Bacteriophage-based synthetic biology

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Abstract

The biology is complex and there is a sort of organisms that interacts worldwide. In that big system, there are the viruses, organisms that are not “alive” while they are out of the host organism. When viruses enter inside the host cell, immediately start to use their own molecular machinery in combination with the host one, in order to produce the molecules that are encoded in their genes. The first interactions between the host (inner medium, cell cycle state, etc.), and the viruses affects to behaviour will develop the virus. The behaviours are: produce copies of the viruses (lytic state) or be inserted to the host genome and be silenced until dangers for the virus became (lysogenic state). The aim of this project is to develop new applications that allow controlling λ-phage virus (bacteriophage) in several scenarios. The control was reached with synthetic genetic construct that interacts with the wild type genome of the virus in order to control the transition between the lysogenic and lytic states. This study was a computational approach where we use the simple model from Hasty [1] that describes CI-CRO genetic circuit, responsible of the virus bistability, and we add in this system the parts that describes our synthetic circuits. Using this approaches we have finally obtained circuits that make the bacteria immune to the λ-phage virus infection, lyses the cells using an external effector and finally a population control of infected cells via coupling Quorum sensing [2] with the regulation of the viral genome. For all these systems we have agent-based simulations using Netlogo. These simulations and the simplicity of the synthetic circuits give us good perspectives in order to be implemented in the wet-lab.

Keywords: Synthetic Biology, λ-phage virus, CI-CRO bistability, Quorum Sensing, Netlogo.
Introduction

Since Louis Pasteur explained the existence of microbiology, there had been many trials to avoid their undesired effects (like the pasteurization to eliminate bacteria in food). Afterwards, studies demonstrated that humans need some of this microbial to survive (as the ones that are part of our microbiome). Finally, in the last years, we have learned to modify them for our proposals. We have bacteria that are able to produce drugs, other are used to produce beer, and even we use them to clean environmental disasters as oil leaks. But, even though the progress that we have made, there are a lot of problems in the biomedical field that still need a new approach to be solved. Some examples of these problems are: low efficacy of bacterial transformation in laboratories, the lack of control of synthetic populations that have finished the job for which they were designed, population control in biological reactors, control of viral infections and control of the wild bacterial populations. All the possible current solutions available have too many undesired side-effects. Hence, we need new approaches in order to solve some of these problems.

One of this new approaches could be to take advantage of what the nature by the evolution have already produced. The idea is to use these natural mechanisms and give to them a new application. For instance, now we can use epidemics to make the host immune to other infection. According to this, we hypothesise that combining one carrier that is “accepted” by the environment of the problem and one engineered virus that is able to infect, we can produce desired changes in the wild type system. The property that is interesting in order to use viruses is that they can develop a chain reaction. This mean that a few viruses in the starting point can produce a huge amount of infected cells, a sort of pandemics in that environment.

The science that does modifications in organisms in order to add new functions to organisms is the Synthetic Biology. This science use the knowledge about the genetic circuits that have been obtained by the systems biology to design and implement new synthetic genetic circuits that have the properties that are desired for the application [3].

In this study, we make a proof of principles in the simplest and realistic condition: the carrier is *Escherichia Coli* and the virus is the *Bacteriophage λ*. Engineering these two organisms we achieve one theoretical new approach that can be applied to solve multiple actual problems and improve procedures that are currently done in lowest efficient way. This is done by using synthetic gene circuits that combines parts of the genome of bacteriophage λ and regulatory elements in order to be expressed in the right moment.
I. Escherichia Coli

Escherichia Coli (E.coli) is one of the most used bacteria in the research wet-labs. These bacteria are present in the warm-organism gut where are in symbiosis with the host being part of the bacterial flora. These organisms are able to live outside the host and depending on the strain can be poisonous.

The biological characteristics of E.coli are:

- The chromosome has around the 4 \(10^6\) bp.
- At least, it is have one plasmid (but is able to carry multiple).
- Its optimal growth temperature is at 37ºC
- It is able to live in many substrates because of the multiple metabolic pathways that have programmed in its genome.

II. Bacteriophage Lambda

The bacteriophage lambda is a model and fundamental virus in research. It is said, in the last lines of the discussion, “Despite the changing fashions in research, the vicissitudes of NIH grant funding, and the changing cast of characters studying them, these phages have been a remarkably consistent and productive source of new biological insight that remains an important experimental model system in the vanguard of biological science. This seems to us unlikely to change any time soon” [4]. With that affirmation they remark, not only the importance that these have had, but also that these viruses will be important for the future research. This virus has become an interesting organism because it has short but very well regulated genome.

\(\lambda\)-phage genome

The genome of this bacteriophage is 48.49 kb of double stranded lineal DNA, that have sticky ends in order to be circularized inside the host cell. This circularization confuses the “immune system of the cell” due to the similarity to the plasmids. This genome, after the infection, is inserted in the E.coli chromosome [5]. The phage genome can be segmented in four regions [6]:

1. Lysogenic-lysis decision system.
2. Early left operon (proteins that are needed for the insertion in to the host chromosome).
3. Early right operon (replication machinery and promoters for the lytic genes).

4. Late operon (there are the capsid proteins and the ones needed to evoke the lysis).

Depending on the interactions that occurs in the first region the other regions will be activated or repress. This first region is a toggle-switch that has two fix points, the lytic state and the lysogenic state. This toggle-switch is known as CI-CRO bistable switch [7] because this are two molecules have mutual inhibition, if one is in high concentrations, the other is in low concentrations.

![Bacteriophage lambda genome map](from [6]). Labelled genome with colorized proteins from the capsid.

**Regulatory architecture**

The complexity of the regulatory system of the λ-phage genome comes from the different regulatory layers. There are molecules that compete for binding in regulatory parts of promoters. This binding will produce a folding of DNA that can repress or activate a set of genes. Also there are promoters that are activated by a heterodimer (CII-CIII). Moreover, in another layer of regulation, there are the antiterminators (Q and N) that acts like switches. If the molecule is present, the next proteins in the messenger RNA will be expressed. And finally, there is the

![Schema λ-phage genetic regulatory system](Arrows are the six promoters that are regulated and important in the regulation of the phage behaviour (Pi, PL, Prm, Pr, Pre and Pr'). The squares are the codified genes that play a role (CIII, N, Cl, CRO, CII, O|P and Q). Also there are specified the regulatory places where there are binding of proteins in blue the regions for CII+CIII, in red the ones for CRO2 and CII2 (OR and OL). And with circles where there are the terminators regulated by antiterminators (Q and N).)

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5
antiCRO-RNA that is the complementary RNA of CRO’s RNA. It is able to bind to the mRNA$_{CRO}$ and then produce the dsRNA that will be degraded.

The genetic architecture of the λ-phage has almost all kinds of promoters. There are promoters that are activated for dimers (like the Pi and Pr). Some others are repressed by dimers (as PL, Prm and Pr that are repressed if there are binding on OL1, OR3 and OR1 respectively). And also there is the Pr’ that is a constitutive promoter what it means that it is always active.

All this complicated regulatory system, in early stages of the research, they conclude that could be explained by the bistability between CI and CRO. Because at the end of the decision process, they are completely determinant: high concentrations of CRO leads to lysis of the cell, and high of CI means silencing.

Table 1: Promoters of λ-phage genetic regulatory system

<table>
<thead>
<tr>
<th>Promoter</th>
<th>Promote</th>
<th>Activated</th>
<th>Repressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pi</td>
<td>DNA integrases</td>
<td>Dimer CII + CIII</td>
<td>-</td>
</tr>
<tr>
<td>PL</td>
<td>Antiterminator N and (if there is N) CIII</td>
<td>CRO in low concentrations</td>
<td>CI and CRO in high concentrations</td>
</tr>
<tr>
<td>Prm</td>
<td>CI</td>
<td>CI in low and medium concentrations</td>
<td>CRO and CI in high concentrations</td>
</tr>
<tr>
<td>Pr</td>
<td>CRO, CII, Q, P and (if there is N) Q</td>
<td>CRO in low concentrations</td>
<td>CI and CRO in high concentrations</td>
</tr>
<tr>
<td>Pre</td>
<td>(if there is N) anti-CRO (RNA) and CI</td>
<td>Dimer CII + CIII</td>
<td>-</td>
</tr>
<tr>
<td>Pr’</td>
<td>Lytic and viral proteins (if there is Q)</td>
<td>Continuously</td>
<td>-</td>
</tr>
</tbody>
</table>

CI-CRO bistable switch

The balance between CI and CRO determines which behavioural state of the λ-phage infection (lysogenic or lytic state) is the system in each moment.

Both proteins have very similar behaviours; they are autoinduced when the concentration is low and autorepressed when it is high. This means, if you isolate these two systems both will reach a concentration of protein and it will remain constant in time (homeostasis) with some alterations due to noise because is a biological system. This is the fix point of the isolated systems.
The CI or CRO isolated systems can be described mathematically as:

\[
\frac{d(\text{Protein})}{dt} = \text{Nonregulated production} + \text{Regulated production} - \text{Dimerization} + \text{Unbinding} - \text{Protein degradation}
\]

\[
\frac{d(\text{Dimerized protein})}{dt} = \text{Dimerization} - \text{Unbinding}
\]

- The nonregulated production is named leakage. This is due to the control of the promoter have some issues that make it unperfected. Mathematically, it is described by a constant \((a_0)\) because this production is independent of the conditions.
- The dimerization and the unbinding of the proteins have constants that give us the relation between the production and the existing proteins.

\[
\text{Dimerization} = K_{\text{dimerization}} \cdot \text{Protein}^2
\]

\[
\text{Unbinding} = K_{\text{unbinding}} \cdot \text{Dimerized Protein}
\]

- The degradation of the proteins uses to be a constant ratio. This means, the degraded proteins are a fraction \((\delta)\) of the whole protein number. In the case of CI, this degradation can be increased depending on the state of the cell. In order to add this tuneability, there is a new parameter \((\phi)\). This is dependent on the UV radiation, the oxidation, the need of nutrients and all the factors that put the life of the virus in danger (because the protein RecA is produced by E.coli that is in danger).

\[
\text{Protein}^{\delta \cdot \phi} \rightarrow \emptyset
\]

\[
\text{Protein degradation} = \delta \cdot (1 + \phi) \cdot [\text{Protein}]
\]

- Finally, the regulated production can be modelled as the product of two Hill functions; one for auto-activation (in red) and one for auto-repression (in blue).

\[
\text{Regulated Production} = \frac{V \cdot K_{\text{dim}} \cdot \text{Protein}^2}{K^1 + K_{\text{dim}} \cdot \text{Protein}^2} \cdot \frac{V'}{K^2 + K_{\text{dim}} \cdot \text{Protein}^2}
\]

The whole model:

\[
\frac{d(\text{Protein})}{dt} = a_0 + \frac{V \cdot K_d \cdot \text{Protein}^2}{K^1 + K_d \cdot \text{Protein}^2} \cdot \frac{V'}{K^2 + K_d \cdot \text{Protein}^2} - K_d \cdot \text{Protein} \cdot \text{Protein} + K_d \cdot \text{Protein} \cdot \text{Protein} - \delta \cdot (1 + \phi) \cdot \text{Protein}
\]

\[
\frac{d(\text{Dimerized protein})}{dt} = K_d \cdot \text{Protein} \cdot \text{Protein} - K_d \cdot \text{Protein}^2
\]
However, CI and CRO are not isolated in the non-modified organisms. In the reality, they have independent promoters that are overlapping with three regulatory regions. They can either repress or activate depending on which molecule is binded in each of the three positions. This dual effect is possible because each protein induce a different folding of the DNA. Both molecules can be binded in three positions (OR1, OR2, and O3) but have a higher affinity for the positions that are closer to the other promoter [8]. This means that the affinity for CRO is OR3>OR2>OR1 [9] and for CI in the other way around (fig.4). But also means that these promoters can be saturated for either an overexpression of CI or CRO. In fact, this means they can exhibit either mutual-inhibition, auto-activation or auto-inhibition. The first one occurs in low concentrations, the second one in medium concentrations and finally, the third one in high concentrations.

This wild-type toggle-switch was widely studied, and nowadays is the most commune example of toggle-switch for the gene circuit designers [1]. In 2001, Jeff Hasty published the simplest model possible that is able to describe the CI-CRO bistability. The model is a non-dimensional system of two ordinary differential equations.

\[
\begin{align*}
\dot{x} &= m_x \frac{(1+x^2+\alpha \sigma_1 x^4)}{Q(x,y)} - y x \\
\dot{y} &= m_y \rho_y \frac{(1+y^2)}{Q(x,y)} - y y \\
Q(x,y) &= 1 + x^2 + \sigma_1 x^4 + \sigma_2 x^6 + y^2 + (\beta_1 + \beta_2) y^4 + (\beta_1 \beta_3) y^6 + \\
&+ \sigma_1 \beta_4 x^4 y^2 + \beta_5 x^2 y^2
\end{align*}
\]

This model take into account the auto-activation of CI (x) when it is bind in OR1 and OR2, and auto repression when the three positions are occupied. Also it take into account the positive feedback that CRO (y) have when a dimer of it binds to the OR2.

Using the parameters given in the article, which are optimized in order to have a similar behaviour to the real one, the system exhibits bistability (stable point + unstable fix-point + stable point). One fix point is high CRO and negligible CI (lytic state), and the other point, high CI and zero CRO (lysogenic state).

In a previous article [10], they use the reactions for CRO production as an example of the role that the noise have in biological systems. They show that this system can ends in both fix-points.

**Diagram 3: Graphical representation of CI-CRO bistable switch.** The diagram shows the process of: Transcription, Translation, maturation and multimerization of the functional proteins. Furthermore, the binding affinities are represented by the wind of the arrows.
due to the stochastic processes at the first steps of infection. This is the regular behaviour of the bacteriophage $\lambda$ infection in wet-lab experiments.

In deterministic simulations, when there is an infection, the final state is the lytic state. This makes that simulations with that model in a virtual Petri-dish ends with the extermination of the whole population.

This system was tested also in agent-based simulations with Netlogo using stochastic properties for the reactions [11]. In that study, they found four final states of the culture even thought all have the same initial conditions. The states are:

1. Failure of the infection.
2. Death of the population.
3. Homogeneous lysogenic population.
4. Heterogeneous lysogenic and non-infected populations.

These results fits better the real situations that occurs in the experiments with this two organisms (viruses and bacteria).

III. Quorum Sensing

The Quorum Sensing (QS) is the genetic circuit that regulates the expression levels of some genes depending on the concentration of its population [2]. This is one of the communication systems that are present between cells [12] and it is very important for bacteria to produce biofilms (most effective collective defence mechanism of bacterial populations). Depending on the species and strains, they can produce different communicative proteins; this add complexity to the communicative process. For example, the bacteria produce different transmission molecules and modulate the growth rates depending on the state of the biofilm [13]. This adaptive system gives to the colony a better situation in order to resist external aggressions [14]. In medicine, these biofilms are a huge problem. One of the newest strategies to attack them is disrupting this bacterial communication system with inhibitors [15].

The Quorum Sensing system consist in a constant production of one little molecule that will be diffused in the medium (effector), and one sensor molecule that is active with the binding of the effector. The effector is produced via an auto-inducer synthase ($\text{LuxI}$). This effector molecule makes able the cells to know the concentration of bacteria in the colony. More concentration of
effector implies more concentration of cells. This molecule also enters into the bacteria and raises the internal concentration of that molecule. The other part of the QS system are one kind of proteins (LuxR family) that have binding domains for the effector. They act as internal receptors of the cell, for this reason they are being produced continuously (via a constitutive promoter). If there are more molecules of effector, there is more probability to form complexes with internal receptor. These complexes can be repressors or activators of some genes, even the same complex can be activator of some promoters and repressor of others.

This system is very extended over the bacteria populations, where each one uses their own receptors and effectors. For example, different vibrios have different number of receptors and diffusive molecule in order to detect combinations of factors. The numbers on WT species go from 1 molecule in the system of *Vibrio vulnificus*, to 3 systems in *Vibrio anguillarum*. The concentration of these molecules will lead to, usually, mortality or modification in the growth velocity [16].

This communicative system is being used in a lot of applications as: population control [17], pattern formation [18] and synchronization of synthetic oscillators [19, 20].

In order to model the Quorum sensing system we have used the next equations [18].

\[
\frac{d\text{Complex}}{dt} = \rho_{\text{Complex}}[\text{LuxR}]^2 \text{Effector}^2 - \gamma_{\text{Complex}} \text{Complex}
\]

\[
\frac{d\text{Protein}}{dt} = \frac{\alpha \text{Complex}^\eta}{\theta_{\text{complex}}^\eta + \text{Complex}^\eta} - \gamma_{\text{Protein}} \text{Protein}
\]

In this equations, the parameter Effector is the internal is referent to concentration of the effector. In that case the effector molecule is a Lactone (AHL).

Here we have two ordinary differential equations that are modelling the two variable that are in this system. One is the complex Lactone, or effector, and LuxR. And the other is the expression of the protein that must be produced when there is a high concentration of cells in the culture. For this reason, the second equation has an activator Hill function and a lineal protein degradation. If the circuit repress the production of the protein when there is a high concentration of bacteria, the equation for the protein would be:

\[
\frac{d\text{Protein}}{dt} = \frac{\alpha}{\theta_{\text{complex}}^\eta + \text{Complex}^\eta} - \gamma_{\text{Protein}} \text{Protein}
\]

This repressive equation is an example of an inhibitory Hill function where depends on the concentration of cells in the culture.
Methodology

I. Stability Analysis (xppaut)

In order to be able to use the model described by Hasty [1] we have first replicate the dynamical analysis. And we compare our results (fig. 5 A) with the provided in the article (fig.5 B). The analysis was done using the xppaut software, where we visualize the nullclines and the flow around the three fix-points in order to be sure of the stability of each point (fig. 5 C, D and E).

![Figure 5: Stability analysis of the simple model [1] with xppaut.](image)

(A) Nullclines of the ODEs CI-CRO model (plotted with the xppauto). (B) Nullclines from the original article [18]. (C) First stable fix point, this is the one that corresponds to lytic phase (high CRO and low CI). (D) Unstable fix point. (E) Second stable fix point, the correspondent to lysogenic phase (low CRO and high CI).
Ones we had replicated the results from the CI-CRO model’s article, we tested how variations of some parameters modifies the trajectory of the nullclines. Specifically, we have test the increase of the degradation parameter of CI, because this is what happens when RecA is activated.

This procedure was also used to test and optimize the rates of constant production of CI, and eventually CRO, for the synthetic circuits that we have designed during the project.

II. ODEs integration (iPython notebook)

In order to test the model over time (integrated) and also test parameters, we have used iPython notebook. For instance, we have used it to see the behaviour of the population in the early stages of infection.

Figure 6: Nullclines analysis in a range of values for CI degradation. The orange nullclines decrease his maximum high as the degradation parameter increase. Then the bifurcation occurs and only survives the fix-point from lytic phase.

Figure 7: Temporal evolution of concentrations in log-log scale. (A) This is a simulation of the model of Hasty with the addition of the equations that model the variation of the antiterminator Q. This give high levels of CRO and Q, Lytic phase. (B) The model has a tinny expression of CI constitutively that starts with the infection.
Another application was to test if the position (in the viral genome or in the bacteria) where the synthetic circuit of Cl production is located is important for its behaviour. In other words, if the circuit must be in the cell before the infection or can be spreaded with the viruses.

![Steady state for different values of $K_0$ + Infection (green)](image)

**Figure 8:** Temporal evolution comparison of the levels of Cl with constant production in the cell or being part of the $\lambda$-phage genome. The coloured lines are the trajectories of logarithmic levels of Cl when the circuit is in the cell before the infection. In black the trajectories if the production starts with the infection. In green the infection period.

Furthermore we tested transient situations. For example, we put a pulse of lactone (emulating an increase of the population) when the bacteria already have the quorum sensing system coupled to the production of the antiterminator Q.

![Behaviour of the system with $K_0 = 0.01$ and a pulse of $AHL = 0.1$](image)

**Figure 9:** Time course of Cl, CRO and Q, with a pulse of Lactone. The quorum sensing system is linked to the expression of Q, when there is a pulse of AHL, the concentrations of Cl and CRO doesn’t change but Q increase. Because of the $\lambda$-phage genome this increase of Q would produce the lysis of the cell.
III. Agent-based simulations (Netlogo)

The agent-based simulations are the key point of this work. This kind of simulations give us the opportunity to take into account the 2D space and randomize processes as the virion movement. These simulations are useful because they give the opportunity to use single agents (“turtles” in Netlogo language) and the environment that surrounds them (“patches”).

In our case, we have two kinds of turtles: the cells and the viruses. Viruses are simple, only have two processes: they are produced in side a cell when it dies (lyses), and they have a random movement when are in the environment. This movement is similar to the diffusion process. Statistically, the viruses will go to places (patches) where there are less of them. The cells have a label that gives the information about which state is in each moment. This states are non-infected, infected, lysogenic, lysis or death (in the last application there are modified too). Moreover, in this cells there are all the molecules that are in the model, LuxR, AHL, CI, CRO and Q. In order to integrate over time, the equations that govern the trajectories of the molecules of the system are integrated with the Euler method.

\[
P_{t+1} = P_t + \Delta t \frac{dP}{dt}
\]

Image 3: Netlogo designed interface. This is a final interface where there are all the created buttons during the research. Moreover, in this picture we can see the plots, monitors, and the visual simulation window. This is a good visual example of the all possibilities that Netlogo have in order to do simulations.

Diagram 4: Simulated agents. The colors are the same as the used in the Netlogo simulations.
In the patches, we have the concentration of AHL. In these elements there is the diffusion of the Lactone that is necessary in order to have quorum sensing. With this finality, we have used the function 8-neighbours that is used to ask to the other neighbours to do something such as sum AHL concentration of the patches. The colour of the patches can be used to show the concentration of AHL or viruses there, or both in the same time using RGB colour where we normalize AHL levels for red colour and the number of viruses in the blue colour.

The interaction between turtles and patches is crucial. When we model the growth in a Petri dish (supl.3.2) because the cell can’t divide if it is crowded; in case of division must end in the free place near to the original one. This is a typical example of what can’t be done in conventional simulations due to the dimensions and the boundary interactions. Netlogo make us able to test experiments in cell culture (2D-Petri dish) and culture in liquid phase (3D), cells can overlap, using the same file (the code).

With Netlogo, we have obtained information of the cell death under different conditions and stored in a matrix (several trials). This give us the possibility to take a look when all the simulations have been finished and make statistical analysis.

Figure 10: Mortality depending on constitutive production of CI. There is a sharp change between $10^{-3}$ and $10^{-2.5}$. Even though here there are 10 trials plotted there is no variation in the results. (Ploted with Microsoft Excel)
Results

I. Whole $\lambda$-phage model

There is not published models that have as many species and regulatory elements as the ones that are described in the literature of the bacteriophage $\lambda$ genome. This is because is very difficult to take realistic parameters of a big set ODEs. For this reason, we have done a complex model with the same number of promoters and proteins that are really involved in the fate decision of the phage. This model was trained in order to be dimension–free and follow the rules that are described in the literature.

- **Promoters:**

  \[
  P_i = \alpha_i \frac{\text{CHI-CII}}{K_i + \text{CHI-CII}} \quad Pr_e = \alpha_r \frac{\text{CHI-CII}}{K_{re} + \text{CHI-CII}} \quad Pr' = \alpha_r
  \]

  \[
  Pr = \alpha_r \frac{\text{CII} + \text{CRO}^2}{K_i^2 + \text{CII} + \text{CRO}^2 + K_i^2 + \text{CII} + \text{CRO}^2 + K_i^2 + \text{CII} + \text{CRO}^2}
  \]

  \[
  Prm = \alpha_{rm} \frac{\text{CII} + \text{CRO}^2 + \text{CII} + \text{CRO}^2 + \text{CII} + \text{CRO}^2 + \text{CII} + \text{CRO}^2}{K_i^2 + \text{CII} + \text{CRO}^2 + K_i^2 + \text{CII} + \text{CRO}^2 + K_i^2 + \text{CII} + \text{CRO}^2}
  \]

- **RNA:**

  \[
  m\text{CI} = Prm + \frac{N}{K_N + N} Pr_e - \delta_m \text{CI} \quad m\text{CRO} = Pr - \delta_m \text{CRO} m\text{CRO} - \delta_m \text{mantiCRO}
  \]

  \[
  \text{mantiCRO} = \frac{N}{K_N + N} Pr_e - \delta_m \text{CRO} \text{mantiCRO} - \delta_m \text{mCRO} \text{mantiCRO}
  \]

- **Proteins:**

  \[
  \text{CI} = m\text{CI} - \delta_c \text{CI} - \delta_{\text{RecA}} \text{CI} \text{RecA} \quad \text{CRO} = m\text{CRO} - \delta_c \text{CRO} \text{CRO}
  \]

  \[
  \dot{N} = Pr \left( \frac{N}{K_N + N} \right)^2 - \delta N \dot{Q} = Pr \left( \frac{N}{K_N + N} \right)^2 - \delta \dot{Q}
  \]

  \[
  \text{CI} = Pr \frac{\text{N}}{K_N + \text{N}} - \delta_c \text{CII} \quad \text{CII} = P_i \frac{\text{N}}{K_N + \text{N}} - \delta_c \text{CII} \text{CII} \quad \text{IN} = \text{P}
  \]

  \[
  L\text{ys} = Pr' \frac{\text{Q}}{K_Q + \text{Q}} - \delta L\text{ys} \text{Lys} \quad V\text{P} = Pr' \frac{\text{Q}}{K_Q + \text{Q}}
  \]

Figure 11: Expression levels of different viral elements depending on CI and CRO Levels. That is a 3D representation of the complex model equations (in Matlab).
II. Immunity to λ-phage infection via synthetic circuit

Taking into account the model, we decided to add a constitutive production of CI (K₀) and test if this give immunity to the future infections of the bacteriophage lambda. Following the results in the nullclines analysis (fig. 12), we observe that using a promoter one hundred less active that the wild type, when it is active, it gives immunity because the system only have the lysogenic fix point.

These results were replicated by the statistical analysis of mortality vs K₀ done using data from the simulations (fig. 10).
Moreover, the results of the simulations results told us that this immunity will prevent the development of lysis process even if the CI synthetic production is silenced when there is the infection.

**III. Lysis inducible by Lactone in heterogenic culture**

The use of a constitutive promoter for LuxR in the cells makes them able to react to concentrations of lactone (AHL). This system was taken from quorum sensing [17] but now we put under the regulation of the promoter PLuxI the production of the antiterminator Q instead to the Kill protein. This makes the infected engineered cells able to produce the virus in presence of Lactone. This is because now we avoid the CI-CRO balance and producing directly the antiterminator making possible the production of the lytic machinery. This leads to the start of the lytic phase in the same moment that AHL and the virus are present in the cell. There is a loss of immunity even if there is the constitutive production of CI in the modified cells. Using a

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Figure 13: Temporal evolution of disconnected immunity. If the phage silences our circuit, the immunity can remain if before the infection we are immune. (Obtained with iPython notebook)

Diagram 6: Temporal evolution of disconnected immunity. (A) Diagram of the WT system. (B) Diagram of infected modified cells, the immune cells. These modifications are: the constant production of CI and the coupling Quorum Sensing and the viral production. The lines of AHL and LuxR symbolizes the constant production of the receptor (LuxR) and the extern pulse of lactone. (C) The equations of the B diagram. The color terms: purple the immunization via constant production of CI, and in orange the QS terms, the formation of the active complex (LuxR+AHL) and the active production of the antiterminator Q.
IV. Immune cells by constant production of CI and lysis inducible by Lactone

This system is engineered in order to have all the cells immunized with the constant production of CI. In order to make the simulations easier all these cells have constant production even before the infection** (orange). But in the culture we also put some infected cells. All the cells (infected and healthy) have the circuit that makes them able to produce antiterminator Q in presence of Lactone (AHL).

In this case, we obtain a system that maintains both populations overtime even if we induce lysis with pulses of AHL. These pulses will determine how many cells will change its state and how

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Figure 14: Time-lapse of heterogeneous bacterial culture induced with lactone at the centre. The purple cells are the modified cells and the orange the simulated WT. Here we can observe the start of lysis when modified cells sense the AHL and production of virion. These virion start to infect the WT cells. These WT cells enter in lytic phase and lysis. At the end only are alive the cells that where modified and doesn't sense the AHL induction.
many viruses will be released. Finally, this fact will affect the relative change between infected and non-infected populations.

This system is a good one to take control of how many cells you transform, because is directly linked to the inducer (AHL) injection.

**All this simulations have the same results if the synthetic circuit is started when there is the infection, using the virus as a vector.**

Diagram 7: **Genetic architecture of inducible production of viruses.** This diagram is a different view circuit of the previous diagram 8 C. The external lactone binds to LuxR and then activate the Plux promoter. This means production of viruses because of the production of antiterminator Q.

Figure 15: **Time-lapse of heterogeneous bacterial culture induced with lactone at the centre.** All cells have constant production of the Cl molecule and have inducible production of Q antiterminator by AHL. This gives to this system a very robust lysis control, but is sensible to the presence of lactone. This ends in a final state that is completely dependent on the concentration of AHL that is used to induce the population.
V. AND gate of viruses and Lactone with amplification of Lactone

In this system, we put under the control of the Quorum sensing promoter the gene LuxI, which is the necessary molecule to synthesize Lactone. This is an auto-positive-feedback loop. This system have the peculiarity that needs an induction of AHL to start, but when this system is on, there is no manner to stop it and will produce the chain reaction at the population level. Once one cell starts to produce, all the culture will produce it.

Then, the complex AHL/LuxR also activates the production of Q that in presence of infection will produce lysis. For this reason the lysis is regulated for an AND logic gate.

This system could be a control system when bacteria have to be killed when they ends their job.

Figure 16: Time-lapse from the AND gate simulation with input lactone (with amplification) and be infected. The background is the combination between the red of Lactone and the blue of number of viruses. (I) Set-up with the whole solid plate full of cells. In the red region there is an input of Lactone. On the other side there is an injection of viruses. (II) The viruses have infected a few cells and the Lactone is being amplified and diffused in the red region. (III) The Lactone is really close to the infected cells but not enough to induce the behavioural change. (IV) The most peripheral infected cells have induced the lysis. (V) The viruses are being spreading over the active cells that were producing lactone. (VI) The wave of infection is reaching the other side of the petri dish. (VII) All the lactone was degraded and there are only viruses all over the dish.
VI. Population control: Quorum sensing and $\lambda$-phage

Now, the cells are producing constantly CI, in order to be immunes; LuxI produce Lactone, to communicate their presence; and LuxR, to sense the concentration of Lactone. This whole system, make possible the autoregulation of the population. When there are too many cells, some will go to lytic phase and will die (fig. 17). This is an auto-negative feedback loop for the density of cells.

If we test it in a heterogeneous culture, mixture of WT cells and modified, we see that the modified cells start growing as fast as the WT. Then, when there are too many modified cells, some of them start to lyses and the viruses transform WT into modified cells. At the end, the system reach the equilibrium. This state have only modified cells, some in lytic and some in lysogenic phase.

![Diagram 9: Complete circuit coupling Quorum Sensing with the viral genome. Constant production of AHL and LuxR.](image)

**Figure 17:** Population over time with the quorum sensing linked to $\lambda$ system. (A) The start is a homogeneous culture of infected and engineered cells. (B) The starting point is a heterogeneous culture of infected and engineered cells and Wild Type. The viruses turn all the WT cells into infected and engineered cells. (C) The starting point is the same as B, but now the viruses give different circuit that the progenitor cells.
On the one hand, the system can also be used as a vector factory (massive production of viruses). If we engineer the viral genome that will be transmitted, this system convert all the WT in new modified cells and these ones grow transformed. In this culture, there are also a remaining population of factory cells that will remain producing vectors that ac be used for other cultures. If we take a portion of the whole population, now this is able to recover all the system.

On the other hand, this genetic system could be implemented in order to produce transformed cells with a cheap and fast procedure. Only engineer one cell that will spread their own circuit by division and the second one by viral vector.
Conclusions

The bacteriophage $\lambda$ has been exhaustively studied, since its discovery. Thanks to this research, for example, nowadays we understand the recombination and how to use specific-site recombinase. It is also a tool, because it is used as vector for bacterial transformations. We have shown that the whole regulatory system of the $\lambda$-phage is more complex than the balance between CI and CRO. Additionally, we have done a model that takes into account all the regulatory elements that this complicated system have. It was not possible to make the model quantitative because it was not possible to measure all the needed parameters for this model. Rather than use our own model, we have used one that is simpler (only considers CI-CRO toggle switch) and we have added the regulatory elements that we need to be able to tune the behaviour of the bacteriophage. This tuning can be done in a huge variety of ways. Here we have tested completely immunity, which is possible due to constitutive production of CI because it blocks the toggle switch in lysogenic phase. Also we induce lysogenic-lytic transition with an external effector, for which we have used the quorum sensing machinery but without production of lactone (because was our inducer). Furthermore, there is the auto-destruction mechanism that is powered by the amplification of the external lactone signal and few infected cells. And finally the complete auto-control of the population of the whole culture, including non-modified cells, using the complete quorum sensing system coupled to the production of viruses.

Moreover, we have implemented agent-based simulations of all these systems. We have tested that in order to achieve conditions that can be observed in the wet-lab. With these simulations we were doing mix between the deterministic equations and the stochasticity of the movement, infection rates and division processes. These give us the ability to control the fate decision and give to the simulation some freedom degrees that also have in the real experiments.

All this work is a great example of what can be done if we use the pieces that were being published over the years and we put them together.
Discussion

Bacteriophage $\lambda$ is the model organism for bacterial infections. By the study of this virus have been discovered several biological mechanisms as the DNA insertion to the genome. Additionally, this phage has been used as a vector to introduce foreign DNA to bacteria and use them for many proposes. It has a highly regulated genome that classically have been explained and modelled by the CI-CRO balance. However this genome has two antiterminators, RNA interferent, and at least four regulatory proteins that are modifying the expression of different genes. In the last review in Nature [4], they put in the centre of the diagram the CII (fig 18) as the key molecule that is on the top of the regulatory system of fate decision of $\lambda$ phage. This is a huge change in our thinking about the bistability of the Lambda phage. In the same way, in this project we have done the complete model of the system. However, that model needs a lot of test with real data due to be entrained to be quantitative.

In order to build the complex ODEs model we have performed a literature research that make us able to understand how this regulatory machinery can be tuned. The idea of use the production of antiterminator Q to change the fate from lysogenic to lytic, comes from the observation of the fact that the viral RNA is produced constantly, but only with Q can be translated. This is the key protein in the inducible circuits of lysis that we have performed.

Our circuits are very suitable to be tested in the wet laboratory, because we have obtained positive results in the simulations that are designed to be as realistic as possible. But also is important to take into account that all the pieces are commune in the synthetic biology labs. All of our circuits have direct applications in the real world. For example, bacteria that are engineered to produce some drug is needed to be as constants as possible. In order to do this, we can use the immunity construct (constitutive production of CI) to avoid the infections of bacteriophages. Moreover, we can add some of the cells that continuously produce virion to avoid the contamination of the culture with some other bacteria (VI -A).
Another application of this system could be to detect the concentrations of lactone that could be from another bacteria. Using the system of amplification (V) we can disturb the quorum sensing communication of the pathogens; for instance, in cholera infection, it would make that the vibrios population will kill themselves as a control population mechanism because they detect more population than the real one. And finally, the system that allow us to transform wild type populations into modified cells (VI-C) can be a solution for some alimentary intolerances. We can use the microbiome of the gut as a factory of enzymes that processes the intolerated substance, only putting a few modified cells.

This systems could have more applications if we find similar mechanisms in viruses that infects eukaryotes. It could be an artificial update of the immune system if we can engineer human cells that have specific viruses and they can infect a type of cells, for instance cancer cells, if they are present in the organism. Using the control population model, we can have a reservoir of this attacking cells living in the organism until it dies and only active when the nondesired cells appear.

Nowadays there are several organisms that are available for bioremediation. However this organism needs to be under control because they can affect the environment modifying its properties. For this reason the induced autodestruction and the population control are important in this field.

This synthetic symbiosis between cells and its pathogens (the viruses), could be one of the future tools that will be used in order to improve the defence of the organism.
References


I. CI-CRO model (from Hasty [1]):

In this article the variable x is CI and y is CRO.

The reactions:

\[
\begin{align*}
X + X & \rightleftharpoons X_2 \\
D + X_2 & \rightleftharpoons D_1^X \\
D_1^X + X_2 & \rightleftharpoons D_2^X D_1^X \\
D_1^Y + D_2^Y + X_2 & \rightleftharpoons D_3^Y D_1^X \\
D_1^X + Y_2 & \rightleftharpoons D_1^X D_3^Y \\
D_1^X + Y_2 & \rightleftharpoons D_1^X D_3^Y \\
D_1^X + Y_2 & \rightleftharpoons D_1^X D_3^Y \\
D_1^X + Y_2 & \rightleftharpoons D_1^X D_3^Y
\end{align*}
\]

The equations:

\[
\begin{align*}
\dot{x} &= \frac{m_X(1 + x^2 + \alpha \sigma_1 x^4)}{Q(x,y)} - \gamma_X x \\
\dot{y} &= \frac{m_Y \rho_y(1 + y^2)}{Q(x,y)} - \gamma_Y y
\end{align*}
\]

Where:

\[
Q(x,y) = 1 + x^2 + \sigma_1 x^4 + \sigma_2 x^6 + y^2 + (\beta_1 + \beta_2) y^4 + (\beta_1 \beta_3) y^6 + \\
+ \sigma_1 \beta_4 x^4 y^2 + \beta_5 x^2 y^2
\]

And the used parameters:

\[
\begin{align*}
\gamma_x &= 0.004 & \text{Degradation rate of CI} \\
\gamma_y &= 0.008 & \text{Degradation rate of CRO} \\
\rho_y &= 62.92 & \text{Differential production of CRO respect to CI} \\
\alpha &= 11 & \text{CI production increase when CI is binded to OR1+OR2.} \\
m_x = m_y &= 1 & \text{Translation rate from RNA.} \\
\sigma_1 &= 2 & \text{Affinity of binding of 4 CI to the OR1+OR2} \\
\sigma_2 &= 0.08 & \text{Affinity of binding CI to OR3} \\
\beta_1 = \beta_2 = \beta_3 &= 0.08 & \text{Inhibition strength when CRO is binded} \\
\beta_4 = \beta_5 &= 1 & \text{Inhibition strength when CI and CRO are binded to OR1 and OR3}
\end{align*}
\]
II. Quorum sensing:

All the following is from the reference [18].

The equations:

\[
\dot{C} = \alpha_c R \eta^3 - \gamma_c C \quad \dot{R} = \rho R [LuxR]^2 [A]^2 - \gamma_R R
\]

In this case C is a model protein.

And the used parameters:

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \gamma_R )</td>
<td>0.0231</td>
<td>Degradation rate of the complex R.</td>
</tr>
<tr>
<td>( \gamma_c )</td>
<td>0.0692</td>
<td>Degradation rate of the protein C.</td>
</tr>
<tr>
<td>( \rho_R )</td>
<td>0.5</td>
<td>Fraction of dimer production.</td>
</tr>
<tr>
<td>( \alpha_c )</td>
<td>1</td>
<td>Maximum velocity of production C.</td>
</tr>
<tr>
<td>( \theta_R )</td>
<td>0.01</td>
<td>Binding affinity of the complex R to the promoter.</td>
</tr>
<tr>
<td>( \eta^3 )</td>
<td>1</td>
<td>Multimerization parameter</td>
</tr>
</tbody>
</table>

Plasmid design of this system (from [18]):
III. Codes

Xppaut Code

The code:

\[
\begin{align*}
\frac{dx}{dt} &= \left( m_x \cdot (1 + x^2 + alfa \cdot sigma1 \cdot x^4)/(1 + x^2 + sigma1 \cdot x^4 + (sigma1 \cdot sigma2 \cdot x^6) + y^2 + (B1 + B2) \cdot y^4 + (B1 \cdot B3) \cdot y^6 + sigma1 \cdot B4 \cdot x^4 \cdot y^2 + B5 \cdot x^2 \cdot y^2) \right) - (gamma_x \cdot x) + K0CI \\
\frac{dy}{dt} &= \left( m_y \cdot p_y \cdot (1 + y^2)/(1 + x^2 + sigma1 \cdot x^4 + (sigma1 \cdot sigma2 \cdot x^6) + y^2 + (B1 + B2) \cdot y^4 + (B1 \cdot B3) \cdot y^6 + sigma1 \cdot B4 \cdot x^4 \cdot y^2 + B5 \cdot x^2 \cdot y^2) \right) - gamma_y \cdot y + K0CRO \\
\end{align*}
\]

params gamma_x=0.004, gamma_y=0.008, p_y=62.92, alfa=11
params m_x=1, m_y=1, sigma1=2, sigma2=0.08
params B1=0.08, B2=0.08, B3=0.08, B4=1, B5=1
params K0CI=0, K0CRO=0

@ xp=x, yp=y, xlo=0, xhi=50, ylo=0, yhi=50, nmesh=500
@ total=2500, dt=1, bound=100
done

Images from the test of constant production of CRO (K0 is the rate). At the end there is only the fix point of lytic state.
Cell realistic division

globals [lpop length_lpop]
spread [Cells cell]
spread [virus virus]
cells-own [100 to life time_div]

to set-up
  ask patch 0 0
  [ sprout-cells 1
    set life 1
    set time_div random-normal 20 dev_div
    set shape 'cell'
    set color pink]
  ]
tick
end

to grow
  ask cells ; ask to the cells that are capable to divide
  [ if life = round (time_div) ;
    ch-dr
    carefully
    [ ask item round (length_lpop - random-float 1.00) lpop
      [ sprout-cells 1
        [ set life 0
          set time_div random-normal 20 dev_div
          set shape 'cell'
          set color pink
        ]
        set lpop remove-item 0 lpop
      ]
    ]
  ]
  [ set life 0
    set time_div random-normal 20 dev_div
  ]
ask cells ; [ set life life + 1]
tick
end
IV. Supplementary results

Matlab figure of the complex model: [http://nubr.co/ykKCsT](http://nubr.co/ykKCsT)

Movie about AND gate (results V): [http://nubr.co/mQ8UGu](http://nubr.co/mQ8UGu)
V. Effect of modified cells / WT population with autoactivation-inducible Lactone circuit

Here we can observe how the final state of the petri dish depends on the relation between WT and modified cells at the start.

The results show that all the WT cells die and the modified cells also. But the last ones only die if they sense AHL around them.

Diagram 10: Complete circuit with QS and viral genome coupled.