



Heavy metal
scavengers
with a vertical
gas drive

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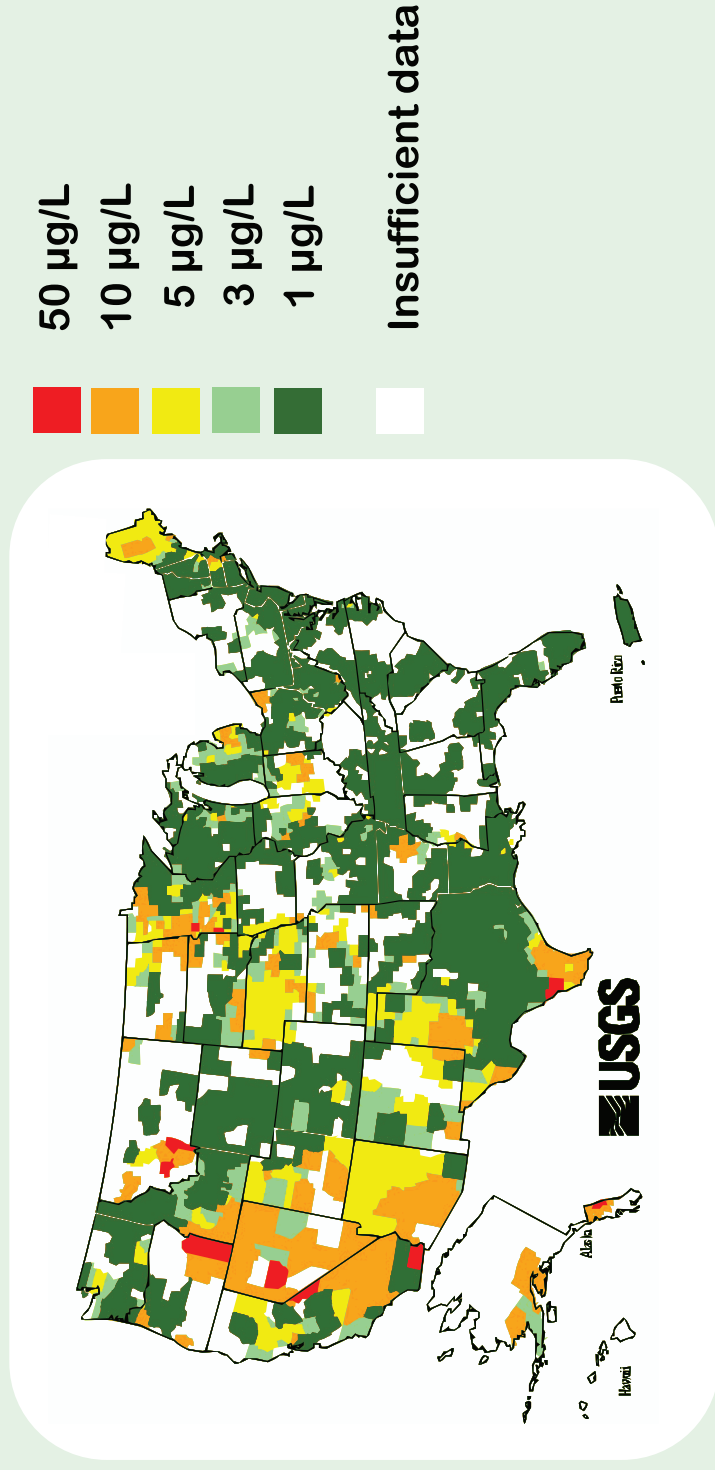
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Dirk Jan Slotboom

Bert Poolman



Arsenic in our Drinking Water



- Over 137 million people in more than 70 countries
- Causes skin, liver and lung cancer
- Maximum dose 10 µg/L (=0.13 µM)

Vision

Water/Sludge cleaner

Heavy or toxic metal removal from water or sludge, after which floating provides easy removal

Aim

Cheap and efficient method for cleaning sediment and water

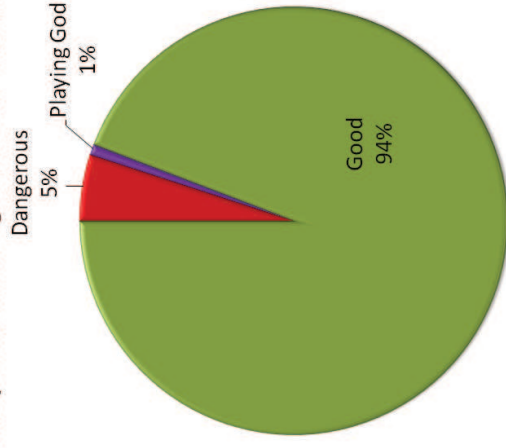
Ethics

Ethical issues

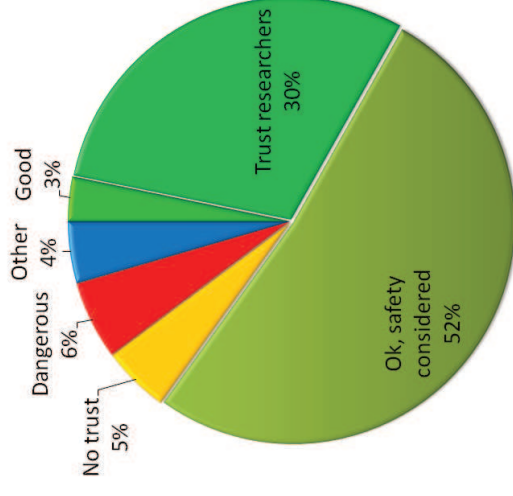
- Is it safe to use a genetically modified bacterial system in practice?
- Is there a risk of misuse?

Survey 262 respondents (147 man, 115 female)

Dutch opinion on using GMO's in research



Dutch opinion on using GMO's in our application



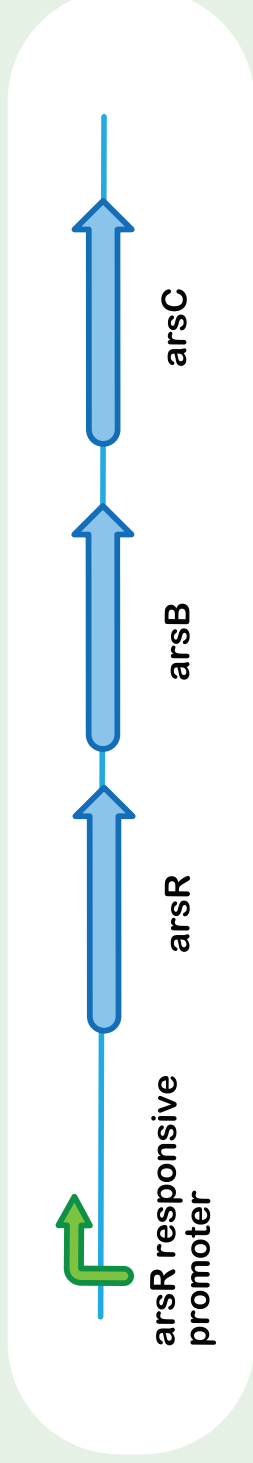
- There is no significant difference between people who know what synthetic biology is and those who do not.
- Small risks, like spreading of genes were considered.



Chassis

E. coli DH10B (Invitrogen TOP10 cells)

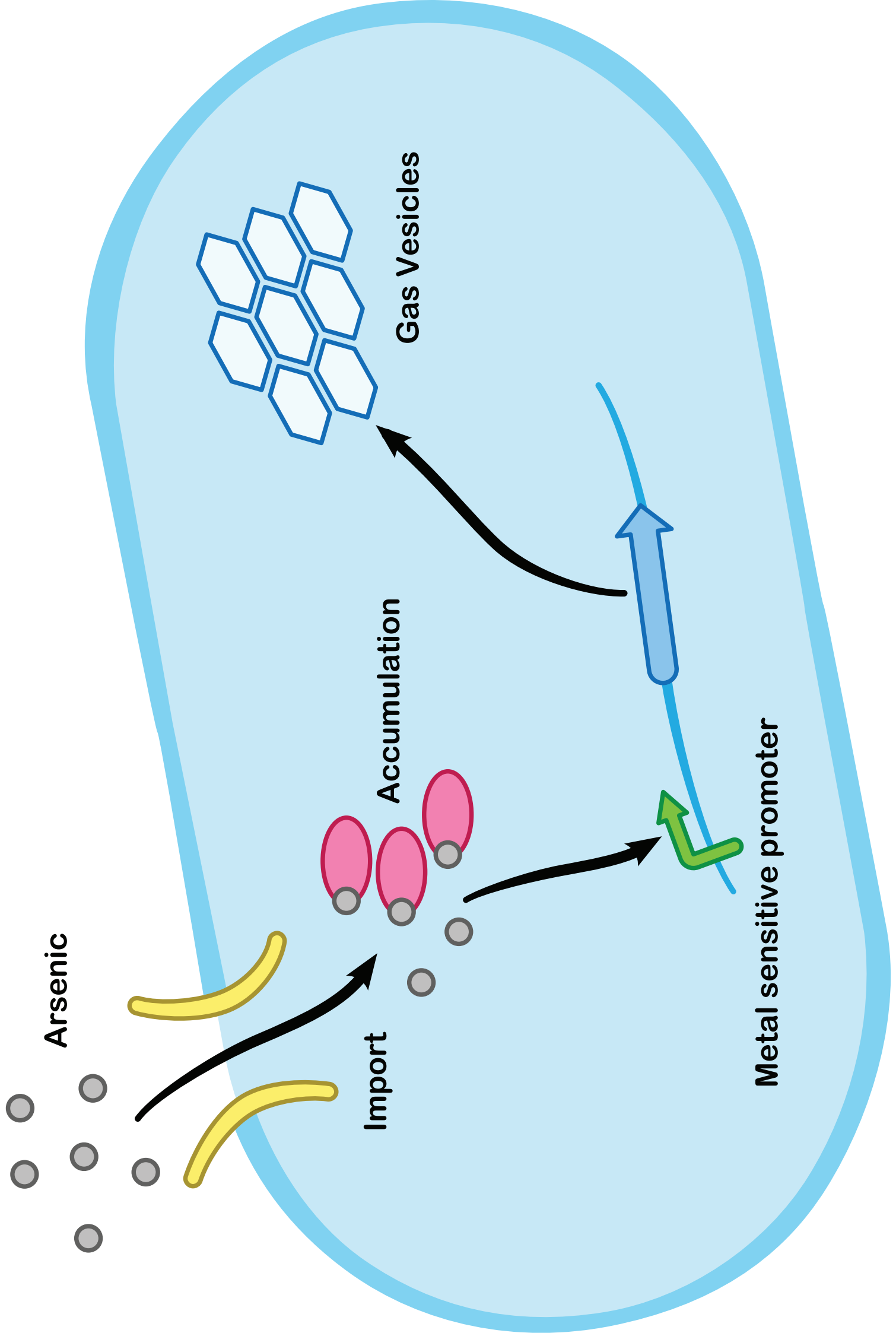
Ars operon



arsR - Ars (III) Transcriptional regulator
arsB - As(OH)₃/H⁺ antiporter
arsC - Ars(V) reductase; As(V) → As(III)

GlpF

Aquaglyceroporin



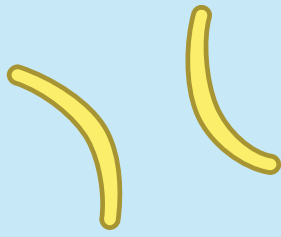
Arsenic

Import

Accumulation

Gas Vesicles

Metal sensitive promoter

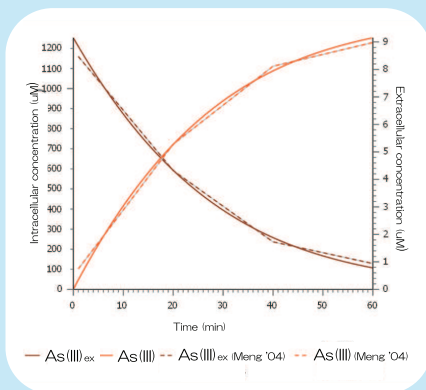


Import

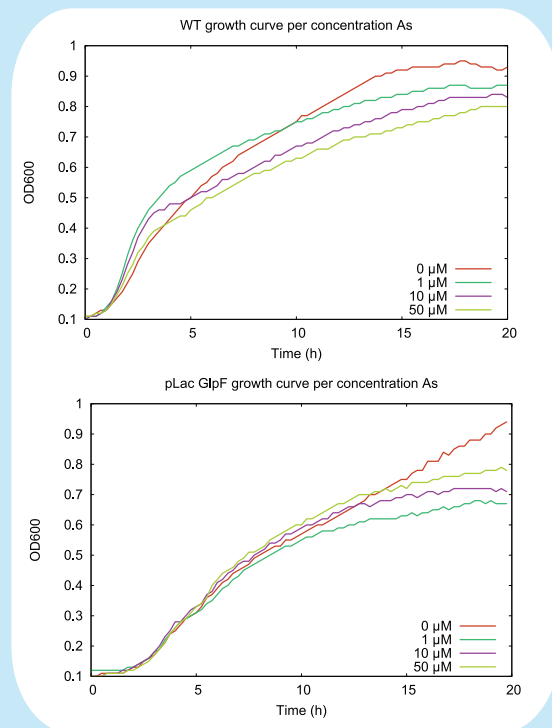
GlpF

- Aquaglyceroporin (Singh et al., 2008)
- Metal sensitivity assay

Modelling



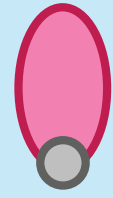
Testing



References:

Singh *et al.* 2008, *Microbiology* 74(9): 2924–2927

Meng *et al.* 2004, *Journal of Biological Chemistry* 18(279):18334–18341



Accumulation



fMT

- Metallothionein from *Fucus vesiculosus* (Morris et al. 1999)
- fMT binds 5x As(III) and As(V)

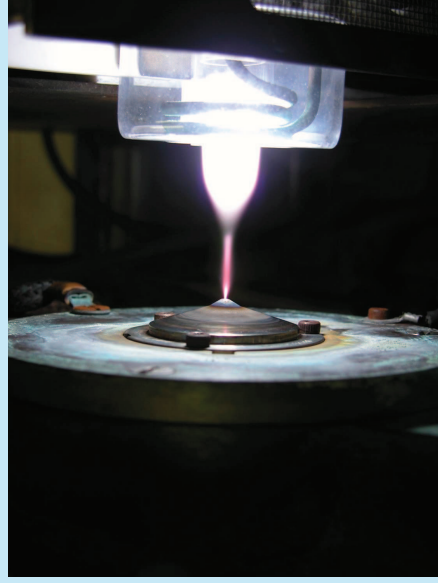


ArsR-MBP

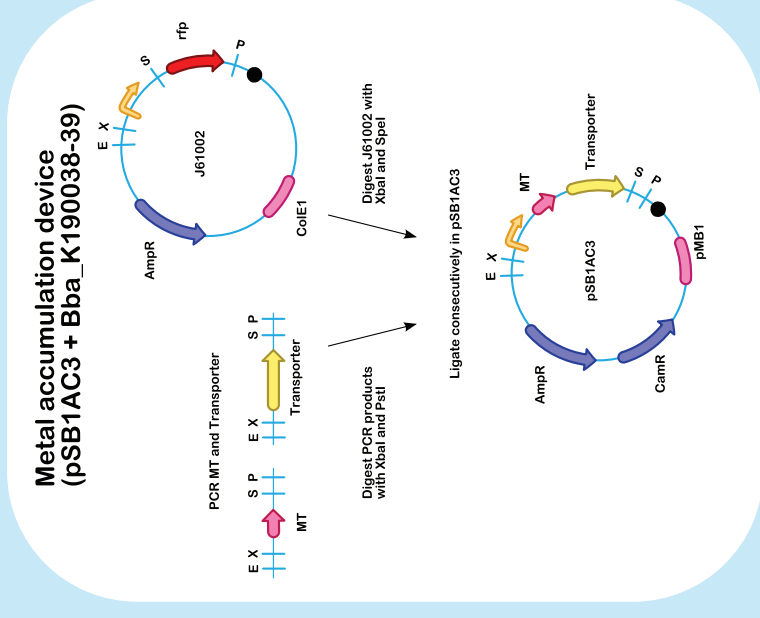
- ArsR is a transcriptional regulator from *E. coli*
- ArsR-fusion protein is known to accumulate As(III) (Kostal et al. 2004)

Testing

- Using Inductively Coupled Plasma - Mass Spectrometry
- Accumulation up to 2 nmol/mg dry cells (in agreement with results found by Kostal et al. 2004)



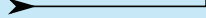
Accumulation device



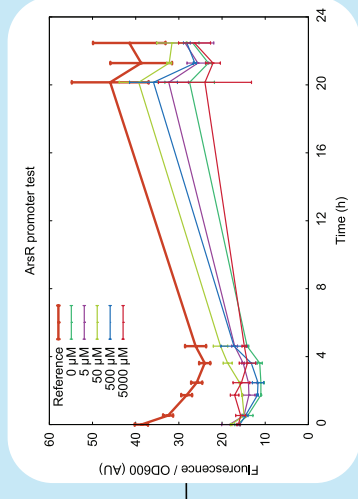
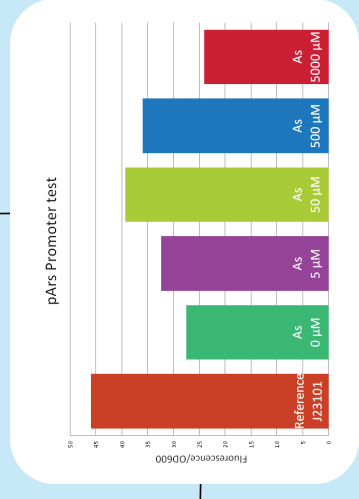
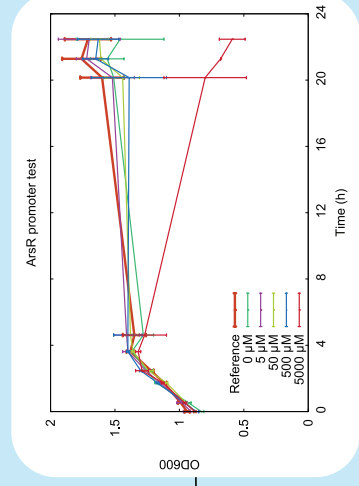
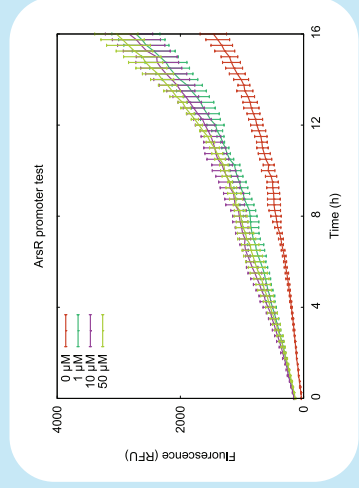
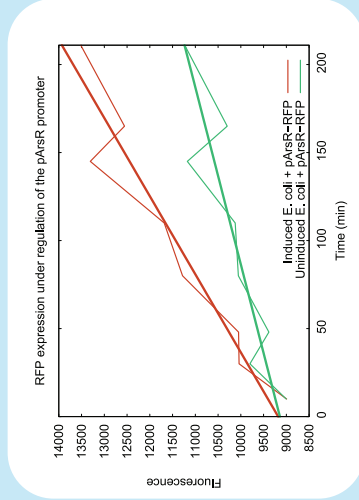
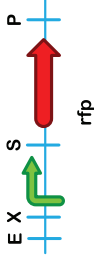
Metal sensitive promoter

Arsenic promoter

- Negatively regulated by ArsR
(Summers 2009; Kostal *et al.* 2004)

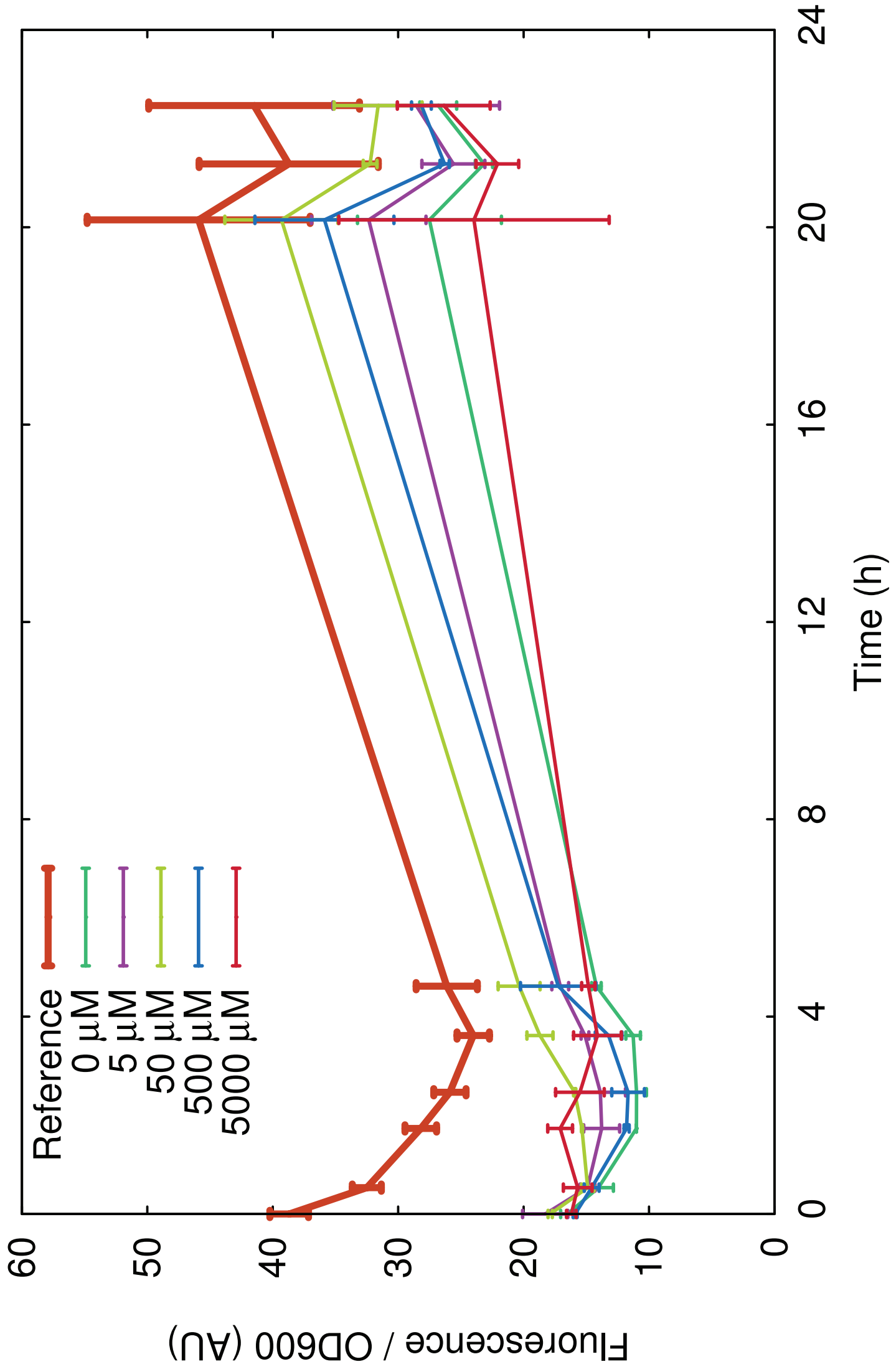


Promoter measurement device
(J61002 + Bba_K190022-24)

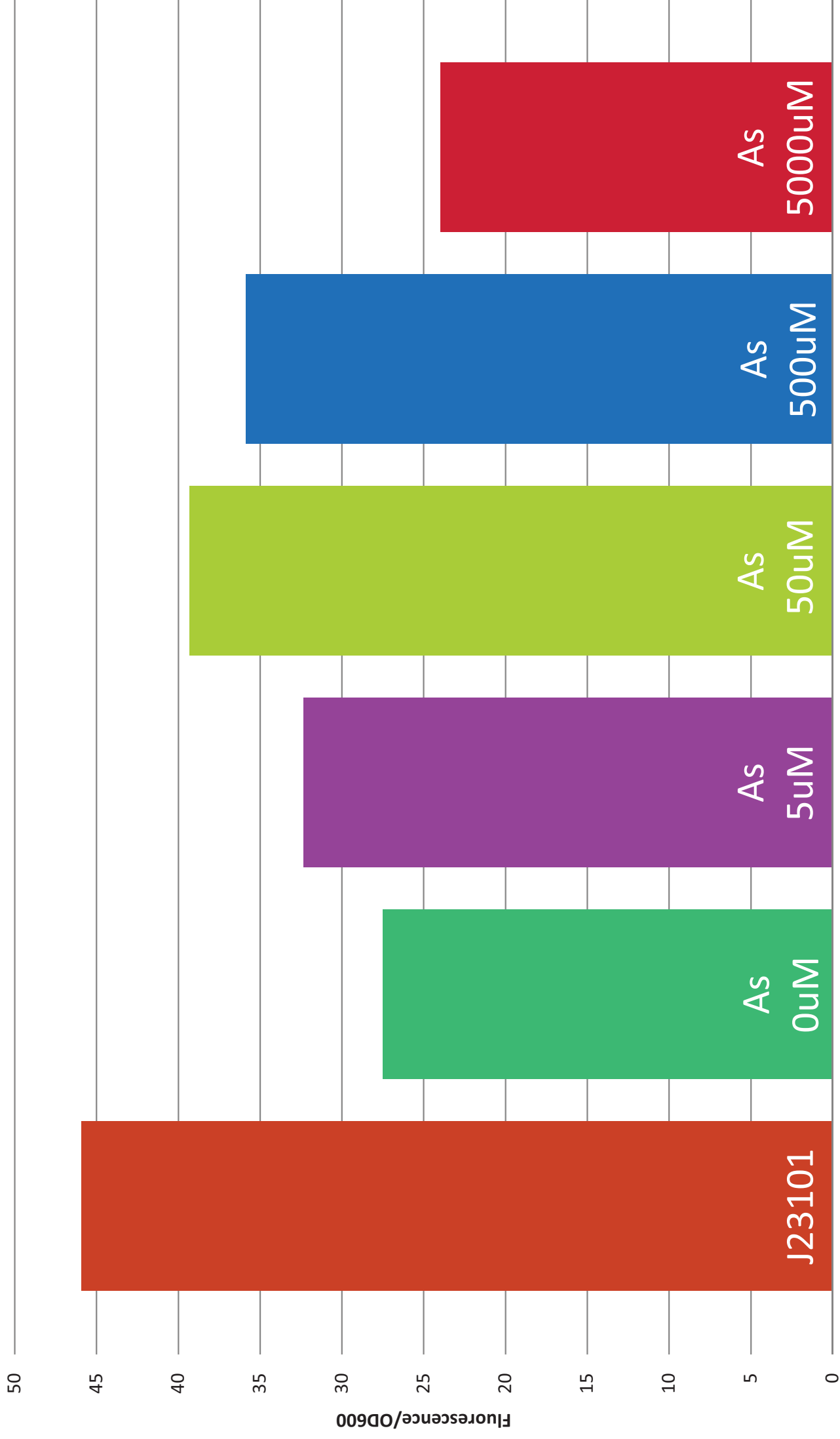


References:
Kostal *et al.* 2004, *Applied and Environmental Microbiology* 70(8): 4582-4587
Summers 2009, *Current Opinion in Microbiology* 12(2): 138-144

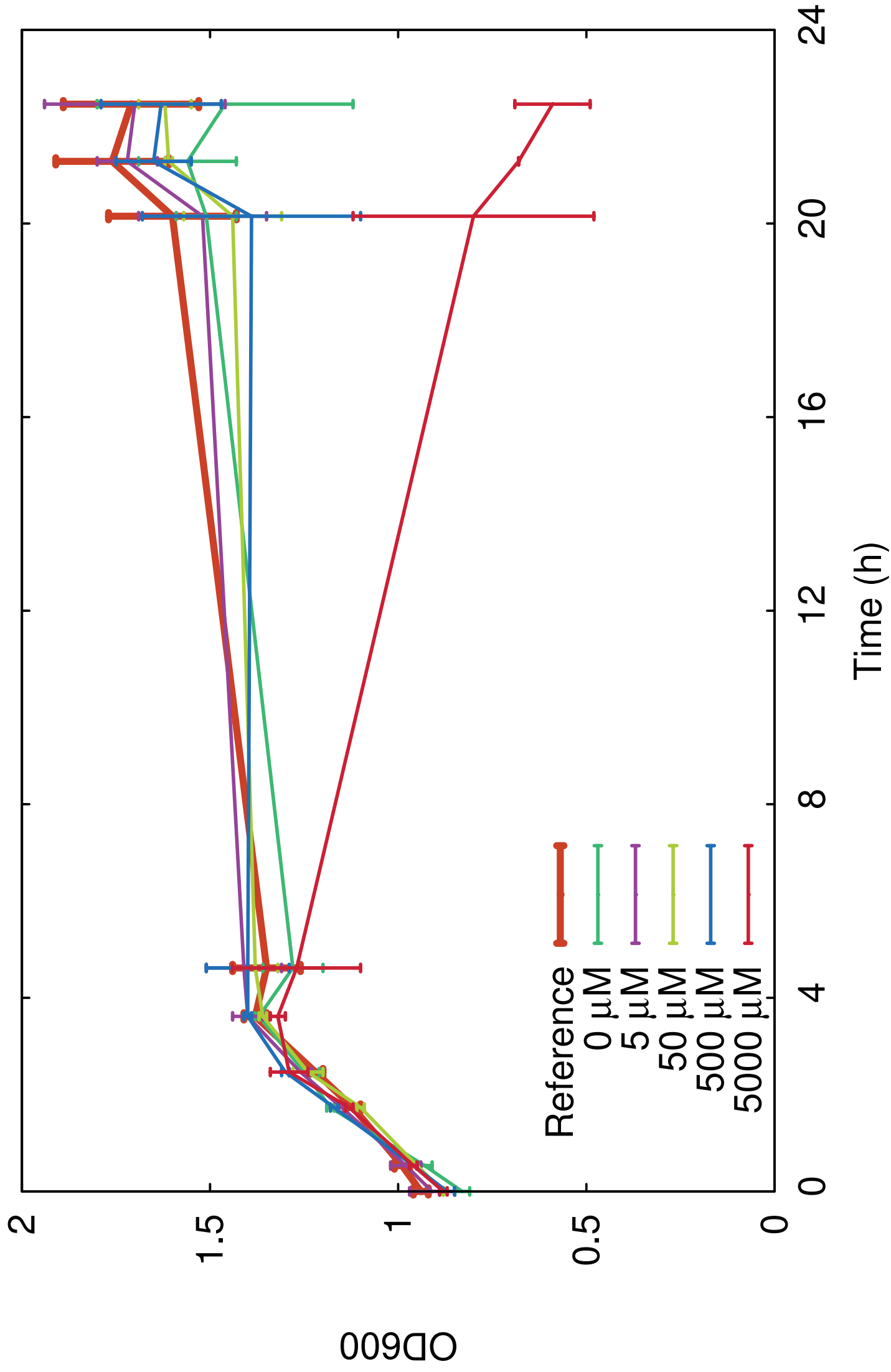
ArsR promoter test



pArs Promotor test



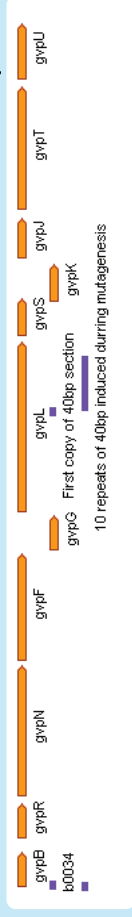
ArsR promoter test



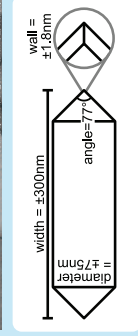
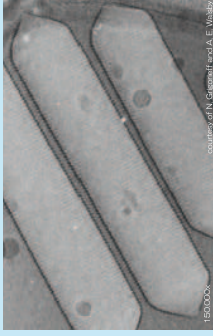
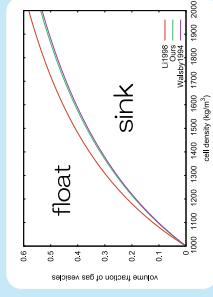
Gas vesicles

Gas vesicle gene cluster

- Buoyancy phenotype after expression of GVP from *Bacillus megaterium* in *E. coli* (Li & Cannon, 1998)
- Use BioBrick with GVP from Melbourne 2007 (BBa_I750016)

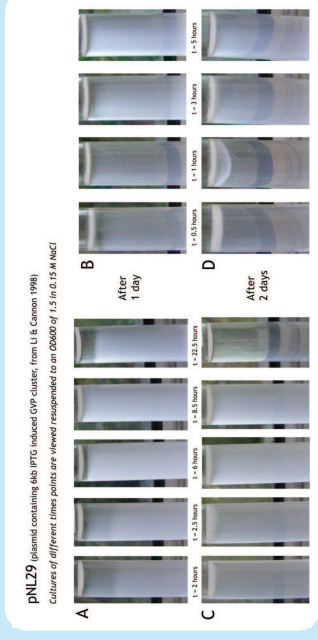
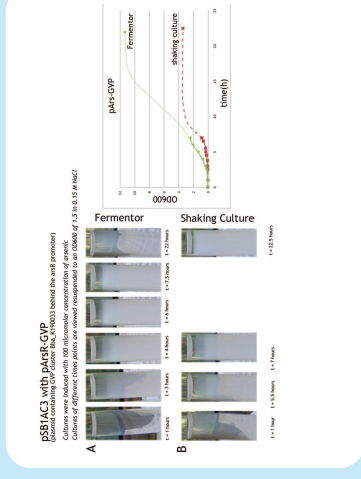
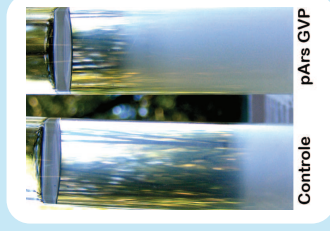


Modelling

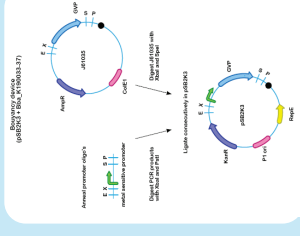


Results

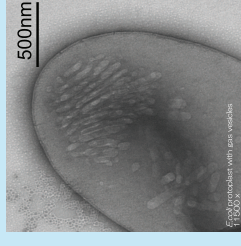
Buoyancy test

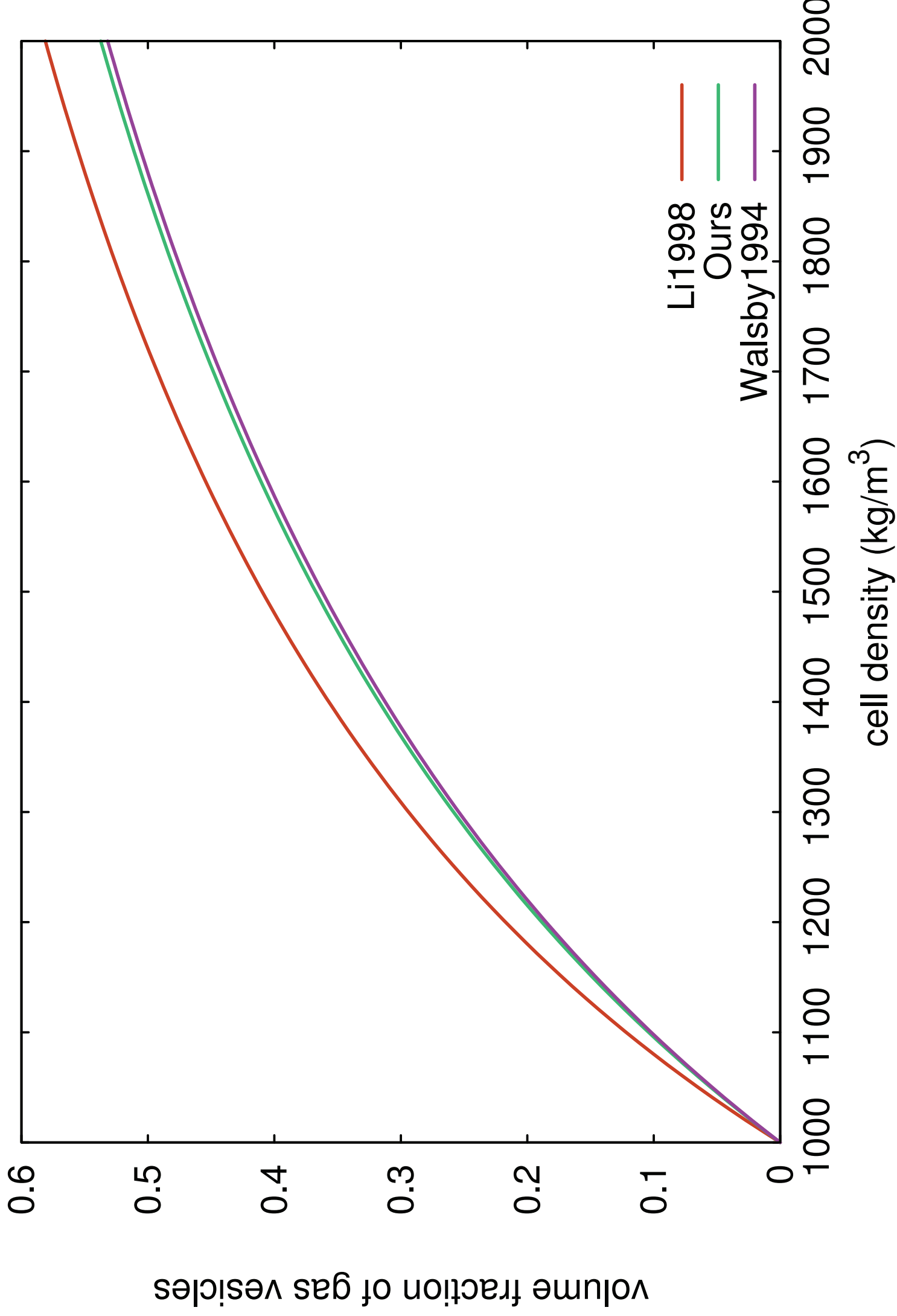


Buoyancy device



Transmission electron microscopy





Modelling our system

Starting with one part

Import 



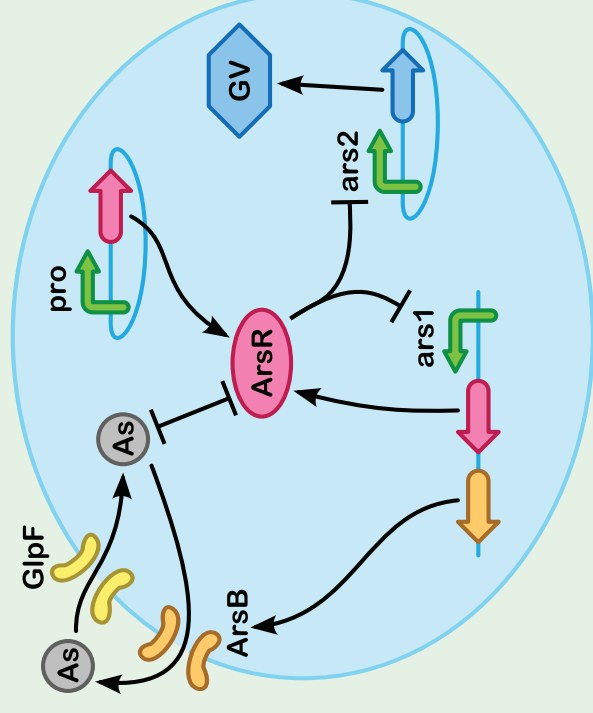
Time derivatives

GlpF-As, As(III)ex, GlpF

Optimization through
Simulated Annealing

- **More experiments can be added**
- **System independent**
- **Interactive on wiki**

To create the whole system

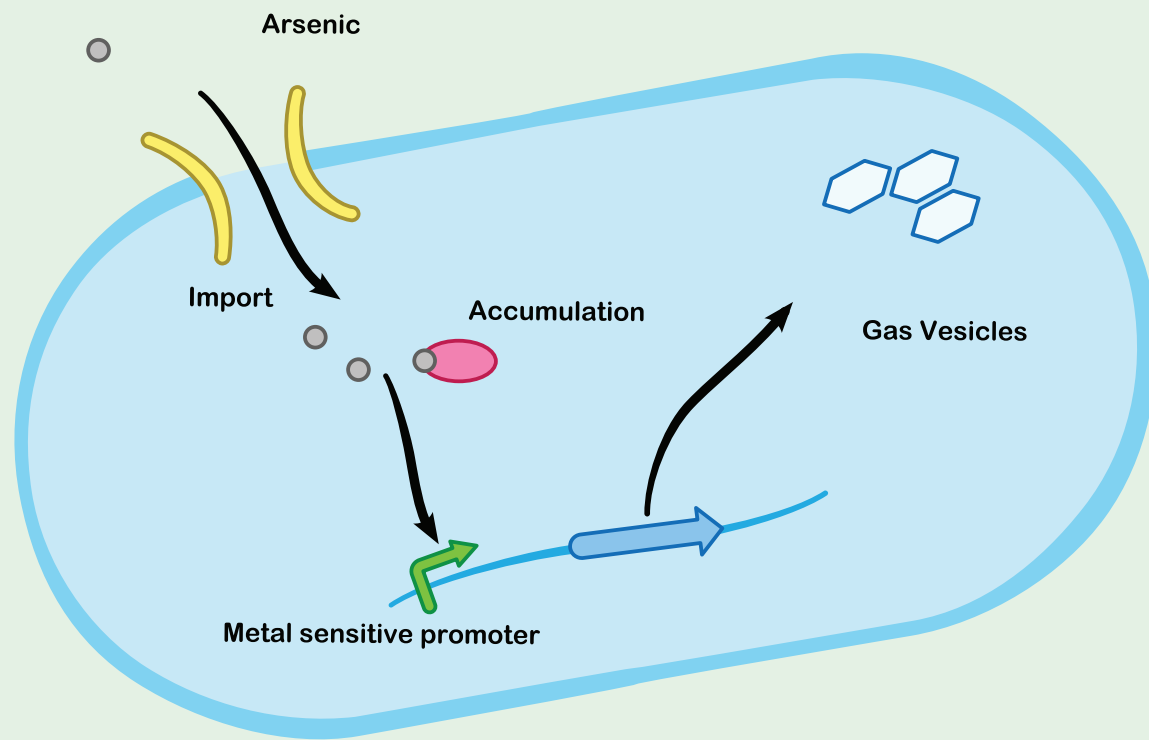


Characterizing our Parts

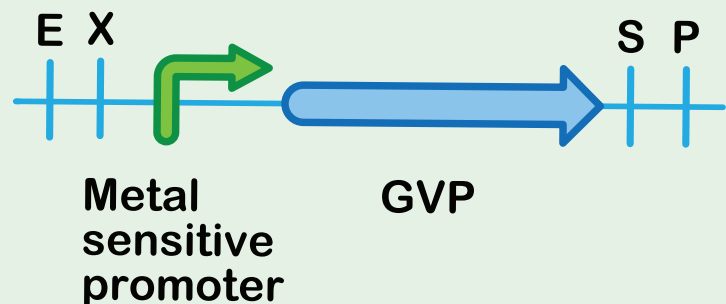
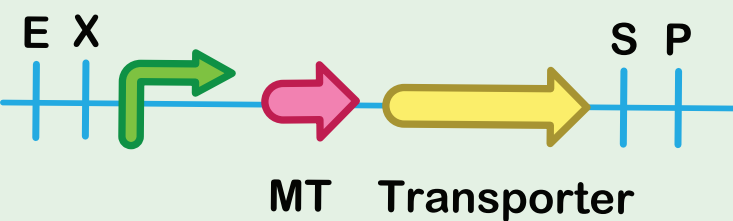
- **What are our production and degradation rates?**
- **Can we determine the RPU of our part?**

Whole system

Overview



Our devices



Modular system

Adaptation for almost any type of 'scavenger'

Cu
Copper

Zn
Zinc

Au
Gold



Transporter



Storage proteins



Promoter

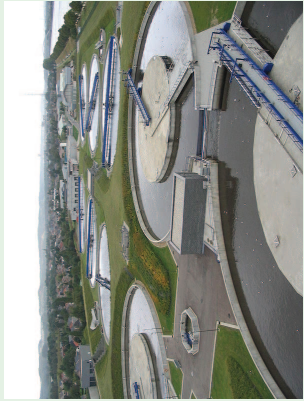


Regulator



Gas vesicles

Applications



**Drinking
Water**



**Sludge
Purification**



Mining

Acknowledgements

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Elly Wijma, RIKILT**



**Rudi Anakotta,
Food & Consumer
Product Safety Authority**



Thank you for your attention!

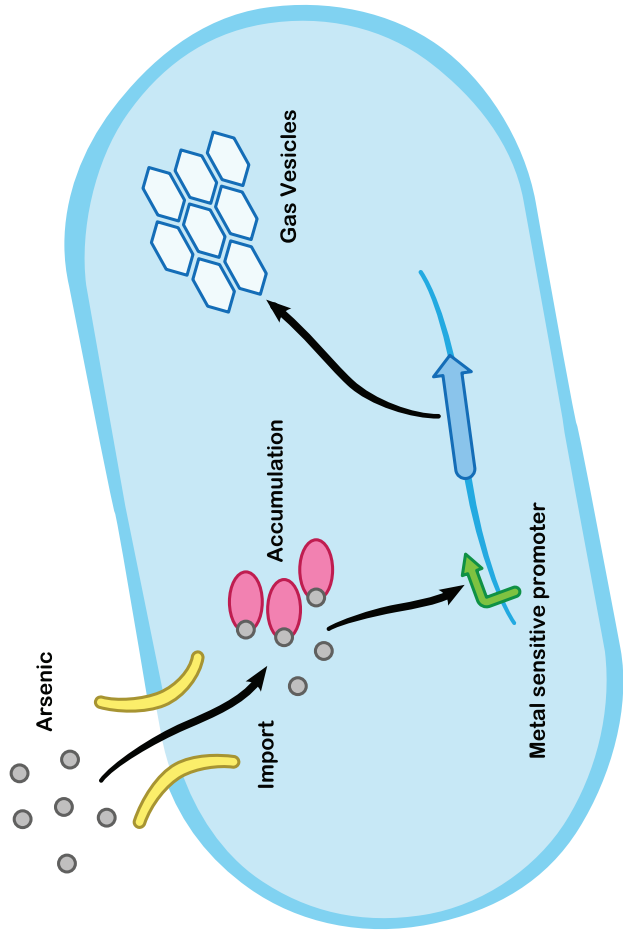
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Heavy metal scavengers with a vertical gas drive

iGEM Groningen 2009



Thank you for your attention!