TOXiMOP

Splash! And the toxin’s gone ...
Perfect synthetic biology project

- Idea
- Community project
  - Present
  - Evaluate
  - Improve
- Final product
- Something beautiful!
Our problem: Algal Blooms

- Explosion in the growth of blue-green algae (cyanobacteria)
- Production of toxins
  - Hazardous to humans, pets and livestock.
- Local problem
  - E.g. Reservoirs, lakes/lochs
- Global problem

Clatto reservoir, Dundee
Microcystis aeruginosa

Picture courtesy of Pauline Lang, SEPA.
Liver toxin: Microcystin
PP1 & Microcystin

- Binds covalently to Protein Phosphatase 1
- Blocks the active site
- PP1 is an essential regulatory protein

Goldberg et al. (1995) Nature 376, 745-753. PDB code: 1FJM
Current methods for dealing with algal bloom toxicity are far from ideal

- Existing methods target the blue-green algae not the toxin
- We were tasked with directly targeting the toxin
Our idea: a biological ‘ToxiMop’
Who’s packing?  

**E. coli** vs **B. subtilis**

\[
PP1_{\text{number}} = \frac{V_{\text{peri}}}{V_{PP1}} \\
= \frac{6.27 \times 10^{-20}}{7.54 \times 10^{-25}} \\
= 83 000
\]

\[
PP1_{B.\text{sub}} = PP1_{\text{cylinder}} + PP1_{\text{hemi}} \\
= 35 400
\]
Transport across inner membrane

PP1 (sp) → PP1 → Sec

PP1 → Tat
Engineered PP1 proteins

Tat-targeted

TorA_{sp}-PP1

Sec-Targeted

MalE_{sp}-PP1
TorA_{sp}-PP1 localises to the periplasm
TorA_{sp}-PP1 localises to the periplasm
$\text{TorA}_{\text{sp}}\text{-PP1 localises to the periplasm}$
E. coli TorA<sub>sp</sub>-PP1 cells will make up our ‘ToxiMop’

TorA<sub>sp</sub>-PP1 localises to the periplasm
How much PP1 would a Tat transporter transport if a Tat transporter could transport wood?

~200 PP1 in the periplasm
~1000 left in the cytoplasm
Predicting Transport Rates

Number of PP1 in cytoplasm vs. Time (s)

Number of PP1 in periplasm vs. Time (s)
Testing the ability of our ‘ToxiMop’ cells to clean up the toxin.
ToxiMop assay

TorA<sub>sp</sub>-PP1  NarG-PP1  Vector Only

No cells control
ToxiMop in action

Incubate for 1 h at room temp.

Centrifuge cells and take the supernatant

E. coli cells
Catching microcystin using the human PP1 protein

ToxiMop ELISA

70% of periplasmic PP1 is bound to microcystin

0.26 nM Microcystin
Are these concentrations of microcystin relevant?

Alberta, Toxic drinking water 4.3 nM

WHO safe-level is below 1 nM
Presenting to community leaders
Suggested addition to our project

To develop a biological detector using synthetic biology.
Our idea: Biological toxin detector
**E. coli** osmolarity sensor: EnvZ

Expression of OmpR regulated genes
Region to be engineered

Expression of OmpR regulated genes

GFP expression controlled by OmpR
GFP reporter construct

OmpC: BBa_R0083
RBS: BBa_B0034
GFP: Bba_E0040
The OmpC-GFP reporter responds to environmental osmolarity in an EnvZ-dependent manner
Discussion: Algal Blooms, Clatto and Synthetic Biology

We held a discussion allowing us to present our project to scientists and environmentalists.
Concerns raised:

1. How can we deploy our detector?

2. We have overlooked the root causes of the problem.

3. How can we use the ToxiMop without releasing GMMs?
Real-time lake monitoring and early warning systems

Biological Detector

Light

Temperature

Humidity

pH

Dissolved O₂

Webcam
Concerns raised:

1. We have overlooked the root causes of the problem.

2. How can we deploy our detector?

3. How can we use the ToxiMop without releasing GMMs?
Prototype devices

Toxi-Teabag

Toxi-Pump
The ToxiMop Works

Problem

We would need 70 g of cells to clean up 200 mL of microcystin contaminated buffer!
Potential solution

3500 PP1: Potential for ToxiMop Cells

~18 Fold Increase

200 PP1: Basic ToxiMop Cells
What have we achieved?

- We have made a biological mop for an environmental toxin
- Built the components for a biological detector
- A project shaped by the community
The Future of ToxiMop

Exploring Commercialisation

• Expanding the ToxiMop

• Moptopus Mk. 2
Splash! And the toxin’s gone ...

Raw Data: Buoyancy Test
The Mop Campaign

Brian Cox helps clean up

In praise of ... the iGEM competition

Entries range from a project aimed at defeating algae blooms to a plan to fight legionnaire's disease with a modified E.coli cell.

Using E.coli to mop-up toxic algal blooms

31 October 2013 Last updated at 11:08 GMT

Students in Dundee have devised a new method to clear toxins caused by algal blooms in lochs and reservoirs.

A "Toximop" uses genetically engineered E.coli, which binds to toxins and clears the water before the poison reaches our bodies.

The work of the multi-disciplinary team has already been recognised in the International Genetically Engineered Machine (iGEM) competition.

Open to undergraduates, the 2012 winners devised a Food Warden that detects meat spoilage.

BBC Scotland's science correspondent Kenneth Macdonald reports.

Read more

Reservoir bugs - Dundee students develop 'cure' using E.coli.

If the Toxi-Mop project is considered one of the 16 best projects on offer there, they will then be through to the finals in Portland, in November.
The Mop Campaign

- We’re in the papers!
- Partnership with Friends of the Earth
The Mop Campaign

• We’re in the papers!
• Partnership with Friends
• Comic Book
The Mop Campaign

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- Toximop Videogame
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- Toximop Videogame
- Life Science undergraduate iGEM practical
- Stand-up comedy
Motivated by local issues with global reach

BBC NEWS

STV News
Motivated by local issues with global reach
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Joe Fitzpatrick MSP
Andrew Llanwarne
George Potts and team
Competitive ELISA

1. Add your sample into the well.
Competitive ELISA

1. Add your sample into the well.

2. Add your primary antibody
Competitive ELISA

1. Add your sample into the well.

2. Add your primary antibody

3. Competition between microcystin at bottom of the well and in the sample for binding of the primary antibody.
High microcystin concentration in the sample

Add sample and primary antibody to well

Wash

Add secondary antibody to well

The small number of HRP-conjugated secondary antibodies bound to the bottom of the well results in a slight colour change.
Low microcystin concentration in the sample

Add sample + primary antibody to well

Wash

Add secondary antibody to well

More HRP-conjugated secondary antibody at bottom of the well results in a **strong** colour change
Catching microcystin using the human PP1 protein
• Don’t delete.