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

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**

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

\[
PP1_{B.\text{sub}} = PP1_{\text{cylinder}} + PP1_{\text{hemi}} = 35,400
\]

*Winner!*
Transport across inner membrane

PP1 (sp) → Sec → Tat → PP1
Engineered PP1 proteins

Tat-targeted

TorA_{sp}-PP1

TorA_{sp} PP1

Sec-Targeted

MalE_{sp}-PP1

MalE_{sp} PP1
TorA_{sp}-PP1 localises to the periplasm
TorA<sub>sp</sub>-PP1 localises to the periplasm
TorA_{sp}-PP1 localises to the periplasm
TorA<sub>sp</sub>-PP1 localises to the periplasm

*E. coli* TorA<sub>sp</sub>-PP1 cells will make up our ‘ToxiMop’
How much PP1 would a Tat transporter transport if a Tat transporter could transport PP1?

~196 PP1 in the periplasm
~980 left in the cytoplasm
Predicting Transport Rates

Number of PP1 in cytoplasm

Number of PP1 in periplasm

Time (s)

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
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 improvement to our project

To develop a biological detector using synthetic biology.
Our idea: Biological toxin detector

Detected microcystin with engineered EnvZ
**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 promoter RBS GFP

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

MOPTOPUS

- 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
PROBLEM!

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

Number of PP1 in periplasm

Time (s)

TatB-C Complexes:TatA Assemblies
- 15:30
- 30:60
- 50:100
- 100:200
- 150:300
- 200:400
- 250:500
- 300:600
What have we achieved?

- We have made a biological mop for an environmental toxin
- Built the components for a biological detector
- Prototype technologies for deploying these
Brian Cox helps students clean up

University rector meets up with local under-graduates

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Dundee University rector
Brian Cox is supporting undergraduates from Forfar and Kirriemuir taking part in an international competition.

Rachel Findlay from Forfar and Kyle Buchan from Kirriemuir are part of the university team which has entered the highly competitive, worldwide, International Genetically Engineered Machine (iGEM) Competition aimed at undergraduate university students.

The 2013 Dundee inter-collegiate team comprises 10 undergraduate students: Kyle Harrison (applied computing), Nasir Ahmad (physics), Craig Johnston (mathematics), Rachel Findlay (mathematics), Sarah Rollo (biology), Kristie Davidson (biology), Christian Smart (biology), Matthew Szczepanek (biology), Ross Cameron (biology) and Niki Treloar (biology).
The Mop Campaign

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

- We’re in the papers!
- Comic Book
- Toximop Videogame
The Mop Campaign

• We’re in the papers!
• Comic Book
• Toximop Videogame
• Life Science undergraduate iGEM practical
The Mop Campaign

• We’re in the papers!
• Comic Book
• Toximop Videogame
• Life Science undergraduate iGEM practical
• Stand-up comedy

• John Allan - A Fungi to be With
Motivated by local issues with global reach
Motivated by local issues with global reach
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Dr Grant Buchanan

Actor - Brian Cox

ToxiMop comic Avril Smart (iGEM 2012)

ToxiMop game Digital Janitors

Everyone at University of Dundee

Prof Tricia Cohen – PP1 antibody.

Prof Carol MacKintosh – PP1 plasmid.

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

More HRP-conjugated secondary antibody at bottom of the well results in a **strong** colour change

Add sample + primary antibody to well

Wash

Add secondary antibody to well
Catching microcystin using the human PPI protein

Delta \textit{tat} strain

- Whole cells
- Periplasm
- Sphaeroplast
Catching microcystin using the human PPI protein
• Don’t delete.