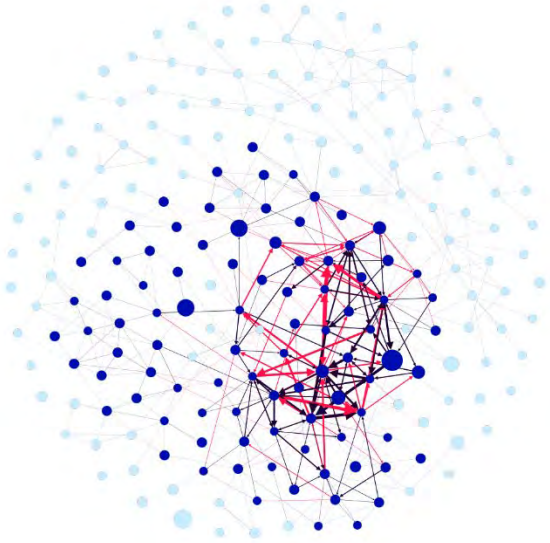
Devices to Systems



Optimization to Data



Synthetic to Endogenous



Biological Inspiration

Acknowledgments

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Ron, Lila, Patrick, Jingjing, Saurabh, Noah, Jon

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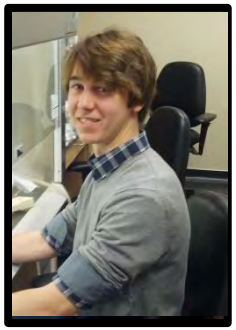

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An NIGMS-funded National Center for Systems Biology

MTL microsystems technology laboratories massachusetts institute of technology

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MITei

ICB

ebrc Engineering Biology Research Consortium

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Thank You!

Questions?

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Toolkit Questions?

ATCC-YTK@mit.edu