ALEX LEATH BNFO 300 RESEARCH PROPOSAL ROUGH DRAFT 1

INTRODUCTION

**CUT OUT MAYBE HALF OF INTRODUCTON**

**PUT MORE BACKGROUND EXPLANATION IN THE INTRO**

**SO READER WILL ARRIVE AT THE EXPERIMENT WITH A BETTER IDEA**

**OF THE TERMINOLOGY**

Amid the countless “Red Revolutions” all over the world it was declared in 1970 that there had as well been a “Green Revolution” 1. The climax of that period coincided with the peace movements and the protests against the Vietnam War. In many ways, while the Age of Aquarius and its neologisms spawned what clearly amounted to a federally funded pursuit of pseudoscience, the actual reproducible science of those years propagated an awareness much like today that science in the United States Congress is viewed as one of countless institutions and bureaucracies in competition for time and funding attention. 2,3

The economies of the world have for over 100 years been starkly painted as one of two parallel ideologies: communism/command style economies and the familiar hybrid socialism/capitalism system which exists in the United States today. It is the ideas underlying these economic structures that have defined and reinforced the divisions of a large share of the political and military conflicts that have occurred in the post war era; although advancements in scientific discoveries in that period are unrivaled in the entire history of human existence 4, science is also a flawed creation of human minds and has as a result failed to maintain provide an independence space scientists are pressed to choose between their funding or the independence to self-assess if their work is safe to publish 5.

The study of life which we call biology, appeared during the middle part of the “Green Revolution” to be on the precipice of curing hunger and mitigating disease on a global level, yet by the time it reached its final decade, biology too had become a victim of the divisive conflagrations and revolutions across the globe 6. The alleviation of famine and starvation in China became an economic incentive and a tool of diplomacy. Instead of the immediate transfer of the tools required for researching and developing a “Green Revolution”, the more pressing concern for China was to simply have enough food. With less emphasis placed at that time on ensuring long term food security through technological investments agricultural and genomic remained limited. Through extensive investments and funding of biotechnology research facilities this has changed dramatically from the 1970s. This transition is evidenced most recently by ChemChina’s purchase of Syngenta. 7

The revolutions brought with them political instability, agricultural and financial inequality, destructive militias and death squads, terrorism against civilians, massive wealth redistributions, and international proxy wars. While the products of the “Green Revolution” and the subsequent advances in genomics were beneficial, they left many behind and little was accomplished in many of the places where communist revolutions had instead taken hold.

More recent conflicts have come to be defined by perpetual diasporas and a global refugee crisis that is projected to continue well into the 21st century. These mass migrations can partially be blamed on war in areas subjected to dwindling fresh water supplies and slow or stagnant growth in crop yields with conflict over water for irrigation only expected to intensify in the coming decades new approaches using modern biology has the potential to aid in solving not just hunger but also alleviation conflicts caused by demands for freshwater and good farm land. It is in these same areas of the globe where food and freshwater are severly limited that have seen the least benefit from the advances of the “Green Revolution”.

When the 1970s came to a start, humanity stood divided between two incompatible outcomes: one that sought to achieve global revolutions at any cost and another that sought to prevent a nuclear apocalypse at all costs. When the planet and its people were most in need of a “Green Renaissance”, the outcome was instead a “Revolution” born out of initiatives and projects that sought to ensure that global access to cutting edge science remained limited as a means of constricting access to nuclear materials and agrochemical dual-use technologies.

The “Green Revolution” faltered in achieving its egalitarian goals in part due to relentless efforts to guard the secrets of a bold new biological science from international adversaries who it was believed would seize the technology to develop sophisticated biological weapons in a world where international policies were set by brinksmanship, mutually assured destruction, and the security dilemma. The massive technological and scientific paradigm shifts of the previous four decades showed the world in the 1970s that it was possible to use genomics to optimize crop outputs, but what the “Green Revolution” failed miserably at was showing those who were most in need of revolutionary methods in agricultural practices how to achieve the radical possibilities afforded by the scientific progress of the era.

The 21st century offers biology a chance as a science to achieve not just a “Green Revolution”, but a far reaching and transformational “Green Renaissance” that will empower people around the world toward agricultural and medicinal discovery. As democratization of knowledge through computational discovery makes once difficult methods in science more trivial, biology must confront the issue of dual use technology in a way that does not stymie access to life saving scientific tools as happened in the 20th century. Today, the knowledge required to automate science remains occulted both within the ivory towers of academia and behind the razor wires of military bases and national research laboratories. This is for the time being a good thing for the world considering that before synthetic biology should be democratized, safety and predictability must first be demonstrated within the confines of scientific establishments where safety and training compliance are required and reviewed. The United States, in part due to the use controversial electronic surveillance programs could feasibly monitor its citizen scientists in do-it-yourself labs in homes and garages as a means of hazard mitigation and counter-terrorism prevention, but the established institutions of scientific research remain by far the safest and best options for the incubation of dual use technologies that could provide much-needed boosts to the global agricultural output and the medical problems the world will face in the coming decades.

Whether heavily surveilled do-it-yourself labs provide a societal benefit or whether surveilling them is a morally appropriate or constitutional approach to take in democratizing the biological sciences is controversial and while it is beyond the scope of this paper, the reader is urged to consider whether a world of dramatic biological paradigm shifts multiple times per decade may require fundamentally rethinking laws regarding publication and also regarding what expectations of privacy scientists should reasonably expect to have in the laboratory. Keeping places beyond the lab safe while still taking advantage of progress in areas like agricultural biotechnology and environmental microbial engineering for reclamation presents many significant challenges and maybe just as many creative potential solutions. These potential solutions comprise a significant and necessary piece of background for understanding the research that is proposed in the remainder of this paper. Understanding how orthogonal life can improve the safety of synthetic organisms used outside of secure labs or in places without environmental regulations is presented as a study of one potential application.

AGRICULTURAL APPLICATIONS

In a world where the very definition of life itself may soon need to be reassessed, investigating the flexibility of the information coding patterns conserved across all the natural biological systems has begun to deliver results that have aided in the construction of genomes for orthogonal biological organisms.

Explain what orthogonal Biological systems are…

Further research continues to bring orthogonality to additional cellular cycles such as rexod systems9. Possible uses for orthogonality in biocontainment can be found within the laboratory, in agricultural applications, and in the environment through reclamation projects. 10,11,12

The agricultural industry will likely be quick to adopt advances in orthogonal biology. This is a result of a number of factors: public relations and image rehabilitation by spinning the the new technology as an investment in ensuring that consumers are safe from the dangers of legacy era genetically modified organisms, as a result of increased investment and decreased patent competition resulting from consolidations, and also because these technologies may provide a safer tool in reaching previously unattainable increases in crop outputs to meet the ever growing world populations need for food.

The holy grail of agricultural biotechnology as it has been framed, involves the transfer of nitrogen fixation into cereals as a means of increasing crop production and eliminating the need for nitrogen based fertilizers that rely on the Haber-Bosh process. While such a technology has been a topic of intense research interest since the 1970’s it has remained elusive and difficult to implement in higher plants. The advances of genomics seen in the last two decades brings this goal much closer to becoming a viable commercial possibility and if combined with the right biocontainment solutions could simultaneously solve concerns related to horizontal gene transfer present in today’s genetically modified crops. While any breakthrough in incorporating effective nitrogen fixation into cereals is a spectacular achievement, the first generation of simple products and methods that result from this research will remain dogged by the same potential hazards that traditional genetically modified organisms present. Additional hazards likely remain unidentified or unimagined and must be sought out to better understand the unintended consequences that might result by use of engineered bacteria on farms and fields around the world.

A great variety of current research involving biocontainment concerns the use of orthogonal biological systems with recoded genomes and non-canonical amino acid incorporation.

INFO ABOUT ORTHOGONAL BIOLOGY

INFO ABOUT tRNA/aaRS AND INCORPORATION OF NON-CANONICAL AMINO ACIDS

This remainder of this research proposal will focus on the description an experiment with the potential to contribute to a deeper understanding of what role t-RNA nucleotide modifications could facilitate when designing for the biocontainment of plant endosymbionts whose genomes have had one more codons replaced at all locations across the entire genome. Additional information about t-RNA modification patterns could find practical use in designing of synthetic t-RNA/aaRS pairs for non-canonical amino acid incorporation into specific locations in bacterial proteins. An additional goal of this proposal is to address a basic scientific question in biology: Does the removal of specific codons from an entire bacterial genome result in a correlated change in t-RNA modifications characterized by Liquid Chromatography-Mass Spectrometry. Subsequent investigations would be directed toward measuring cellular stress response induced t-RNA modification in organisms where one or more codon possibilities has been eliminated from the entire genome. This question will become experimentally possible to address due to recent work in which every instance of 7 codons are to be removed from the genome. Assembly of this organism is currently in progress by George M. Church’s laboratory with funding from the Department of Energy (DOE) and the Defense Advanced Research Projects Agency (DARPA). **13**

INFO ABOUT tRNA NUCLEOTIDE MODIFICATIONS AND THEIR ROLE

DESCRIPTION OF HYDROLYSIS OF tRNA, SEPARATION, LC/MS

EXPLAIN HOW LC/MS WORKS FOR A BIOLOGY STUDENT AUDIENCE

CHARACTERIZATION OF tRNA MODIFICATIONS USING LC-MS

Hydrolysis of t-RNA in a solution containing sodium acetate, ZnCl2, nuclease P1, RNaseA, coformycin, tetrahydrouridine, deferoxamine mesylate, butylated hydroxytoluone, pMol[15N]5-2’-deoxyadenosine-([15N]5-dA).

Incubate at 37 C for 3h.

Add augmented solution containing sodium acetate, alkaline phosphatase, phosphodiesterase I

Incubate at 37 C overnight

Proteins removed by centrifugal ultrafiltration

Aliquot of filtrate containing ribonucleosides is loaded on HPLC and ribonucleodies were eluted with a gradient of acetonitrile in ammonium acetate.

HPLC column coupled ot an Agilent 6410 Triple Quadrupole LC/MS Mass Spectrometer.

LS-MS SECTION SOURCES

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