Genetic circuit modelling in synthetic biology

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Learning goals

After this lecture, you will be able to

- Explain what are gate response functions
- Describe why the gate response functions are useful
- Suggest how to model circuit dynamics

Reading material

Nielsen et al. (2016) Genetic circuit design automation. Science. 352:aac7341. doi: 10.1126/science.aac7341.

Dynamic modelling part: Moser et al. (2018) Dynamic control of endogenous metabolism with combinatorial logic circuits. Mol Syst Biol. 14:e8605. doi: 10.15252/msb.20188605

Article

TRANSPARENT PROCESS

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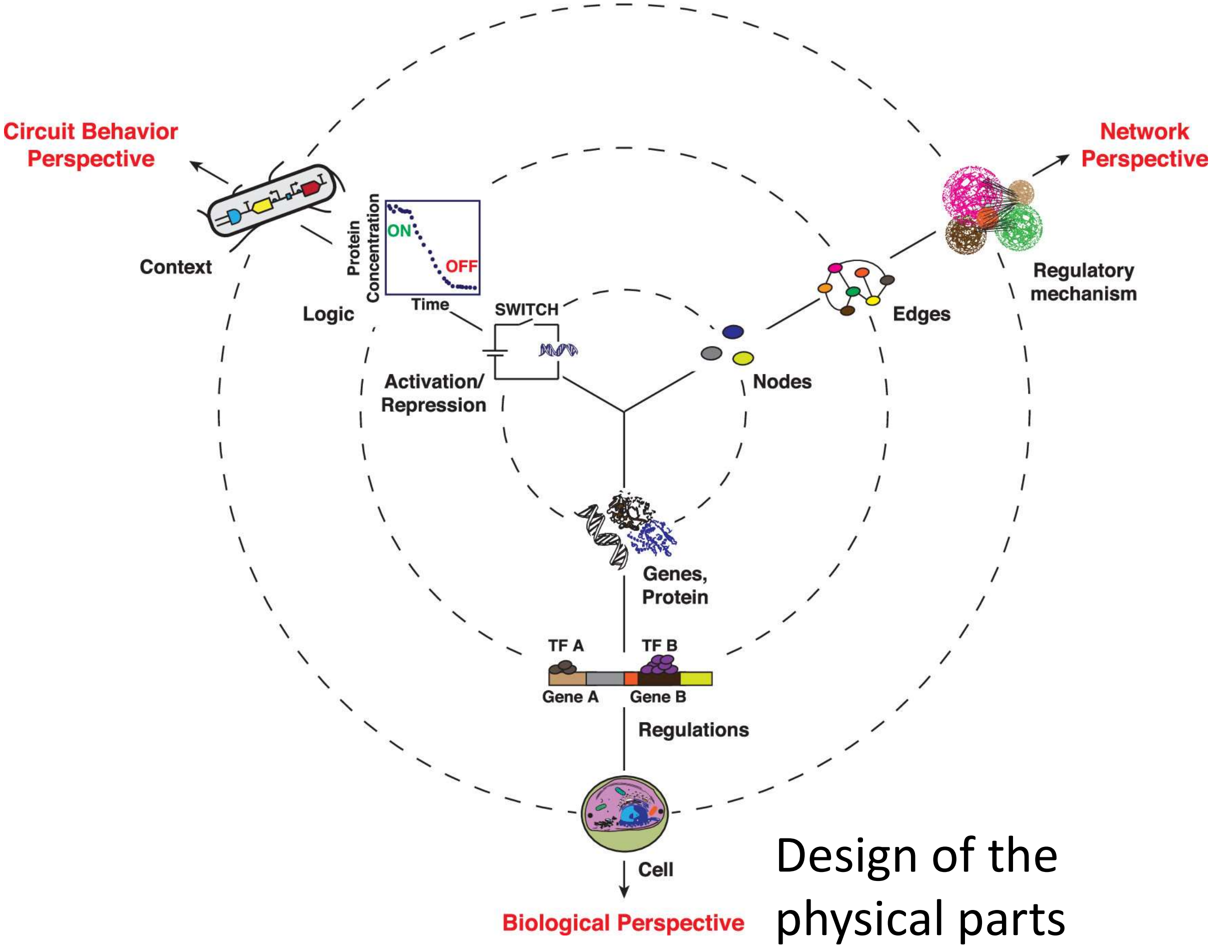
molecular systems biology

Dynamic control of endogenous metabolism with combinatorial logic circuits

Felix Moser, Amin Espah Borujeni, Amar N. Ghodasara, Ewen Cameron, Yongjin Park & Christopher A. Voigt*

Genetic circuit design perspectives



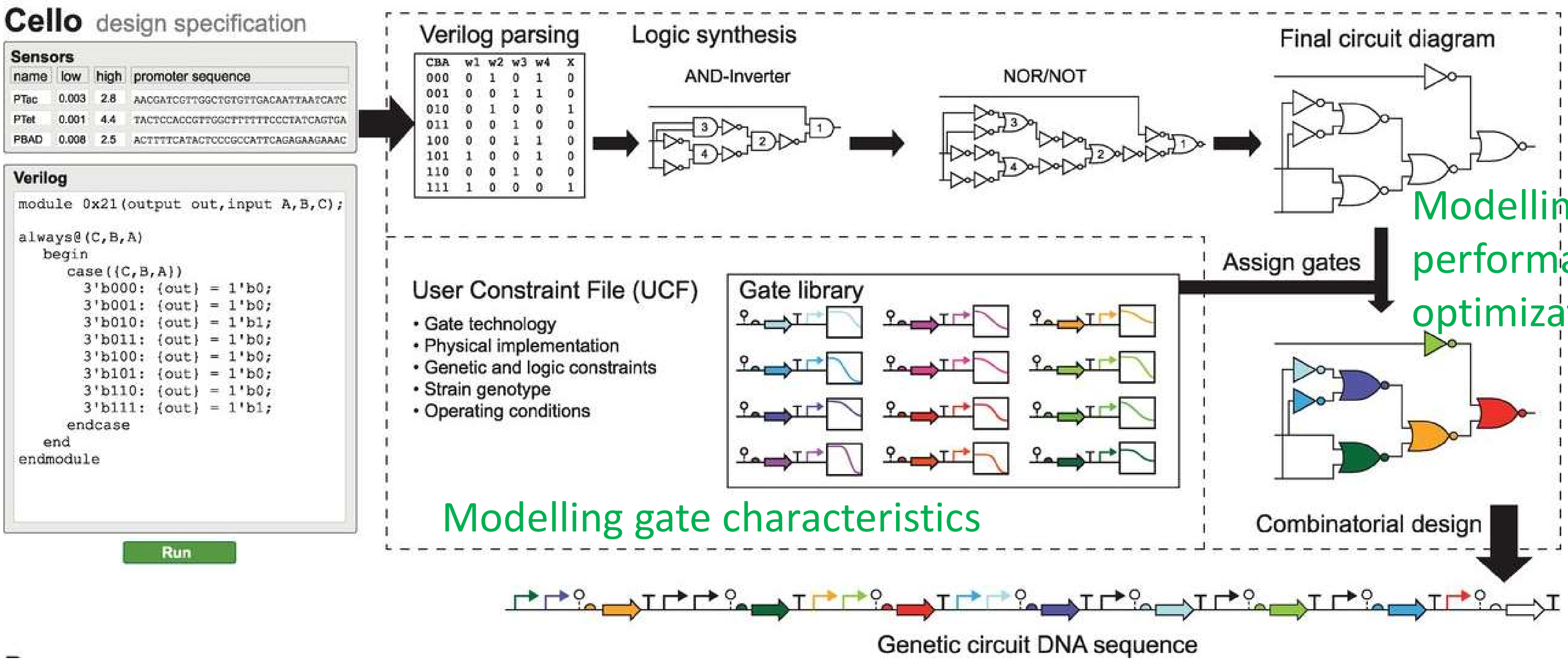
Design of the circuit composition and performance

Design of the circuit topology

Design of the physical parts

Chakraborty et al. (2022) ACS Synth Biol. 11:1377-1388. doi: 10.1021/acssynbio.1c00557.

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



Sensors

name	low	high	promoter sequence
PTac	0.003	2.8	AACGATCGTTGGCTGTGTTGACAATTAATCATC
PTet	0.001	4.4	TACTCCACCGTTGGCTTTTTTCCCTATCAGTGA
PBAD	0.008	2.5	ACTTTTCATACTCCCGCCATTCAGAGAAGAAAC

Verilog

```
module 0x21(output out,input A,B,C);

always@(C,B,A)
   begin
      case({C,B,A})
        3'b000: {out} = 1'b0;
        3'b001: {out} = 1'b0;
        3'b010: {out} = 1'b1;
        3'b011: {out} = 1'b0;
        3'b100: {out} = 1'b0;
        3'b101: {out} = 1'b0;
        3'b110: {out} = 1'b0;
        3'b111: {out} = 1'b1;
      endcase
   end
endmodule
```

Verilog parsing

CBA	w1	w2	w3	w4	X
000	0	1	0	1	0
001	0	0	1	1	0
010	0	1	0	0	1
011	0	0	1	0	0
100	0	0	1	1	0
101	1	0	0	1	0
110	0	0	1	0	0
111	1	0	0	0	1

User Constraint File (UCF)
- Gate technology
- Physical implementation
- Genetic and logic constraints
- Strain genotype
- Operating conditions

Modelling circuit performance + optimization

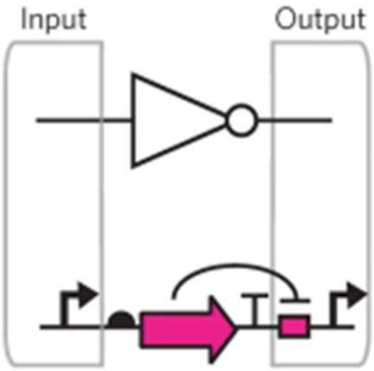
Modelling gate characteristics

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

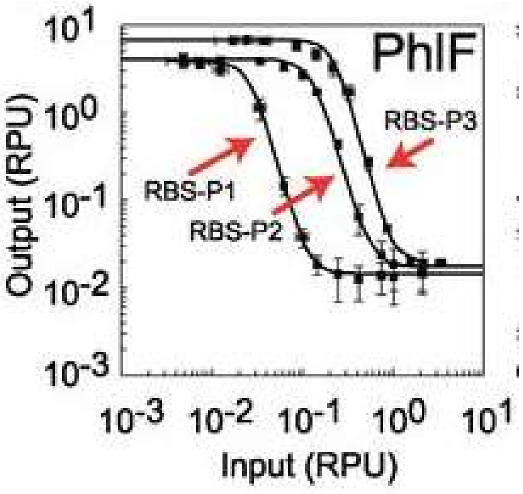
E. coli: Nielsen et al. (2016) Science 352:aac7341. doi: 10.1126/science.aac7341.

Gate characteristics described by a response function

NOT-gate



Response function



RPU (relative promoter unit)
RBS (ribosome binding site)

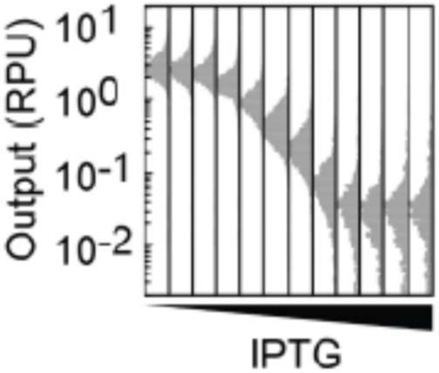
Response functions of gates needed for designing circuit(s)

Nielsen et al. (2016) Science 352:aac7341. doi: 10.1126/science.aac7341.

Modelling gate characteristics as a response function

- Standard promoter: *E. coli* BBa_J23101 constitutive promoter, output of 1 RPU
- Fluorescence measured under a range of inducer concentrations from strains in which
  1. Fluorescence protein is expressed from the standard promoter $\langle YFP \rangle_{RPU}$
  2. Autofluorescence control without fluorescence protein $\langle YFP \rangle_0$
  3. Fluorescence protein is expressed from the input promoter
  4. Gate controls fluorescence protein

Example for strain 4, IPTG as inducer



Nielsen et al. (2016) Science 352:aac7341. doi: 10.1126/science.aac7341.

Gate characterization and input-output normalization RPUs

- Convert the fluorescence readouts to RPUs for both
  1. Fluorescence protein is expressed from the input promoter
  2. Gate controls fluorescence protein

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

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

- Plot output as a function of input at each concentration of inducer
- Fit Hill function to the response curve

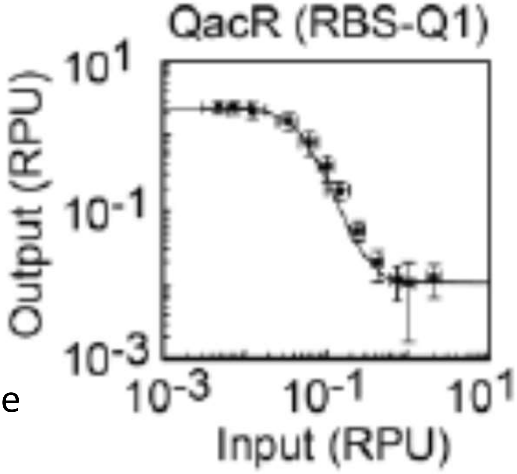
$$y = y_{min} + (y_{max} - y_{min})\frac{K^n}{K^n + x^n}$$

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



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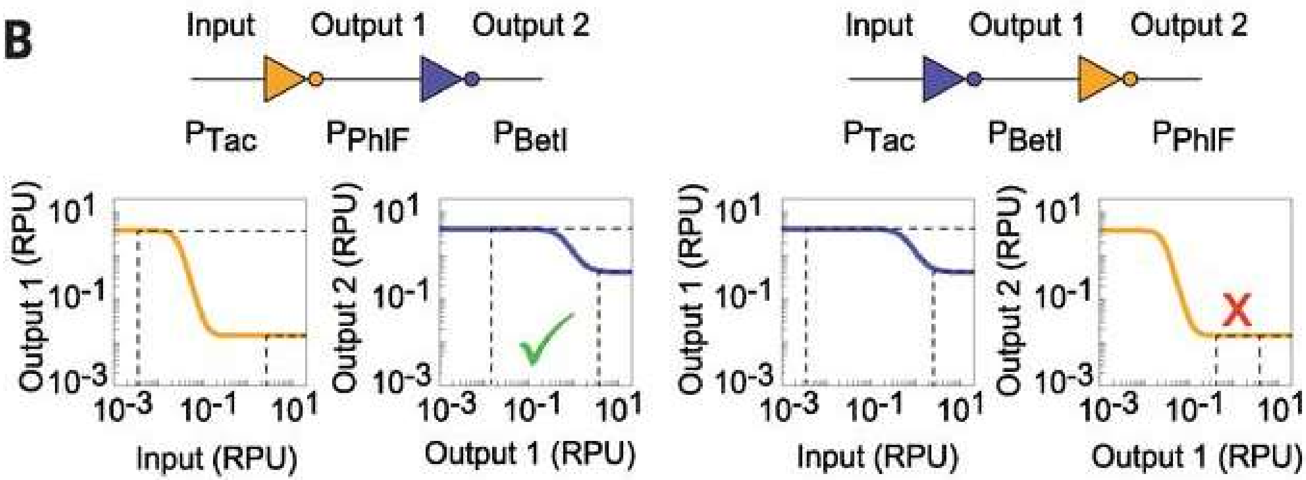
Response functions are essential for combining gates into functional circuits



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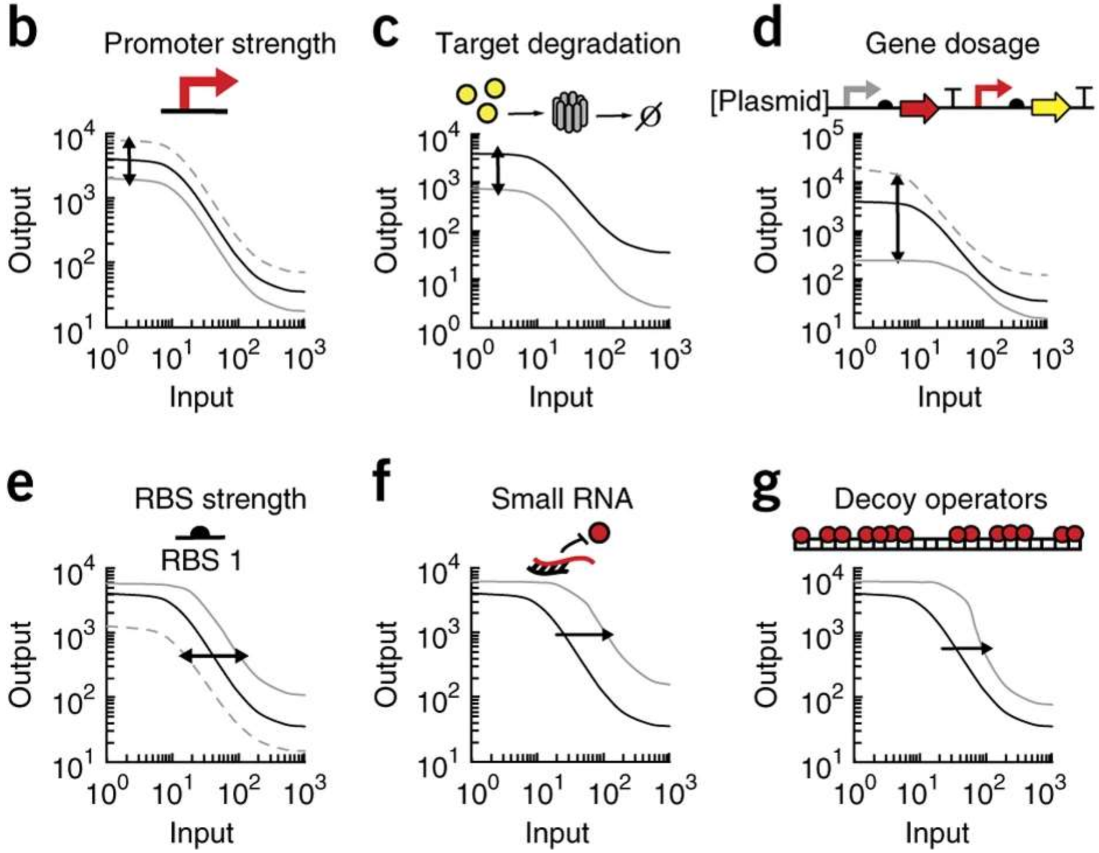
Circuit tuning shifts the response function

Relative promoter units (RPU)



UP-
DOWN
shift

LEFT-
RIGHT
shift

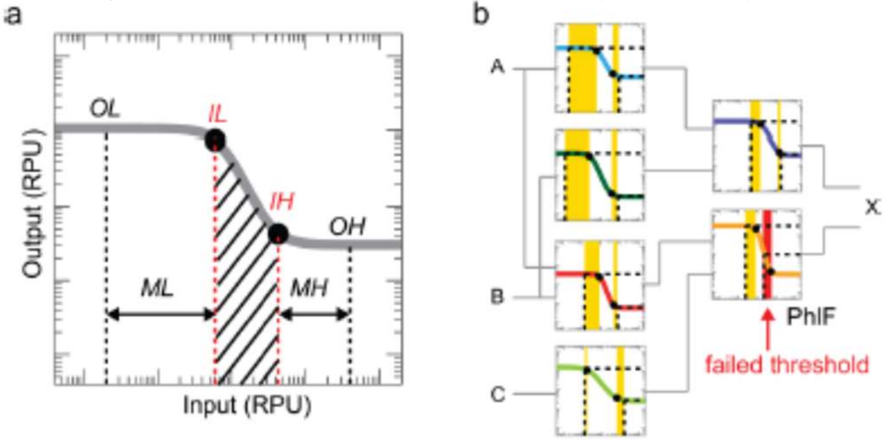
Brophy and Voigt, 2014

Gate assignment is an NP-complete optimization problem

Cello uses simulated annealing algorithm to search gate assignments maximizing circuit score S:

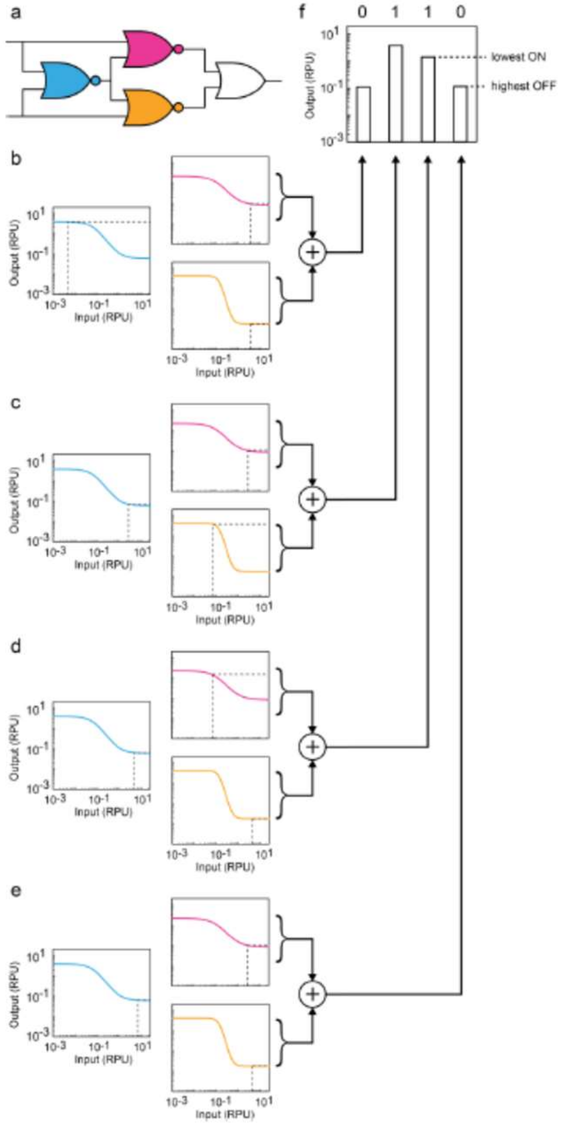
$$S = \frac{\min(ON)}{\max(OFF)}$$

The gate connections must pass input threshold analysis



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

E. coli: Nielsen et al. (2016) Science 352:aac7341. doi: 10.1126/science.aac7341.



Circuit dynamics

- If circuit input is dynamic, modelling circuit dynamics is useful for *in silico* screening of circuit designs
- From response functions to ODEs of dynamic responses

NOT

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

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

AND

$$y = y_{min} + (y_{max} - y_{min})\frac{x_1{x_2}^2}{K + x_1{x_2}^2}$$

$$\boldsymbol{\frac{dy}{dt} = \alpha(y_{max} - y_{min})\frac{x_1(t)x_2(t)^2}{K + x_1(t)x_2(t)^2} - \gamma(y(t) - y_{min})}$$

ANDN

$$y = y_{min} + (x_1 - y_{min})\frac{K}{K + x_2}$$

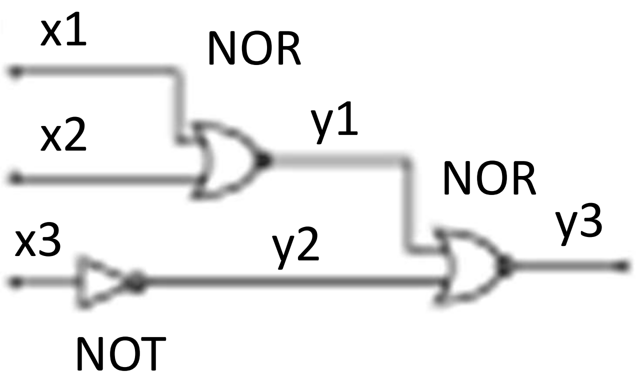
$$\boldsymbol{\frac{dy}{dt} = \alpha(x_1(t) - y_{min})\frac{K}{K + x_2(t)} - \gamma(y(t) - y_{min})}$$

- **Parameters from the response function**
- Rate constants α and γ of turning the gate ON and OFF, respectively, 1/h (Tabor et al. 2009; Moon et al. 2012)

Adopted from: Moser et al. (2018) Mol Syst Biol. 14:e8605. doi: 10.15252/msb.20188605.

Example: ODE model for a three gate circuit

Parameters from the response function, and rate constants α and γ of turning a gate ON and OFF



$$\frac{\mathrm{d}y_1}{\mathrm{d}t} = \alpha\left(y_{\max,1} - y_{\min,1}\right)\frac{{K_1}^{n_1}}{{K_1}^{n_1} + [x_1(t) + x_2(t)]^{n_1}} - \gamma\left(y_1(t) - y_{\min,1}\right),$$

$$\frac{\mathrm{d}y_2}{\mathrm{d}t} = \alpha\left(y_{\max,2} - y_{\min,2}\right)\frac{{K_2}^{n_2}}{{K_2}^{n_2} + x_3(t)^{n_2}} - \gamma\left(y_2(t) - y_{\min,2}\right),$$

$$\frac{\mathrm{d}y_3}{\mathrm{d}t} = \alpha\left(y_{\max,3} - y_{\min,3}\right)\frac{{K_3}^{n_3}}{{K_3}^{n_3} + \left[y_1(t) + y_2(t)\right]^{n_3}} - \gamma\left(y_3(t) - y_{\min,3}\right)$$

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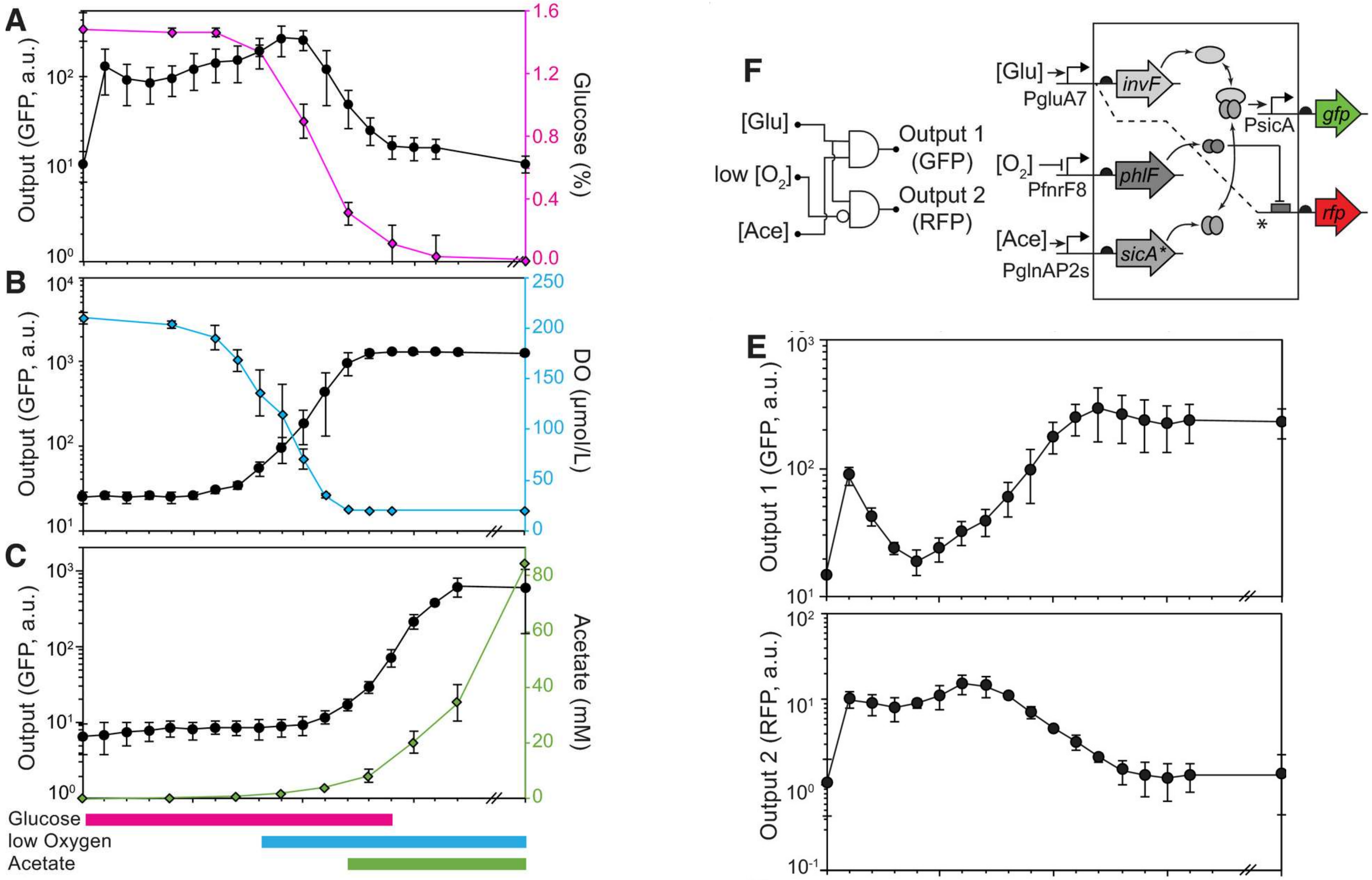
Glucose, oxygen and acetate sensors’ controlled circuit dynamics predicted for *E. coli* batch culture



- ODE system solved discretely
- In each time step, the corresponding empirical values for the output activity of glucose, oxygen, and acetate sensors were assigned to the inputs

Adopted from: Moser et al. (2018) Mol Syst Biol. 14:e8605. doi: 10.15252/msb.20188605.