Increasing the Production of Encapsulated Double Stranded RNA for Biological Pesticides
(Technical Paper)

Societal Implications of Ethical Ambiguity in Biohacking
(STS Paper)

A Thesis Prospectus Submitted to the

Faculty of the School of Engineering and Applied Science
University of Virginia • Charlottesville, Virginia

In Partial Fulfillment of the Requirements of the Degree
Bachelor of Science, School of Engineering

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Fall, 2020

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On my honor as a University Student, I have neither given nor received
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Introduction

Synthetic biology is an emerging field that has the power to transform society. In the agriculture industry, synthetic biology drives the creation of novel biopesticides that utilize RNA interference (RNAi) technology. Chemical pesticides are currently the most prevalent crop protection strategy, despite the numerous human health and environmental problems associated with their use (Nicolopoulou-Stamati et al., 2016). In contrast, RNAi biopesticides are much safer for humans, and are more sustainable for the environment. RNAi biopesticides have not yet been successful in replacing chemical pesticides because the technology is not yet translational to be effective in the field. The problems surrounding the use of chemical pesticides have created a need for a more viable RNAi biopesticide to advance the agriculture industry. AgroSpheres is a biotechnology company poised to develop the first commercially available RNAi biopesticide because of their unique RNA delivery mechanism. However, AgroSpheres' current technology still exhibits deficiencies in both protein expression and fermentation processes, and will benefit from further investigation and improvement. In partnership with AgroSpheres, the final deliverable of the technical project will be an optimization of fermentation processes and characterization of the protein expression system to create a more scalable and effective RNAi biopesticide.

Synthetic biology also holds the potential to cause harm to society if misused. People across the world engage in "biohacking" or "DIY-bio." Biohacking is a movement in which hobbyists conduct their own synthetic biology research, often in makeshift home labs (Landrain et al., 2013). Biohackers believe in democratizing synthetic biology as an open scientific platform for discovery (Keulartz & van den Belt, 2016). However, biohacking is not formally bound by any institutional code of ethics or regulatory oversight, making it the "wild west of synthetic biology" (Blazeski, 2014). The ethical obscurity within the DIY-bio community impacts the health and safety of both the individual and society as a whole. Therefore, the goal of the STS research will be to identify and analyze the risks to society associated with biohacking.

Technical Topic

About 5.6 billion pounds of chemical pesticides are used globally each year, and 1 billion pounds are used annually by the United States alone (Alavanja, 2009). However, the widespread usage of chemical pesticides has produced significant negative impacts on human health, the environment, and crop protection itself. As the world population continues to grow, agriculture will also have to be scaled up to feed increasing numbers of people, amplifying pesticide use even further. Therefore, it is crucial to create alternative biological pesticides to address the myriad of problems associated with chemical pesticides.

Chemical pesticides have been linked to a number of serious health conditions in humans. These conditions include problems relating to endocrine, respiratory, reproductive, neurological, and gastrointestinal functions (Nicolopoulou-Stamati et al., 2016). The World Health Organization reports that many scientific studies have classified chemical pesticides as carcinogenic, neurotoxic, and teratogenic substances (*Food Safety*, n.d.). In addition, there are multiple ways that humans can be exposed to the harsh chemical pesticides, including inhalation, ingestion, or skin contact (Nicolopoulou-Stamati et al., 2016). Residues on food, in water, and in the air all contribute to the accumulation of pesticides within the human body (Nicolopoulou-Stamati et al., 2016). Exposure to these chemicals is toxic to the humans, necessitating the development of a safer solution.

Numerous environmental effects also result from the continued use of chemical pesticides. Since many of these pesticides are synthetic, they persist in the environment because microorganisms have not evolved mechanisms to break these chemicals down (Gavrilescu, 2005). Persistence becomes a global environmental issue when coupled with the transport of pesticides through the air, soil, and water due to emission, leaching, and runoff (Gavrilescu, 2005). These methods of environmental pesticide transport cause these chemicals to spread far beyond the farms they originated from. With the expansion of the agricultural industry to feed larger populations, it is clear that the continued use of chemical pesticides will exacerbate the destruction of the environment. Furthermore, many organisms targeted by current pesticides have evolved genetic resistances to the chemicals used, rendering them ineffective. When a pesticide is consistently applied, a population of pests will eventually develop resistance over time as an evolutionary response to the continued exposure (*Understanding Resistance – Pesticide Environmental Stewardship*, n.d.). As pests continue to develop resistances, chemical pesticides are becoming progressively inferior in their crop protection abilities.

Suitable biopesticides must be developed to replace chemical pesticides and mitigate their harmful effects. RNA interference (RNAi) is a technology that causes gene knockdown in organisms (Mamta & Rajam, 2017). RNAi uses double stranded RNA (dsRNA) sequences to bind to messenger RNA (mRNA) transcripts, therefore blocking translation of mRNA into proteins within the cell (Kim & Rossi, 2008). Standard chemical pesticides are dangerous for human consumption because they target enzymes in pests which are often homologous to human enzymes, causing harm in both organisms (Coman et al., 2013). However, the genetic sequences targeted using RNAi are specific to the pest, not similar to human sequences. This specificity to harm only the pest makes RNAi pesticides much safer for human consumption. In addition, RNA breaks down easily in the environment and poses no risk of persistence or contamination (Fletcher et al., 2020). RNAi is also an effective method for overcoming genetic resistances pests have evolved to current chemical pesticides, because unique genetic sequences are targeted in these organisms for which resistances have not yet been evolved. Despite the many advantages of using RNAi biopesticides, significant barriers exist that have prevented widespread adoption by the agriculture industry.

One of the major obstacles in creating a viable RNAi biopesticide is the tendency of RNA to degrade rapidly in the environment, leaving crops vulnerable (Fletcher et al., 2020). In order to prevent degradation, topical field applications of RNAi biopesticides would have to be performed much more often, which is inefficient for farmers. Another challenge is that RNAi biopesticides must be scaled up to match the industrial production capabilities of chemical pesticides. The biotechnology company AgroSpheres has made significant strides in developing an RNAi biopesticide technology that is both commercially feasible and is more stable in the field. AgroSpheres utilizes bacterial minicells to protect the dsRNA inside so the biopesticide can remain effective in protecting crops. However, AgroSpheres' platform technology must be further optimized before entering the market. In order to increase the efficacy of this RNAi biopesticide, the technical team will focus on improving both the fermentation production processes and the expression of the dsRNA binding protein. Specifically, the intended outcome will be an increase in dsRNA concentration that is encapsulated within the minicells after optimization of these factors. This research is already being pursued by the capstone group in the Fall 2020 semester, and will continue over the course of the Spring 2021 semester.

The dsRNA binding protein is expressed along with the dsRNA itself, and acts to increase stability of the dsRNA within the minicell. However, preliminary investigations within AgroSpheres show that the dsRNA binding protein is not being successfully overexpressed in bacteria. In addition, a significant portion of the dsRNA binding protein is being expressed in the insoluble protein fraction, meaning that it cannot bind to the dsRNA expressed in the soluble fraction. Therefore, increasing the concentration of dsRNA binding protein expressed, especially focusing on improving expression in the soluble fraction, will prevent degradation of dsRNA within the minicells. This will result in an increase in total dsRNA yield, making the biopesticide more effective in the field.

Optimizing fermentation is important in ensuring and improving the scalability of production, and several aspects of the fermentation process will be isolated for improvement. This includes implementing an exponential feed rate within lab-scale bioreactors, which will result in a higher level of cell growth by providing nutrients proportional to the growing bacterial population. In addition, adjusting batch phase minimal media components through small-scale growth studies will increase growth at this stage of production. Most importantly is optimizing the conditions around induction, since this is the step in the bioprocess which shifts the metabolism of the cells from growing to producing dsRNA binding protein and dsRNA. An analysis of substrate uptake before and after induction is essential because studies have shown this parameter to be essential for increasing the production of recombinant protein products (Wechselberger et al., 2012). Implementing these methods will be an innovative approach to improving AgroShperes' RNAi biopesticide technology.

STS Topic

Synthetic biology research has traditionally taken place within the confines of either an academic institution or a commercial laboratory at a biotechnology company, collectively nicknamed "Big Bio" (Delfanti, 2011). Biological research is very expensive, and Big Bio organizations have access to extensive funding. Big Bio has created a system whereby access to synthetic biology technologies is often dominated by economic incentives. Breakthrough medical treatments and other technologies are often inaccessible or not affordable for many people because they are controlled exclusively by major biotechnology institutions. Biohackers instead advocate to democratize science and reduce inequities in synthetic biology created by Big Bio through engaging in their own private biological research (Delfanti, 2011).

The synthetic biology research within Big Bio institutions is controlled by strict codes of ethics and government regulations. In contrast, biohackers are largely unbound by any formal ethical or governmental restrictions. In 2011, two biohacking congresses met in both North America and Europe to produce an informal code of ethics that encourages biohackers to adopt values such as respect and responsibility in their work ("Codes," 2011; Eggleson, 2014). However, the existing code of biohacking ethics is weak, non-specific, and is not enforced. In addition, the two separate European and North American congresses created two different codes, meaning biohackers in different parts of the world operate under different ethical standards (Eggleson, 2014). Biohackers from all over the world have also become more connected through the internet to exchange experimental data, procedures, and resources (Landrain et al., 2013). The lack of cohesive ethical oversight and the thriving global networks promote dangerous ideologies and create ambiguity in distinguishing what is ethical in synthetic biology.

The potential dangers of biohacking are illustrated by biohackers that engage in reckless experiments with little regard for ethical implications. In one such case, Aaron Traywick, a prominent leader in the biohacking community, injected a patient with an experimental HIV gene therapy treatment. Traywick streamed the injection on Facebook Live, and hundreds of people watched him administer the untested treatment (Bromwich, 2018; Lussenhop, 2017). Extreme public stunts like this show how one instance of unethical biohacking influences many other biohackers to also disregard ethics. Unethical and uncontrolled biohacking creates significant risks to society related to the abuse of synthetic biology technologies.

Synthetic biology becomes progressively more “de-skilled” as access scientific information and resources increases (Tucker, 2011). More people are becoming involved in synthetic biology research with less formal scientific training. The combination of de-skilling and the practice of DIY-bio together create biosecurity and bioterrorism threats to society (Keulartz & van den Belt, 2016; Schmidt, 2008; Tucker, 2011). In many ways, the current rise of biohacking compares closely to computer hacking. The de-skilling of programing has allowed people to build their own software. Over the years, hackers have created numerous malware programs and caused cybersecurity incidents with significant societal ramifications (Schmidt, 2008). If biohacking follows the same path, the impacts on society will be similarly consequential.

However, as an emerging field the risks to society created by biohacking and synthetic biology technologies have not yet been well defined. According to Ulrich Beck, “manufactured uncertainties” are risks resulting from rapid modernization of technologies in society (Beck, 2009). They are the result of both increased technical knowledge and simultaneous unawareness of the potential effects of the technology (Beck, 2000, Chapter 12). Manufactured risks are reliant upon human decisions and are hybrids of many factors including politics, technology, ethics, and culture (Beck, 2009). Beck also defines world risk society as a state in which technological innovation creates internal risks that are increasingly difficult to control (Jarvis, 2007). Some critics view Beck’s outlook on modernity and world risk society as too extreme and exaggerated (Jarvis, 2007). However, world risk society should not be construed as a hopeless society completely unable to control the uncertainties of technological progress. Instead, analyzing the manufactured risks created by technology is a tool for society to change itself and evolve new strategies to avoid catastrophic events, which Beck calls reflexivity (Beck, 2000, Chapter 12). Biohacking is a manufactured uncertainty in the modernization of synthetic biology in society, especially because of the lack of ethical regulation. Therefore, it is imperative to gain a better understanding of how these ethical shortcomings contribute to the gravity of the manufactured risks associated with biohacking. Identifying manufactured uncertainties will promote reflexive modernization of biohacking and synthetic biology technologies in society.

Methodologies

Research Question: What are the societal implications of ethical ambiguity in biohacking?

In order to answer this question, concepts from the Risk Analysis framework will be used to investigate biohacking as a manufactured uncertainty. First, background will be provided relating to the rise of synthetic biology in society and how this modernization has fueled the development of both Big Bio institutions and the biohacking movement. A thorough discussion of ethics related to biohacking will follow, which is important in defining the problem being addressed. A review of the Risk Analysis STS framework will also be included and the concepts will be applied to explore the risks to society created by biohacking. The Risk Analysis method supports the research question because it contextualizes how ethical obscurity in biohacking actually impacts society through the creation of various risks. Specific examples and cases of unethical biohacking will be used to illustrate the various ways that manufactured risks arise specifically from the lack of ethical oversight in DIY-bio, which will be key evidence to support the argument. The paper will finish with an explanation that connects how gauging the manufactured risks will prepare society to make reflexive changes in response. In this analysis, various sources will be used, including news articles reporting about specific biohacking incidents and journal articles related to ethics in biohacking. These different sources will allow quality examples to be derived and contribute to a comprehensive discussion the role of ethics in DIY-bio. The works of Ulrich Beck will be particularly important STS resources, because he pioneered many of the concepts related to the Risk Analysis framework. The STS research will be completed during the Spring 2021 semester.

Conclusion

The field of synthetic biology will be explored in both the technical and STS research projects. In the technical project, the team will improve the efficacy of AgroSpheres' current RNAi biopesticide technology through optimizing both the protein expression system and the fermentation processes. Increasing expression of the dsRNA binding protein will lead to better stability of the dsRNA encapsulated within the minicells. In addition, improvements to the fermentation process will make the RNAi biopesticide better suited for production on an industrial scale. The final outcome of this project will be an increase in the dsRNA yield encapsulated within the bioparticles. This will signify a major advancement of AgroSpheres' novel crop protection technology, one step closer to a world free of toxic chemical pesticides.

In addition, the impacts of synthetic biology on society will be evaluated in relation to biohacking. Currently, ethical standards in DIY-bio are extremely vague and largely unenforced. This leads to the development of numerous threats to society, which will be investigated in the context of the Risk Analysis framework. The STS research will aim to anticipate the manufactured risks associated with the ethical uncertainties of DIY-bio. Identifying the manufactured risks helps promote changes in society that will reduce the chances of a catastrophic event related to biohacking.

References

Alavanja, M. C. R. (2009). Pesticides Use and Exposure Extensive Worldwide. *Reviews on Environmental Health*, *24*(4), 303–309.

Beck, U. (2000). Risk society revisited: theory, politics and research programmes. In B. AdamU. Beck & J. van Loon (Eds.), *The risk society and beyond: Critical issues for social theory* (pp. 211-229). London: SAGE Publications Ltd doi: 10.4135/9781446219539.n12

Beck, U. (2009). World Risk Society and Manufactured Uncertainties. *Iris. European Journal of Philosophy and Public Debate. 1*(2)*:* 291-299

Blazeski, G. (2014). The Need for Government Oversight Over Do-It-Yourself Biohacking, the Wild West of Synthetic Biology. *Law School Student Scholarship.* 411.

Codes. (2011, March 29). *DIYbio*. https://diybio.org/codes/

Coman, G., Farcas, A., Matei, A. V., & Florian, C. (2013). Pesticides Mechanisms of Action in Living Organisms. In L. I. Simeonov, F. Z. Macaev, & B. G. Simeonova (Eds.), *Environmental Security Assessment and Management of Obsolete Pesticides in Southeast Europe* (pp. 173–184). Springer Netherlands. https://doi.org/10.1007/978-94-007-6461-3_16

Bromwich, J. E. Death of a Biohacker. (2018, May 19) *The New York Times*. Retrieved October 15, 2020, from https://www.nytimes.com/2018/05/19/style/biohacker-death-aaron-traywick.html

Delfanti, A. (2011). Hacking genomes. The ethics of open and rebel biology. *International Review of Information Ethics, 15*, 52-57.

Eggleson, K. (2014). Transatlantic Divergences in Citizen Science Ethics—Comparative Analysis of the DIYbio Code of Ethics Drafts of 2011. *NanoEthics*, *8*(2), 187–192. https://doi.org/10.1007/s11569-014-0197-7

Fletcher, S. J., Reeves, P. T., Hoang, B. T., & Mitter, N. (2020). A Perspective on RNAi-Based Biopesticides. *Frontiers in Plant Science*, *11*. https://doi.org/10.3389/fpls.2020.00051

Food safety: Pesticide residue. (n.d.). Retrieved November 1, 2020, from https://www.who.int/news-room/q-a-detail/food-safety-pesticide-residue

Gavrilescu, M. (2005). Fate of Pesticides in the Environment and its Bioremediation. *Engineering in Life Sciences*, *5*(6), 497–526. https://doi.org/10.1002/elsc.200520098

Jarvis, D. (2007). Risk, Globalisation and the State: A Critical Appraisal of Ulrich Beck and the World Risk Society Thesis, *Global Society*, *21:1*, 23-46, doi: 10.1080/13600820601116468

Keulartz, J., & van den Belt, H. (2016). DIY-Bio – economic, epistemological and ethical implications and ambivalences. *Life Sciences, Society and Policy*, *12*(1), 7. https://doi.org/10.1186/s40504-016-0039-1

Kim, D. H., & Rossi, J. J. (2008). RNAi mechanisms and applications. *BioTechniques*, *44*(5), 613–616. https://doi.org/10.2144/000112792

Landrain, T., Meyer, M., Perez, A. M., & Sussan, R. (2013). Do-it-yourself biology: Challenges and promises for an open science and technology movement. *Systems and Synthetic Biology*, *7*(3), 115–126. https://doi.org/10.1007/s11693-013-9116-4

Lussenhop, J. (2017, November 21). Why I injected myself with an untested gene therapy. *BBC News*. https://www.bbc.com/news/world-us-canada-41990981

Mamta, B., & Rajam, M. V. (2017). RNAi technology: A new platform for crop pest control. *Physiology and Molecular Biology of Plants*, *23*(3), 487–501. https://doi.org/10.1007/s12298-017-0443-x

Nicolopoulou-Stamati, P., Maipas, S., Kotampasi, C., Stamatis, P., & Hens, L. (2016). Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture. *Frontiers in Public Health*, *4*. https://doi.org/10.3389/fpubh.2016.00148

Schmidt, M. (2008). Diffusion of synthetic biology: A challenge to biosafety. *Systems and Synthetic Biology*, *2*(1), 1–6. https://doi.org/10.1007/s11693-008-9018-z

Tucker, J. B. (2011). Could Terrorists Exploit Synthetic Biology? *The New Atlantis*, 31, 69-81.

Understanding Resistance – Pesticide Environmental Stewardship. (n.d.). Retrieved November 1, 2020, from https://pesticidestewardship.org/resistance/understanding-resistance/

Wechselberger, P., Sagmeister, P., Engelking, H., Schmidt, T., Wenger, J., & Herwig, C. (2012). Efficient feeding profile optimization for recombinant protein production using physiological information. *Bioprocess and Biosystems Engineering*, *35*(9), 1637–1649. https://doi.org/10.1007/s00449-012-0754-9

Wolinsky, H. (2009). Kitchen biology. *EMBO Reports*, *10*(7), 683–685. https://doi.org/10.1038/embor.2009.145