After 100 million years of doing what they were *made* for

*E. coli* will soon discover what they were *meant* for…
FLOW-E
A Microbial Flow Inducer
Inspiration


http://www.rsc.org/chemistryworld/_denial.asp

The Idea

**RETINAL GENERATOR**
Converts β-carotene to retinal

**PHOTOTAXIS**
Introduces sensitivity to blue light

**HYPERFLAGELLATION**
Overexpresses flagella
Modelling

The System

- Very narrow channel, 15 µm
- The liquid behaves like syrup
- High bacterial density, ~ 2 µm distance

The Flagella

- Highly flexible polymer
- Multi-unit coordination
- Complex flow with vorticity

**Modelling**

**Assumptions**
- Flowfield from only one source
- Ignore steric effects
- Rigid flagella system

**Model**
- Regular array of rigid arms
- A harmonic preferred angle
Modelling

Results

- No dynamic behaviour
- Strong dependence of the start configuration
Hyperflagellation

The Flagella

Synthesis is controlled by three classes of genes

- **Class I**: *flhDC* master operon
- **Class II**: Basal body proteins, $\sigma^{28}$
- **Class III**: Filament proteins

Transcription of *flhDC* is tightly regulated by environmental factors
Characterization

Phase contrast microscopy

- K343004 in pSB3K3 were compared to the wild type MG1655
Motility assay

- *E. coli* MG1655 expressing K343004 in pSB3K3 was compared to wild type MG1655 and positive control H10407.
Bacterial motility

- Resembles random walk
- Smooth swimming interrupted by brief tumbles
- Tumbling is used to reorient the cell
- Tumbling will reduce/terminate the flow

Images modified from *Microbe* by Moselio Schaechter, John L. Ingraham, and Frederick C. Neidhardt, 2005.
Bacterial motility

The graph shows the dynamics of CheA-P, CheB-P, and CheY-P over time. The peaks indicate changes in phosphorylation levels of these proteins, which are crucial for bacterial motility. The x-axis represents time, and the y-axis shows the concentration of the proteins, with different colors for each type.
Photosensor

- SopII-HtrII-Tar couples itself to E. coli’s chemotaxis pathway.

- Exposure to blue light results in less CheY-P and therefore a lowered tumbling frequency.

- Faster response time than “traditional” light receptors.
Photosensor
Results
Motility assay
• The bacteria containing BioBrick show a modified motility pattern when exposed to blue light.
• Light seems to act as an attractant stimulus on the SopII-HtrII-Tar complex.
Retinal synthesis

The Purpose

- Retinal is needed for the photosensor to work

The BioBrick

- Encodes β-carotene 15,15’-monooxygenase
- Converts β-carotene to retinal
- Coding sequence of *ninaB* from *D. melanogaster*
- β-carotene is provided by K274210 (Cambridge, 2009)
Retinal synthesis

Characterization

UV-vis showed that

- MG1655 produces
  - β-carotene
  - Retinal?

- TOP 10 produces
  - β-carotene
  - Retinal?

HPLC experiments were inconclusive

MG1655 w. K343006 + β-carotene (added to media)
TOP10 w. K343006 + K274210
MG1655 control + β-carotene (added to media)
MG1655 w. K343006 + K274210
The BioBricks

- Flagella overexpression
  - Works!
- Photosensor chimera
  - Works!
- Retinal generator
  - Inconclusive results
Biosafety

- Safety in selection of project
  - The genes were BLASTed to find their function and known homologs

- Researchers
  - Risk-assessment guidelines (available on our wiki!) were made based on european standards

- Society
  - Functions of genes were researched, described and considered according to the questions asked in our risk-assessment paper

- Environment

- Prevention of malign use
**Biosafety**

**Watermarking BioBricks**

**Benefits:**
- Easy identification
- Quick access to information
- Increased safety

**Cross-reference to:**
- Team/Lab/Company
- Name of the part
- Description of the part
- Risk-assessment of the part
- How to neutralize an organism holding this part
Biosafety

Watermarking BioBricks

Criteria:
- Creators’ ‘license’
- No interference
- Persistency
- Easy to find
- Easy to insert

We propose:
- 12 nucleotide sequence

AGCTCG

TTATCG
The Checklist

- Provided information on 8 new BioBricks
- Submitted DNA for 8 new BioBricks
- Showed 2 tested BioBricks work as expected
- Characterized the new BioBricks
- Further characterized an existing BioBrick
- Helped another iGEM team
- Developed a new approach in human practices
- Added information to partsregistry
Credits

Thank you!

Learn more on 2010.igem.org/Team:SDU-Denmark