The “Bacuum” Cleaner - Intelligent Self-propelling Cleaner

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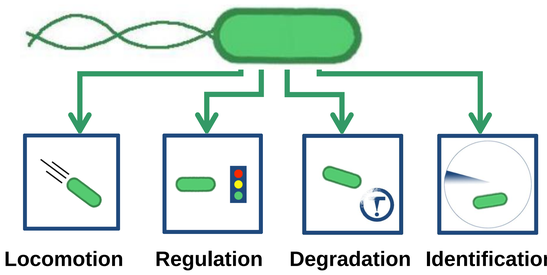
Introduction

Tailing ponds used to store discarded waste from oil refineries pose a major environmental dilemma. Our goal is to create modified *Escherichia coli* capable of seeking out and degrading toxic aromatic pollutants created during the oil refinery and mining processes. Our “bacuum” cleaner will respond to a toxic compound through interaction with programmable riboswitches. The riboswitches will control translation at varying concentrations of target ligand, thus altering the induced signal. At low concentrations, we intend to have our riboswitch express the motility protein CheZ in *E. coli*, directing the bacterium towards higher concentrations of our target molecule. Once it reaches a threshold concentration, a catabolic pathway capable of degrading our target pollutant will be activated.

The “Bacuum” Cleaner

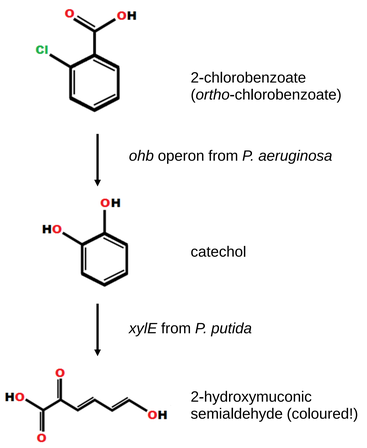


Degradation

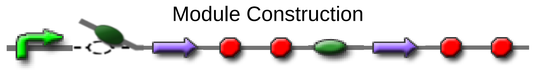
One critical step in the degradation of PCBs involves the elimination of chlorine substituents. In order to show that it is possible to degrade such compounds we have engineered a novel pathway that degrades 2-chlorobenzoate.

Tsoi *et al*. (1999) successfully isolated and characterized a pathway which degrades *ortho*-halobenzoates in strain 142 of *Pseudomonas aeruginosa*. This metabolic pathway, comprising of the *ohb* genes, catalyzes the degradation of 2-chlorobenzoate to catechol.

We aim to combine this system with the *xylE* gene from *Pseudomonas putida* which encodes for catechol 2,3-dioxygenase (Ingram *et al*., 1989). By combining these systems we will further break down catechol to the nontoxic 2-hydroxymuconic semialdehyde. This is an intense orange coloured compound and thus can be used as a reporter system for our novel degradation pathway.



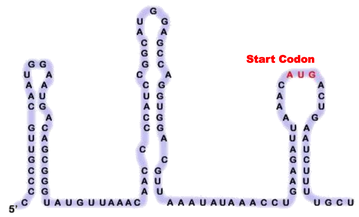
Module Construction



Identification

Translation initiation regions (TIRs) are elements found in the 5′-UTR of various mRNAs that facilitate translation initiation by noncanonical and initiation factor independent interactions (Hellen, 2007). In order to override translation initiation we are going to incorporate the TIR from the *rpsA* gene, encoding ribosomal protein S1 of *E. coli* into our “bacuum” cleaner. This results in constitutive expression of the gene of choice. For characterization purposes we have chosen to use green fluorescent protein. This provides a means of monitoring our “bacuum” cells under conditions of minimal resources, and serves as a unique “watermark” for our “bacuum” cleaner.

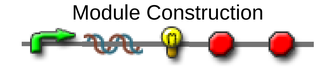
Alternatively, a repressible promoter could be used to control expression of a self-destruct, or suicide gene which encodes a compound which is highly toxic to the cell. In the event, that no target ligand is present, the repressor is removed, translation occurs, and the cell expires.



The TIR from the *rpsA* gene

- Initiates S1 translation
- Lacks Shine-Dalgarno
- High intrinsic activity
- Unique watermark

Module Construction

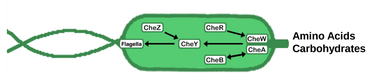


Locomotion

In order to control locomotion we will regulate the expression of CheZ (Topp and Gallivan, 2007).

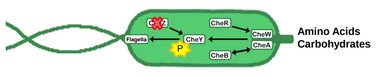
CheZ is present: CheY is dephosphorylated

Bacteria are in running mode



CheZ absent: CheY remains phosphorylated

Bacteria are in tumbling mode

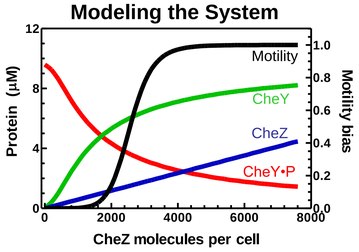


Motility Assay

Positive Control
E. coli WT (K12)

Negative Control
E. coli Δ*cheZ* (RP1616)

Modeling the System



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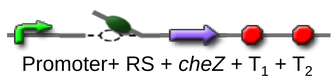
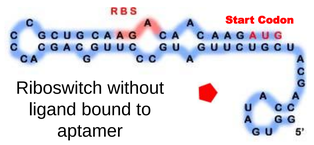


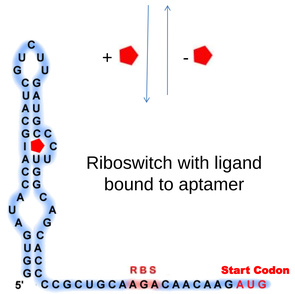
BCT Modeling Software from Bray *et al.* 2007.

Regulation

To regulate gene expression on the translational level we have chosen to use riboswitches. This will allow us to control translation initiation. In order to create a “bacuum” cleaner with a “search and destroy” mechanism we intend to engineer riboswitches, which will respond to different levels of target ligand concentration. At low levels of the riboswitch will bind the ligand and induce the production of CheZ causing the “bacuum” cleaner to move up the concentration gradient. At higher concentrations of ligand, the riboswitch controlling metabolism will be activated resulting in ligand degradation. The theophylline riboswitch was chosen because it is 75 nucleotides in length and theophylline is structurally similar to our target compound 2-chlorobenzoate. The theophylline riboswitch can be easily synthesized and modified to create a riboswitch which will bind to 2-chlorobenzoate using SELEX.

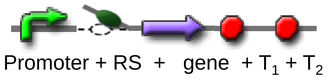


Riboswitch without ligand bound to aptamer



Riboswitch with ligand bound to aptamer

Module Construction



Conclusion and Future Directions

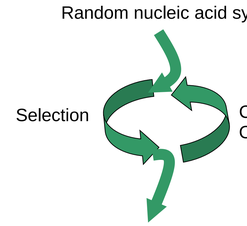
Parts Made:

- •*cheZ* – Biobrick Part Bba_K147000 (Locomotion)
- •*ohbA* – Biobrick Part Bba_K147003 (Degradation)
- •*xylE* – Biobrick Part Bba_K147002 (Degradation)
- •Theophylline riboswitch – Synthesized (Regulation)
- •*rpsA* TIR – cloned into pGEM T-easy (Identification)
- •*ohbB, ohbC, ohbR* (Degradation)
- •2-chlorobenzoate riboswitch (Regulation)

Future Directions:

- •Continue with construct formation
- •Modeling of reprogrammed “bacuum cleaner” motility
- •Generate novel aptamer using SELEX
- •Characterize novel riboswitch function
- •Assemble and test the system!

SELEX (Systematic Evolution of Ligands by Exponential Enrichment)



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Acknowledgements

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