A new generation of glue

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Abstract

Whether you want to stop a leaking ship's hull, or repair a fractured bone, you need a strong adhesive. Our project aims at producing a new generation of glue. In contrast to most glues, our glue is natural, biodegradable, efficient on wet surfaces and is composed of polysaccharides naturally produced by the *Caulobacter crescentus* bacterium. Using BioBrick™ standard biological parts, we engineered a synthetic *Escherichia coli* strain which synthesizes this adhesive material. To improve our expression system, we plan to use a new plasmid stabilization technique, the Staby™ system. This system stabilizes expression plasmid without using antibiotics, which is of major concern in large-scale production of biological materials.

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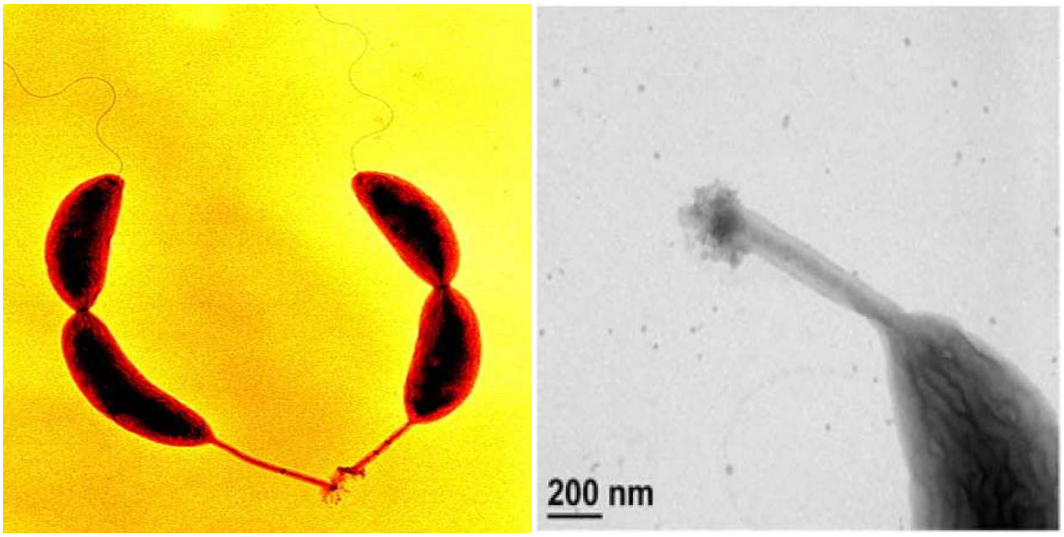
Contents

1 Introduction

Caulobacter crescentus is a gram-negative bacterium studied for the properties of its cell cycle [2]- [7].This bacterium is widely spread in aquatic environments and exists either as a motile cell or as a sessile cell Figure 1(a). In the sessile stage, *C. crescentus* sticks to surfaces by synthesizing adhesive at the stalk, a polar organelle shown in Figure 1(b). At the tip of this appendage, a complex holdfast structure is assembled.



(a) (b)

Figure 1: (a): *C. crescentus* motile and sessile cells [13] (b): *C. crescentus* stalk [11]

This material is an elastic, gel-like adhesive substance composed of both protein and polysaccharide. The N-acetylglucosamine polymer has been identified as the main polysaccharide component [8]. This holdfast shows unprecedented adhesive properties: the adhesive can adhere with a strength up to 68 N/mm^2. In comparison, commercial superglue can withstand a force of $18 - 30$ N/mm^2 [9], [10], [11]. Since this adhesive adheres to wet surfaces, many industrial applications are foreseen. The glue has already been isolated but the challenge is to produce large amounts of glue without it sticking to the material where it is produced.

A model of holdfast biosynthesis, export and attachment in *C. crescentus* has already been characterized, see Figure 2, [11].

The hfsE hfsF hfsG hfsH genes are organized in operon and their products are required for the minimum repeat unit holdfast synthesis while the polymerization of the repeat unit of the holdfast substance needs the expression of hfsC and hfsI genes.

The products of the 3 adjacent hfsD hfsA and hfsB genes are involved in the holdfast export. The anchoring of the holdfast polysaccharide is carried out by the hfa gene products. [12]

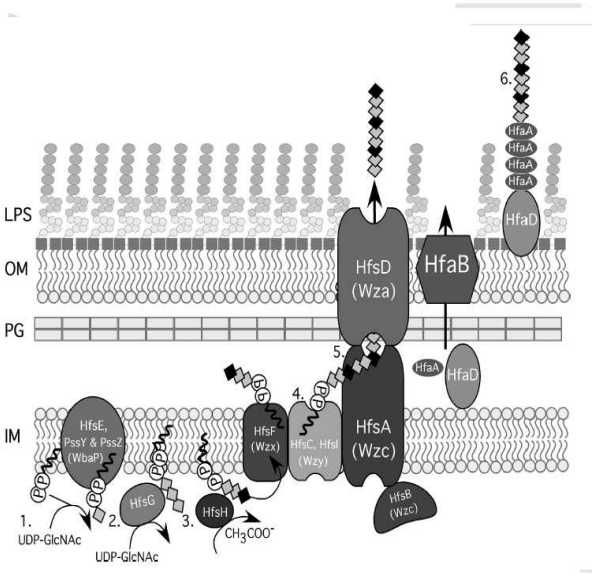


Figure 2: Model of holdfast biosynthesis, export, and anchoring in *C. crescentus*. Protein names in parentheses are the *E. coli* homolog proteins. The inner membrane HfsE protein initiates glycosyltransferase by transferring N-acetylglucosamine (NAG) from UDP−NAG to a lipid carrier. HfsG, a second glycosyltransferase protein, transfers NAG subunits to the growing polysaccharide chain. HfsH deacetylates one or more NAG residues. The HfsF protein translocates the polysaccharide chain linked to the lipid carrier across the inner membrane. The polymerases HfsC and HfsI proteins link the NAG repeat units together. The holdfast polysaccharide is transferred across the outer membrane by HfsA, HfsB and HfsD proteins. Hfa proteins mediate the polysaccharide attachment to the cell. [11]

Our aim is to find a way of mass-producing the glue. For this purpose, we planned to transfer the *C. crescentus* holdfast polysaccharide biosynthesis pathway to *Escherichia coli*.

As *Escherichia coli* possesses similar genes (Appendix A) involved in the production and the export of the holdfast in *C. crescentus*, we only inserted the hfsG and hfsH genes in an *E. Coli* strain : GluColi was born.

The problem of stickiness to the material of the container used to produce the glue was not investigated. In fact, we thought it was more relevant to synthesize the glue whenever and wherever it is required, instead of focusing on the extraction of the adhesive. For this purpose, we designed a theoretical circuit using chemotaxis and quorum sensing.

GluColi started producing an adhesive material and we begun to characterize the glue in the ”Physical and chemical properties of our glue” section. The mathematical modeling will also be considered.

2 Biological model

2.1 Materials and methods

On the Figure 3 we show the plasmid we created in order to insert the hfsG and hfsH genes.

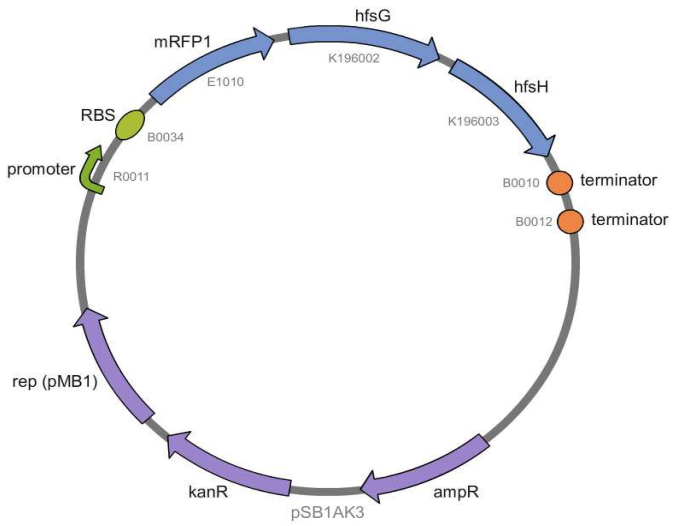


Figure 3: pSB1AK3 final plasmid

The following bricks come from the BioBricks partsregistry[1]. We chose a lactose inductible promoter so that we could easily control the expression of the genes of interest. This promoter is the BBa R0011 brick (located in the pSB1A2 ampicillin resistant plasmid).

The RBS (ribosome binding site) and the RFP bricks are located on the same plasmid (BBa K093005). RFP is a reporter gene. If the glue is not released (or we can not detect it), we will know the genes are expressed.

We chose a commonly used terminator, namely BBa B0015 (located on the pSB1AK3 kanamycin resistant plasmid). The replication origin is a pUC19-derived pMB1 (copy number of $100-300$ per cell) The final plasmid pSB1AK3 is ampicillin and kanamycin resistant. Seeing the importance of hfsG ans hfsH in our project, they were optimized and synthesized[2]. Their sequences have been optimized for two reasons:

- First they are compatible with all assembly standards
- Secondly the use of codon is not the same in *Caulobacter crescentus* and in *E. coli*. This could limit the rate of translation of our genes.

[1] http://partsregistry.org
[2] http://mrgene.com

The hfsG and hsfH sequences show the restriction sites and the optimized codons (Appendix B).

Transformation:

To increase the amount of plasmidic material, we performed transformations with DG1 *E. coli* competent cells from Delphi Genetics[3]. Its genotype is the following one:

mcrA Δ(mrr−hsdRMSmcrBC, modification−, restriction−) Φ80lacZΔM15 ΔlacX74 rec A1 araD139Δ(ara − leu)7697 galU galK rpsL endA1 nupG

For the full protocol see the StabyExpress^{TM} T7 Kit Manual, transformation using chemically competent cells[4].

Ligations:

All the ligations were achieved in the Assembly standard 10^5. We followed the assembly protocol recommended by New England BioLabs[6]. The first ligation concerns promoter, RBS and RFP. We cut the promoter plasmid with the restriction enzymes EcoR1 and Spe1. We cut the RBS+RFP plasmid with EcoR1 and Xba1. This one was dephosphorylated to prevent it from self-ligating without any inserts. Seeing that both plasmids are ampicillin resistant, we cut the promoter plasmid with AflIII and ScaI. This will prevent any promoter plasmid from being transformed. At this stage we have promoter, RBS and RFP in the RBS−RFP ampicillin resistant plasmid (Figure 4).

We transformed DG1 *E.coli* competent cells with this construction. The culture was left overnight (37°C) on ampicillin resistant medium. We selected the appropriated colonies and put them back at 37°C in liquid medium.

We proceeded with the extraction of the plasmid using the GenElute Plasmid Miniprep Kit[7]. In order to verify our construction, we cut upstream (EcoR1) and downstream (Pst1) of the Promoter+RBS+RFP. Then we proceeded with the electrophoresis of the digested product. We tested the construction by adding IPTG to a sample and this one was analyzed by fluorescent microscopy.

The second ligation concerns hfsG, hfsH and the terminator. Seeing that both hfsG and hfsH plasmids were ampicillin resistant and that we did not have appropriate enzymes to cut one of them, we chose to ligate hfsH and the terminator (which have different antibiotic resistances). The terminator plasmid is the destination plasmid and hfsH is the insert. The selection was made with kanamycine (following the above-cited protocol). After that, hfsG was added to this construction. At this stage, we have hfsG, hfsH and terminator in the terminator kanamycin resistant plasmid which is our final destination plasmid, Figure 5. Finally we assembled the first and the second construction to obtain the final plasmid, Figure 3.

[3] http://www.delphigenetics.com
[4] https://www.delphigenetics.com/manuals-48.html
[5] http://partsregistry.org/wiki/images/1/1a/StdAss.png
[6] http://www.neb.com/nebecomm/products/protocolProductE0546.asp
[7] Sigma-Aldrich ref (PNL350)

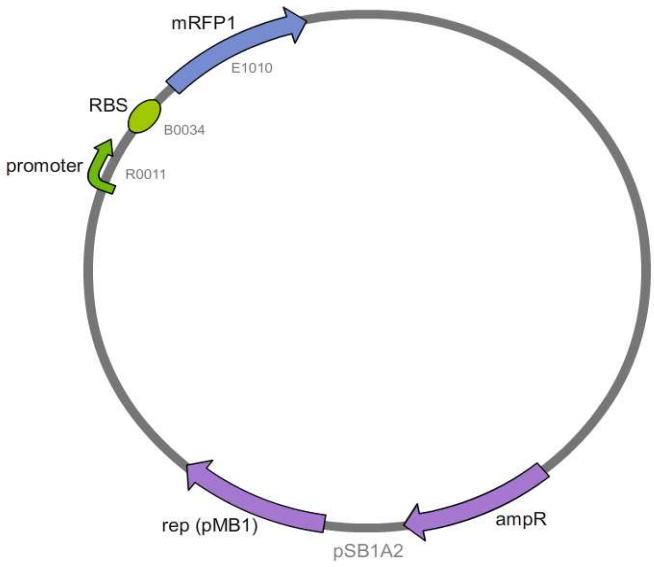


Figure 4: RBS−RFP ampicillin resistant plasmid

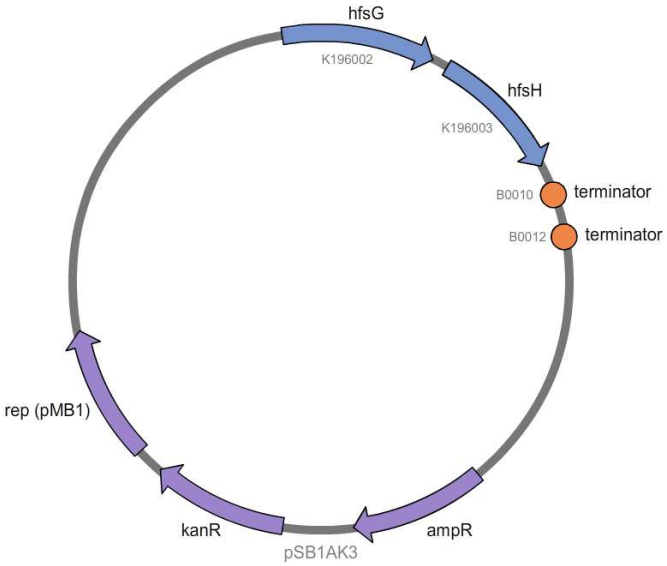


Figure 5: hfsG and hfsH ampicillin and kanamycin resistant plasmid

2.2 GluColi, engineering a biological circuit

For our first participation in the iGEM competition, we aimed at producing a new biological material. Finding a new way to produce a biological and biodegradable glue became our objective. Once we found a way to produce this, it was interesting to think about how we could have this glue produced where it is needed.

We designed a biological circuit composed of three main parts:

1. Bacteria produciong glue are attracted to the target point to be repaired.
2. Glue production at the leaking point, the crack or the fracture.
3. Preventing GluColi proliferation.

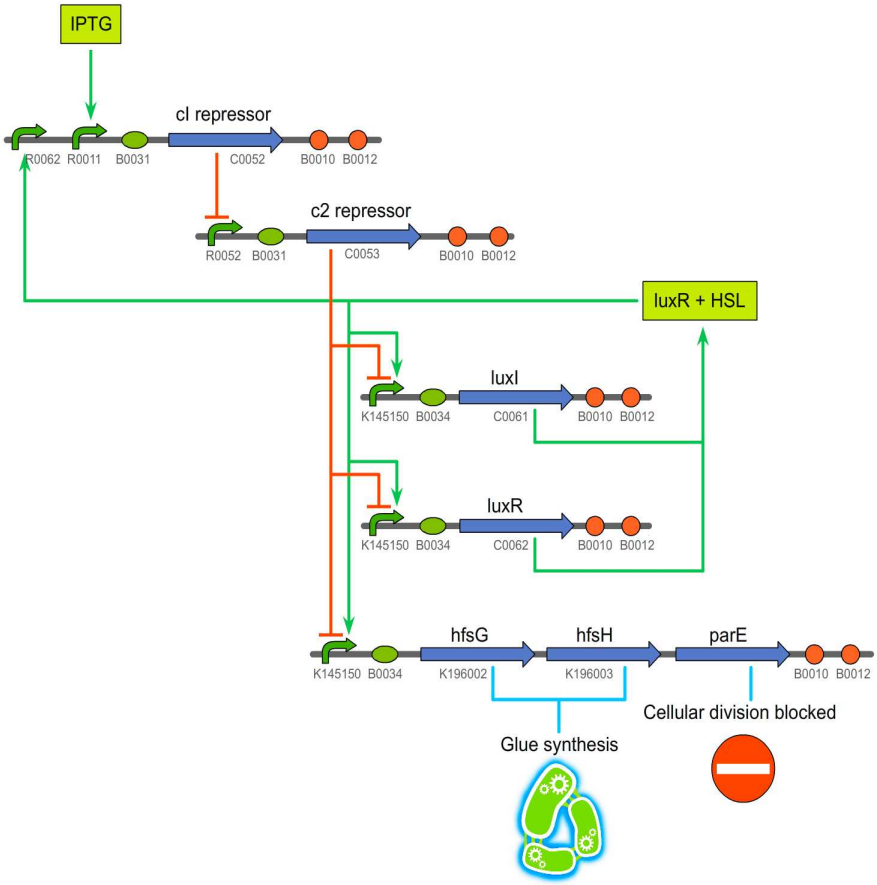


Figure 6: Schema of the biological system

Escherichia coli has a natural system of chemotaxis (Appendix C). Thanks to its flagella, it can swim towards a local chemical gradient. In order to guide GluColi to its target, we thought that the leaking should use aspartate as we know it is a good chemoattractant for *E. coli*. As it is a natural system found in *E. coli* we did not intend to add bricks for chemotaxis.

After that, a certain bacterial density is needed to produce enough glue. We engineered a glue production system controlled by quorum sensing, (Appendix D). As long as there are not enough bacteria at the target point, glue will not be produced. The quorum sensing mechanism senses the population density and coordinates gene expression. Glue production will be triggered only at high GluColi density.

The suggested circuit relies on a cascade of promoter repression/expression that will result in a positive feedback. Phage and quorum sensing promoter are used.

IPTG (Isopropyl β-D-1-thiogalactopyranoside) would permit the transcription of the downstream genes comprising cI434 which codes for a repressor issued from bacteriophage 434.

When there is no leaking, thus no IPTG, the quorum sensing is OFF because of the promoter placed in front of the genes involved in it. This promoter is a hybrid promoter proposed by last years KULeuven team and works as follows : it is repressed by c2 P22, (from phage 22), and activated by the complex LuxRHSL. If there is neither c2 P22 nor LuxR$-$HSL, then there will be a low background expression of the downstream genes. The activation by LuxR$-$HSL permits this device to be fully activated. The repression by c2 P22 is provided by the expression c2 P22 which is under control of the cI434 promoter. This promoter is constitutive. So, as long as there will be no IPTG, c2 P22 will be produced and there will be no quorum sensing and no glue. As soon as IPTG is present, cI434 is produced in order to repress cI434 promoter, c2 P22 will not be expressed anymore and the quorum sensing can take place. Once LuxR and HSL dimerize, they can also fully activate the production of *Caulobacter crescentus*'s enzymes needed for the glue synthesis. We also added a lux box in front of the cI434 device to ensure that c2 P22 is well repressed when the glue needs to be produced.

Now that the glue is produced, as we are not sure it will kill our bacteria and with the intention of not letting the bacteria proliferate, we intended to introduce a toxin that would inhibit cell growth without really killing the bacteria. We thought about parE which target is *E. coli* gyrase, [1]. By lack of time, we could not test our circuit. Here under, we describe how we thought to implement it into *E. coli*.

We would use four different plasmids. The first plasmid would be the cI434/c2 P22 generator depending on the presence or absence of IPTG. The second one would be the LuxR generator, following by the LuxI generator plasmid. The last one would be responsible for the glue synthesis and the "death" of the bacteria.

The first plasmid includes a lac promoter, which is a strong promoter (BBa R0011) induced by IPTG and repressed by LacI. The lac promoter controls the expression of the repressor cI434 from the cI434 generator (BBa P0152) consisting of a RBS, the repressor itself and a terminator. As said above we also added a Lux box promoter (BBa R0062).

The second plasmid contains a very strong promoter controlled by the CI434 repressor (BBa R0052). This promoter controls the expression of the c2 P22 repressor gene included in the c2 P22 generator (BBa P0153). In that way, if there is IPTG, there will be no c2 P22.

The third plasmid includes a hybrid promoter (BBa K145150) consisting of the lux box and binding sites for c2 P22. Transcription from this promoter can be activated by a LuxR$-$HSL

complex and repressed by c2 P22. The downstream gene codes for the LuxR protein (BBa I0462) which can sense $3OC_6$HSL (Appendix C). When there is IPTG, as there is no c2 P22, the LuxR protein is therefore synthesized at a low rate, the quorum sensing takes place and enhances itself.

The fourth plasmid includes the same hybrid promoter (BBa K145150) and the LuxI generator (BBa K092400) that allows the synthesis of $3OC_6$HSL. As well as the LuxR protein, this occurs after activation by the presence of IPTG.

The glue device itself is also under control of the hybrid promoter (BBa K145150) and allows the expression of hfsG (a glycosyltransferase) and hfsH (a polysaccharide deacetylase) genes and the toxin parE.
This circuit remains theoretical. The mathematical model is discussed in the section "Mathematical modeling".

Further, another aspect we should bear in mind is what would happen if the bacteria do not reach the target. A solution involving the synthesis of a toxin "after a while" should be considered.

2.3 Circuit validation

In order to validate experimentally our biological circuit and to characterize some of its parameters, some tests should be carried out. We divided the circuit in different parts.

Circuit I

This circuit[8] (Figure 7) allows us to characterize and verify the performance of the C0056 inhibitor. For this purpose the C0053 gene needs to be substituted by a GFP. Without IPTG no fluorescence should be detected. Chemical kinetics illustrating the fluorescence rate should be measured after adding IPTG to the substrate.

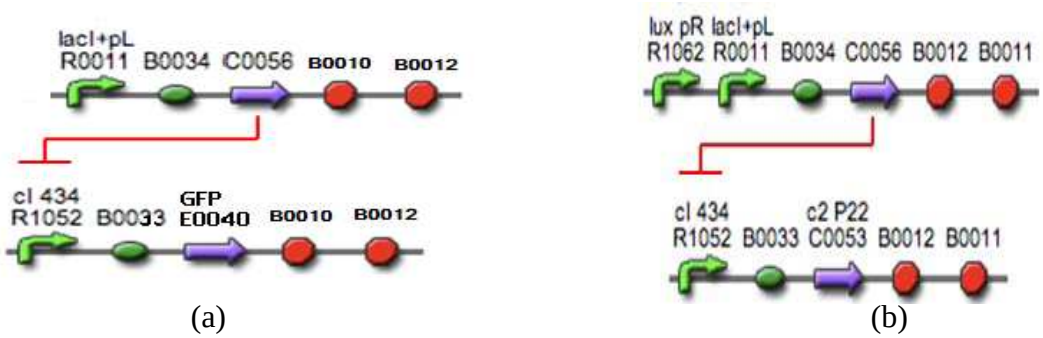
Circuit II

Another experiment should also be carried out to test the influence of the C0053 inhibitor on the K145150 promoter, Figure 8[9].

The promoter upstream from the inhibitor is induced by arabinose.

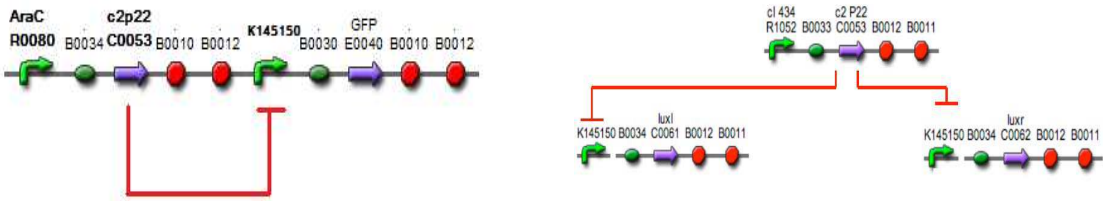
[8]Experimental model:Construction : Bba R0011+BBa P0152 (http://partsregistry.org/wiki/index.php?title=Part:BBa P0152); Part: BBa R10522 (http://partsregistry.org/wiki/index.php?title=Part:BBa R1052) + Part: BBa E0240) (http://partsregistry.org/wiki/index.php?title=Part:BBa RE0240

[9]Experimental circuit: Construction: BBa $R0080$ (http://partsregistry.org/Part:BBa R0080), BBa $C0053$, BBa K145150 (http://partsregistry.org/Part:BBa K145150), Part: BBa E0240



(a) (b)

Figure 7: (a): Experimental model, (b): Theoretical model

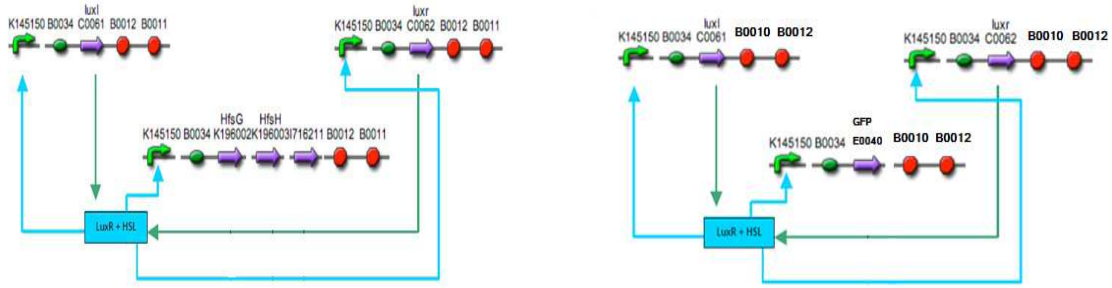


(a) (b)

Figure 8: (a)Experimental circuit. Repression by c2p22, (b)Theoretical quorum sensing circuit

Circuit III

The Quorum sensing should also be tested (cf. Figure 9): we use promoters whose regulation can be controlled by LuxI and LuxR. The fluorescence rate would be measured according to time and population density. Then, we can determine the time necessary for the bacteria to reach a certain density allowing them to produce the LuxR-HSL complex.



(a) (b)

Figure 9: (a): Experimental quorum sensing circuit, (b): Theoretical quorum sensing circuit

3 Physical and chemical properties of our glue

In order to characterize the glue, a series of tests have been carried out such as:

3.1 Phenotypic description of the transformed bacteria

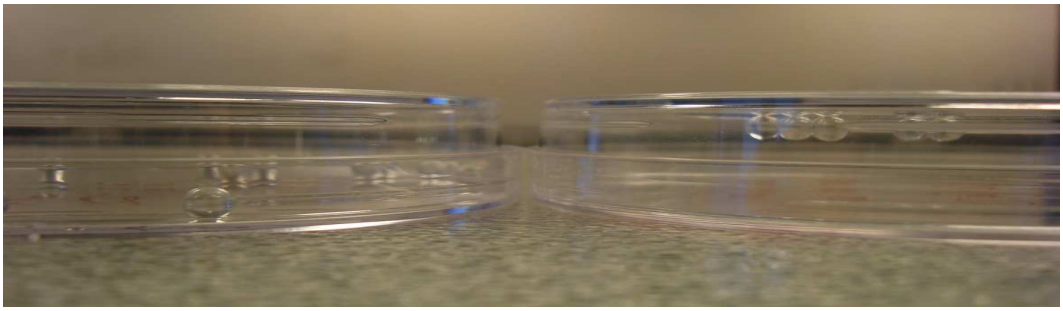


Figure 10: glass beads adherence to a Petri dish

Transformed bacterial colonies showed a filamentous aspect when put back in a liquid culture. Because of the glue, the whole colony is taken as they stick together. We let the bacteria grow at $37°$C overnight in a shaking incubator. A strand originating from the bottom of the tube was observed.

The first tests were done to see if our glue actually stuck. A colony from a Petri dish was isolated and spread on a sheet of paper. After that, the paper has been placed on a glass substrate during a short period of time (1 to 2 hours) under a 2N pressure. We noticed a slight adherence and a bright spot where the bacteria had been spread on the sheet of paper.

A second phenotypic test has been carried out in a liquid culture with glass beads. The beads have been dipped into the liquid culture and placed on a plastic medium. After 7 hours of incubation, we observed a perfect adherence to the plastic medium (even after a huge shock). (Figure 10)

3.2 Fluorescence microscopy

Only a few bacteria showed expected fluorescence properties (due to the RFP sequence).

Furthermore, it seems that the glue is synthesized in a vacuole. Indeed vacuoles were visible in transformed bacteria. (Figure 11)

This is a significant feature of our modified strain of *E. coli*. We also observed a higher bacteria aggregation at $27°$C in comparison to $37°$C (Figure 12).

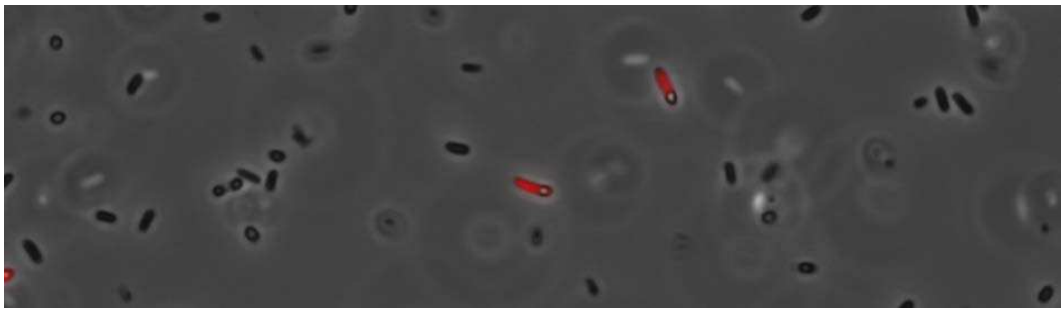
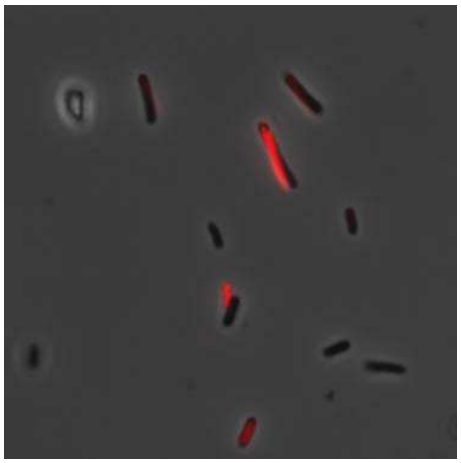
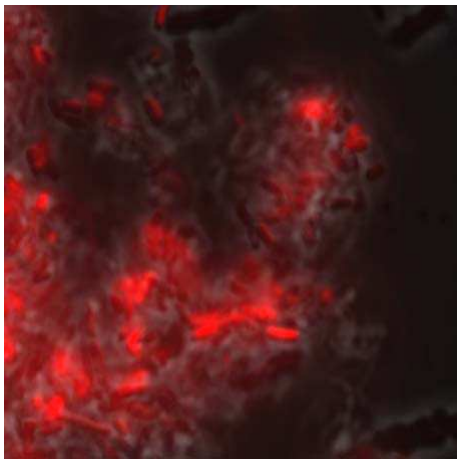


Figure 11: vacuole in transformed bacteria



(a)



(b)

Figure 12: (a):incubation at 37°C, (b): incubation at 27°C

3.3 Biofilm-forming abilities

A test on a 96-well microtiter was carried out to highlight biofilm-forming abilities. Unfortunately, this test was not conclusive; the bacteria did not stick to each other. *Caulobacter crescentus* bacteria might stick to nearly all substrates but they also synthesize a matrix of extracellular polymeric substance to adhere to each other.

3.4 Wet strength

Water and ”growth medium (LB)” inhibit the adhesive effect. Moreover, water separated glass beads. This result was not what we expected given what is observed for *C. crescentus* living in aquatic environments. It would be advisable to specify that glass beads stick together again when water is removed.

First it is possible that the similar genes found in *E.coli* (hfsE, hfsF, hfsC, hsfI, hfsA, hfsB and hfsD genes) do not work in an identical way than in *C. crescentus*.

Secondly the two exogenes (named hfsG and hfsH) are overexpressed compared to the other holdfast biosynthesis and export genes. Indeed, we used a high copy plasmid with a strong promoter. A failure during sugar polymerization might explain the ineffectivineness in water environment.

Thirdly, *C. crescentus* synthesizes its complex holdfast structure at the tip of a stalk. The anchoring proteins might be required to ”protect” the adhesive against the water.

Finally, apart from the polysaccharides, the composition of the adhesive remains unknown. Other essential components might be implicated in the water and solvent strengths.

Complementary approaches are foreseen to differentiate these assumptions:

- The other genes (hfsE hsfF, hsfC hsfI, hsfD hsfA and hsfB) should also be transferred to *E. Coli* with the same transcription rate.
- As the anchoring genes may play a role in the glue composition, hfaA, hfaB and hfaD genes should be inserted in *E.Coli* too.
- We need to have more information about the adhesive composition. A proteomic approach with *C.crescentus* mutants that do not produce the glue might be undertaken.

3.5 Tensile strength

Two different tests have been carried out in order to characterize the tensile strength of the glue. It is important to note that the glue used in these experiments has not been purified. It consists of bacteria lysate.

In the first test, liquid cultures of transformed bacteria are tested on different materials. To enhance the adhesion of these materials, different attempts have been made to minimize the impact of the ”wet part” of the liquid culture. Indeed, previous experiments showed the adhesive to be ineffective in water.

On one hand different materials stuck together have been placed in a box filled up with salt. On the other hand, a sample of $300\ \mu$l of liquid culture has been spread on LB solid medium (Petri dish). The bacteria then grow on the surface while the LB solid medium absorbs the remaining liquid. To collect the glue on a specific material, it has been rubbed against the Petri dish. In fact, this is how we first showed our glue was functional.

Unfortunately none of these experiments were successful. Two hypotheses can be put forth. Firstly the glue is still too liquid and because of that ineffective. Secondly there are proportionately more bacteria than glue. So there is not enough glue to stick different materials together.

In the second test, different ”GluColi” colonies have been taken from solid cultures and directly put on the materials. This experiment showed better results than the previous one. Several materials such as plastic, wood, cork, paper and CD-ROM have been successfully stuck.

In order to measure the tensile strength, two plastic materials have been stuck together on a surface of 1.5 cm^2. The dynamometer[10] showed a tensile strength of 9N which is $0,06$ N$/$mm^2, Figure 13. This result is far less than the one found in other laboratories with *C. crescentus* (up to 68 N$/$mm^2). One explanation, as previously stated, is that our glue is not purified.

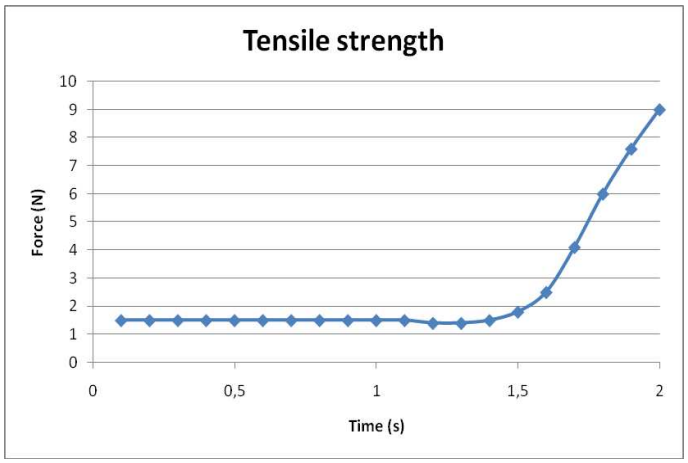


Figure 13: Tensile strength measured with the dynamometer

4 Mathematical modeling

4.1 Introduction

Synthetic biology is a recent development in biology which aims at producing useful material via biological agents. In this context, a biological system can be seen as a complex network composed of different functional parts ([14]). Mathematical tools allow one to make prediction on the dynamical behaviour of a given bio-system. We aim at studying ft the system which has been built in the second section of this report. We already know from our experiment that our system is able to produce glue in the presence of IPTG inductor. We now address the following question: what is the influence of the different parameters on the global dynamics (degradation rate, production rate, level of initial quantity for the C2P22 repressor,...) with and without IPTG. In order to achieve this task, we will consider three different models: the first one shows the basal property we are looking for: a system able to produce glue. The two next models can be seen as two different steps of improvement. The purpose of these models is to improve the

[10]Force sensor, Pasport, PS-2104

experimental control of the glue production. Parameters values result from the literature and previous iGEM team wiki's. We know these remain qualitative and that lab work should be carried out to specify them more precisely.

We start from the global biological system established in the first section (cf. Fig.14(a)). In order to be able to obtain the dynamical equations for the global behaviour of our system, we have to make some assumptions and simplifications. Firstly, we completely neglect the detailed composition of the biobricks. Each of them is considered as a simple block. The next step of our mathematical design is the modeling of the interactions between all the different blocks. The regulation network of our system is modelized using Hill functions for a repressor and an activator. [14]

- Hill function for activator

$$H_a([x]) = \frac{\alpha\,[x]^p}{[x]^p + k^p} \tag{1}$$

- Hill function for repressor

$$H_p([x]) = \frac{\alpha}{[x]^p + k^p} \tag{2}$$

In these expressions, $[x]$ is the concentration of activated genes, p is called the Hill coefficient, k is the activation coefficient and α is the maximum expression level of the promotor.

For each block of the system, we can obtain a dynamical equation by considering its interactions with the other blocks of the system. For each block we can build an equation of the following form:

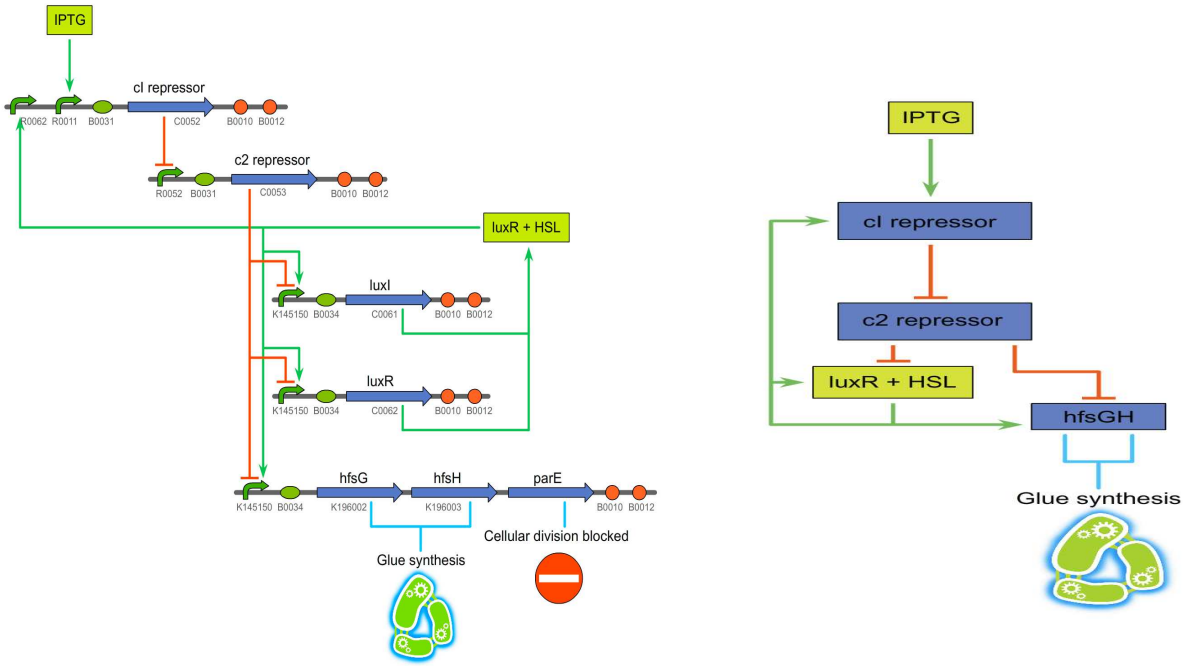
$$\frac{d\,[x]_i}{dt} = \mathcal{R}(H_a([x]), H_p([x])) - \gamma_i\,[x]_i \tag{3}$$

Where, $[x]_i$ is the concentration of the gene i, $\mathcal{R}(H_a([x]), H_p([x]))$ is the regulating function which is a combination of Hill functions. The second term of the right side is the destruction term, where γ_i is the maximum destruction rate of the gene i.

We must bear in mind, however that the robustness of a given operational regime with respect to external perturbations strongly depends on the value of the Hill coefficients. [16] In particular, the robustness is expected to increase with the value of the hill coefficient. The cooperativity behaviour is also a function of the Hill coefficient. For these reasons we will consider a situation for which $p = 2$.

4.2 A first simplified model

In this first step, we present a simplified model of our system. We make the following assumptions: we consider that the LuxR+HSL complex is formed quickly at the beginning of the dynamics. This assumption, allows us to modelize the quorum sensing system by considering the complex LuxR+HSL only. The simplified schema is shown on Figure 14(b): the effect of the LuxI and LuxR is represented by the autoregulation arrow on the box of the complex LuxR+HSL. We also neglect the effect of the block parE in this first approach. For the



(a) (b)

Figure 14: (a): Schema of the system from the biological point of view (b): same system but from the mathematical modeling point of view

system shown in Figure 14(b) we can obtain the following equations (writing here $[L]$ for the LuxR+HSL concentration:

- Equation for the $c1$ repressor block (designed by $c1$ in the equation):

$$\frac{d\,[c1]}{dt} = \beta + \frac{[L]^2}{[L]^2 + k_L^2} - \gamma_1\,[c1] \tag{4}$$

In this equation, the parameter β has the following explicit form:

$$\beta = \frac{1}{1 + [LacI]\,/(1 + [IPTG])^2} \tag{5}$$

In our case, $[LacI]$ can be considered as a constant, then we have $\beta = \beta([IPTG])$. In this first model we consider only the situation with IPTG inside the system: $\beta \neq 0$.

- Equation for the $c2$ repressor block (designed by $c2$ in the equation):

$$\frac{d\,[c2]}{dt} = \frac{\alpha_1}{[c1]^2 + k_1^2} - \gamma_2\,[c2] \tag{6}$$

- Equation for LuxR+HSL block (designed by L in the equation):

$$\frac{d\,[L]}{dt} = \frac{\alpha_2 k_2^2}{k_2^2 + c_2^2}\frac{[L]^2}{k_L^2 + [L]^2} - \gamma_L\,[L] \tag{7}$$

- Equation for the glue production (designed by Gl in the equation, or by hfsGH in the text)

$$\frac{d\,[Gl]}{dt} = \frac{\alpha_5 k_2^2}{k_2^2 + c_2^2}\frac{[L]^2}{k_L^2 + [L]^2} - \gamma_{Gl}\,[Gl] \tag{8}$$

We choose a set of values for the different parameters:

$$\alpha_i = 1, \quad (i = 1, 2, 3) \quad \gamma_1 = \gamma_2 = \gamma_L = 0.1 \quad \gamma_{Gl} = 0.02 \quad k_L = 0.5, k_1 = 0.5, k_2 = 0.5 \tag{9}$$

4.2.1 Stationary state

The first step of our analysis is the study of the stationary point. In order to do that we consider the following algebraic system:

$$\begin{cases} \beta + \frac{[L]^2}{[L]^2 + k_L^2} = \gamma_1\,[c1] \\ \frac{\alpha_1}{[c1]^2 + k_1^2} = \gamma_2\,[c2] \\ \frac{\alpha_2 k_2^2}{k_2^2 + c_2^2}\frac{[L]^2}{k_L^2 + [L]^2} = \gamma_L\,[L] \\ \frac{\alpha_5 k_2^2}{k_2^2 + c_2^2}\frac{[L]^2}{k_L^2 + [L]^2} = \gamma_{Gl}\,[Gl] \end{cases}$$

By solving this system of equations for the previous set of parameters we can find different sets of stationary states. So we have (considering only the states with real values):

$$\begin{cases} \text{set one: } \{[L]_s = 9.97337 \quad [c1]_s = 19.9749 \quad [c2]_s = 0.00626178 \quad [Gl]_s = 99.7337\} \\ \text{set two: } \{[L]_s = 0 \quad [c1]_s = 10 \quad [c2]_s = 0.025 \quad [Gl]_s = 0\} \\ \text{set three: } \{[L]_s = 0.0251248 \quad [c1]_s = 10.0252 \quad [c2]_s = 0.0251248 \quad [Gl]_s = 0.251248\} \end{cases}$$

4.2.2 Linear stability analysis

To check the stability of the stationary states, we perform a linear stability analysis. To this end, we assume that the configuration of the system is perturbed by:

$$\delta\vec{x} = \vec{A}e^{\omega t} \tag{10}$$

ω is the growth rate of the perturbation.

$$\delta\vec{x} = \begin{pmatrix} \delta\,[c1] \\ \delta\,[c2] \\ \delta\,[Gl] \\ \delta\,[L] \end{pmatrix}$$

is the vector representing the perturbation of each concentration. Linearizing the equations, we can rewrite them in the following form [17]:

$$\frac{d\vec{x}}{dt} = \mathcal{L}(\gamma_i, \alpha_i).\vec{x} \tag{11}$$

where

$$\vec{x} = \begin{pmatrix} [c1] \\ [c2] \\ [Gl] \\ [L] \end{pmatrix}$$

and

$$\mathcal{L}(\gamma_i, \alpha_i) = \left(\frac{\partial\vec{F}}{\partial\vec{x}}\right)_{\vec{x}=\vec{x}^s} \tag{12}$$

is the Jacobian matrix. Taking into account the form of the perturbation (26), we can write the equation (11) as follows:

$$\mathcal{L}.\vec{x} = \omega\vec{x} \tag{13}$$

and the eigenvalues ω satisfy the characteristic equation:

$$\det|\mathcal{L}_{ij} - \omega\delta_{ij}| = 0 \tag{14}$$

Given the roots ω_i of the above equation, we can easily deduce the steady state. From (26) it is easy to see that a given state is unstable if any of the ω_i has a positive real part. In our case we obtain the following expression for $\det|\mathcal{L}_{ij} - \omega\delta_{ij}|$:

$$(\gamma_{Gl}+\omega)\left[\frac{12\alpha_2\,[L]_s\,[c1]_s\,k_L^2k_2^2k_3^2\,[c2]_s}{([L]_s^2+k_L^2)^3([c1]_s^2+k_1^2)^2(k_2^2+[c2]_s^2)^2} - (\gamma_1+\omega)(\gamma_2+\omega)\left\{\gamma_6+\omega-\frac{2\alpha_3\,[L]_s\,k_L^2k_2^2}{([L]_s^2+k_L^2)^2(k_2^2+[c2]_s^2)}\right\}\right] \tag{15}$$

Equation (14) is a polynomial of 4th order and its first root is of the form:

$$\omega = -\gamma_{Gl} \tag{16}$$

The others are the roots of a polynomial expression of the third order. We could obtain these roots analytically but their expressions are too complicated to be useful. So, we solve numerically the equation for the previous sets of parameters and we put into the equation (15) the corresponding values for the different sets of stationary states. We have:

- set one $= \{\omega_{1,2} = -0.100036 \pm 0.0002i, \omega_3 = -0.0994, \omega_4 = -0.01\}$

- set two $= \{\omega_1 = -0.1, \omega_2 = -0.1, \omega_3 = -0.1, \omega_4 = -0.01\}$
- set three $= \{\omega_{1,2} = -0.1014 \pm 0.0240i, \omega_3 = -0.01, \omega_4 = 0.1023\}$

Two states are stable and the last one is unstable. The system can stay near its initial configuration, which corresponds to the second set of values or it can also go to another state for which we have glue production (first set). To learn more about the dynamics of this system, we need to study its complete behaviour.

4.2.3 Global dynamics

In order to have an overview of the dynamics of the system we perform a numerical integration of the dynamical equations with the set of previous values for the different parameters. When we solve the system, we see that the system travels from its initial configuration to the nearest stationary stable state which is the second set, corresponding to a state without glue production. We have to stress that we do not find any different situation by varying the set of parameters. We have always a stationary state with no glue production: the system chooses this final configuration. In order to force the system to go to the other state, we add a little basal production term (κ) in the dynamical equation for the complex LuxR+HSL:

$$\frac{d\,[L]}{dt} = \frac{\alpha_2 k_2^2}{k_2^2 + c_2^2} \frac{[L]^2}{k_L^2 + [L]^2} - \gamma_L\,[L] + \kappa \tag{17}$$

In this case, as we can see on the Figure (15), the system reaches the first stationary state, for which we have a glue production.

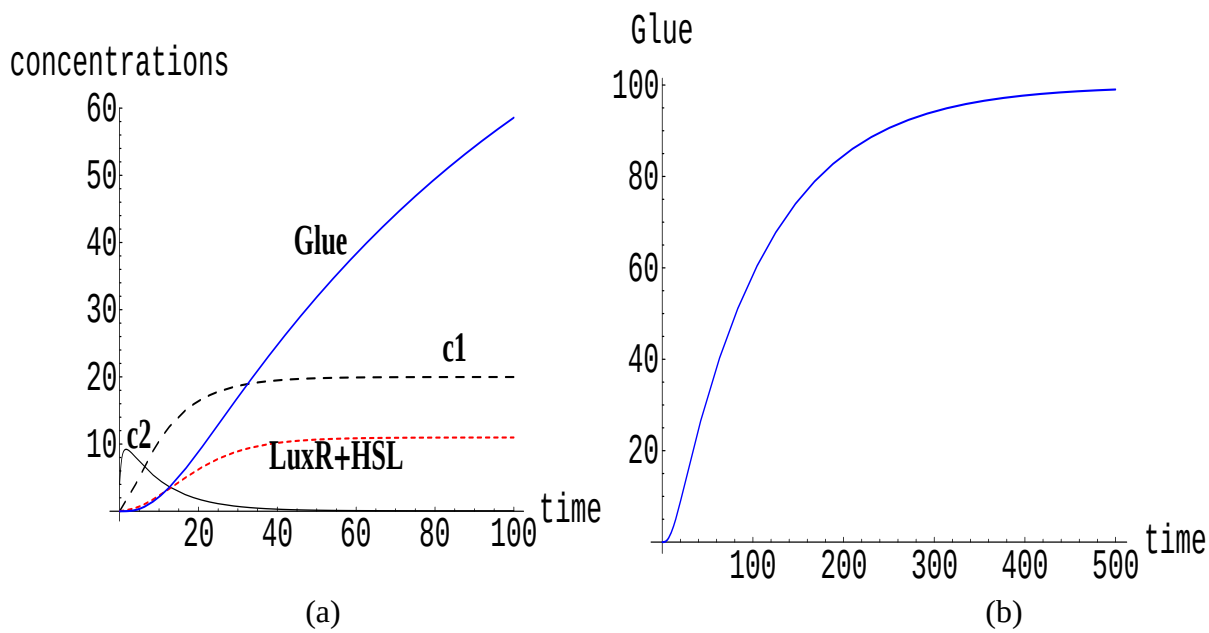


Figure 15: (a): Dynamical evolution of the concentrations (b): Dynamical evolution of the glue concentration

4.2.4 Discussion

In this first attempt to obtain a mathematical model of our biological system, we have been able to build a model for which we can obtain stable stationary states with or without glue production. The problem is the following one: the system shows a sensitiveness to the values of the different parameters and the only way to obtain the glue is to introduce a basal production term. The presence of this term is not unrealistic but it is clear that an improvement of this model is needed in order to obtain a dynamical system for which the switch from a state without glue to a state with glue is regulated by the value of β, meaning by the concentration of the inductor in the system (which is the control parameter from the experimental point of view). This is not the case with this simplified model because with $\kappa \neq 0$ we have a glue production independently of the value of β.

4.3 Detailed model for the Quorum Sensing system

The aim of this section is to present a more realistic system in order to solve the problem pointed out in the last section. The main difference in this new model is the following one: as we can see on the Figure 16(b), we consider a more complete description of the quorum sensing system by taking into account the presence of the LuxR and LuxI blocks. We also consider the effect of the parE block.

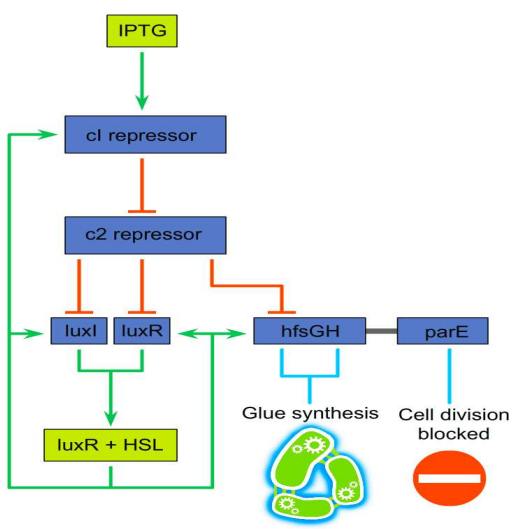


Figure 16: Mathematical model of the biological system including the detailed dynamics of the quorum sensing system

For the system shown on Figure 16 we have the following equations (writing here $[Li]$ for the LuxI concentration and $[Lr]$ for the LuxR concentration):

- Equation for the $c1$ repressor block, which is the same as in the previous model:

$$\frac{d\,[c1]}{dt} = \alpha_6 \frac{[L]^2}{[L]^2 + k_L^2} + \beta - \gamma_1\,[c1] \tag{18}$$

- Equation for the $c2$ repressor block, which is the same as in the previous model:

$$\frac{d\,[c2]}{dt} = \frac{\alpha_1}{[c1]^2 + k_1^2} - \gamma_2\,[c2] \tag{19}$$

- Equations for the LuxI block and LuxR block: this is the mean improvement in this model.

$$\frac{d\,[Li]}{dt} = 1 + \frac{\alpha_2\,[c2]^2}{[c2]^2 + k_2^2}\left(\frac{\alpha_6\,[L]^2}{[L]^2 + k_L^2} - 1\right) - \gamma_{Li}\,[Li] \tag{20}$$

$$\frac{d\,[Lr]}{dt} = 1 + \frac{\alpha_2\,[c2]^2}{[c2]^2 + k_2^2}\left(\frac{\alpha_6\,[L]^2}{[L]^2 + k_L^2} - 1\right) - \gamma_{Lr}\,[Lr] \tag{21}$$

- Equation for parE block, this is a new equation:

$$\frac{d\,[parE]}{dt} = 1 + \frac{\alpha_2\,[c2]^2}{[c2]^2 + k_2^2}\left(\frac{\alpha_6\,[L]^2}{[L]^2 + k_L^2} - 1\right) - \gamma_{parE}\,[parE] \tag{22}$$

- Equation for the HfsGH block (Gl in the equation), it is almost the same form as before, but there is the effect of the parE block:

$$\frac{d\,[Gl]}{dt} = \left[1 + \frac{\alpha_2\,[c2]^2}{[c2]^2 + k_2^2}\left(\frac{\alpha_6\,[L]^2}{[L]^2 + k_L^2} - 1\right)\right]\frac{\alpha_7}{[parE]^2 + k_{parE}^2} - \gamma_{Gl}\,[Gl] \tag{23}$$

- Equation for LuxR+HSL block:

$$\frac{d\,[L]}{dt} = \frac{\alpha_4\,[Lr]^2}{[Lr]^2 + k_{Lr}^2}\frac{\alpha_3\,[Li]^2}{[Li]^2 + k_{Li}^2} - \gamma_L\,[L] \tag{24}$$

  There is no explicit auto regulation in this model.

In the two next sections of this study we will consider the following set of values for the different parameters which appear in our model:

$$\begin{cases} \gamma_1 = \gamma_2 = \gamma_{Li} = \gamma_{Lr} = \gamma_{Gl} = \gamma_L = 0.0001 \\ \gamma_{parE} = 0.02 \\ \alpha_i = 1 \quad (i = 1, ..., 7) \\ k_1 = 0.76 \\ k_2 = 0.01 \\ k_{Li} = k_{Lr} = k_{Gl} = 1 \\ k_L = 0.0001 \\ k_{parE} = 1 \end{cases}$$

Considering this set of values, we will study the dynamical behaviour of our system in absence of IPTG and when the inductor is added. We will focus mainly on the influence of the initial value of the $[c2]$ concentration and on the quantity of IPTG which is added in the system. These two concentrations are the main values which can be controlled from an experimental point of view. In the last section of this part, we will discuss the behaviour of the system when some of the intrinsic parameters are modified.

4.3.1 Dynamics of the system without inductor

Steady state

In absence of an inductor ($\beta = 0$), the system is expected to stay at rest and not to produce a large amount of glue. We then consider the following initial conditions:

$$[c1]^s = 0 \quad [c2]^s = x_{initial} \quad [Li]^s = [Lr]^s = [Gl]^s = [L]^s = [parE]^s = 0 \tag{25}$$

Due to the complexity of the system we do not expect that it will stay at rest with these initial values but that at least this state will be stable. Indeed, if we try to obtain the steady state by considering the set of algebraic equations obtained by assuming that the time derivatives of all concentrations are set to zero:

$$\begin{cases} \alpha_6 \frac{[L]^2}{[L]^2+k_L^2} + \beta = \gamma_1 [c1] \\ \frac{\alpha_1}{[c1]^2+k_1^2} = \gamma_2 [c2] \\ 1 + \frac{\alpha_2 [c2]^2}{[c2]^2+k_2^2} (\frac{\alpha_6 [L]^2}{[L]^2+k_L^2} - 1) = \gamma_{Li} [Li] \\ 1 + \frac{\alpha_2 [c2]^2}{[c2]^2+k_2^2} (\frac{\alpha_6 [L]^2}{[L]^2+k_L^2} - 1) = \gamma_{Lr} [Lr] \\ \left[1 + \frac{\alpha_2 [c2]^2}{[c2]^2+k_2^2} (\frac{\alpha_6 [L]^2}{[L]^2+k_L^2} - 1)\right] \frac{\alpha_7}{[parE]^2+k_{parE}^2} = \gamma_{Gl} [Gl] \\ \frac{\alpha_4 [Lr]^2}{[Lr]^2+k_{Lr}^2} \frac{\alpha_3 [Li]^2}{[Li]^2+k_{Li}^2} = \gamma_L [L] \\ 1 + \frac{\alpha_2 [c2]^2}{[c2]^2+k_2^2} (\frac{\alpha_6 [L]^2}{[L]^2+k_L^2} - 1) = \gamma_{parE} [parE] \end{cases}$$

It can be seen (for the set of values which has been chosen) that the system will reach a steady state near the initial configuration[11] ($[c2]$ increases to a very high value and the other variables go to almost zero).

Linear stability analysis

As previously explained, we assume that the configuration of the system is perturbed by

$$\delta \vec{x} = \vec{A} e^{\omega t} \tag{26}$$

with

$$\delta \vec{x} = \begin{pmatrix} \delta [c1] \\ \delta [c2] \\ \delta [Li] \\ \delta [Lr] \\ \delta [Gl] \\ \delta [L] \\ \delta [parE] \end{pmatrix}$$

[11] It has to be noticed that the glue (hfsGH or Gl in the model) continues to oscillate near zero then it is numerically impossible to obtain a true stationary state for which $\frac{d[Gl]}{dt}$ is strictly zero.

and

$$\vec{x} = \begin{pmatrix} [c1] \\ [c2] \\ [Li] \\ [Lr] \\ [Gl] \\ [L] \\ [parE] \end{pmatrix}$$

The eigenvalues ω satisfy the characteristic equation:

$$\det |\mathcal{L}_{ij} - \omega\delta_{ij}| = 0 \tag{27}$$

In this case we obtain the expression:

$$(\gamma_1 + \omega_1)(\gamma_2 + \omega_2)(\gamma_{Li} + \omega_3)(\gamma_{Lr} + \omega_4)(\gamma_{Gl} + \omega_5)(\gamma_L + \omega_6)(\gamma_{parE} + \omega_7) = 0 \tag{28}$$

This is a polynomial of 7th order and all its roots are of the form:

$$\omega_i = -\frac{1}{\gamma_i} < 0 \tag{29}$$

As we can see, the expression is much more simpler that the one we obtained in the first model. From the linear analysis point of view, the system can reach any stationary state.

Results

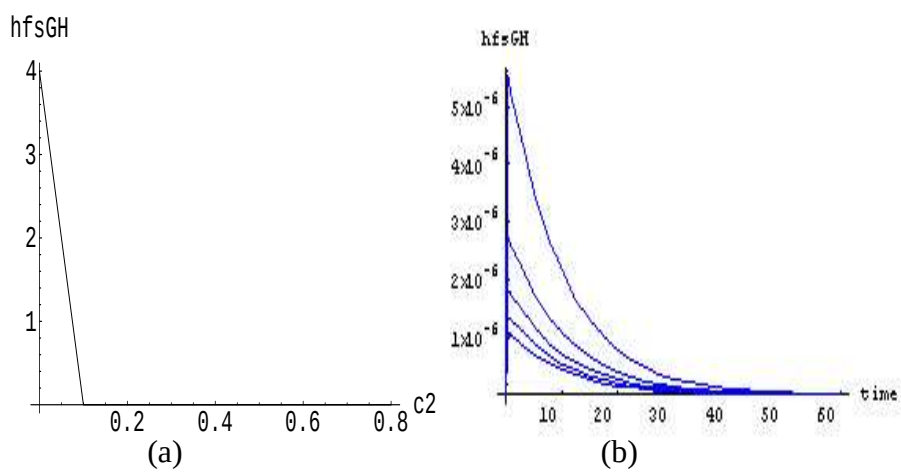


Figure 17: (a): Bifurcation diagram in function of the initial value of $[c2]$ (b): Time evolution of the glue concentration, for values of $[c2]\,(t = 0) = 10, 20, 30, 40, 50$, with $\beta = 0$

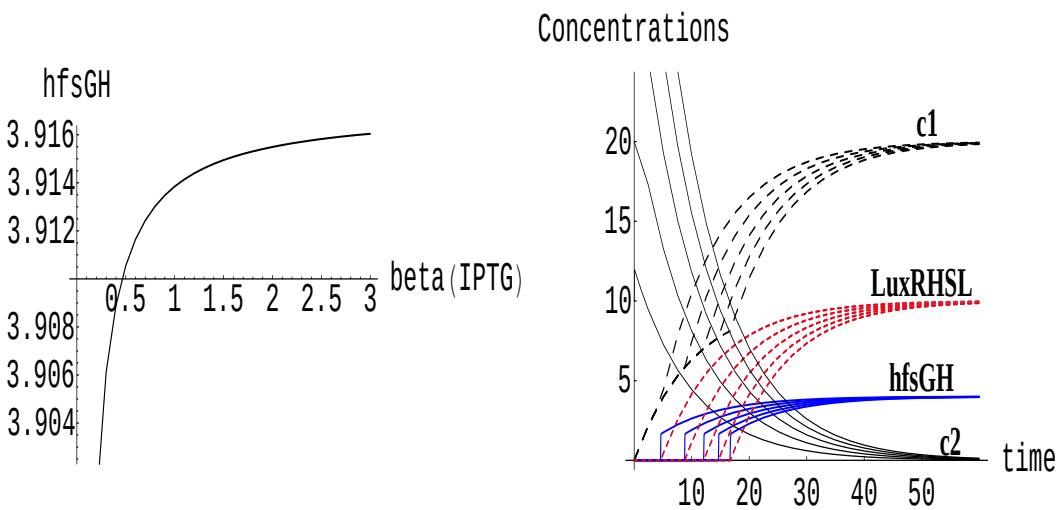
On Figure 17(b) we show the time evolution of $[hfsGH]$ for different initial values of $[c2]$, we see that a higher value of $[c2]$ tends toward a diminution of the maximum value of $[hfsGH]$ concentration. As we can see its value is very low, there is just a small increase at the beginning. During the same time the $[c2]$ concentration reaches its steady state. Its concentration is very high; this is mainly due to the fact that the other variables as $[L]$ or $[c1]$ (which can repress $[c2]$)

go to a insignificant value.

Existence of a critical value for $[c2]$

We have to stress that there is a minimum value for $[c2]$ which has to be found in the system at the beginning. Indeed, as it can be seen on Figure 17(a), when the initial concentration $[c2]$ is too small, the production of glue can start without inductor. The initial concentration $[c2]$ has to be sufficiently high so that the $c2$ repressor can really represses the other blocks in the system. The critical value for $[c2]$ depends on the parameters of the components of the system so that α_i, γ_i and k_i: $[c2]_c = [c2]_c(\alpha_i, \gamma_i, k_i)$.

4.3.2 Dynamics of the system with inductor



(a) (b)

Figure 18: (a):Diagram in function of the amount of inductor for different initial values of $[c2]$, (b): Evolution of the different concentrations for $[c1]$, $[c2]$, $[hfsGH]$ and $[L]$ inside the system with IPTG (model without toggle switch), for initial value of $[c2]$: $10, 20, 30, 40, 50$ (quantities have been rescaled)

When IPTG is added, the system leaves its initial configuration (25) and starts producing glue. Eventually, the system settles down in a steady glue producing state. On Figure 18(b) we show the dynamical evolution of the system in the presence of IPTG. As we can see the production of the glue begins when the repressor $[c2]$ reaches its final value. During the decrease in $[c2]$, $[c1]$ symmetrically increases. When the production of the glue begins, $[c1]$ rapidly increases to reach its steady value. In this system only a few amount of IPTG is enough to start the process (independently of the initial value of $[c2]$), as we see on Figure 18(a). We also have to notice that the concentration of $[Li]$ and $[Lr]$ follows the same temporal evolution as the $[L]$ complex,this is due to the symmetry of the system. The initial value of $[c2]$ concentration does not have any influence on the final amount of produced glue , but the initial value of $[c2]$ has an influence on the rate of the global process: if we increase $[c2]$, the system will take more time

to begin the glue production.

In this new model, we see that the glue production can be controled by adding IPTG. But, the system is now too sensitive to the presence of the inductor. In the next section we show how this problem can be solved.

4.4 Detailed Quorum Sensing system with integrated Toggle Switch

4.4.1 Sensibility to the initial condition

In the previous section, we have seen that the production process can start with only a few concentration of IPTG inside the system. This characteristics can be a problem from a practical point of view. If we look at the situation from a commercial or industrial point of view it is obvious that it is mandatory to implement a control system in order to avoid a non desired production of glue. In this section we present a theoretical improvement of the actual system which can solve this problem.

4.4.2 An improvement proposal

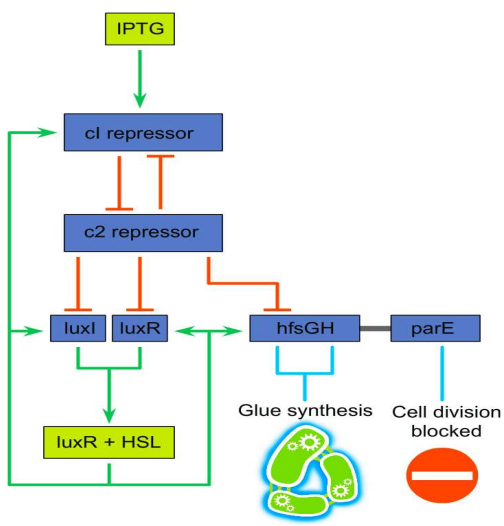


Figure 19: Model with integrated toggle switch

A natural way to improve our system is to use the well known toggle switch system in order to reduce the sensitiveness of our system. The toggle switch system is described in [15].
The schema for this new model is shown on Figure 19. The only difference with the model in the Figure 16 is the presence of a negative regulation of the $c2$ repressor on the $c1$ repressor. The equation 18 is then modified as follows:

$$\frac{d\,[c1]}{dt} = \left[\alpha_6 \frac{[L]^2}{[L]^2 + k_L^2} + \beta\right] \frac{\alpha_2}{[c2]^2 + k_2^2} - \gamma_1\,[c1] \tag{30}$$

Let focus on the two first equations of the system. Assuming that we start from an initial state in which $[L](t=0)=0$, the two first equations reduce to (see the corresponding diagram on Figure 20):

$$\begin{cases} \frac{d[c1]}{dt} = \alpha_2 \frac{\beta}{[c2]^2+k_2^2} - \gamma_1 [c1] \\ \frac{d[c2]}{dt} = \frac{\alpha_1}{[c1]^2+k_1^2} - \gamma_2 [c2] \end{cases}$$

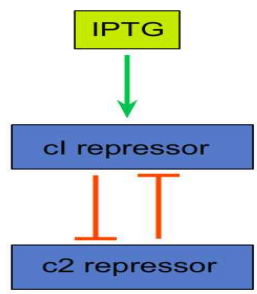


Figure 20: Configuration of the toggle switch for the initial condition

This is similar to the dynamical system described in [15]. The difference is that in the first equation the initial value of β appears. If we choose the parameters α_1 and α_2 so that the system is initially in the bistability region, we can put the system in a state for which $[c2]$ dominates the global dynamics. In that way, we are sure that the system will stay in its initial configuration.
We can see that in this case, we have a strong connection between the initial concentration of $[c2]$ and the concentration of IPTG we have to add in order to switch to the production mode. It means that the system will not start to produce glue if a too small amount of inductor is added inside the system (by accident for example).

4.4.3 Dynamics of the system without inductor

When IPTG is lacking ($\beta = 0$), we find the same behaviour as in the previous model (figure 21), the results from the previous linear stability analysis remain valid. On the Figure 21(a) we see that as previously we need to put a minimal value of $[c2]$ to stay in the initial configuration without inductor. However, it can be seen on the Figure 21(b) that we recover the same influence of the initial value of the $[c2]$ concentration on the amplitude of the initial perturbation.

4.4.4 Dynamics of the system with inductor

In this case we focus on the correlation which has been established between the minimal amount of IPTG which is needed to start the glue production and the initial value of the $[c2]$ concentration. The bifurcation diagram is shown on Figure 22. If we compare with the analog diagram of the previous model (Figure 18(a)), we see that in this new model, there is an obvious correlation between the initial value of $[c2]$ and the quantity of IPTG which has to be added in order to produce the glue. We also notice that the needed IPTG values are higher. On Figure

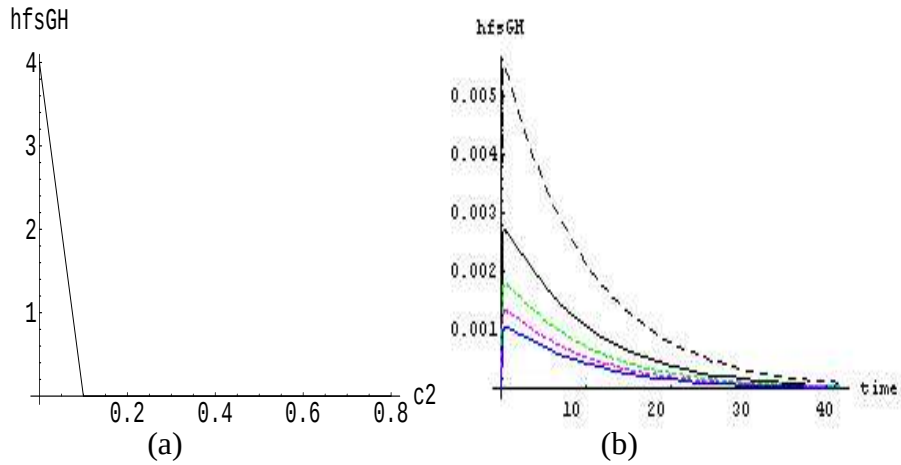


Figure 21: (a): Bifurcation diagram in function of the initial value of $[c2]$ in the system with integrated toggle switch (b): time evolution of the glue concentration in absence of IPTG (integrated toggle switch model)

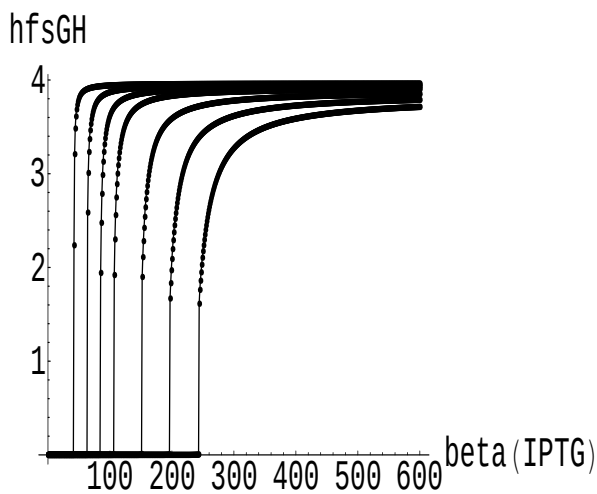


Figure 22: Bifurcation diagram for different initial values of $[c2]$(from left to right: $[c2] = 10, 20, 40, , 50, 70, 100, 300, 500$)

23 we observe the time evolution of the different concentrations when the glue production is activated. About the glue production we have the same behaviour as for the model without toggle switch. The main difference lies in the dynamics of $[c1]$: it increases rapidly to a very high value. This is due to the very high initial concentration of $[IPTG]$ which is added in this case. When $[c2]$ varies we observe the same type of influence on the time evolution as for the case without toggle switch. We have to notice that, like in the previous model, when the glue production process starts, the final amount of glue which is produced is not influenced by the value of IPTG concentration or $[c2]$ concentration.

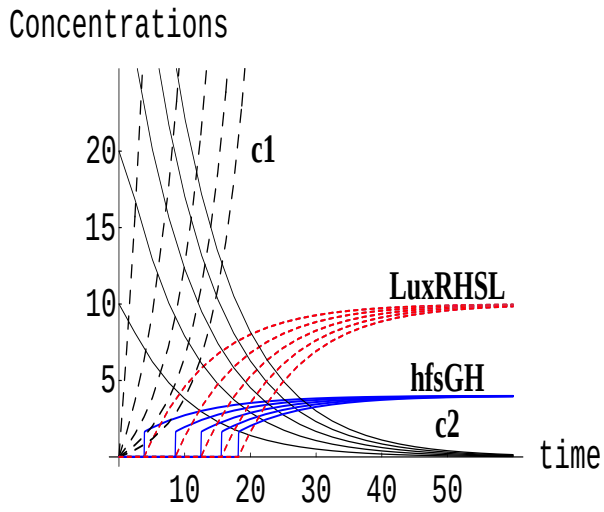


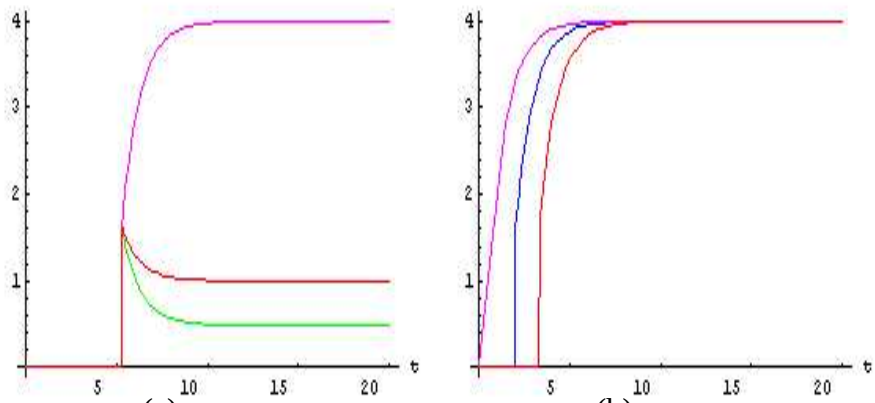
Figure 23: Time evolution of the concentrations for $\beta = 600$ for $[c2] = 10, 20, 30, 40, 50$

4.5 Discussion and conclusion

In this part, we described our biological model from a dynamical point of view. Our aim was to identify the function of the main biobricks components present in the model. We showed that the system is able to switch from a stage of zero glue production to a stage where a steady state of glue production is reached. In our last model, this transition is entirely regulated by the quantity of IPTG inductor added in the system. Lacking such an inductor we observed that the system reaches a steady state for which there is no glue. But we saw that if the initial concentration $[c2]$ is too low, the glue production can start without inductor. In the second configuration of our model, the glue production can start for a very small quantity of IPTG. This can be a problem from a practical point of view. Because of this high sensitiveness, the glue production could start just by accident. In order to increase the robustness of our system in regard to the IPTG concentration, it is useful to improve the current system by adding a toggle switch system between the $c1$ repressor and the $c2$ repressor biobricks. Indeed, with this last improvement, there is a minimal quantity of IPTG which is needed to start the glue production, This minimal quantity is strongly correlated to the concentration of $[c2]$ initially found in the system. Moreover an increase in the initial quantity of $[c2]$ leads to a diminution of the disturbance amplitude in the glue concentration if there is no IPTG. In the presence of inductor, the decrease in the initial concentration $[c2]$ leads to an increase in the growth rate of the glue production.

From a more theoretical point of view, we can also address the following question: for this global system, which genes should we choose in order to obtain an optimal equilibrium between robustness and glue production? To answer that we studied the behaviour of our theoretical model with integrated toggle switch when the parameters values (such as activation concentration, degradation rates which are intrinsic to the genes) are modified. Firstly, an increase in the $[c2]$ degradation rate (γ_2) leads to an increase in the glue production rate when

IPTG is added. Secondly, when we lower the value of the k_{Lr} and k_{Li} constant, an increase in the glue production rate is also observed (see Figure 24(b)). Thirdly, a decrease in the k_1 constant value leads to a crash of glue production even in presence of IPTG. As we said in the previous section, the total amount of produced glue depends also on the intrinsic characteristics of the biobricks: a decrease in the γ_{parE} coefficient leads to a lower quantity of glue when the stationary state is reached (see Figure 24(a)), α_7 has also a similar influence.



(a) (b)

Figure 24: (a): evolution of the glue concentration for different values of the γ_{parE} coefficient: (red: $\gamma_{parE} = 0.01$, purple: $\gamma_{parE} = 0.02$, green: $\gamma_{parE} = 0.007$), with $[IPTG] = 2000$ and $[c2]\,(t = 0) = 50$(b): evolution of the glue concentration with different values for the k_{Lr}, k_{Li}:blue: $k_{Lr} = k_{Li} = 0.00001$, red: $k_{Lr} = k_{Li} = 0.001$, purple: $k_{Lr} = k_{Li} = k_2$, with $\gamma_{parE} = 0.02$

5 Perspectives

GluColi: a future alternative to toxic glues?

Glue has become a material of everyday life but we have to know that common glues contain Volatile Organic Compounds (VOC). These substances are air pollutants and toxic. [19] [20] [21] For example, cyanoacrylate commonly sold under trade names like super glue can cause various toxic issues depending on their precise composition, [22].

According to Yves Brun, Indiana University biologist, concerning *Caulobacter crescentus*'s glue: "This natural, non-toxic glue [...], has the strongest adhesion force of any known natural material. Unlike commercial super glues, which are often toxic, it sticks well under water, even salt water, and, thus, has a wide range of potential applications ", [13].

Indeed, succeeding in the production of our GluColi could offer a wide range of useful applications. That could cover fields ranging from medical purposes to ship repairing and automotive or aeronautic industry.

Bone reconstructive surgery has lead to a large demand for bone graft, [26], [27]. Consequently, synthetic bone−graft substitutes have been developped with mixed success and surgical acceptance. Bone engineering has still lots of challenges to cope with. Surgical adhesives are part of this field of research but toxic issues are sometimes reported. Looking for new adhesives is today of great interest [28]. In that, GluColi may play an interesting role.

GluColi and a free-antibiotics alternative

There is no Ethic Comity at the Institute of Molecular Biology and Medecine. However the governing body of the ULB supported us in the achievement of our project.

In this work, we intended to be as far as possible aware of some ethical issues, in particular the use of antibiotics and the toxicity of the current adhesives. Antibiotics are used to select all types of plasmidic vectors in bacteria which is a significant concern in industrial cultures producing recombinant proteins or DNA. As plasmid-free cells grow faster than plasmid-carrying cells, the yield and the production reproducibility of recombinant molecules are significantly lowered in the absence of plasmid selection by the appropriate antibiotic.

To overcome the plasmid instability, many antibiotic resistance genes are used as selectable markers in fermentation processes. Antibiotics are expensive and they pose safety problems: they contaminate the production product. [23] [24] The Belgian company Delphi Genetics has developed a strategy called Staby^{TM} system which relies on the use of poison-antidote genes.
The StabyTM system is based on the poison-antidote ccd module. Poison-antidote modules are found in natural plasmid in which they serve to the plasmid maintenance. The CcdB protein (poison) is cytotoxic and poisons DNA-gyrase complexes. Expression of this gene in the

absence of its cognate ccdA antidote leads the death of the bacteria. The product of the ccdA gene (antidote) antagonizes this toxic activity by forming a poison-antidote protein complex. If a plasmid carrying the ccd module is lost at cell division, the plasmid-free bacteria contain poison and antidote proteins in their cytoplasm. Since the antidote is unstable and degraded by a host protease, the poison will be free and able to poison DNA-gyrase complexes. This will lead eventually to cell death.

In theStaby^{TM} system,the antidote gene (ccdA) is introduced in the plasmid DNA under the control of a weak constitutive promoter: the mob gene promoter, which originates from a broad host range plasmid (pBHR1). On the other hand, the toxic gene (ccdB) is introduced in the *E. coli* chromosome of the bacteria. Expression of the poison gene is under the control of a promoter strongly repressed in the presence of the plasmid. Practically, 100 % of the bacteria will carry the vector. If they lose the vector, they will not obtain a growth advantage, but will die. Upon induction, all the bacteria will start producing the recombinant protein. It will lead to higher yields of the recombinant protein and less background caused by unwanted proteins. Therefore, higher plasmid stability means higher protein of interest production.

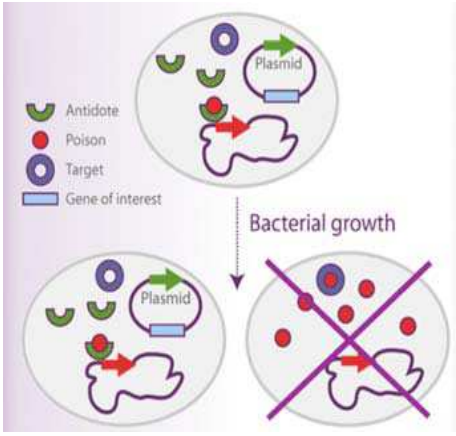


Figure 25: Staby^{TM}; www.delphigenetics.com

We think it could be relevant to implement this antibiotic free expression strategy in our project. This is the reason why we designed 2 bricks containing the ccdA gene. In order to carry out transformation, bacteria should be furnished with the ccdB gene in their genome. (i.e. by Delphi Genetics)

6 Conclusions

In this report, we describe a new way to produce a strong bioadhesive. We transferred part of Caulobacter crescentus's holdfast system in *Escherichia coli* which produced an adhesive and was able to stick.

We conducted a few experiments in order to characterize the glue. The glue did not show all the expected adhesive properties. However they could be improved by purifying the glue.

In addition, we designed a biological circuit that would allow us to integrate the glue synthesis into a useful application. The circuit described in this report should activate the glue synthesis only in specific spots where repairs are needed. This is achieved by attracting GluColi bacteria to the break and inducing the glue synthesis once the adequate cell density is reached (i.e. thanks to quorum sensing).

For the mathematical modeling part of our project we built a theoretical system which is able to produce the glue. However, we found two main improvements to increase the robustness of the system. Firstly, by considering the complete dynamics of the Quorum Sensing system, we showed that it is possible to controle the glue production only with the quantity of inductor. Secondly, we increased the robustness of our system by adding a toggle switch. This last improvement establishes a connection between the initial concentration of the $c2$ repressor and the IPTG value we put in the system to start the glue production process.

GluColi has been created, but still needs more research. In particular, further research should focus on:

- the analysis of the composition of the glue,
- the understanding of the differences between the characteristics of our glue and the original glue from *Caulobacter crescentus*,

Indeed, the glue should be resistant to water in order to repair ship's hull without the use of toxic chemicals.

Finally, to allow a precise characterization of the system, the modeling should be refined once the theoretical circuit is implemented in *E. coli*.

A Homolog genes

A.1 Homolog genes similarities

Comparison of sequence similarities between genes implied in holdfast biosynthesis in *Caulobacter crescentus* [37] and their homologs in *Escherichia coli* [36] after sequence alignment [35].

Genes	Number of matches	Percentage of total length	Percentage of common segment
hfsA−wzc	291	13.1	44.7
hfsB−wzc	623	27.9	43.2
hfsC−wzy	451	34.0	38.3
hfsD−wza	164	14.1	23.5
hfsE−wbaP	304	19.4	21.7
hfsF−wzx	461	30.9	38.0

A.2 Sequence alignment

Sequence alignment between homolog genes in *Caulobacter crescentus* CB15 genome (GenBank id: 19172958) and *Escherichia coli* K30 (GenBank id: 8469170). Stars indicate exact matches between sequences.

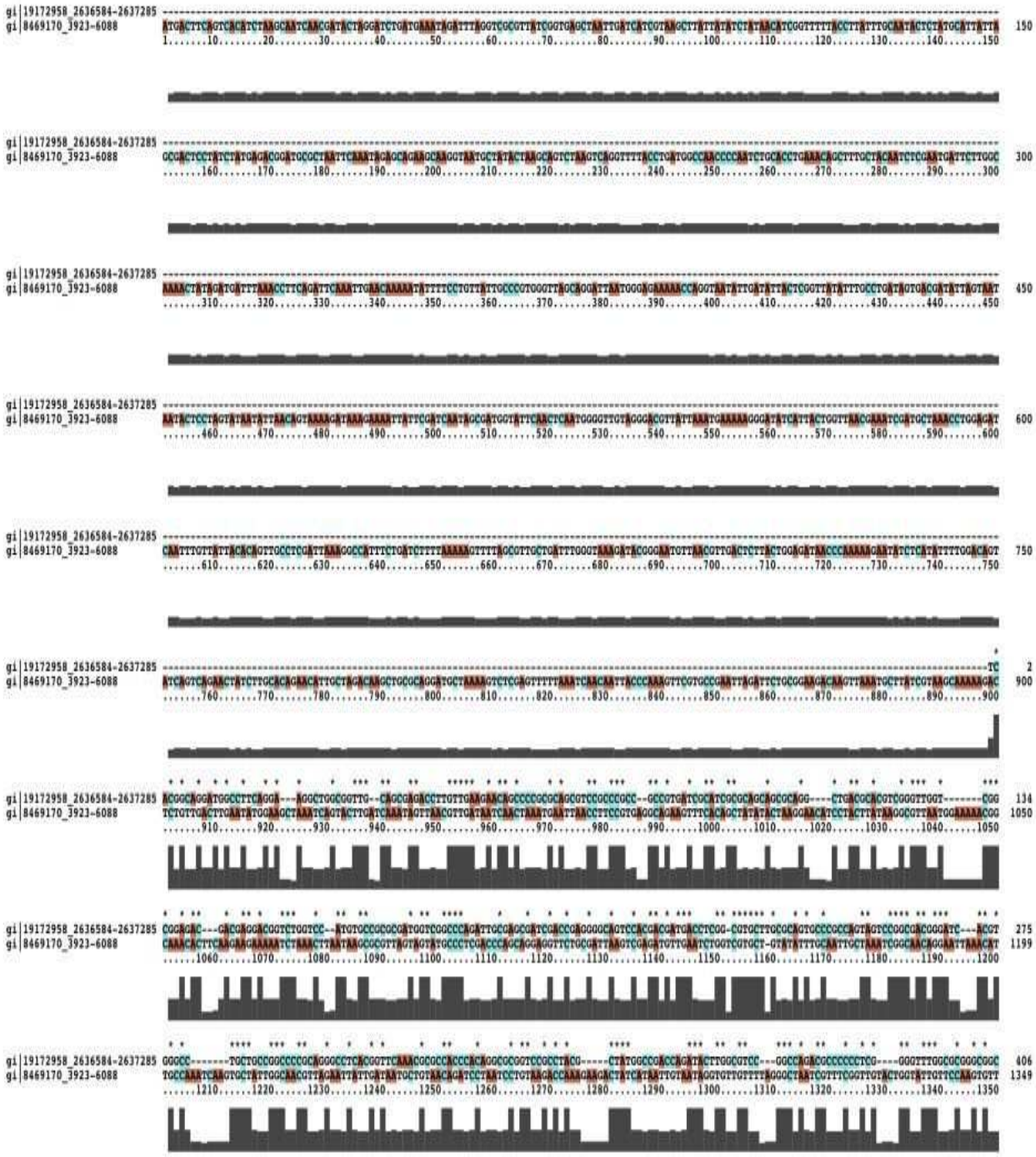


Figure 26: hfsA−wzc,part 1

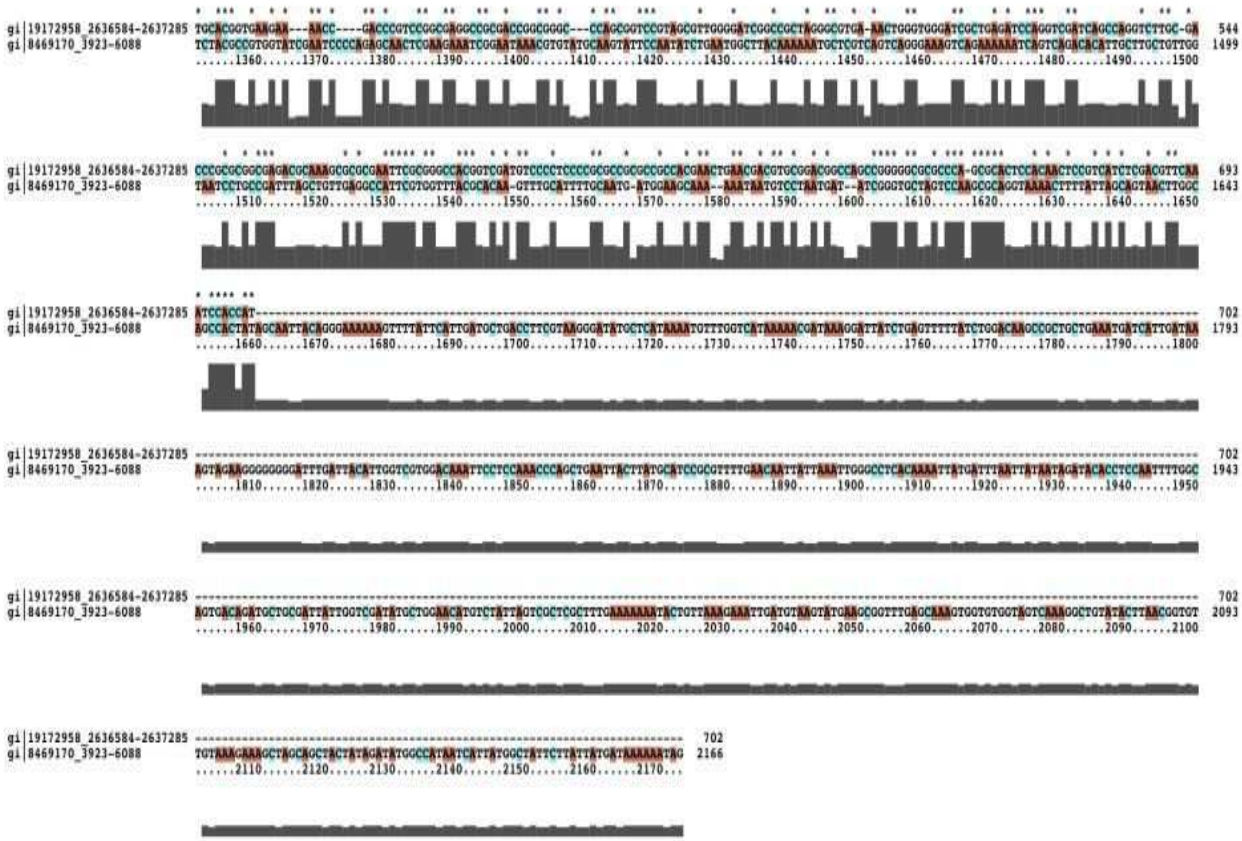


Figure 27: hfsA−wzc,part 2

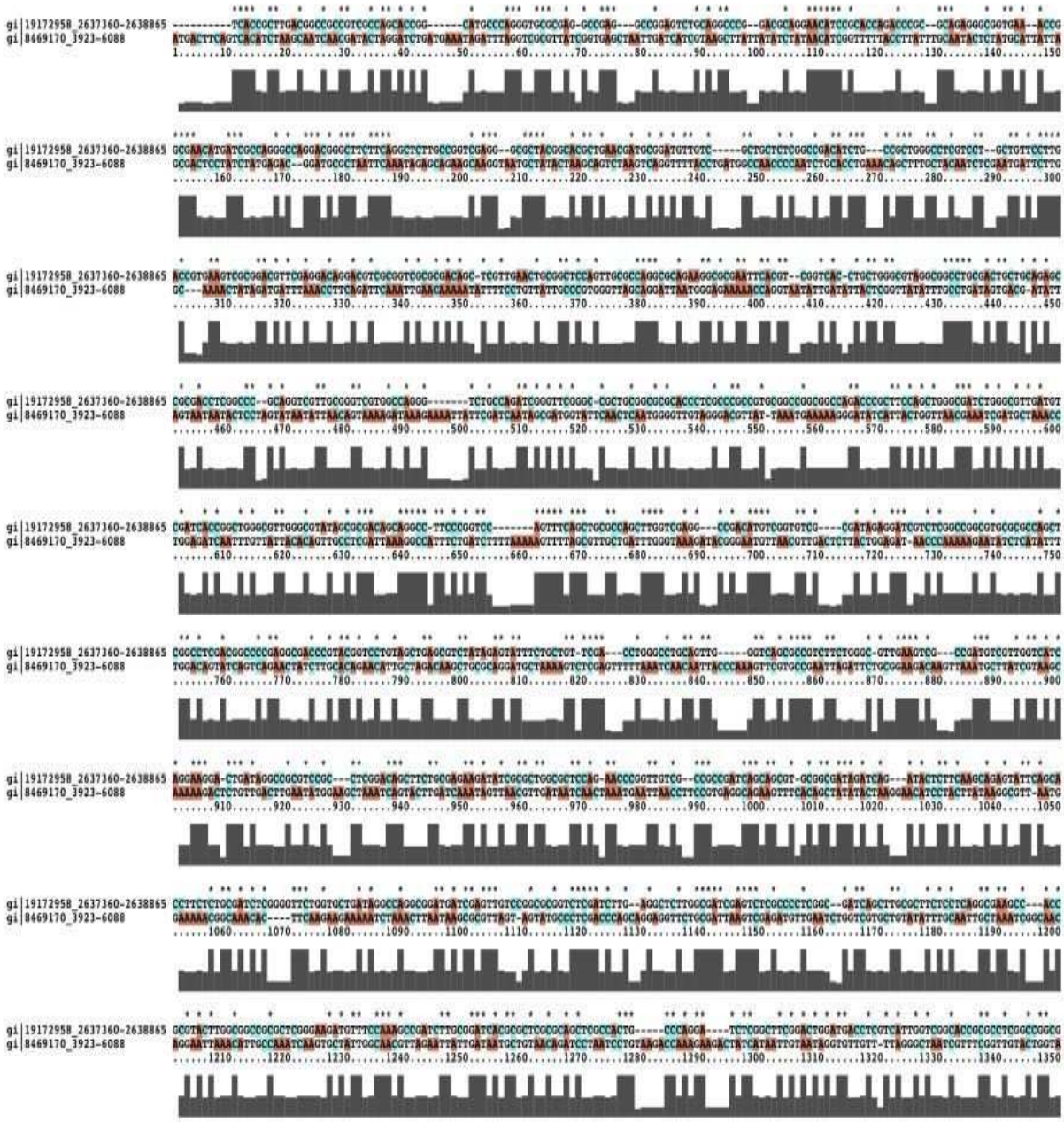


Figure 28: hfsB−wzc, part 1

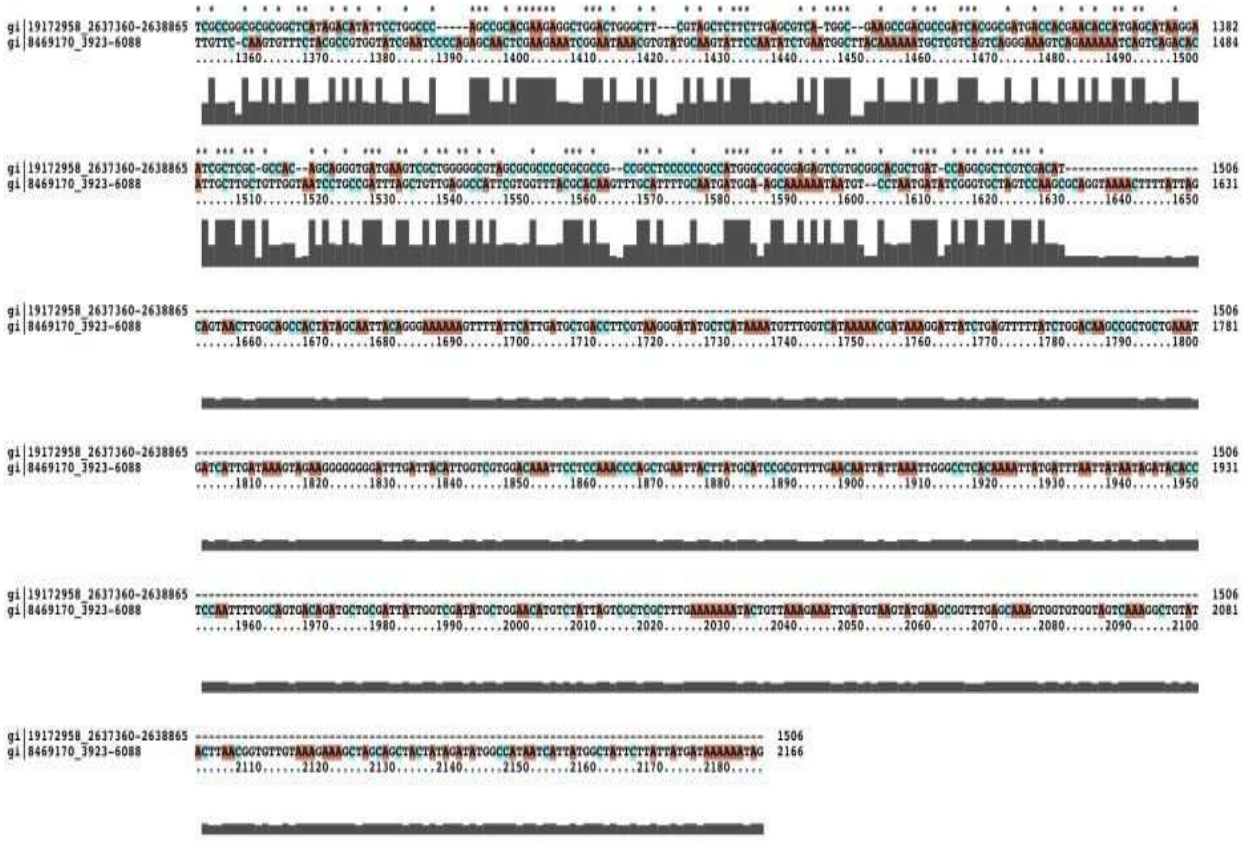


Figure 29: hfsB−wzc, part 2

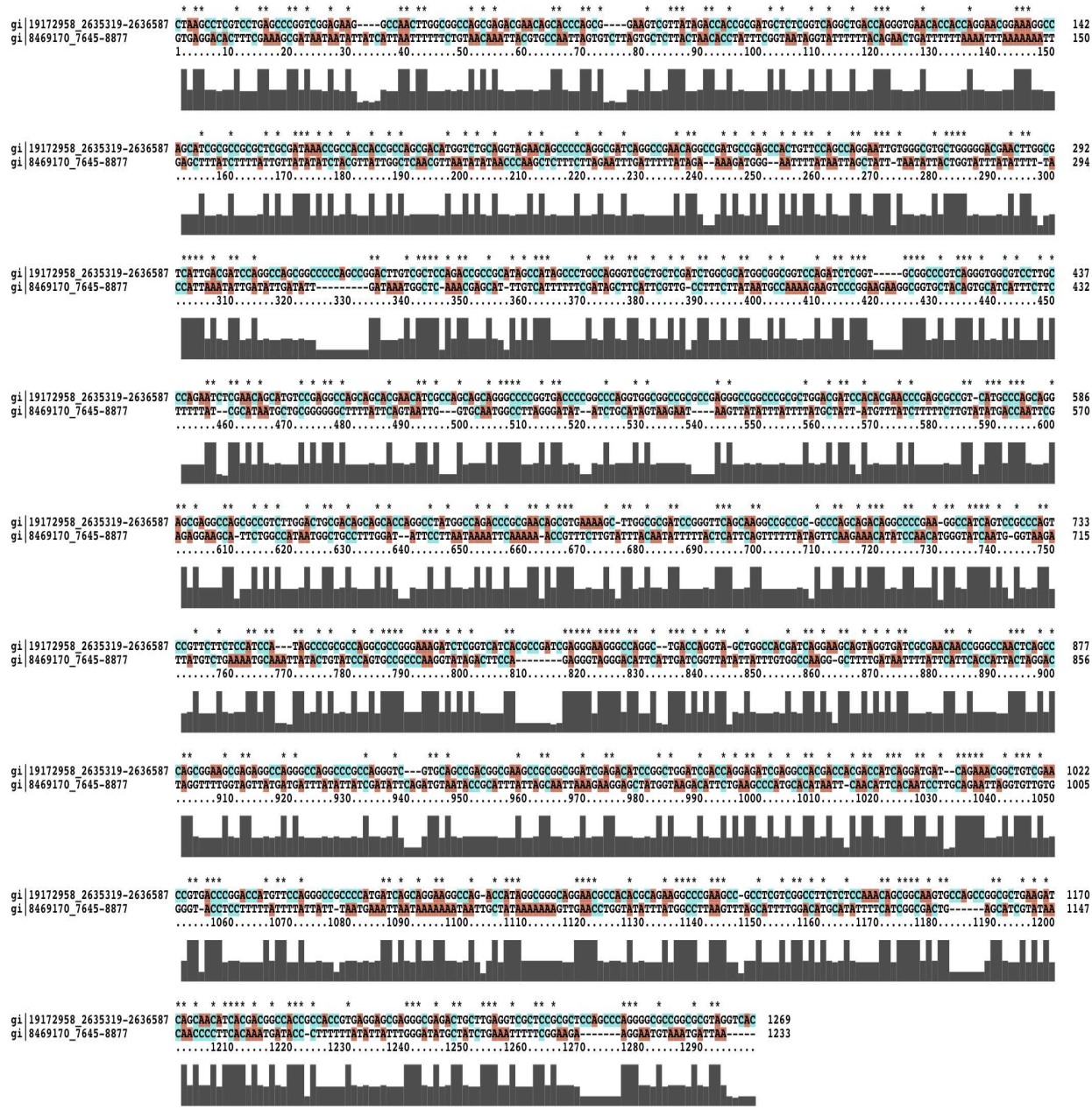


Figure 30: hfsC−wzy

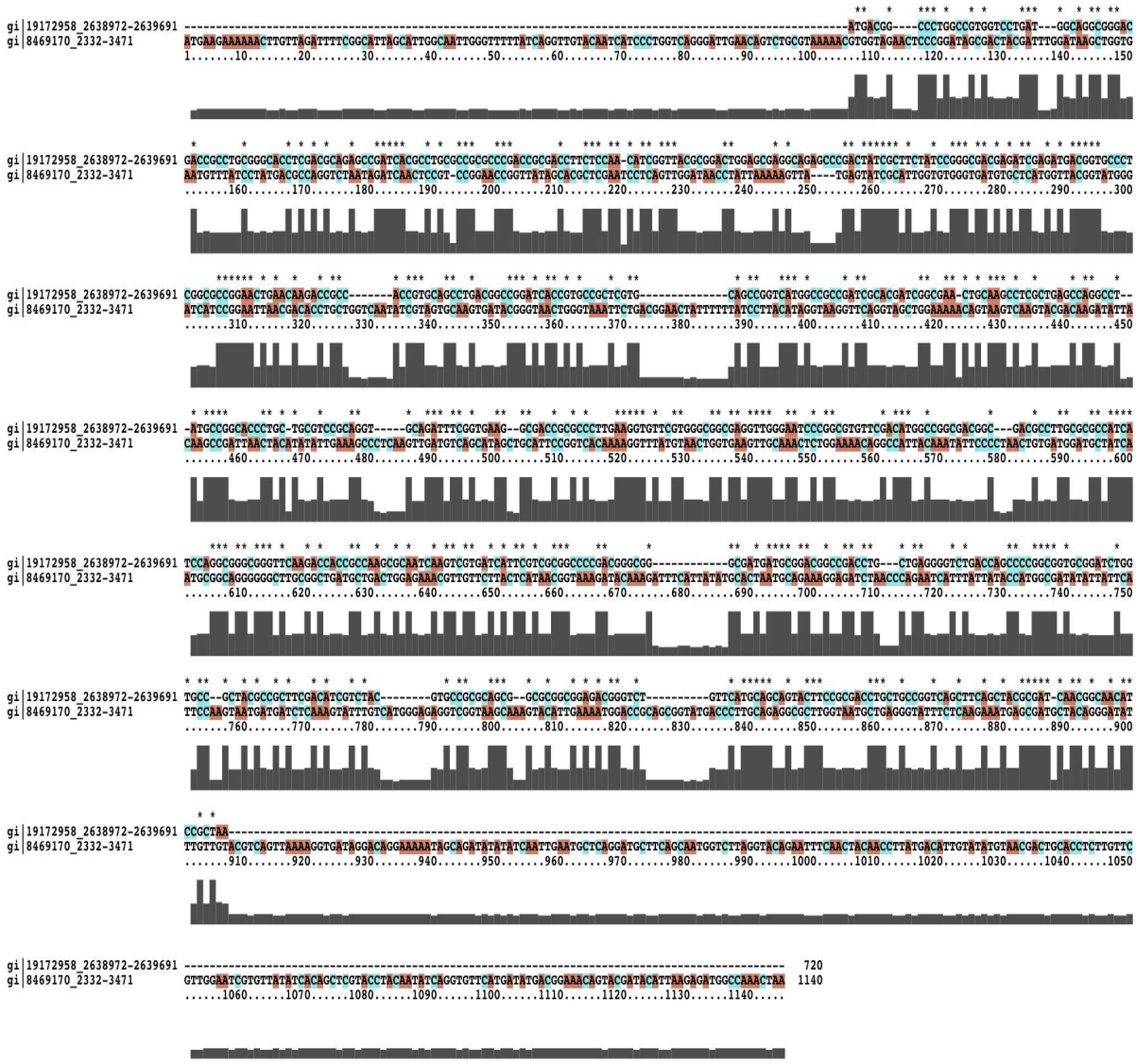


Figure 31: hfsD−wza

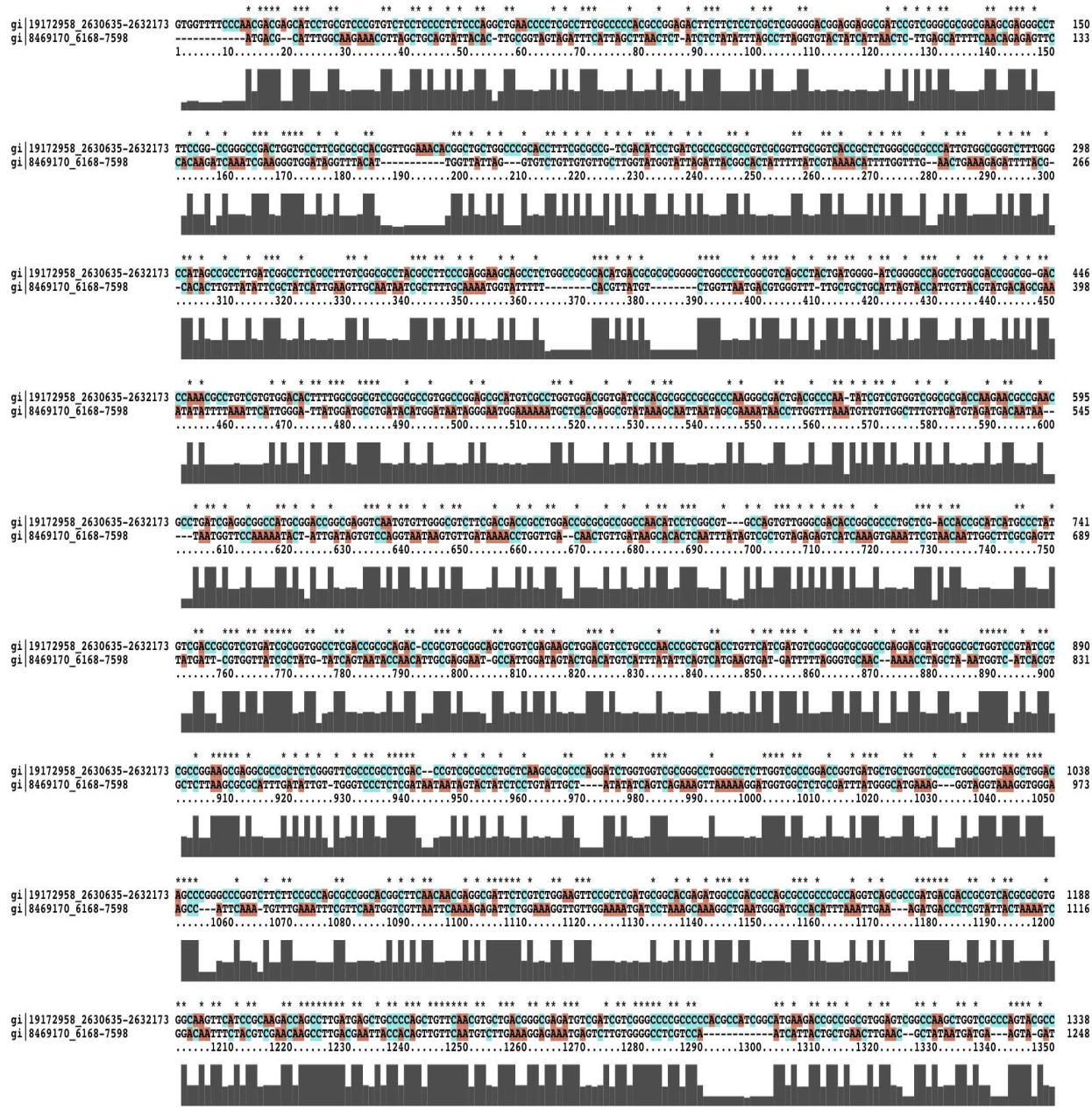


Figure 32: hfsE−wbaP, part 1

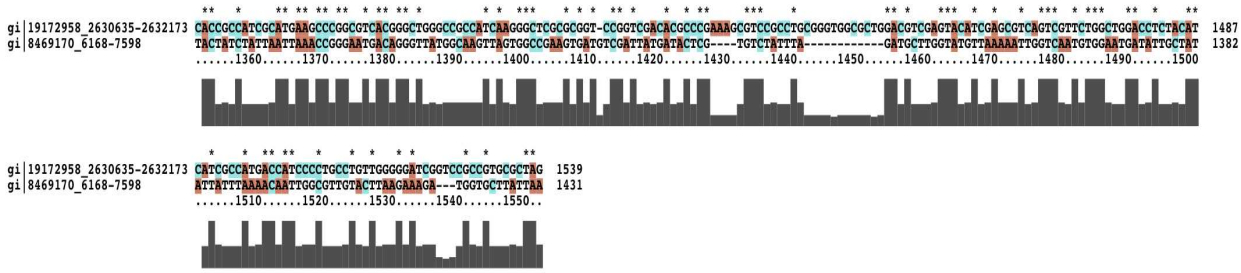


Figure 33: hfsE−wbaP, part 2

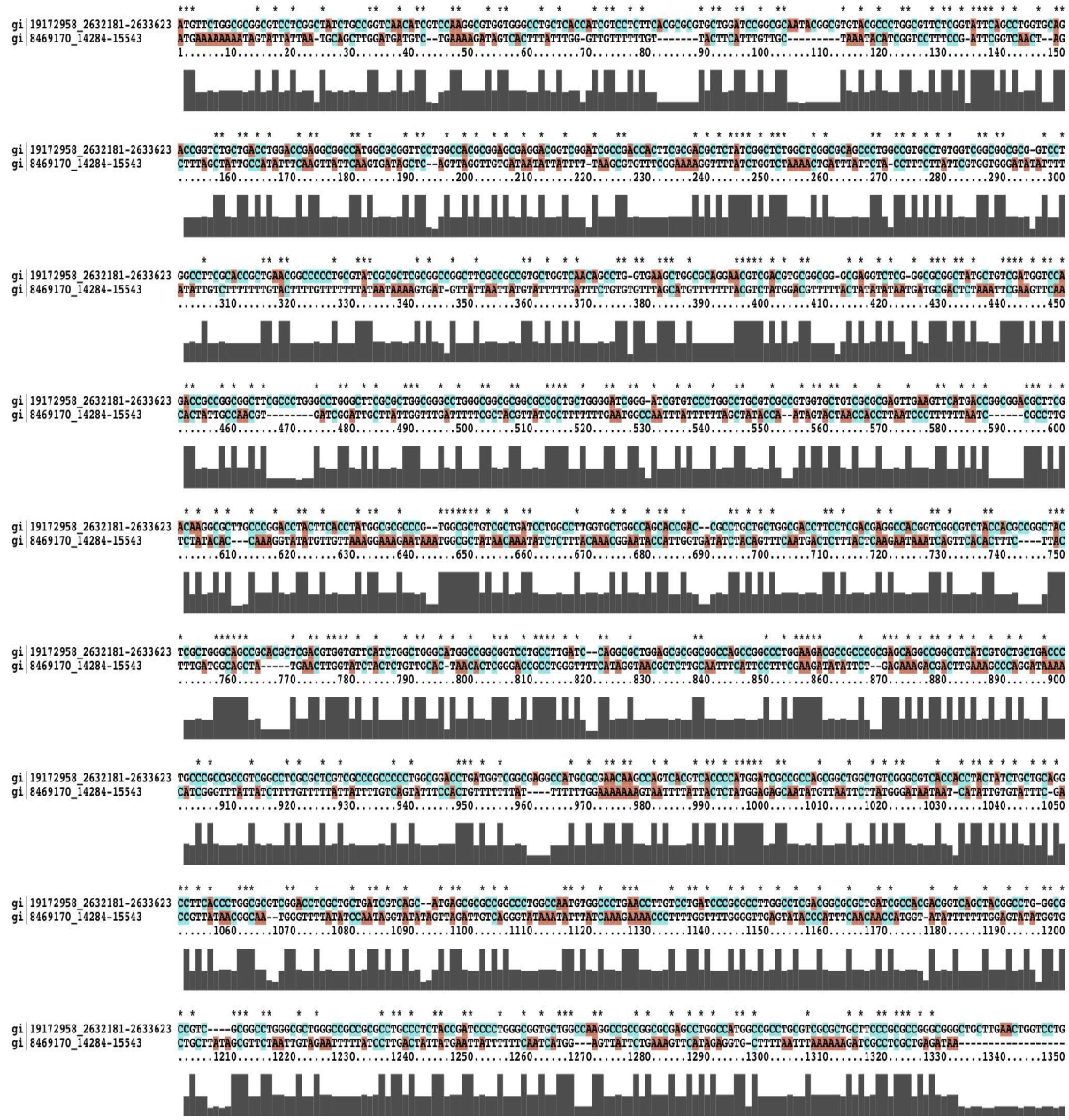


Figure 34: hfsF−wzx, part 1

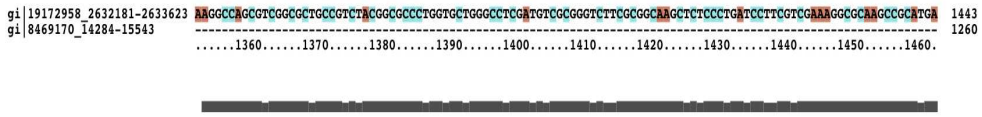


Figure 35: hfsF−wzx, part 2

B Sequence optimization and Standard compliance

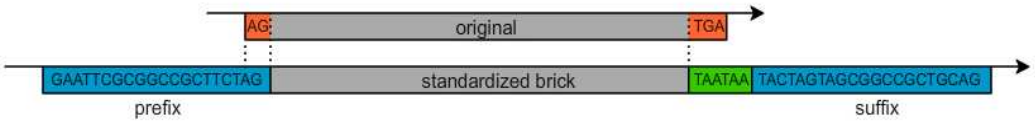


Figure 36: Modifications applied to the sequences in order to be compliant with BioBrick prefix and suffix. Blue and green elements are added to the gene sequence, orange parts are removed

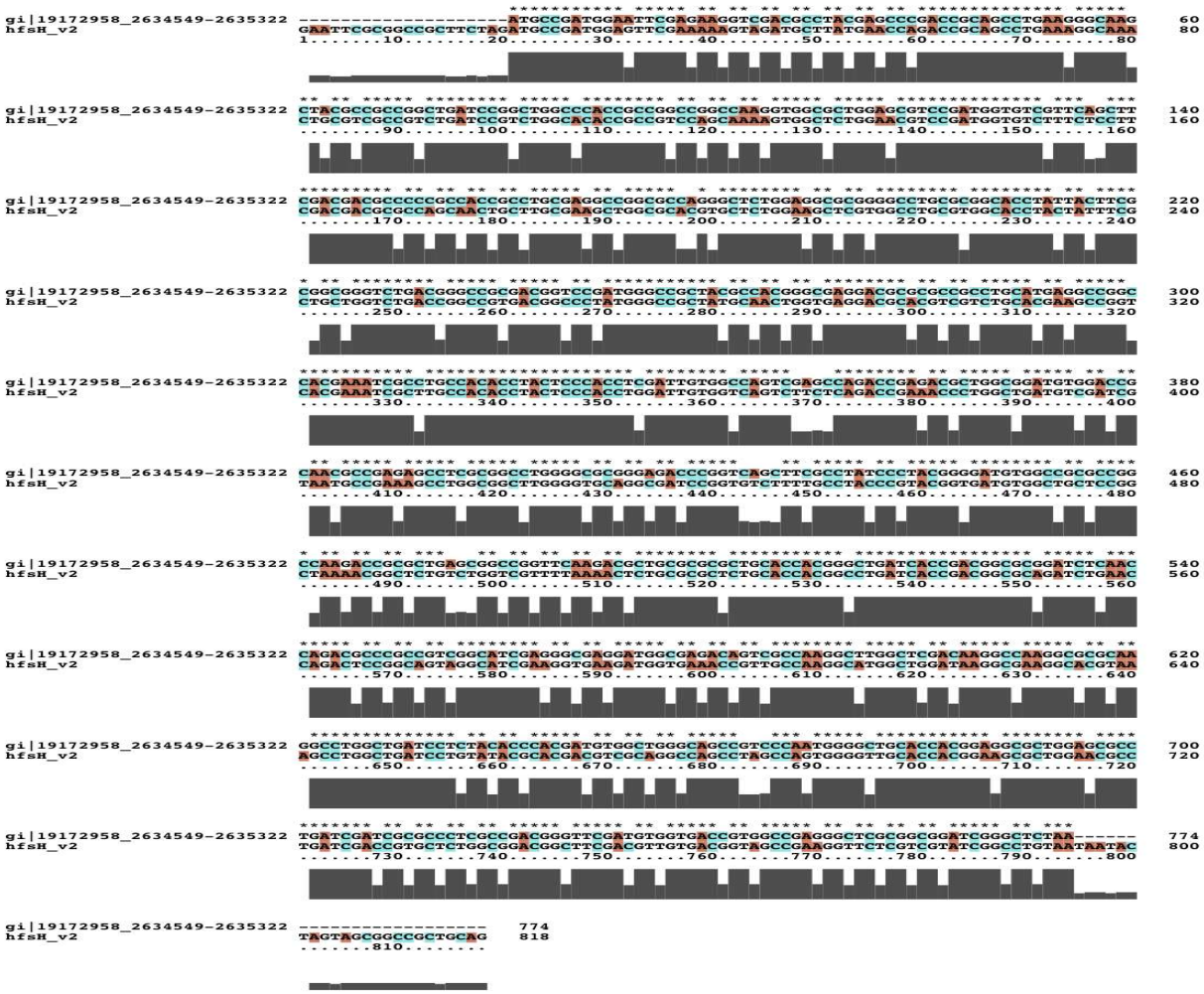


Figure 37: Sequence comparison between wild-type hfsH from *Caulobacter crescentus* [37] and our optimized gene for *Escherichia coli*. This sequence includes addition of prefix and suffix as well as removal of restriction sites recommended by BioBrick assembly standart 10.

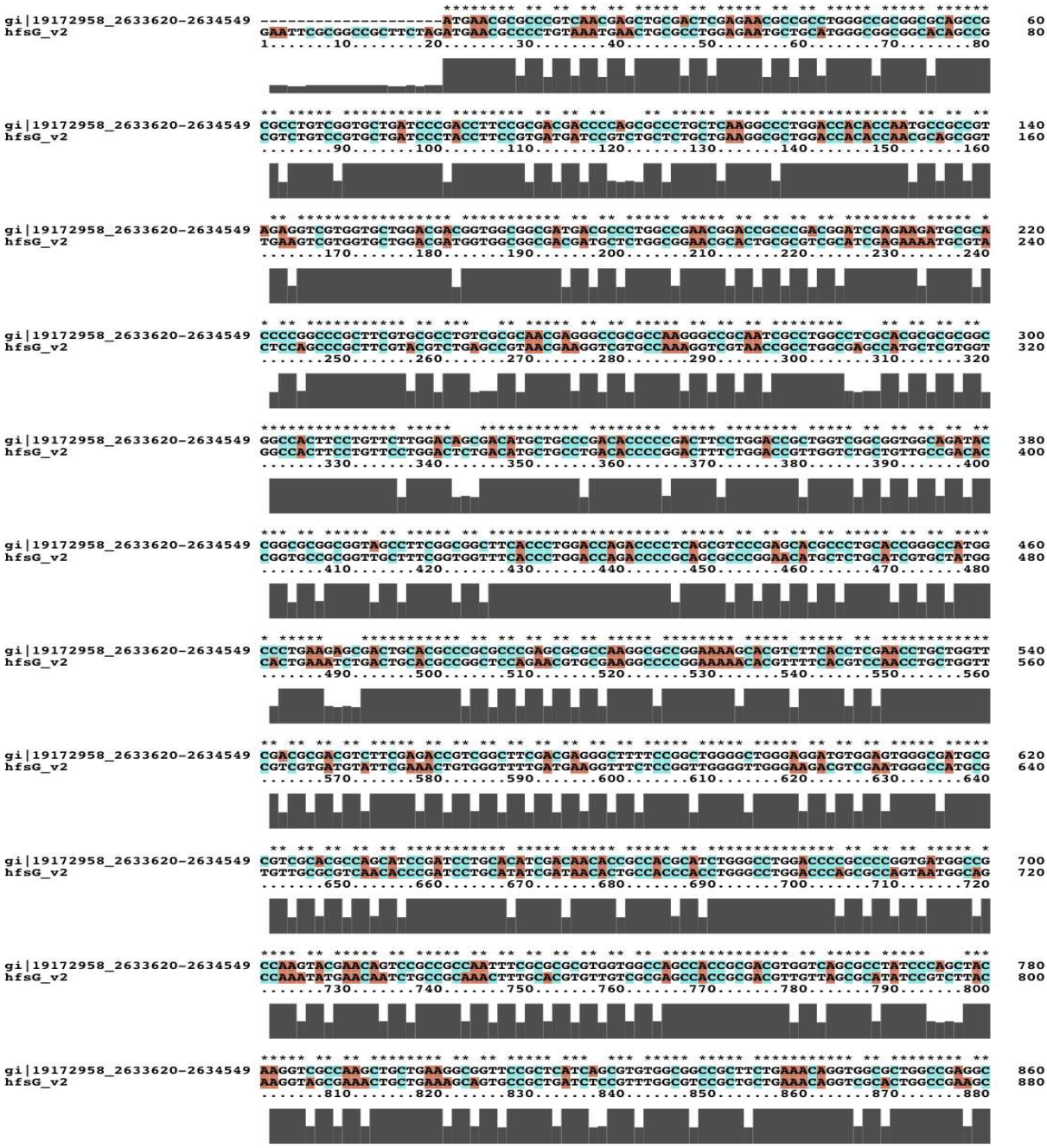


Figure 38: Sequence comparison between wild-type hfsG from *Caulobacter crescentus* [37] and our optimized gene for *Escherichia coli*. This sequence includes addition of prefix and suffix as well as removal of restriction sites recommended by BioBrick assembly standart 10.

C Bacterial Chemotaxis

In order to respond to changing environment, bacteria developed sensory systems. One way to respond may be to move. And that is achieved by the action of small phosphorylated molcules binding to the flagellar rotor. There are many different sensory pathways in eukaryotes as well as in prokaryotes. Prokaryotes commonly use a histidine−aspartate phosphorelay (HAP) system consisting of a histidine protein kinase (HPK) and a response regulator (RR). The environmental signals can be concentrations of nutrients or toxis, oxygen levels, pH, osmolarity, light,...

The HAP systems works as follows: the HPK (e.g. in *Escherichia coli*, CheA interacting with

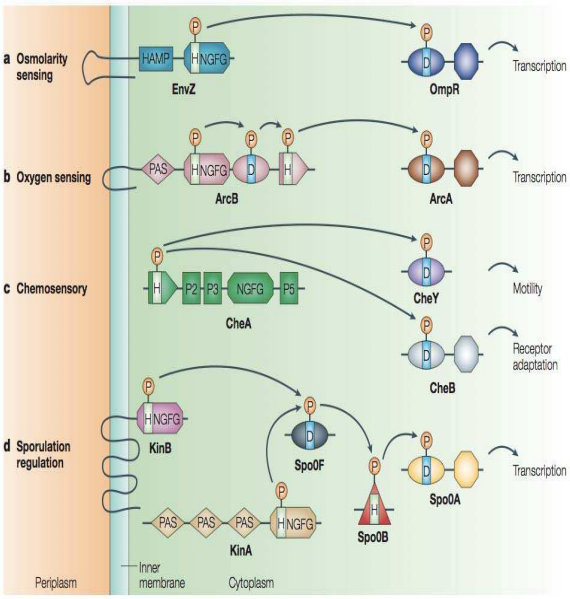


Figure 39: The domain organization of selected histidine-aspartate-phosphorelay systems. [30]

transmembrane receptors) is trans−autophosphorylated on a His residue. The phosphoryl group is then transferred to an Asp residue situated on a RR that becomes therefore activated and able to respond to the signal. In the chemosensory pathway of *E. coli*, there are two RRs competing for the phosphoryl group: CheY and CheB. The first one controls flagellar motor switching: by binding to the flagellar motor, it causes the switch to clockwise rotation, the cell tumbles and swims off in a new direction. The second one is a methylesterase that controls the adaptation of the methyl−accepting chemotaxis proteins or chemoreceptors (MCPs). [30]

E. coli possesses at least four MCPs: Trg for ribose and galactose; Tar for aspartate; Tsr for serine; Tap for peptides. A fifth one may be a redox detector. [31]

In the absence of gradients of chemoattractants, the cells walk randomly. They evaluate the changes in concentrations by comparing the number of bound receptors over the past 1s with

the number during the past 3s. When this number increases they are expected to rotate counterclockwise and therefore be propelled forward. When they move away from the cells cluster, they tumble in order to come back. The tumble rate and the clusters boundaries are thus determinated by the sensory memory of cells. [32]

D Quorum Sensing

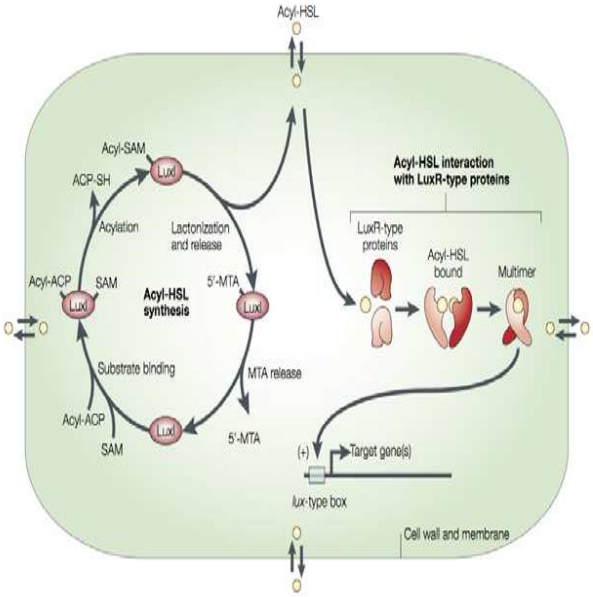


Figure 40: Model of acyl−HSL quorum sensing in a single generalized bacterial cell. [30]

Quorum sensing was first identified in *Vibrio harveyi* and *Vibrio fischeri*, two bioluminescent marine bacteria, and was then identified as a highly conserved regulatory system among the Proteobacteria.
Quorum sensing is a community behaviour that enables microorganisms to communicate, perceive and monitor population density, and modulate gene expression by producing and responding to diffusible signal molecules. There are different signalling systems but the bestcharacterized mechanism is the one relying on the acylated homoserine lactones (acyl−HSL, e.g. $3OC_6$HSL).
Acyl−HSLs are synthesized (at a low level) by acyl−HSL synthases like the LuxI protein. These signal molecules are then removed from the cell by diffusion. When the population increases, the signal concentration elevates and at a threshold level the acyl−HSLs come in interaction with transcription factors like the LuxR proteins. That multimer is now able to activate the quorum sensing−regulated genes by binding the Lux box. These target genes can include the LuxI coding region in order ton make a positive feedback loop. [33]
Reaching the critical level of quorum sensing molecules enables therefore a coordinated expression of specific genes, for example the luminescence genes in V. fischeri or the competence in S. pneumoniae. This cell−to−cell communication also plays a role in biofilm development. [34]

We implemented the LuxI−LuxR mechanism into our GluColi in order to monitor the glue production. So the target genes are in our case hfsG and hfsH coding for the Caulobacter enzymes needed to synthesize the glue.

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