Bac-Man: Sequestering Cadmium into *Bacillus* Spores

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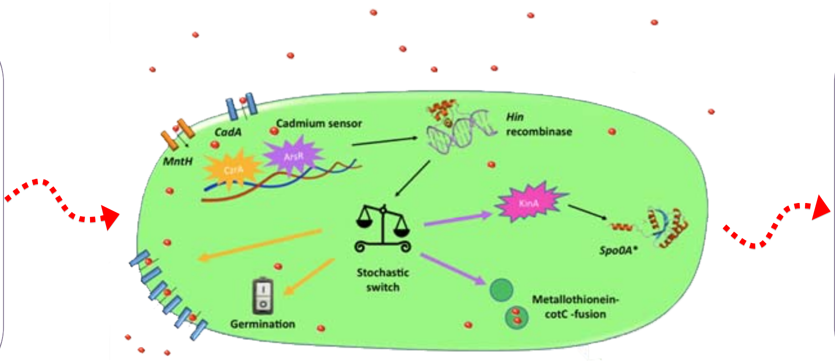
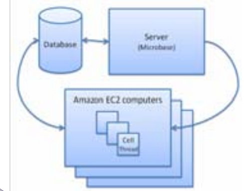
Introduction

Cadmium contamination is a serious problem for the environment. Our system addresses this issue by engineering ***Bacillus subtilis*** to sense and isolate cadmium ions from the **soil** environment, sequestering them into highly resilient spores thus rendering cadmium **bio-unavailable**. Spore germination is disabled, making retrieval of the cadmium unnecessary. We computationally simulated the **life cycles** of individual cells and entire cell populations to estimate the parameter values necessary to maintain **sustainable populations** of sporulating, germinating and vegetative cells. Our design required us to **engineer stochastic differentiation** processes at a single cell level.



Population Model

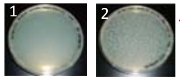
We considered the effects of modifying the bacterial life cycle on the dynamics of the population. We developed an **agent-based model** in Java, in which each cell makes its own life decisions and runs computational models using cell-specific parameters.



Since the model is complex and CPU-intensive, we developed a modelling environment using the **Amazon Elastic Cloud**, to spread computation over multiple CPUs worldwide.

Chassis

It is critical to disable germination to prevent release of the sequestered cadmium back into the environment. We are using spores which are **germination deficient** due to inactivation of the genes *sleB* and *cwlJ*.

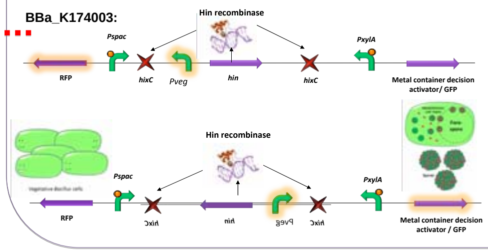


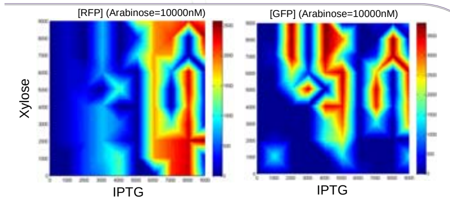
Under default conditions we complement Δ*sleB*Δ*cwlJ* to allow germination. A metal container decision from the stochastic switch turns off complementation.

A germination deficient chassis: (1) Δ*sleB*Δ*cwlJ* spores fail to germinate (2) after treatment for recovery

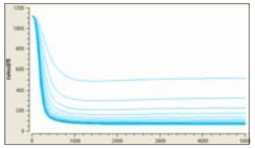
Stochastic Switch

This device is a tuneable, heritable, stochastic switch that can be **biased** using two inducible promoters and by controlling the **rate of degradation** of the protein responsible for the switching, Hin invertase. The device can be tuned to control cell differentiation and fate.





Hin expression is controlled by IPTG and Xylose via *Pspac* or *PxylA* promoters. Upstream *rfp*, or downstream genes, are expressed by the *sigA* promoter.

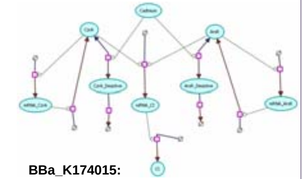


Above: Gfp/Rfp expression vs. IPTG vs. xylose.
Opposite: Hin degradation vs. time vs. arabinose

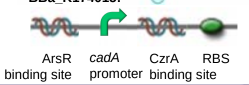
Cadmium Sensing

We used metal binding ArsR and CzrA repressor proteins, to selectively sense cadmium in **an AND gate configuration**.

Metal Sensor	Metals Sensed			
ArsR	As(III)	Ag(I)	Cu	Cd
CzrA	Zn	Co	Ni	Cd

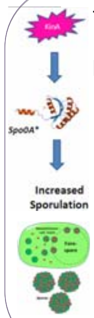


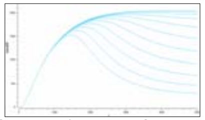
Operator binding sites for these proteins were combined so that downstream genes are only expressed in the presence of cadmium.



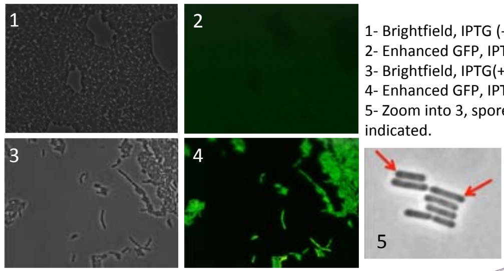
Sporulation Tuner

The sporulation rate is **differentially** altered by the signal from the stochastic switch. Upregulating *kinA* **triggers** the phosphorylation of Spo0A via a multi-component phosphorelay. The rate of sporulation is enhanced by gradually increasing Spo0A~P.





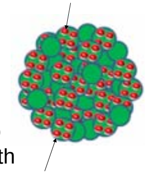
SpoOA~P vs. time vs. IPTG concentration



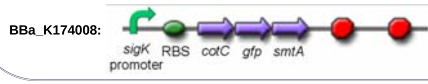
1- Brightfield, IPTG (-)
2- Enhanced GFP, IPTG(-)
3- Brightfield, IPTG(+)
4- Enhanced GFP, IPTG(+)
5- Zoom into 3, spores indicated.

Metal Sequestration

SmtA metallothionein soaks up intracellular cadmium. By **translationally fusing** *smtA* with *cotC* encoding spore coat protein from *B. subtilis*, SmtA can be **localized to the spore coat**, trapping heavy metals into bacterial spores. SmtA is also fused with Gfp to facilitate the visualisation of spores using fluorescent microscopy.



Cadmium sequestering metallothionein-CotC fusion protein



Conclusion

Our project was ambitious and so we demonstrated the application of each subproject separately. We devised some novel and reusable concepts within synthetic biology, which can be taken forward in future projects. With further time and development our work could result in implementation of our original design to reduce environmental cadmium pollution.

References

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