Design Brief

Photosynthetic enhancement of algae to increase food production*

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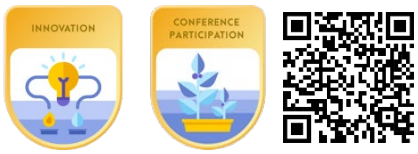
Currently, approximately nine million people die of hunger annually. This number will only rise in the coming years due to global warming, limited farm area, rapidly increasing population size, and other factors of the modern world. While many researchers have focused on genetically enhancing traditional crops to increase food production, others have looked to modifying more environmentally sustainable food sources, such as algae. Algae is rich in proteins, lipids, fatty acids, and vitamins essential to boosting an organism's rapid growth rate. It has high energy density and greater photosynthetic and water efficiency, which make it a sustainable food source for enhancement. Our team aims to improve the efficiency of the algae Nannochloropsis oculata *to cheaply produce large quantities of starch. The systems designed promise improved light capture, carbon fixation, and starch production through three distinct but interdependent mechanisms. First, phycobilisomes—large protein complexes—will be expressed for their incredibly efficient light-harvesting ability. Next, a multicopper oxidase, regulated by a negative feedback loop, will be leveraged to reduce O_2 into water, thus increasing the proportion of CO_2 to O_2 and freeing RuBisCO, an enzyme present in chloroplasts and involved in fixing atmospheric carbon during photosynthesis, to operate more efficiently by conducting less photorespiration. Finally, overexpression of CmGLG1, which initiates starch and glycogen synthesis, and its regulator (target of rapamycin or TOR) are both predicted to harness the increased available light energy. With development, such systems may circumvent the causes of famine that arise from traditional farming methods—such as crop spoilage and damage, dependency on fragile food networks, and artificial scarcity—or contribute to famine relief efforts per the United Nations' World Food Programme. This project is inspired by the UN's Sustainable Development Goal, "Zero Hunger," which aims to achieve innovative forms of sustainable agriculture.*

Keywords: Photosynthesis, sustainable agriculture, famine, algae, United Nations



Famine and food shortages are ubiquitous problems that have plagued humans for millennia and still adversely affect humans today. These crises are more relevant than ever, further exacerbated by record-breaking human population growth, diminishing agricultural space, global conflicts, and climate change. Moreover, the effects are likewise more glaring than ever; today, food shortages cause an estimated nine million deaths annually, which is inevitably bound to increase in the future (Beasley, 2021). Although this problem is only underscored when deaths are associated with it, a large portion of the world's population is food insecure, which tends to be disregarded.

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The Food and Agriculture Organization of the United Nations (FAO) defines food insecurity as a "lack of regular access to enough safe and nutritious food for normal growth and development and an active and healthy life" (United Nations, 2021). During 2021, around 2.3 billion people worldwide (29.3%) were thought to be moderately to severely food insecure, with that number thought to be increasing by 600 million each year (World Health Organization, 2022).

Moreover, as ultra-processed foods become more prevalent and cheaper, more families will turn to these food sources in lieu of healthier alternatives. For most families, this choice is a must, either because healthier alternatives are unavailable or because prices for organic foods have substantially increased. This shows how traditional ideas may lead one to incorrectly conclude that food insecurity is only associated with famine and food shortages, when, in actuality, it is also associated with a lack of healthy and nutritious food.

We also live in precarious times, where isolated conflicts can have massive impacts on the food supply chain. For example, the war between Ukraine and Russia has had major effects on the global supply chain, especially since both countries are major producers of wheat, which is depended on by many Asian and African countries (General Secretariat of the Council (GSC) web communication team, 2023). Specifically, 36 of the 55 countries throughout the world already suffering food crises depend on exports from Ukraine and Russia (Filho et al., 2023). In fact, in the modern day, famine often arises as a result of human failings, a situation we posit unique systems could address, as explored further in the Discussion section (Hasell & Roser, 2017).

As a result, achieving the UN's Sustainable Development Goal, "Zero Hunger," by 2030 will fall short, especially given the large task ahead, if drastic change is not taken, but novel approaches may facilitate this large-scale change. In recent decades, a new challenge has emerged in food production: how do we curb the effects of famines and food shortages without compromising nutritional value?

Synthetic biology has led to advancements in crop resistance, combatting the effects of climate change, and maintaining high crop yields. In the mid-20th century, society was suffering extreme food shortages due to the Cold War and other factors. The usage of high-yield crops and disease-resistant strains greatly increased during this time (Chawla et al., 2023). More recently, with the arrival of the Genome Revolution, biotechnology has been used to genetically enhance crops to increase resistance to modern environmental instability factors, such as rapid temperature changes (Chawla et al., 2023). Additionally, certain genetic enhancements have increased nutrition within crops, leading them to stay in circulation in the food supply chain. However, amid all these efforts, most of the focus has been placed on traditional crops rather than algae (Ameen & Raza, 2017).

Contrarily, our team's primary initiative was to use novel environmentally friendly methods to maximize food production output. We specifically chose algae as our ideal organism to produce large quantities of food that are sustainable, cost-effective, nutritious, and replicable globally.

Although this article only describes a design, we hope that further research and experimentation will bring about a real-world solution for the problems described above.

Detailed advantages of our system are further elaborated on in the Discussion section of the article.

Systems level

In complement with the challenges and desires mentioned in the Background section, we have designed an algal system that aims to take the fullest possible advantage of resources in order to quickly produce large quantities of starch.

This system is visualized as being cheap and efficient enough—employing low-tech vertical growth methods further discussed in the Next Steps section—to integrate effectively with current solutions, principally emergency food assistance, the most effective way to fight famine (*Fighting famine*, 2024). Furthermore, because—as described in our background—famine is often at least partly the result of artificial limitations, a system such as that described

may be able to approach the problem in a uniquely effective way, a topic of elaboration in the Discussion section.

Our plan involves increasing the efficiency of two parts of photosynthesis: light absorption and the catalyzation of carbon dioxide through RuBisCo. The two processes will increase the overall efficiency of photosynthesis, which will help the third step to increase the production of starch.

The first stage in increasing starch production involves increasing the absorption of light using structures called phycobilisomes, large and intricate protein complexes, which are estimated to transfer energy with 95% efficiency (Glazer, 1985). Though the precise mechanisms underlying the phycobilisome's incredible efficiency are still unknown, it is believed that it is due to the protein complex's structure, which is arranged in such a way that it gathers a wide variety of wavelengths, from 380 to 700 nanometers, with the energy of the wavelength absorbed decreasing as the location moves "downstream", closer to photosystem II (Sohoni, 2023). However, the increase in light absorption will not cause an increase in starch production unless other systems are also improved.

For our second stage, we plan to use a multicopper oxidase in order to reduce O_2 levels to prevent oxygenation from RuBisCo, which allows the enzyme to bind to more CO_2 and causes an increase in carbon fixation levels. This novel device should increase the efficiency of RuBisCo and allow us to utilize the increase in light capture from stage one.

Finally, the third stage will use the overexpression of CmGLG1, a glycogenin analog which can, under these circumstances, increase the production of starch up to 4.7 times by initiating glycogen production in the latter's stead (Pancha et al., 2018). With all three of these parts working together, there should be an increase in overall starch production from our algae, which can be used to achieve the goals mentioned above.

Device level

We plan to transform the algae *Nannochloropsis oculata*, not only because of the innate advantages of algae, but also because the genus' inner workings and the procedures for its modification are comparatively well understood (Li et al., 2014). The algae will be transformed with three key devices: the light capture mechanism, the carbon fixation mechanism, and the starch production mechanism.

The light capture mechanism aims to improve the efficiency of light capture by providing more efficient mechanisms: namely, phycobilisomes. In particular, the pigments phycoerythrin, phycocyanin, and allophycocyanin are able to increase the absorption of light by algae. However, when used for photosynthetic enhancement in isolation, their dramatic ability is detrimental; the devices, which have evolved for darker conditions, have been found to absorb far more energy than can be utilized, resulting in debilitating photooxidation. This has traditionally been addressed by removing components of the phycobilisomes to hinder their productivity (Luan et al., 2020). Pursuing an alternative path, we have elected to try addressing this concern by removing the light-harvesting system from its isolation.

We hope to offset the buildup of excess energy by providing enough organic matter to absorb the excess energy in the service of our ultimate goal. The carbon fixation device we have designed should also, of course, serve the primary purpose of fixing enough carbon dioxide that the lack does not bottleneck our system, a principal challenge in photosynthetic enhancement. These processes terminate in an upregulation in starch production, as induced by our final device.

The increased starch production is brought about by the overexpression of CmGLG1, which initiates glycogen production but is inhibited by the target of rapamycin (TOR)—specifically TOR complex 1—which, though it regulates cell metabolism as well as starch production, shares this function with TOR complex 2, which is not inhibited by rapamycin (Pancha et al., 2018). This decentralization may explain why this device did not notably hinder the development of modified algae in the past. Free of this concern, we have decided to use rapamycin and FKBP12 to inhibit TOR and allow the overexpression of CmGLG1.

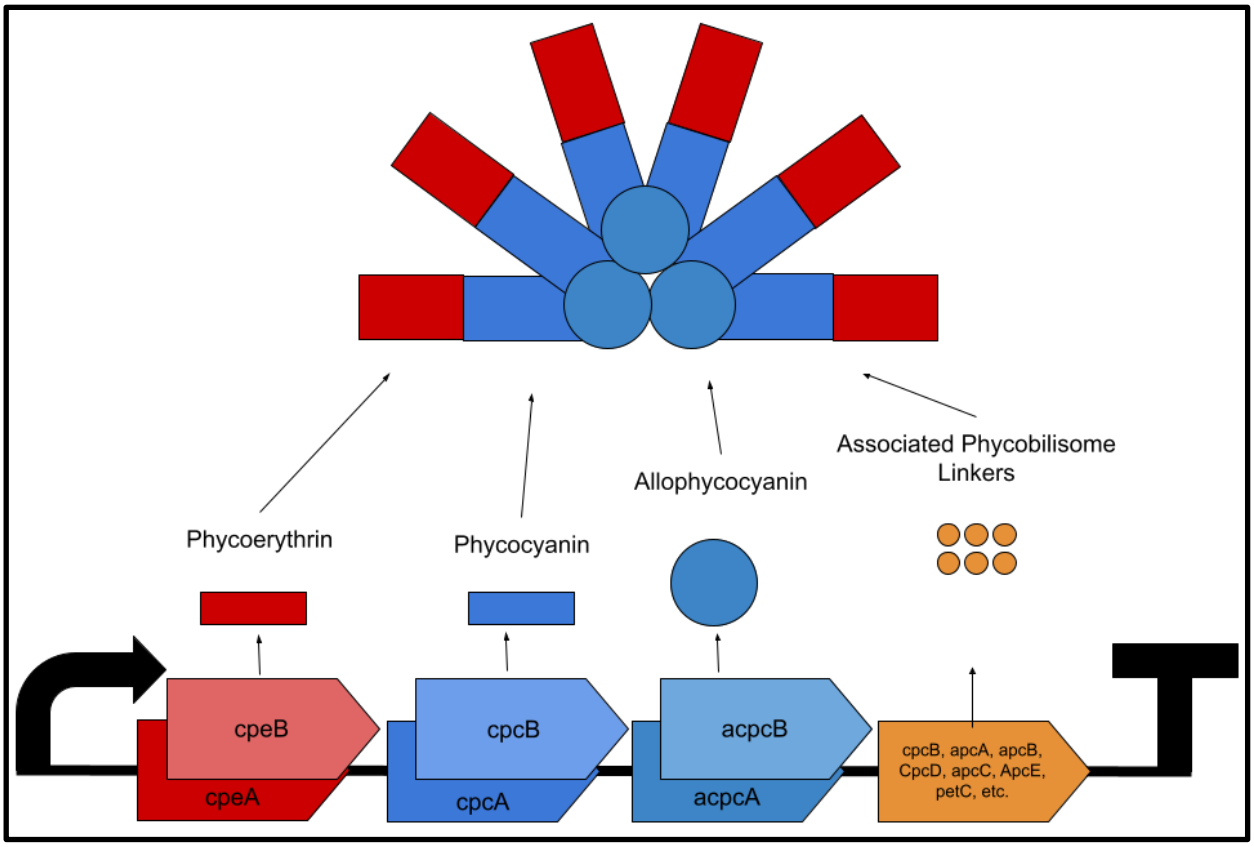

Figure 1. The distribution of phycoerythrin, phycocyanin, and allophycocyanin within the phycobilisome. When the transcription of the device begins, phycoerythrin, phycocyanin, and allophycocyanin will be created. Aided by assorted linkers, the A and B, or α and β, subunits bond to form αβ heterodimers, then $(\alpha\beta)_3$ trimers.

Parts level

As mentioned, the phycobilisomes of the light-harvesting device are large protein complexes; these hemi-discoidal supramolecular structures can come in a variety of forms depending on their subunits. The subunits consist of phycobiliproteins bound by phycobilisome linkers. Phycobiliproteins, pigments categorized as phycoerythrin, phycocyanin, and allophycocyanin, play key roles in light harvesting, absorbing high-, middling-, and low-energy light, respectively. A wide array of phycobilisome linkers—key examples being cpcB, apcA, apcB, CpcD, ApcC, ApcE, and petC—serve to facilitate their bonding and to transfer the captured light energy (Figure 1). Each phycobiliprotein comes in α and β variants, sometimes represented as a and b; thus, phycoerythrin is coded for by the genes cpeA and cpeB, phycocyanin by cpcA and cpcB, and allophycocyanin by acpA and apcB. In the construction of the phycobilisomes, pairs of these α and β subunits form αβ heterodimers, which go on to form $(\alpha\beta)_3$ trimers (Chang et al., 2014). These arrangements—along with the linker proteins—go on to bind further into their respective portions of the rods or core; this may be due to their being acidic and basic, respectively (Mishra, 2018). In the fully assembled phycobilisomes, captured energy flows through the system until it is transferred by state transition to both photosystems II and I, though the systems are thought to bind to the former (Chang et al., 2014). In this way, the photosystems should integrate their captured light energy directly into the photosynthetic systems of our chassis.

When designing our carbon fixation

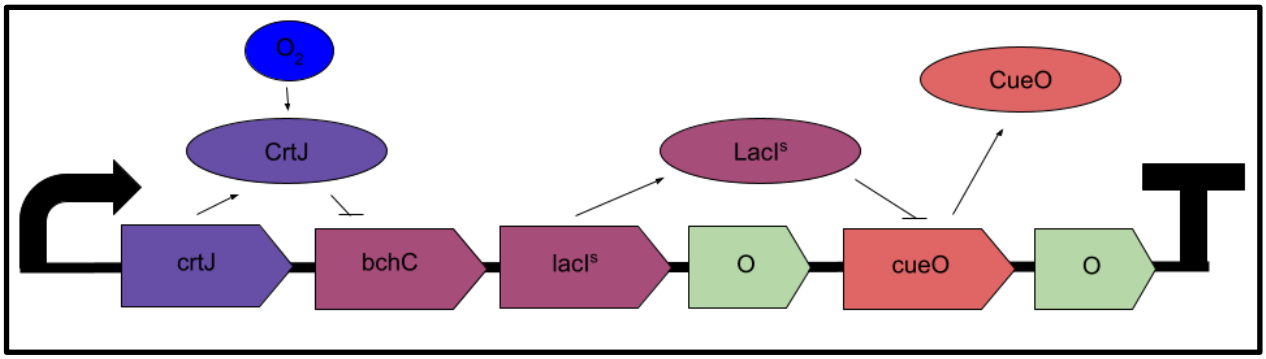

Figure 2. A molecular system regulating the production of the multicopper oxidase CueO in accordance with molecular oxygen levels. The crtJ gene is always transcribed into CrtJ, a repressor that inhibits the bchC operon when activated by molecular oxygen. This will end the production of the lacI^S mutant inhibitor, allowing the expression of the multicopper oxidase CueO.

device, we planned to integrate a C4 system—a less common carbon fixation cycle used typically as an adaptation by plants in warmer climates. These plants have leaves that contain unique structural features that aid in reducing photorespiration, as less oxygen is accidentally captured by RuBisCo. Thus, the C4 system is commonly analyzed by researchers attempting to enhance photosynthesis. Upon finding out that C4 photosynthesis is very difficult to engineer, we took inspiration from one of the key characteristics of C4 photosynthesis—the manipulation of CO_2 and O_2 levels to decrease RuBisCO's photorespiration—and designed a prototype device in that vein (Schuler et al., 2016). The device utilizes CrtJ, an aerobic repressor that is used to stop transcription in the presence of molecular oxygen (Masuda et al., 2002). This repressor will bind to the bchC operon, inhibiting it (Ponnampalam & Bauer, 1997). This inhibitory effect should extend to the lacI^S gene downstream, which codes for a mutant of the LacI repressor notable for always being activated (Leacock, 2021). LacI^S goes on to inhibit the production of whatever is between two lac operators by folding the affected DNA into a loop; in this case, the gene in question codes for the tentatively selected multicopper oxidase CueO, which would reduce molecular oxygen into water (Nickle & Barrette, 2022; Siegbahn, 2020). This arrangement should result in a negative feedback loop that reduces molecular oxygen into water when concentrations rise too high, restoring the efficiency of the chassis' native RuBisCO, but does not expend resources if unnecessary (Figure 2).

The third device, which aims to improve starch production and fixation, relies on a web of interconnected interactions relating to the regulation of algal metabolism (Figure 3). The system depends principally on the overexpression of CmGLG1, a glycogenin

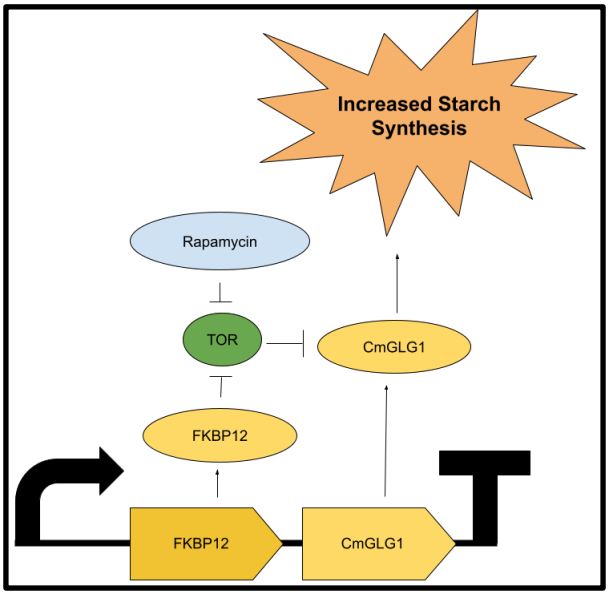

Figure 3. System for upregulating algal starch production. The overexpression of CmGLG1 results in increased starch production, though its transcription is not the only aspect necessary. Instead, rapamycin must be added to inhibit its regulator, target of rapamycin (TOR). FKBP12 is expressed to make the algae susceptible to rapamycin.

analog whose overexpression has been found to increase starch content up to 4.7 times. However, CmGLG1 expression alone is not sufficient because it is phosphorylated by TOR, preventing it from accumulating starch. We also plan to add the protein kinase rapamycin; the algae will be made susceptible to this by expressing the protein FKBP12 (Pancha et al., 2018). Rapamycin will inhibit TOR, which will allow CmGLG1 to be overexpressed. The interactions of this device should result in the initiation of glycogen synthesis. While TOR also has an important role in cell growth, TOR is found in two protein complexes, TORC1 and TORC2. TORC1 is the complex used in starch production and is affected by rapamycin, while TORC2 is not involved in starch production and is only minorly impacted by rapamycin. As such, only starch production should be impacted by this system.

As discussed in the Next Steps section, our selection and optimization of regulatory mechanisms will depend to a great extent on the optimal interactions of the parts they regulate and have not been selected due to inadequate experimental evidence of sufficient precision; we hope that preliminary trials will enable us to share more worthy information in this category.

Safety

The most important safety concern is the algae outcompeting other plant species due to overproduction, especially due to its nature, as a deliberate attempt to push efficiency beyond natural bounds. If the algae is not properly contained in the device which we envision and communicate in the Next Steps section, it is reasonable to fear that it may quickly grow and take over other plant species; even in death, it could destabilize the ecosystem by prompting eutrophication as it decomposes. However, this concern may be nullified by the limitations of the ways in which the algae will be enhanced; our plan does not currently involve the algae gaining the ability to gather all required nutrients or vitamins proportionally. While it leads to some drawbacks that will also be addressed in the Next Steps section, such as a decreased ability of the hypothetical product to address malnourishment in tandem with malnutrition, the algae's inability to gather certain nutrients like phosphorus and nitrogen at higher than natural rates should limit the growth of the algal populations without limiting biomass production, as the access to nutrients necessary to create starch should not be able to replace nutrients necessary for algal growth, preventing our creation from becoming a massively successful invasive species. However, to verify this, we plan to run extensive trials in a contained environment such as a lab, making sure that the effects of the algae on surrounding plant species is negligible.

Discussions

To implement this device, we must consider real-world implications, which include addressing benefits, potential challenges, and future improvements. *N. oculata* can be cheaply produced, is nutrient-rich, and has capabilities for high starch production and rapid biomass production. The vital benefits that *N. oculata* provides are due to its efficient usage of light and high rates of carbon fixation, which may enable quick and effective production of raw starch in times of crisis. However, these benefits come with a cost. The highly efficient light capturing and carbon fixation lead to a build-up of oxygen, which may impede carbon fixation and photosynthesis rates. Also, the highly efficient system might produce excess energy from the excess oxygen, and harmful oxidation may occur because the energy cannot be immediately disposed of or put to use in any way. Another area of concern is that the excess energy may be only used for higher starch production, which will result in the algae not having sufficient energy for growth or survival. Additionally, the algae's nutrient makeup, biomass production, and other behaviors may be affected by density-induced shade avoidance. This trait arises due to the organism's struggle to overcome the shade of other plants. Trying to enhance the algae's components by introducing a new plasmid also presents challenges, such as the current target electroporation method being unpredictable and not yet completely planned

out. Like the lack of clarity on our method of electroporation, there is also a significant lack of in-depth research on the effects of C4-photosynthesis on algae, which have also been observed to be very difficult to redesign. Despite the possible difficulties with solving these major problems and the lack of in-depth research, it is not impossible to find solutions to improve the system. The first improvement would be making the algae capture more of the electromagnetic spectrum to have more light-level options. Being able to photosynthesize in more types of light conditions would allow for optimal photosynthesis to go on for a longer time to produce starch and grow. This could help us with another improvement: improving efficiency in taking up nutrients, even in dry phosphorus-deficient soils.

Beyond the biological aspect of famine, we consider that a system possessing the characteristics we envision may offer unique ways to circumvent some of the occurrences' causes. For example, while real or predicted food shortages can prompt traders to increase the price of or hoard their wares—whether motivated by desire for profit or by fear—the knowledge of a readily available food supply may discourage such behavior. Likewise, a disruption in supply chains, no matter the source, can render them unable to supply the expected resources, but the predicted function of our system could mean that local populations would be able to quickly receive far more food than was imported (Hasell & Roser, 2017). The uniqueness of this approach may also provide benefits even where the cause of famine is entirely or partly of organic origin. For instance, populations made vulnerable by the fragility of subsistence farming may benefit from access to a simple and reliable method of diversification. Such diversification may serve to increase communal resilience against threats such as infestation and infection (Concern Worldwide U.S., 2022).

Next steps

The type of algae used will be *N. oculata*, which is both light efficient and has high starch production. With it, our next steps are to modify the algae to increase its starch production through enhancement of photosynthesis to provide an affordable and efficient food source. To maximize its growth potential, we will be using a vertical growth system indoors, which has been developed to produce algae faster and more efficiently than open pond growth. With vertical growing, algae is placed in a clear plastic bag so it can be exposed to natural light on two sides; natural light will be simulated by artificial lights to control for any outdoor irregularities. The bidirectional light exposure will increase the productivity rate of the algae, which will increase the amount of algae that can be used to produce starch as a food source (Newman, 1970). Freshwater that is between 5 and 35 degrees celsius will be used to simulate the aquatic environment that the algae grows in. Furthermore, algae require essential nutrients for growth, such as phosphorus and nitrogen. To initialize algae growth, we will use between 0.15 mg/L and 15 mg/L of phosphate-containing compounds, as this range is the optimal concentration for maximum algae growth. Normally, nitrogen levels are harder to control, because nitrogen is abundant in the atmosphere; however, our closed system will be better able to regulate nitrogen levels and prevent excess nitrogen uptake. Generally, for algae growth, 0.2 mg/L or greater is optimal. Moreover, the World Health Organization declares that acceptable pH values for algae growth are between 6.5 and 8.5. With the optimal baseline set, we can adjust the concentrations of these nutrients over time, approaching more realistic values so we can measure the importance of potentially addressing this discrepancy. With the algae in a proper environment for growth, we will begin the testing phase by designing a plasmid (pNOC_hfnCas12a-Nlux) and, by electroporation, introducing it into the algae for transformation. Once algae has gone through the transformation, the next step will be to optimize the new system's components. We will use tagged proteins to calculate the promoter's strength so we can determine if it will properly overexpress the components in an efficient ratio; for example, CmGLG1, whose overexpression has been found to produce as much as 4.7 times regular starch production in algae, may need to be upregulated if the algae is displaying excess

energy and material resources or downregulated if the opposite is true (Pancha et al., 2018). After calculating the promoter's strength from the data provided by the tagged proteins, we will optimize the system to express proteins under promoters and ribosome binding sites of different strengths. After optimization of the system, we will induce transcription factors to best fit the data until the result of the maximum efficiency ratio (O_2/quanta) is achieved. The optimization of the new system will result in improved carbon fixation, light harvesting, and starch production. Hopefully, this system will be able to cheaply increase the production of starch to help combat food insecurity and famine in the world.

Author contributions

A.R. and H.T. initiated the project idea and took the lead in conducting research to develop the idea and proposed design mechanisms after conducting a literature review. A.R. and H.T. took the lead in writing the paper and making the video. Z.D., K.D., Z.K., A.L., A.M., M.V., and E.W. contributed equally to the research process and drafting of certain sections of the paper. All authors provided critical feedback and helped shape the research, analysis, and manuscript.

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