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FNSNF

MICROFLUIDIC CHIPS TO MEASURE BACTERIAL CHEMOTAXIS IN A BIOSENSORY PERSPECTIVE

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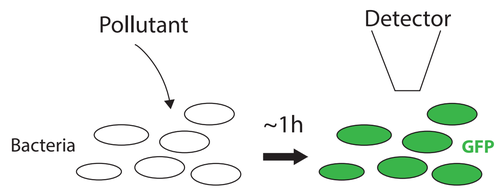
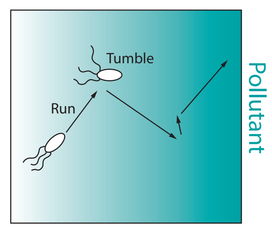
Conventional bacterial bioreporters are based on the induction of a reporter gene (e.g. GFP) in presence of a specific inducer (e.g. pollutant). Inducible bioreporters work very well but it requires one hour to get a significant signal.
In the ENVIROBOT project, we aim to develop reporters with faster response time. Our idea is to use chemotaxis, the behaviour of swimming in direction of an attractant or away from a repellent, because chemotaxis is based on decisions by cells at a time scale of 100-500 ms. Furthermore, chemotaxis exists even towards toxic compounds, which could be exploited as reporters.



Transcription based bacterial bioreporter



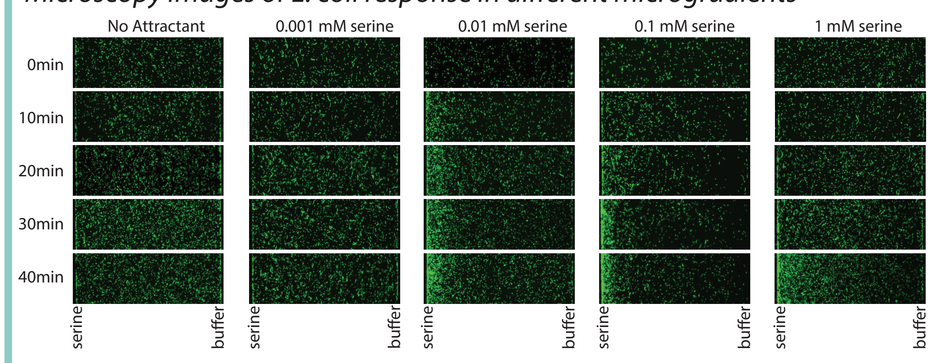
Chemotaxis based bioreporter

AIM: Develop bioreporters based on chemotaxis with a shorter time response → APPROACH: Measure the directionnal movement of bacteria in microgradients inside microfluidic chips

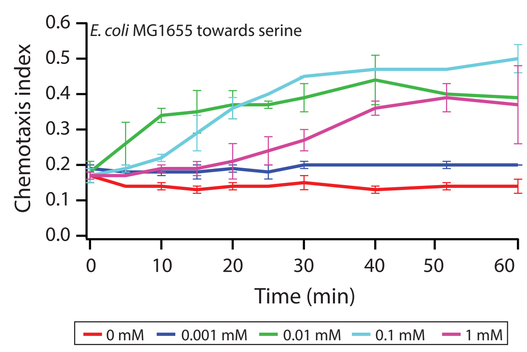
Quantification of chemotaxis from the response of a population of cells

A microfluidic chip was designed to quantify chemotaxis of *Escherichia coli* towards serine.

Microscopy images of E. coli response in different microgradients

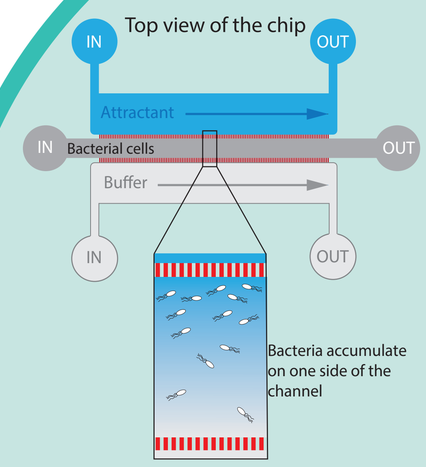


Quantification of the chemotaxis index over time



The *chemotaxis index* is the proportion of cells that localize in the 100 microns closest to the source of attractant.
--> We can easily quantify *E. coli* chemotaxis over time. Cells already react significantly after 10 min with 0.01 mM of serine.

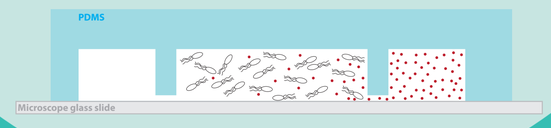
Principle of the chip



The PDMS chip is composed of 3 parallel channels linked by filters of only 700 nm height, which prevent the passage of the cells.

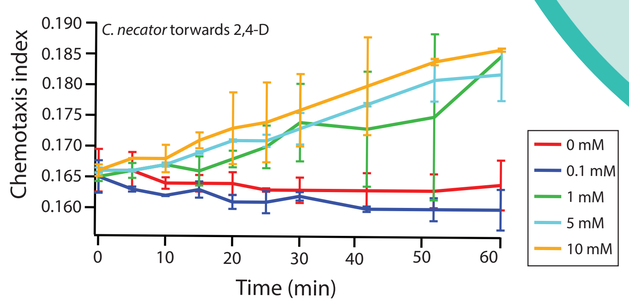
A gradient is generated in the middle channel by flowing attractant and buffer in the side channels. Bacteria are inserted in the middle channel and are attracted on one side of the channel.

Side view of the chip



We also used *Cupriavidus necator* JMP134, which shows chemotaxis towards the herbicide, 2,4-dichlorophenoxyacetate (2,4-D).
--> We can also quantify chemotaxis of *C. necator* towards 2,4-D but the response has a lower amplitude and the strain is less sensitive than *E. coli* and serine.

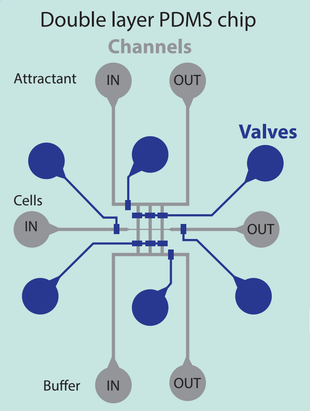
Quantification of the chemotaxis index over time



With our microfluidic chip, we can generate micro-scaled gradients and quantify chemotaxis from the response of the population of bacteria. The use of species naturally chemotactic to toxic pollutants demonstrates the biosensory perspective of the device.

Quantification of chemotaxis from the response of individual cell

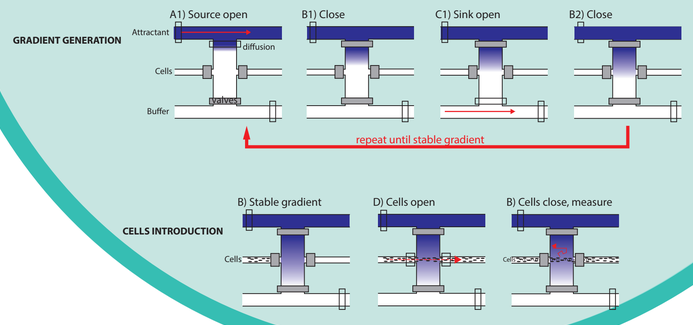
Principle of the chip



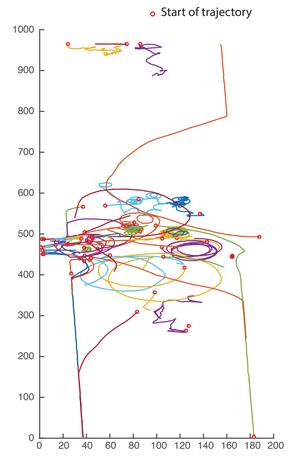
The double layer PDMS allows a control of the flow by the addition of valves inside the PDMS structure.

The gradient is generated by alternating valve opening. A group of bacteria is introduced in the middle and their responses are followed in real time.

Generation of the gradient by alternating valve opening



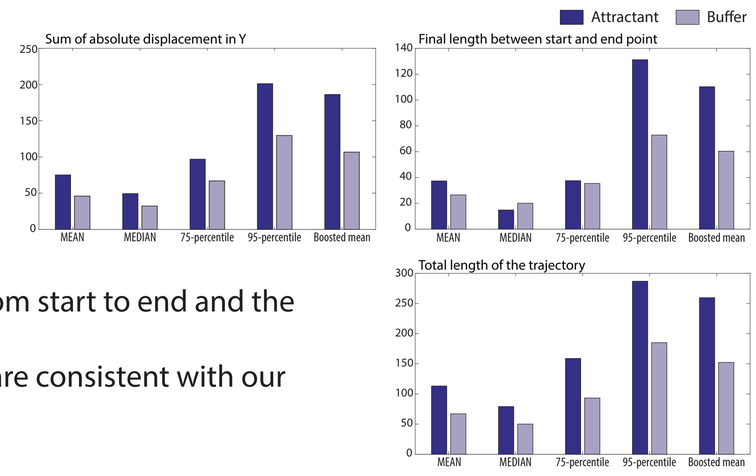
Trajectories extracted from 30s movie



We used *E. coli* chemotaxis towards serine to test the principle of the chip.

Hypothesis:
In presence of a gradient, the bacteria swim more straight in direction of the source of attractant.

Bacteria are filmed during the first minutes of exposure to the gradient. The trajectories of individual bacteria are extracted from the movies and their characteristics are analysed



For each trajectory, we measured the length, the distance from start to end and the displacement in Y.
--> The 75-percentile, 95-percentile or the boosted average are consistent with our hypothesis.

With this microfluidic chip, we can follow individual cell behavior directly after exposure to a gradient. More experiments have to be performed to identify the most consistent descriptor(s) of bacterial response according to our hypothesis

Conclusion and perspectives

Two types of microfluidic chips were developed, that generate microgradients and allow quantification of bacterial chemotaxis, either at the population or individual cell level.
The use of species that show chemotaxis towards toxic molecules can be used to detect the presence of pollutant in the water.

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