Edinburgh iGEM 2006

Jamboree presentation

11 - 4 - 2006

Intro to our team

- **Team members**
- Engineering:
  - Kim de Mora
  - Bryony Davidson
  - Jen Wilson
- Biology:
  - Judith Nicholson
  - Farid Bizzari
  - Jelena Aleksic
- Informatics:
  - Sreemati Lalgudi Seshasayee
  - Patrick Cai
  - Sergii Ivakhno
- Faculty:
  - Dr Chris French
  - Dr Alistair Elfick
  - Dr Hongwu Ma
  - Laszlo Kozma-Bognar

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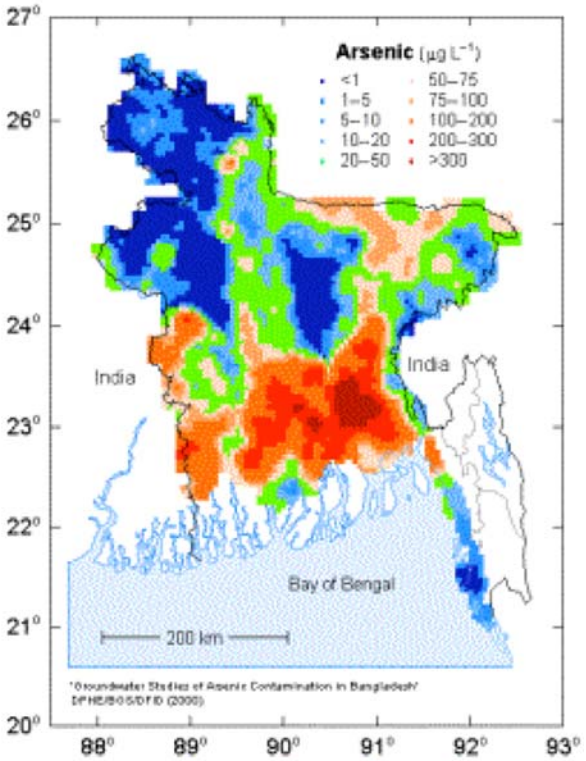
Why detect arsenic?

Bangladesh

Another cruel twist of fate…



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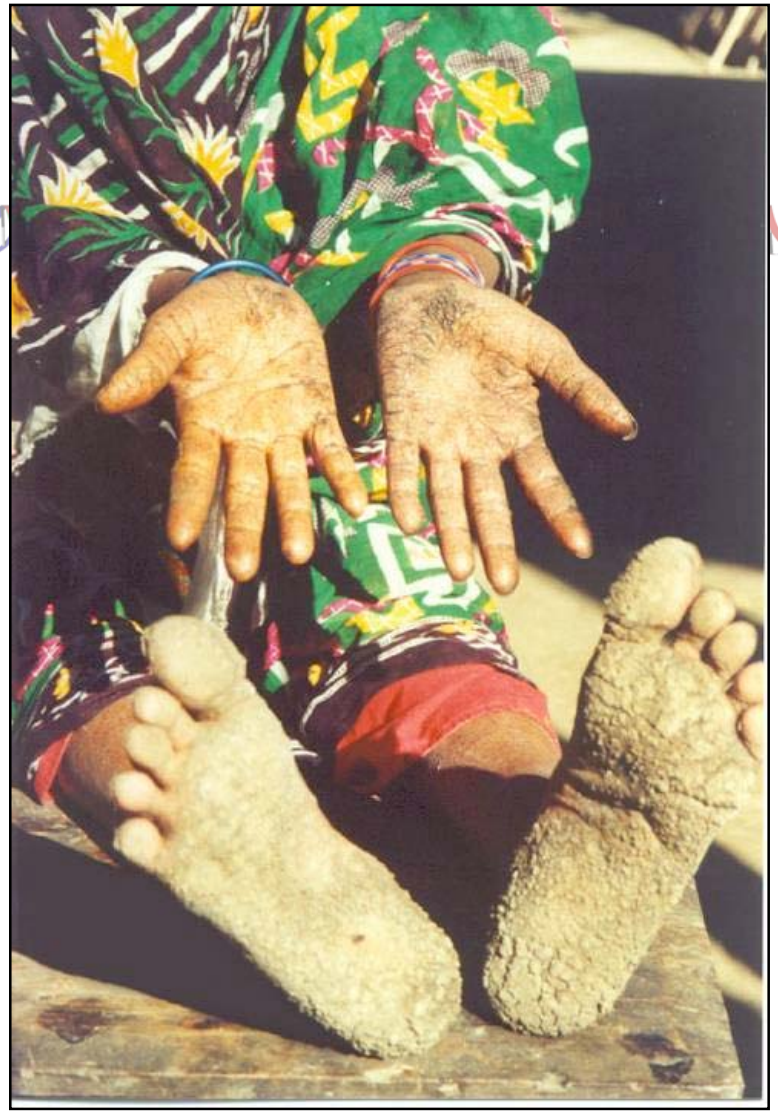

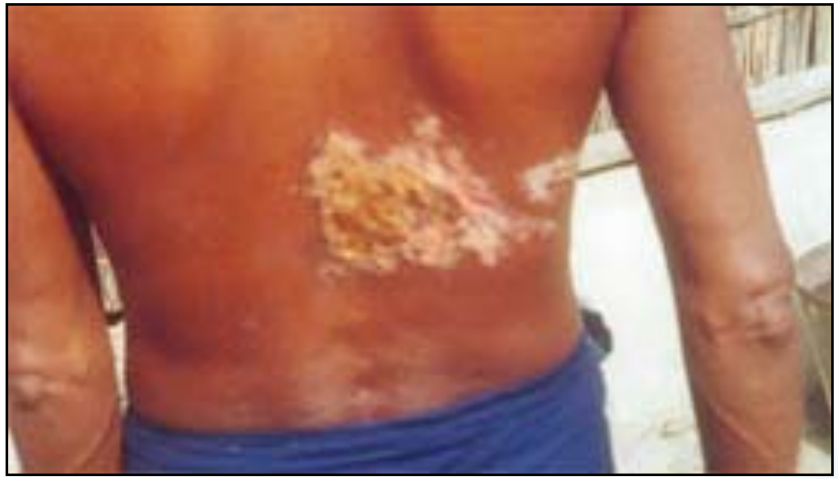

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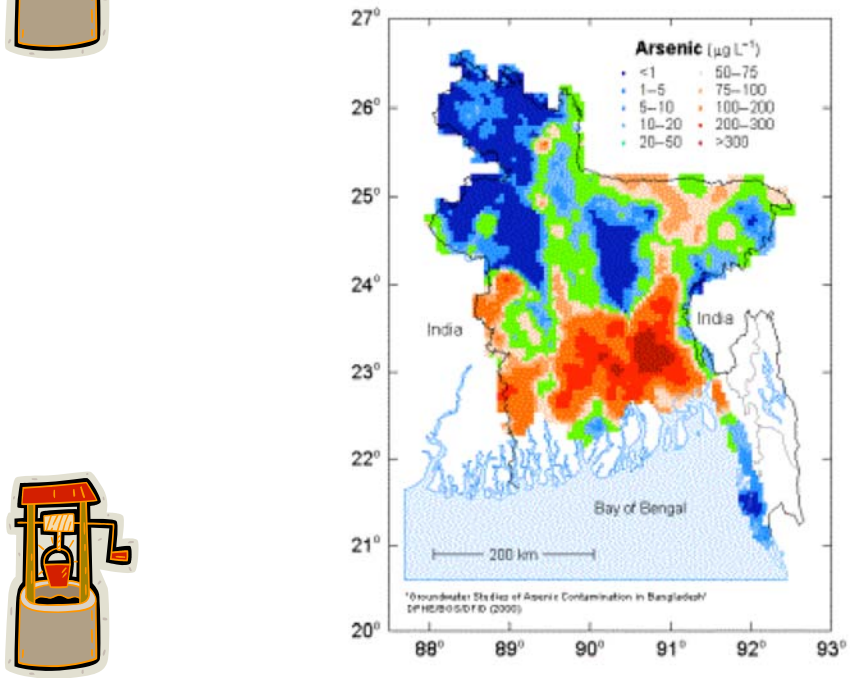
33-75 million people affected

?



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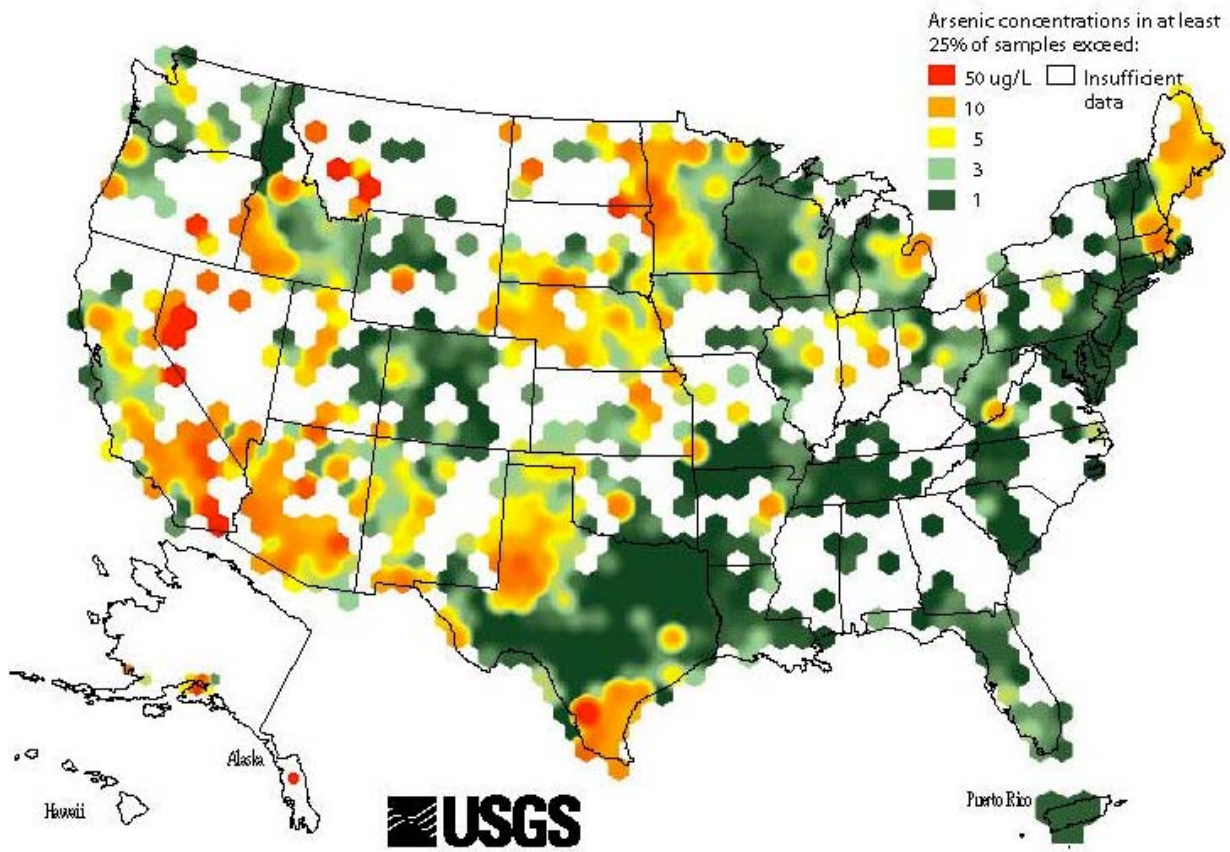
100 million people worldwide

And not just in the developing world either…



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Arsenic detection method

- Current field technology: the Gutzeit Method

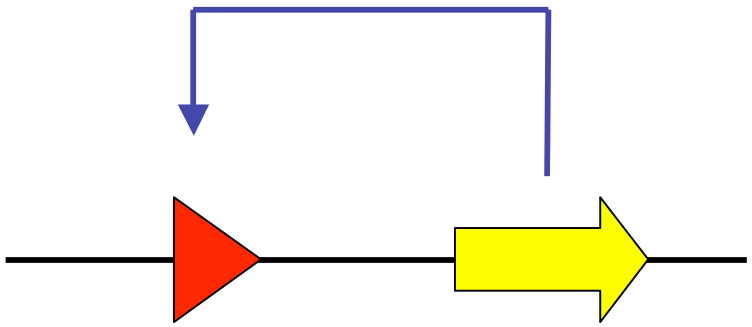
- Expensive and unreliable (max sensitivity of 50 ppb & 33% false negatives)

Our device

Arsenic-sensing bacteria with a pH output

- Very sensitive
- pH requires an indicator, litmus paper or a simple electrode
- It is possible to engineer a quantitative response:
  - ureABC → urease → pH ↑
  - *lacZ'* → lactose fermentation → pH ↓

ArsR mechanism in E. coli



ArsR sensitive promoter

arsR gene

Key:

Gene

Promoter

Binding site

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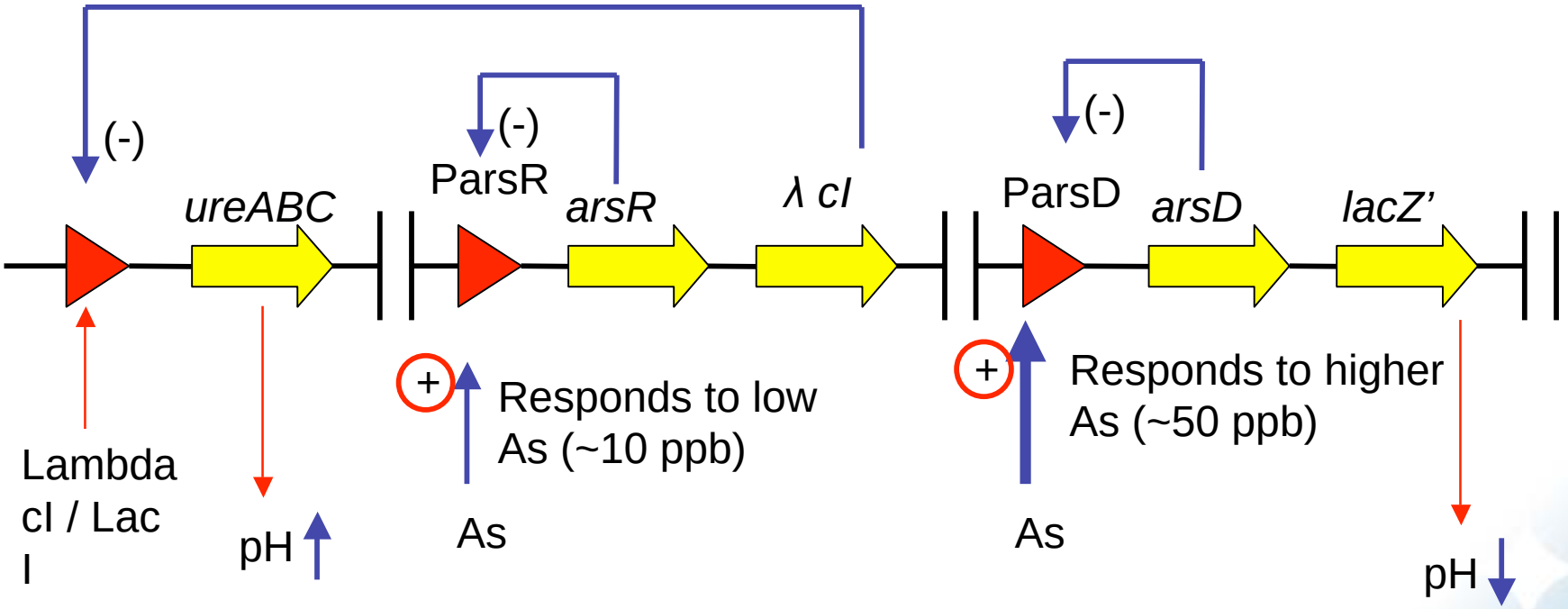
System gene circuit diagram



3 step response:

0 ppb As = pH 9

10 ppb As = pH 7

50 ppb As = pH 4

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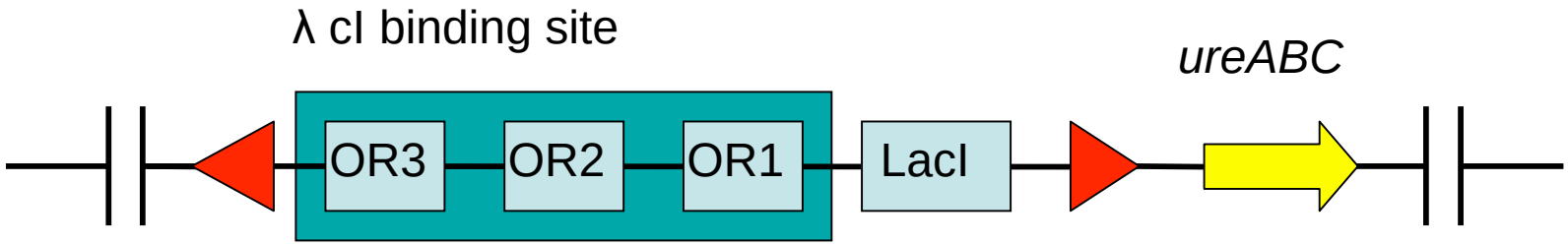
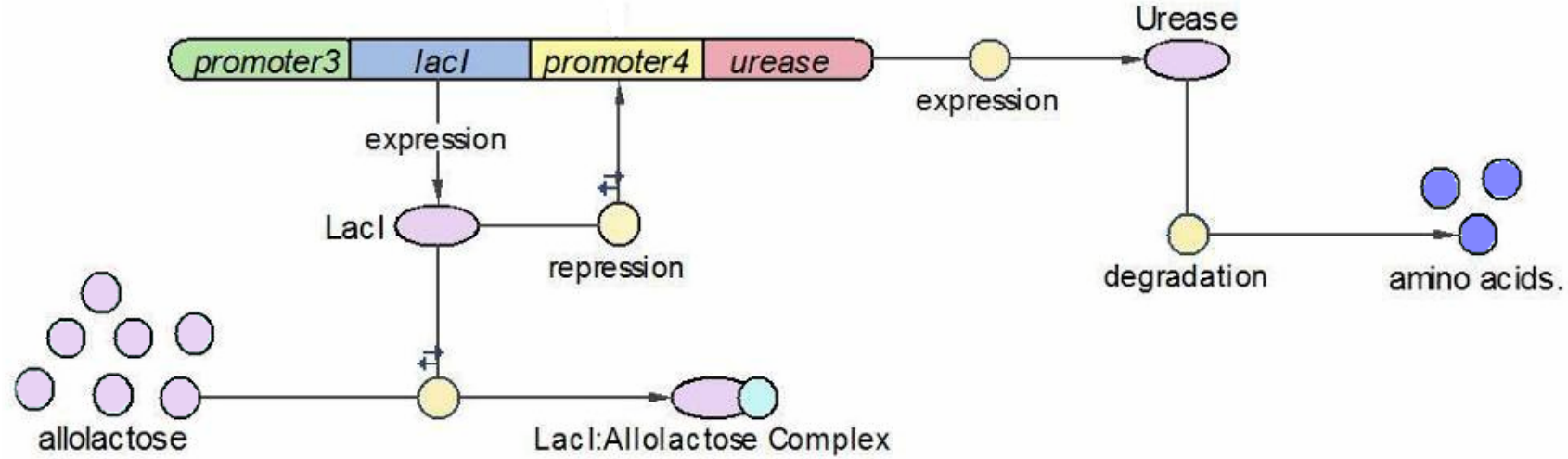
λ cI / LacI hybrid promoter detail

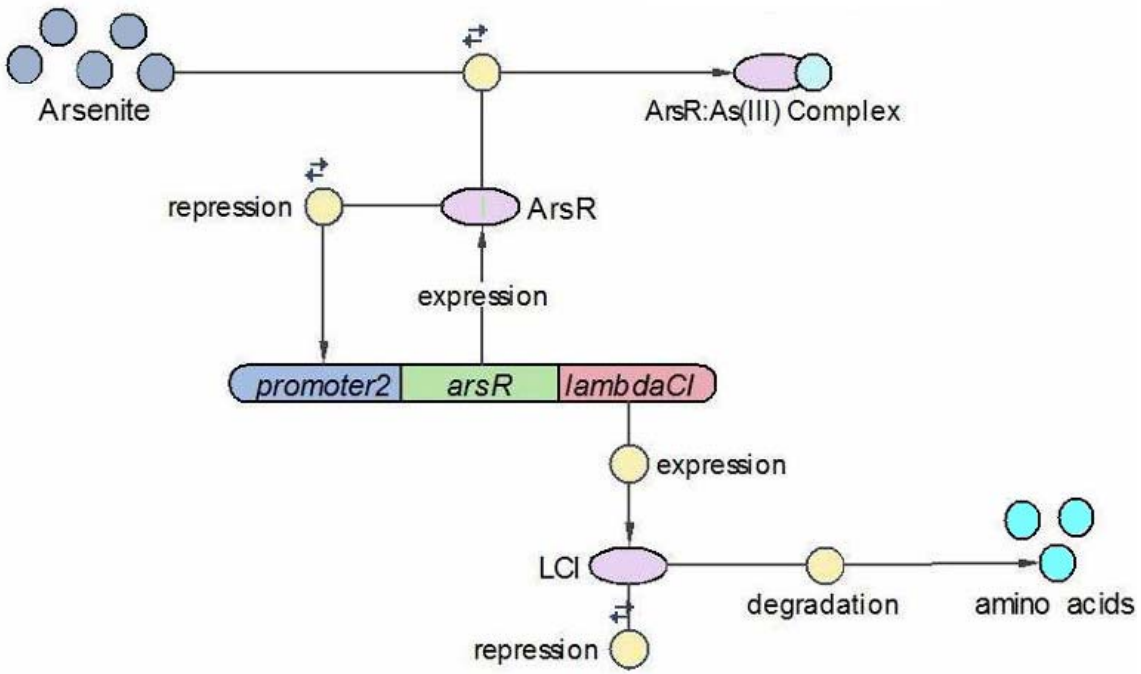


The model explained: Urease operon



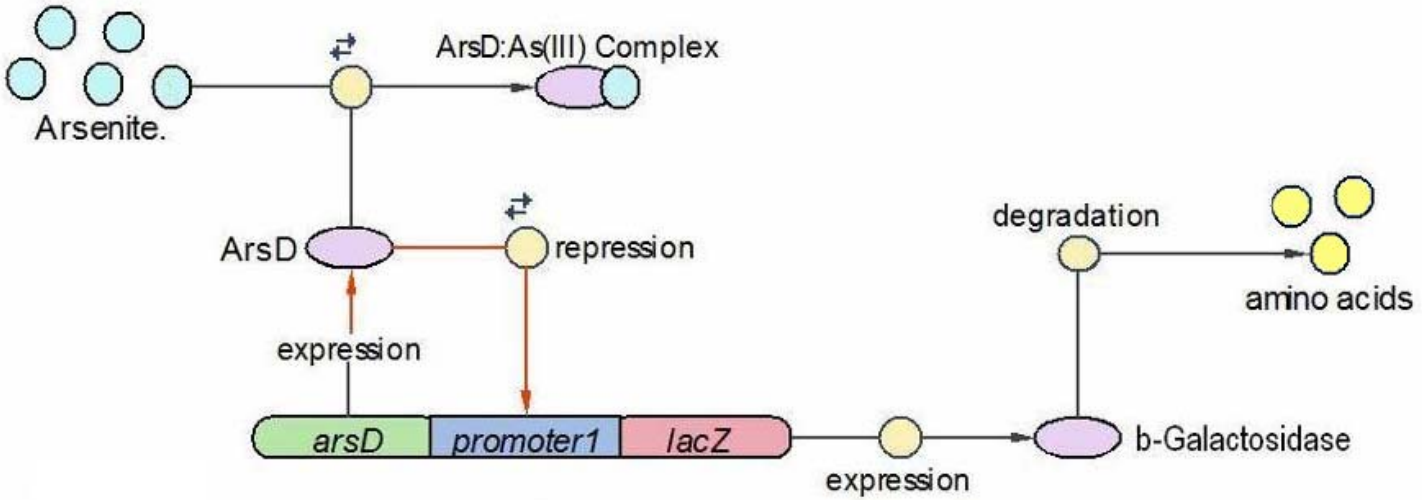
No.	Name	Equation
1	LacI production	promoter3->promoter3+LacI
2	LacI binding to allolactose	LacI+allolactose=LacI-allolactose
3	LacI binding to promoter4	LacI+promoter4 = LacI-promoter4
4	Urease production	promoter4->promoter4+Urease
5	LacI degradation	LacI->null
6	Urease degradation	Urease->null

The model explained: λCI operon



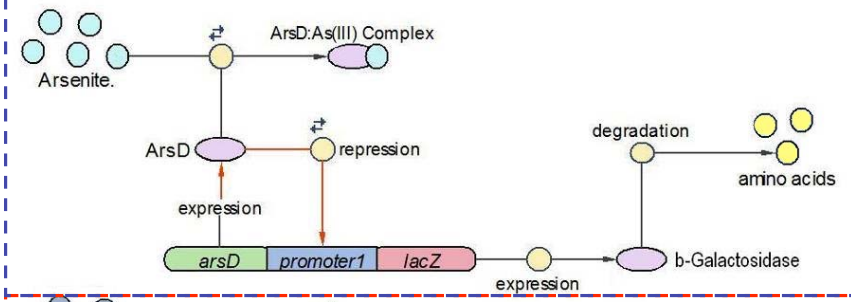
No.	Name	Equation
7	ArsR production	promoter2->promoter2+ArsR
8	ArsR binding to Arsenic	ArsR+2As(III)=ArsR-2As(III)
9	ArsR binding to promoter2	2ArsR+promoter2=2ArsR-promoter2
10	LCI production	promoter2->promoter2+LCI
11	LCI binding to promoter 4	LCI+promoter4=LCI-promoter4
12	ArsR degradation	ArsR->null
13	LCI degradation	LCI->null

The model explained: *lacZ'* operon

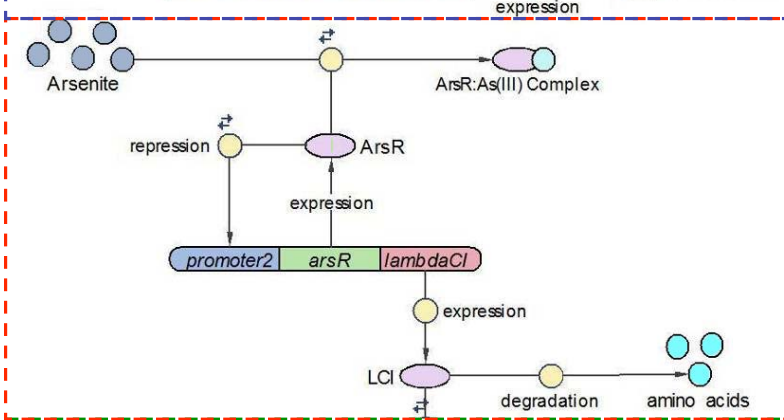


No.	Name	Equation
14	ArsD production	promoter1->promoter1+ArsD
15	ArsD binding to Arsenic	ArsD+2As(III)=ArsD-2As(III)
16	lacZ production	promoter1->promoter1+lacZ
17	ArsD binding to promoter1	2 ArsD+promoter1=2ArsD-promoter1
18	ArsD degradation	ArsD->null
19	lacZ degradation	lacZ->null

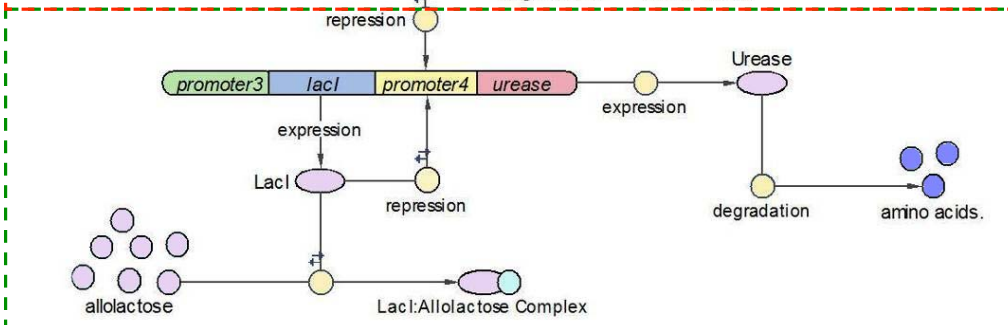
The model explained: the whole system



- Operon 1
  - 6 Reactions
  - 10 Parameters

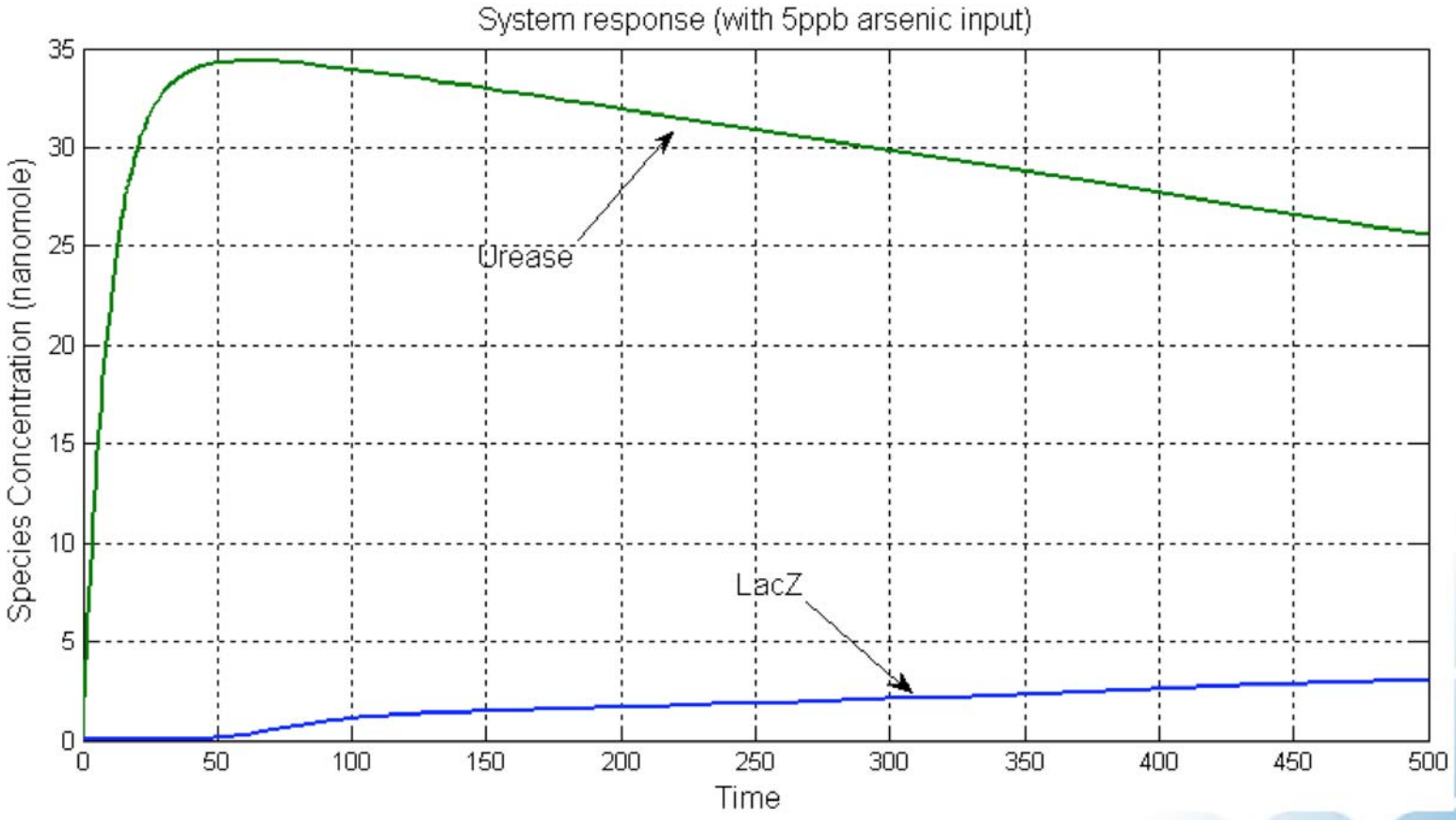


- Operon 2
  - 6 Reactions
  - 10 Parameters

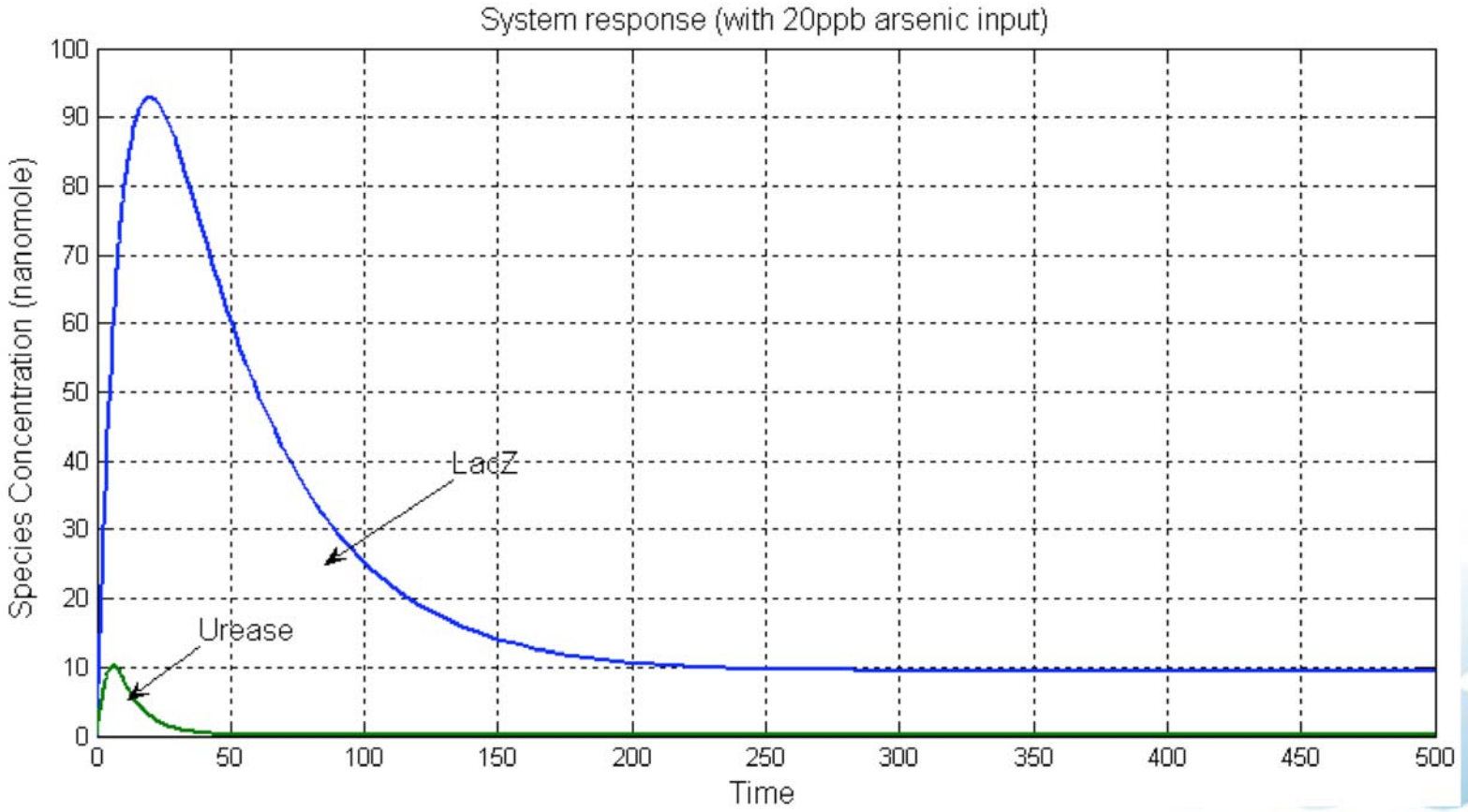


- Operon 3
  - 7 Reactions
  - 12 Parameters

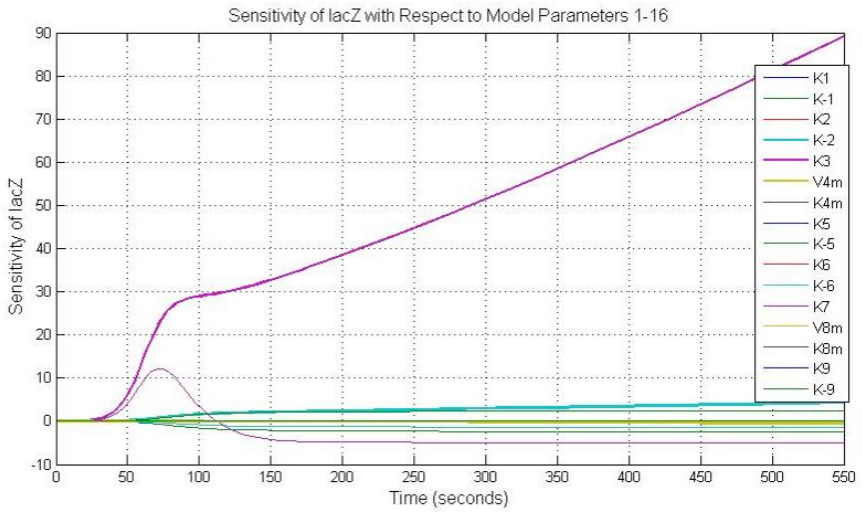
Modelling result: low arsenic

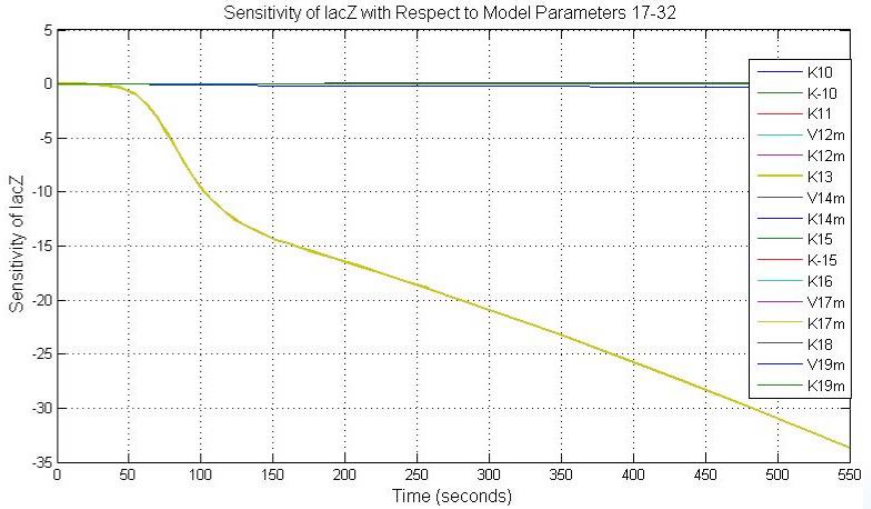


Modelling result: high arsenic



Parameters sensitivity analysis

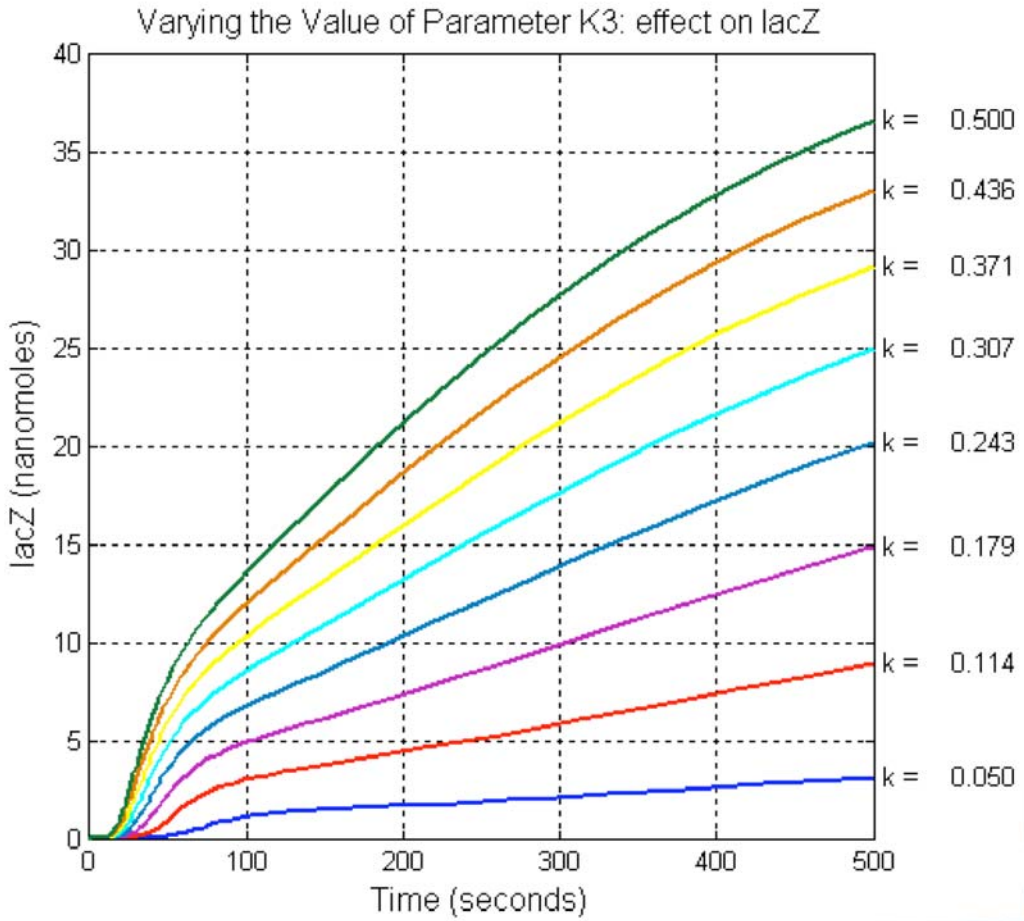




The most sensitive parameters affecting lacZ

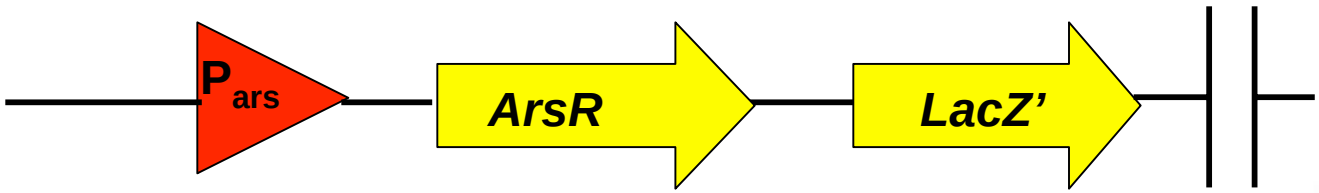
Nanme	Description	Peak value of sensitivity	Normal value
K-1	ArsD-2AS(III) dissociate rate	-2	0.65/s
K-2	2ArsD-promoter1 dissociate rate	4	0.65/s
K3	ArsD degradation rate	90	0.05/s
K7	ArsR degradation rate	12	0.05/s
K13	LacZ degradation rate	-35	0.1/s

Parameter scanning



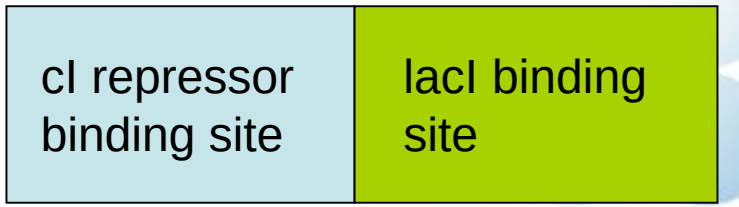
Building the biosensor

- To prove our concept and provide a starting point for the larger device, we built a simple construct
- To test arsenic sensitivity and magnitude of pH change (if any!)



Biobricks

- Putting new parts in the registry one of our project aims
- Created ArsR and Ars promoter parts from *E. coli*, with *B. subtilis* for comparison
- Also built new *lacZ'*
- Hybrid promoter built and urease planned (site-specific mutagenesis on urease)



Urease part ideas

- Secondary idea - 3D Structure Builder
- Links to biosensor model by using the urease part
- Tissue engineering, bioremediation, etc.

$$Urea + H_2O \rightarrow CO_2 + NH_3$$

= pH =

Calcium carbonate

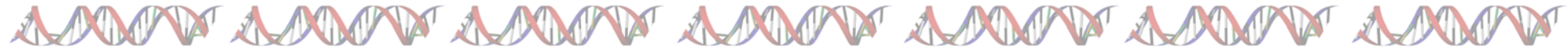
Outcomes

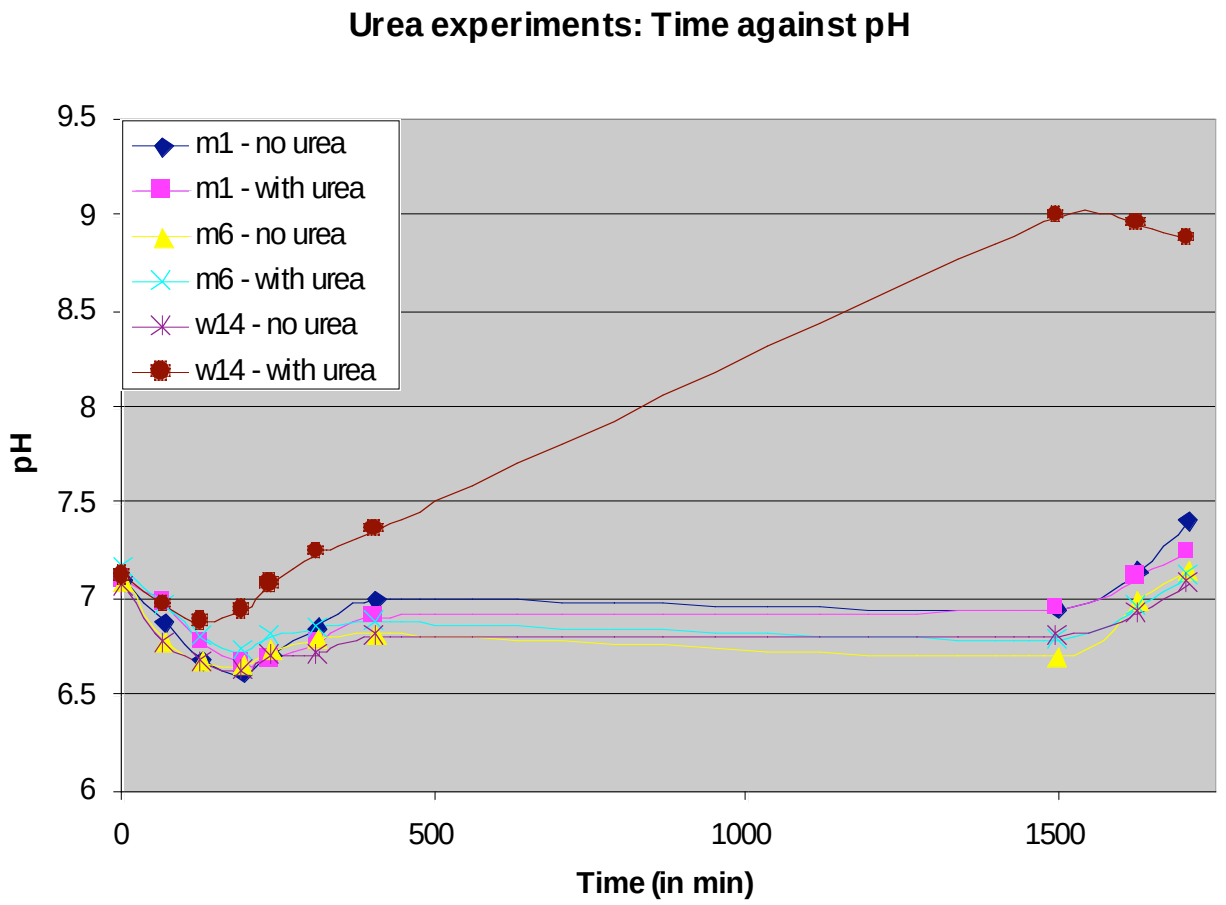
- Result: Simple construct built and ready to be characterised with pH experiments
- Several parts sequenced for registry
- Future: Test hybrid promoter and urease device, work towards full device

Characterization procedure

- Now we have the engineered constructs, we need to test them
- Aims:
  - to measure the scale of the pH response
  - To test the sensitivity of the *B. subtilis* and *E. coli* Arsenic promoters

Urease characterization

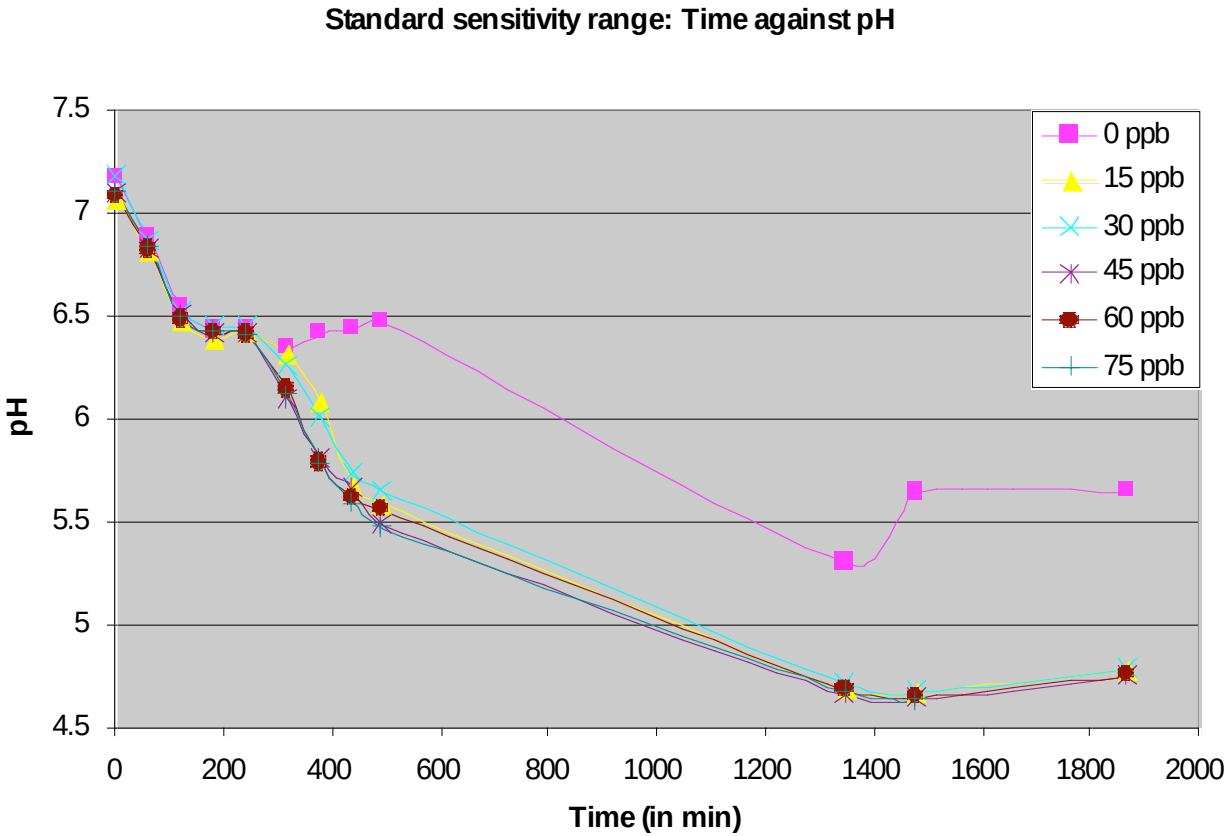




Note: only 1 response from wildtype urease

- The Wildtype urease contains 2 forbidden restriction sites
- The strain with the restriction sites removed failed to change the pH (i.e. the mutagenesis failed)
- Only the wild type Urease operon produced a change in pH

E. coli biosensor characterization



- Only testing a yes/no response here
- arsR promoter begins to function after about 300 min
- Difference of 0.6 pH units between control and average readings

Note: successful response at all concentrations of arsenic

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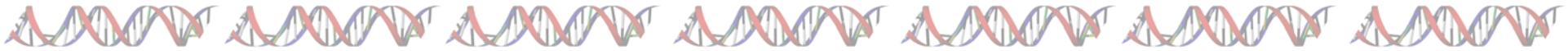
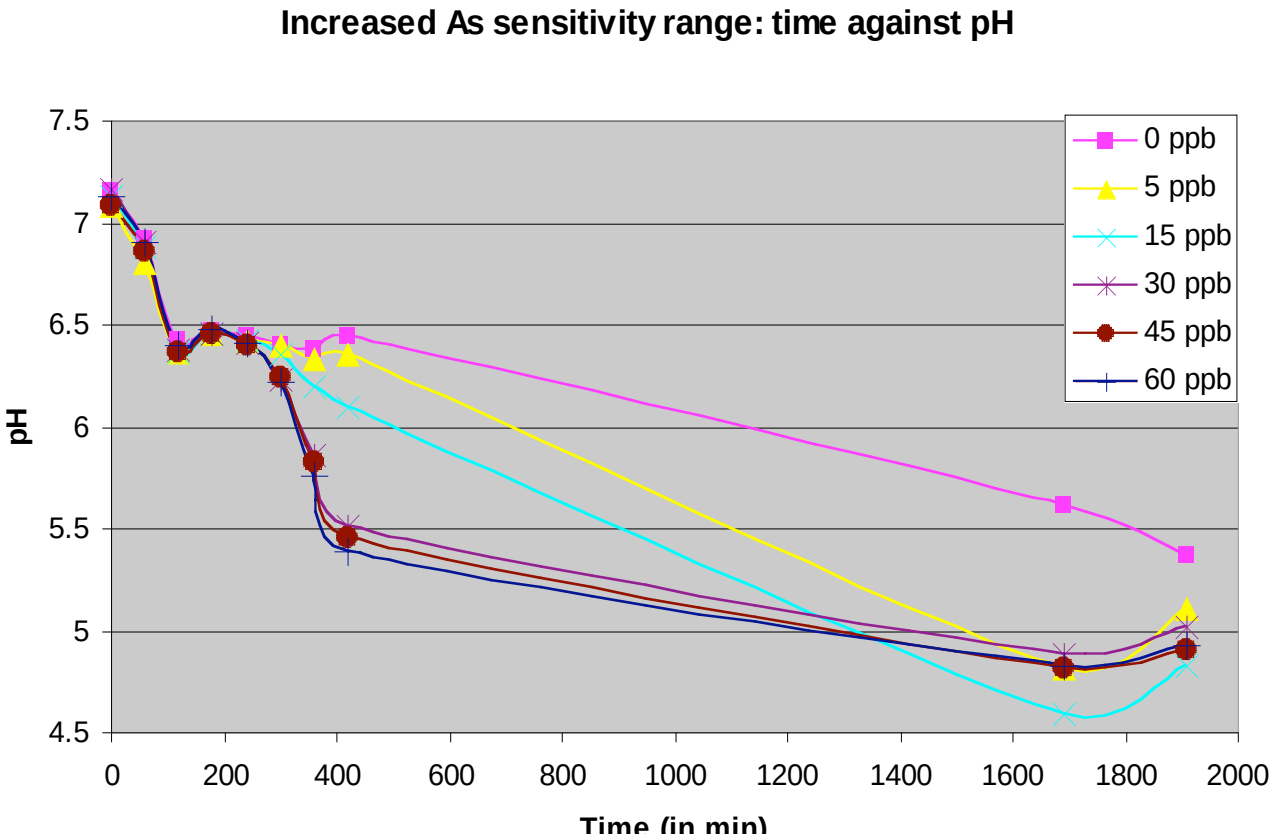
E. coli biosensor characterization





- Non-optimised growth medium conditions (response could be faster)
- Average overnight difference of 0.81 pH units
- Despite slower response, 5 ppb within sensitivity of promoter
- Can detect WHO guideline levels of Arsenic (arsenate)

Note: successful response at all concentrations of arsenic

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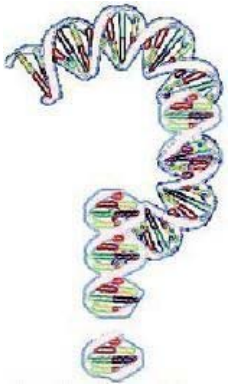
The desired end result

- Cheap, reliable, robust field test device
- Foolproof to operate and get accurate results
- Can be produced for less than 1$ in mass volumes



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The field test device

- A test tube could contain all the necessary components: Freeze dried bacteria, growth medium, indicator powder, Ampicillin salt, etc…

•These tubes could then be given to local villagers to monitor their own water quality themselves

•A good alternative to the widely used Gutzeit method

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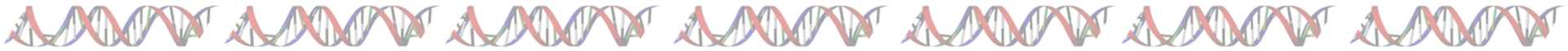
Conclusions



- Successfully designed and modelled complex device
- Proved a measurable pH response could be obtained with As concentration to WHO standards
- Successfully biobricked a variety of parts to deposit in the registry
- With further work, our device could potentially help millions worldwide

We would like to thank our sponsors

- Funding assistance:

The Gatsby Charitable Foundation

bbsrc
biotechnology and biological sciences research council

SYNBIOCOMM

EPSRC Engineering and Physical Sciences Research Council

- Sysbio 2.0 toolbox licenses:

The MathWorks

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Questions?

Edinburgh Castle (on the only day this year it wasn't raining)

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