**Dr. Coli**

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**Sensor**

The sensor system can detect disease markers. When it does, it activates the drug production and the filter.

Since detecting real disease markers would have led us too far, Dr. Coli is a proof of concept. So the system was replaced by a simpler one where only the filter is constitutively produced. This represents the filter and the drug production. Disease detection is simulated and the response is released by adding CO. This way the filter and drug production are activated.

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**Drug**

In a medical application the input would be a certain disease marker that results in the production of a specific medical drug with GPF.

If we let Dr. Coli act as the sensor, there are two major questions: Is Dr. Coli sensitive enough to respond to the presence of the disease marker? Can Dr. Coli produce a drug in a proof of concept and we replaced these specific medical drug with GPF.

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**Memory**

The memory is a vital part of Dr. Coli. It prevents the activation of the timer and cell death until the first disease markers are detected. This way Dr. Coli can be produced and enter the body without killing itself.

The memory consists of two repressors: cFP22 and cH34. cFP22 represses the cH34 GPF transcription and cH34 GPF represses the cFP22 transcription. The system is like this: if one repressor is high or the other is low, the system is active. When the repressor is low, the system is inactive. During H34 overproduction of cH34, which represses cFP22 as well, this happens when disease markers are detected. The memory turns on and at low cell concentrations, enables the cell death system.

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**Cell Death**

When no disease markers have been detected for some while, Dr. Coli is no longer needed and the cell death system will be induced.

Initially, this system is disabled by cFP22. cFP22, from the memory, represses the hybrid promoter so no CFP22 is produced. When the bacteria arrive disease markers for the first time, cFP22 is repressed and cH34 is produced. cH34 is also produced by cH34 when the disease markers are detected. cH34 directly activates the cell death system. The hybrid promoter functions as a doxide gate: once cH34 is produced, cFP22 and cH34 are produced at the same time. cH34 does not only activate the cell death system but also cFP22, thus activating the cell death system. cFP22 and cH34 are produced at the same time. The cell death system is active if the cell is in the presence of disease markers.

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**Filter**

The filter was put in our system to filter out background signals. It ensures that only a certain concentration of a signal can activate the InverTimer and reset.

The filter will only produce T and T2 when disease markers are detected for a sufficiently long time. The reset and the InverTimer that react on the filter. The concentration of T needs to be high enough to affect the InverTimer and reset. When the signal is too short the reset will not be active, and the InverTimer will not be reset.

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**InverTimer**

The InverTimer starts timing when the concentration of the signal is above the threshold.

When the timer reaches a critical point, the signal will activate cell death. To make the timer more robust, the input signal or disease markers has to pass the filter.

When no disease markers are detected, CFP22 is produced. CFP22 forms a complex at a certain concentration. When the concentration of CFP22 is high enough, the gene is activated.

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**Reset**

This system resets the timer when disease markers are detected again after a disease-free period.

When disease markers are no longer detected, the system will quickly decay. If this continues, it will eventually trigger cell death. During a disease-free period, when HSL concentrations are low, Dr. Coli must be able to react itself to the disease markers. This is done by producing antibiotics which blocks HSL, resetting the timer.

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**Modeling**

Modeling is a technique that is applied to many different kinds of systems. Our model is based on ordinary differential equations (ODEs) that describe translation, transcription, diffusion reactions, occurring in the system.

Based on the design of the system, simulations can be done to check whether the system is working as intended. Without these simulations, we wouldn’t have found the errors in our original design, e.g., the memory control wasn’t working. In the lab, it is much harder to test the system. Therefore, we have to simulate it on the computer. Simulation is crucial for designing the system, as it allows us to test our design and to optimize the system before building it in the lab. This will save a lot of our precious time and has effectively reduced the number of design errors.

To perform these simulations, we extensively used CellDesigner and Simbiology. Without these numerical solutions, it’s practically impossible to solve the system of ODEs. CellDesigner is a visual simulation tool, whereas Simbiology is a modeling tool that allows us to separate theory from program code and allows us to model the system separately before linking it at the entire system. This methodology had a positive effect on the efficacy of the modeling team because different subunits could work on different projects.

After finishing the entire system, a sensitivity analysis showed that if we did not critically dependent on many parameters and hence is robust. This is necessary because for many of these parameters the exact values are unknown and the model is critical for the success of the system. For a parameter, the less important is a precise value for this parameter is.

We are proud to announce that we have written our own software tool: Multi Cell toolbox which allows the simulation of the interactions between different cells.

The figure at the left is the MATLAB Simbiology representation of the Dr. Coli system. The subplots: substrate, memory, filter, cell death, novel (production) and InverTimer are indicated by a blue square.

In the representation five parts are used:

- gene
- mRNA
- antisense mRNA
- degradation product
- protein
- reaction
- reaction arrow from a reactant to a or reaction product
- reaction arrow indicating a catalysis