

Genetic network generating spatial patterns through cell-cell communication and controlled information processing

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Introduction

Biological systems are unique in their ability to combine information and energy to generate complex entities. Genetically encoded networks drive many of these patterning processes. Furthermore, developmental studies have highlighted the importance of gradient formation and cell-cell communication for the generation of cellular patterns in the early stages of life. It has been shown that simple networks can form both static and dynamic patterns, but a system whose pattern formation is dependent on combinations of multiple signals has yet to be demonstrated. Here we address this by designing a network, involving two different quorum-sensing based signaling mechanisms. Using a pre-defined set of rules which was selected on its ability to generate spatial patterns, the cell can then express its final state by emitting red or green fluorescence and transmit its state to its neighbors. In the future this system can be applied to not only understand many aspects of fundamental biology but also to provide applications in medical diagnostics and therapeutics.

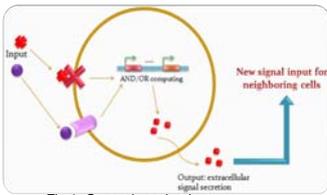


Fig.1: General mechanism

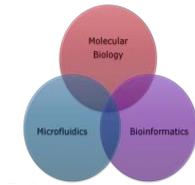


Fig.2: An interdisciplinary approach

Plasmid construction and Characterization

Our system combines a new plasmid and a pre-existing system (R. Weiss et al., Nature, vol.434, April 2005) which detects different levels of the Lux-associated quorum sensing molecule and gives a specific response according to the concentration received. To allow integration of a second signalling system (Rhl), the Weiss configuration was modified so that *rhlII* (Rhl-associated quorum sensing being our second) is co-expressed with GFP.

The new plasmid contains the additional parts of the genetic circuit, according to fig. 6a). Also, we added an extra copy of the *lacI*m gene so that it can be tested independently.

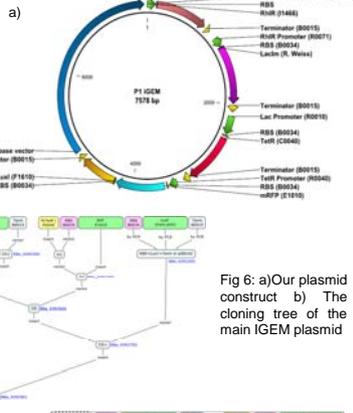


Fig 6: a) Our plasmid construct b) The cloning tree of the main IGEN plasmid

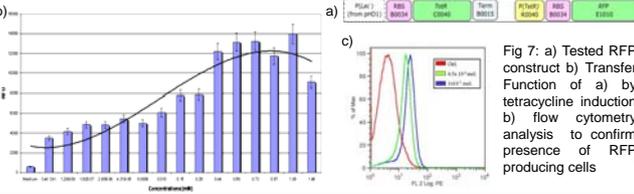


Fig 7: a) Tested RFP construct b) Transfer Function of a) by tetracycline induction b) flow cytometry analysis to confirm presence of RFP producing cells

Team EPFL

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Lausanne is a beautiful city near the Leman Lake and the Alps, in Switzerland. We are from the Life Sciences department, and we enjoy a beautiful campus, with infrastructures in many engineering departments, which we take advantage of for interdisciplinary projects.

Acknowledgements

We thank the Faculty of Life Sciences EPFL for their support.



Genetic circuit

We designed our genetic circuit to be able to respond variably depending on the input received. As there are three channels of possible input arrival (chip design), we programmed rules to be able to respond accordingly. Below two of the four routes of action within the circuit are shown.

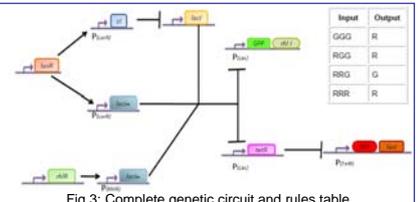


Fig.3: Complete genetic circuit and rules table

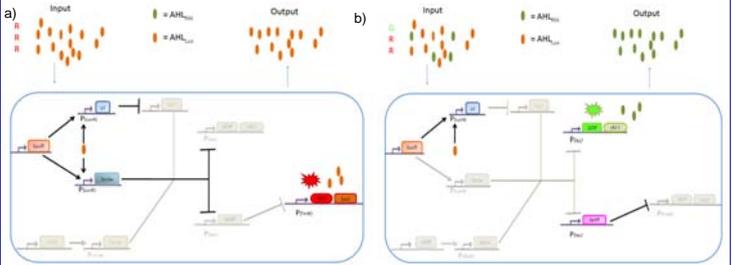


Fig.4: Examples of response mechanisms a) to a RRR input b) to a GRR input

Modeling

Here we describe a semi-quantitative model of the Lux band-pass detector and the Rhl high-pass detector. The model describes each protein and sending molecules involved in the system. At this stage, we test the ability of the system to give a constant input of green and red (ALux or ARhl) and choose an output state, either red or green.

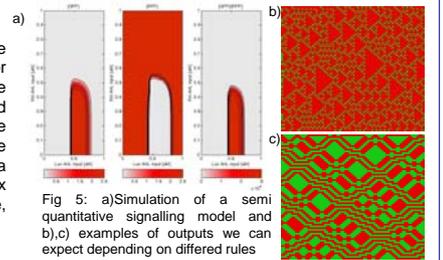


Fig 5: a) Simulation of a semi-quantitative signalling model and b) c) examples of outputs we can expect depending on differed rules

Microfluidics and microbiologic

The Chip we use was designed to meet the needs of our experiment: to provide a matrix divided into chambers in which cells can grow. These chambers are connected by channels that will allow cell-cell, or in our case chamber-chamber communication.

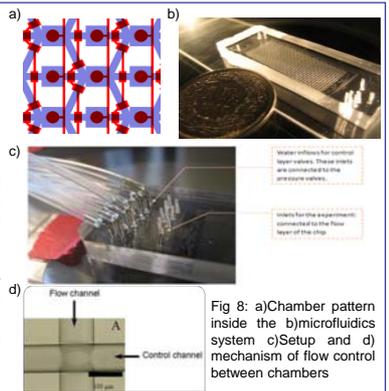
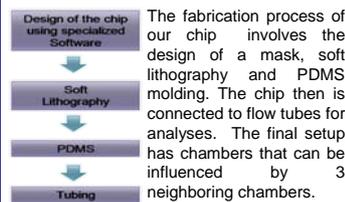


Fig 8: a) Chamber pattern inside the microfluidics system b) Setup and c) mechanism of flow control between chambers

Integration of bacteria in microfluidic system

We conducted several experiments with cells containing a plasmid with RFP under control of a TetR-inducible promoter in order to characterize it. We were able to establish a controlled flow within the chambers and expected to observe the onset of RFP fluorescence when the trigger concentration of TetR would be reached.

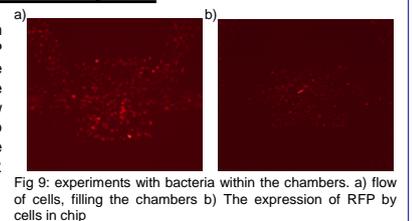


Fig 9: experiments with bacteria within the chambers. a) flow of cells, filling the chambers b) The expression of RFP by cells in chip

Conclusion and Future Applications

We were able to complete the plasmid constructs but were not able to submit it on time. We were able to characterize a part and see that it is indeed functional and also tested the microfluidic chambers for the functionality it was designed for. We have obtained promising results and we are confident that our project can have a big potential.

In the future, our system could be further developed to function as a diagnostics device detecting disease-related biomarkers in bodily fluids. Each first column chip well would contain specialized bacterial cells engineered to detect specific biomarkers, allowing the simultaneous sampling of hundreds to even thousands of patient samples. Depending on which samples contain the biomarker of interest, a specific pattern would be generated. Using our algorithm, we would have a priori defined all possible patterns. Simple scanning and analysis by computer would provide a high-throughput mechanism to analyze patient samples.

References: [1]Basu, S. et al., Nature 434 (7037), 1130 (2005), [2] Weiss, R., Nature 428 (6985), 868 (2004)