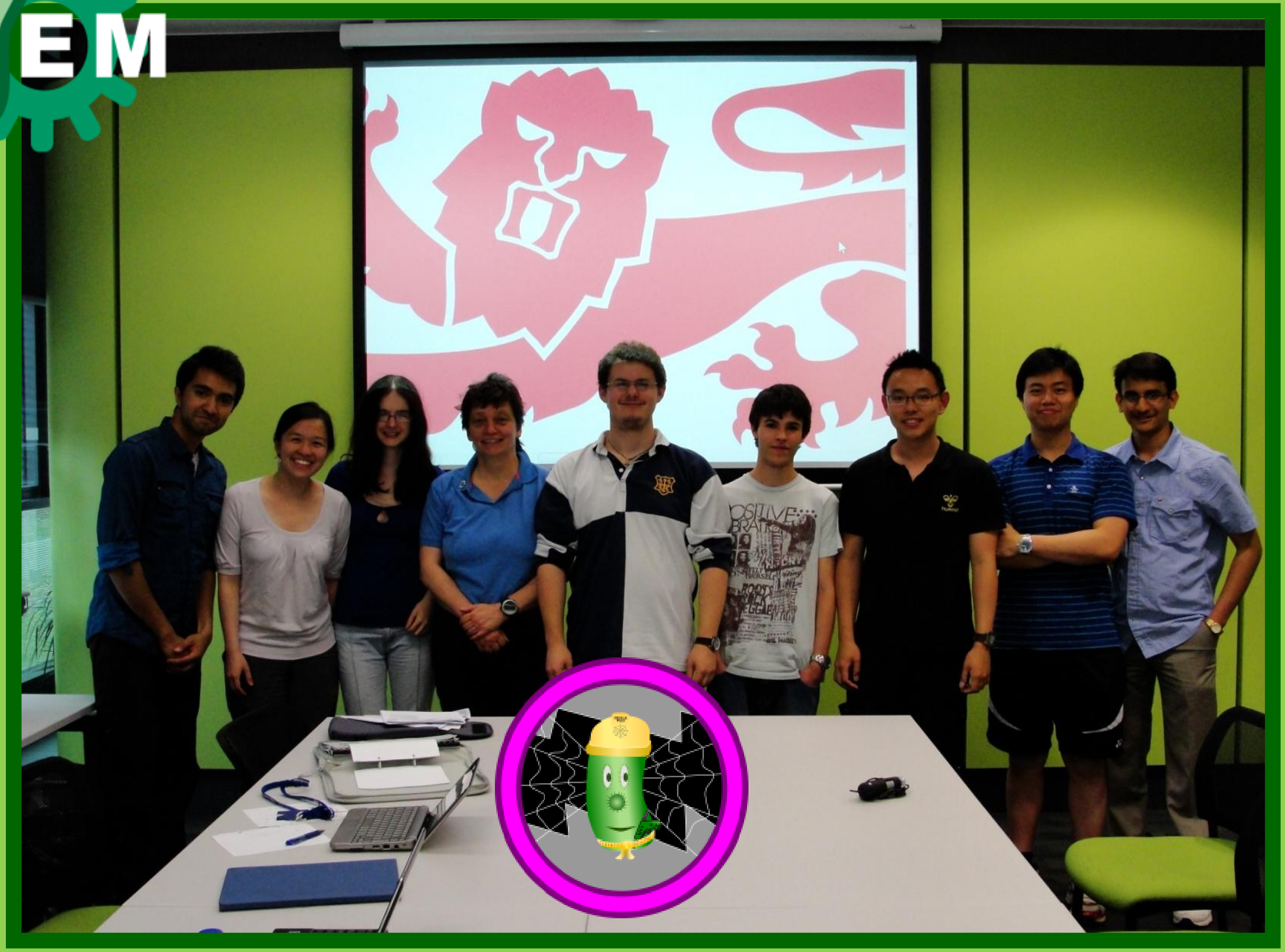
Team Newcastle



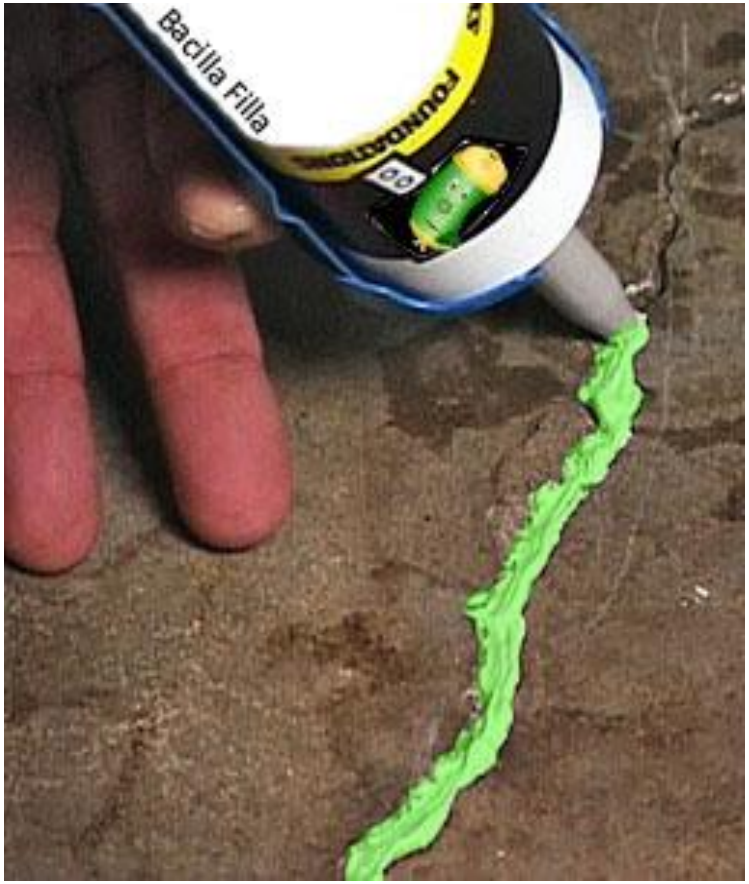
BacillaFilla

(Younus, Deena, Rachel, Jannetta, Phil, Steven, Alan, Da, Harsh)

http://twitter.com/Newcastle_iGEM

Our Project

Filling up the cracks in concrete



Reasons cracks are bad!

- Allows water to reach and corrode steel reinforcements
- Weakens concrete structures
- Difficult to repair

How our project helps?

- Reduces corrosion rate of the steel reinforcements
- Reduces the need to demolish and rebuild concrete structures
- Reduces cement production

Earthquakes

We also aim to fill up small cracks caused by earthquakes.

The Concrete Timeline

1840 — Portland cement invented

1995 — Gollapudi *et al.* First introduce using bacteria to fix cracks

2001 — Bang *et al.* Immobilise bacteria in polyurethane to combat high pH in concrete

2004 — Bottcher *et al.* Immobilise bacteria in silica gel

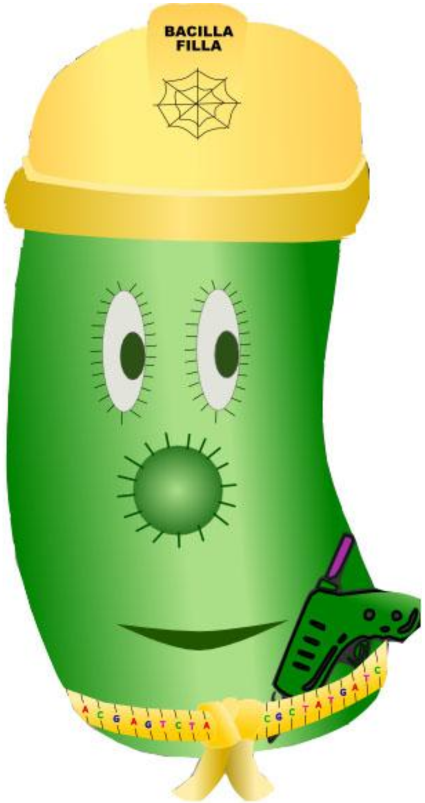
2007 — Jonker *et al.* Introduce spores into concrete

2010 — **iGEM Team Newcastle BacillaFilla**

Why *Bacillus Subtilis*?

- *B.subtilis* has many states
  - **Spore**
  - **Filamentous**
  - Motile
  - Vegetative
  - Chain

- Natural expression of urease



General Outlook of the Project

Cells in the Bioreactor

Induced sporulation of live cells

Storage of spores in containers and their transportation to the site of construction or repair

Spraying of the spores by hand operated sprayers

Spores stick to the concrete slab

Spores escape into the environment

Spores stick to the concrete slab

Germination of the spores

Sensing the cracks in the concrete slab

Swarming into the cracks

End of the crack signaling/ Quorum sensing

Calcium carbonate precipitation + Glue production

Death after certain amount of time due to nutrients limitation

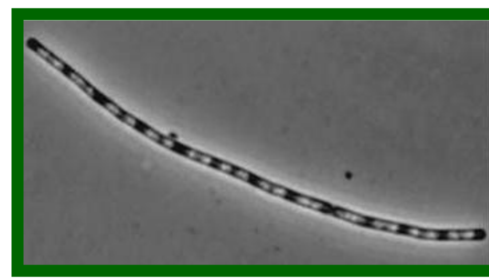
Spores escape into the environment

Germination of the spores

Non- environmental kill switch induction

Death of the bacterium

Filamentous cell formation



Pictures adapted from: Y. Kawai, S. Moriya and N. Ogasawara. 2003. Identification of a protein, YneA, responsible for cell division suppression during the SOS response in *Bacillus subtilis.* Mol Microbiology **47**: 1113-1122.

BioBricks

❶ **Swarming** (*sfp* & *swrA*)

❷ **Subtilin** (production & immunity)

❸ **Urease** (*SR1* & *rocF*)

❹ **LacI**

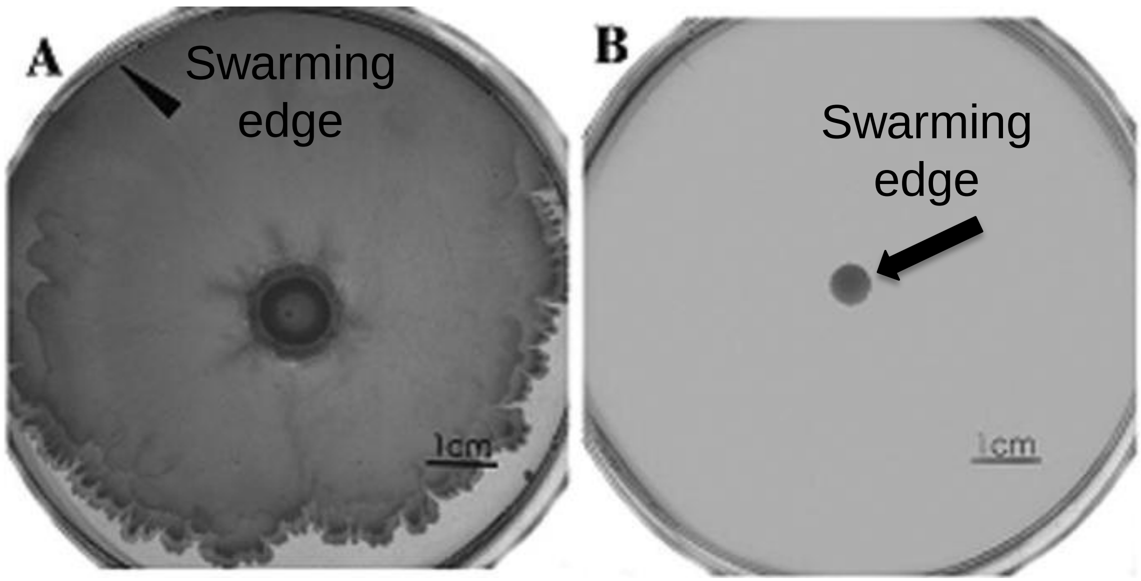
❺ **Filamentous cells** (*yneA*)

❻ **Kill-switch**

Swarming BioBrick

The problem: *Bacillus subtilis*168 is unable to swarm on solid surface

B. subtilis **3610**
(wild type)



B. subtilis **168**
(lab strain)

Reason 1
Frameshift mutation in *sfp,*
involved surfactin production

⬇

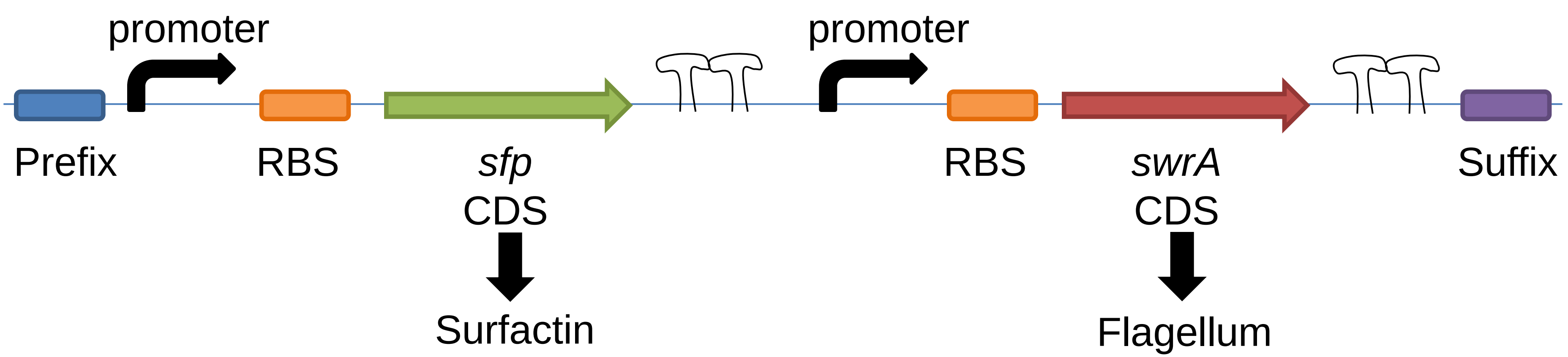
The function
Post translational modification
of the immature surfactin
peptide

Reason 2
Frameshift mutation in *swrA*,
involved in flagellum
biosynthesis

⬇

The function
swrA acts on transcription
factor for genes required in the
late flagellum biosynthesis

The solution



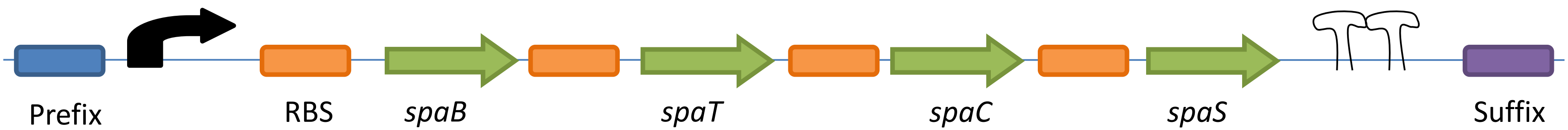
BacillaFilla swimming down the cracks!

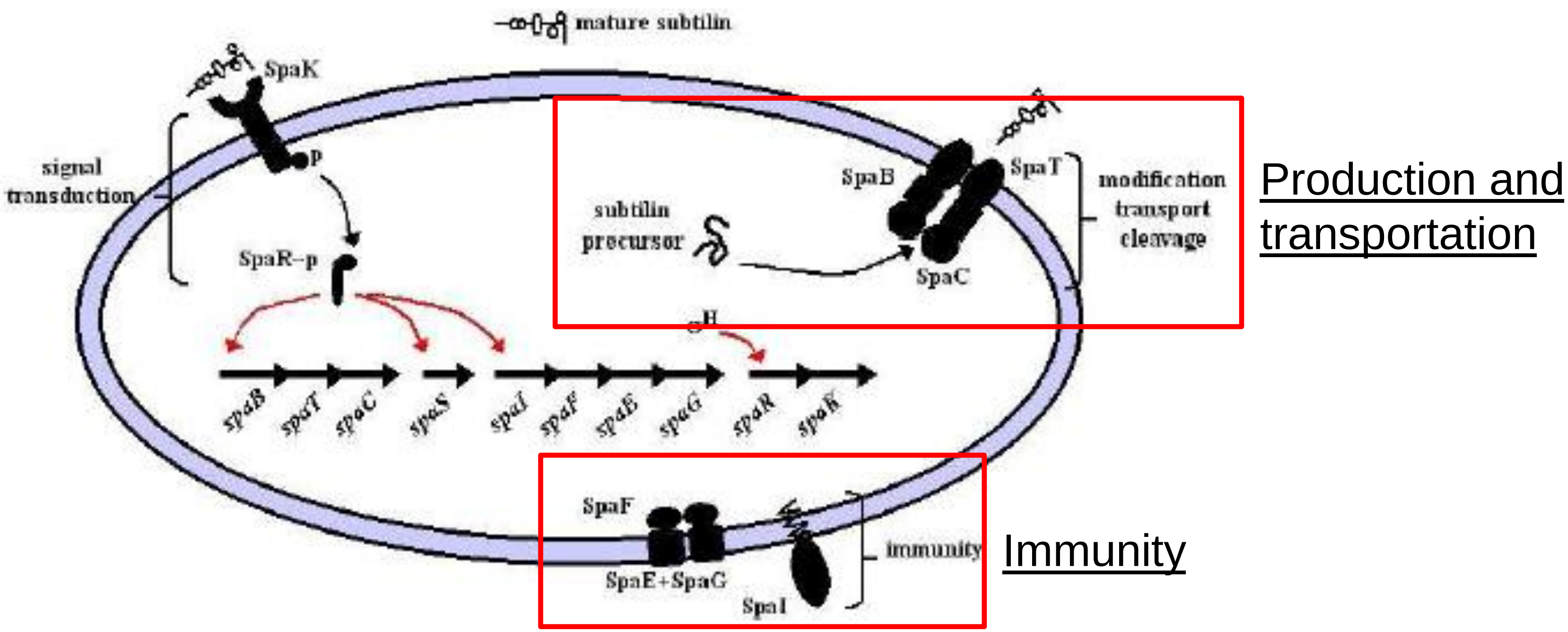
Pictures adapted from: **Julkowska D., Obuchowski M., and Holland B.** 2005. Comparative analysis of the development of swarming communities of Bacillus subtilis 168 and a natural wild type: critical effects of surfactin and the composition of the medium. J. Bacteriol. **187:65-76**.

Subtilin cell-signalling system

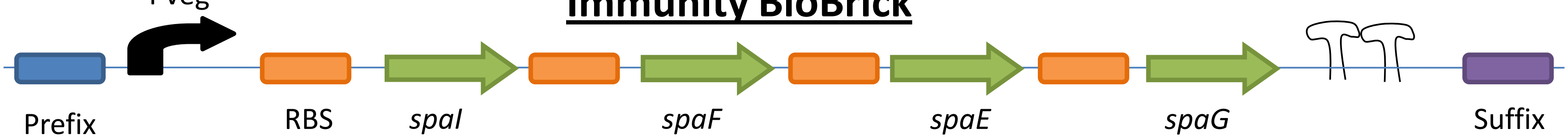
The problem: A signalling system to trigger $CaCO_3$ precipitation and filament formation once our bacteria have reached a sufficient density inside a microcrack.

Production BioBrick





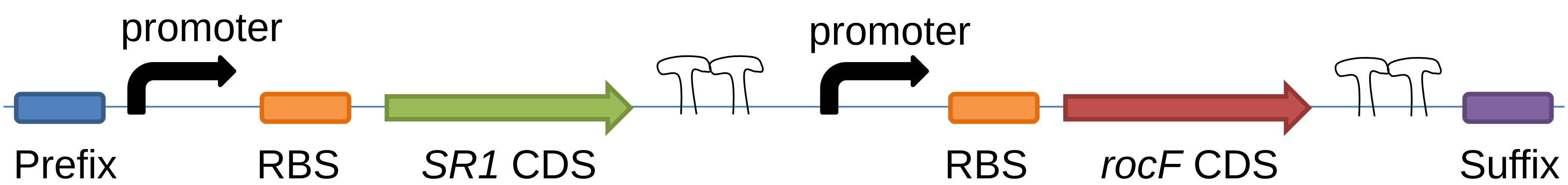
Immunity BioBrick

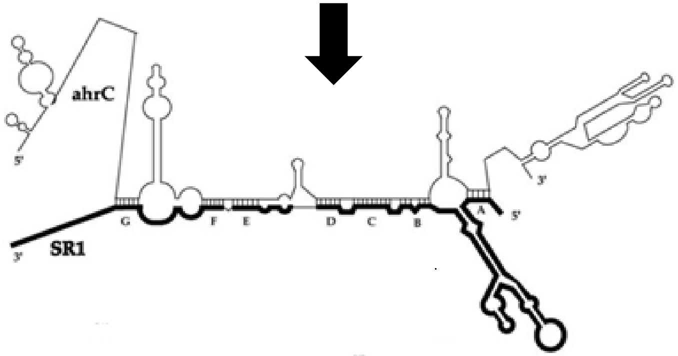


Urease BioBrick

The problem: *Bacillus subtilis* 168 needs to increase its $CaCO_3$ production to fill up cracks – this requires the cells to generate carbonate at a high pH (pH8-9). This can be done by increasing the production of carbamate and ammonium from arginine via urea production

The solution





rocF codes for the arginase enzyme that breaks down arginine to ornithine and urea

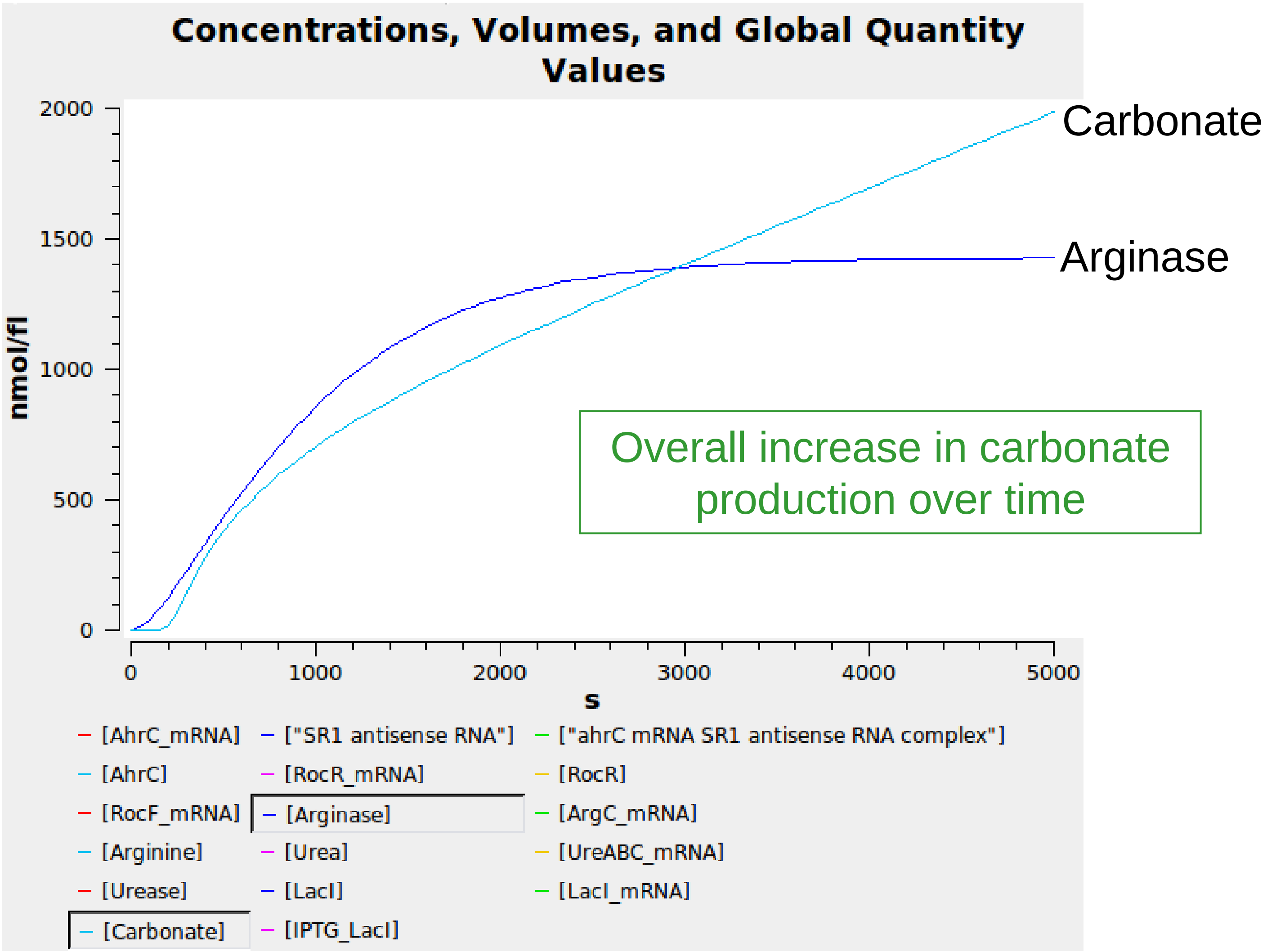
↑ Arginase ↑ Urease

↑ Arginine → Urea + H_2O → CO_2 + $2NH_3$

Ornithine

BacillaFilla filling up cracks with $CaCO_3$

Computational Model of Urease Production



Written in SBML and simulated from Copasi v4.5 (build 30)

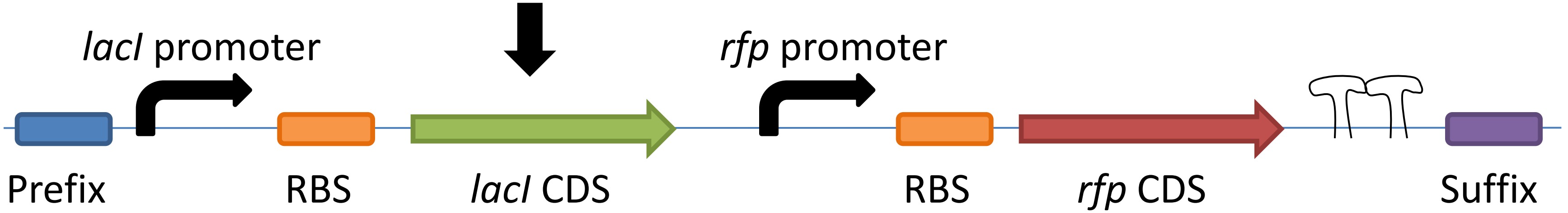
LacI BioBrick

The problem: To allow characterisation of BioBricks as it puts gene expression under our control

Repesses lac-based promotors such as pspac or hyperspankoid

The function

Allows to increase gene expression by adding IPTG



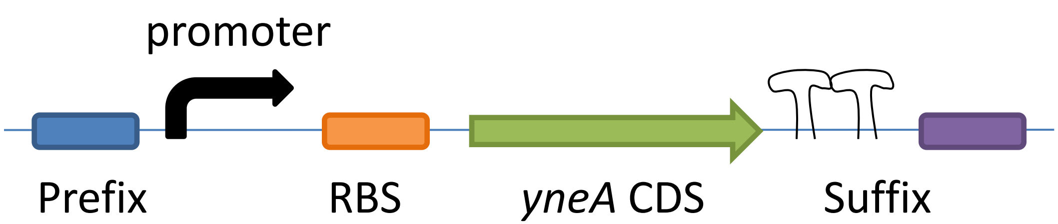
LacI

Rfp

yneA BioBrick (Filamentous cells)

The problem: To allow filamentous cell growth which will be used as a reinforcement to $CaCO_3$ used to fill the crack

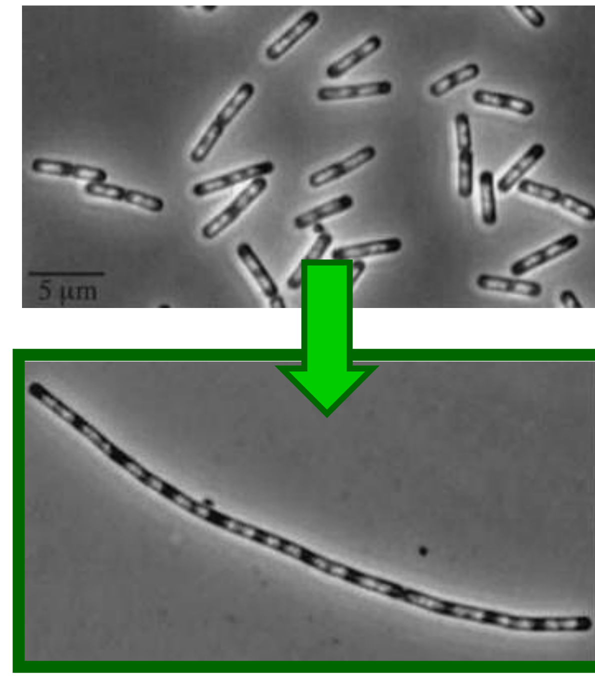
- Filamentous cells are formed under stress.
- YneA reduces FtsZ ring formation.



YneA protein

1. Inhibits FtsZ ring formation

2. Inhibits cell division



Results in filamentous cell formation!

Pictures adapted from: Y. Kawai, S. Moriya and N. Ogasawara. 2003. Identification of a protein, YneA, responsible for cell division suppression during the SOS response in *Bacillus subtilis.* Mol Microbiology **47**: 1113-1122.

What next?...

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Facebook fanpage: Newcastle iGEM 2010

Acknowledgments

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6. Mr. Goksel Misirli

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Questions?

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