Valencia Team

iGEM 2007

The E.coliRuler

Calibrating promoters with a biological comparator

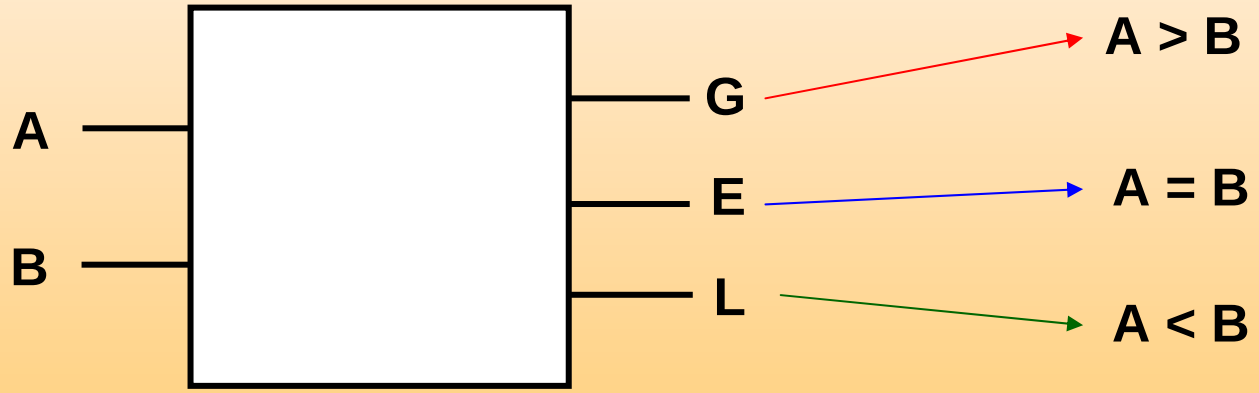
- Introduction
- Comparator
- E.coliRuler
  - in silico work
  - lab work
- Further applications
- Conclusions

The idea

- Electronic comparator
  - small, useful device
- Biological comparator
  - few genes
  - highly modular
- E.coliRuler: Promoter calibrator

What is the Comparator?

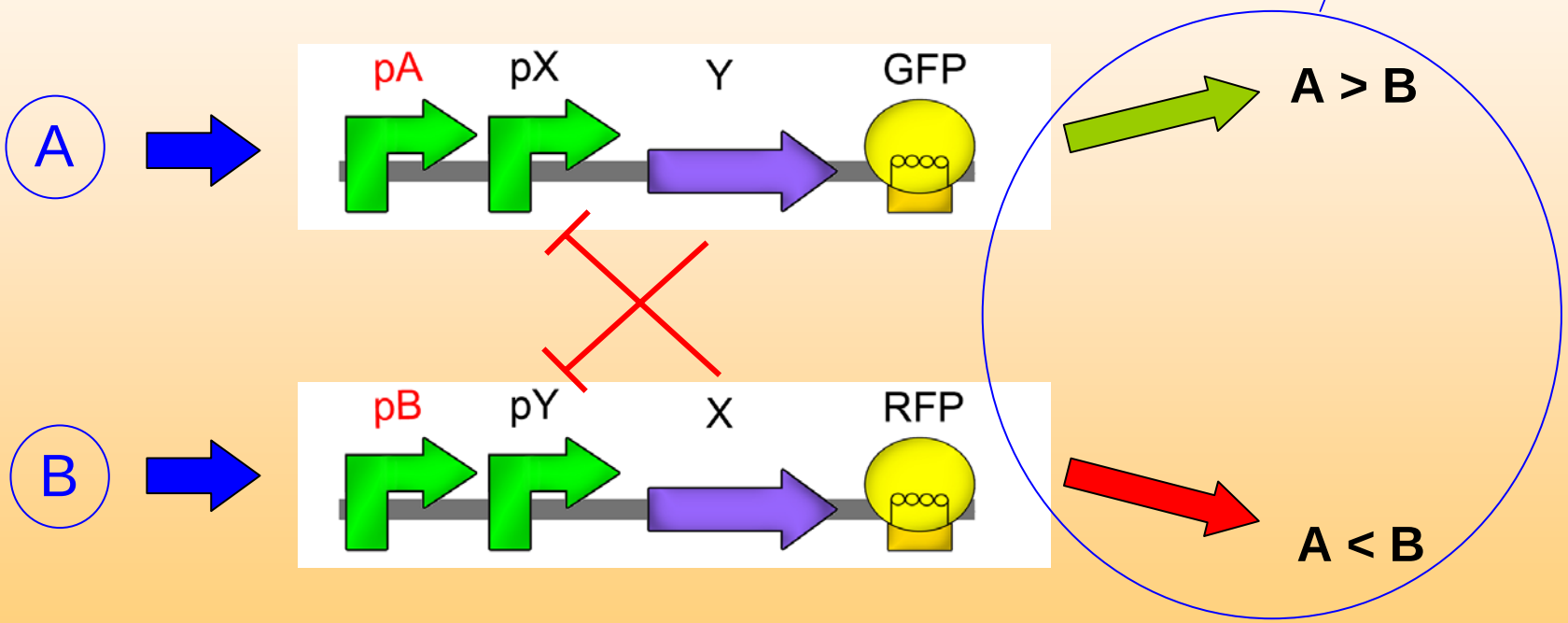
Its electronic counterpart:



What is the Comparator?

In biological language:

Let’s go prove it with a simple simulation



Compares two inputs and amplifies the bigger one.

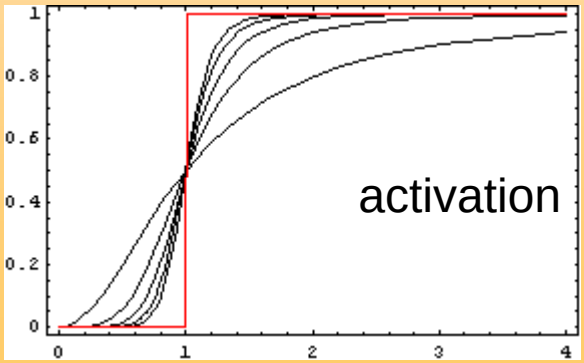
Outputs can be fluorescence proteins or another kind of proteins.

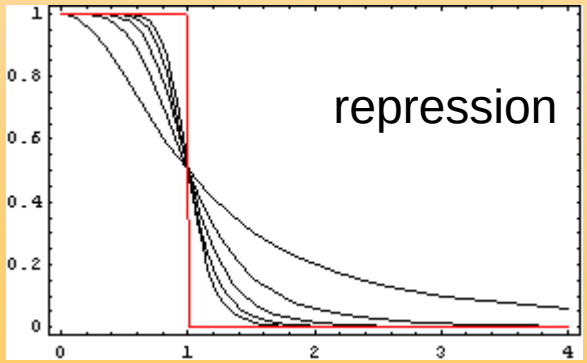
Comparator

The effective model:

$$\frac{dx}{dt} = \alpha_x \frac{(A/k_A)^{n_A}}{1+(A/k_A)^{n_A}} \cdot \frac{1}{1+(y/k_y)^{n_y}} - \beta_x x + \gamma_x$$

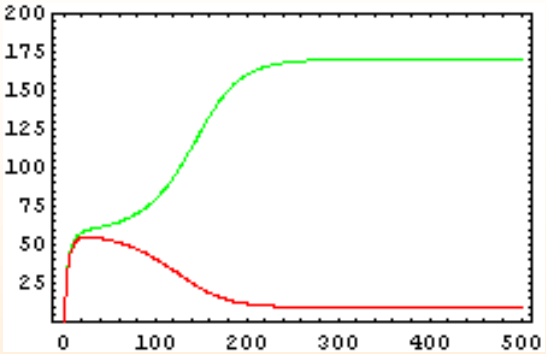
$$\frac{dy}{dt} = \alpha_y \frac{(B/k_B)^{n_B}}{1+(B/k_B)^{n_B}} \cdot \frac{1}{1+(x/k_x)^{n_x}} - \beta_y y + \gamma_y$$





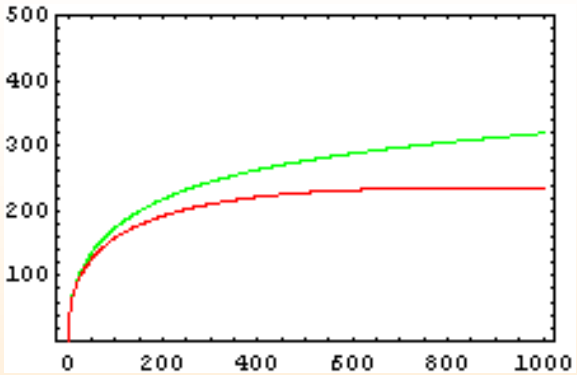
Comparator

alpha



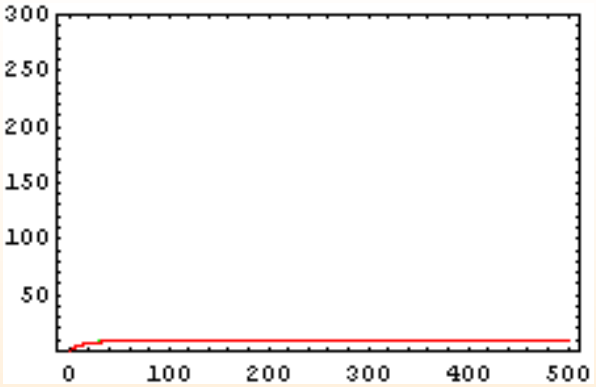
transcription rate

beta



decay rate

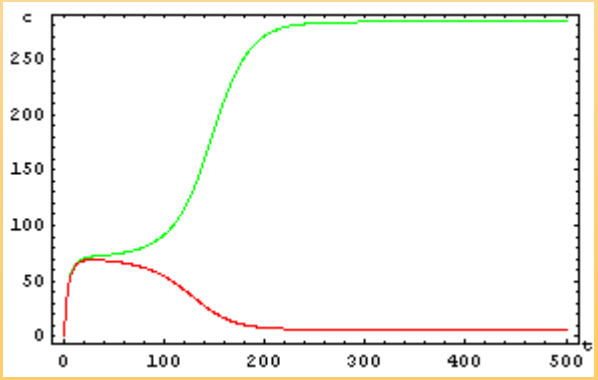
gamma



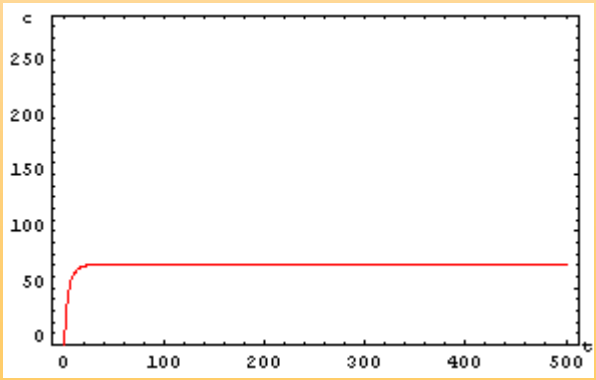
basal state

Simulations with typical part parameters:

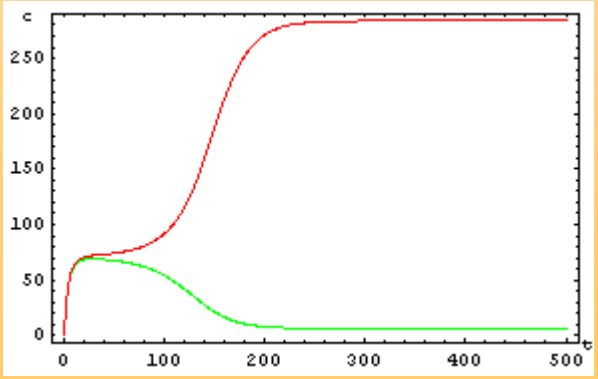
A > B



A = B

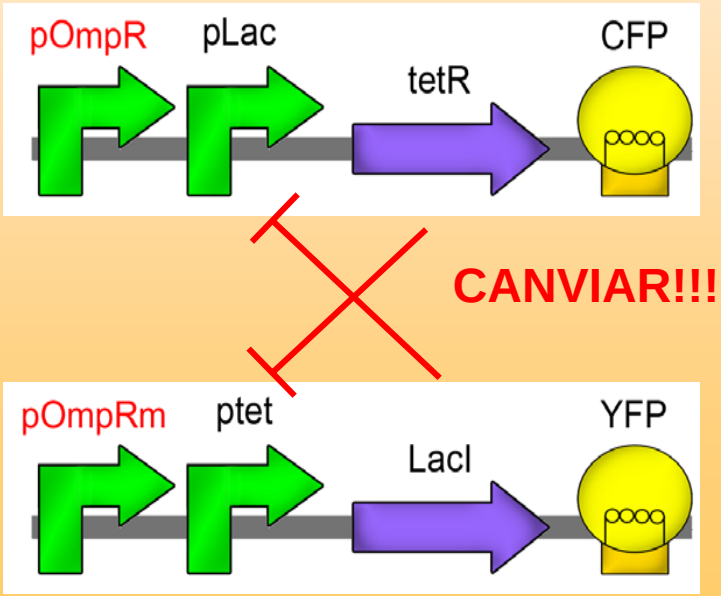


A < B



E.coliRuler

- Calibrating promoters with a biological comparator.
- Can be used to:
  - Compare **relative strenghts of diffetent promoters**



pOmpR vs. pOmpRm

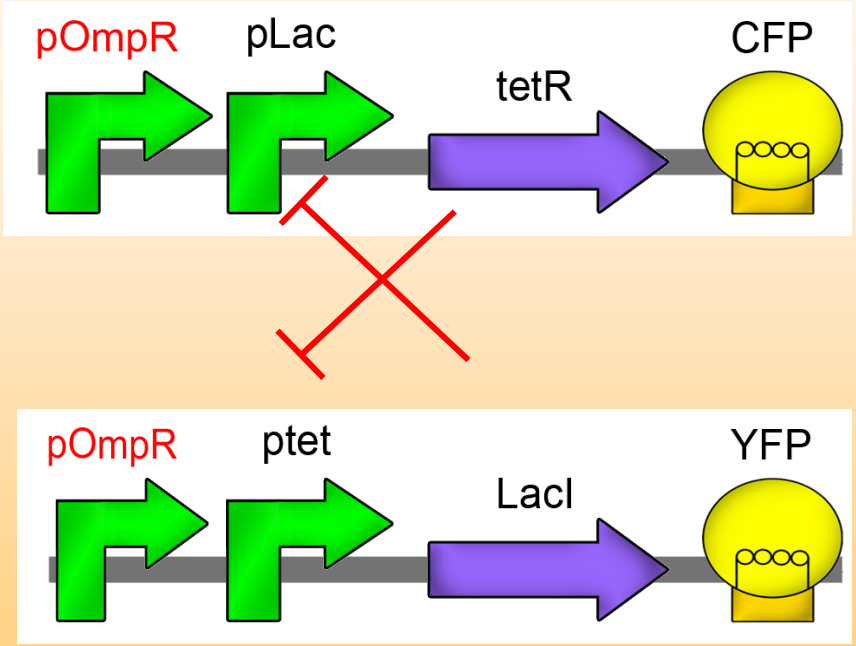
- pOmpR:
  - Promoter of the gene for OmpC in *E.coli*
  - 3 binding sites
- pOmpRm:
  - only 1 binding site
- Regulated by osmolarity
  - EnvZ-OmpR regulatory system
- pOmpR is stronger than pOmpRm

EXPERIMENTAL PROCESS

Two types of experiments:

1) Promoters A and B

pOmpR



EXPERIMENTAL PROCESS

Two types of experiments:

1) Promoters A and B

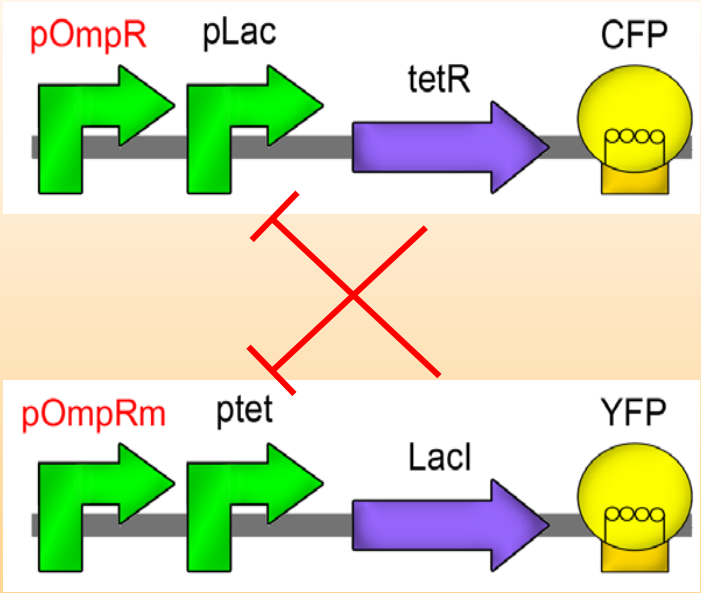
pOmpR

2) Promoter A

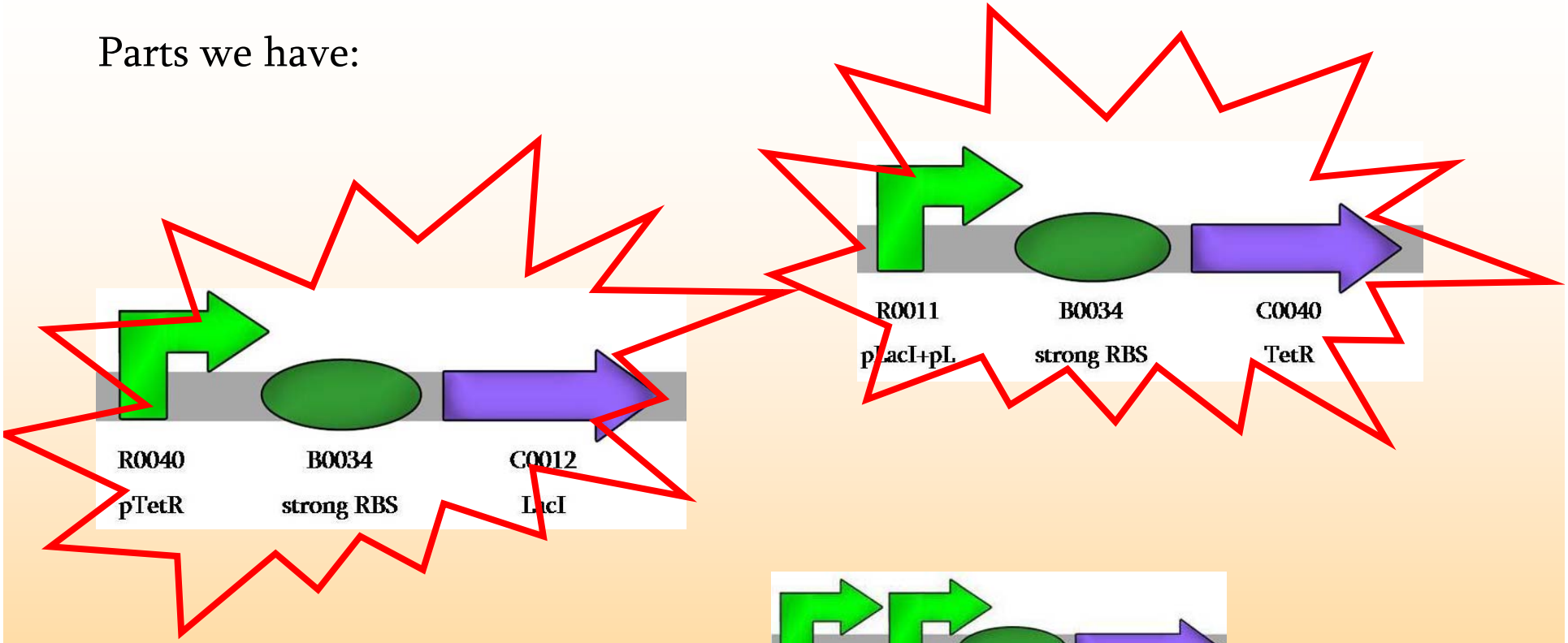
pOmpR

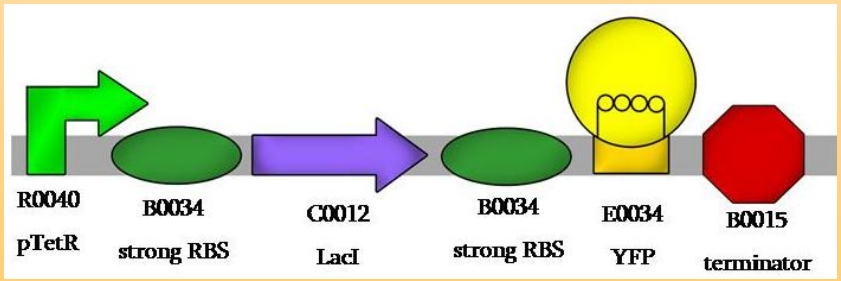
Promoter B

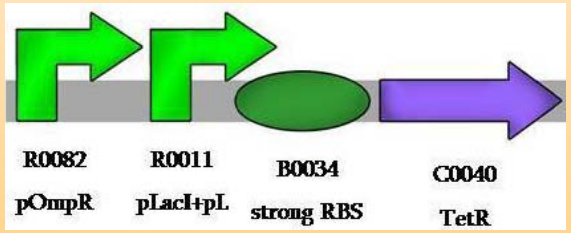
pOmpRm

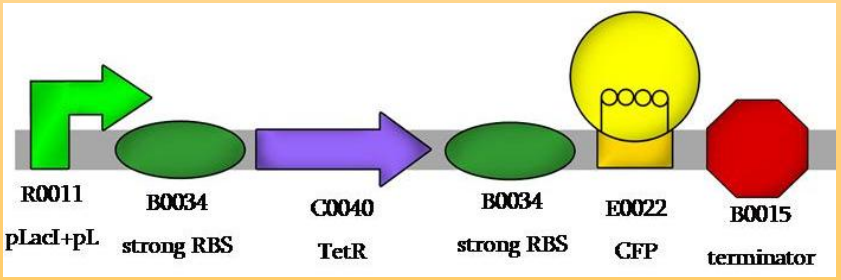


Parts we have:









Future Work

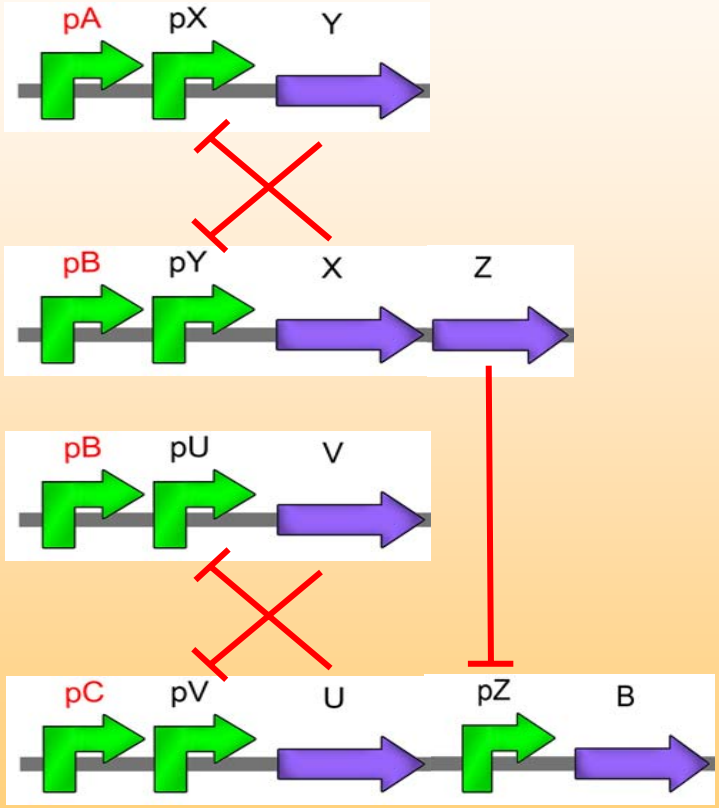
- complete the constructs –next week
- FACS experiments
  - different osmolarity at steady state
  - dinamical measurements at a given osmolarity
- Estimate the specific parameters of our system

Further applications

- Controller
- Filters
- Discrete level detector: *happy bacteria*

Controller

upper threshold



lower threshold

A controller is a device which maintains a protein value given two thresholds

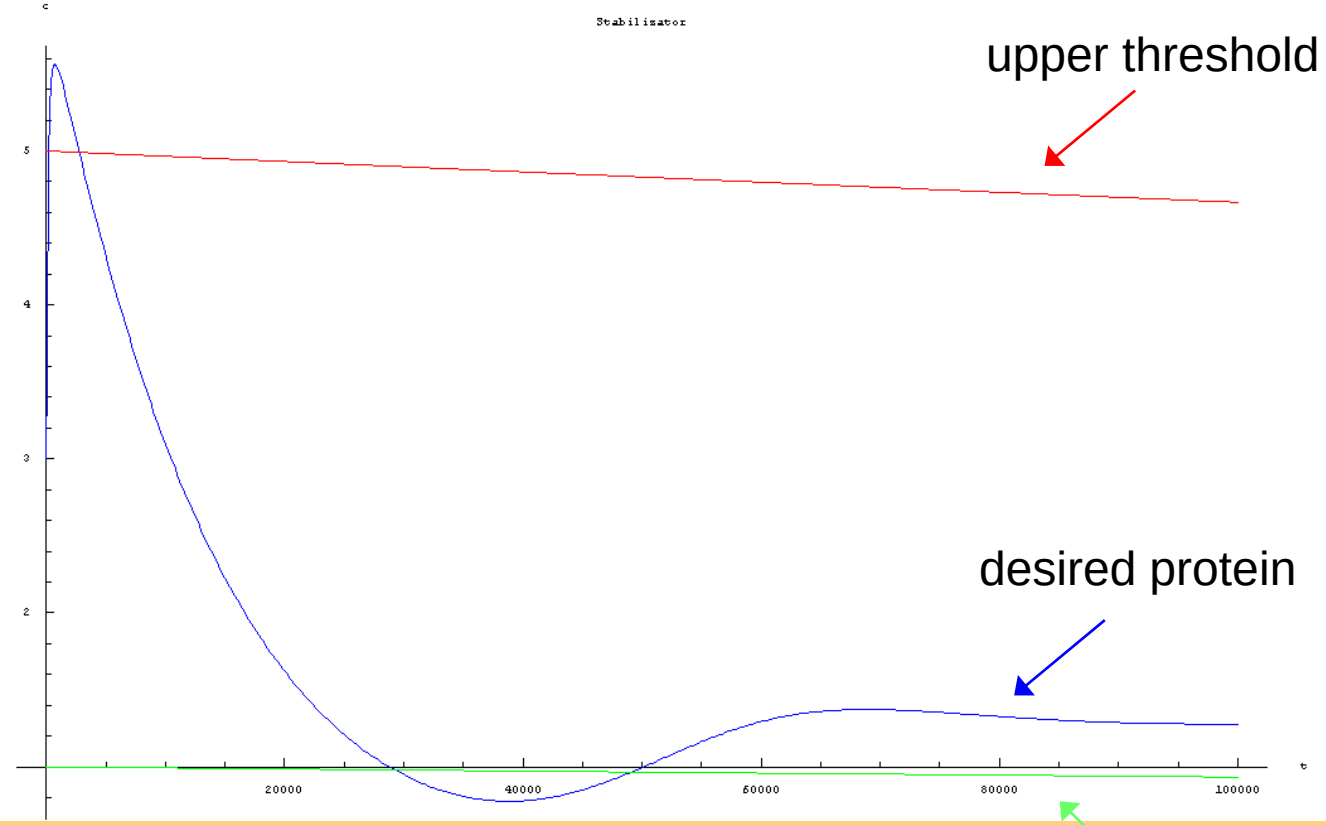
↓

mathematical modelling

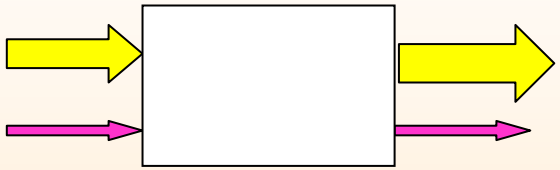
↓

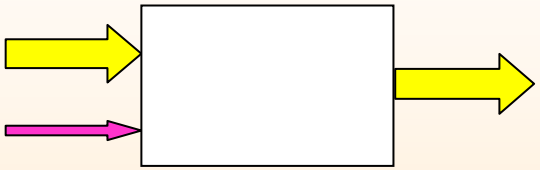
Let’s go simulating…

And here it is!



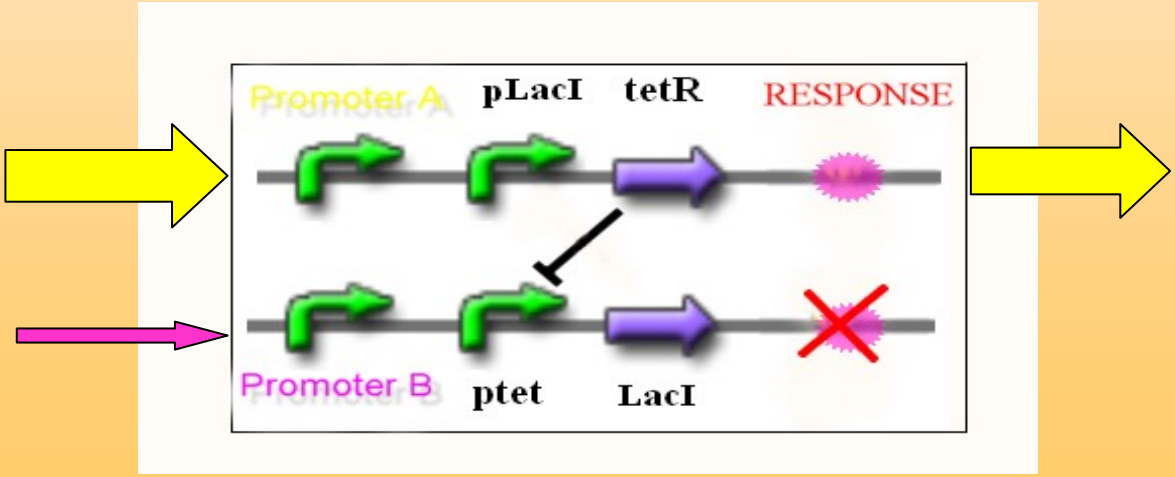
Filter



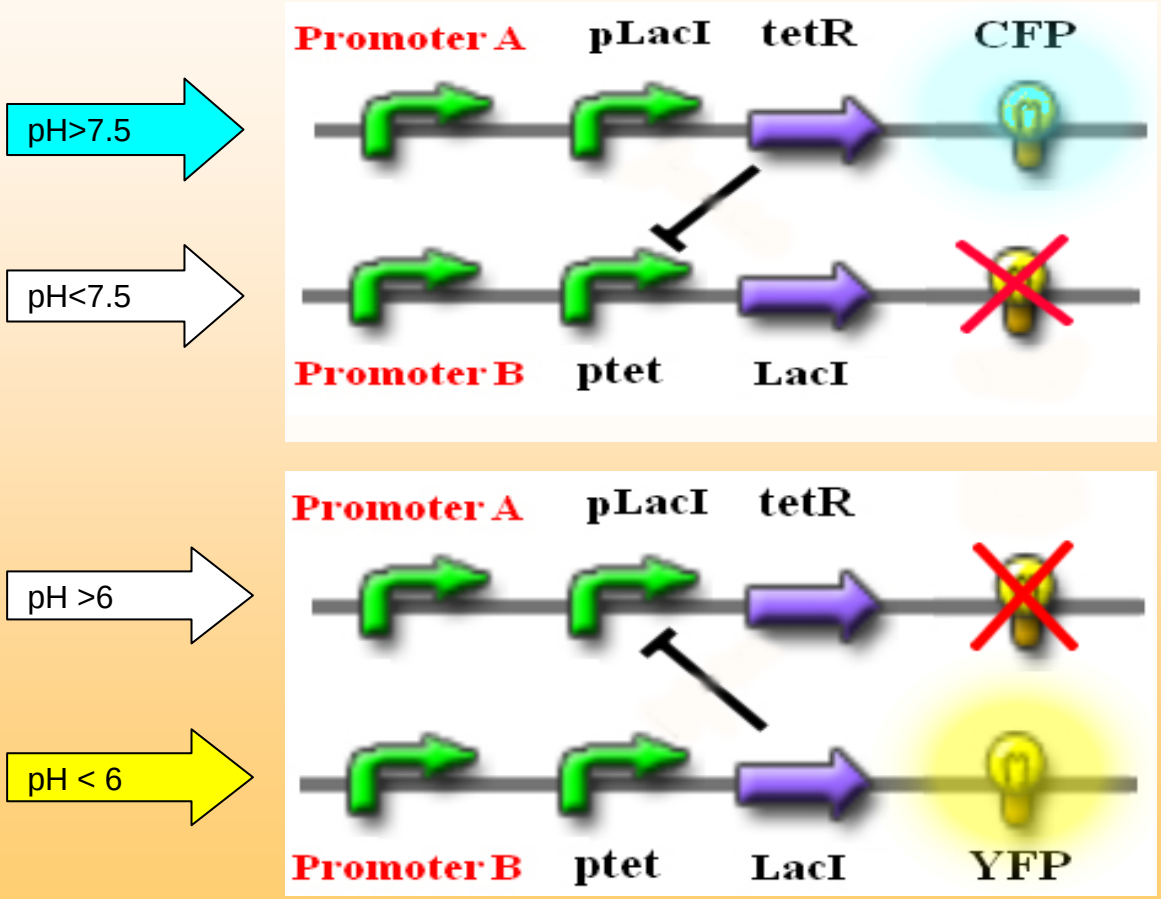


Normal Cell Response

Filter



Discrete level detector: *happy bacteria*



Conclusions

- Biological comparator: simple, robust, useful
  - implemented *in silico*
  - *in vivo* built and sequenced
  - we have tested two applications, but your imagination is the limit
- E.coliRuler: promoter calibrator
  - developed *in silico*
  - 75% implemented *in vivo*
- Controller:
  - elaborated *in silico*… soon *in vivo*

Our team:

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The *E*.nd