

## Biological Decaffeination

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Caffeine is the most commonly used drug in the world. People often consume food and beverages containing caffeine to stimulate their nervous systems and provide an “energy” jolt to make them feel more alert. However, caffeine can have negative effects on the body, including causing an increased heart rate, blood pressure, urination, insomnia, and a mild dependence on the drug. Current methods for removing caffeine from products are arduous and result in products of inferior quality compared to the original. We propose to solve this problem by constructing a synthetic biological system to accomplish this task. The transgenic system described here would use the baker’s yeast, *Saccharomyces cerevisiae*, to produce decaffeinated beverages on an industrial scale. It utilizes a feedback loop to first detect and then remove caffeine from the drink. A Caffeine Level Indicator (CLI) would function as a molecular indicator to visually show whether or not caffeine is present. In the presence of high levels of caffeine, the cell will make Red Fluorescent Protein (RFP) and fluoresce red. As levels of caffeine drop, the cells will make Green Fluorescent Protein (GFP) and the red signal would be switched off, making the cell fluoresce green. Removal of caffeine would be mediated by a “Caffeine Destruction Device” (CDD). This would be accomplished by utilizing the endogenous PKC1-MAPK signaling pathway to sense the presence of caffeine in a solution and activate expression of a heterologous CYP1A2 gene (Bschorer 2013). The enzyme encoded by CYP1A2 is normally found in liver cells and is responsible for metabolizing drugs. To get the CYP1A2 enzyme out of the cell and into solution, an export signal sequence will be fused to the N-terminus of the protein. We believe “Biological Decaffeination” would be more environmentally friendly, cheaper to perform, and produce a more consistent product.

**Keywords:** Decaffeination, Detoxification Enzymes, PKC-MAPK cascade, feedback loops/signal transduction, Synthetic Biology/Bio Design

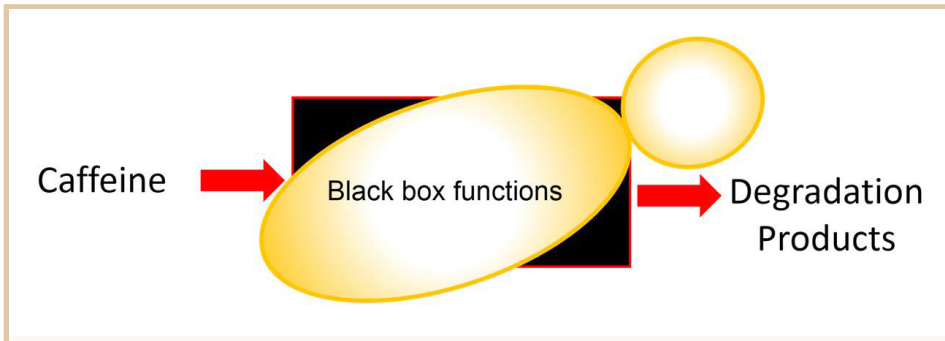
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Decaffeination has been an issue that has been attacked from a variety of standpoints ever since the decaffeinated beverage craze began in the 1980s. From that period on, decaffeination had begun taking shape as a scientific innovation that links itself to the American capitalist market in trying to make a consistent product that can grant consumers a near identical

product to their favorite coffees and teas without the harmful effects of caffeine and act as a great way to begin a solid kick of a caffeine addiction (Gonzalez de Mejia 2014). The methods scientists have used to achieve these results have ranged from chemistry, hybridization, breeding, genetic engineering, and mutagenesis. The only problem is these methods have been

sub par, inaccurate, and outdated. They’re time consuming and dangerous. Chemistry is very dangerous, stripping some of the goods away from the bean and leaving chemicals that could be harmful for the body, not to mention the process is long, which could result in inconsistencies. Hybridization is another method, but it’s highly difficult to perfectly select and discard traits



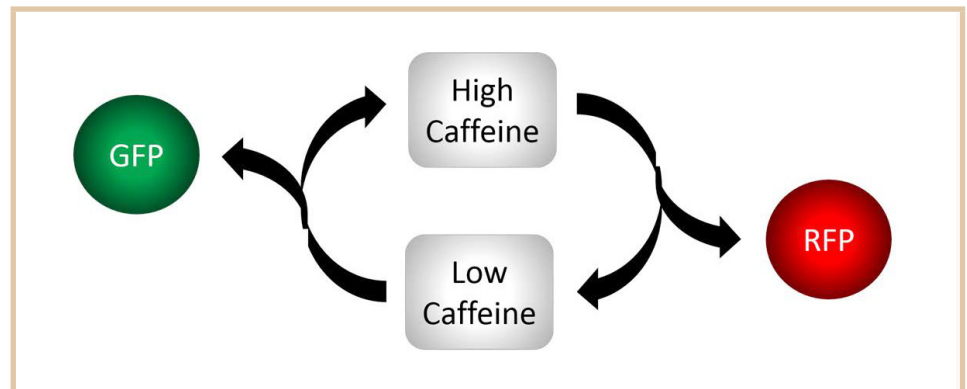
**Figure 1.** This diagram shows our project from abstraction, which at the system level is to input caffeine through our biological yeast system to get three degradation products.

from other plants and mutants to get out a consistent product that is acceptable. Breeding, very similarly to hybridization, will hold the same problems, and it has been extremely difficult to find a caffeine-free strain of coffee beans. Genetic engineering and mutagenesis are also very time consuming because they involve screening, and if there's any problems in screening then the product can't be consistently released (Borrell 2012)! Overall, we need to find a way to take on this issue with a newer approach.

Taking on the issue of decaffeination as stated by many professionals in the field is a task many scientists have tried in a variety of fields via a variety of attempts, but never has decaffeination been touched by the new field of synthetic biology, and that's what this experiment is proposing. Synthetic biology building off both biological functions and the designs of engineering is fascinating to the point where you could claim it to be science fiction, but it's revolutionary because it's also technology (Kuldell 2015). The idea is

set behind the fundamental idea that the human race has made amazing machines and in our bodies, especially at the cellular level, there are fasci-

precedence in the beverage industry, and the eukaryotic system has pathways that can build off more than a prokaryote like *Escherichia coli*) by building off the previously discovered Protein Kinase Cascade 1-Mitogen Activated Protein Kinase (PKC1-MAPK) as a city may build off its subways to make extensions (Kuranda et al. 2006). Our "extensions" will add genes that will power our little cellular machines such as Red Fluorescent Protein (RFP), Green Fluorescent Protein (GFP), CYP1A2, and the LexA Bacterium Repressor. These modifications that will be explained in full design will make a tiny group of Saccharomyces into a powerhouse for a cheaper and more accurate way of industrial decaffeination.

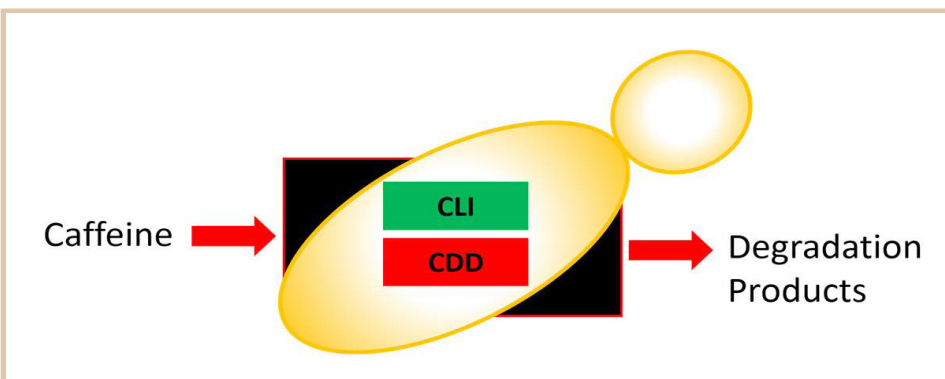


**Figure 2.** This diagram depicts the goal of detecting the caffeine and determining whether the system is working, which is a feedback loop that would make Red Fluorescent Protein (RFP) in high levels of caffeine and Green Fluorescent Protein (GFP) in low levels of caffeine.

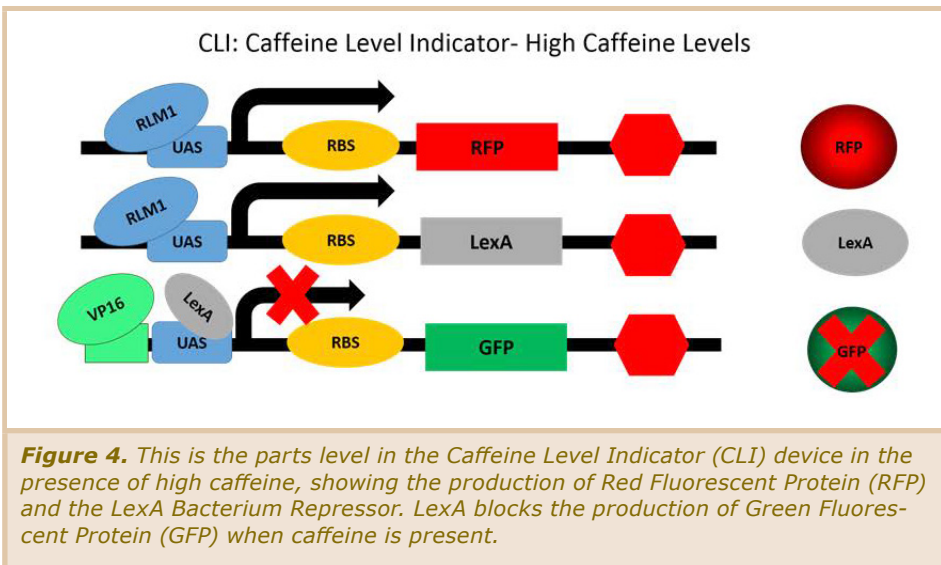
nating organic machines. Combining these two foundations can only begin to provide marvelous breakthrough across all fields of science in the near future. How does this relate to caffeine? Well, our experiment is to engineer a yeast cell (because yeast cells have

### System Level

This whole effort is built around the idea of developing a system. System down to devices down to parts is the philosophy of synthetic biology. Part of that philosophy is in the black box philosophy of turning the cell into a working organism that can receive an input and produce an output (see Figure 1). Our system is biological decaffeination, and the goal of it is to detect and degrade caffeine (using caffeine as an input in hopes of receiving three degradation products as an output) in a solution using a feedback loop (see Figure 2). To go back to the analogy of an extension, this feedback loop would be like making a street into a rotary. The electrical signals caffeine would send down the PKC1-MAPK cascade would then kickstart the Caffeine Lev-



**Figure 3.** The two devices present in our device level in this system are the Caffeine Level Indicator (CLI) and the Caffeine Destruction Device (CDD).



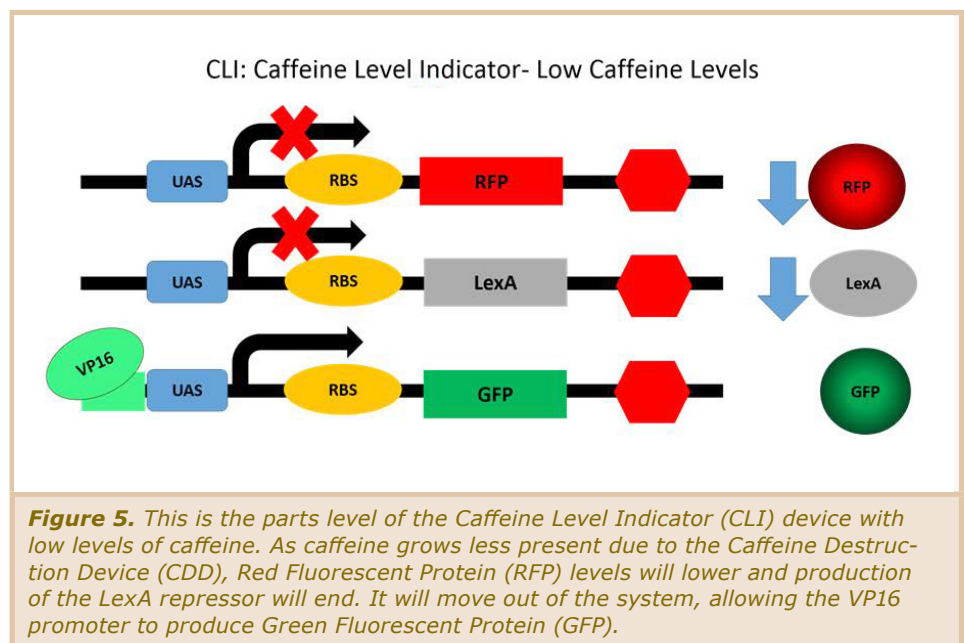
el Indicator (CLI) and Caffeine Destruction Device (CDD), causing a fluorescent color detection of caffeine that will be in constant flux based on the enzymatic degradation caused by the inserted CYP1A2 enzyme that gets exported to the N-terminus of the cell in the presence of high caffeine levels along the outer cellular membrane.

### Device Level

This system works by being utilized by two devices, the CDD and CLI as previously stated. The first device—the CDD—will regulate the CLI because as low levels change to high levels, the CDD exports the enzyme CYP1A2 to the N-terminus of the cell to degrade the extracellular caffeine. As the caffeine is degraded, the CLI will change based on the change in caffeine levels and signals received by the PKC1-MAPK cascade. The PKC1-MAPK cascade is activated when caffeine is present around the cell. Caffeine induces phosphorylation of MAPK in a dose-dependent manner. When caffeine is present, the epidermal growth factor receptor (EGFR) on the outside of the cell membrane acts as an “on/off” switch and is left in the “on” position when bound. This activates the tyrosine kinase activity of the receptor, which will promote the removal of guanosine-5′-diphosphate (GDP) from a member of the Ras subfamily and binds to guanosine-5′-triphosphate (GTP), which then activate the Ras. This starts the PKC. The activated Ras will induce the protein kinase action of the rapidly accelerated fibro-

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sarcoma (RAF) kinase (a proto-oncogene serine/threonine-protein kinase). Phosphorylated RAF will activate MEK (MEK1 and MEK2)—MAPK kinases—which will then activate MAPK. MAPK, in turn, phosphorylates and activates MPK1, another mitogen protein kinase (Okano 2008). MPK1 binds to the RLM1 promoter which binds to the upstream activation sequence (UAS) to activate our CLI. The CLI is a reverse stoplight where our yeast cell will constantly fluoresce green (due to GFP) in the presence of no or low levels of caffeine, but then as the caffeine levels rise or caffeine is exposed to the cell, the GFP production will be shut down and RFP will be made to set the awareness visually that there is a high level of caffeine. This device is important because



it allows us or any potential user to visually see if the system/mechanism is working without the need to run any post-tests. (See Figure 3).

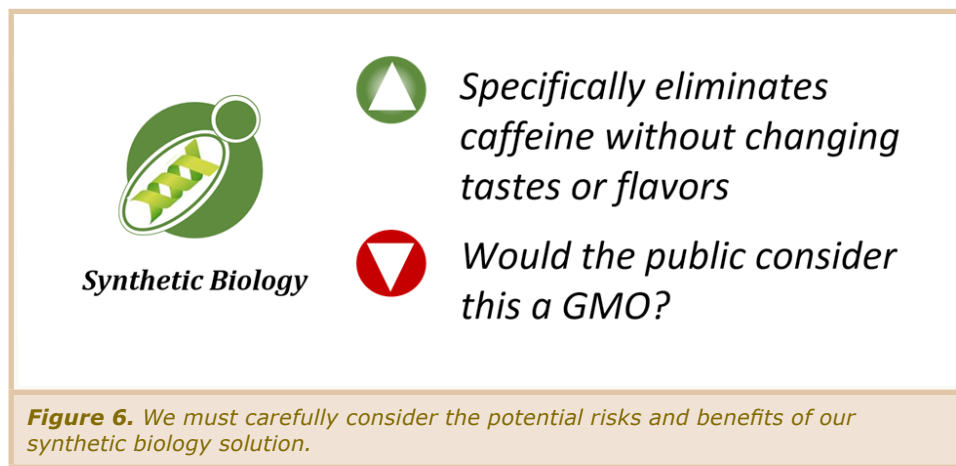
### Parts Level

For a system to work, devices are needed, and for the devices to work, parts are needed, like the rails that make up our subways. Both the CDD and the CLI are designed from parts that may not always have been put together. For the CDD, we started with a promoter to start the process of transcription with a regulating sequence that will transcribe the CYP1A2 gene. This has an included RBS (Ribosome Binding Site) for efficient translation, and the resulting CYP1A2 enzyme being produced will be our own version with an N-terminal cell export tag to transport the enzyme out to the cell membrane to degrade extracellular caffeine. The CLI gets a bit more complicated, as it has more parts and more transcriptional factors. The CLI is built around three transcriptional factors that are in constant flux based on what the CDD is doing and how much caffeine is present. The three transcriptional factors themselves are the transcription of RFP, the transcription of GFP, and the transcription of the LexA Bacterium Repressor (see Figure 4). These transcriptional factors work differently based on two different conditions—high caffeine and low caffeine. In the pres-

ence of high caffeine, the transcriptional process is to begin producing RFP and LexA to signal visually that there's a high presence of caffeine, and the LexA is produced to block the production GFP, which is constantly promoted by a VP16 promoter (Sadowski 1998; see Figure 5). Now, if the CDD is working and the CYP1A2 enzyme is breaking down caffeine, then the levels will change and that flux will lower the production RFP and LexA. The lower production of LexA will cause the repression of VP16 and GFP to stop and the transcriptional factor would occur, showing visually that the caffeine is gone or at a very low level—meaning that our system has worked.

### BioSafety and Discussion

With synthetic biology being a growing field, there's also growing concerns that we as scientists have to address. Bioethics has been crucial in the development of synthetic biology, as it address the idea that just because we can do something, does that mean we should do it (Figure 6)? Another concern is whether or not we as humans should have the right to engineer life forms and act as a creator? That also grows the debate of backlash from religious groups. Specifically of concern, however, for our project is the Genetically Modified Organism (GMO) debate, especially in the food and beverage industry! GMOs in our foods is a growing debate, because there are strong points for both sides but there's also a lot of confusion and unspecified matters in this debate, which are drawing uneducated opinions that cloud people's judgment. Our project is promoted by being more environmentally friendly to the coffee industry and by being cheaper to perform and quicker to achieve a more consistent product. This project hopes to dispel any GMO concerns because yeast is already



**Figure 6.** We must carefully consider the potential risks and benefits of our synthetic biology solution.

heavily used in both the baking industry and in the beverage industry for alcohol production, and the product itself is not a GMO. If these concerns are addressed to the public clearly with honesty, then we can be promoting both our project and the bioethics within synthetic biology with a good role model on how future experiments and projects should be conducted, reducing the public stigma of the synthetic biology field.

### Acknowledgements

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