# Genetically Engineering a Heavy Metal Biosensor Using *Bacillus subtilis*


Cornell University Genetically Engineered Machines

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## Abstract

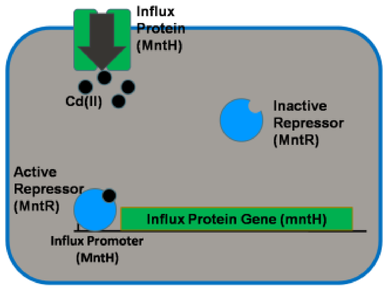

The goal of our project is to create a whole cell cadmium biosensor by attaching cadmium responsive promoters in *Bacillus subtilis* to fluorescent reporter proteins. Cadmium is a toxic heavy metal which has no known biological function. Ingestion of cadmium contaminated water can induce bone fractures and severe renal damage. Major sources of cadmium contamination include fertilizers, sewage sludge, manure and atmospheric deposition. cadmium contaminated sewage is often used for irrigation purposes in many parts of the world, especially in developing nations. Crops grown in these contaminated soils are then sold in markets without any detoxification treatment. Current analytical methods such as atomic absorption spectroscopy, though highly sensitive, are significantly more expensive than bacterial biosensors and are unable to measure the amount of bioavailable cadmium.


## The Chassis: *Bacillus subtilis*

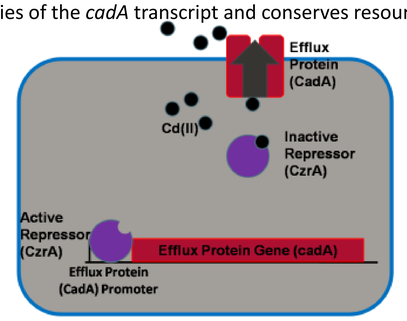
*Bacillus subtilis* is a well-characterized gram-positive soil bacteria. It is naturally competent, allowing for simple transformation procedures. *B. subtilis* was chosen as our chassis because it has a complex metal ion homeostasis system that can be utilized in our biosensor design.

## Metal Ion Homeostasis

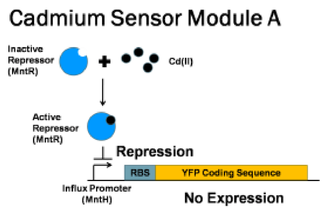
The *mntH* gene codes for manganese influx pump, however cadmium is able to enter the cell through this pump as well. The expression of this gene is repressed by the MntR repressor. This gene must be tightly regulated because the cell requires manganese for its cellular processes, high levels of manganese are pernicious. When cadmium or manganese is present, the repressor is active and inhibits protein expression. The repressor is inactive in the absence of cadmium, thus allowing *mntH* to be expressed. Thus when cadmium enters the cell through the MntH protein, expression of the protein is repressed to prevent additional harmful cadmium from entering the cell. However, when there is no cadmium, the *mntH* gene continues to be expressed in order to allow manganese into the cell.




The *cadA* gene codes for the cadmium efflux pump. The gene is repressed by the repressor CzrA, which is active in the absence of cadmium and inactive in the presence of cadmium. When cadmium binds the repressor, it becomes inactive and *cadA* is transcribed. Thus when there is a high intracellular concentration of cadmium, the repressor is inactive and the CadA efflux protein continues to be produced, to quickly rid the cell of the harmful cadmium. However, when there is low intracellular concentration of cadmium the cell does not produce as many copies of the *cadA* transcript and conserves resources.




## Design

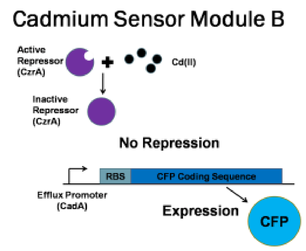



### Cadmium Sensor Module A

We construct the first cadmium sensing module by fusing a YFP sequence to the 3' end of a *mntH* promoter and *B. subtilis*-specific ribosome binding site. After insertion into the genome of the bacteria, fluorescence will be observed in the absence of cadmium. This is different from many other traditional sensing devices in that the decrease in fluorescence activity gives us an indication of the concentration of our signal. Since *mntH* is also responsive to manganese, we must ensure that our sample contains no manganese. Any additional manganese in our growth media can be corrected for using the internal reference module described below.

### Cadmium Sensor Module B

We construct the second cadmium sensing module by fusing a CFP sequence to the 3' end of a *cadA* promoter and *B. subtilis*-specific ribosome binding site construct. After insertion into the genome of the bacteria, fluorescence will increase with cadmium concentration, since the gene is repressed in the absence of cadmium. Since our two modules fluoresce at different wavelengths by comparing the output from our two modules with the internal reference module, we can obtain a more accurate picture of the amount of cadmium in our sample.

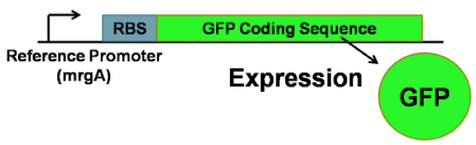



### Internal Reference Module

As an experimental control, we can compare the activity of Modules A and B to that of an in vivo reference module—an internal reference. An internal reference is necessary to control for different growth conditions and to check viability of the cells. For example, if bacteria cultures are left to grow for different periods of time in an incubator, their fluorescent activities will reflect not only the concentration cadmium but also the incubation time. Incubation temperature can also affect the health of cells and impacts their fluorescent activity as well. Additionally, differences in the nutritional content in of growth media can cause variations in cell growth and therefore fluorescence. An internal reference promoter will control for variations in these growth conditions and to indicate that our cells remain viable in a sample with potentially high cadmium concentration.

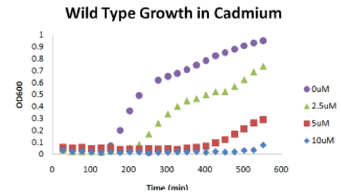
We will use the metal-regulatory gene A (*mrgA*) promoter as our internal reference standard. This promoter was taken from the mutant strain HB1266, in which the first half of the inverted repeat (extending from bases -12 to -20) upstream of the -35 region has been deleted. This deletion mutation led to a thorough de-repression of the *mrgA* gene and thus caused the promoter to constitutively transcribe gene products.

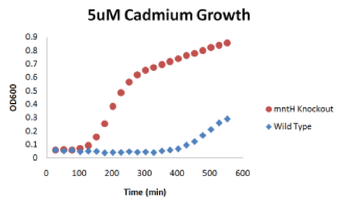
We construct our internal reference module by combining the constitutive *mrgA* internal reference promoter, a *B. subtilis* RBS, and a GFP reporter. By comparing the fluorescence of Module A and Module B to the fluorescence of the Internal Reference Module we can control for variations in growth conditions.

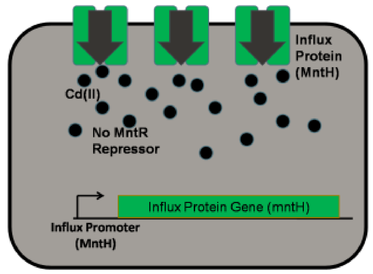



## Characterizing Chassis

In order for our sensor to function, *B. subtilis* must be healthy in a cadmium rich environment. Work was done to characterize wild type *B. subtilis* growth in cadmium at various concentration to determine concentrations that our cells would remain viable at.




### Tuning Cadmium Influx

To make *B. subtilis* remain viable at high cadmium concentrations, we can knock out *mntH* which codes for the cadmium influx protein. Alternatively , knocking out the gene *mntR* will eliminate repression of *mntH* influx protein. This will increase the number of influx proteins through which cadmium can enter the cell. With more cadmium entering the cell, our sensor modules will be more sensitive to external cadmium concentrations.

By tuning the promoters of *mntH* and *mntR*, we can increase or decrease the amount of cadmium that enters the cell. This allows us to find the right balance between the competing needs of keeping *B subtilis* healthy and our sensor functional and attaining high sensitivity.

## Achievements and Future Work

**Achievements:**

-Designed cadmium biosensor constructs
-Succesfully created and sequenced multiple Biobrick parts: cadA, mrgA promoter mrgA rbs
-Successfully created and sequenced construction intermediates
-Collected and analyzed data on viability of chassis in heavy metal conditions

**Future Work:**

-Complete cadmium sensor modules
-Transform into *B. subtilis*
-Compare sensitivity of modules in different tuned chassis : mntH null, mntR null, etc
-Incorporate additional metal sensitive promoters: copZ, arsR, etc

## Sponsors


Cornell University
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