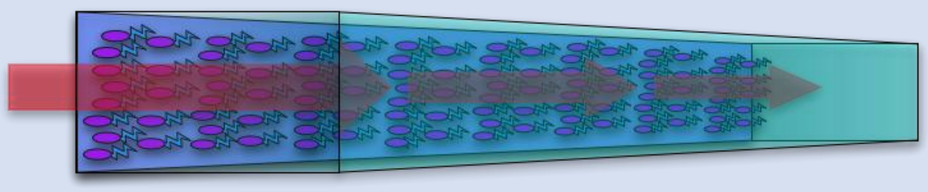
FLOWIE

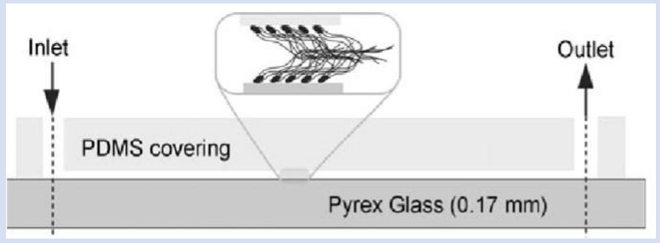
The project in one minute

We want to modify a strain of *E. coli* so that it will be able to establish a liquid flow through a micro tube. Furthermore, we want to control the flow in terms of strength, direction and duration. This will be achieved through phototaxis and upregulated expression of flagella.



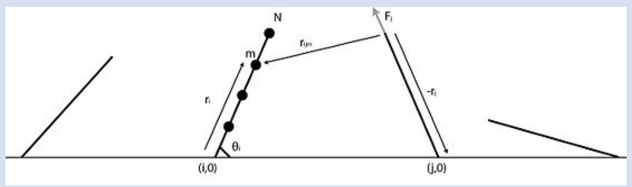
Inspiration

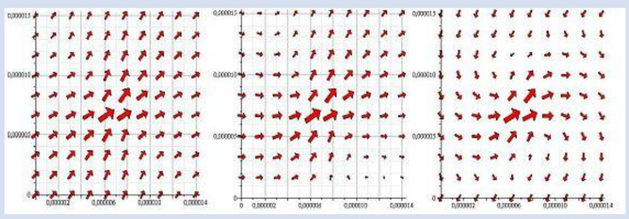
The inspiration for this project came from an article by Kim *et al.*[1], who discovered that it was possible to generate a flow through a capillary tube using a bacterial carpet as driving force. Deeply fascinated, we decided to make a similar system, only better! So we introduced a way of controlling the flow by phototaxis.



Modelling

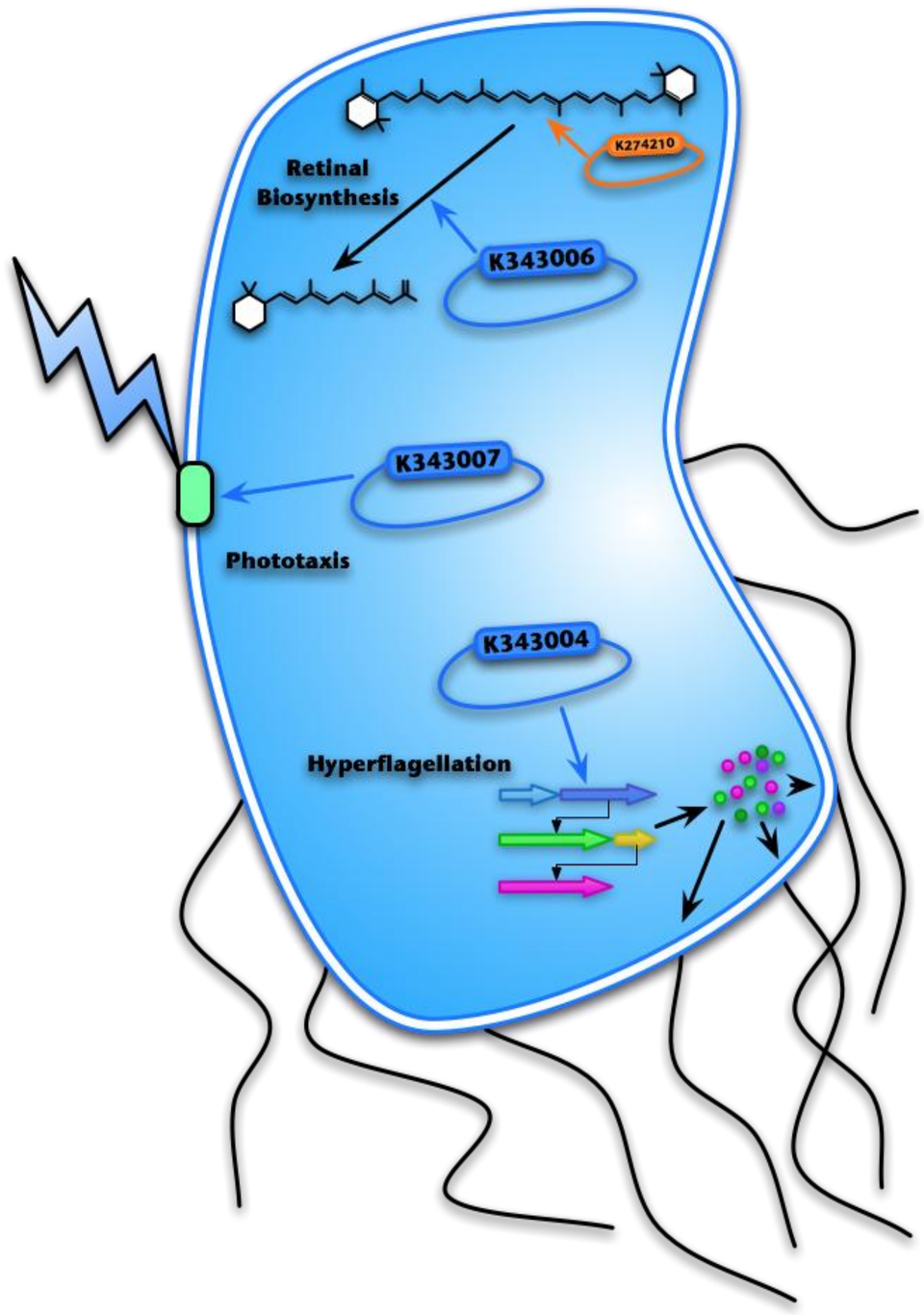
Our physical model shows flows created in our system. Flagella are represented by rods creating a point force.





No walls One wall Two walls

Flow fields are calculated by point forces. The flow and position of the flagella is observed to change over time.



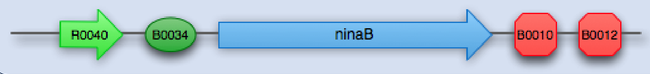
Retinal

We have created a BioBrick that makes our bacteria produce retinal from β -carotene. The *D. melanogaster* gene *ninaB* encodes the protein β-carotene 15,15'-monooxygenase. β-carotene 15,15'- monooxygenase cleaves a double bond in β-carotene under the consumption of oxygene and thereby produces two molecules of retinal.

β-carotene is provided by the brick K274210.

Serial dilution of retinal. Concentrations: 1 mM, 100 µM, 10 µM and 1 µM

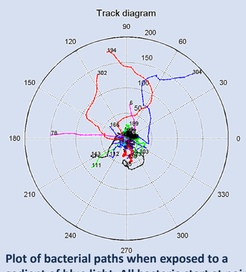
Serial dilution of β-carotene. Concentrations: 1 mM, 100 µM, 10 µM and 1 µM



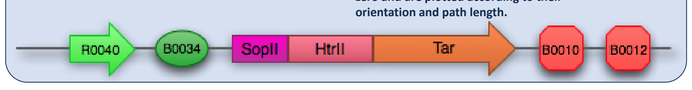
Phototaxis

To introduce control into our system we chose to make our bacteria sensitive to light, so that they will change their motility pattern when exposed to light. For this we created a BioBrick with the SopII-HtrII-Tar fusion, chimera protein, which couples a halobacterial photosensor to E.Coli's chemotaxis pathway [2].

The effect is that the bacteria will exhibit a lowered tumbling rate when exposed to blue light, as the light acts as an attractant stimulus.

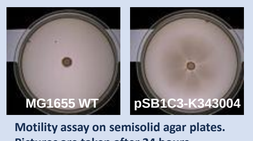


Plot of bacterial paths when exposed to a gadient of blue light. All bacteria start at point zero and are plotted according to their orientation and path length.

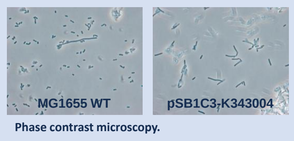


Hyperflagellation

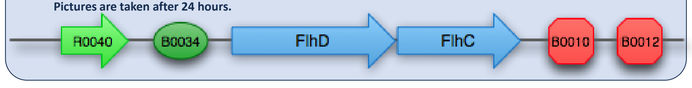
One of the major parts of bacterial motion is the turning of the bacteria's *flagella*. Flagellation is a motility system that exists in many microorganisms, including *E. coli*. In *E. coli*, the synthesis of flagella is regulated by the master operon *flhDC*. The synthesis of flagella is tightly coupled to the bacteria's environment since an abundance of nutrients will render motility superfluous and thus discourage the synthesis of flagella[3].
In our system, we put the existing *flhDC* master operon under a constitutively active promoter to increase flagella synthesis. The results are shown below.



Motility assay on semisolid agar plates. Pictures are taken after 24 hours.

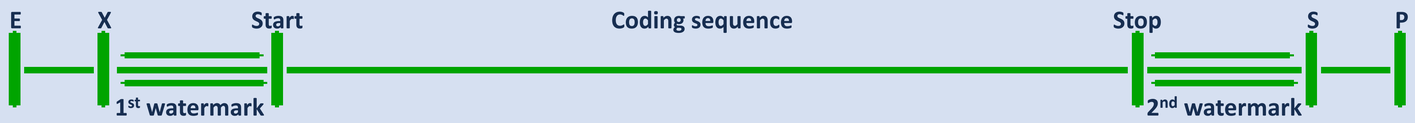


Phase contrast microscopy.



Human practices

We have proposed a *new safety standard* using watermarking[4] of parts, to ease the identification of bacteria, in case it escapes a safe environment (e.g. the lab). The watermark will be placed between the cutting sites and the start and end of the coding sequence (see figure below). The watermark links a BioBrick to its creatiors, and the parts' name, description, risk assessment and neutralizing information will then be available from an online database. The *safety* of our project was considered by making a risk assessment of each BioBrick, as well as an overall assessment of the project.



Conclusions and future prospects

We have shown that two of our three BioBricks work as expected. The bacteria containing the photosensor are reacting to blue light and the hyperflagellated bacteria are more motile than the wildtype. For the retinal brick, further experiments are needed to determine if retinal is produced or not.
The next challenge would be to assemble a brick coding for all these functions and thereby producing a bacteria that has all three functions at once. Afterwards the real physical experiment would have to be conducted, where we coat a tube with this strain and see if we can establish and control a microflow.

As for the human practices part, the next step would be to introduce the watermarking standard to parts registry so that iGEM can set an example of how to use this. Necessarily, an online database should be created and licenses should be made available for labs world wide.
Why is it even relevant to create the micro flow? This could be used in a 'lab on a chip' to mix fluids or to create flows in very narrow tubes that wouldn't normally be possible to have.

References: [1] Kim MJ, Breuer KS, *Microfluidic pump powered by self-organizing bacteria*, Small 4, 111-118. (2007). [2] Jung KH, Spudich EN, Trivedi VD, Spudich JL, *An Archaeal Photosignal-Transducing Module Mediates Phototaxis in Escherichia coli* J Bacteriol. 2001 Nov;183(21). [3] Gauger EJ, Leatham MP, Mercado-Lubo R, David C. Laux, Conway T, Cohen PS. *Role of Motility and the flhDC Operon in Escherichia coli MG1655 Colonization of the Mouse Intestine.* Infection and Immunity. 2007 Jul;75(7) p. 3315-3324. [4] Inspired by J. Craig Venter.