The BacteriO'Clock

By Paris iGEM Team 2008

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Abstract

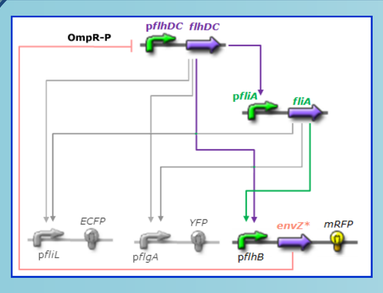
The **BacteriO'clock** is a simple test tube containing modified bacteria that gives you the time, directly from living organisms, the hours of the day being color-coded, and oscillations ensuring the repeated periodic behavior. An efficient encoding of the hours of the day is ensured by a **First-In/First-Out (FIFO)** expression of fluorescent genes.

The FIFO behavior is used by nature to optimally build its sofisticated machines (*e.g.* flagella) as well as commonly by engineers**.** We based ours on a special **Feed-Forward Loop (FFL)** network motif where output genes are under the combinatorial control of two genes, FlhDC and FliA, the second being activated by the first.
Achieving a synthetic FIFO and controlling its temporal parameters will provide a chassis for optimising the *in vivo* assembly of any genetic machine from its BateriO'clock expressed parts.

Oscillations can in principle be obtained by **adding a single negative feedback loop**. Yet our modeling work and a very recent publication (Stricker, 2008) suggest that this can be achieved only if delays are implemented within the network **to avoid an equilibrium state to be reached**. Our models suggest that such a delay could be achieved by using the well-known **quorum-sensing system**. Additionally, this provides us an elegant **synchronization mechanisms** for our oscillations.

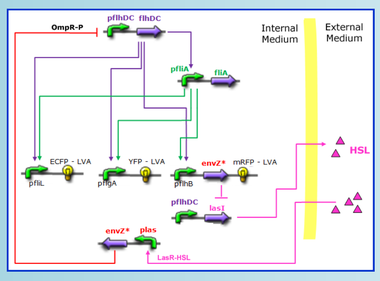
Here we present how we have
- **build models from experimentally-measured data** and obtained informative predictions that have helped us to improve our initial design
- **constructed the complete system and obtained preliminary experimental data** showing that it should work as predicted
- set up an integrated workflow for the **experimental characterization of promoter activities** and **system's modeling** based on a bottom-up approach

Design of the Genetic Network

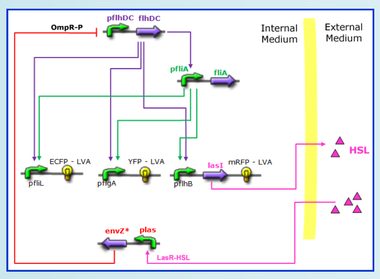


The first system we design consists in a **FIFO organization of three genes**, to which we add a negative feedback. Besides, the **FFL motif** underlies this construction.

Simulations shows that **it does not oscillate** !



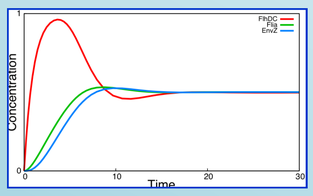
To improve our initial system we **coupled it** with **a well known HSL mediated oscillator** using a modular approach. This design just shows **damped oscillations**.



Finally, we rewire the coupled oscillator system to produce a **single loop oscillator** based on the same genes but a different topology.

This system shows **sustained oscillations** and allows synchronization by construction!

Models and Simulations



Using the parameters we obtained **from biological experiments**, the system reaches **a steady state**, following the equations :

$$\frac{d[FliA]}{dt} = -\gamma_{FliA} \times [FliA] + \beta_{FliA} \times [FlhDC] + \beta'_{FliA} \times [FliA]$$

$$\frac{d[CFP]}{dt} = -\gamma_{CFP} \times [CFP] + \beta_{CFP} \times [FlhDC] + \beta'_{CFP} \times [FliA]$$

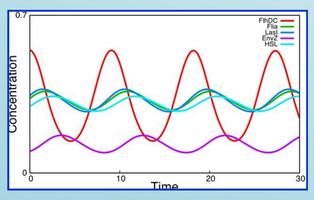
$$\frac{d[YFP]}{dt} = -\gamma_{YFP} \times [YFP] + \beta_{YFP} \times [FlhDC] + \beta'_{YFP} \times [FliA]$$

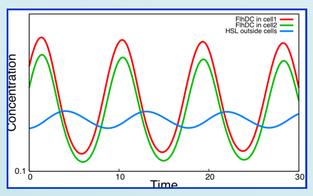
$$\frac{d[EnvZ-RFP]}{dt} = -\gamma_{EnvZ-RFP} \times [EnvZ-RFP] + \beta'_{EnvZ-RFP} \times [FlhDC] + \beta_{EnvZ-RFP} \times [FliA]$$

$$\frac{d[flhDC]}{dt} = -\gamma_{FlhDC} \times [FlhDC] + \beta_{FlhDC} \times \frac{\theta_{EnvZ}^{n_{EnvZ}}}{\theta_{EnvZ}^{n_{EnvZ}} + [EnvZ]^{n_{EnvZ}}}$$

By modifying specific interaction strength we only obtained **damped oscilations**.

Sustained oscillations are only obtained when we included an increased delay obtained via quorum sensing synchronization





Population modeling allows us to conclude that synchronization and oscillations cannot be dissociated.

Let's test this hypothesis experimentally!

Besides, we use an **Akaike Criteria** to decide if a model can be used, given a limited number of experiments to estimate the unknown parameters.

Prospects

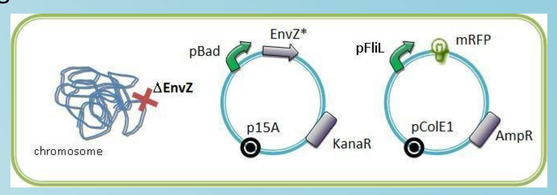
We have found two potential applications of our FIFO system.

Control of injectisome construction: The injectisome is a protein complex that can inject proteins across eukaryotic cell membranes. Because of the similarity of this structure with the flagellum, we could use our FIFO system to control the injectisome assembly.

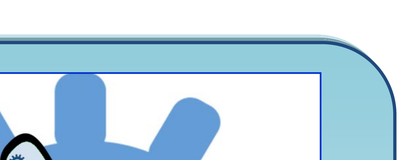
Optimisation of biosynthesis pathways: For each pathway such that (1) intermediate products are used in alternatives competing metabolic pathways and (2) only the final product is of interest, we have shown that a FIFO expression of the pathway genes would improve the global throughput. This is for example the case of the polyhydroxyalkanoate biosynthesis pathway, an environmentally friendly plastic material.

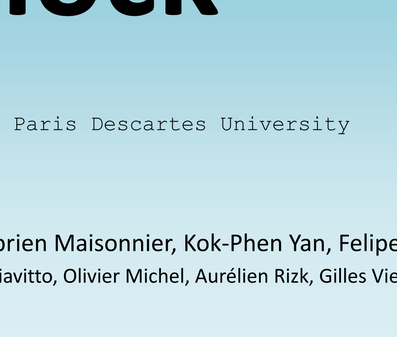
Constructions and Results

The following constructions allow the characterization of our FIFO system.



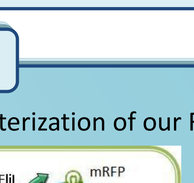
This **motility assay** shows that the pBAD-envZ construct is working (also it is leaky). When expressed, **EnvZ represses the flagella expression** (and motility).

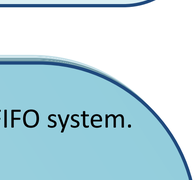




EnvZ (expressed from the pBAD) naturally represses flhDC through the activation of OmpR. FlhDC is the master regulator of the feed forward loop leading to the expression of the class 2 genes (fliL, flgA, flhB...). We measure here their activity. Awaiting more quantitative results on the FIFO behaviour, here are already the **"on" and "off" states**.

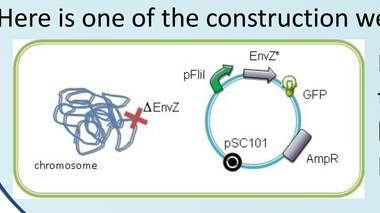
The **expression of pFliL and pFlgA** was measure through fluroscence microscopy under the expression of envZ (0.2% arabinose) or it's repression (1% glucose). As expected (Kalir, 2004), the mean level of expression of **pflgA is lower than that of pfliL**.





Off step of pFliL. The strain was grown with glucose and plated on arabinose. Its fluorescence was then followed by timelapse microscope.

Here is one of the construction we made in order to follow the oscillations:



Experiments are in progress... We still hope that the modelers were wrong ! Nevertheless, we already started constructs for the HSL-mediated oscillator.

Virtual Lab by Characterizations

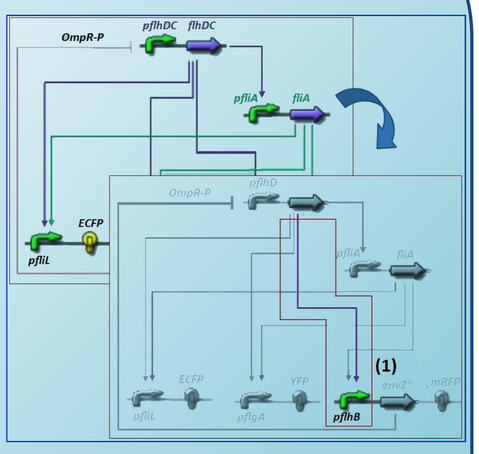
We outline here an idea of *Systematic Characterization Plan*, attempting to reach the dream of all modelisators : a *modular* and *predictive* model of the System.

(1) Decomposition of the final system in small and independant modules, treated as «Black Boxes» : outputs= *functions* of inputs

(2) Prediction of given *Analytic Expressions* for these *functions* by the *Bio-Mathematical Model*

$$(FlhDC)_{n_4} \bowtie pFlgB]_{eq} = \frac{[FlhDC]^{n_4}}{K_4^{n_4} + [FlhDC]^{n_4}} \cdot [pFlgB^{total}]$$

$$\frac{d[FP3]}{dt} = \beta_{30} \cdot \frac{[FlhDC]^{n_5}}{K_5^{n_5} + [FlhDC]^{n_5}} \quad \textbf{(2)}$$

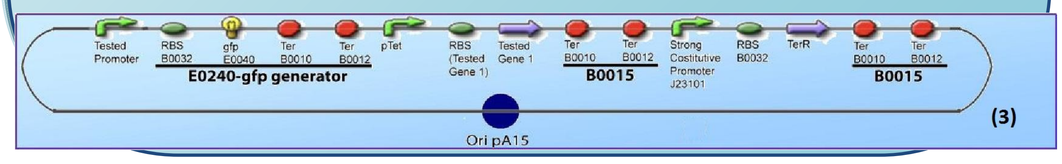


(3) Conception of *Specific Constructs* and *Experiments*

(4) Treatment of Experimental Results by *Optimisation Algorithms*

```
function opt_param=find_FP(X_data, Y_data, init_param)
% search for the fittest parameters involved in f
% X_data = [flhDC]i ; Y_data = measured values of f
  function output = act_pflhB(param, X_data)
    for k = 1:length(X_data)
      output(k)=param(1)*hill(X_data(k),param(2),param(3));
    end
  end
opt_param = lsqcurvefit( @(param, X_data) act_pflhB
    (parameters, X_data),init_param, X_data, Y_data ); (4)
```

Since most of the *Characterizations* consist in measuring promoter activities as a function of their regulators we developed a generic, BioBrick-compatible plasmid.
To improve predictability of the resulting model, these constructs are used in our experimental conditions… but could be adapted to the iGEM standard measurement conditions.



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