sb2cl Lab

UNIVERSIDAD POLITECNICA DE VALENCIA

Synthetic Biology and Biosystems Control Lab
Valencia UPV

iGEM 2020 Measurement

Modeling: Modeling circuits with ODEs and experimental data

Section 1: Composing circuit models from Hill Functions

by Alejandro Vignoni (alvig2@upv.es)

An iGEM Measurement Committee Webinar
Week 3a, June 30th, 2020

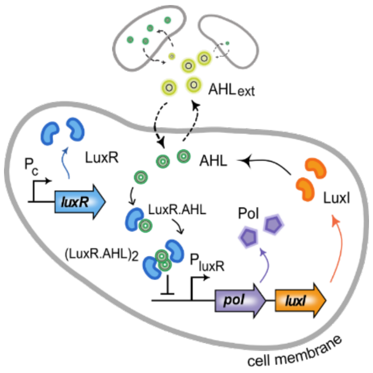
Today Webinar's Topics

- Section 1: **Composing circuit models from Hill functions** (15 min)
- Section 2: Relating parameters and data (15 min)
- Section 3: Example: Incoherent feed-forward loop (model & data) (15 min)
- Q&A – (at the end of each 15 minutes block, total 15 min)

iGEM 2020 Measurement

Remember our journey: but now going directly to reduced models

Schematic → Biochemical Reactions → Reduced Mathematical Model



$$\xrightarrow{C_R} mR$$
$$gPI \xrightarrow{k_{e_I}} gPI + mPI$$
$$mR \xrightarrow{p_R} mR + R$$
$$mPI \xrightarrow{p_I} mPI + PI$$
$$I \xrightarrow{k_A} A + I$$
$$R + A \underset{k_{-1}}{\overset{k_{-1}/k_{d1}}{\rightleftharpoons}} (R \cdot A)$$
$$2(R \cdot A) \underset{k_{-2}}{\overset{k_{-2}/k_{d2}}{\rightleftharpoons}} (R \cdot A)_2$$
$$gPI + (R \cdot A)_2 \underset{k_{lux}}{\overset{k_{lux}/k_{dlux}}{\rightleftharpoons}} gPI \cdot (R \cdot A)_2$$
$$gPI \cdot (R \cdot A)_2 \xrightarrow{\alpha k_{e_I}} gPI \cdot (R \cdot A)_2 + mPI$$
$$A \underset{DV_c}{\overset{D}{\rightleftharpoons}} A_{ext}$$

$$\dot{n}_1^i = \frac{C_I p_I}{d_{m_I}} \left(\frac{k_{dlux} + \alpha n_3^i}{k_{dlux} + n_3^i} \right) - d_I n_1^i$$
$$\dot{n}_2^i = \frac{C_R p_R}{d_{m_R}} + k_{-1} n_6^i - \left(\frac{k_{-1}}{k_{d1}} n_4^i + d_R \right) n_2^i$$
$$\dot{n}_3^i = \frac{k_{-2}}{k_{d2}} (n_6^i)^2 - (k_{-2} + d_{RA_2}) n_3^i$$
$$\dot{n}_4^i = k_{-1} n_6^i + k_A n_1^i + D \left(\frac{n_5}{V_c} - n_4^i \right) - \left(\frac{k_{-1}}{k_{d1}} n_2^i + d_A \right)$$

From
Y. Boada (2018)

Modeling a genetic circuit: What do you want to do?

- Biosensor
- Promoter

- Logic (Inverter)
- Memory
- Level detection

- Reporter
- Enzyme
- SM Signal
- Therapeutic

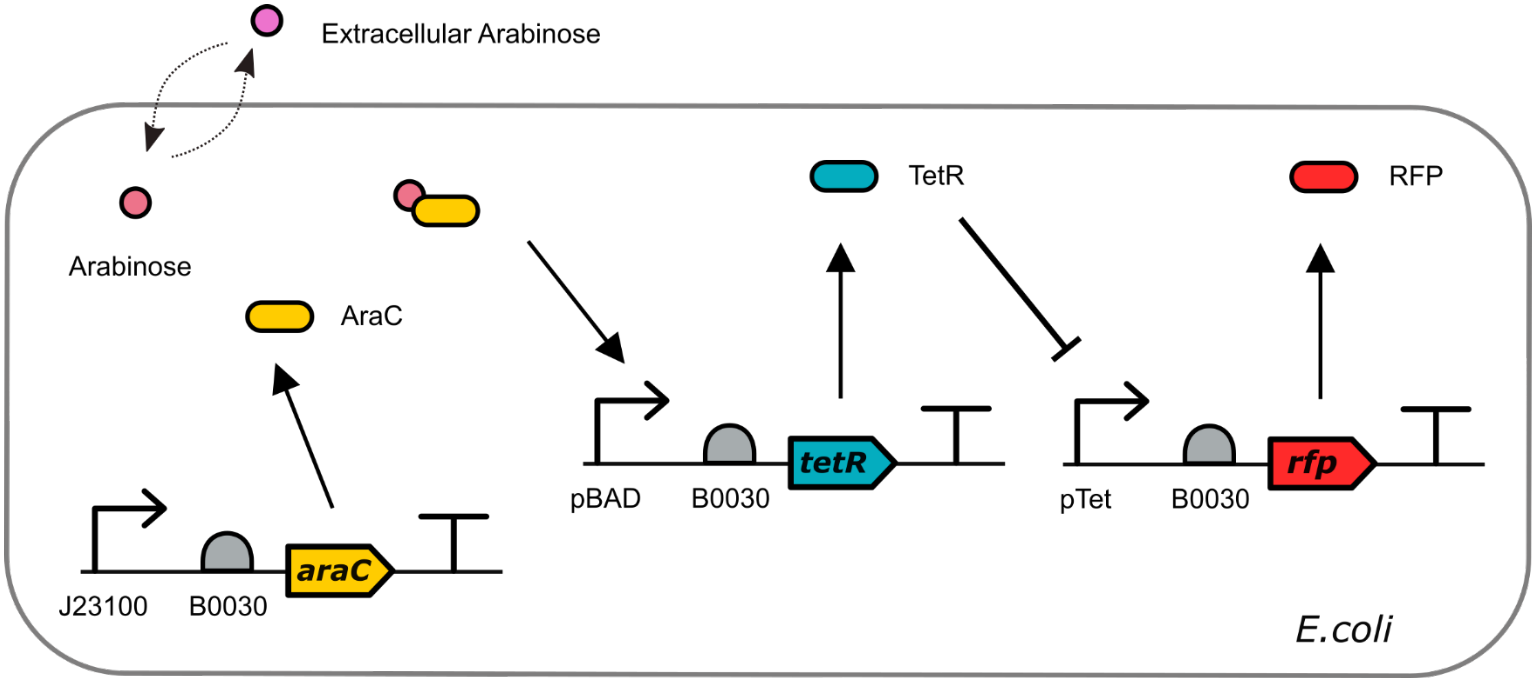
Example: Detect Arabinose

Inverter (TetR)

Fluorescence Protein (RFP)

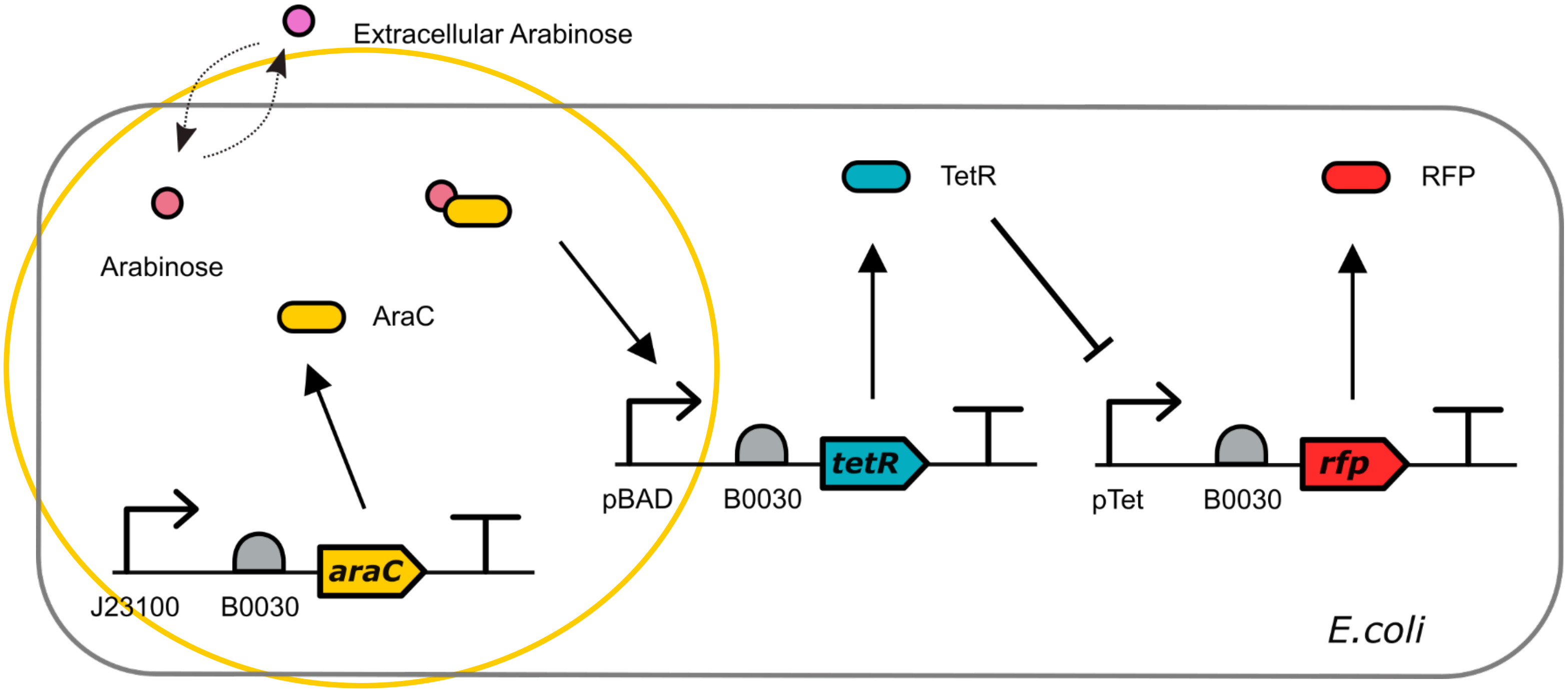
Modeling a genetic circuit

Example Sense-Compute-Act



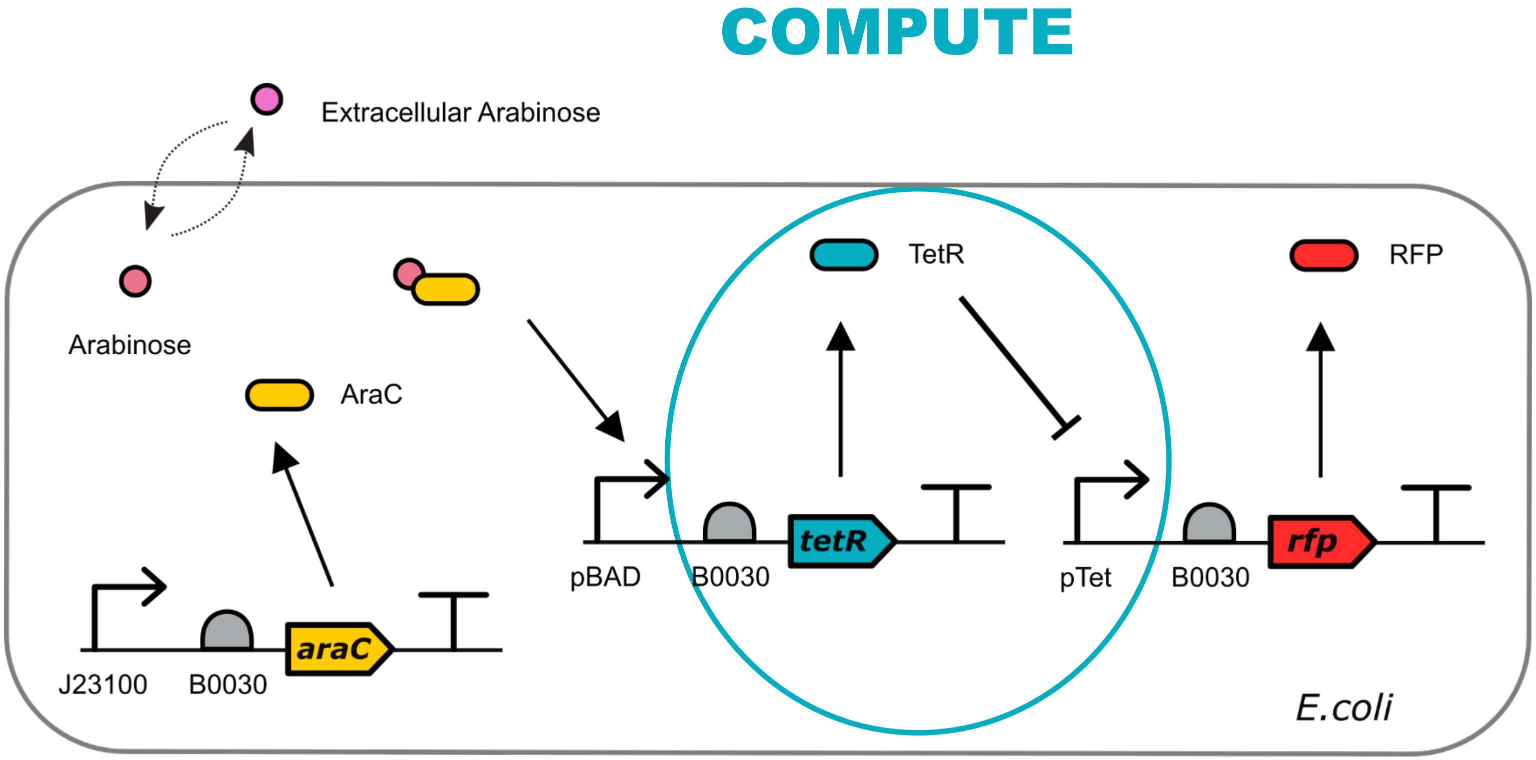
Modeling a genetic circuit
Example Sense-Compute-Act

SENSE



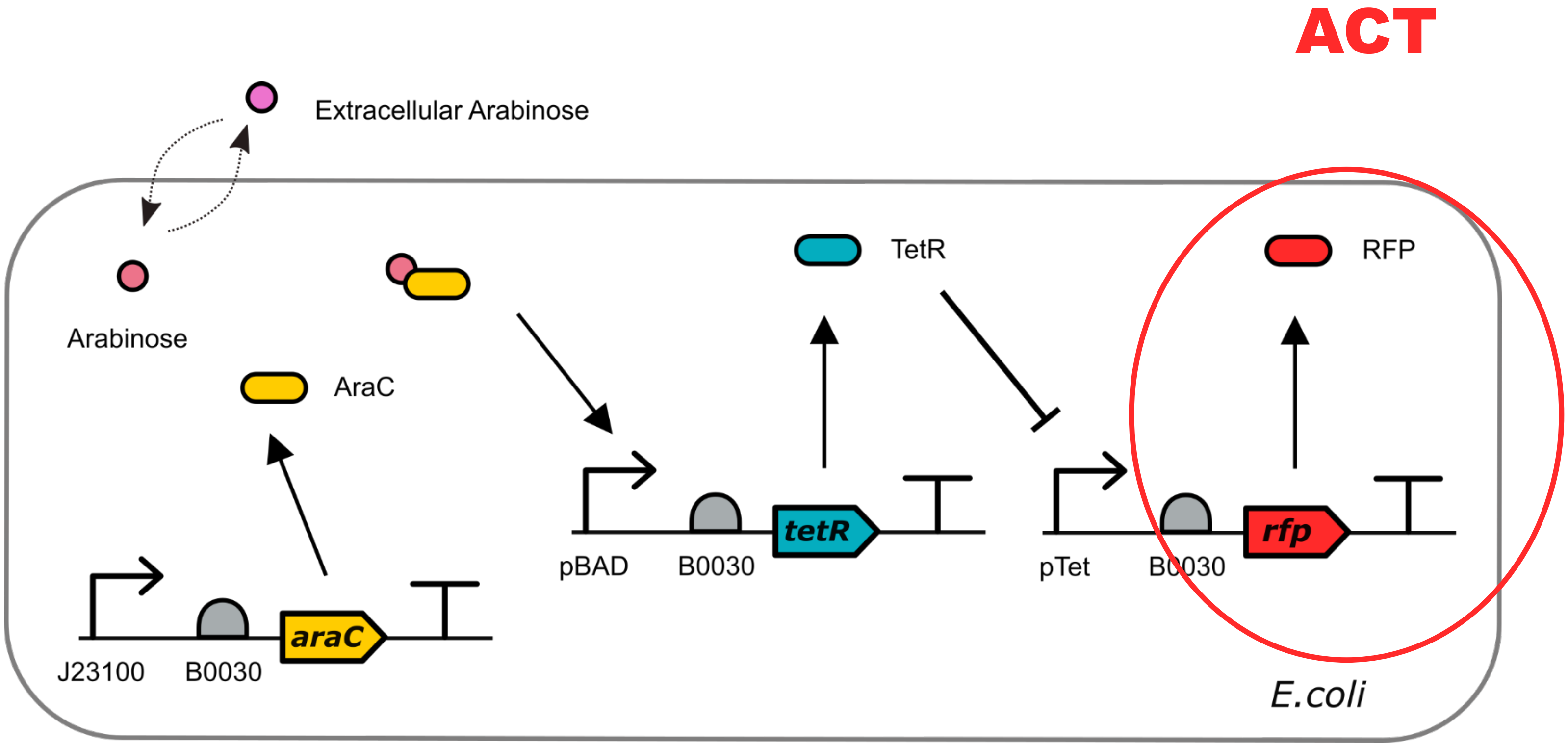
Modeling a genetic circuit

Example Sense-Compute-Act



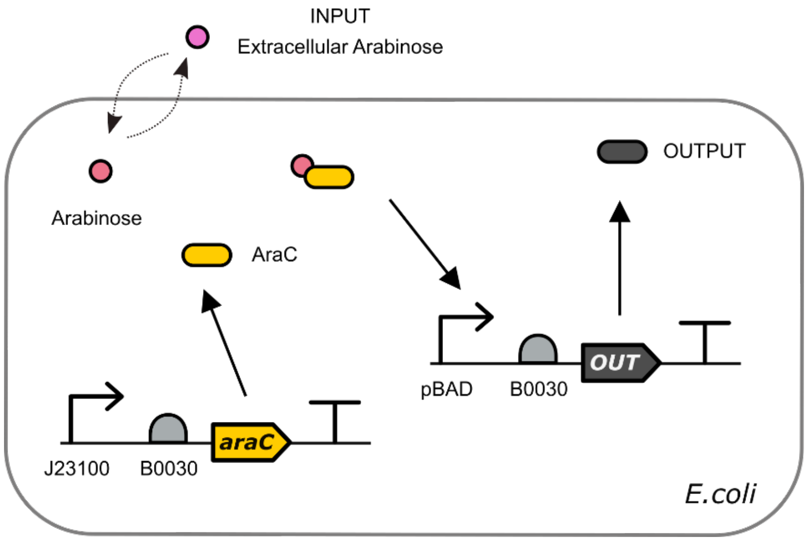
Modeling a genetic circuit

Example Sense-Compute-Act



Modeling a genetic circuit Example Sense-Compute-Act

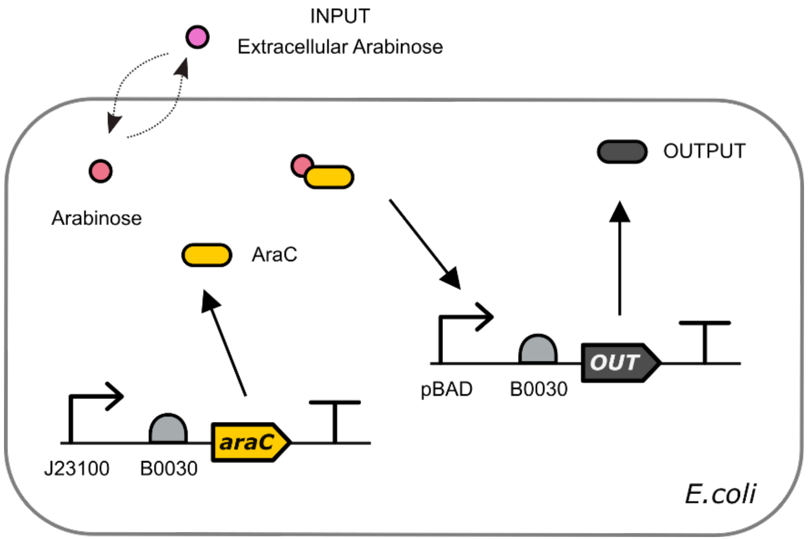
SENSE



$$[\text{OUTPUT}] = \frac{\alpha_{\text{pBAD}}}{d_{\text{OUT}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\text{Arab}]^{n_a}}{\left(K_{d_{\text{pBAD}}}\right)^{n_a} + [\text{Arab}]^{n_a}}\right)$$

Modeling a genetic circuit Example Sense-Compute-Act

SENSE



$$[\mathrm{OUTPUT}] = \frac{\alpha_{\mathrm{pBAD}}}{d_{\mathrm{OUT}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

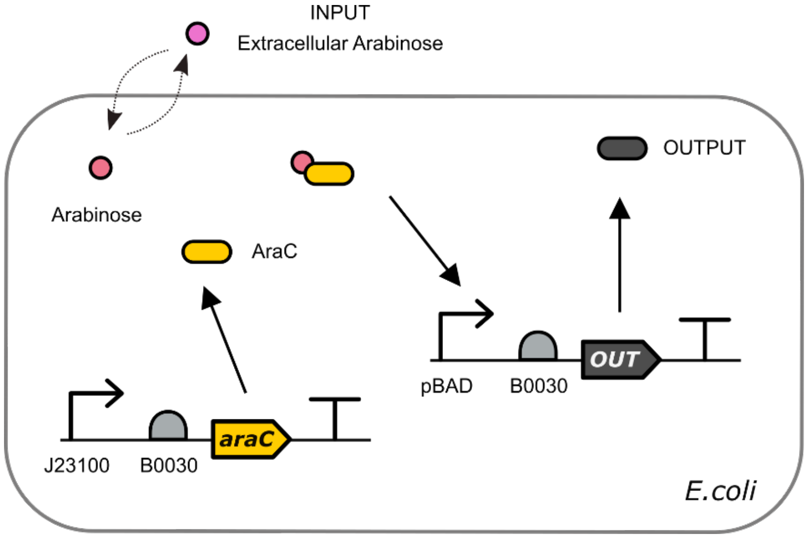
$$\alpha_{\mathrm{pBAD}} = \frac{k_{2_{\mathrm{OUT}}}}{d_{m_{\mathrm{OUT}}}} k_{1_{pBAD}} C_N$$

$$K_{d_{pBAD}} = \frac{K_d K_{dis}\, C_N}{[\mathrm{AraC}]^{n_A}}$$

Trabelsi, H., Koch, M., & Faulon, J. L. (2018). Building a minimal and generalizable model of transcription factor–based biosensors: Showcasing flavonoids. Biotechnology and bioengineering, 115(9), 2292-2304.

Modeling a genetic circuit Example Sense-Compute-Act

SENSE



$$[\mathrm{OUTPUT}] = \frac{\alpha_{\mathrm{pBAD}}}{d_{\mathrm{OUT}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

$$\alpha_{\mathrm{pBAD}} = \frac{k_{2_{\mathrm{OUT}}}}{d_{m_{\mathrm{OUT}}}} k_{1_{pBAD}} C_N$$

$$K_{d_{pBAD}} = \frac{K_d K_{dis} C_N}{[\mathrm{AraC}]^{n_A}}$$

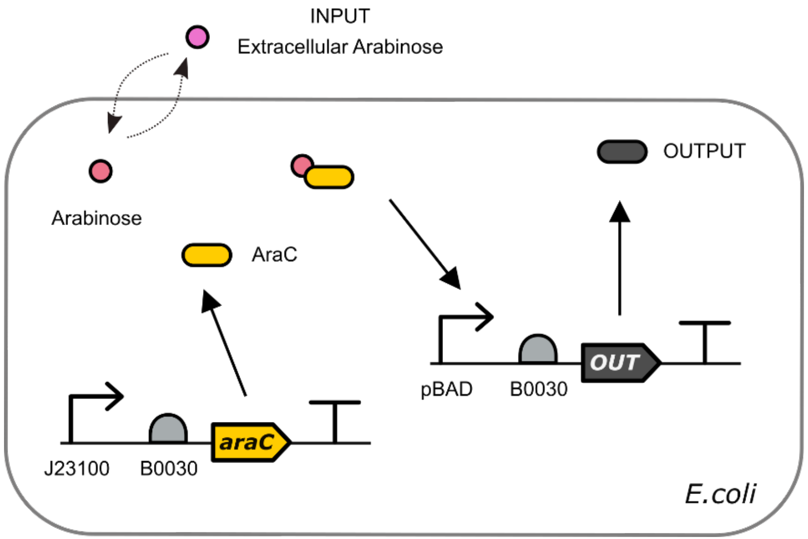
pBAD

AraC

Trabelsi, H., Koch, M., & Faulon, J. L. (2018). Building a minimal and generalizable model of transcription factor–based biosensors: Showcasing flavonoids. Biotechnology and bioengineering, 115(9), 2292-2304.

Modeling a genetic circuit Example Sense-Compute-Act

SENSE



$$[\mathrm{OUTPUT}] = \frac{\alpha_{\mathrm{pBAD}}}{d_{\mathrm{OUT}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

$$\alpha_{\mathrm{pBAD}} = \frac{k_{2_{\mathrm{OUT}}}}{d_{m_{\mathrm{OUT}}}} k_{1_{pBAD}} C_N$$

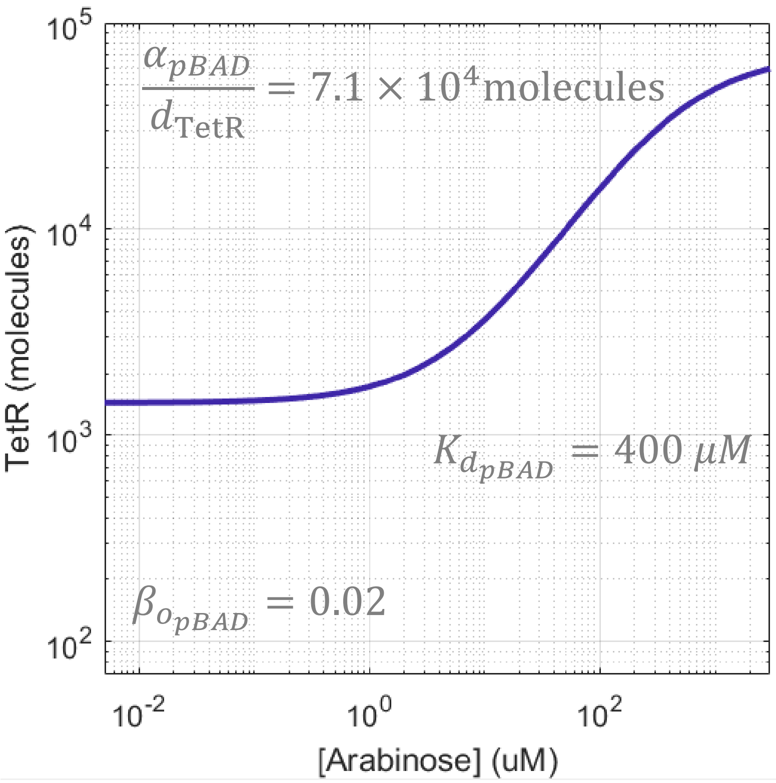
OUTPUT

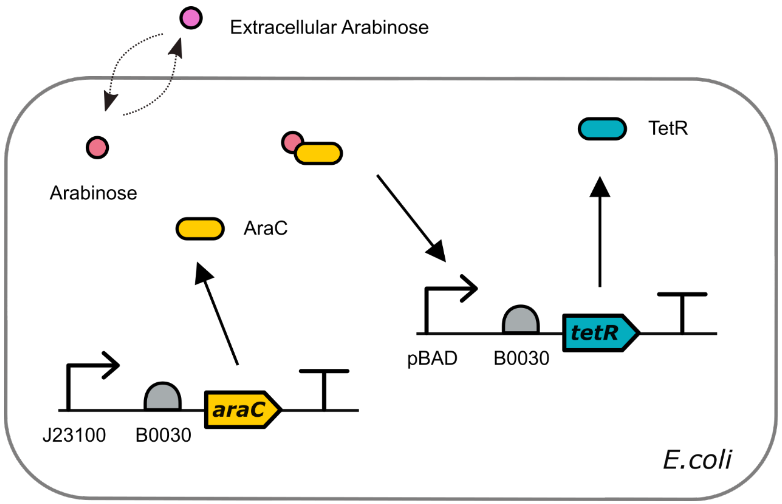
$$K_{d_{pBAD}} = \frac{K_d K_{dis}\, C_N}{[\mathrm{AraC}]^{n_A}}$$

Trabelsi, H., Koch, M., & Faulon, J. L. (2018). Building a minimal and generalizable model of transcription factor–based biosensors: Showcasing flavonoids. Biotechnology and bioengineering, 115(9), 2292-2304.

Modeling a genetic circuit Example Sense-Compute-Act

SENSE

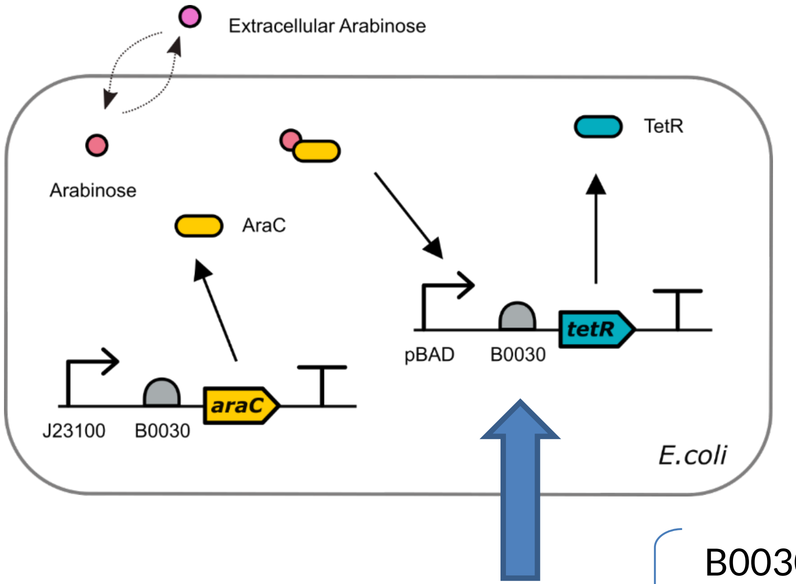




$$[\mathrm{TetR}] = \frac{\alpha_{\mathrm{pBAD}}}{d_{\mathrm{TetR}}} \left(\beta_{o_{pBAD}} + \frac{\left(1 - \beta_{o_{pBAD}}\right) [\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}} \right)$$

Modeling a genetic circuit Example Sense-Compute-Act

SENSE



$$[\mathrm{TetR}] = \frac{\alpha_{pBAD}}{d_{\mathrm{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

$$\alpha_{pBAD} = k_{2_{\mathrm{TetR}}}\frac{k_{1_{\mathrm{mTetR}}}}{d_{m_{\mathrm{TetR}}}} C_N$$

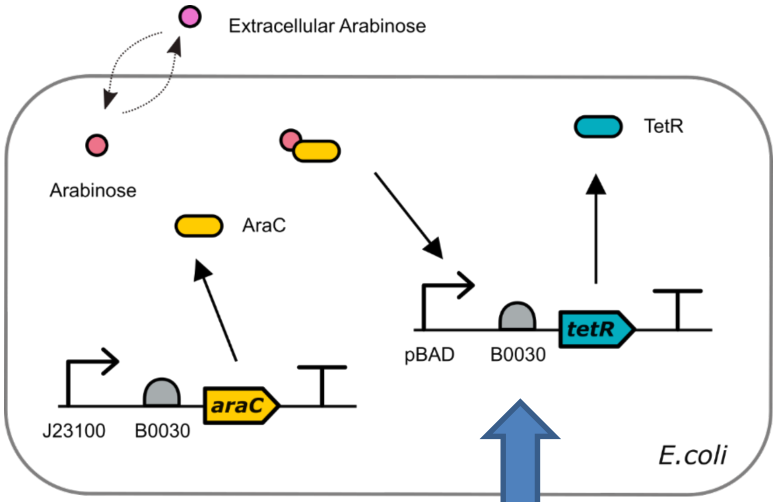
Let us try with different RBS

- B0030
- B0034
- B0032

What effect does it have in the hill function?

Modeling a genetic circuit Example Sense-Compute-Act

SENSE



$$[\text{TetR}] = \frac{\alpha_{pBAD}}{d_{\text{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\text{Arab}]^{n_a}}{\left(K_{d_{\text{pBAD}}}\right)^{n_a} + [\text{Arab}]^{n_a}}\right)$$

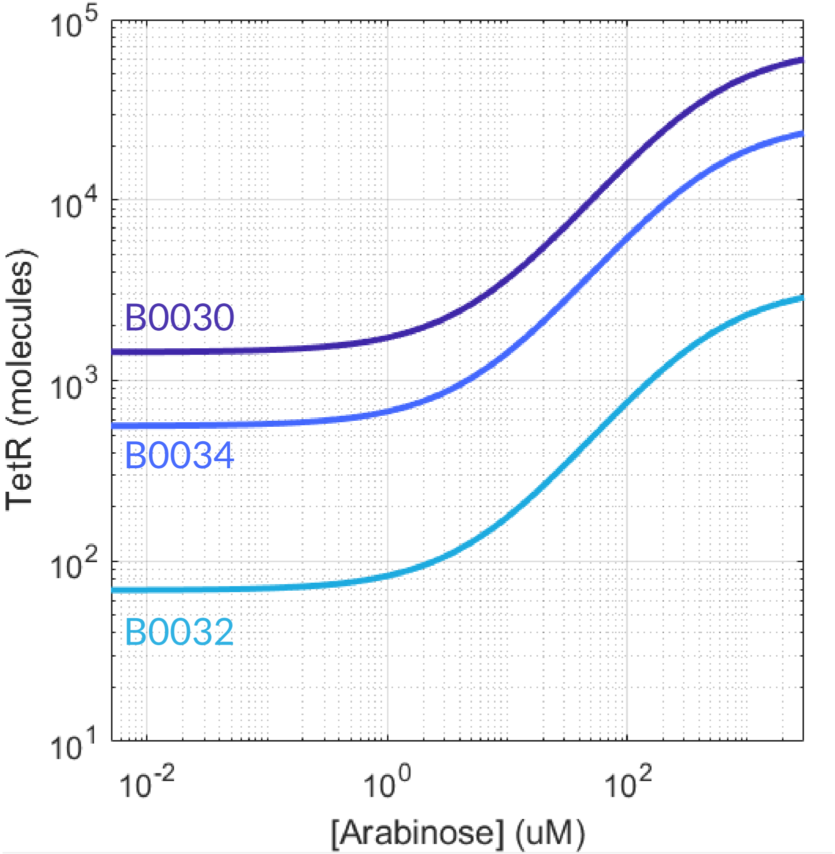
$$\alpha_{pBAD} = k_{2_{\text{TetR}}} \frac{k_{1_{\text{mTetR}}}}{d_{m_{\text{TetR}}}} C_N$$

Let us try with different RBS

B0030
B0034
B0032

Modeling a genetic circuit Example Sense-Compute-Act

SENSE



$$[\mathrm{TetR}] = \frac{\alpha_{pBAD}}{d_{\mathrm{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

$$\alpha_{pBAD} = k_{2_{\mathrm{TetR}}} \frac{k_{1_{\mathrm{mTetR}}}}{d_{m_{\mathrm{TetR}}}} C_N$$

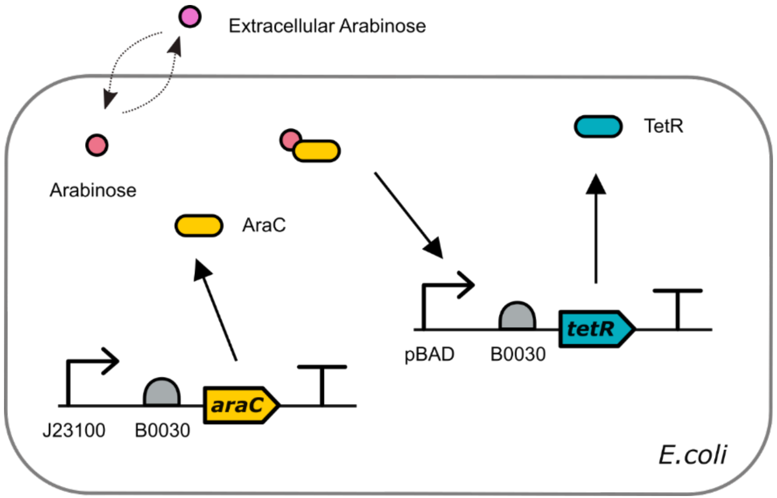
B0030: $\alpha_{pBAD} \approx 7.1 \times 10^4$ molecules

B0034: $\alpha_{pBAD} \approx 2.5 \times 10^4$ molecules

B0032: $\alpha_{pBAD} \approx 3.3 \times 10^3$ molecules

Modeling a genetic circuit Example Sense-Compute-Act

SENSE



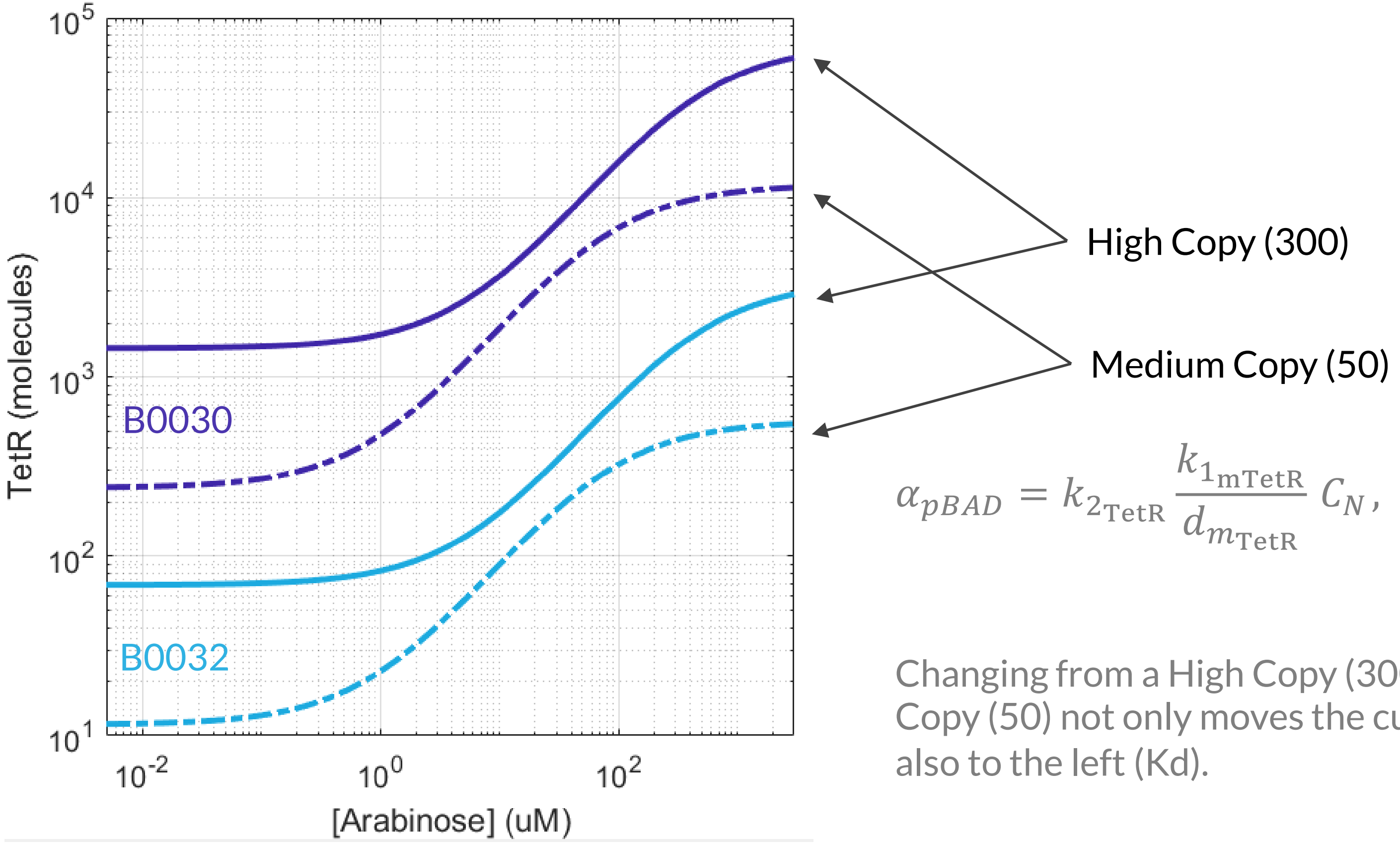
$$[\mathrm{TetR}] = \frac{\alpha_{pBAD}}{d_{\mathrm{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

$$\alpha_{pBAD} = k_{2_{\mathrm{TetR}}}\frac{k_{1_{\mathrm{mTetR}}}}{d_{m_{\mathrm{TetR}}}} C_N$$

Now let us try with different Plasmid Copy Number (High/Medium)

Modeling a genetic circuit Example Sense-Compute-Act

SENSE

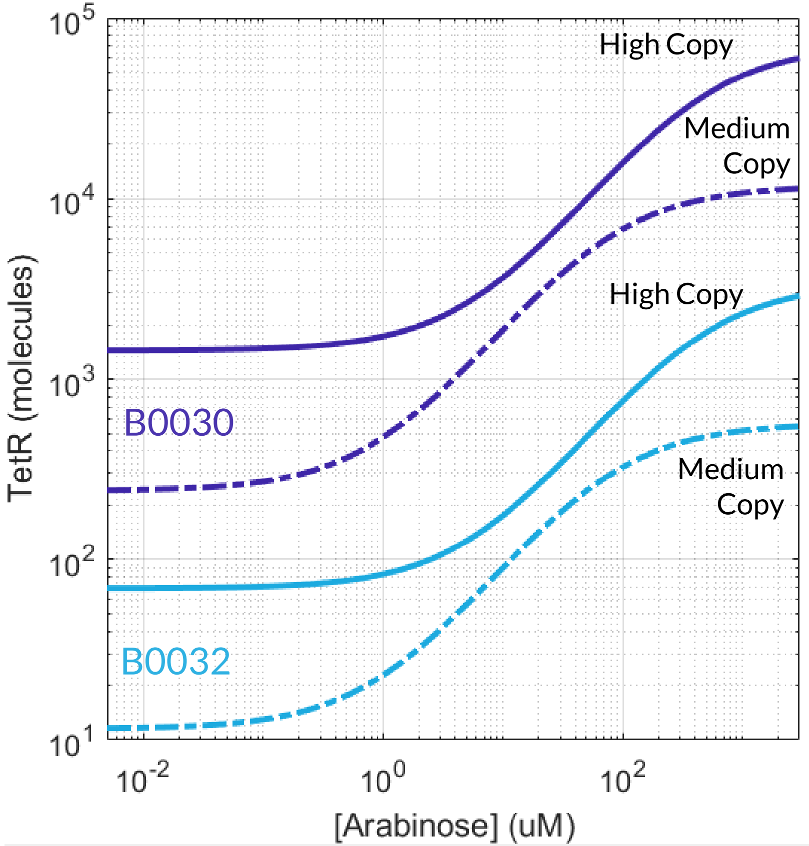


$$\alpha_{pBAD} = k_{2_{\text{TetR}}} \frac{k_{1_{\text{mTetR}}}}{d_{m_{\text{TetR}}}} C_N, \quad K_{d_{pBAD}} = \frac{K_d K_{dis} C_N}{[\text{AraC}]^{n_A}}$$

Changing from a High Copy (300) to a Medium Copy (50) not only moves the curve down (α), but also to the left (Kd).

Modeling a genetic circuit Example Sense-Compute-Act

SENSE



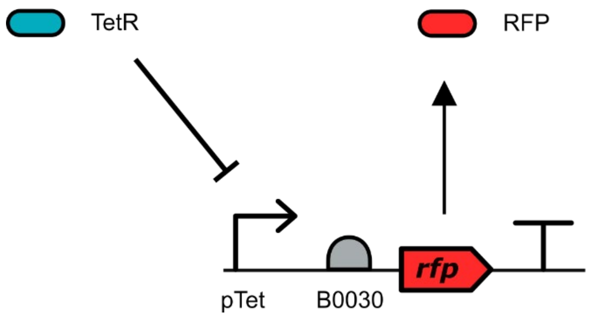
α_{pBAD}	High Copy	Medium Copy
B0030	7.1×10^4 molecules	1.2×10^4 molecules
B0032	3300 molecules	560 molecules

	High Copy	Medium Copy
$K_{d_{pBAD}}$	440 μM	14 μM

Changing from a High Copy (300) to a Medium Copy (50) not only moves the curve down (α), but also to the left (Kd).

Modeling a genetic circuit Example Sense-Compute-Act

COMPUTE - ACT



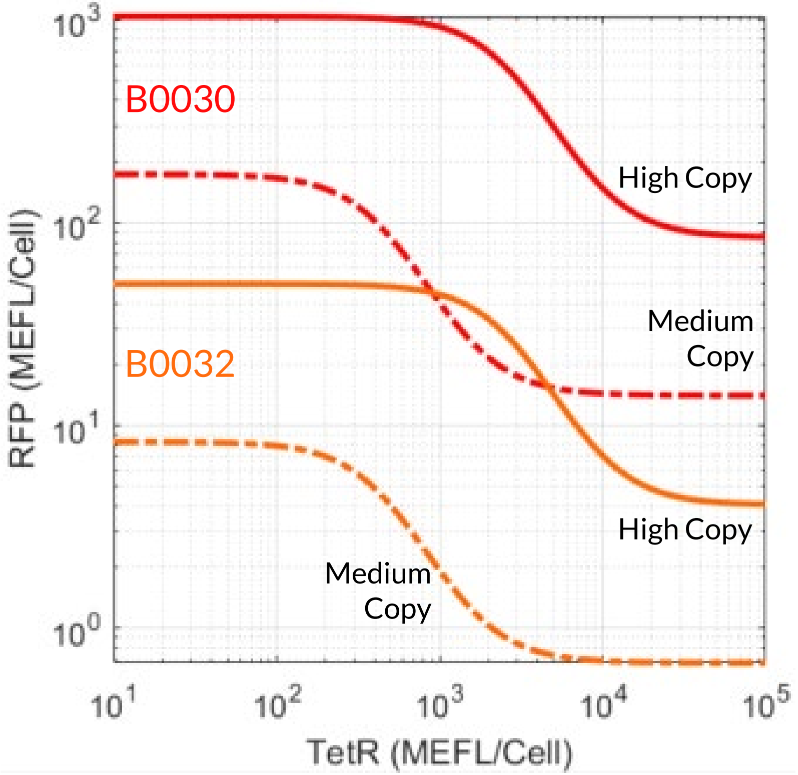
$$[\mathrm{RFP}] = \frac{\alpha_{\mathrm{pTet}}}{d_{\mathrm{RFP}}}\left(\beta_{o_{\mathrm{pTet}}} + \frac{\left(1-\beta_{o_{\mathrm{pTet}}}\right)\left(K_{d_{\mathrm{pTet}}}\right)^{n_t}}{\left(K_{d_{\mathrm{pTet}}}\right)^{n_t} + [\mathrm{TetR}]^{n_t}}\right)$$

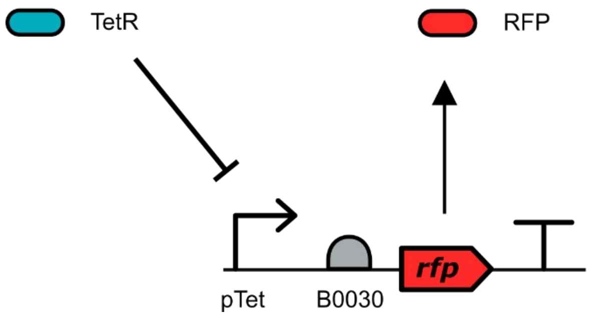
$$\alpha_{\mathrm{pTet}} = k_{2_{\mathrm{RFP}}} \frac{k_{1_{\mathrm{m_{RFP}}}}}{d_{\mathrm{m_{RFP}}}} C_N$$

$$K_{d_{\mathrm{pTet}}} = K_d\, C_N$$

Modeling a genetic circuit Example Sense-Compute-Act

COMPUTE - ACT



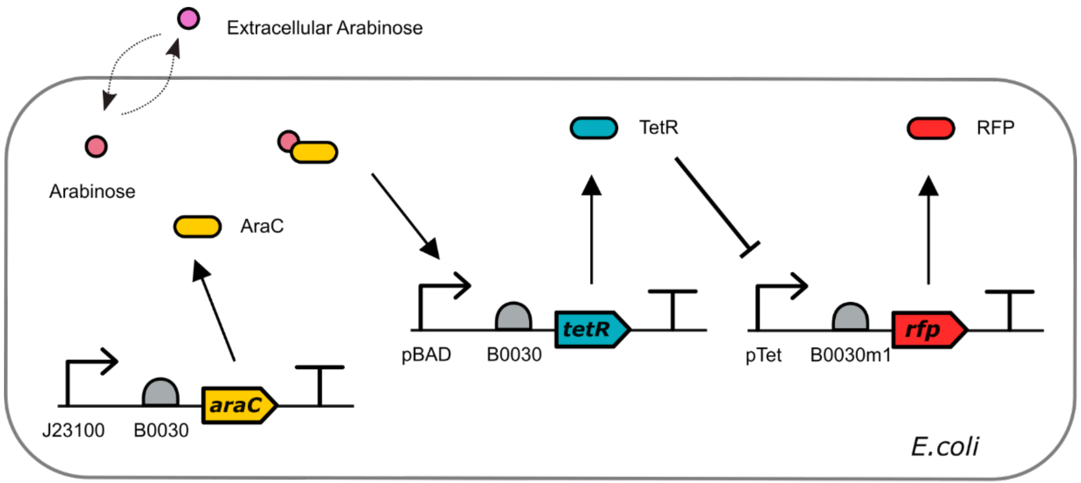


$$[\mathrm{RFP}] = \frac{\alpha_{\mathrm{pTet}}}{d_{\mathrm{RFP}}}\left(\beta_{o_{\mathrm{pTet}}} + \frac{\left(1-\beta_{o_{\mathrm{pTet}}}\right)[\mathrm{TetR}]^{n_t}}{\left(K_{d_{\mathrm{pTet}}}\right)^{n_t} + [\mathrm{TetR}]^{n_t}}\right)$$

Let us try with different RBS and Plasmid Copy Numbers

Modeling a genetic circuit Example Sense-Compute-Act

SENSE - COMPUTE - ACT

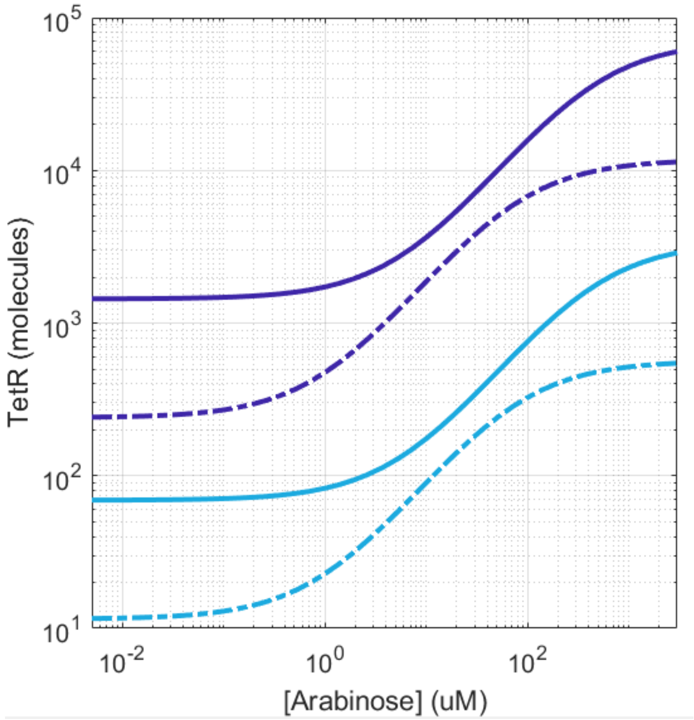


$$[\mathrm{TetR}] = \frac{\alpha_{pBAD}}{d_{\mathrm{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

$$[\mathrm{RFP}] = \frac{\alpha_{\mathrm{pTet}}}{d_{\mathrm{RFP}}}\left(\beta_{o_{\mathrm{pTet}}} + \frac{\left(1-\beta_{o_{\mathrm{pTet}}}\right)[\mathrm{TetR}]^{n_t}}{\left(K_{d_{\mathrm{pTet}}}\right)^{n_t} + [\mathrm{TetR}]^{n_t}}\right)$$

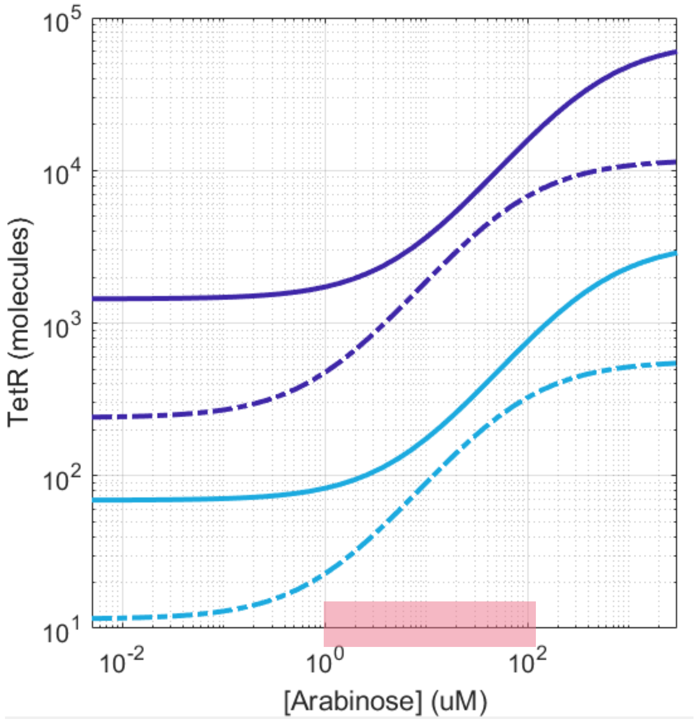
Modeling a genetic circuit Example Sense-Compute-Act

SENSE - COMPUTE



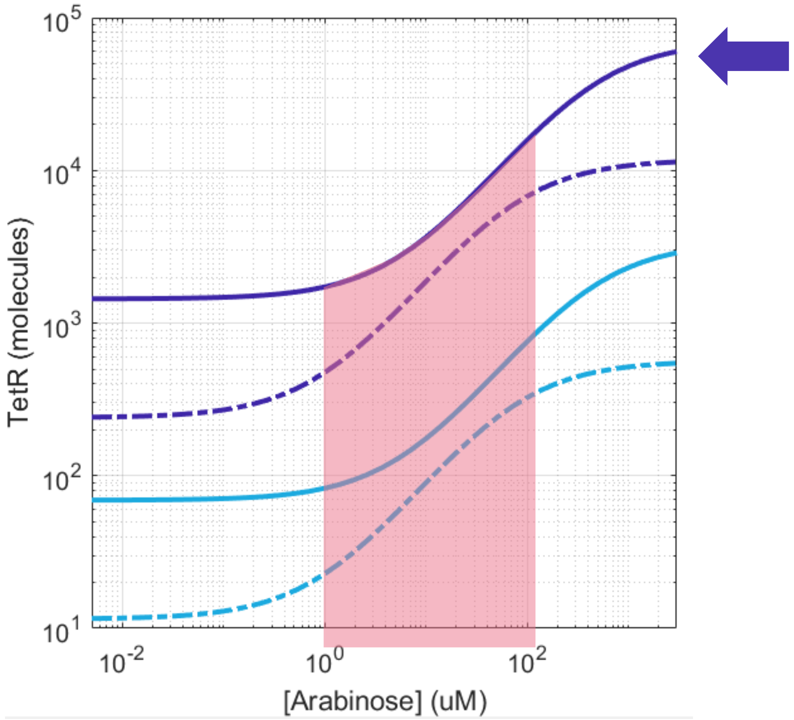
Modeling a genetic circuit Example Sense-Compute-Act

SENSE - COMPUTE



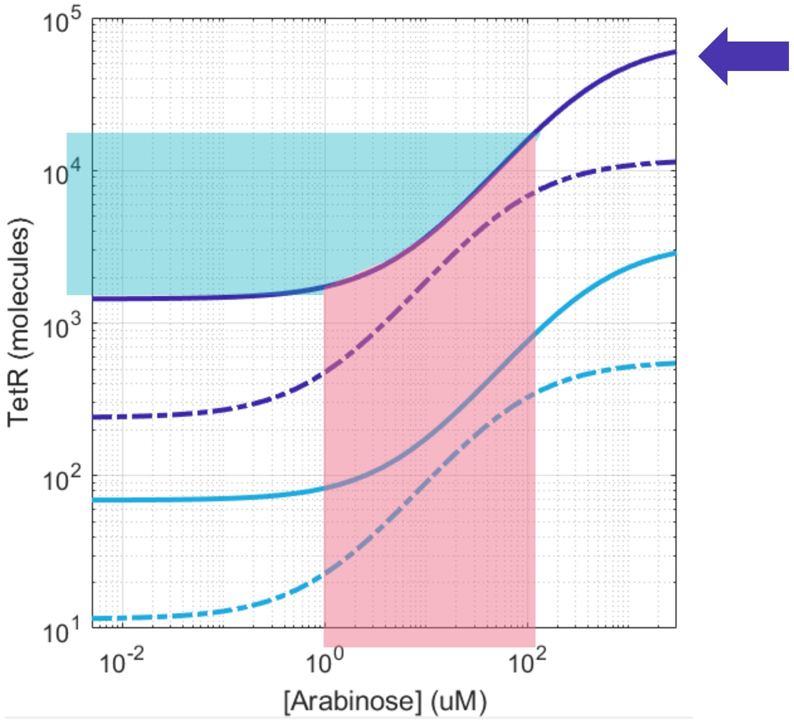
Modeling a genetic circuit Example Sense-Compute-Act

SENSE - COMPUTE



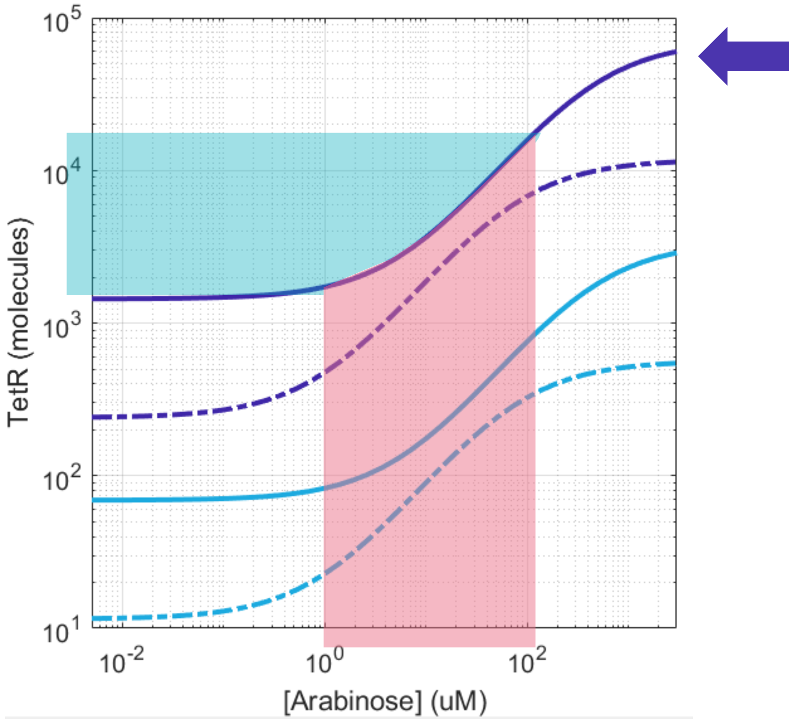
Modeling a genetic circuit Example Sense-Compute-Act

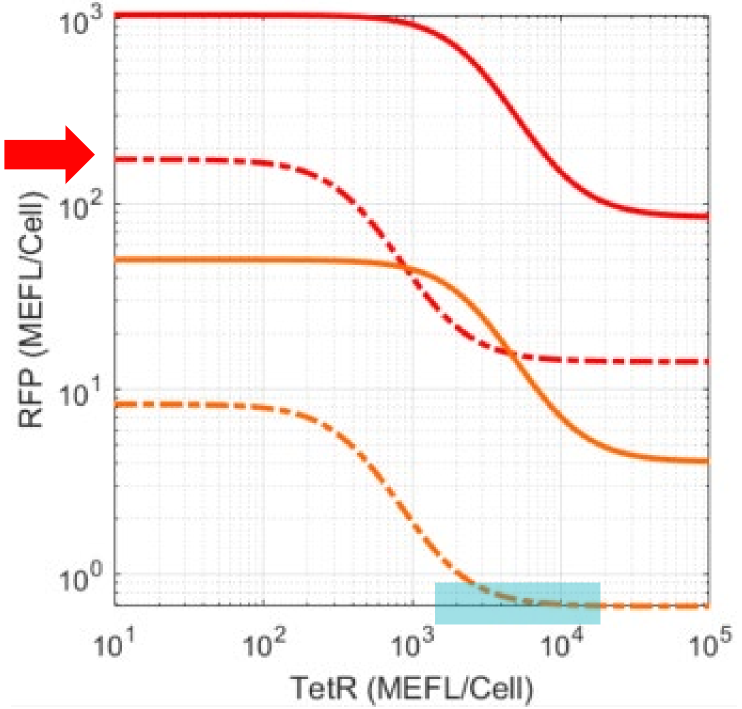
SENSE - COMPUTE



Modeling a genetic circuit Example Sense-Compute-Act

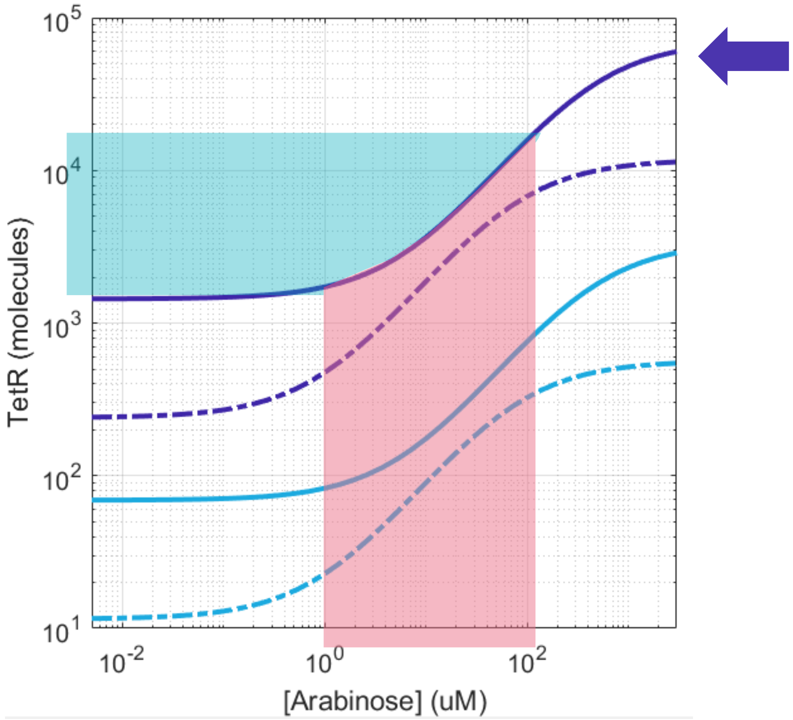
SENSE - COMPUTE - ACT

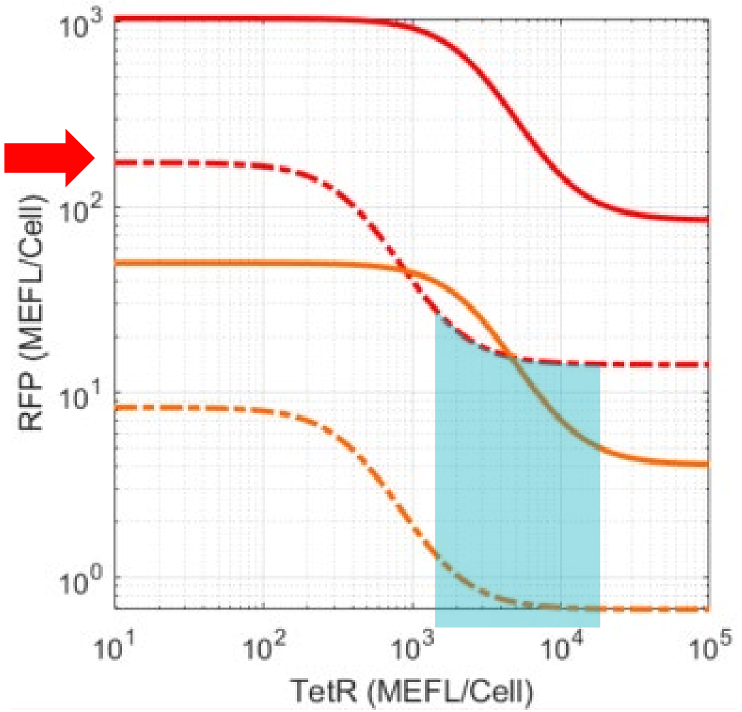




Modeling a genetic circuit Example Sense-Compute-Act

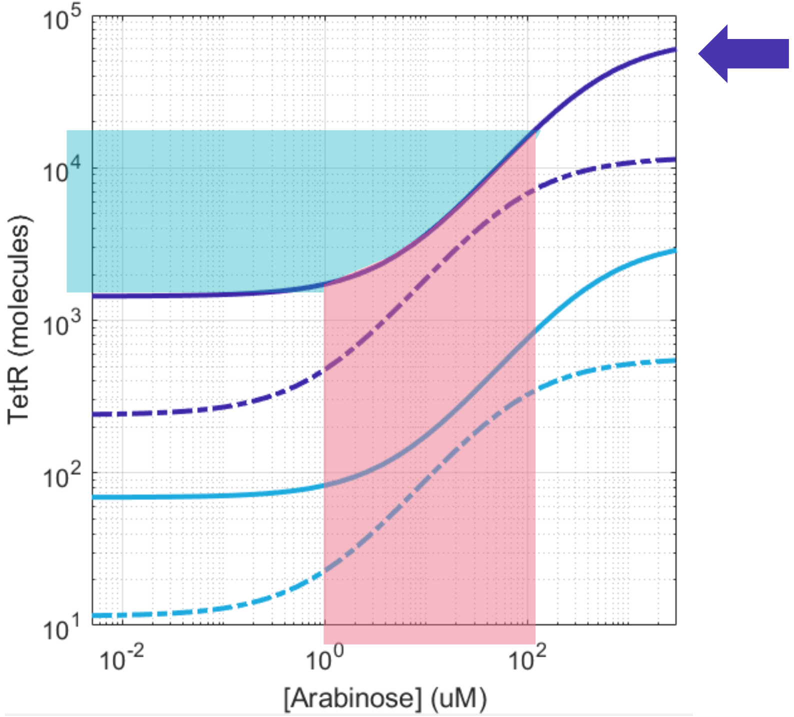
SENSE - COMPUTE - ACT

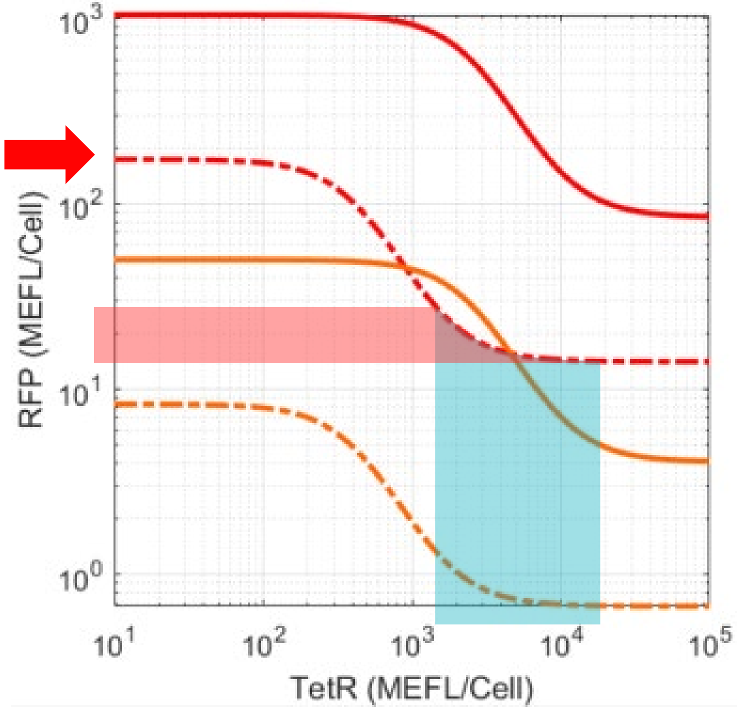




Modeling a genetic circuit Example Sense-Compute-Act

SENSE - COMPUTE - ACT





Questions?
Ask writing in the chat or contact me by email (alvig2 [at] upv [dot] es)

Stay tuned, next Section 2:

Relating parameters and data

Modeling a genetic circuit Example Sense-Compute-Act

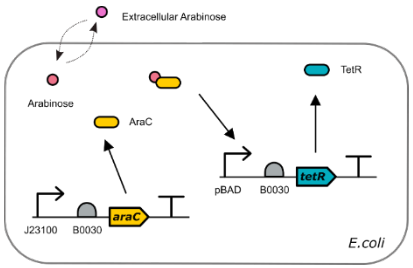
Basal Expression

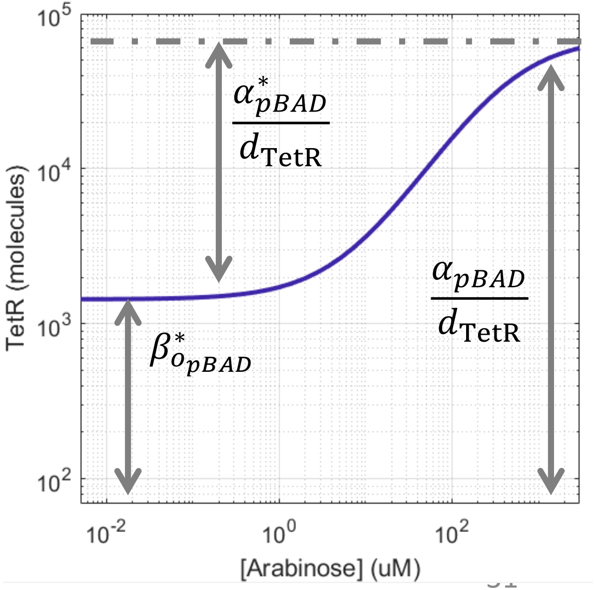
$$[\text{TetR}] = \frac{\alpha_{pBAD}}{d_{\text{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\text{Arab}]^{n_a}}{\left(K_{d_{\text{pBAD}}}\right)^{n_a} + [\text{Arab}]^{n_a}}\right)$$

$$[\text{TetR}] = \beta_{o_{pBAD}}\frac{\alpha_{pBAD}}{d_{\text{TetR}}} + \left(1-\beta_{o_{pBAD}}\right)\frac{\alpha_{pBAD}}{d_{\text{TetR}}}\frac{[\text{Arab}]^{n_a}}{\left(K_{d_{\text{pBAD}}}\right)^{n_a} + [\text{Arab}]^{n_a}}$$

$$[\text{TetR}] = \beta^*_{o_{pBAD}} + \frac{\alpha^*_{pBAD}}{d_{\text{TetR}}}\frac{[\text{Arab}]^{n_a}}{\left(K_{d_{\text{pBAD}}}\right)^{n_a} + [\text{Arab}]^{n_a}}$$

$$\beta^*_{o_{pBAD}} = \beta_{o_{pBAD}}\frac{\alpha_{pBAD}}{d_{\text{TetR}}} \qquad \frac{\alpha^*_{pBAD}}{d_{\text{TetR}}} = \left(1-\beta_{o_{pBAD}}\right)\frac{\alpha_{pBAD}}{d_{\text{TetR}}}$$





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UNIVERSIDAD POLITECNICA DE VALENCIA
Synthetic Biology and Biosystems Control Lab
Valencia UPV

iGEM 2020 Measurement

Modeling: Modeling circuits with ODEs and experimental data

Section 2: Relating parameters and data

by Alejandro Vignoni (alvig2@upv.es)

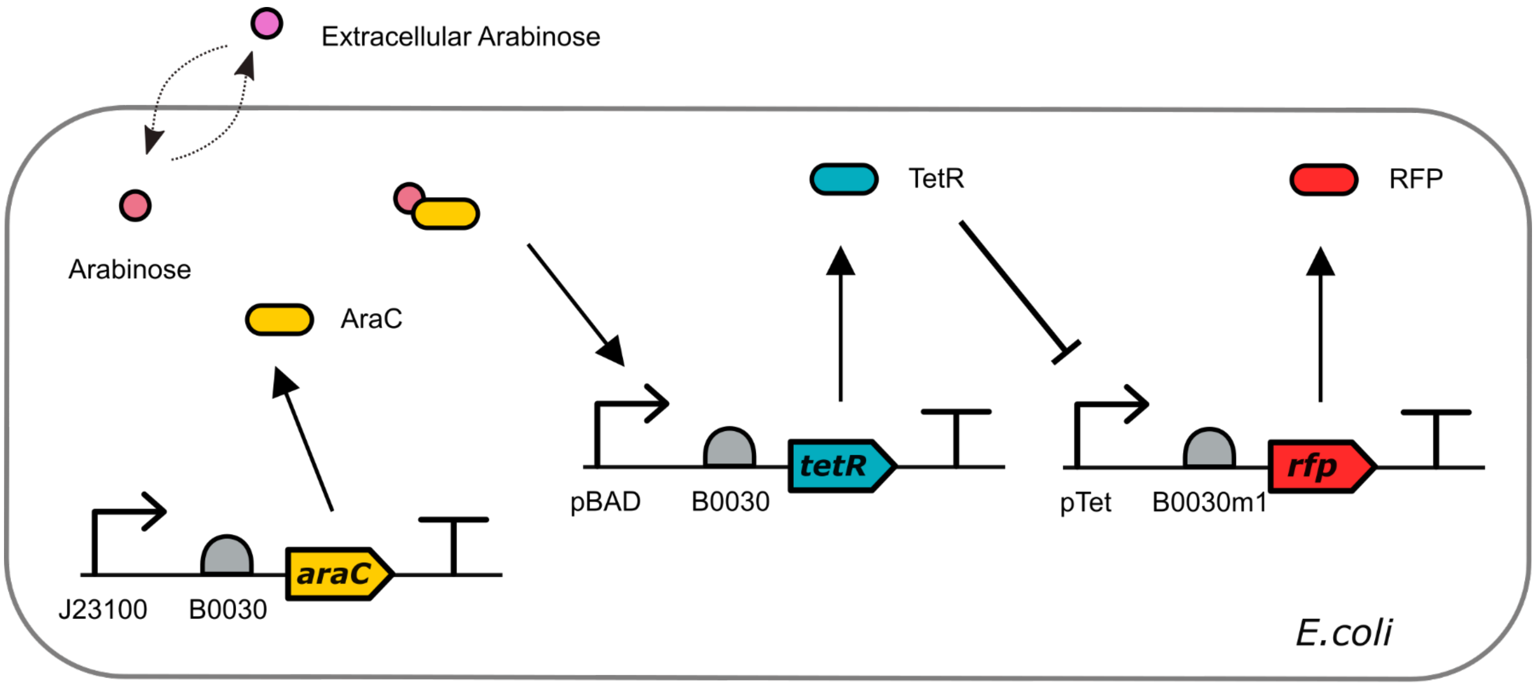
An iGEM Measurement Committee Webinar
Week 3a, June 30th, 2020

Today Webinar's Topics

- Section 1: Composing circuit models from Hill functions (15 min)
- Section 2: **Relating parameters and data** (15 min)
- Section 3: Example: Incoherent feed-forward loop (model & data) (15 min)
- Q&A – (at the end of each 15 minutes block, total 15 min)

Relating model parameters and data

Example Sense-Compute-Act

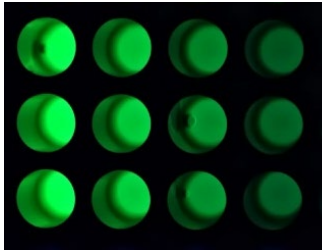


But first we need to get experimental data:

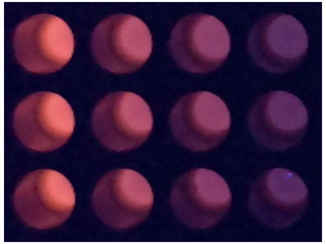
Measurement -> Calibrated measurement

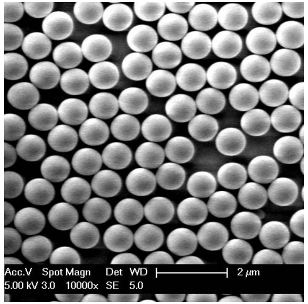
Plate Reader

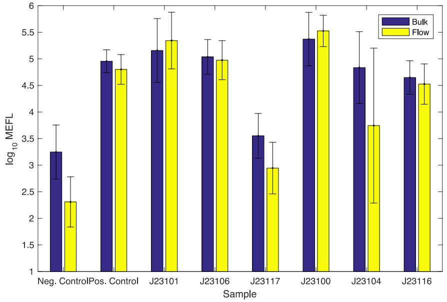
Fluorescein



Texas Red

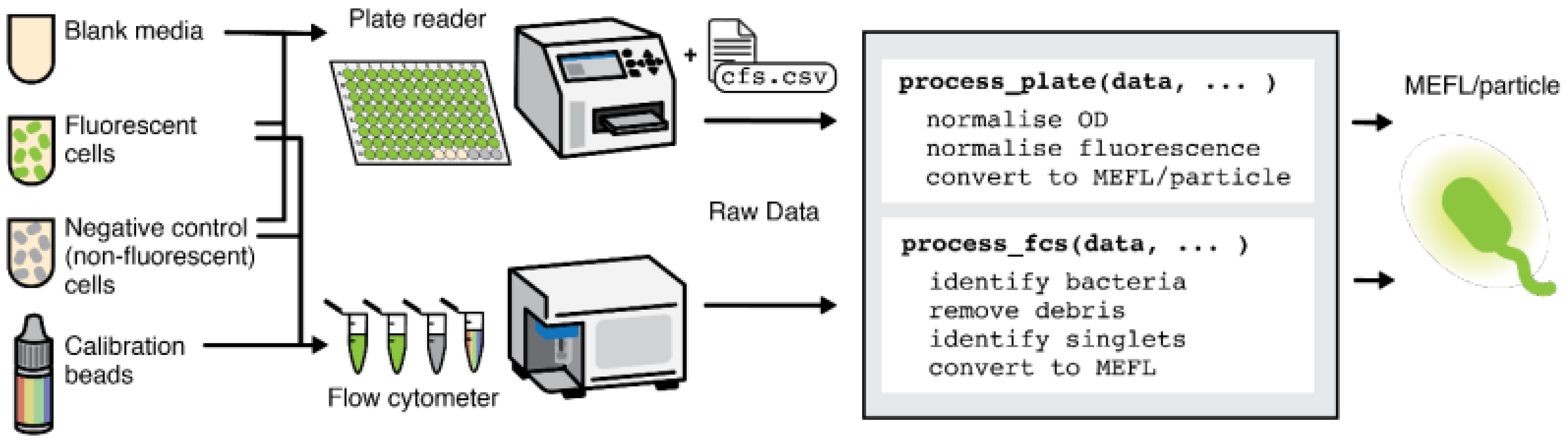






https://2020.igem.org/Measurement/Protocols#validation

Measurement Calibration

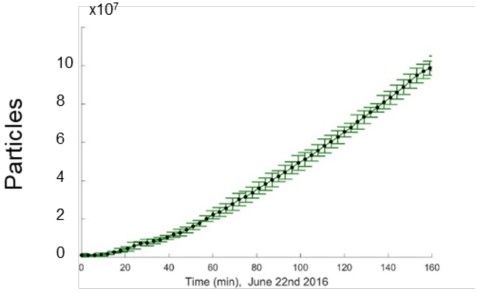


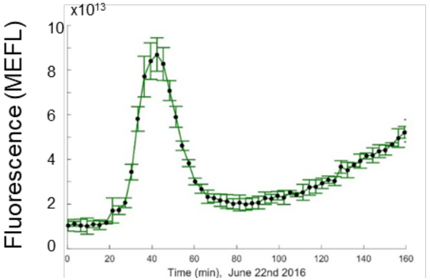
Stay in tune for Measurement Committee Webinars about Calibration:

Week 5 - Tuesday July 14th - 7am EDT - Quantifying fluorescence and cell count with plate readers
Week 6 - Tuesday July 23rd - 7am EDT - Quantifying fluorescence and cell phenotypes with flow cytometry

(Robinson et al, 2020, DOI: 10.1101/2020.06.01.127084)

Why? Because it is exactly what we get from the model



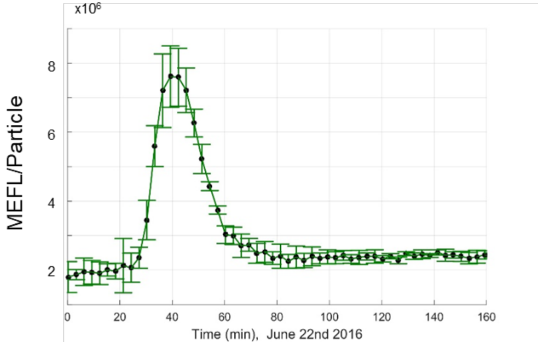


$$\frac{d[R]}{dt} = \frac{\mathrm{p_R C_N k_R}}{\mathrm{dm_R} + \mu} - (\mathrm{d_R} + \mu)[R]$$

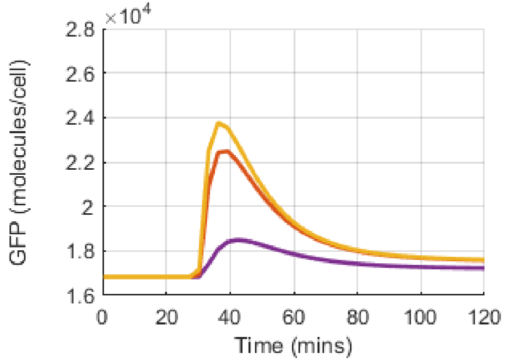
$$\frac{d[cI]}{dt} = \frac{\mathrm{p_{cI} C_N k_{cI}}}{\mathrm{dm_{cI}} + \mu}\left(\alpha + (1-\alpha)\frac{\frac{1}{\mathrm{k_{dlux}}}\left(\frac{[R][A]}{\mathrm{k_{d2} C_N}}\right)^2}{1 + \frac{1}{\mathrm{k_{dlux}}}\left(\frac{[R][A]}{\mathrm{k_{d2} C_N}}\right)^2}\right) - (\mathrm{d_{cI}} + \mu)[cI]$$

$$\frac{d[GFP]}{dt} = \frac{\mathrm{p_G C_N k_G}}{\mathrm{dm_G} + \mu}\left(\alpha + (1-\alpha)\frac{\frac{1}{\mathrm{k_{dlux}}}\left(\frac{[R][A]}{\mathrm{k_{d2} C_N}}\right)^2}{1 + \frac{1}{\mathrm{k_{dlux}}}\left(\frac{[R][A]}{\mathrm{k_{d2} C_N}}\right)^2}\frac{1}{1 + \frac{[cI]^2}{\mathrm{k_{dcI} C_N}}}\right) - (\mathrm{d_G} + \mu)[G]$$

$$\frac{dN}{dt} = \mu N\left(1 - \frac{N}{\mathrm{N_{max}}}\right)$$



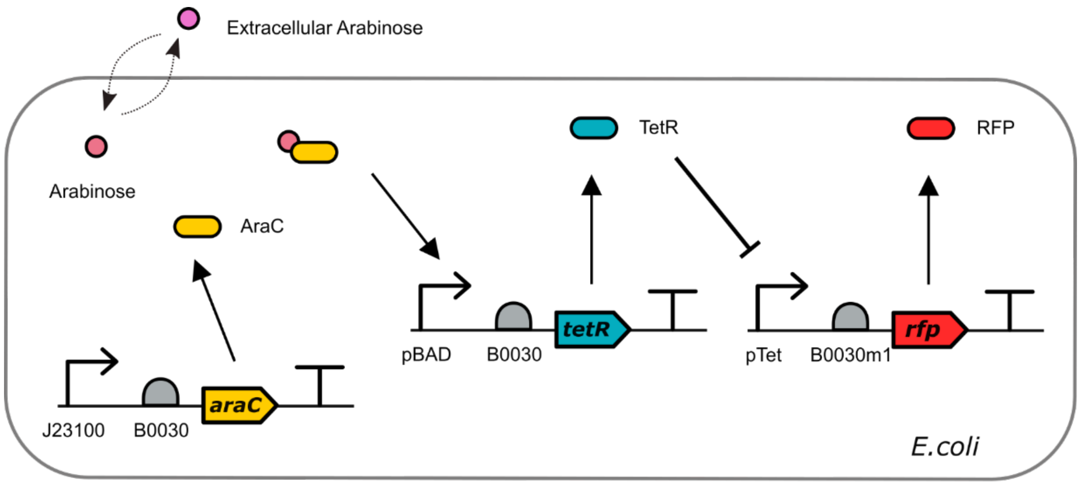
$\equiv$



MEFL/Particle unit is equivalent to number of molecules/cell from the mathematical model

Measuring a genetic circuit Example Sense-Compute-Act

SENSE - COMPUTE - ACT

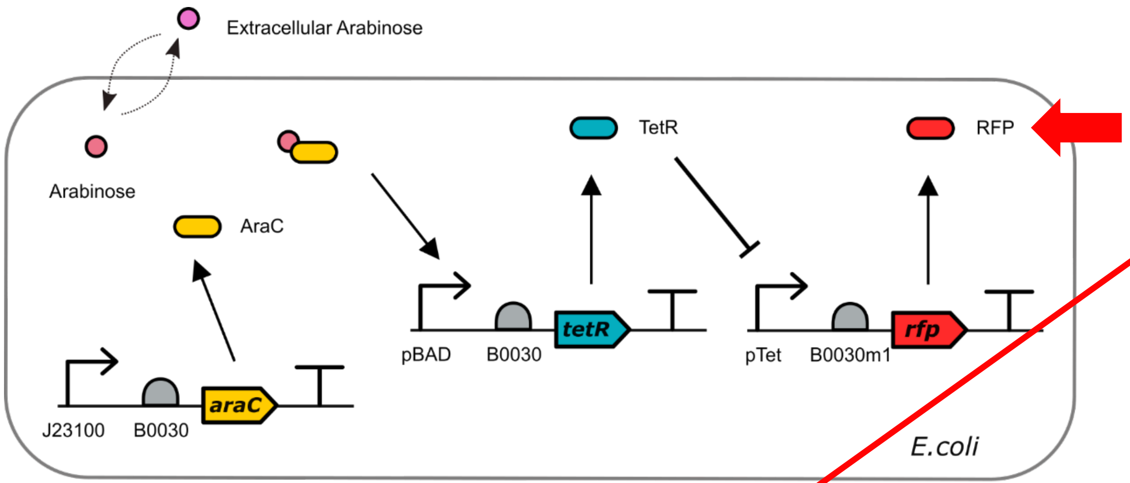


$$[\mathrm{TetR}] = \frac{\alpha_{pBAD}}{d_{\mathrm{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

$$[\mathrm{RFP}] = \frac{\alpha_{\mathrm{pTet}}}{d_{\mathrm{RFP}}}\left(\beta_{o_{\mathrm{pTet}}} + \frac{\left(1-\beta_{o_{\mathrm{pTet}}}\right)[\mathrm{TetR}]^{n_t}}{\left(K_{d_{\mathrm{pTet}}}\right)^{n_t} + [\mathrm{TetR}]^{n_t}}\right)$$

Measuring a genetic circuit Example Sense-Compute-Act

SENSE - COMPUTE - ACT



We can measure only RFP

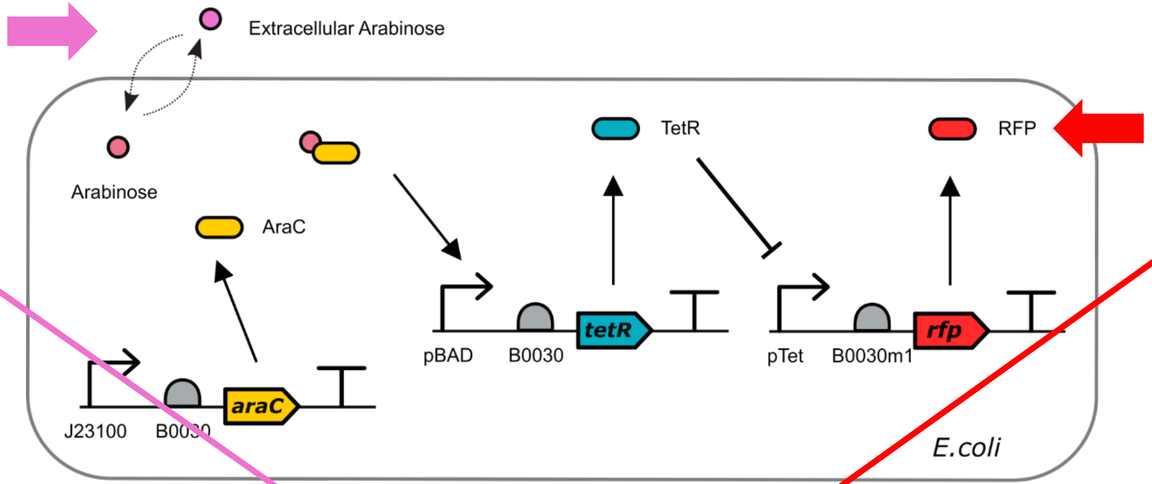
$$[\mathrm{TetR}] = \frac{\alpha_{pBAD}}{d_{\mathrm{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

$$[\mathrm{RFP}] = \frac{\alpha_{\mathrm{pTet}}}{d_{\mathrm{RFP}}}\left(\beta_{o_{\mathrm{pTet}}} + \frac{\left(1-\beta_{o_{\mathrm{pTet}}}\right)[\mathrm{TetR}]^{n_t}}{\left(K_{d_{\mathrm{pTet}}}\right)^{n_t} + [\mathrm{TetR}]^{n_t}}\right)$$

Measuring a genetic circuit Example Sense-Compute-Act

SENSE - COMPUTE - ACT

We can only change the amount of Arabinose



We can measure only RFP

$$[\mathrm{TetR}] = \frac{\alpha_{pBAD}}{d_{\mathrm{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

$$[\mathrm{RFP}] = \frac{\alpha_{\mathrm{pTet}}}{d_{\mathrm{RFP}}}\left(\beta_{o_{\mathrm{pTet}}} + \frac{\left(1-\beta_{o_{\mathrm{pTet}}}\right)[\mathrm{TetR}]^{n_t}}{\left(K_{d_{\mathrm{pTet}}}\right)^{n_t} + [\mathrm{TetR}]^{n_t}}\right)$$

Measuring a genetic circuit Example Sense-Compute-Act

SENSE - COMPUTE - ACT

We can only change the amount of Arabinose

We need more!!

What can we do?

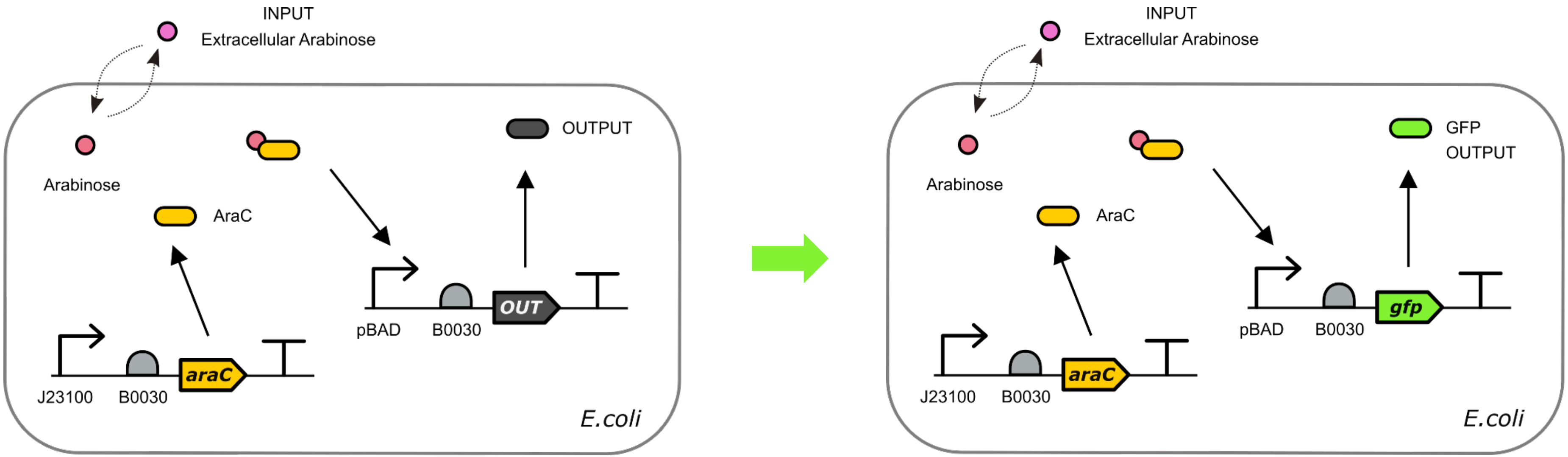
We can measure only RFP

$$[\mathrm{TetR}] = \frac{\alpha_{pBAD}}{d_{\mathrm{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\mathrm{Arab}]^{n_a}}{\left(K_{d_{\mathrm{pBAD}}}\right)^{n_a} + [\mathrm{Arab}]^{n_a}}\right)$$

$$[\mathrm{RFP}] = \frac{\alpha_{\mathrm{pTet}}}{d_{\mathrm{RFP}}}\left(\beta_{o_{\mathrm{pTet}}} + \frac{\left(1-\beta_{o_{\mathrm{pTet}}}\right)[\mathrm{TetR}]^{n_t}}{\left(K_{d_{\mathrm{pTet}}}\right)^{n_t} + [\mathrm{TetR}]^{n_t}}\right)$$

Measuring a genetic circuit Example Sense-Compute-Act

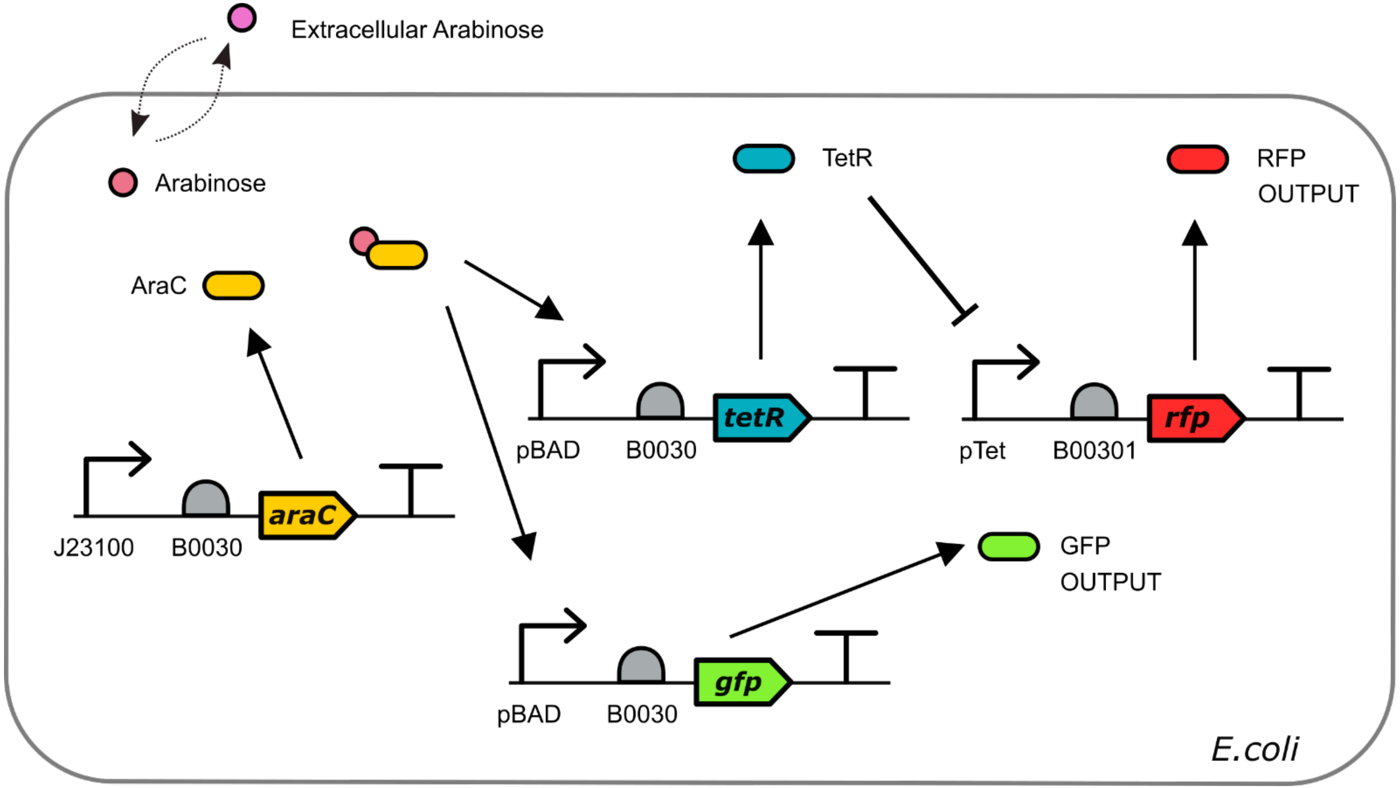
SENSE



We can make another construct, with GFP as OUTPUT.

Measuring a genetic circuit Example Sense-Compute-Act

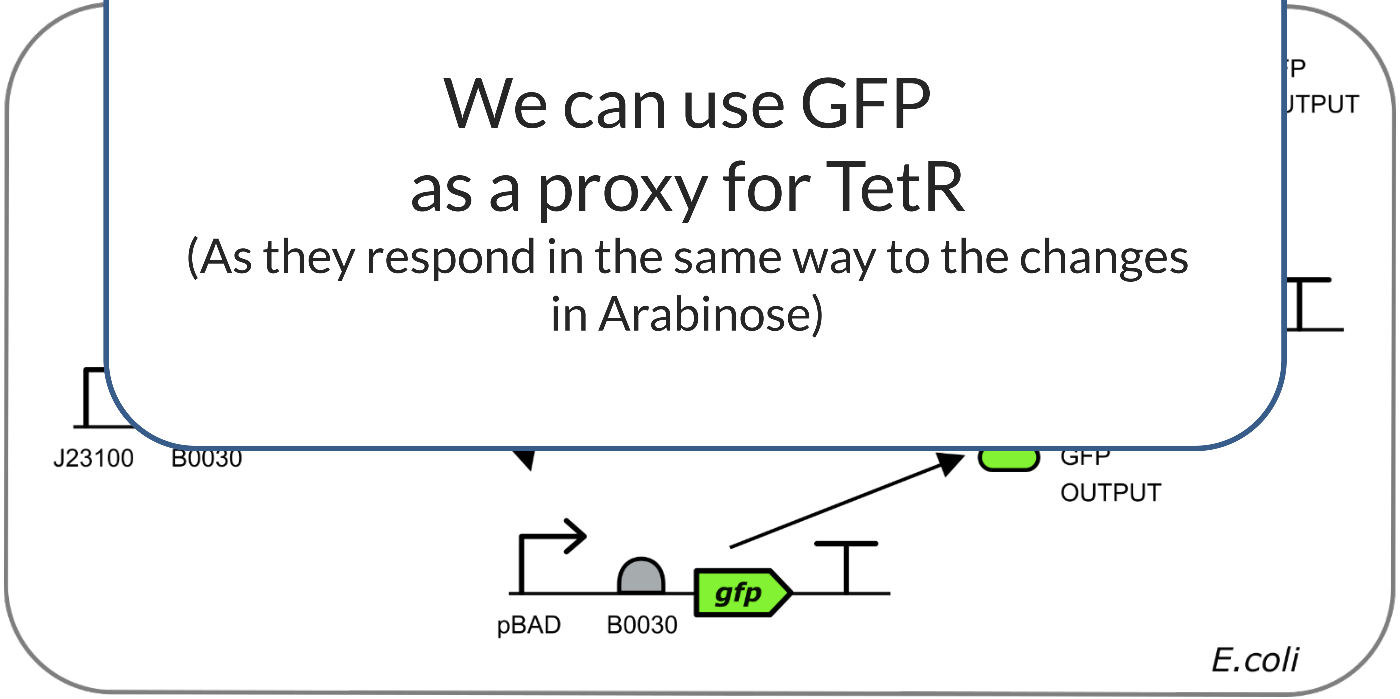
SENSE - COMPUTE - ACT for measurement



Measuring a genetic circuit Example Sense-Compute-Act

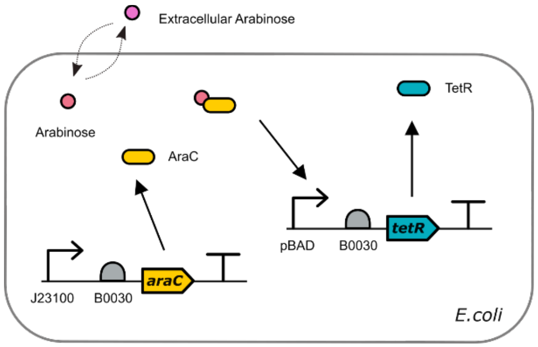
SENSE - COMPUTE - ACT for measurement

We can use GFP
as a proxy for TetR
(As they respond in the same way to the changes
in Arabinose)

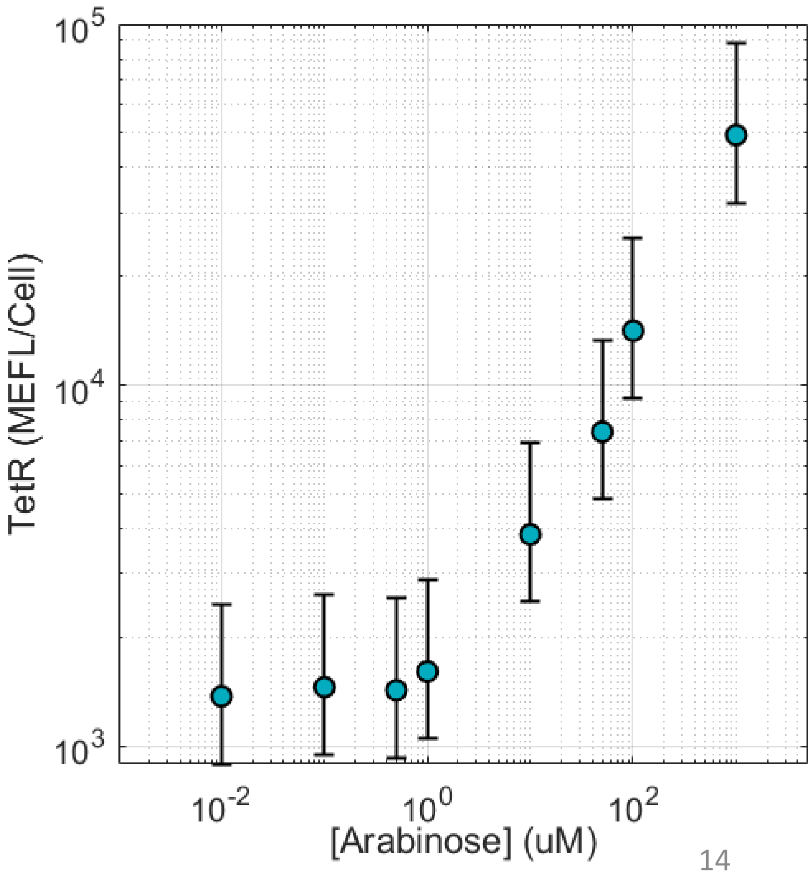


Modeling a genetic circuit Example Sense-Compute-Act

SENSE

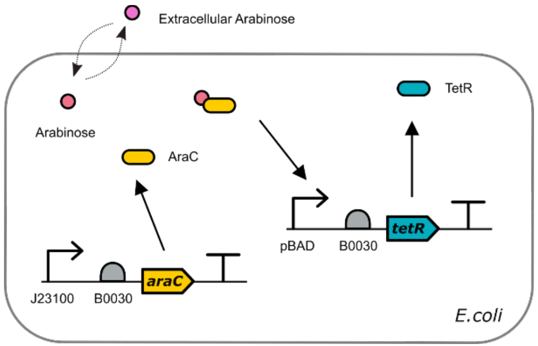


We make experiments with 8 different levels of Arabinose induction, measure GFP (TetR) in a flowcytometer and calibrate the mesurement.



Modeling a genetic circuit Example Sense-Compute-Act

SENSE

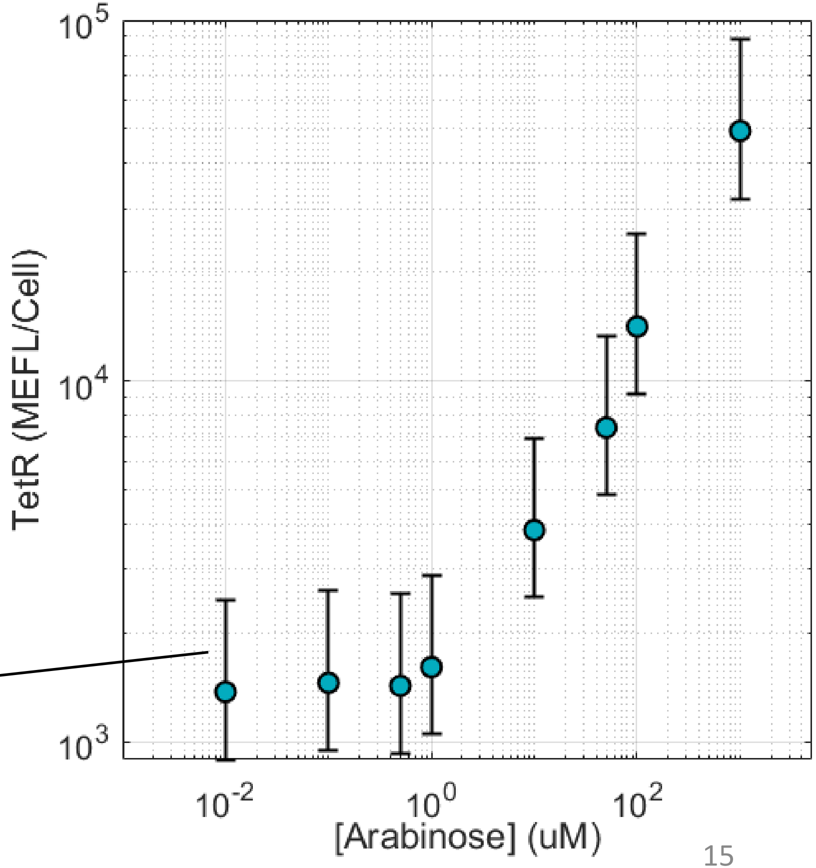


$$[\text{TetR}] = \frac{\alpha_{pBAD}}{d_{\text{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\text{Arab}]^{n_a}}{\left(K_{d_{\text{pBAD}}}\right)^{n_a} + [\text{Arab}]^{n_a}}\right)$$

$$\text{Error} = \frac{1}{m}\sum_{i}^{m}\left([TetR]_{model,\,i} - [TerR]_{measured,\,i}\right)^2$$

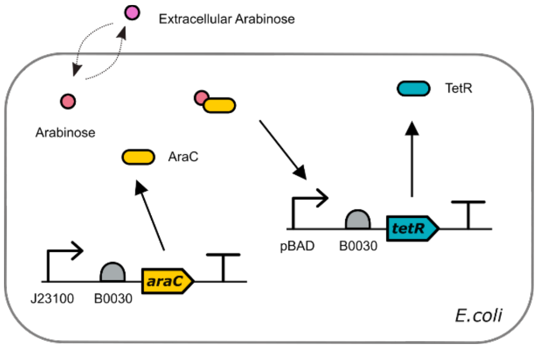
For the m different concentrations of Arabinose.

Then we minimize the error…



Modeling a genetic circuit Example Sense-Compute-Act

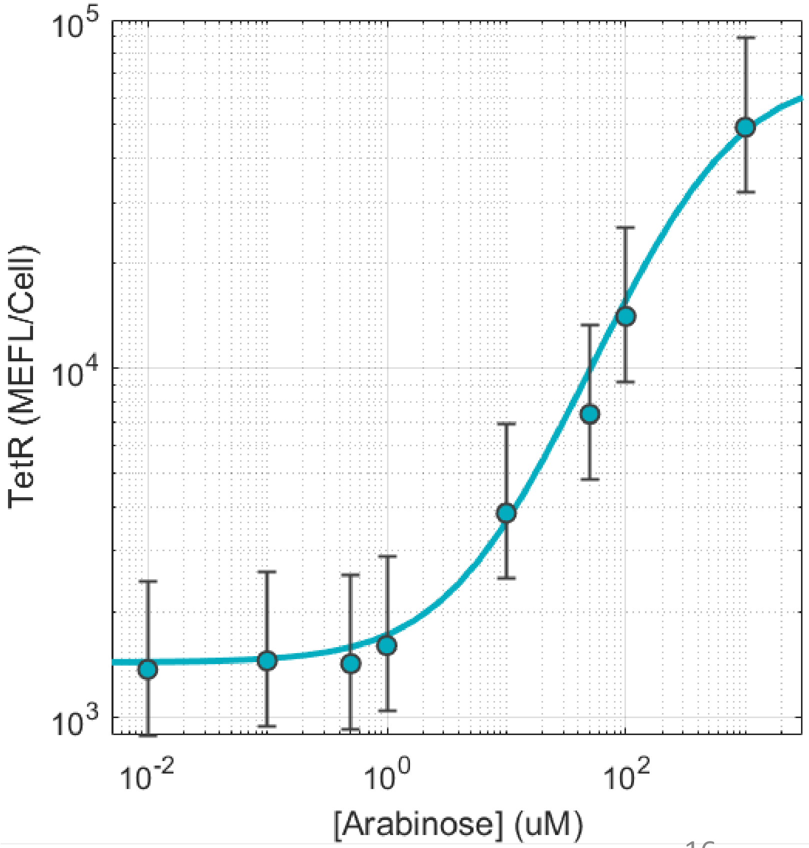
SENSE



$$[\text{TetR}] = \frac{\alpha_{pBAD}}{d_{\text{TetR}}}\left(\beta_{o_{pBAD}} + \frac{\left(1-\beta_{o_{pBAD}}\right)[\text{Arab}]^{n_a}}{\left(K_{d_{\text{pBAD}}}\right)^{n_a} + [\text{Arab}]^{n_a}}\right)$$

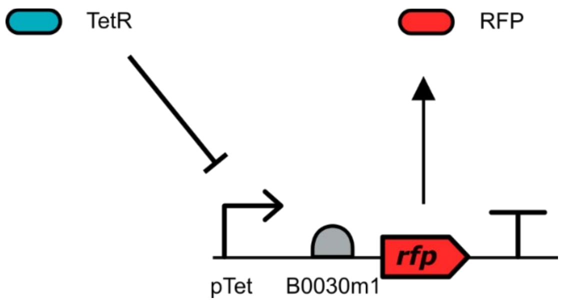
$$\frac{\alpha_{pBAD}}{d_{\text{TetR}}} = 7.056 \times 10^4 \text{molecules}$$

$K_{d_{pBAD}} = 444.5\mu M$ $\beta_{o_{pBAD}} = 0.02$ $n_a = 1$



Modeling a genetic circuit Example Sense-Compute-Act

COMPUTE - ACT



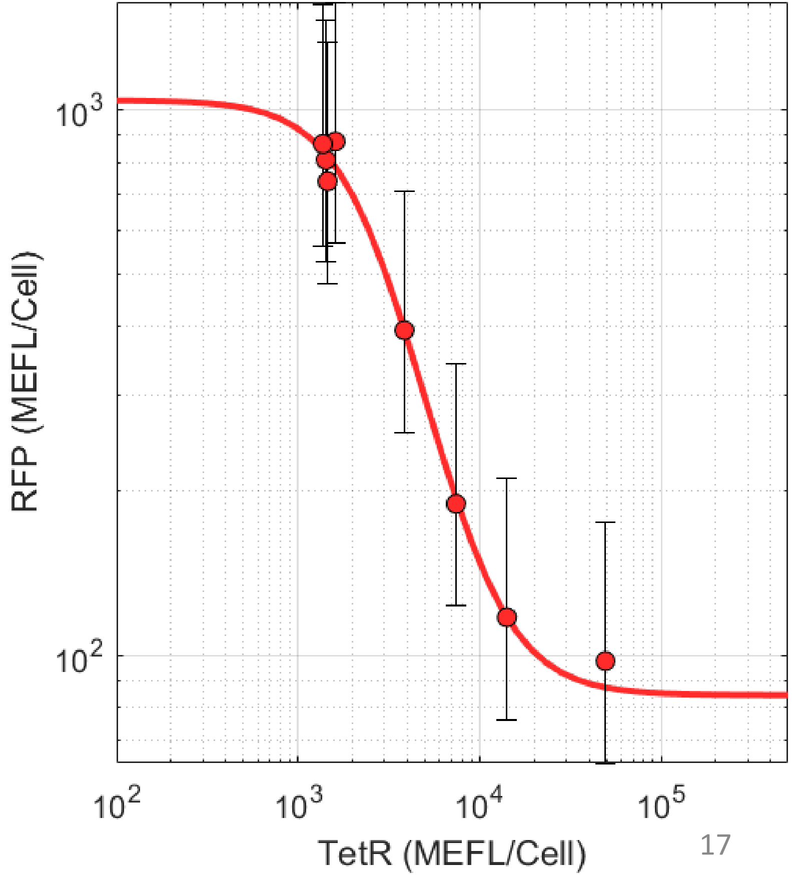
$$[\mathrm{RFP}] = \frac{\alpha_{\mathrm{pTet}}}{d_{\mathrm{RFP}}}\left(\beta_{o_{\mathrm{pTet}}} + \frac{\left(1-\beta_{o_{\mathrm{pTet}}}\right)[\mathrm{TetR}]^{n_t}}{\left(K_{d_{\mathrm{pTet}}}\right)^{n_t} + [\mathrm{TetR}]^{n_t}}\right)$$

$\frac{\alpha_{pTet}}{d_{\mathrm{RFP}}} = 1039$ molecules

$\beta_{o_{pBAD}} = 0.08$

$K_{d_{pTet}} = 2668$ molecules

$n_t = 2$



Questions?
Ask writing in the chat or contact me by email (alvig2 [at] upv [dot] es)

Stay tuned, next Section 3:

Example: Incoherent feed-forward loop (model & data)

sb2cl Lab

Synthetic Biology and Biosystems Control Lab
Valencia UPV

iGEM 2020 Measurement

Modeling: Modeling circuits with ODEs and experimental data

Section 3 Example: Incoherent feed-forward loop (model & data)

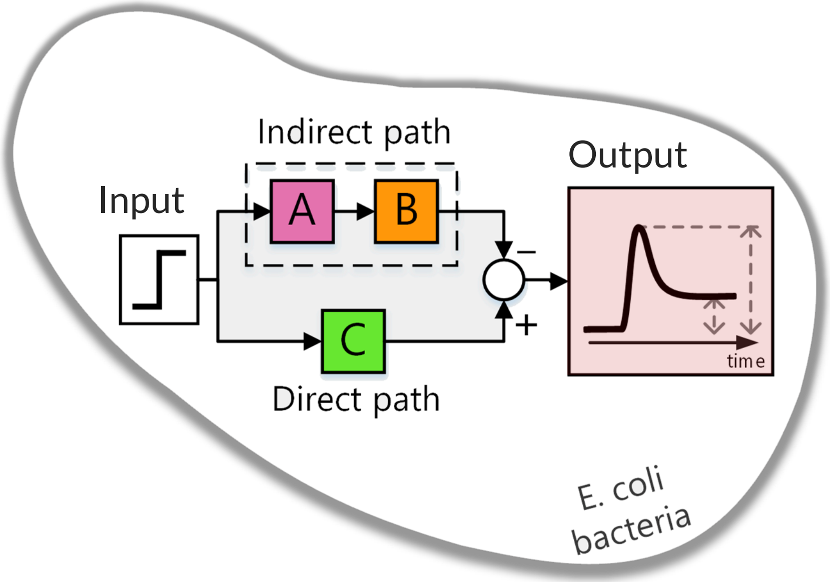
by Alejandro Vignoni (alvig2@upv.es)

An iGEM Measurement Committee Webinar
Week 3a, June 30th, 2020

Today Webinar's Topics

- Section 1: Composing circuit models from Hill functions (15 min)
- Section 2: Relating parameters and data (15 min)
- Section 3: **Example: Incoherent feed-forward loop (model & data)** (15 min)
- Q&A – (at the end of each 15 minutes block, total 15 min)

Incoherent type1 feedforward circuit (I1-FFL)

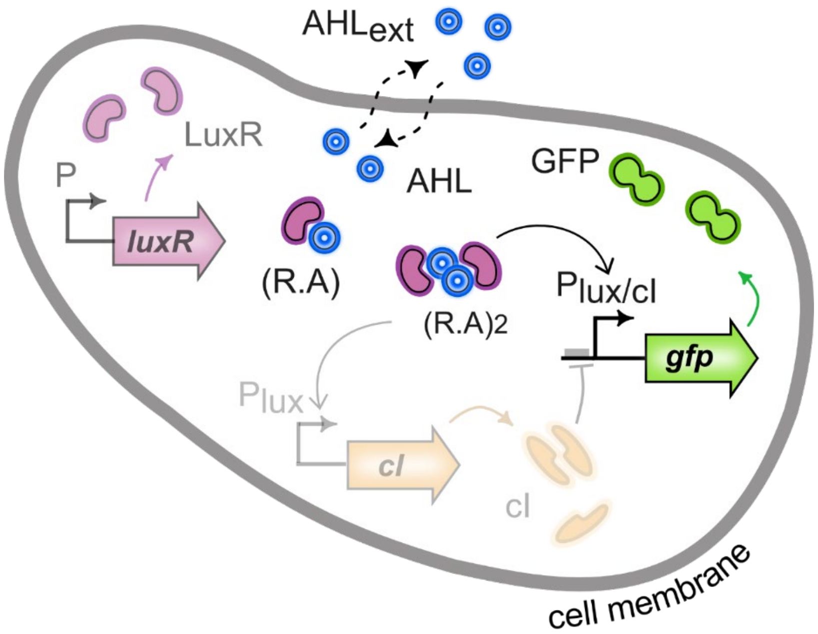


Change-fold detector

Responds to a change in its input and returns to the value it had prior to the stimulus.

In biology, this behavior is called *adaptation*

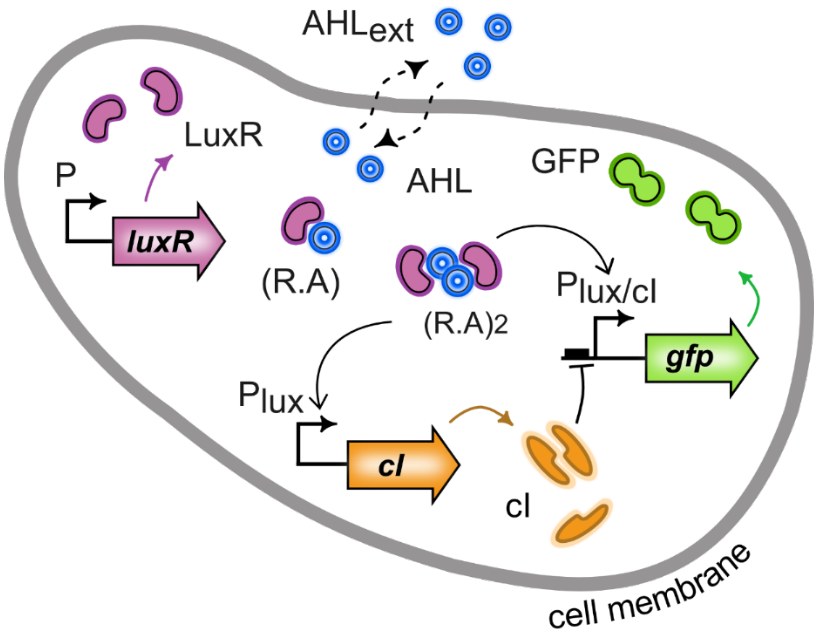
Structure of a design for the I1-FFL gene circuit



Direct path

Input AHL_{ext} diffuses across the cell membrane. AHL together with LuxR protein activates the output protein GFP.

Structure of the I1-FFL gene circuit



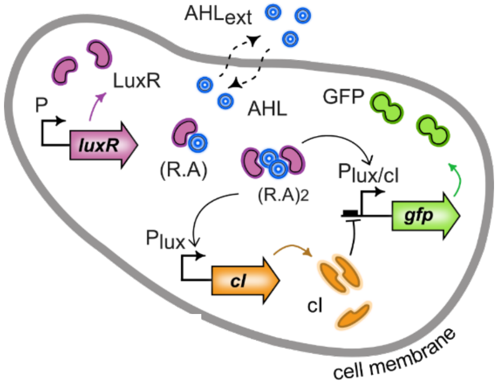
Direct path

Input AHL_{ext} diffuses across the cell membrane. AHL together with LuxR protein activates the output protein GFP.

Indirect path

AHL together with LuxR proteins also activate cI protein. After some time, cI represses the output protein GFP.

Model of the I1-FFL gene circuit



$$\frac{d[R]}{dt} = \frac{\mathrm{p_R C_N k_R}}{\mathrm{dm_R} + \mu} - (\mathrm{d_R} + \mu)[R]$$

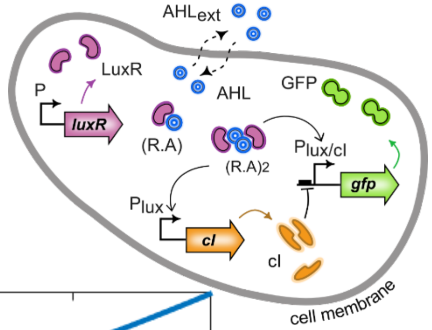
$$\frac{d[cI]}{dt} = \frac{\mathrm{p_{cI} C_N k_{cI}}}{\mathrm{dm_{cI}} + \mu}\left(\alpha + (1-\alpha)\,\frac{\frac{1}{\mathrm{k_{dlux}}}\left(\frac{[R][A]}{\mathrm{k_{d2} C_N}}\right)^2}{1 + \frac{1}{\mathrm{k_{dlux}}}\left(\frac{[R][A]}{\mathrm{k_{d2} C_N}}\right)^2}\right) - (\mathrm{d_{cI}} + \mu)[cI]$$

$$\frac{d[GFP]}{dt} = \frac{\mathrm{p_G C_N k_G}}{\mathrm{dm_G} + \mu}\left(\alpha + (1-\alpha)\,\frac{\frac{1}{\mathrm{k_{dlux}}}\left(\frac{[R][A]}{\mathrm{k_{d2} C_N}}\right)^2}{1 + \frac{1}{\mathrm{k_{dlux}}}\left(\frac{[R][A]}{\mathrm{k_{d2} C_N}}\right)^2}\,\frac{1}{1 + \frac{[cI]^2}{\mathrm{k_{dcI} C_N}}}\right) - (\mathrm{d_G} + \mu)[G]$$

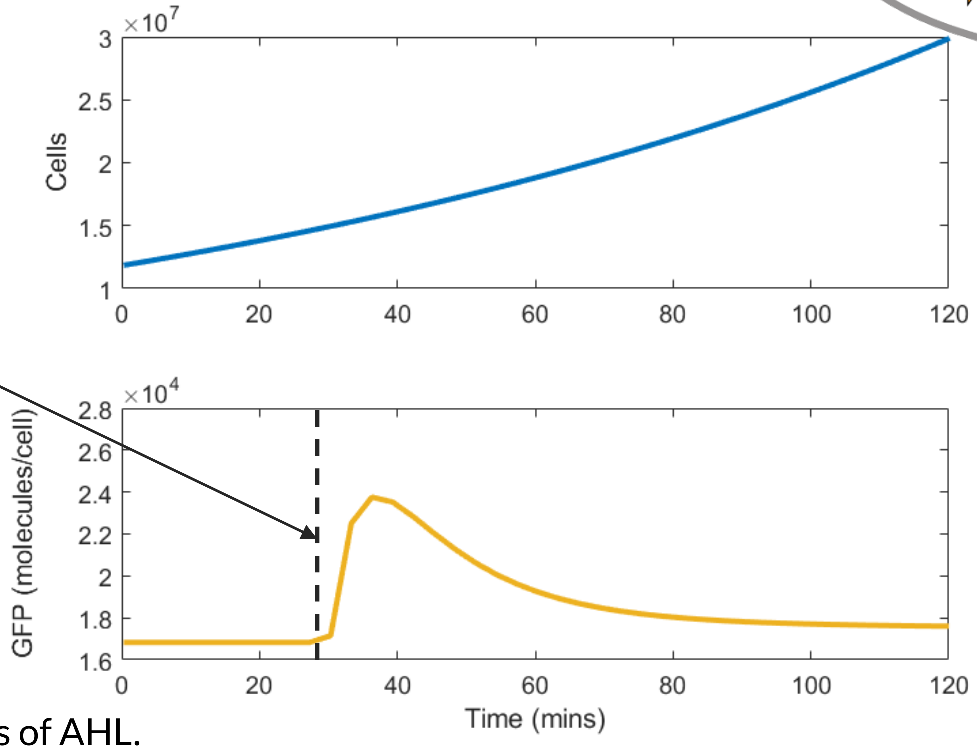
$$\frac{dN}{dt} = \mu N\left(1 - \frac{N}{\mathrm{N_{max}}}\right)$$

Y. Boada, G. Reynoso-Meza, J. Picó, and A. Vignoni. Multi-objective optimization framework to obtain model-based guidelines for tuning biological synthetic devices: an adaptive network case. BMC Syst Biol, 10(1):27, 2016b.

Model of the I1-FFL gene circuit



Simulation of a construct

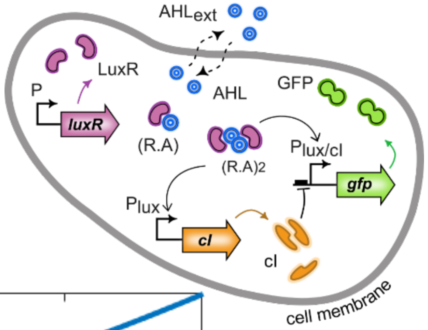


Different induction levels

But only one peak!

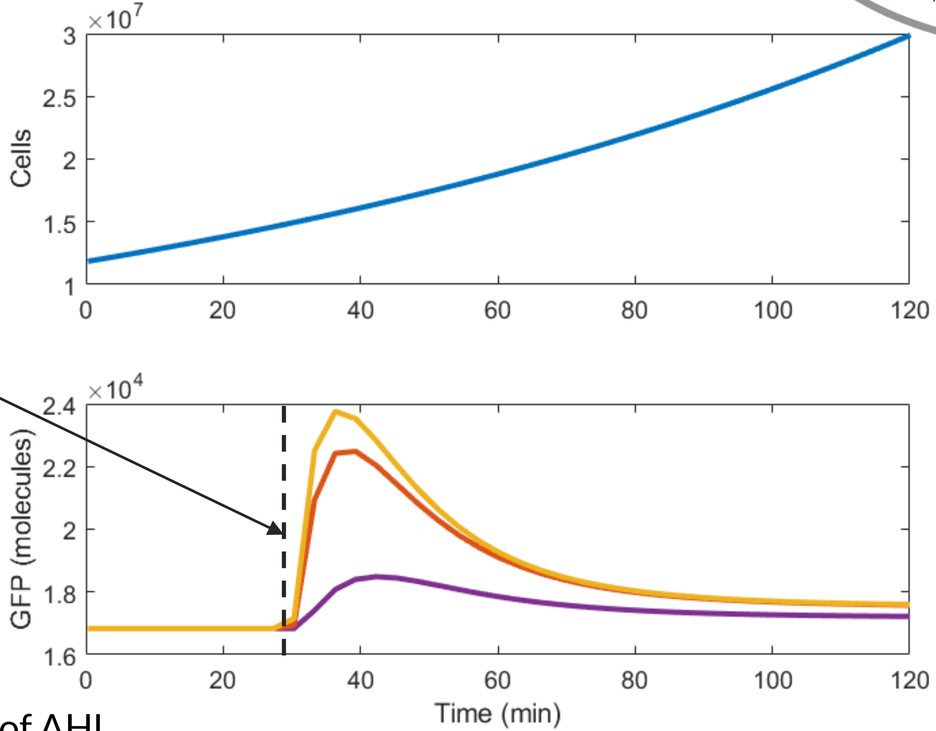
The system responds,
but is insensitive to the different levels of AHL.

Model of the I1-FFL gene circuit



Simulation of another construct

Increasing the C_N of the Hybrid promoter (to increase the Kd)



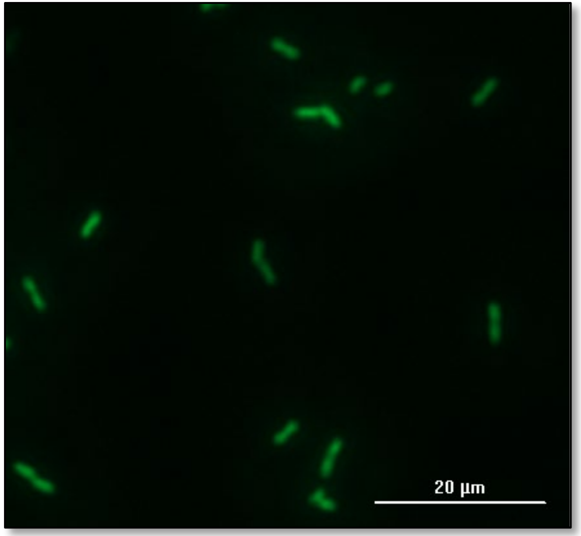
Different induction levels

Different peaks maxima!

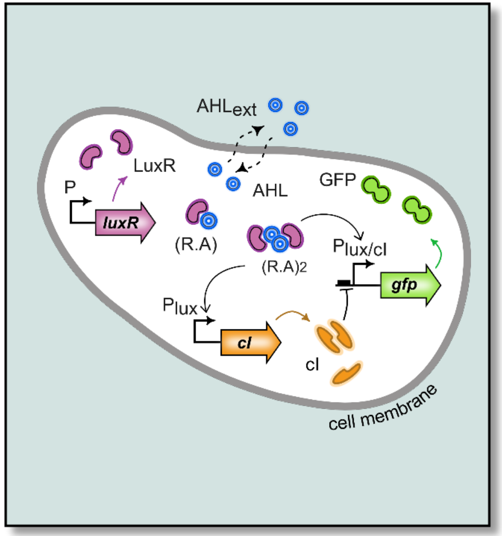
Now the system responds and changes the peak maximum with different levels of AHL.

In vivo implementation of one version of I1-FFL circuit

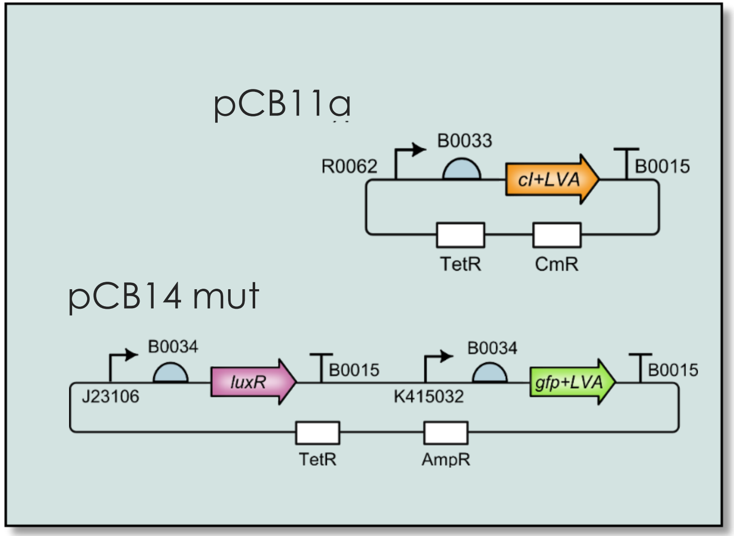
E. coli bacteria



GFP protein after AHL_{ext} induction.



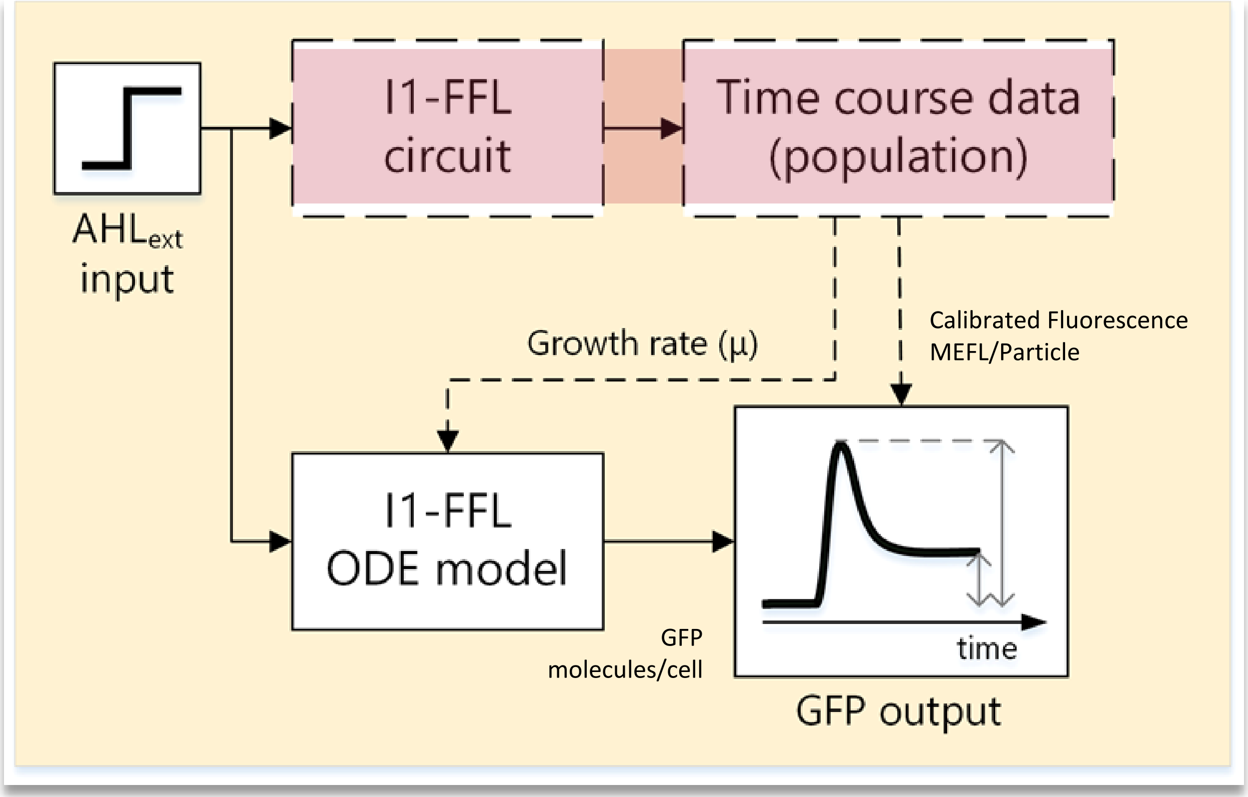
I1-FFL circuit with its biochemical reactions.



DNA sequences of the three gene circuits *cI*, *luxR* and *gfp*.

Source: Image x20, SB2CL Lab UPV 2019.

Model parameter estimation of the I1-FFL circuit



Cost function of the I1-FFL circuit

5 experimental scenarios

Mean squared error (MSE)

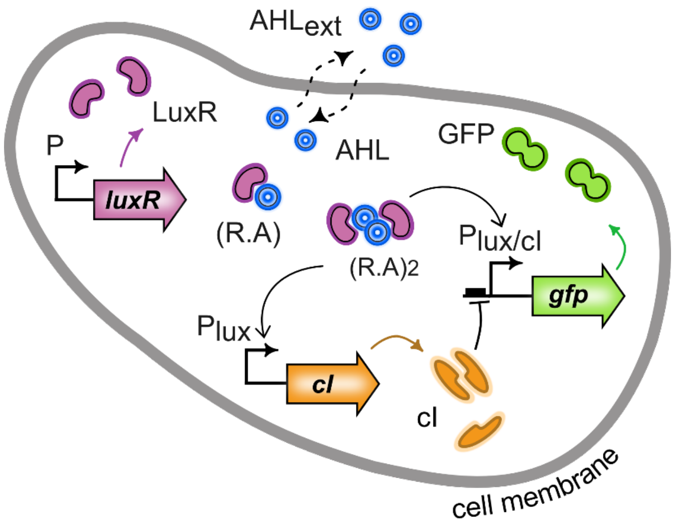
$$J_{[i=1,\ldots,5]}(\theta) = \frac{1}{n}\sum_{q=1}^{n}\frac{1}{m}\sum_{k=1}^{m}\left(x^{m}_{10_{iq}}(k) - x_{10_{iq}}(kT)\right)^2$$

$$\min_{\theta\in\Re^{17}} J(\theta) = [J_1(\theta), \ldots, J_5(\theta)] \in \Re^5$$

subject to: I1-FFL model (5.1)

17 decision variables $\boldsymbol{\theta} \in \mathbb{R}^{17}$

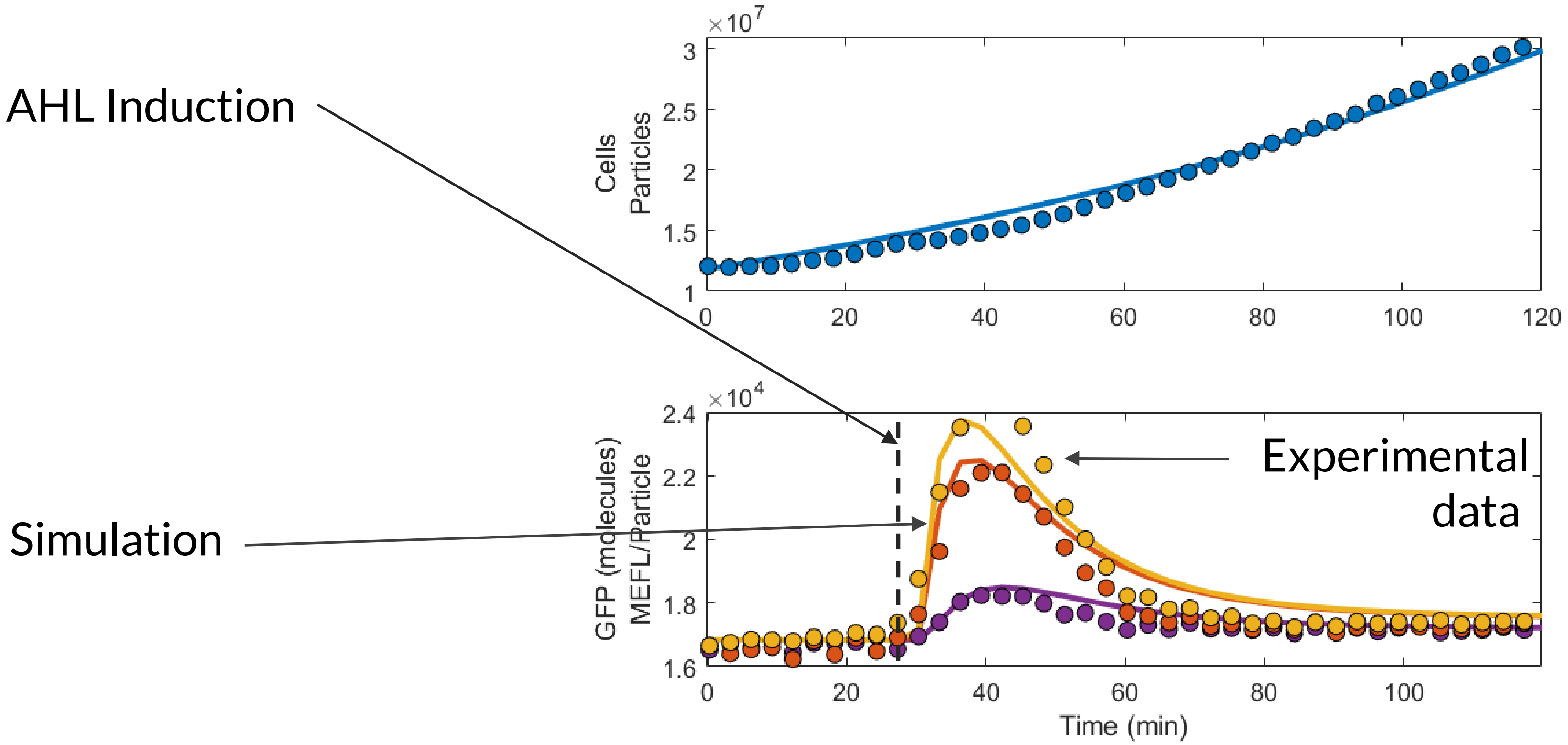
Unknown Parameter	Description	Range of values
$d_{\text{cI}}, d_{\text{GFP}}$	cI, GFP degradation rate	[0.01 0.3] min^{-1}
γ_1	pLux Promoter Hill constant	[50 100] nM
γ_3	Hybrid pLuxR/cI promoter coefficient	[0.0001 0.5]
γ_4	Hybrid pLuxR/cI promoter coefficient	[0.0005 5]
γ_5	Hybrid pLuxR/cI promoter coefficient	[1 100]
$k_{p_{\text{cI}}}, k_{p_{\text{gfp}}}$	cI, GFP translation rate	[1 60], [1 100] min^{-1}
β_1	Hybrid promoter basal expression	[0 0.01]
β_2	Hybrid promoter leakiness	[0 0.01]
$k_{m_{\text{cI}}}, k_{m_{\text{gfp}}}$	cI, gfp transcription rate	[0.1 75], [0.1 25] min^{-1}
k_{-2}, k_{-3}	Monomer and dimer dissociation rate	[0.05 0.3], [0.1 1] min^{-1}
k_2, k_3	Monomer and dimer association rate	[0.0006 0.06] min^{-1}
k_{mat}	GFP maturation time	[20 120] min



Parameter estimation based on MOOD

spMODE algorithm (http://matlabcentral/fileexchange/39215)

Comparison between model and data for the I1-FFL circuit
Parameter estimation



Questions?
Ask writing in the chat or contact me by email (alvig2 [at] upv [dot] es)

Scripts and files in the Git Repository

https://github.com/iGEM-Measurement-Tools/Modeling-Tutorials