Genetic circuit design in synthetic biology

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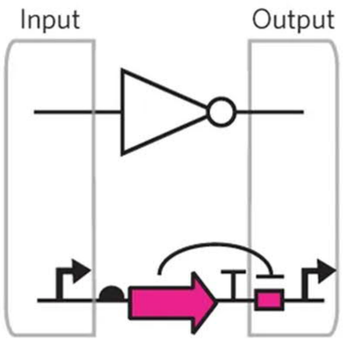
Genetic circuit design

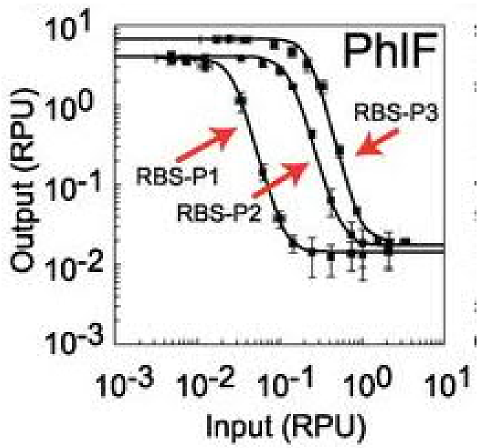
Nielsen et al. Genetic circuit design automation. *Science* (2016) 352:6281. DOI: 10.1126/science.aac7341
Moser et al. Dynamic control of endogenous metabolism with combinatorial logic circuits. *Mol Syst Biol* (2018)14:e8605. DOI: 10.15252/msb.20188605
Brophy and Voigt. Principles of genetic circuit design. *Nat Methods* (2014) 11:508-20. DOI: 10.1038/nmeth.2926

Gate is characterized by determining the response function

NOT-gate



Response function

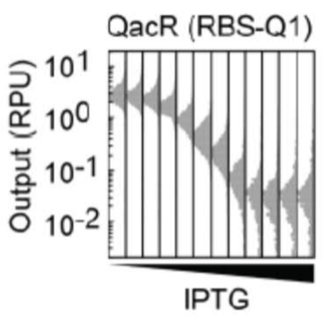


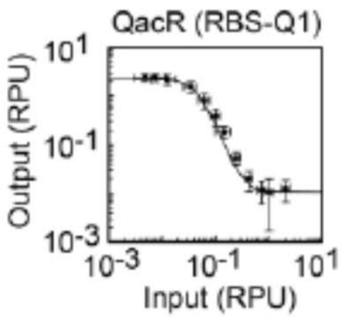
RPU (relative promoter unit)

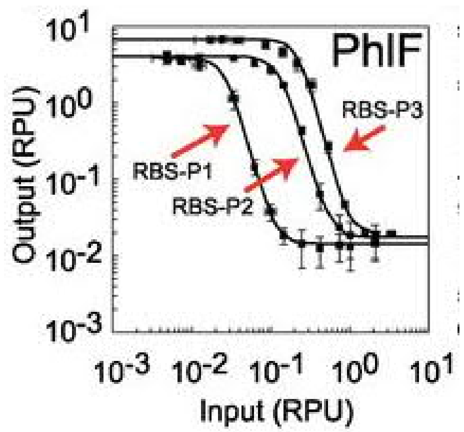
Gate characterization and input-output normalization RPUs

- *E. coli* BBa_J23101 constitutive promoter adopted as a standard with output of 1 RPU
- Four strains for gate characterization with:
  - 1) **gate** controlling fluorescence protein,
  - 2) RPU standard promoter expressing fluorescence protein (<YFP>$_{RPU}$),
  - 3) autofluorescence control without fluorescence protein (to normalize all other <YFP>$_0$),
  - 4) **input promoter** expressing fluorescence protein
- Measure fluorescence from all under a range of inducer concentrations
- Convert the strains 1 and 4 fluorescence readouts to RPUs:
- Plot output as a function of input at each concentration of inducer
- Fit Hill function to the response curve

$$RPU = \frac{\langle YFP \rangle - \langle YFP \rangle_0}{\langle YFP \rangle_{RPU} - \langle YFP \rangle_0}$$







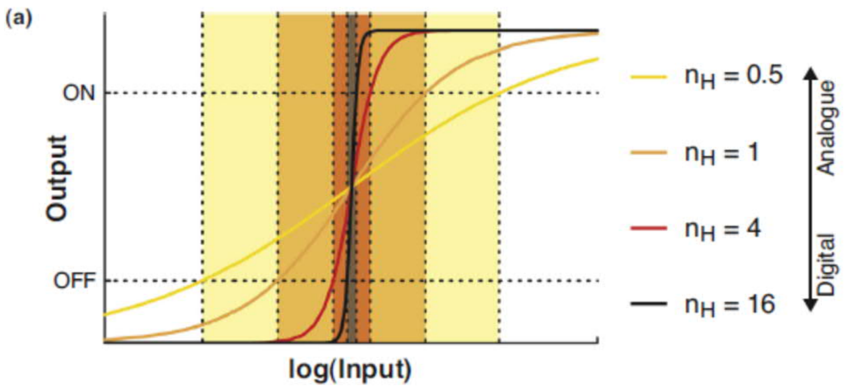
Response curve has Hill function shape

NOT gate response data can be fitted to Hill function: $$y = y_{min} + (y_{max} - y_{min}) \frac{K^n}{K^n + x^n}$$
where

n is the Hill coefficient

K is the threshold input level where the output is half maximum

y_{min} and y_{max} are the minimum and maximum output values from the gate



Bradley et al. 2016: https://doi.org/10.1016/j.mib.2016.07.004

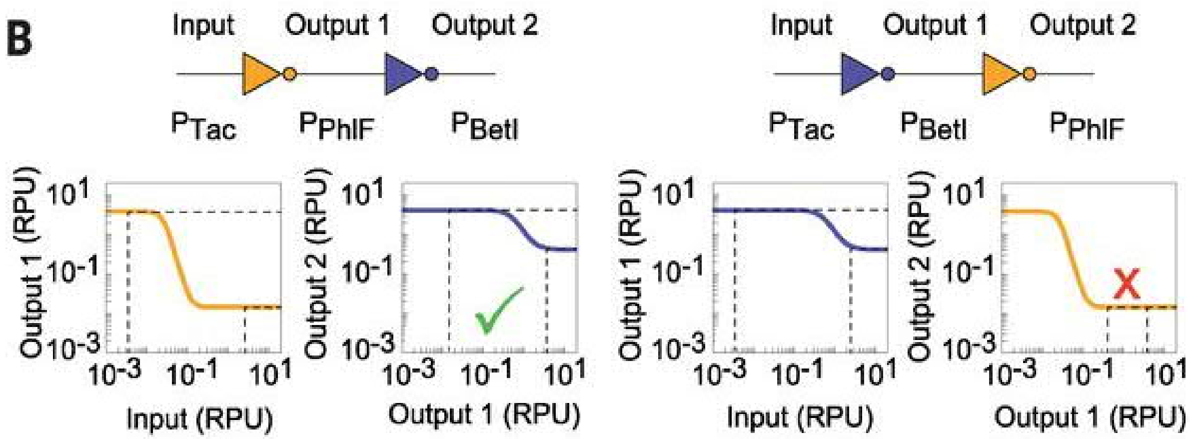
Task:

If n is bigger, how does the function of the NOT gate change?

a) it becomes more switch like
b) it looses the functionality

$$y = y_{min} + (y_{max} - y_{min})\frac{K^n}{K^n + x^n}$$

Response functions are essential for combining gates into functional circuits



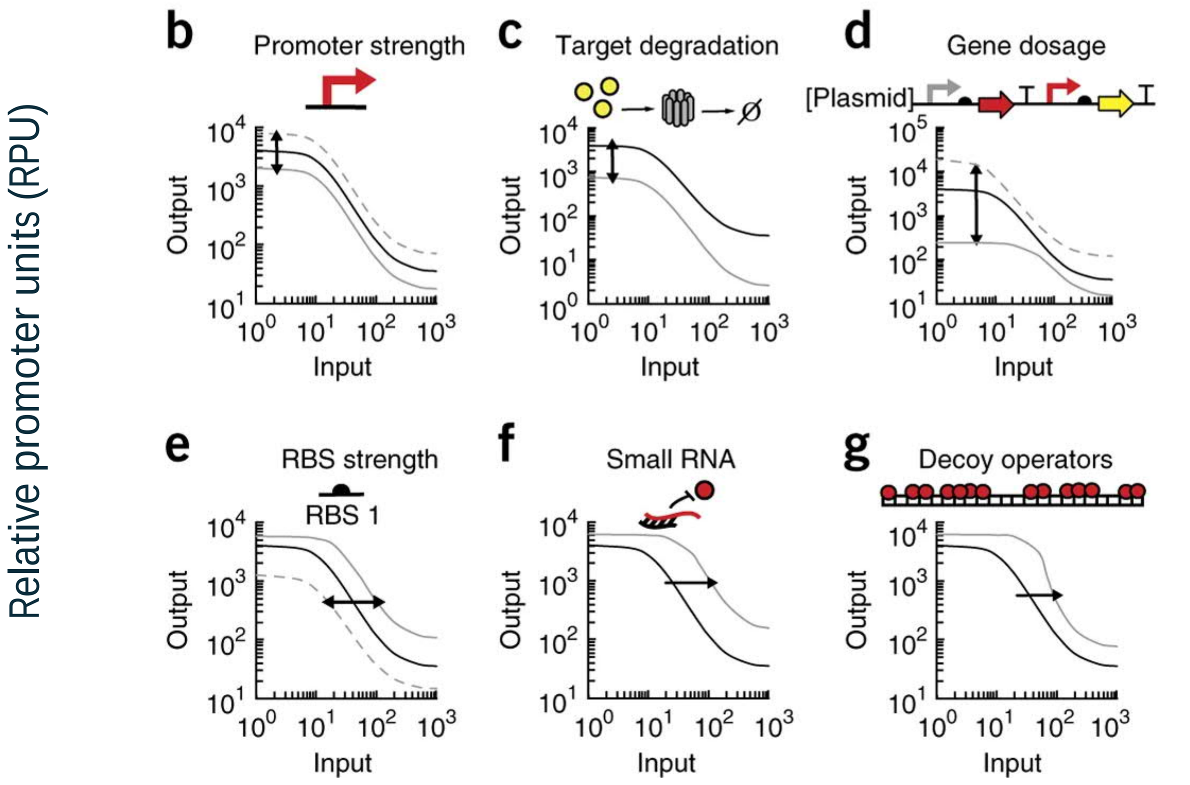
Nielsen et al. 2016

Task:

Why is the conversion to RPUs needed?

a) the gate becomes more stable
b) gates can be compared and combined

Circuit tuning shifts the response function

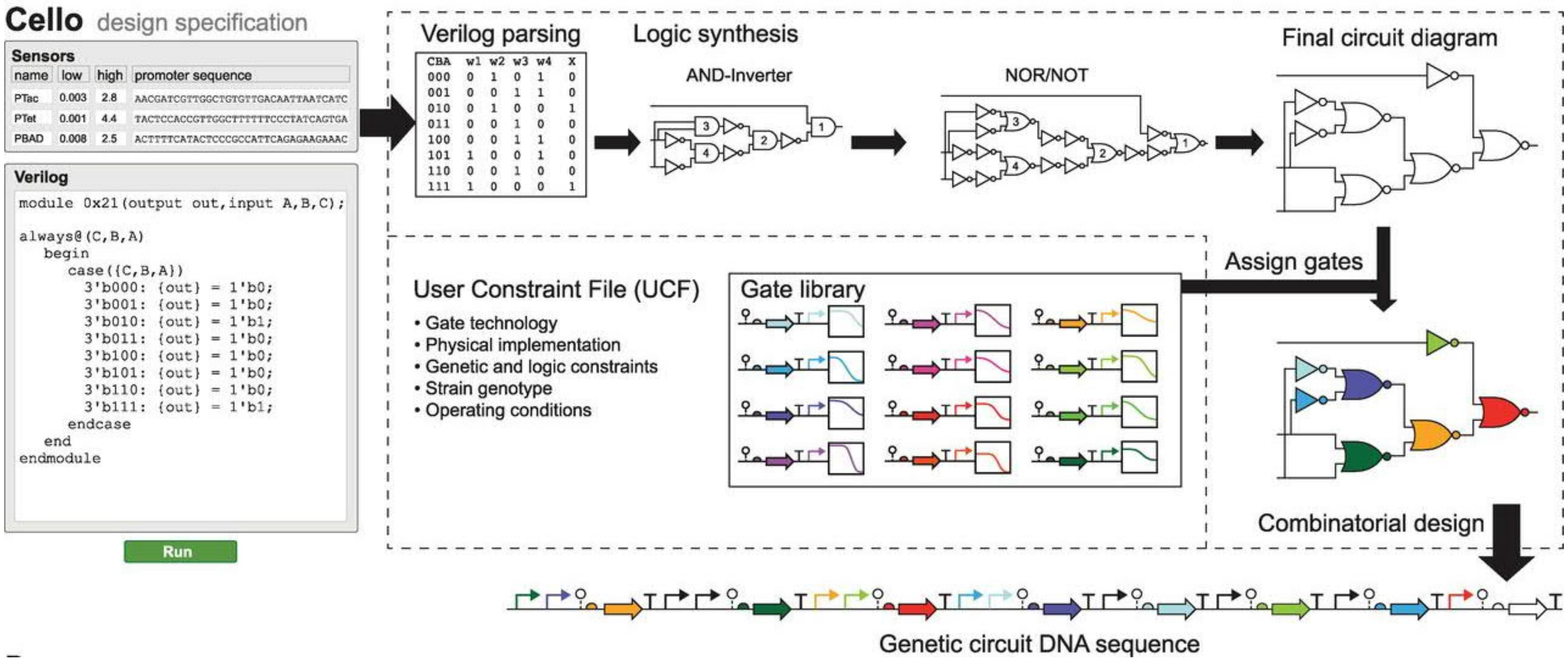


UP-DOWN shift

LEFT-RIGHT shift

Brophy and Voigt, 2014

Cello automates genetic circuit design in *E. coli and S. cerevisiae*

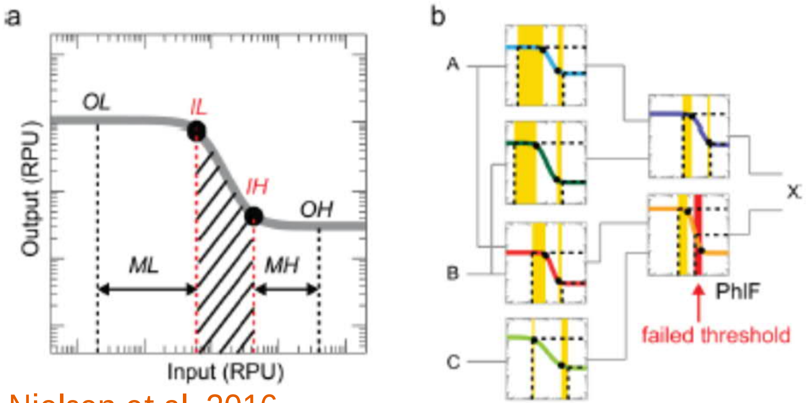


Nielsen et al. 2016 *S. cerevisiae*: Chen et al. Nat Microbiol. 2020 Nov;5(11):1349-1360. doi: 10.1038/s41564-020-0757-2.

Gate assignment is an NP-complete optimization problem

Cello uses simulated annealing algorithm

Input threshold analysis



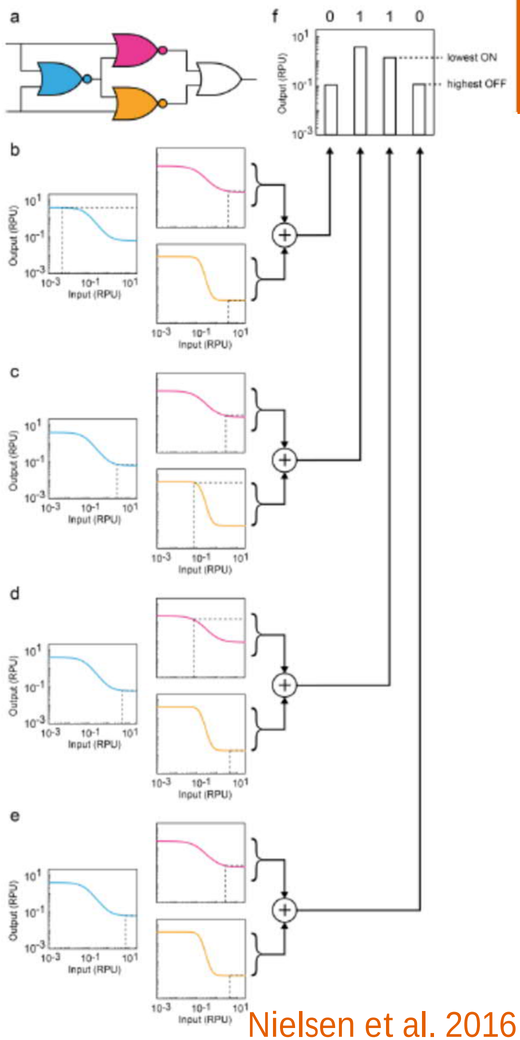
Nielsen et al. 2016

OL and OH from previous gate output have to leave positive margins when compared to next gate's IL and IH.

Circuit score

$$S = \frac{\min(ON)}{\max(OFF)}$$

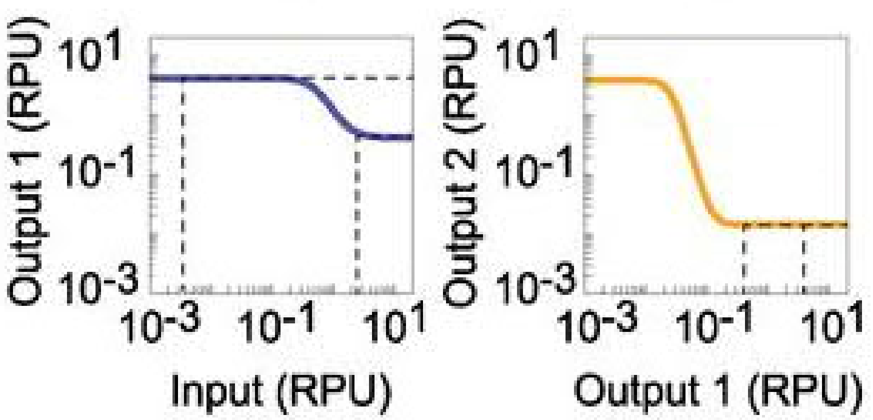
Optimal gate assignment maximizes S



Nielsen et al. 2016

Tasks:

Can the gates below be connected? a) yes, b) no

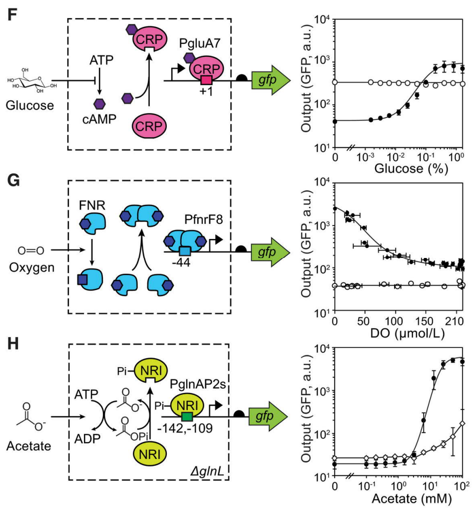


Dynamic control of endogenous metabolism with combinatorial logic circuits, Moser et al. 2018

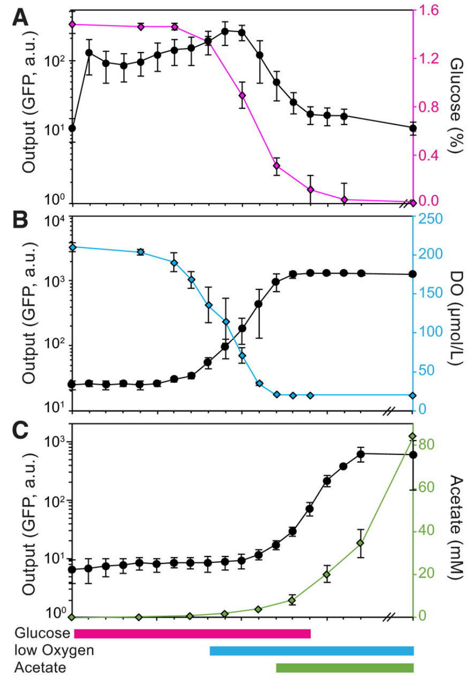
https://www.embopress.org/doi/full/10.15252/msb.20188605

- During the growth of Escherichia coli in a batch culture, the oxygen and glucose become depleted and acetate is accumulated
- Oxygen, glucose, and acetate sensors will have dynamic input
- Can these sensors coupled to gates be used to reduce acetate accumulation?

Sensors

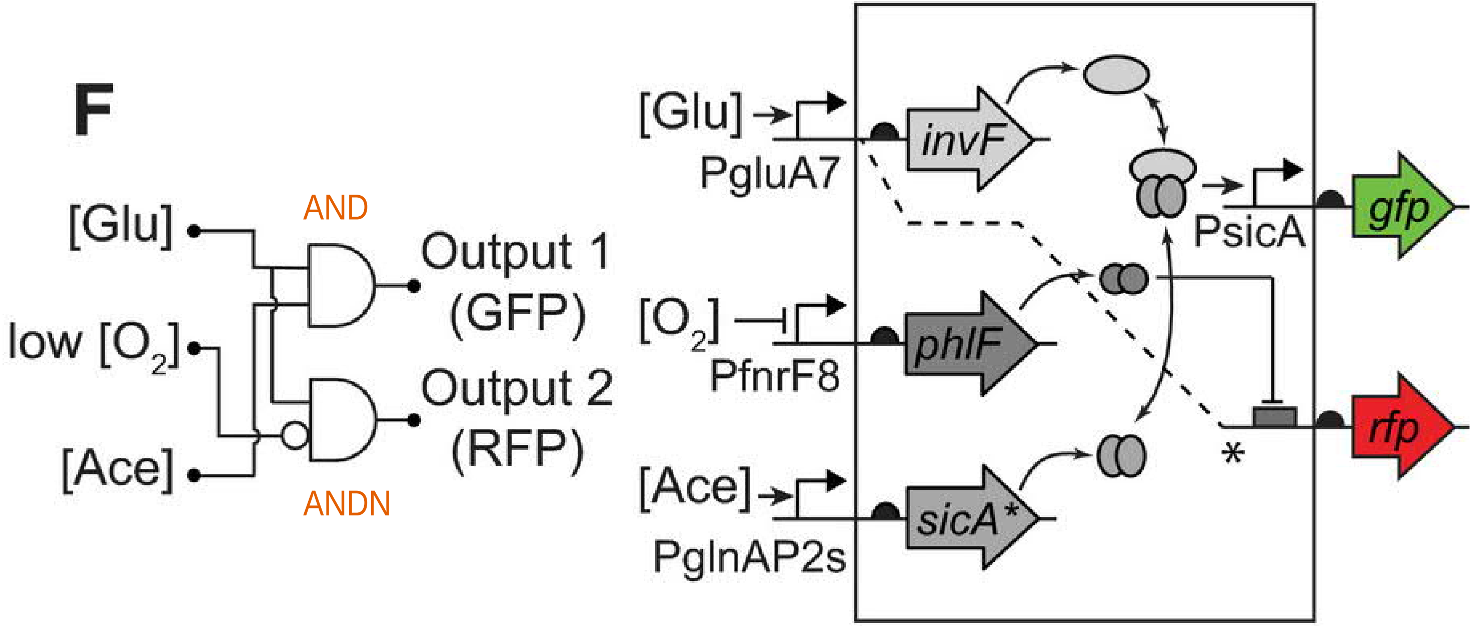


Inducer dependent outputs



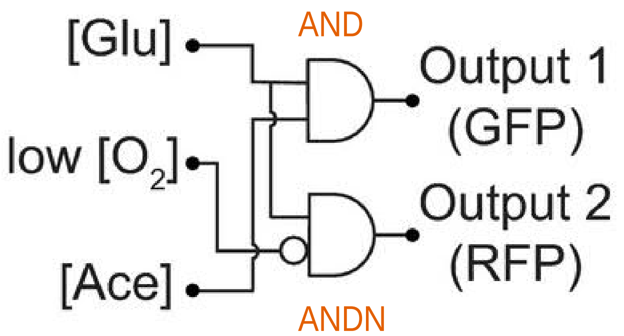
Responses in cultures

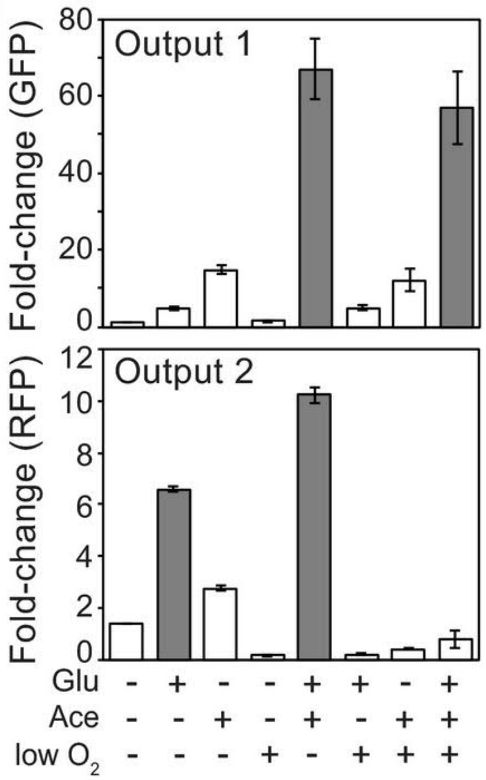
Experimentally built circuit with the sensors



Moser et al. 2018

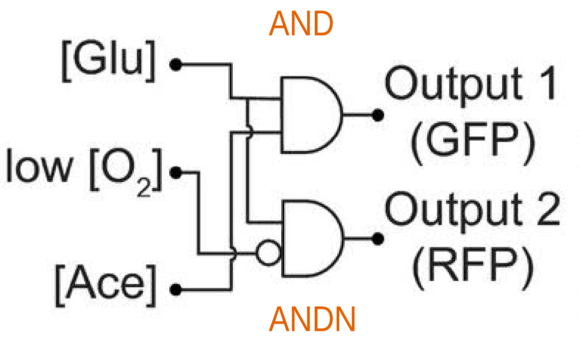
Response to different combinations of stimuli

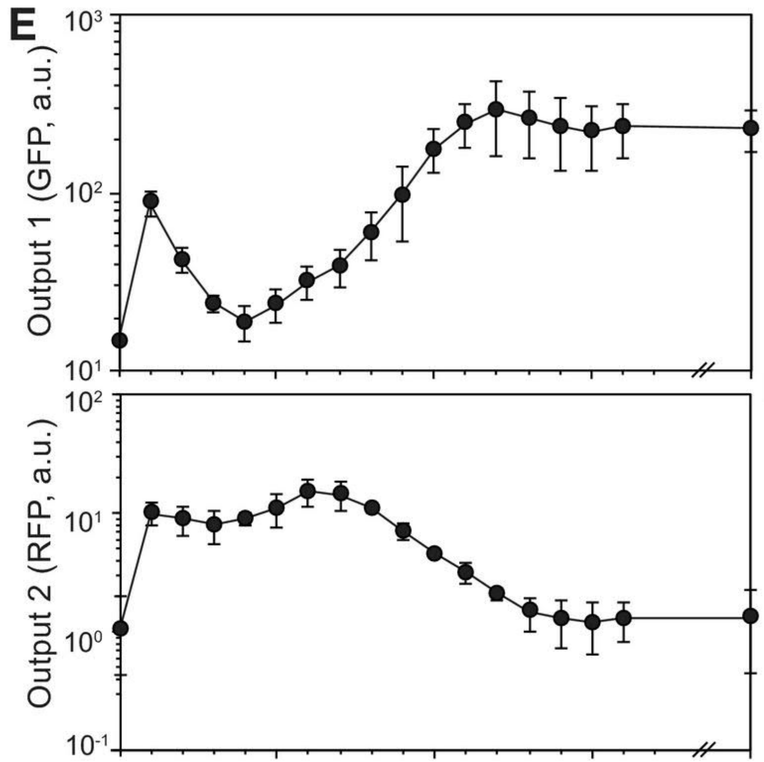




Moser et al. 2018

Response in cultures (simulated and experimental)





Moser et al. 2018

ODE models of circuit dynamics

Moser et al. 2018

NOT gate response function: $y = y_{min} + (y_{max} - y_{min})\frac{K^n}{K^n + x^n}$

Change in NOT gate output activity: $\frac{dy}{dt} = \alpha(y_{max} - y_{min})\frac{K^n}{K^n + x(t)^n} - \gamma(y(t) - y_{min})$

α and γ are the rate constants for turning a gate ON and OFF, respectively

AND gate response function : $y = y_{min} + (y_{max} - y_{min})\frac{x_1 {x_2}^2}{K + x_1 {x_2}^2}$

Change in AND gate output activity: $\frac{dy}{dt} = \alpha(y_{max} - y_{min})\frac{x_1(t)x_2(t)^2}{K + x_1(t)x_2(t)^2} - \gamma(y(t) - y_{min})$

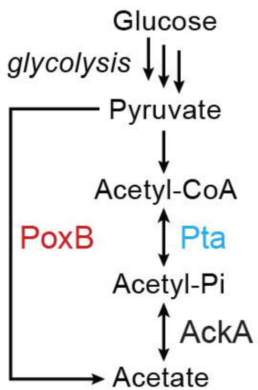
ANDN gate response function : $y = y_{min} + (x_1 - y_{min})\frac{K}{K + x_2}$

Change in ANDN gate output activity: $\frac{dy}{dt} = \alpha(x_1(t) - y_{min})\frac{K}{K + x_2(t)} - \gamma(y(t) - y_{min})$

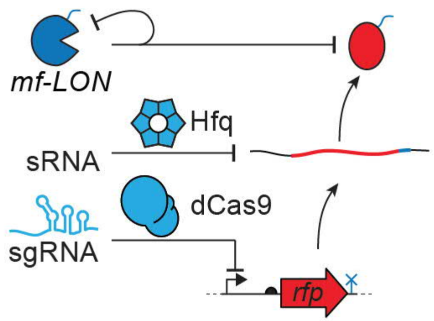
Acetate accumulation could be dynamically controlled and decreased with a circuit

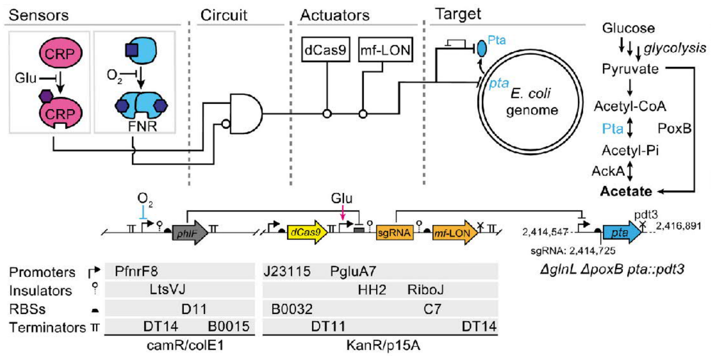
pta encodes phosphate acetyltransferase
pta contributes to acetate generation in exponential phase

D

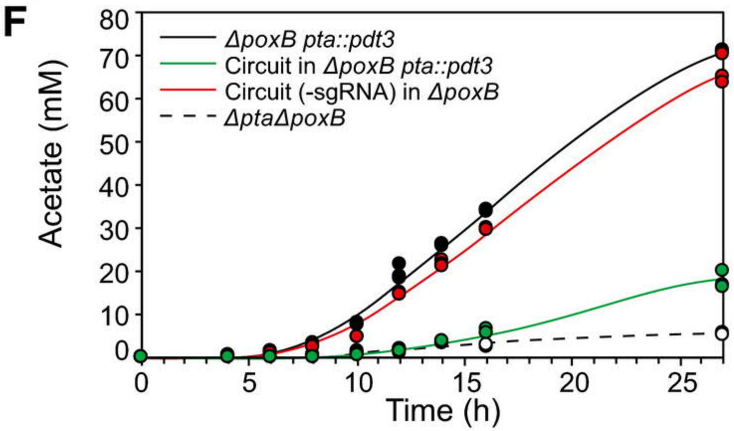


A





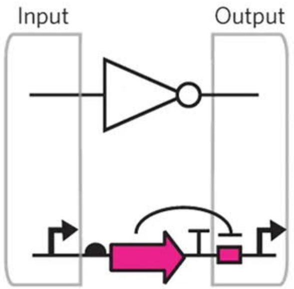
F

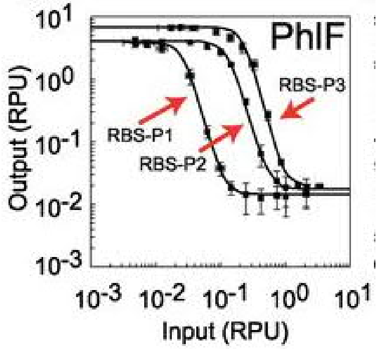


poxB encodes pyruvate oxidase (dehydrogenase)
poxB contributes to acetate generation in stationary phase

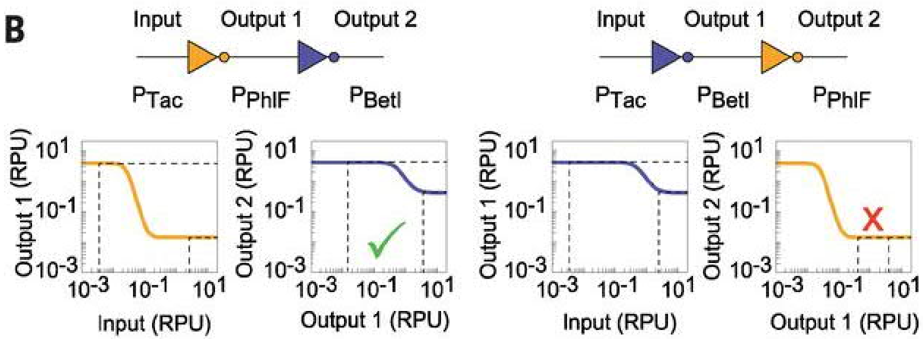
Moser et al. 2018

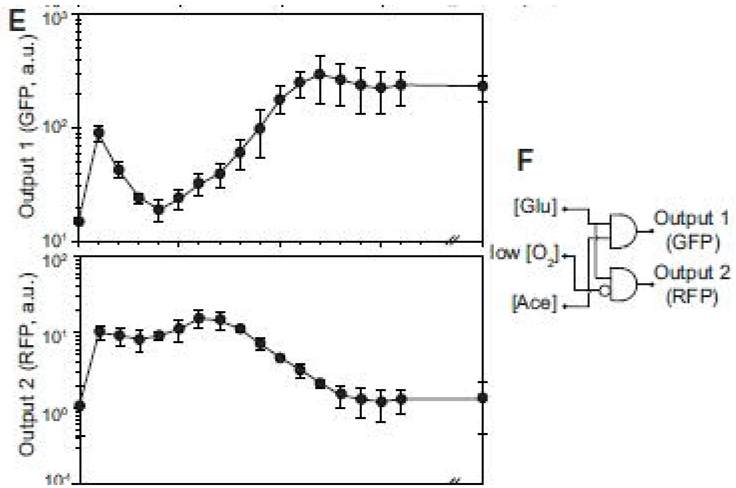
Summary





$$y = y_{min} + (y_{max} - y_{min})\frac{K^n}{K^n + x^n}$$





Nielsen et al. 2016

$$\frac{dy}{dt} = \alpha(y_{max} - y_{min})\frac{K^n}{K^n + x(t)^n} - \gamma(y(t) - y_{min})$$

Moser et al. 2018