Cadmium is Badmium: Designing a Biological Device to Detect the Presence of Cadmium

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Cadmium is a heavy metal present in the environment (in polluted air, rivers, and soil) and in man-made objects (paints, plastics, batteries, and tobacco). It can cause cancer, high blood pressure, and other adverse health effects when inhaled or ingested. Since such deadly side-effects are a problem in Asia, it would be beneficial to have an efficient yet easily-utilized detector. Our team decided to create a cadmium-sensitive bacteria biosensor. We researched and designed a recombinant plasmid which encodes a red fluorescent protein in the presence of cadmium, before transforming *Escherichia coli* bacteria with it. The transformed bacteria was then tested with dilutions of cadmium chloride and dilutions of cadmium sulfate. Preliminary results show promise in demonstrating that the presence of cadmium can be detected with our transformed bacteria. The next steps for our biosensor is to further test in cadmium solutions and later test foods that may contain dangerous levels of cadmium (e.g. rice from certain areas of China). We also plan to continue transformation protocols with different *E. coli* strains to test the efficiency of protein production. A practical and reliable method of detecting cadmium based on our cadmium biosensor can help people avoid cadmium poisoning and enhance the overall health and safety of mankind.

Keywords: cadmium, biological device, biosensor, heavy metal detection

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Cadmium is a toxic heavy metal found naturally in the environment in compounds. Its exposure causes issues by polluting the air and water, affecting the ecosystem and the health of animals. Though cadmium can rarely be found in its pure form in nature, it has many different compounds. Of those compounds, cadmium chloride and cadmium sulfate readily dissolve in water, allowing for the uptake of cadmium in rivers and irrigation systems. In irrigation systems it can readily be absorbed into vegetation, and when said vegetation is consumed, it can be found in animals (Dartmouth c2017). Human activities such as mining metals (e.g. zinc, copper, and lead), burning fossil fuels, battery production, waste incineration and steel production can result in an increase of cadmium (a bioproduct) in the environment surrounding the area (Dartmouth c2017). It is estimated from 4,000 to 13,000 tons of cadmium are released into the environment every year as a result of human activities (Dartmouth c2017). Cadmium poisoning is an issue posed especially in areas that have or have had lax environmental policies on pollution and industrial waste. A study conducted in Shanghai, China, showed that of those tested, 6.6% of the Shanghainese population faced health risks from dietary cadmium consumption. The study also discovered that 27.5% of cadmium consumption is a result of tobacco, 20.2% of cadmium consumption comes from vegetables, and 14.6% from rice (He et al. 2013) A case in the Toyama Prefecture, Japan, in the 1950s led to the first documented mass outbreak of cadmium poisoning in the world, named "Itai-Itai" (meaning "ouch-ouch" for the pain it induced in the victims), as a result of heavy mining during World War II; the runoff cadmium entered the irrigation system and the rivers, leading to the death of many fish and to the absorption of cadmium into vegetation (Rasnake 2009). Besides living in areas such as these, smoking also increases an individual's exposure to cadmium.

Approximately 2-6% of cadmium ingested is absorbed, but when inhaled around 30-64% of cadmium is absorbed; this poses an increased risk of cadmium side effects to smokers (as a cigarette as an average of 1-2 µg) or anyone who comes into contact with cigarette smoke (ICdA 2016). A study conducted in Germany, a country with strict regulations on industrial waste, found that the average German citizen had an intake of 30-35 µg of cadmium and that the average smoker had an additional 30 µg (Godt et al. 2006). Comparing this to the WHO's level for tolerable weekly intake of 7 µg per kg of body weight (ATSDR 2013), the average person is not at a great risk of cadmium poisoning. However, when habitual smoking, environment, and occupation are factored in, there is a risk. Adverse effects on humans as a result of exposure to cadmium in the environment consist of pulmonary irritation, bronchiolitis and emphysema (if inhaled), build-up of cadmium in the kidneys, potential kidney disease (such as proteinuria), decrease in glomerular filtration rate, increased frequency of kidney stone formation, problems with bones (e.g. bone weakening and softening), and cancer. In animals, long-term inhalation or oral exposure to cadmium results in harmful effects on the kidneys, liver, lungs, bones, immune system, blood, and the nervous system (Huff et al. 2007).

As a result of its toxicity, scientists have worked hard on minimizing the exposure of cadmium and on detecting cadmium, and we have decided to join in. With all of these disastrous results when creatures come into contact with cadmium, having an efficient and cheap method of testing for the presence of cadmium would obviously be beneficial. The idea of combining synthetic biology – a rapidly growing field of science – with the detection of heavy metals intrigued us. Thus, the goal of this project would be to facilitate the research for a more affordable heavy-metal detector by designing and fabricating a device that expresses a red fluorescent protein in the presence of cadmium. With this device we hope to continue with our research and insert the device into a plant in the hope of creating a facilitated way to detect cadmium in areas with pollution problems. If inserted into a plant, the user would only have to water the plant and see if a red hue is created. However, having the device in *E. coli* is a less complicated process (though there is concern about contamination and spilling the bacteria as the plasmid would have an antibody resistant gene to insure the bacteria has the plasmid after transformation).

System Level

A biological system in synthetic biology fulfills a certain function by accepting a specific input item and producing a desired output. Since our goal was to produce a system that would detect cadmium, we insured that the input item we needed was, well, cadmium. To finish the system we had to figure out an efficient and easy way for the user to tell whether or not the solution our bacteria was in contained cadmium. We finally decided to have

Figure 1. The grand scope of what our biological system does. The insertion of cadmium ends up with red color (produced by the red fluorescent protein).

the system end with a red color. To summarize our biological system (Figure 1): the input was cadmium metal and the output was red fluorescent protein. When cadmium was present in the environment, the system would recognize and activate the device to notify by turning red.

Figure 2. The basics of our device.

Device Level

Our device (Figure 2) was similar to the whole system, since we have only inserted one device in our system. When the user places the bacteria in a solution, the presence of cadmium ions (if there are any) turns on our cadmium detecting device (CDD). Once the device has been turned on, it will activate the promoter and start the translation and transcription process and express the reporter sequence we have inserted into the device, which results in red color.

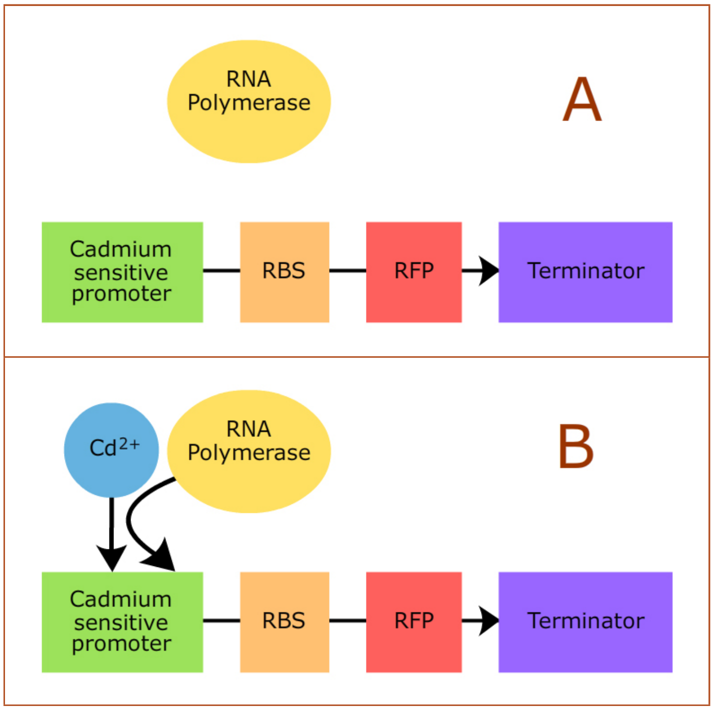

Figure 3. (A) Without cadmium, RNA polymerase (which starts the transcription of the device) will not bind to the system and thus does not allow for the expression of the red fluorescent protein. (B) When cadmium is added to the solution it allows RNA polymerase to bind to the device and start the transcription of the red fluorescent protein.

Parts Level

In order to create our genetic device, we had to plan out each part that the device would require. We utilized four basic parts (Figure 3): first we used a cadmium-sensitive forward promoter (this promoter only allows for the transcription of the device to be undergone when the bacteria is in the presence of cadmium); following our cadmium-sensitive promoter is our chosen ribosome binding site (RBS), which we added to ensure efficiency during translation; following the RBS we inserted an open reading frame that included a red fluorescent protein reporter (this allowed for the scientist/viewer to more readily see if there is cadmium in the solution the bacteria is in); finally we included a terminator which concluded the device (and marked where transcription should stop).The combination of these parts resulted in a genetic device that would create a red glow when in the presence of cadmium.

Safety
While working in the lab, we made sure to follow all proper lab protocols. When making agar, plating bacteria, and placing bacteria into tubes, we either used Bunsen burners or a fume hood to ensure that there would be no contamination of our materials or the lab facilities. For disposal, we put all materials that may have come into contact with our bacteria through the autoclave. During the cadmium-solution-preparation section of our protocol, we handled everything to do with cadmium under the hood while using gloves, goggles, masks (since we were working with cadmium powder, and cadmium inhalation results in more absorption than cadmium ingestion), and lab coats. For disposal, we sent all materials that may have come into contact with cadmium to a facility managed by an outside, government-backed agency.

Discussion
Synthetic biology is a recently developed field of study, and thus has great potential power. Our team has aimed to continue such innovation with our cadmium biosensor, as to harness the functions of living bacteria to serve the practical purpose of detecting environmental cadmium pollution. However, because our biosensor has not been fully examined, our team is unsure about potential risks it might bring. Incorporating this device into bacteria or other living organisms (for example, plants) could result in side-effects that might be more serious if the technology was implanted on a larger scale (as we hope to do in order to provide a widely useable cadmium sensor)

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References
Agency for Toxic Substances and Disease Registry (ATSDR) [Internet]. Atlanta (GA): ATSDR [updated: 2017 June 30]. Cadmium toxicity; [updated 2008 May 12; cited 2017 May 8]. Available from: http://bit.ly/2umMKu9

Dartmouth toxic metals superfund research program [Internet]. Hanover (NH): Dartmouth College; c2017. The facts on cadmium; [updated 2010 Nov 18; cited 2017 Mar 6]. Available from http://bit.ly/2umOuUd

Godt J, et al. The toxicity of cadmium and resulting hazards for human health. Journal of Occupational Medicine and Toxicology [Internet]. 2006 Sept 10 [cited 2017 May 8];1(22). Available from http://bit.ly/2uLgymX doi:10.1186/1745-6673-1-22.

He P, Lu Y, Liang Y, et al. Exposure assessment of dietary cadmium: findings from shanghainese over 40 years, China. BMC Public Health [Internet]. 2013 [cited 2017 Mar 6]; 13(1). Available from http://bit.ly/2uMbXk3 doi:10.1186/1471-2458-13-590

Huff J, et al. Cadmium-induced cancers in animals and in humans. International Journal of Occupational and Environmental Health [Internet]. 2007 Apr-Jun [cited 2017 Mar 6]; 13(2):202-12. Available from http://bit.ly/2uTFKrG doi:10.1179/oeh.2007.13.2.202

ICdA. [Internet]. Brussels (Belgium): International Cadmium Association; 2016. Cadmium exposure and human health; [cited 2017 May 2]. Available from: http://bit.ly/2ulaqRe

Rasnake, J. Itai-Itai Disease: A puzzling example of metal toxicity [Internet]. Charlottesville (VA): University of Virginia; 2009 [cited 2017 Apr 20]. Available from http://bit.ly/2uLuo8W