

Strategies for Designing, Testing and Demonstrating Safety:
What Synthetic Biology Can Learn From Retrospective Cases

by

Neelima Yeddanapudi

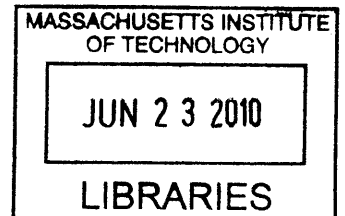
B.S., Electrical Engineering and Computer Science (1999)
M.Eng., Electrical Engineering and Computer Science (2002)
Massachusetts Institute of Technology

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Signature of Author:.....

Technology and Policy Program
August 15, 2009

Certified by:

Kenneth A. Oye
Professor of Political Science and Engineering Systems
Thesis Supervisor

Accepted by:

Dava J. Newman
Professor of Aeronautics and Astronautics and Engineering systems
Director, Technology and Policy Program

Strategies for Designing, Testing and Demonstrating
Safety in Synthetic Biology

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Neelima Yeddanapudi

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ABSTRACT

Synthetic biology is an emerging technology field within the realm of genetic engineering, differing from traditional genetic engineering in that it focuses on the modularization of genetic parts and the creation of de novo organisms. Significant concerns over safety have been expressed. This research explores traditional engineering and biotechnology practices for overarching principles of design, testing and demonstration that address safety concerns. The information is used to assess the current state of design, testing and demonstration in current synthetic biology projects addressing safety. Component and system design literature provide an engineering backbone of safety systems however, biological attributes such as mutation, growth, and multiplication create safety gaps, where biological engineering practices are needed. These principles are organized into categories of design and testing, and testing and demonstration to gain greater insight on where gaps in the literature might lie. Retrospective cases of traditional engineering and current cases of biotechnologies provide external validation and further illustrate which practices address which design, testing and demonstration needs. While most of the traditional engineering cases addressed safety through design and testing, when they were faced with questions of safety, they presented specific efforts to gain public confidence. The probiotics case was different in that the safety concerns came from the scientific community since history is being used as the convincing demonstration of safety. The three synthetic biology research projects cross the divide between traditional engineering and biotechnologies, but these efforts are firmly in the area of design and testing. These efforts begin to show the tradeoff between implementing safety and faster technical results. Strategies for further research are explored.

Thesis Supervisor: Kenneth A. Oye

Title: Professor of Political Science and Engineering System

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Chapter 1: Introduction

Synthetic biology is an emerging technology field within the realm of genetic engineering. It holds the promise of developing sustainable biofuels, better pharmaceuticals, and advanced medical therapies. However, as with any biological research, there are safety and environmental concerns. In particular, there is a fear that the accidental or intentional release, administration, or consumption of genetically engineered products or organisms could potentially endanger public health and the environment. The 1975 Asilomar conference provided a platform on which the safety of biological engineering could be addressed. It resulted in the establishment of biological safety laboratories with various levels of safety, as well as standards of practice that included working with weaker organisms. Still there remains substantial uncertainty regarding what types of threats are possible, whether concern for future occurrences could affect the safety of current research practices, and whether established safety guidelines can handle emerging practices in genetic manipulation. This uncertainty combined with public distrust of scientists illustrates how the anticipation and mitigation of risks is becoming a fundamental challenge in designing biotechnological products and practices. As a pre-emptive approach in addressing these concerns, the National Science Advisory Board for Biosafety (NSABB) and the National Institutes of Health Recombinant DNA Advisory Committee (NIH RAC) Roundtable on Synthetic Biology expressed interest in efforts to “engineer containment into synthetic systems/organisms” and asked synthetic biologists to take on the this challenge. [NSABB 2007]

A report by the Woodrow Wilson Institute found in their “survey among 1,003 adults” that, when provided with a description of the potential risks and benefits of synthetic biology, the proportion of those inclined to believe that “the risks would outweigh the benefits” exceeded the proportion of those inclined to believe the reverse. [Peter D. Hart Research Associates 2008] In synthetic biology, scientists address issues of risk mitigation and safety by learning to predict and control viability, horizontal gene transfer, and genetic stability in complex and uncontrollable environments. While scientists perfect engineering these strategies into their biological designs, they are also looking for ways to assure the broader, non-expert community that their safe designs are reliable. It will take substantial testing and demonstration to overcome the asymmetry in knowledge between what the scientists assert and what the public believes or will most likely believe. Assurances of safety may have no credibility without proper engagement of the stakeholders, engagement that not only seeks to inform but also to improve designs and tests through feedback from those broader communities. This research investigates what methods are used to implement safety and gain public confidence, both currently and in the past, and asks how those methods are being or can be applied in synthetic biology to help advance the field safely. This introduction serves to provide additional context to these questions, the motivation for asking them, and the scope and approach taken in this investigation.

1.1 Background

1.1.1 Biotechnology Concerns

As a starting point, it seems most logical to discuss safety concerns as they relate to biotechnologies. Despite the contributions of genetic research such as DNA sequencing, human understanding of the underlying mechanisms and “laws” governing biological systems is incomplete. In fact, greater knowledge and the resulting increase in biotechnological development have introduced new areas of uncertainty with respect to safety.

One clear threat to human safety involves work with extremely dangerous and infectious diseases such as Spanish influenza, polio, or smallpox. Driven to develop improved methods of fighting disease scientists collect or resurrect viral or bacterial contagions for purposes of study and experimentation. This leads to fears of accidental exposure and release similar to the 1978 case of accidental laboratory exposure to smallpox in Birmingham, England. [Fenner, Henderson et al. 1988]. Other sources of human exposure with uncertain consequences include genetically modified foods and pesticides, gene therapies, or simply working with or modifying biological organisms in a laboratory or factory. In fact, it is the practice of creating or modifying that emphasizes the amount of uncertainty present, as in the 2001 case of the mouse pox virus, that was modified to serve as a contraceptive, but became a highly potent pathogen instead. [Jackson, Ramsay et al. 2001]

Uncertainty with respect to safety also refers to environmental threats. George Church highlights a number of instances where animal and plant populations were

affected for the worse by human interference. [Church 2005] He and others discuss how the introduction of new or non-indigenous species can disturb an ecosystem. While the ecosystem will probably reach equilibrium eventually, it may become a new ecosystem, unfriendly to the original specie inhabitants. Such environmental alterations could result from bioremediation, genetically modified crops or pesticides, or the unanticipated release of a genetically created or modified microbe.

Exposure can be intentional, such as with a genetically modified pesticide or a gene therapy; or it can be unintentional, as with accidental disease exposure or microbe release. In the case of synthetic biology, the purpose of a genetically modified or created microbe will dictate whether it should survive in various environments and determine for how long. Such careful design should explicitly account for the limits of current human knowledge regarding the mechanisms at work in biological systems and how they behave over time.

As Ellenberg and Chen state, “we would all like such products to pose zero risk of adverse effects. Unfortunately, this goal is not achievable for any pharmacologically active product.” [Ellenberg and Chen 1997] Since safe design can only minimize the number of potential dangers inherent in a particular biotechnology, it is important to communicate: how the safety is only reliable within a particular degree or threshold value, what that threshold value is, and consequently what behavior complies with the limitations of the provided safety. Managing actual safety is the first step in handling fear. However, if the existing fear is not addressed, it has the potential to self-sustain and persist on its own. Therefore mishandling or misunderstanding fear can lead to additional problems.

Past mistakes and accidents have damaged credibility of safety assurances in existing biotechnologies, including assurances from the technology wielders and the institutions and companies they work for. While harm may be unintentional, various other forces may play an unwitting part in undermining the implementation of safe design and testing. Similarly, the push for progress may all but eliminate motivation and efforts to engage in credible demonstrations.

Medicine is an area where biotechnologies hold great potential to contribute both positively and negatively. Unfortunately, the push for results stemming from a company's pipeline depletion, medical urgency, or the need to achieve tenure or produce publications may hurry safe design and testing to their detriment. In 1999 Jessie Gelsinger volunteered as a healthy individual and was killed by the administration of an experimental gene therapy. The viral vector used to carry the gene therapy caused his death. [Stolberg 1999] Perhaps further tests for safety or interference with the patient system were warranted. Another problem is that the push for immediate or positive results may have undermined implementing safe design and testing. Needless to say, this damaged public trust in researchers, clinical studies and gene therapies. Boston University's initial disregard of the Boston community in building their Bio Safety Level (BSL) 4 laboratories, [Lawler 2005] did little to mitigate that pattern of distrust.

Public trust in companies or industries is no greater. In 1985 Advanced Genetic Sciences Inc. neglected to notify the community or gain their acceptance before going ahead with plans to conduct field tests of ice-minus bacterium. [Sun 1986] This failure to communicate or involve the community enraged the public and provoked a controversy on

field tests. This type of behavior promotes the belief that dollar signs are of primary interest to corporate biotechnology, and that interest will outweigh incentives to minimize risk and protect their communities and their customers.

1.1.2 Effects of Concerns

While the products of biotechnology may have deleterious effects that one must protect against, safe design is not enough. Just as important is testing those designs and demonstrating the safety provided by those designs. The effects of fears can create just as many research, economic, and societal hurdles as the dangers inherent in biotechnology.

Fears, whether founded or unfounded, can slow or prevent technological progress and development. They can lead a government to take steps that hinder, suspend or eliminate future research, as in 1976 when the city of Cambridge tried to prohibit experiments with recombinant DNA. [J. Dyson 1981] Existing fears push for government interference that may generate unnecessary obstacles in the way of progress. Not only does this reduce scientists' ability to function, it harms their faith in public interaction and government authority, thus becoming a social divider. This may cause a flight of researchers to alternative countries without technological oversight, leading to uncontrolled, unrestricted research. A less extreme move might involve abandoning academia for the corporate sector, (an area with less regulation over research), however litigation over accidental injury from biotechnologies may contribute to the decline of companies engaged in important biotechnology research such as producing vaccines.

Controversy over genetically modified organisms (GMOs), more specifically food or pesticides has lead to regulatory inconsistencies across international lines and subsequent

economic consequences. Fear of GM foods, or foods treated with GM pesticides can influence public purchasing negatively, regardless of the lack of evidence supporting claims of negative affects. Furthermore, depending on the protective philosophy of governments, it has led to trade inconsistencies that make it difficult to import and export food and medications across international lines. Europe takes a caution first approach to GMOs increasing the labeling requirements or preventing the import or sale of many genetically modified foods, which can create trade difficulties, especially for US food exporters. In the US, GMOs are considered substantially equal unless proven differently.

Essentially differences in trade restrictions create barriers based on individual countries priorities and intentions to protect their public. These differences in safety standards create inefficiencies in the market where companies must cater to multiple sets of regulations causing a slower global market with increased inertia and an inability to adapt. Furthermore, in addition to hassles of litigation, there is the cost of litigation and uncertainty in regulatory frameworks that make commercial interests less likely to invest in biotechnologies. This could lead to additional safety issues where the distrust of medical therapies from genetic engineering leads to increased lawsuits, increasing prices of the therapies, discontinued research or production, and a subsequent shortage in crucial medical solutions when they are most needed.

This highlights the importance of ensuring that research and products of genetic engineering are designed and tested for safety, and that convincing, credible demonstrations are utilized to apprise the public of that safety. Fears built on little evidence can not only cause problems for corporate interests and others such as farmers using that

technology, they can also affect the arbitration of scientific proof on the part of jurors in civil cases. Additionally, in the case of vaccines and other medical therapies, fears of therapies that overshadow fears of the disease lead to increased cases of disease, which in turn elevate health threats to society. [Offit 2007] Furthermore, fears causing regulatory action may result in rescission of approval for necessary remedies, like vaccines to prevent major diseases in cattle, swine, and sheep. [Warren 1986]

1.1.3 Current Public Perceptions and Discourse

One step to understanding public perceptions and discourse is to consider what the general public understands or cares about. For instance, current issues reveal that some of the most common concerns include health, financial conditions and employment, cheaper living necessities such as fuel, leaving less of a carbon footprint, and less reliance on hostile nations. Biotechnologies can benefit these causes with potential contributions to health technologies that: facilitate cheaper health care, more efficient and targeted medical therapies, easier and less painful therapies, and corrective rather than treatment therapies. They can help broaden the jobs market with associated sectors that support biotechnology development, mass production or provide complimentary technologies; and they can facilitate improvements in cheaper fuels and environmentally sound products.

On the other hand, biotechnologies also have the potential to produce health hazards such as biohazard accidents, emergent side effects of medical therapies, allergies to GMOs, and bacterial resistance to existing antibiotics. They can also contribute to a loss of employment by replacement industries and create more expensive fuels; or they may

contribute additional environmental concerns with emergent properties of biofuels or their byproducts and waste, or the escape of genetically modified entities.

A survey sponsored by the Woodrow Wilson Institute explored public knowledge and perceptions of synthetic biology. [Peter D. Hart Research Associates 2008] While the methods and information used to administer this survey could be questioned with respect to expressed biases, some aspects of public perceptions and biases regarding synthetic biology were relatively clear. First, the public seemed mostly unaware of synthetic biology and the associated research. The survey reveals a preponderance of risk-averse feeling since providing information about synthetic biology caused more people to believe that the risks would outweigh the benefits. Many of those who had never heard of synthetic biology were willing to guess and provide opinions based on those perceptions, which reveals a tendency for people to make associations with concepts they are already familiar with. Again, leaning towards risks reveals association with biotechnology consequences rather than benefits. These points highlight the importance of communication and demonstration in informing the public, alleviating unfounded fears, allowing the public to contribute founded concerns and input, and fostering an understanding of what to reasonably expect in terms of benefits and perceived dangers. An informed public allows for productive involvement and provides for more voluntary interaction with biotechnologies.

If Institutions and Industry continue with their research, even if practiced safely, while disregarding ongoing fears and failing to reassure stakeholders and the interested public, obstacles will continue to hinder their progress. Interest groups who advocate less or no progress in biotechnologies will have greater influence as their message associates

more clearly with caution and protection of the public. As they tend to be comprised of members of the “general,” non-expert public and claim to be representative of the public, their message will gain greater support.

1.1.4 Current Regulatory Frameworks

Historically, regulatory frameworks that deal with safety seem to be responsive efforts rather than pre-emptive efforts. Many regulations, created by the Food and Drug Administration (FDA), were created in response to adulterated foods and medical remedies promoted by snake oil salesmen. Clinical research protocols were developed in response to poorly tested medications or testing that harmed subjects. The National Childhood Vaccine Injury Act (NCVIA) passed in 1986 was developed in response to cases where patients developed the actual disease in full form instead of developing immunity.

With the advent of recombinant DNA techniques, scientists and legislators chose to be pro-active because the possibility of creating a new disease or other type of hazardous critter had dangerous implications. The Asilomar conference in 1975 was an attempt by scientists to meet and discuss the technical implications, possibilities and dangers that may result from rDNA research. They chose to institute a voluntary moratorium on research until they could determine how to proceed safely. The Asilomar conference directly resulted in the NIH guidelines for biotechnology research, including stipulations for biosafety laboratories specifying research criteria that associated the research with the necessary biosafety levels. In addition, they recommended one type of inherent safety design – working with weaker organisms. [Rogers 1975]

Unfortunately, NIH guidelines could only be required of recipients of federal funding. This meant that groups conducting genetic research with federal funding were directly responsible for following the guidelines, while scientists working for corporations enjoyed more freedoms. In 1986 the Coordinated Framework for Regulation and Biotechnology [Unknown 1986] utilizing existing legislation such as FIFRA, TSCA, FDCA, OSHA and APHIS, was devised to manage the issues associated with biotechnologies since both their developmental processes and uses spanned a variety of areas covered by existing US regulatory bodies. For instance, biotechnologies that result in pesticides are subject to safety requirements under FIFRA and TSCA, while food and medical therapies are subject to the guidance of the FDCA. Groups such as the CDC can require corporate compliance with NIH guidelines in order to receive pathogens for the purpose of testing or development of medical therapies. Subsequently, corporations that require the cooperation and assistance of these organizations become subject to their requirements, expanding the coverage of the NIH guidelines. As of yet there have been no known biological catastrophes in the US, which suggests that the NIH guidelines have been successful. Yet safety is still a concern, as the political reaction to biotechnologies especially in the case of Europe, has shown.

1.2 Defining Synthetic Biology

1.2.1 What is Synthetic Biology?

Three advances in biological studies can be considered a significant part of the foundations of synthetic biology. First, recombinant DNA (rDNA) technology, developed in the 1970s, utilizes various restriction enzymes and ligases to cleave DNA fragments from

one organism and insert them into a DNA vector that can replicate in another organism. Essentially, it was a method developed to generate sequences of DNA derived from multiple sources. [F. Lodish, Berk et al. 2007] Second, systems biology, a method of studying biology that has had recent popularity, explores the complex interactions of multiple integrated components in living systems from a higher level, systemic perspective. Potential research practices involve removing components of a system and observing the resulting behavior, which could be cell death, loss of specific function or weakening. Finally, the development of newer, faster DNA sequencing techniques, the process by which the order of DNA bases is determined in a segment of DNA, has increased the amount of genetic information available to scientists. “By mid-2008, complete genomes were available for 719 prokaryotes (microorganisms that lack nuclei) and 23 eukaryotes (“higher” organisms with nucleated cells, including humans). An additional 446 eukaryotic genome sequences are in varying stages of completion.” [Mukunda, Oye et al. 2009] The combination of rDNA technologies and information contributed by systems biology and DNA sequencing has created a fertile bed for the emergence of activities labeled synthetic biology.

Where traditional genetic engineering has involved combining DNA sequences cleaved from an existing genome, synthetic biology, utilizing knowledge of the sequences, aims to construct specific DNA segments from scratch before combining them. These segments are referred to as “parts.” When those “parts” are assembled through biological reactions to perform a specific function (defined by the engineer), the combination can be referred to as a “device,” and devices can be integrated to perform processes in a system.

Thomas Knight notes that, “the key notion in the design of our strategy is that the transformations performed on component parts during the assembly reactions are idempotent in a structural sense. That is, each reaction leaves the key structural elements of the component the same. The output of any such transformation, therefore, is a component which can be used as the input to any subsequent manipulation.” [Mukunda, Oye et al. 2009] In other words, these systems can ultimately perform functions like circuits, taking a certain input and producing an output, and like circuits, they can be used as parts of a larger system or circuit.

Research in synthetic biology is still in its early stages, therefore it can be considered a combination of two practices. Some types of research can be considered belonging to systems biology, such as the top down (reductionist) approaches that are used to gain information by reducing systems to their smallest pieces, along the way, figuring out how those pieces function. Other types of approaches, such as a bottom up approach, involve building components *de novo*. Some studies have manufactured vesicles with metabolic functions, a possible cell membrane of sorts [Noireaux and Libchaber 2004], while others involve adding components and observing the resulting behavior.

As suggested, the research can take many forms. Some scientists test current understanding of biological systems by designing and constructing their own synthetic biological systems that can model component behavior in natural cells. They also explore the possibilities of using existing organisms such as yeast or *E. coli*, and optimizing these biological systems for specific purposes by removing “unnecessary” or redundant elements. These could serve as mini labs for experimenting with pathways or bodies for creating

novel organisms that fulfill specific purposes. Constructed metabolic pathways, essentially programmed systems, can be used to generate products such as drugs or biofuels, or to perform functions such as filtering contaminated water or delivering medical therapies. One of the most fundamental ways in which synthetic biology differs from traditional genetic engineering is the intention to build parts and standardize them. Moving forward in this thesis, this characteristic will be considered the most distinguishing aspect of synthetic biology. Many synthetic biologists are working to develop foundational technologies that make the construction of synthetic biological systems easier, in other words, a set of biological legos. The purpose is to simplify the assembly of designed systems, with an “emphasis on developing modularized biological parts, protocols for interoperability and standards for parts performance, open parts registries, and routinized methods of assembly for creating biological devices.” [Mukunda, Oye et al. 2009]

1.2.2 Potential Benefits and Safety Concerns

Given the current range of activity in synthetic biology research, one can look forward to many potential benefits. First and foremost the “application of principles of modular design may cut design costs and development times, allow parts outsourcing with resulting scale economies, and allow more rapid diffusion of the methods of biological design.” [Mukunda, Oye et al. 2009] This will provide tools for facilitating faster advances in biotechnology as well as encouraging multiple approaches to managing various health and environmental challenges. There is a potential for alternative biofuels: diesel, ethanol, and non-ethanol, and reducing dependence on non-renewable resources. Further advances could reduce dependence on specific feedstocks whose increased use could cause changes

in environmental conditions as well as displace indigenous species and societies.

Contributions to public health include the development of tumor-seeking bacteria, artemisinin or new vaccines; or broadening knowledge of existing or past diseases through actual experimental study.

However, with synthetic biology emerging as a new biotechnology, it seems prudent to examine how safety concerns differ from those currently studied in traditional genetic engineering. An e-conference conducted by Markus Schmidt found that “Synthetic biology has been considered to be a sophisticated continuation of genetic engineering, implying that biosafety issues would only differ quantitatively and not qualitatively.” [Schmidt, Torgersen et al. 2008] Traditional genetic engineering involves highly skilled and educated scientists who custom design sequences of DNA utilizing complicated and time-consuming methods. However, with the intention to standardize parts and processes along with decreasing time and expense, the assembly of biological systems will be possible for less and less skilled technologists, perhaps creating a qualitatively new challenge.

The evolution of existing organisms required millions of years of development, including trial and error with mutated sequences and adaptive alterations to a constantly changing environment. Though existing biological systems can be considered ad hoc, there may be specific biological reasons for various types of redundancy and “unnecessary” genetic sequences that may result in protection for the organism or protection for those it shares an environment with. Current efforts to optimize organisms may ignore or be unaware of these complexities. Especially since knowledge of biological systems and how and why they function is still expanding, indicating that the knowledge is yet incomplete.

One might argue that an educated scientist would take this into account when designing their entities, however the same cannot be said of unskilled tinkerers with access to easily understandable parts and assembly protocols. This has the potential of endangering an individual working with the parts or the surrounding individuals or environment.

Furthermore, incomplete knowledge of an organism means an incomplete ability to predict how that organism will interact with its environment. Interactions with an environment may cause irreversible alterations to the environment or to the organism itself, perhaps causing it to pose additional danger. There is an uncertainty and unpredictability associated with living systems that makes it difficult to anticipate these types of consequences.

Other concerns that may be shared with traditional genetic engineering include the “Theseus paradox” which questions how many alterations can be made before an organism is no longer associable with the original. In the case of safety, if a host organism is considered harmless, how many alterations can be made before that becomes invalid and re-evaluation is necessary. What if the organism is completely developed from the ground up? Is it still the same? It seems that traditional rDNA work with microbes rendered the microbes incapable of survival outside the laboratory, however since current work involves creating microbes for a particular purpose, that might involve increasing its ability to survive. In this case, environmental concerns with biotechnologies will take center stage, along with questions of deliberate release and organism lifecycles. Critters designed to perform functions in environments outside the laboratory may need survival time limits.

How these types of creations or alterations affect safety will be pertinent when questions of testing for safety come later.

1.3 Scope

1.3.1 Relevant Literature

Given the current reservations felt towards the field of biotechnologies, the focus of this research centers on how to support safe progress in synthetic biology through optimal practices in safe design, testing and demonstration. Given the traditional engineering and biotechnological influences over synthetic biology, it seems prudent to draw on literature from both areas and the various disciplines within them, in order to explore the strengths and weaknesses of approaches in those fields, and to inform the safe practices explored in this thesis. Literature on design and testing has been drawn from these areas, while the demonstration literature discusses the successful diffusion of technology and the practices that influence successful adoption, including utilizing stakeholder involvement.

To provide an element of external validation as well as anecdotal evidence of successfully applied principles in design, testing, and demonstration, retrospective engineering and biotechnology mini cases have been collected. Examples of safety engineering in bridges, buildings and devices as well as safe practices in probiotics and vaccine development contribute both traditional engineering and biotechnological perspectives on safe design.

The second piece of this thesis draws on synthetic biology literature and information gained from interviews with various laboratories working on safe design in synthetic biology. This thesis mainly considers safe chassis design being developed in three

local or “accessible” laboratories: MIT (Thomas Knight), Harvard (George Church), and Lawrence Berkeley (Adam Arkin) laboratories.

1.3.2 Area of Inquiry

It is stated in the beginning of this introduction, this research seeks to explore methods of implementing safe design, testing, and demonstration, both currently and in the past, in order to better facilitate safe progress in synthetic biology research. It assumes there is a benefit to continued research but recognizes the potential for losses, therefore the focus here is to present a different way of thinking about the incorporation of safety in design that will support beneficial advancement and avoid collateral loss.

This thesis will not engage in a risk-benefit analysis focused whether to continue practices in synthetic biology. Nor will it debate probabilistic outcomes of damage or benefits. Rather, given an assumption that synthetic biology will continue to develop, it will ponder what strategies can best mitigate the risks and enhance the benefits.

While it acknowledges the existence of ethical arguments associated with the continued practice of synthetic biology, neither will this thesis debate the ethical implications of continuing this research.

Finally, this thesis will not examine which principles of safe design, testing, and demonstration are better than others. Rather, it will explore safety issues and determine what principles would be useful in providing that safety.

Given the implied relevance of engineering and biology in synthetic biology, this thesis explores practices for safe design, testing, and demonstration in those areas and their applicability to synthetic biology. Moving forward “engineering” shall describe those

practices associated with traditional engineering disciplines such as structural, mechanical or electrical engineering. “Biotechnology” shall describe those practices utilized in biology and chemistry laboratory research as well as biological engineering. These two areas contain insightful literature and germane examples that have the potential to guide future practices in synthetic biology. The strategies explored might hopefully provide guidance for scientists and regulators.

1.4 Approach

As mentioned above, initial steps to this thesis involved studying the field of synthetic biology, as well as exploring extensive literature in safe design, testing, and demonstration across a variety of disciplines. The information gained from this literature review has taken form as overall concepts and categorized principles. Based on the theory presented around these practices, this thesis combines the practices of design and testing (D&T) and testing and demonstration (T&D), in order to tease out methods specifically geared towards establishing safety and those geared towards establishing credibility. While stakeholder input will be touched on in both areas, significant emphasis on stakeholder interaction will be explored in the T&D section. The principles and practices studied in testing seem to extend across the design and demonstration processes and are represented as such. Engineering and biotechnological practices and principles are explored and categorized into these combined areas D&T and T&D. This provides a look at the strengths and weaknesses of engineering principles in these categories with respect to synthetic biology and how principles of biotechnological design are needed to compensate for some of these weaknesses.

Anecdotal evidence is surveyed for principles of safe design, testing, and demonstration that lead to successful development and adoption of particular designs. These principles serve as external informers and validators, and are also categorized along the lines of engineering or biotechnology and T&D or D&T. A design space or diagram will be created to further illustrate these concepts.

Practices in synthetic biology, specifically in safe chassis design will be studied and compared to this design space. The purpose of which is to determine where current practices may fall short and how they can be improved.

1.5 Structure of Thesis

The introduction provides a context that explores the concerns and resulting effects fostered by continued research in biotechnologies. It also presents a description of synthetic biology that reviews the origins, practices and perceptions, as well as the hopes and concerns specific to this field. This is the description that shall be referred to in as this discussion progresses. Chapter 2 explores the concepts of design, testing and demonstration through a framework that categorizes these concepts as design and testing (D&T) and testing and demonstration (T&D). The chapter will review relevant literature in traditional engineering (e.g. structural, mechanical, electrical) and biotechnological practices for theories that might provide useful principles for implementation of these concepts. Chapter 3 provides anecdotal evidence that further explores the concepts of design and testing and testing and demonstration utilizing principles obtained from the literature. This chapter will broaden the analysis of safe design, testing and demonstration and explore these concepts in the context of synthetic biology. The final chapter

concludes with observations and suggestions for future research. It will also mention potential lessons for scientists and regulators that might facilitate safe progress in the field.

Chapter 2: Designing for Safety

2.1 Defining Safety

In discussing safety and ways of incorporating safe design into synthetic biology, it is first important to provide a definition of safety that will function in the context of synthetic biology. Contributions from Webster provide a basic starting point. The Merriam-Webster Online Dictionary defines safety as:

the condition of being safe from undergoing or causing hurt, injury or loss;
where safe is defined as:

- secure from threat of danger, harm, or loss, and
- not threatening danger,

and fail-safe is defined as:

- incorporating some feature for automatically counteracting the effect of an anticipated possible source of failure, and
- having no chance of failure: infallibly problem-free

[Unknown 2009]

Two subtle points are significant here. First, attention should be drawn to the fact that the definition does not specify the entity to which “condition” refers. It could be an object, organism, or any type of system. Second, the implication of the word, “condition” should be underscored. It can be interpreted as a state of being at a moment in time or throughout a specified period of time; an interpretation that highlights the difference between static and dynamic systems. Something can start off safe and become unsafe given time, for instance the Minneapolis I-35W bridge, opened in 1967, remained or was considered safe for almost 40 years when it collapsed due to rusted and cracked supports. This emphasizes the broader concept of dynamic conditions where safety can only be applied to a particular instance in time and must be inferred in future scenarios. The

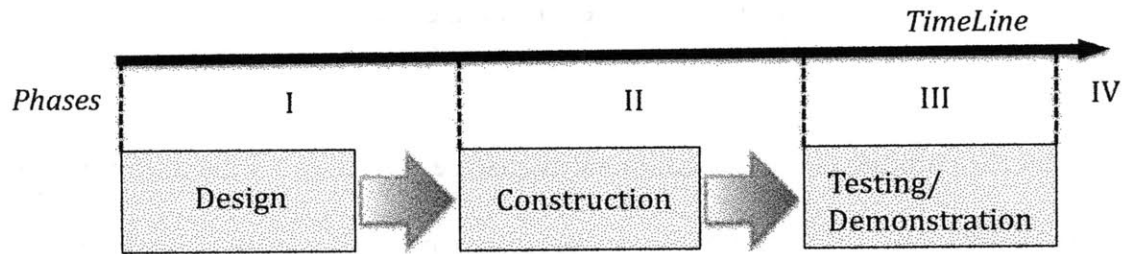
Tacoma Narrows Bridge was believed safe, yet its designers did not consider change in external conditions such as wind. External changes as well as internal changes apply dynamic pressures on an object and affect the state of safety that is assumed at any point in time.

For the purposes of this discussion, safety shall be defined as a dynamic state when a design (object, organism, system) is secure from experiencing or causing a non-desirable event, such as harm or loss. Refining this definition for the purposes of engineering, it is assumed that the design is composed of various components that work together to provide safety, and that the quantity or level of safety is conditional upon the ranges of component function under dynamic pressures.

2.2 Design, Testing and Demonstration

There exists a conventional perception of engineering processes that can be described as design, then construction, then a combination of testing and demonstration, where testing is most closely associated with validating and is many times lumped in with or placed instead of demonstration (Figure 2.2-1). This paradigm can be considered to have four phases where constructing the design (II) takes place with the assumption that the design phase (I) has been completed. Testing and/or demonstration (III) is expected to take place after the system has been designed and built and is ready for validation, where presentation of validation results may be considered demonstration. The final phase of continued performance (IV) takes place afterwards with the assumption that the system will exist and function adequately on its own. Typically the overall process will contain overlap,

but these steps are generally considered as independent processes that usually occur



consecutively.

Figure 2.2-I

Clearly each of these steps is important for a system to function properly. "Functioning properly" will be considered the successful provision of safety in this thesis.

Intuitively, a design should exist before a system is built because planning is essential in optimizing safety. Yet, design doesn't end at the plan. Construction brings realization to the plan, however design should be thought of as an ongoing process during the construction phase in order to incorporate the ability and expectation of adaptation as time reveals additional criteria or altered conditions (Figure 2.2-II)(II).

Similarly, the role of testing should not be confined to a post-design and post-construction phase (Figure 2.2-II)(I-V). The term "testing" can refer to methods of experimentation or methods of validation. Validation can be defined as the "confirmation by examination and provision of objective evidence that the particular requirements for an intended use are fulfilled." [Frey and Dym 2006] Experimentation, on the other hand, can play a substantial role in design and construction as the practice of conceptually testing the design as well as interim testing of the system and components provides ongoing knowledge to the designer. Both are important in testing for safety and do not have to

occur only after a system has been designed and constructed. Experiments provide a method for better understanding a design or parts of a design and they “help to reduce the bias that can exist in less rigorous forms of observation.” [Mcdermott 2002] In addition, Myron Uman suggests predicting the results in an experiment in order to better understand the results of the test as well as evaluate and improve understanding of the system. This is different than a validation approach where specific results are expected because they are the intended results. Simply relying on validation leads to planning ahead based on expected results, a practice that can create difficulties when a system needs actual results to move forward and later provide the expected results. [Unknown 2004] Experimentation can also play a role post-design and construction because it helps determine system limitations when functioning under various circumstances, and monitoring throughout the expected functional lifecycle (V) helps maintain the system and manage any deterioration in safety aspects of the design. Validation on the other hand tends to refer solely to whether a system provides the expected results, however this too can be a process throughout as a system is made up of components whose validation may be essential to the design and construction process. This chapter continues to explore some of the current engineering literature relating to both experimentation and validation.

Demonstration can be a step that is easily skipped but can play a significant role in the success of a design (Figure 2.2-II)(IV). It is especially useful when the success of the design relies on the safety of a design. Having safety mechanisms that are validated does little good when there is no way of providing stakeholders with an assurance of that validation. Submitting measurements or results that speak to a scientist is not necessarily

considered demonstration when the audience is not composed of scientists. Furthermore, it seems unwise to perform a validation test as a demonstration without almost complete assurance that the expected results will occur. A true demonstration, conducted in public in order to build credibility, is undeniably helpful, especially when it comes to safety. Proper attention to demonstration strategies will help the overall acceptance of safe design. Demonstration can also be used as a technique that facilitates interaction with stakeholders. Stakeholder input has the potential to inform design and testing as well as demonstration. Concerns of external non-expert stakeholders may differ from what a scientist believes should be of concern. Interaction and involvement with stakeholders bridges this gap and shares ownership, which facilitates positive acceptance and adoption.

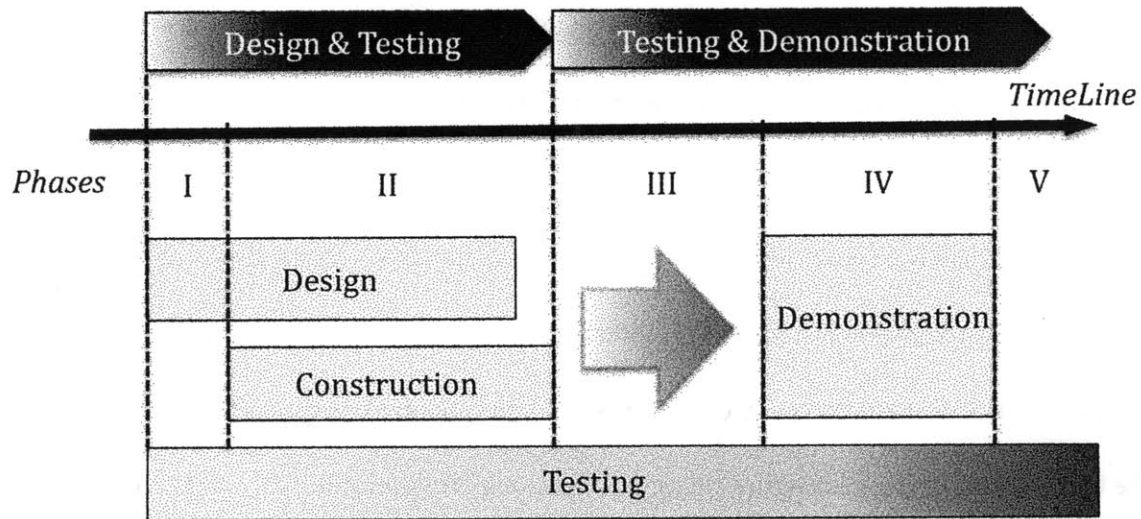


Figure 2.2-II

This diagram illustrates the relationships between these different processes. Phase I covers initial design and conceptual testing. Phase II shows the overlap of design, construction, and testing. Phase III is the validation and learning limitations phase. Phase

IV covers demonstration and additional testing and monitoring, and phase V illustrates ongoing testing.

Clearly, all four of these steps, design, construction, testing and demonstration can play a vital role in the success of a design for safety. Therefore it seems optimal to think about all phases together instead of individually. In addition to considering function as the purpose of the design, the designer might also think about ease in constructing according to specifications, the ability to test to understand and validate the design, and the value of demonstrating the safety of the design. In this case, the designer is designing for the overall success of the design. Taking this a step further, thinking about these processes as integrated rather than concurrent allows for a paradigm where each process enhances the others functioning in a coherent synergistic system. As discussed above, testing facilitates better design and demonstration, and demonstration can support successful adoption and provide stakeholder input. This type of holistic paradigm, more commonly referred to as systems thinking, supports using an integrated engineering design system (the combination of design, construction, testing and demonstration) in order to successfully implement safety in a design.

Due to the overlap in the design, construction and testing phases, this thesis will explore both the integrated process of “Design & Testing” (D&T) where design includes construction, and the integrated process of “Testing & Validation” (T&D) (Figure 2.2-II).

Daniel Frey and Myron Uman strongly stress the advantages of testing throughout design. Uman advises that efforts at design incorporate testing into the design by checking functionality while progressing. [Steuer and Fletcher 1988] This interim testing is common

in systems design where parts need to function properly in order to advance construction. It is also a concept familiar to biologists and chemists. Frey discusses an iterative process that involves design followed by testing with the intention to redesign. Here results of tests are not meant to validate, but to point out what is missing or what changes must be made to achieve the desired goal. [Magee and Frey 2006] Combining these two approaches with alternative design options [Stever and Fletcher 1988] creates a design system that can both manage and exploit interim “failures.” Given the recursive operation of design, construction and testing (Figure 2.2-III)(A), it makes sense to explore strategies for safe design in synthetic biology through the lens of “Design & Testing.”

Similarly, since testing in the form of validation should support demonstration, it also makes sense to think of “Testing & Demonstration” as its own lens. Furthermore stakeholder input, obtained through the demonstration process, can be used to inform testing strategies so that the safety criteria valued by stakeholders is recognized and confirmed, illustrating another recursive relationship between testing and demonstration (Figure 2.2-III)(B). As mentioned before, testing in this phase can be in the form of validation or monitoring.

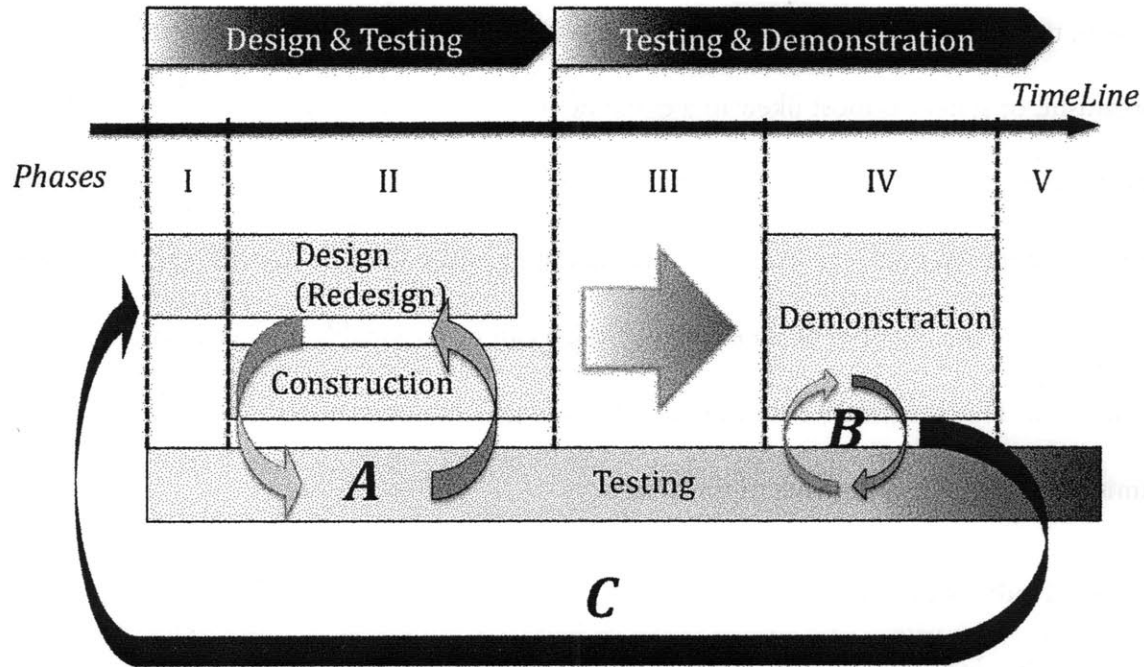


Figure 2.2-III

Despite considering this paradigm as two integrated processes, it is important to realize that together they cover the entire lifecycle of a system from the beginning to the end of function. Besides illustrating the recursive relationships already discussed, the addition of a redesign loop further characterizes the feedback nature of the integrated engineering design system (Figure 2.2-III)(C). For example, surveillance, which can be considered a form of ongoing field-testing or a natural experiment, facilitates testing and demonstration which then facilitates new designing and testing.

2.3 Safety in Design and Testing

In understanding the strategies that go into design and testing for safety, as well as the challenges and limitations, this section will explore the conventional wisdom and contributions of various experts in the field as they touch on different aspects and perspectives of, and approaches to safe design. In determining what is considered safe

design, the definition of safety written above is utilized. Acknowledging that an object, organism, or system is most likely in a dynamic state, and that safety as a functional attribute can only be claimed for a certain range of function, it is logical to characterize safe design as a design that remains “safe” throughout its entire lifecycle. In this case, lifecycle is defined to be the quantity of time when a design can be reasonably predicted to stay functional. This is typically determined by the design’s limitations in function or the limitations of the components in the design.

2.3.1 Traditional Engineering

Lifecycle Engineering

In his book, *To Engineer is Human*, Henry Petroski discusses lifecycles and two related design philosophies:

fail-safe

- those that incorporate structural obstacles to the spontaneous growth of cracks that might escape detection, and

safe-life

- allows for the inevitability of failure well beyond the service life of the structure
- function throughout the life of the structure

[Petroski 1992]

Both of these philosophies acknowledge the existence of dynamic pressures. If “cracks” is replaced by small failures or small damages, his definition of fail-safe can be extended to other engineering disciplines. Implementation of fail-safe design could involve incorporating multiple mechanisms such as redundancies (e.g. multiple shells; inner plugs & outer walls to prevent escape) or safety layers (e.g. airbags & anti-lock breaks). Another method might be increasing robustness by substituting or utilizing error-tolerant or error-resistant materials (e.g. fire-proof paints instead of flammable paints).

Safe-life allows for dynamic pressures to render an object unsafe and confines the definition of safe design to a period of time where function can reasonably be expected. Othmar Käppeli and Lillian Auberson support this approach asserting that safety “cannot be expressed in absolute terms; it is a relative concept more adequately defined in terms of tolerability and acceptability limits.” [Käppeli and Auberson 1997] Utilizing multiple mechanisms or alternative materials that increase robustness can also be of benefit in implementing this design philosophy. Other methods involve: utilizing trial and error and experimentation to determine limiting factors or components, and conducting hazard assessments.

Systems Engineering

Designing for safety can be an extremely complex and thoughtful process. Whether the design is for an object, organism, or system, the relevance of external and inherent factors including temporal dynamics calls for a systemic approach to safety where “safe-life” refers to the functional lifecycle of the entire system, and “fail-safe” refers to the implementation of components in the system. For instance, the following sequence highlights secondary and tertiary emerging threats that might not have been considered initially when designing a water main.

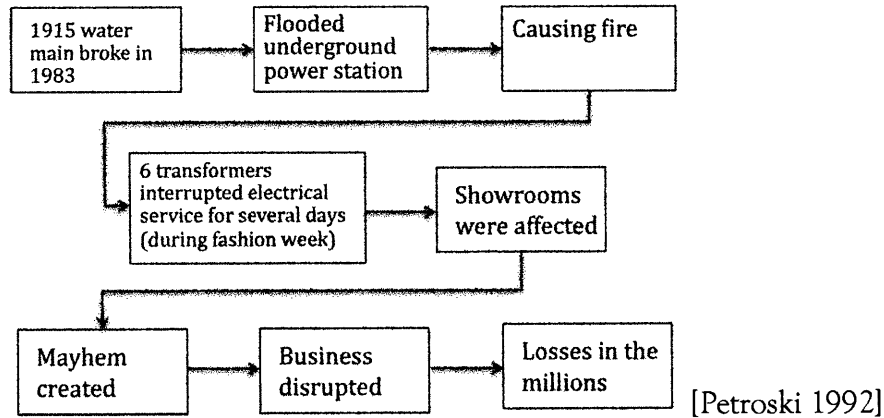


Figure 2.3-I

Since it is the combination of multiple factors that lead to the overall loss, a systemic approach to designing the water main and how it interacts with the existing system might have yielded different design criteria in order to prevent loss. In other words, fail-safes might have been implemented to prevent flooding, or fire, or the interference of electrical service. Clearly, the functional attributes of objects and systems are intertwined; therefore a comprehensive design approach calls for treatment of the design as an individual component and as part of whole system. This approach to engineering is especially valid given that safe design of a chassis in synthetic biology can be considered: the safe design of a device, the safe design of a system that functions as a device, and the safe design of a device component of a larger system. In other words, the chassis as a body in which to build other parts functions as a device. Multiple pathways essential to the function of a chassis indicate it is also a system. Finally, the chassis functions as a device in a system when included in a therapy delivery system or part of a biofuels creation system.

Research by Nancy Leveson on safety systems, identifies safety as an attribute of a system with multiple components. Components in a safety system must be verified reliable in order to confirm overall safety, and understanding the functional ranges of those

components allows the designer to approximate the combined range of function in a system designed for safety. She asserts that, “safety is assured by first identifying hazards and then performing a fault hazard analysis where components are assigned a reliability target such that the system as a whole can reach failure rate requirements.” [Leveson 2003] This can also be considered a method of determining the safe-life of a system.

It is important at this point to distinguish reliability from safety. Reliability refers to whether something works as it is intended. A sharp knife slices and is fairly reliable at achieving that goal. That a sharp knife, working as intended, can slice off a finger while cutting indicates that perhaps a sharp knife is not necessarily safe. Similarly, the failure of a system does not instantly mean it is unsafe. For instance, circuit breakers are meant to cause circuit failures in order to maintain overall safety. Both failure and reliability can be directly related to safety when the components and mechanisms that require reliability are specifically intended for the purpose of ensuring safety. Leveson’s claim that safety is assured by assigning components of a system a required reliability factor in order to ensure compliance with an overall system failure rate is predicated upon the assumption that the system itself is a safety system. Considering safe design from a socio-technical perspective allows the engineer to understand all the known components and their limitations. It is then possible, as Petroski advises, to understand where the weakest links are and design for them to withstand the greatest expected load or pressure.

While understanding safety systems is important in safe design, it is also important to recognize external factors that play an influential role. In order to settle on a particular safe design, basic assumptions regarding the operating environment should be

acknowledged, including: scale, complexity, time, deterioration rates, environmental changes, component failures and component interactions. By understanding when and where in a designed system these play a role, appropriate assumptions regarding the intended operating situation can be made along with efforts that contribute to a safer system. A more effective approach might be to expand the socio-technical system, encompassing these external elements as internal ones in an effort to better understand their roles. In other words, the safety system described above cannot be truly comprehensive unless it incorporates the environment as an additional set of dynamic components. In the context of a biological chassis as a device in a system, considering the influence of these external elements allows a designer to further manage safe function with respect to expected and unexpected situations or use.

Utilizing strategies associated with risk reduction, a general model for safe design seems to be anticipating failure modes using event trees, or other types of extrapolation, usually based on accident analysis techniques. Scenario methods for assessing long-term impacts in advance also benefit from this type of analysis. In general, models and simulations are very useful for this type of conceptual or theoretical testing, not only used to test for the success or failure of a design, but to facilitate choosing between alternative designs. Faisal I Kahn presents three strategies that echo Petroski's philosophies:

- inherent design - reducing or eliminating hazards by using materials and process conditions which are less hazardous
- passive design - reducing or eliminating hazards by process and equipment design features which reduce either incident frequency or consequences without the active functioning of devices
- active design - using engineered features such as controls, safety interlocks, and emergency, emergency shutdown systems to detect potentially hazardous process deviations and to take corrective action

[Khan and Amyotte 2003]

In testing whether safe design can reduce risks and manage uncertainty, be it from the perspective of a component or an entire system, it is impossible to ignore the need for some type of measure or indicators that can help characterize the quantity of risk or uncertainty reduced or the safety provided. Surrogates, attributes of the safety system that may be correlated to the successful function of a safe design, can be used as indicators. Additionally, while indicators of successful safe design might be difficult to identify, there are indicators associated with the components in the system that are more readily measurable. Measuring successful component function and aggregating that information serves to determine the overall success of the safe design, however that relies on accuracy in measuring successful component function. The ability to use indicators such as surrogates as measures greatly enhances the ability to test the system. This is a strategy that proves advantageous in checking functionality of components in systems, especially biological systems. An engineered living system may have multiple outputs whose functioning pathways are inter-connected. Affecting one pathway may affect another providing surrogate indicators.

Utilizing hazard assessment techniques and indicators as measured elements, some approaches to thinking about testing that can facilitate the design process include:

- testing to decide between design alternatives
- testing for conceptual success/failure of a design utilizing failure/hazard analysis
- testing to inform design

- testing to validate components of a system – testing for reliability
- testing response of design to temporal effects or changes

Methods used for testing safe design can differ depending on the reason for testing.

This affects how the inputs and outputs in a test for safety are handled. One way to further explore this concept is to ask the following two questions:

1. What is the approach used for testing?
2. Based on that purpose, what types of methods should be used?

For instance, testing for reliability of a design or of components of a design, as Nancy Leveson recommends, can be considered an approach for establishing safety assuming individual functioning components contributes to the overall safety of the design. As noted before, something can be perfectly reliable and unsafe at the same time. Therefore when using this approach to testing, the effectiveness depends on understanding how each component contributes to the safety provided by the overall design. One method, validating the function of individual components can involve testing: parts before and after installation, testing parts in parallel subsystems, testing parts in serial subsystems, and testing serial connections of redundant units. Another method involves disturbing the function of individual components and observing how the overall design performs at different component failure rates.

As stated earlier in this chapter, another approach is testing to inform design which is suggested by both Myron Uman and Daniel Frey. This approach may involve understanding the behavior of individual components, but it also may involve exploring the behavior and/or characteristics of the materials used, the environment present, or

other conditions associated with the design. Drug development utilizes such testing to determine useful chemical combinations or determine safety issues. Animal models or in vitro samples are used because risk of adverse effects is unacceptable in a human subject when safety information about the drug is unknown. Systems biology utilizes an approach of testing to inform when removing elements from a system in order to identify the usefulness of that element or what system's response behavior might be. Testing to inform is specifically intended to observe elements or conditions of a design for the purpose of understanding and improvement. This also includes utilizing a practice of intermediary testing in order to iterate towards a particularly optimal design. [Magee and Frey 2006]; [Stever and Fletcher 1988]

2.3.2 Biotechnologies

Safety in biotechnology research is most commonly associated with protecting people, animals or the environment. George Church implies that the three best ways of handling safety in biotechnologies is physical containment, biological isolation, and training. [Church 2005] Physical containment can be implemented in laboratory settings utilizing bio-safety levels to determine the type of safety protocols that are necessary to conduct research. Training scientists to understand these biosafety levels and the responsibilities associated with genetic research can only serve to enhance the safety provided by physical containment. Biological isolation on the other hand refers to inherent biological attributes that allow for the isolation of any type of genetically modified or engineered organism in order to prevent unintended interaction with an environment outside of physical containment. Physical containment and training can be considered

external components to manage external factors. This thesis will be confined to safe design as it relates directly to the design of the organism and its internal mechanisms, making the organisms safe to both work with and to create.

The following design methods manage challenges such as survivability, preventing interaction, and preventing mutation away from the design's intended purpose. Two main attributes, fairly unique to biological organisms, that create or contribute to significant safety challenges include the ability to mutate, grow and multiply. Effective biological principles for safe design would ideally manage these attributes. Literature on biological systems, interviews with scientists and past research papers have provided a list of methods that might be used in biological design.

First, the choice of the organism is rather important. If the organism is meant to survive solely in the laboratory a weak (wimpy) organism is chosen. These types of organisms are fully dependent upon the laboratory environment. If they get out into the natural environment, survival is highly improbable because the types of nutrients, combination of nutrients, or conditions of survivability are not immediately available. If the culture becomes contaminated, the organism will most likely die. Establishing growth in a rich synthetic and defined medium, or perpetuating slow growth and reproduction are ways of ensuring that an organism will be nutritionally demanding or die before replicating. To further enhance these attributes, it helps if the organism has low biosynthetic abilities, lacks DNA repair proteins, is non-motile, and has little chance of gaining these attributes through mutation. [Knight 2008]

If the organism is meant to survive in hostile environments, such as one meant to deliver medical therapeutics from within the body, the organism must be fairly robust. In this case, the organism should be stable and virus-resistant, meaning they are less likely to mutate and less susceptible to viruses that work to insert their foreign DNA. These types of organisms are less vulnerable to contaminants in an exposed culture, and less likely to change function through adoption of foreign DNA. Specializing this organism for its intended environment may make it less likely to survive escape. [Church 2008]

Another method of safe design is biological isolation. This can be done a number of ways. Cells can be made to be virus-resistant, similar to above. They can be genetically isolated, meaning they do not use the same codes as other organisms, causing them to resist horizontal gene transfer. In the case that transfer cannot be prevented, codes can also be changes so that they have no affect in other living systems. Incompatibility can also be manufactured by utilizing synthetic nucleotides, designing left-handed DNA, or incorporating 4-base amino acids. Genetic stability can be designed for by removing transposons from cells, resulting in reduced mutation rates. Multiviral resistance can be implemented by changing a single codon that consequently requires a virus to have at least 20 similar, simultaneous changes to its genome. [Church 2008]

Preventing the reproduction or multiplying of biological organisms can be implemented a number of ways. One way is to “castrate” sexual organisms such as yeast by removing any plasmids, which are the main tool of sharing DNA. In addition it keeps the organism from mating or sporulating. Another method would be to reduce the nutrients in

the environment that support reproduction or multiplying, or reduce the ability of the organism to take advantage of the needed nutrients. [Arkin 2009]

One of the attributes of a weak organism is its need for very specific nutrients. Specific nutrient requirements doom these organisms to dying in non-specialized environments. This can be implemented by deleting genes that code for a particularly necessary codon that is extremely rare in the natural world, making the organism dependent on the provision of the resulting nutrient from the laboratory environment. Another method involves creating an addiction to an unnatural amino acid. Still another involves creating organisms with a large number of nutrient requirements that are individually available in the natural environment but rarely available together. In fact, this could be a method of specializing a robust organism to survive solely in its intended environment. It could also be utilized as a method of controlling growth or lifespan by providing a limited amount of necessary nutrient. [Knight 2008]

Kill switches or off-switches are also useful in managing the safe use of living systems. One way this can be done is by programming cell death through self-destruction after a certain period of time or prescribed number of cell cycles, possibly by diluting a vital necessity through multiple generations. Inserting a killing gene that is only expressed by a harmless promoter (existing in nature, controlled, synthetic) will have the same suicidal affect. A killing gene also works by expressing all the time except in the presence of some type of anti-suicide protein that is provided in the laboratory culture. [Knight 2008]

If an organism escapes into the natural environment, those methods that are dependent on an external action will necessitate a method for locating or identifying the

escapee. Utilizing identifiers, such as programming the production of a particular protein that can be detected in alternative environments, is a good way of implementing some type of locating mechanism.

Probably the most useful method of design, one that is expressed as well in the engineering literature is the practice of layering multiple safety mechanisms, for instance, a kill-switch paired with an identifier or layering multiple subsistence requirements. [Church 2008; Knight 2008]

2.3.3 Synthesis of Traditional Engineering and Biotechnology Strategies

While the foundations of synthetic biology stem from biologic origins, many of the practices such as building parts and standardizing components and protocols find their root in traditional engineering, explaining the motivation for drawing from engineering practices. However, since it is a biological system, engineering practices in design and testing may not be completely applicable or offer clear solutions for safety challenges that are mainly biological. The previous sections provide a framework built around concepts of engineering for safety. The previous paragraphs explore biological methods for safety mechanisms. Placing that framework in the context of synthetic biology, the rest of this section looks at where the gaps are and explores biological practices that may add alternative solutions. The section also reviews the challenges, posed by genetic engineering and synthetic biology, which were discussed in the introduction and what biological practices have been suggested to help.

The concept of component or system lifecycles, presented in the Petroski literature, highlights the ideas of fail-safe and safe-life. The idea of fail-safe runs into problems with

biological entities because safety alterations to the biology of any organism that contains DNA for the purposes of safety may not be permanent, in that DNA programmed fail-safe mechanisms have the potential to fail due to mutations or function differently or less effectively through subsequent generations. One way of handling this is to exercise some type of control over factors that can cause mutations, or layer multiple mechanisms so that there are alternative safety mechanisms in place. Layering reduces the probability of the system failing if there are alternative methods for safe function.

Mutation also challenges the idea of safe-life, especially when it comes to figuring out what determines the length of the design's functional lifecycle. Given the different functional needs, lifecycle could pertain to the survival period of an individual organism, or the average survival period of a group of organisms, or the combined survival of an organism and subsequent generations until propagation stops and the organisms die. Each interpretation has different implications and safety challenges. These complexities caused by the timing of a mutation and the type of mutation reduces the ability to predict how a system will respond given passage of time. Assessing the safe-life of living systems becomes complicated and much less reliable than assessing the safe-life of a non-living system.

Drawing from Nancy Leveson's work, another highly applicable engineering concept discussed above is the practice of safety systems engineering. Part of systems engineering is trying to identify and understand those components that have the greatest influence over the intended function of the system, and devising methods of controlling or altering those components in order to support reliable system function. However, this might be more feasible with a bridge or engine than a biotechnological system. The caveat

is that this method of engineering assumes that all relevant or influential components in a system are known and all hazards can be identified and analyzed. The nature of a systems design is dependent on the components, which leaves little room for unanticipated complexities that may not have predictable attributes. The ability to mutate, grow and replicate interferes with applying component and systems methods to living entities.

As stated before, knowledge of living systems is incomplete indicating that some highly significant factors may be overlooked. There is a difficulty in predicting the ability to mutate or how a living system will interact with its environment, intended or not intended. Components that work when tested may not work at a later time. While this is true of inanimate systems, deterioration due to environmental factors is a little easier to anticipate and can possibly be measured unlike mutation. Furthermore, a living system also has the potential to cause genetic changes in its environment, which may subsequently alter the system's function. Biological efforts to increase an organism's stability might involve reducing its ability to mutate by removing elements that support mutagenic processes, such as its potential to genetically affect its environment by transferring DNA or its potential to be genetically affected by its environment by incorporating foreign DNA.

A large amount of genetic engineering concerns involve exposure and damage to an unintended environment. As mentioned above, organisms have the potential to interact with their environments and there are ways to prevent or reduce the potential for those types of interactions. Another danger is an organism's potential to multiply or survive in an unintended environment. One method proposed for managing this is utilizing or creating an organism whose nutrient requirements are rare, or require a rare combination of items.

Another method is to create a kill-switch that can be triggered by a substance that is environmentally harmless or by quorum sensitivity where the large quantity of one of the organism's byproducts is a trigger.

2.4 Safety in Testing and Demonstration

When exploring strategies in testing and demonstration, the types of testing explored here are post design and construction. This type of testing includes testing for validation, testing for credibility, testing across variable applications, environments or target users, testing across variations in executed design, testing for future temporal affects or changes, and again, testing to inform. Of these various approaches to testing, testing for validation tends to line up with demonstration best. The text "diffusion of innovation" states that there is a type of demonstration, usually conducted in private to make sure a design works [M. Rogers 1995]. In the context of safety, this type of demonstration is clearly synonymous with validation; something that one might want to know before "demonstrating" in public. The second type of demonstration is conducted in private in order to communicate or promote an idea or design as well as establish credibility. Presumably, an effective demonstration of safety should be predicated on the successful validation of the safety via testing. When searching for guidance on demonstrating safety, the literature used included general ideas and notes on successful demonstrations, demonstrating to promote technology, and demonstrating safety.

Testing for validation can include testing to a pre-determined set of specifications or a pre-determined confidence level. This type of testing is conducted to verify that a design performs under expected conditions. Tests for validation can be conducted from

component to component, or they can be conducted on the entire system utilizing the intended environment such as a controlled field test. Scientific validation might use in vivo tests with animal models or the actual intended subjects, incorporating methods of randomization and matched controls or utilizing a broad sampling.

Testing for credibility involves testing done to establish the credibility with stakeholders of the testing process. This can be done by utilizing user-defined testing, testing to stakeholder expectations instead of standard specifications, or validating the testing strategy such as examining whether: the strategy addresses the questions being asked; the limits of the experimental system are known; testing interaction effects can be identified and minimized; the testing population represents the intended users; the model is validated; the test failure modes are known; the test itself is altered before or after implementation of the technology; or whether anything else might interfere with readings, measurements, recording, sensitivity, or detection. Some of the challenges that designers and testers must consider involve how to interpret results, and how to account for aspects of the testing design that may have biased the results. For instance, interpreting the meaning of results may be determining what is safe enough? Is a particular drug considered safe if 70% of the subjects survive? If 99% of the subjects survive, how significant are the outlying events? Statistically the 1% of deaths may be considered too low to matter, or the 1% could refer to 100 patients in a sample of 10000, and analyzing those 100 may reveal an unexpected interaction or event that renders the drug dangerous. How much of this interpretation or whether a secondary analysis of the outlying 100 subjects will be influenced by the needs of the designer or tester? Frey also names expectancy effects and

experimenter bias as some of the factors that may alter the credibility of the results reported and therefore the testing process.

Testing across variable environments, applications, designs or target users seems fairly self-explanatory. Essentially, this means testing the technology across situations since they most probably are not standardized or might not otherwise be predicted. Sheldon Krinsky advocates microcosm testing [Krinsky, Wrubel et al. 2007] which can represent an environmental example of in vitro testing. The advantage of using microcosm testing is the ability to mimic potential environments and observe an organism's affect on or behavior in the environment. Some of the user attributes tested for include experience with technology or prior knowledge of the technology or the working conditions.

Testing for variation across executed designs looks at differences in quality, scale or quantity. When discussing testing, one of the requirements of credibility in evaluation is the reproducibility or repeatability of tests. While non-living parts may deteriorate over time, the results of test on a part will typically stay consistent over a shorter and more immediate window. Therefore there is a type of reliability in the results that may not be true for living components. Another problem with testing is that biologics can be used up in the process. Some approaches to addressing such a problem can be found in chemistry where samples of batches are taken and tested. In this case, the question is how broad a sampling must be tested to provide reliable results? In a chemical batch that has not been contaminated, it can usually be assumed that each molecule of that batch is identical. That is not the same for a batch of organisms, as variations exist in multiple organisms of the same type.

Petroski suggests that design, as it is a proposal of a working system, be considered a hypothesis. [Petroski] In this case, the hypothesis that requires validation states that the design will actually be safe. While this type of hypothesis can accumulate numerous instances of verification, it cannot be assumed fact until all conditions under which the hypothesis functions if fulfilled. In other words, a design is only successful until the moment it fails. A scientific hypothesis is tested by comparing its conclusions with the reality of the world as it is. Just one instance of disagreement between the hypothesis and reality is sufficient to make the hypothesis incontrovertibly false. For instance, asserting that a structure will last forever can never be proven true, but will always maintain the possibility to be proven false. Asserting that a structure will last 100 hundred years will only be proven true when the 100 years has elapsed. This emphasizes the importance of specifying conditions for expected success, especially in designing for safety. As Myron Uman suggests, experimentation for knowledge can be used to determine limitations, conditions and ranges of function. [Stever and Fletcher 1988] This includes tests for how and what types of changes take place over time. In other words, these can all be testing to inform which include testing to establish margins of safety over maximum expected operating conditions and the service life which can then be extrapolated to safe-life. Some of this testing can be done through surveillance methods after a design has been adopted. Continuous monitoring can be considered a form of ongoing field-testing or natural experiment. Unfortunately, once a technology is in use, it is outside the controlled conditions and there may be a need for other types of learning to corroborate the data. However, some of the benefits associated with surveillance testing include the acquisition

of data that may support need for more studies or design changes, the ability to rapidly document effects or side effects of the technology, and the ability to generate early warning signs.

Moving on to demonstration, it seems helpful to search history for successful demonstrations outside of demonstrating safety. Edison's "demonstration of the incandescent electric light" and Alexander Graham Bell's demonstration of "his new telephone invention at the New Haven Opera House" are particularly good examples. Edison's demonstration was advertised in order to gather a crowd. Bell's demonstration was in front of faculty and students, utilizing a member of the audience to attest to the success of his invention. [K. Long 2006] These individuals were adept at handling a crowd, picking their audience, and utilizing a volunteer to establish credibility because they understood that a cynical audience would better relate to or trust the experience of one of their own. Magicians are another group of individuals who know how to utilize an audience to establish credibility. In medicine, there are many examples of the power of anecdotal evidence. "Patients who have had a vaccine-preventable disease, like polio, and physicians who worked in polio wards in the 1950s are more likely to be stronger advocates of polio vaccination than patients or physicians who never saw wild polio disease. Personal stories can be a powerful influence and motivator, especially if they are emotionally compelling." [Chen and Hibbs 1998]

Demonstrations that promote technology can be executed a number of ways. An auto show, for instance allows an audience to interact with cars promoting an excitement of the new and shiny. Another method of demonstration is a competition such as a race or

an X-prize that gains notoriety for the winning design, utilizing judges who establish credibility and witnesses who experience the success of the design. Another way to promote successful diffusion of innovation is to use an opinion leader. The text “diffusion of innovation” identifies opinion leaders as individuals who can act as an advocate for a particular technology. This highlights the importance of picking your first audience carefully because if the individual has the power to promote an innovation in technology, they may have the same influential power to defame it. Another important aspect in utilizing opinion leaders is to avoid the appearance of bias, which can negate any bit of potential influence an opinion leader might have had. [M. Rogers 1995]

Indications of a successful demonstration of safety could be the adoption of a process or product. In medicine or food products, it could be FDA approval, adoption by physicians, or positive word of mouth. Possible ways of measuring success could be quantifying media attention in a given period of time. It could be measuring the rate of adoption over a period of time or looking at the extent of use or the lifetime of a design in use. Clearly, assessment of demonstration success seems to be measured in some type of stakeholder response.

In addition to using stakeholders as opinion leaders, stakeholder input can be utilized in testing by determining what must be validated in a design from a user point of view. What the designer may consider an indication of successful design may be different from the views of the relevant stakeholders. One way to do this is to utilize an approach such as Red Teaming in analyzing the safety of a particular design. This gathers input from a group of stakeholders who aim to punch holes or determine ways to undermine a design

in an effort to identify weaknesses. This can be considered a method of testing the concepts and foundations that a design is built on.

It also makes good sense to consider stakeholder needs and interests in designing a demonstration. Stakeholder input allows for the most appropriate and influential aspect of a design to be leveraged in demonstration. Demonstrations that effectively engage stakeholders allow for stakeholders to contribute feedback, whether it be for better designs, increased testing, or more effective demonstrations, all phases can always benefit from improvement. This was phrased very well by an article in popular mechanics that was commenting on the successful implementation of the new I-35W Bridge in Minneapolis, MN. The author describes “a new kind of infrastructure culture, where new ideas are folded into the larger work-in-progress, and no one is waiting for one element to be finished before drawing up plans for the next. And the final product is something that wasn't agreed to in a backroom, or paid for, sight unseen, by a community already rattled by recent tragedy. It was an effort that incorporated decisions from the people who will be driving across it on a daily basis. ” [Sofge 2008]

It should be noted that while the value of stakeholders is recognized as especially important in testing and demonstrating safety, it could also have impacts on the design and testing process. Petroski advocates the use of external input because he thought it was useful to reject conventional wisdom and question prior knowledge used to develop designs. He asserted that “reminders that true causes of failure often take as much of a leap of the analytical imagination as original design concepts,” and that sometimes scientists were not capable of that leap on their own. [Petroski 1992] Edison recognized the

importance of stakeholders as evidenced by his efforts to create an infrastructure and market for his incandescent light bulb since "at the time, the new light was not regarded seriously as a commercial proposition". [K. Long 2006] Companies recognize the importance of stakeholders in design by tasking marketing departments with finding out what their customers want. This process has also seeped into the medical device industry. While a marketing department is used to interact with physicians with regards to developing new products, they rely on other methods such as continuous monitoring and after-market surveillance to obtain feedback from physicians with regard to device failures and needed improvements. In fact, the FDA's Sentinel database can be considered another method of obtaining information on performance of medical therapies and the need for improvement. On the surface this may seem outside the context of design, testing and demonstration, however the nature of continuous monitoring lends itself to being considered an ongoing field test or a natural experiment (study of actual use without experimental controls), which can be considered part of the design - testing - redesign process.

2.5 Organizing Principles

This section reviews and pulls itemized principles from the literature reviewed in the past two sections. This list came together by searching for widespread agreement across the existing literature on concepts, principles and practices that would optimize efforts at safe design, testing and demonstration. Figure 2.5-I shows a diagram that attempts to organize these principles into four quadrants. Design and demonstration principles are organized into their distinct halves while testing methods fall somewhere in between.

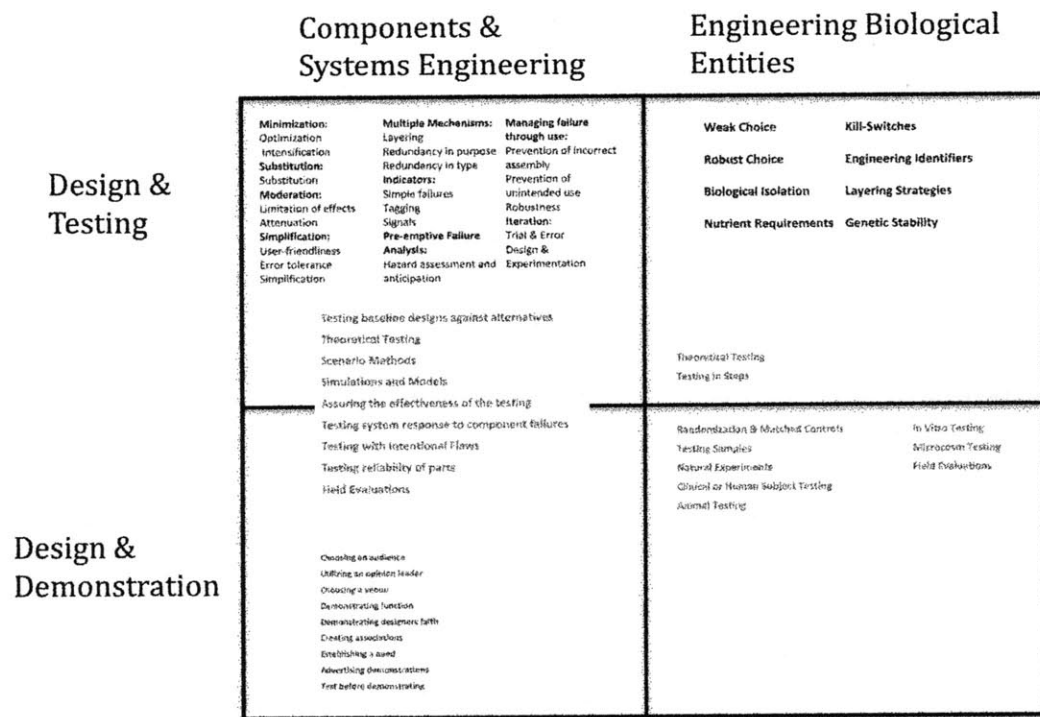


Figure 2.5-I

This diagram presents the principles, as they were collected across the engineering and biology-related literature. Since the demonstration principles were discussed primarily in technology development literature, they were placed in the “Components & Systems Engineering” column. In practice, demonstrations tend to lend themselves to large physical exhibitions, something safe design in microscopic organisms may be more difficult to accomplish. The purpose of this diagram is to show where these practices and principles seem to have the greatest use and relevance. This thesis will continue to explore these principles in practice in order to develop a paradigm that can be applied to synthetic biology. In working towards this goal, it makes sense to consolidate any overlap in applicable principles. The following tables (Table 2.5-A-Table 2.5-B) list engineering principles that can be considered part of both disciplines. Based on these tables, the diagram can be readjusted (Figure 2.5-II).

Table 2.5-A

Design Principles		
Components & Systems Engineering		Engineering Biological Entities
Substitution	Substitution	Weak choice/ Robust choice
Moderation	Limitation of effects	Genetic stability
	Attenuation	Weak choice
Simplification	Error tolerance	Robust choice
Multiple Mechanisms	Layering	Layering Strategies
	Redundancy in purpose	
	Redundancy in type	
Indicators	Simple failures	Weak choice
	Tagging	Engineering Identifiers
	Signals	
Managing failures through use	Robustness	Robust choice

Table 2.5-B

Shared Testing Principles	
Components & Systems Engineering	Engineering Biological Entities
Testing reliability of parts	Testing in steps
Theoretical testing	Theoretical testing
Simulations & Models	In vitro Animal testing Microcosm testing
Field evaluations	Field evaluations Clinical or Human Subject Testing

Components & Systems Engineering

Biotechnological Entities

Design & Testing

Design & Demonstration

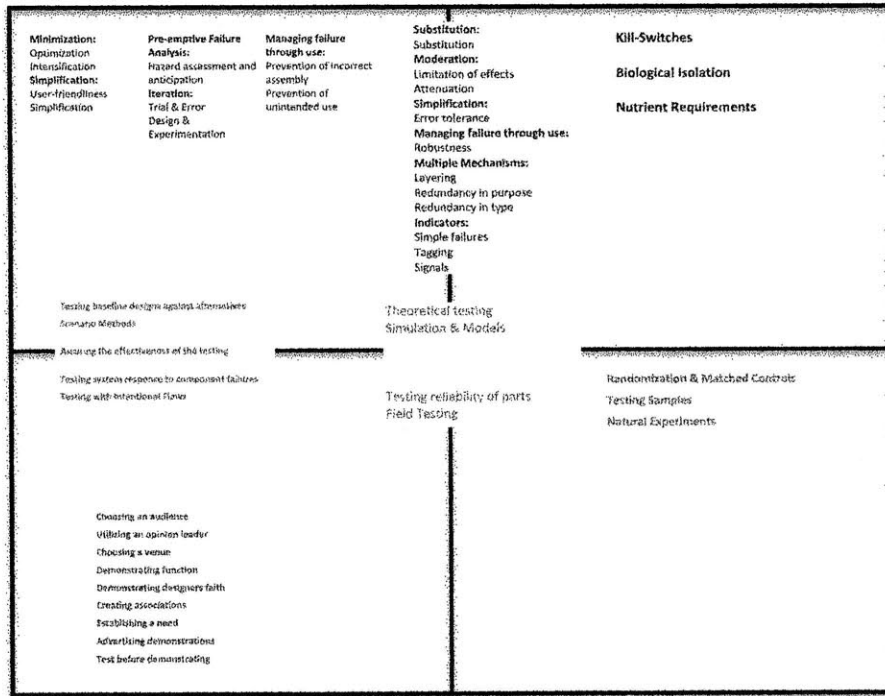


Figure 2.5-II

Chapter 3: Historical Antecedents for Synthetic Biology Research

3.1 Retrospective Cases

The previous chapters discuss theory and frameworks of design, testing and demonstration. They cover traditional engineering design practices in fields such as structural, electrical or mechanical engineering and biotechnological design practices such as those used in biological experimentation and bioengineering. Exploring the different methods used and the types of strategies emphasized, the intention is to consolidate the best of traditional engineering practices honed over time and the new biotechnological design practices currently being developed. The following cases, like the literature, are drawn from both traditional engineering and biotechnology examples. The engineering examples are retrospective cases, used to provide additional insight into D&T and T&D, but also to provide external validation for the principles found in the engineering literature. The biotechnologies, while not quite as old, are meant to serve the same purpose.

Table 3.1–A

	Engineering or Biotechnology	Pre-emptive or Reactive	Example of Safe Design	Example of Testing for Safety	Example of Demonstrating Safety	Example of Stakeholder Interaction
Buildings: Crystal Palace	Engineering	Pre-emptive	X	X	X	
Bridges: Brooklyn Bridge	Engineering	Pre-emptive/Reactive	X	X	X	
Bridges: New 35W	Engineering	Reactive	X	X	X	X
Devices: Pacemakers	Engineering	Pre-emptive/Reactive	X	X	X	X
Vehicles: Elevator Brakes	Engineering	Reactive		X	X	
Probiotics	Biotechnology	Pre-emptive		X	X	
Vaccines	Biotechnology	Pre-emptive/Reactive	X	X	X	

The following cases were chosen for their intended focus on safety during the design, testing and demonstration process. In addition to having this type of focus, these cases are considered successful because they succeeded in maintaining safety over an extended period of time, and the success of their designs have had undeniable impacts on the technological advancement of society.

These cases describe the safety challenges faced and the methods used to address those challenges. They will also discuss why these particular cases are appropriate as anecdotal evidence feeding principles of design, testing, and demonstration in synthetic biology. Buildings and bridges were chosen because they are large structures that can be dangerous to the users if safe design is absent, and they can be subject to external forces

that may not inherently be thought of as hazards. This, specifically, is a similar concern that the entities engineered in synthetic biology may face, especially since a design in both areas can be made more dangerous as a result of unanticipated external circumstances. The specific examples chosen are representative of novel designs to address safety and fairly comprehensive forethought on the part of designers to anticipate hazards and verify the safety of their designs.

The literature associated with traditional engineering concepts discussed above held great emphasis on component and system design. It was pointed out that one of the weaknesses of this approach is that it may not account for external unanticipated complexities. The traditional engineering cases may provide some additional guidance in that area. However, even those approaches do not have to account for uncertainties and complexities associated with the individual components. The biotechnological cases are utilized to fill that gap. They have handled safety with these types of biological complexities in mind.

In considering successful biotechnology cases, both probiotics and vaccines were chosen because they are current biotechnology products that have been and are being developed. With some exceptions and precautions, they are two fairly accepted biotechnologies.

3.1.1 Traditional Engineering Designs

Crystal Palace

The Crystal Palace was a large cast iron and glass hall designed by Joseph Paxton to house the Great Exhibition of 1851. A building built primarily of glass had the potential to

collapse on its visitors, either due to load or inclement weather. Joseph Paxton's attention to detail and design using a number of the principles listed above contributed to the subsequent success of his structure.

First, his specific use of materials included cast and wrought iron as well as glass, all of which were standardized parts readily available. This facilitated construction, allowing for immediate replacements when needed, and more importantly providing the ability to understand and predict the quality and the behavior of the parts. He designed his building with multiple load pathways giving the beams a dual use, as they were also handy in funneling water down from the roof. The roof was built to withstand rain, using sloped glass panels and seamed tarps that moved water quickly away to built in gutters, and the frame was hung in a way that distributed wind load. He even had young boys crawling underneath the wooden floors in order remove the bits of paper that fell through the boards as they posed a fire hazard.

Part of his success was also in the building process. Utilizing principles of design and testing, he tested samples of the wrought iron and even beam of the cast iron to confirm that they would not be weak links in his design. He conducted a preliminary load and dynamic load tests on his platforms before he continued to build more. These involved crowding 300 men on a corner of the platform, having them jump in unison, or running across. He also previewed the exhibition space before Queen Victoria and conducted some of these tests in front of her and her entourage, gaining her endorsement.

Joseph Paxton's design was a novel approach to architecture and one that has been imitated many times since. The novelty of the structure, especially with the use of glass,

lent itself to negative responses and expectations from cynics whose intuitive sense told them that because it has not been done before, it could not or should not be done.

Synthetic biology has had its share of similar responses. It provides a nice example of significant and thoughtful design and testing that resulted in a stable safe successful structure.

[Branchflower and Petit 1995; Petroski 1992; Virginia 2001]

Brooklyn Bridge

The Brooklyn Bridge was another novel design in suspension bridge building that at the time would have been the longest suspension bridge in the world connecting New York City and Brooklyn. It was an ambitious task requiring strong supports and anchors, and faced with a history of many failed suspension bridges.

John Roebling and his son, Washington Roebling, intended the bridge to be able to handle six times the expected load. In building for this expectation, they made their own twisted cables substituting steel instead of iron because the Roeblings believed iron was the reason many bridges were failing and steel was better at sustaining the elements. They utilized stone masonry for the middle towers and sunk the caissons at extremely low depths to ensure better support. In addition to stronger cables for the suspension supports, Washington Roebling added cable stays, which today is considered possible overkill. However, given the changes in dynamic loads, from cars to trains, trolleys, walkers and horse buggies, that extra stability might also be responsible for the survival of the bridge to this day, and consequently the survival of millions of travelers across it.

While the Roeblings did utilize testing, especially when developing methods to properly sink and situate the caissons, they serve as an interesting example of testing and demonstration. When the first cables were strung connecting one bank to the other, one of the <> crossed the distance in a suspended tub in order to prove the stability of the cables to the rest of the workers. This type of demonstration established credibility in the actual possibility of a bridge one day. However, once the bridge was built, soon after it had opened, a woman let out a random exclamation leading to an exit stampede of people who were erroneously convinced the bridge was collapsing. [Unknown 1883] Still opening day had the benefit of a visit from the U.S. President and the then New York governor, who carried clear endorsements. In addition Washington Roebling's wife Emily Roebling was the first to cross the bridge. P.T. Barnum wanted to hold a circus parade across the bridge showing his faith in the structure and was allowed to do so the following year.

The Brooklyn Bridge like the Crystal Palace was structure achieving something grand, in the face of many doubters especially where safety was concerned. Like the previous example, it shows a meticulous effort on the part of the designer to ensure the safety not only of his structure, but of those working on the structure as well. The fact that working on the structure could have been as much of a hazard as interacting with the final structure is similar to situation of researchers and students interacting with novel organisms. In addition, bridges on the whole echo a similar context to synthetic biology in that the public may have very little choice in the design itself or their exposure to it.

[Birdsall 1983; Burns 1982; NYCRoads.com ; Petroski 1992]

New I-35W Bridge

Design and construction of the new I-35W bridge, also known as the St. Anthony Falls Bridge, has been an example of extremely successful efforts at safe design, testing and demonstration. The first I-35W Bridge, built in 1967, collapsed into the Mississippi river unexpectedly in 2007 killing 13 people and injuring more than 100. It was the main artery in and out of the city connecting northern and southern parts of the state. In building a successful bridge FIGG Engineering, the lead designer on the project, needed to overcome community skepticism and disappointment, fears and distrust stemming from the catastrophe. Amongst all the cases presented here, theirs provides the best success stories for both design and testing, and testing and demonstration.

One of the problems of the original bridge was the lack of redundancies in the design. The girders and supports had become rusted and cracked leading to the eventual failure. The new design ensured multiple load pathways none of which were failure critical. In addition the bridge was designed to take future increases in loads in the eventual case that an automated public transportation system might be added. It was also equipped advanced materials and devices such as anti-icing sprayers to manage inclement weather. Another advanced material used was high-strength concrete, which was less permeable and hardened faster than traditional types of concrete. Because the concrete was new, they utilized previous studies conducted by the University of Minnesota, conducted experiments which allowed them to understand the material and how it would harden, and they conducted interim tests during implementation to confirm the strength of the material.

They built the bridge in sections, building the sections at a separate site where they could better control the conditions and test the piece before installation.

Continually through this process their interactions with the community help build both support and trust. They held open meetings to gain community input on the expectations, needs and initial designs. They had a representative available to talk to the community every weekend, who would provide updates and discuss the progress of the bridge. Their interactions with the community demonstrated their willingness to hear stakeholders needs and involve them in the process. They gave the community a sense of ownership in the bridge.

When the bridge was complete, the final test was conducted. Police cars held up traffic at the ends of the bridge until it was sufficiently packed and then led the cars across the bridge demonstrating the ability of the bridge to manage the load. While most would claim that the initial bridge did not fail for 40 years, they cannot claim such for individual components of the bridge. Those types of wear and failures collecting over time caused the overall failure of the bridge. To address these fears, the company installed multiple sensor systems to monitor the traffic on the bridge, the changes in load, and the strength of the bridge. There are also sensors to pick up effects of environmental changes on the bridge.

The I-35W Bridge is a useful example in that it presents a failed design improved through innovation by an extremely safe design. It also contrasts the damage of fatigue over time with the benefits of anticipating those failures as well as implementing monitoring as a form of ongoing field-testing. This bridge provides a successful example of safety in

engineering through the design and testing process as well as the testing and demonstration process.

[McCarthy 2008; Russel 2008; Sofge 2008; Transportation]

Elevator Brakes

This case was chosen for its clear example of testing and demonstration. Originally, elevators were typically used for raising and transporting freight, primarily because lack of dependability on the suspensions posed a huge safety risk for human passengers. While working on freight elevators, Elisha Otis developed the first elevator brakes designed to activate when the ropes suspending an elevator failed. He utilized redundancy by creating a fail-safe. In order to convince people of the safety provided by his innovation, he demonstrated their function at the New York World's Fair in 1854. The demonstration involved Otis standing on a raised elevator while someone cut the suspension ropes. As he intended, his brakes stopped the elevator from falling. This demonstration was successful in convincing people of the safety provided by his elevators, and enabled the building of the tall skylines around the world today.

The story of Elisha Otis and the elevator brakes is as effective in exemplifying a convincing demonstration as the Edison and Bell references in the previous chapter. In considering demonstrations of safety, this example provides a similar context to synthetic biology in that both technologies need to convince a cynical and distrustful public of the safety they are asserting.

[Banuri ; Finder ; Infoplease ; Wikipedia]

Pacemakers

The case of pacemakers straddles the line between methods for engineering safety in devices and methods for managing safety from a biotechnological perspective.

Pacemakers evolved through much iteration by the work of both physicians and engineers. Initial knowledge of safety concerns was incorporated in the design, but the main purpose of the device was solely to keep the heart beating. Many advances were incorporated as greater safety concerns were identified. For example, original pacemakers were external and utilized plugs for gaining power. When one of the innovators observed that if power were lost in a hospital, so would the patients be who were dependent on the devices. This led to the development of the battery powered pacemakers. The pacemaker case provides examples of design and testing as well as testing and demonstration.

Current pacemaker innovations manage biostability and biosafety through the choice of materials for leads and the casings for the device. They are designed to separate the body completely from the function parts of the device. Some leads have steroid eluting tips to reduce inflammation and infection that might occur during implantation of leads. Designing pacemakers involves extensive knowledge of the heart, and programming pacemakers involves additional understanding. Pacemakers are programmed to manage various heart problems and the software running the device needs to be just as robust as the internal workings. Current devices use lithium iodine batteries, which have the benefit of a slow drop-off in power and can be used to indicate when a battery is failing, an ability critical for device dependent patients.

Like all clinical drugs, new devices go through similar testing. An electrical device such as a pacemaker will go through design testing in a laboratory. Animal testing and other pre-clinical testing will follow this, before moving on to clinical studies with human subjects. Once a device has been approved and implantation has been adopted, post market studies have been developing that follow the progress and function of the device.

Companies such as Medtronic make great use of demonstration principles. They have engineers whose sole purpose is to teach physicians about the devices and perform demonstrations. They are also involved in instructing the physician on how to use the device. However, along with this practice is the practice of drawing input from the physicians they service. In fact, marketing departments in companies such as these work with physicians to uncover where the next advances are needed. This information makes its way back to the engineers and scientists designing new and better devices and the cycle begins again.

Pacemakers were chosen simply because they are smaller devices that have the added complexity of interacting with biological environments, similar to medical therapy applications that involve safe chassis design. The uncertainty faced from an external biological environment can be considered similar. In addition, the process of design and improvement can be shown most clearly here through a pacemaker history of continual optimization.

[Jeffrey and Parsonnet 1998; Mallela, Ilankumaran et al. 2004; MOND, SLOMAN et al. 1982]

3.1.2 Current Biotechnologies

Probiotics

Probiotics are “defined as viable microorganisms (bacteria or yeasts) that exhibit a beneficial effect on the health of the host when they are ingested. They are used in foods, fermented dairy products, and in pharmaceutical preparations.” [Salminen, von Wright et al. 1998] Due to a history of safe consumption, most commonly in yogurt, many probiotics have been placed on the GRAS list. Still, many of the bacterial species that constitute probiotics have actually been isolated from infection sites [Ishibashi and Yamazaki 2001] and might pose a danger to immuno-compromised individuals. Additional concerns have been raised about possible side effects such as “systemic infections, risk of deleterious metabolic activities, risk of adjuvant side-effects of immunomodulation, and risk of gene transfer.” [Salminen, von Wright et al. 1998] Recent studies have even shown that a combination of certain probiotics may increase the chance of an individual dying of pancreatitis. [Offit 2007] While none of these have been definitive, it does raise the issue of assuming safety in the case of probiotics especially when new probiotics are being discovered, created, or modified.

Probably the most useful way of ensuring safety is to work with probiotics whose behavior is known. Since probiotic effects are strain specific, the choice of a safe probiotics strain is important. Strain identity helps link strains to a specific health effects and enables accurate surveillance and epidemiological studies.” [Group 2002] Essentially, the use of prior knowledge is key in developing and incorporating safe probiotics into products. As for the question of demonstration, companies who use probiotics rely heavily on

associations with healthy food in order to get people to bypass fears that may arise from realizing that they are consuming bacteria gained from infections.

As an example in this collection of cases, probiotics appears because it involves the consumption of organisms, perhaps even *E. coli*. Current trends are slowly moving from existing microorganisms that are accepted as safe to bioengineered organisms. Like medical therapies that may arise from synthetic biology, individuals may be working with, consuming, or internalizing organisms that have the potential to be dangerous. Also similar to synthetic biology, there have been no overall catastrophes associated with the consumption of probiotics, although there have been plenty of bacterial infections in the past, therefore the current pushes for testing is also a pre-emptive stroke at preventing a health crisis.

Vaccines

“The concept of vaccination is essentially, the introduction of dangerous foreign material into healthy individuals, for the purpose of developing immunity. [Ellenberg and Chen 1997] Vaccines utilize viruses or bacteria in a weakened or dead state. Aside from the potential of organisms to might revert to virulence, [Warren 1986] there are multiple sources of viral contamination resulting from infected animal tissues as a cell source; viruses used to establish the cell line; contaminated biological reagents; contamination during manipulation. [Ellenberg and Chen 1997] Furthermore, vaccines have a shelf life which means a choice between bacterial contamination (aged vaccine) and exposure to minimal amounts of mercury (in preservatives). Preservatives such as thimerosal improve vaccine stability, potency, and safety. However, they may also contain mercury. This has

caused distrust on the part of the public who must trust their children to vaccines. Yet with all these fears people all over the world are vaccinated.

As mentioned before, one of the ways to create a vaccine is to utilize a virus in its weakened or dead state and utilize genetic engineering in bacteria, yeast, or mammalian cells to produce large amounts of antigens/vaccines. [Warren 1986] Another method is to use a less dangerous relation of the target virus such as using cowpox to vaccinate for smallpox. Because of the dangerous nature of vaccines, a great deal of testing is involved. When Jonas Salk first developed the polio vaccine, he tested it on himself and his family and then went on to run a full trial with child subjects. This not only served as a good test, it also served as a good demonstration, which encouraged the trust of parents. Today, vaccines face many of the same clinical trials required for drug testing, and they require FDA approval. In fact, FDA approval serves as a method of demonstrating safety, however, other types of demonstrations may be needed in the future as time continues. "Issues affecting risk perception include the ability to control exposure, whether effects are immediate or delayed, reversibility of effects, level of trust in responsible institutions and media attention. The primary sources of public information on vaccine safety are physicians (especially pediatricians), parenting books and magazines, the Internet, and friends in health care-related fields." [Ellenberg, Foulkes et al. 2005]

Researchers and Physicians overwhelmingly believe that "the risks of vaccine reactions, both the common mild reactions and the rare, more serious reactions, are very much outweighed by the public health benefit conferred by current vaccination practices and policies." One of the most interesting things about vaccines is that despite the possible

health risks, vaccines can be required by a community, school, or by law, which emphasizes a need for guaranteeing safety.

This second biotechnology case was chosen because it involves working with pathogens and mimics potential situations that synthetic biologists might eventually take part in. They are used as medical therapies and therefore must be extremely safe – yet the possibility and uncertainty of a virus reverting is a fear. In the case of the tumor-killing bacteria, the fears may be similar; therefore the methods used for design and testing may be quite informative to synthetic biology. Furthermore, the public health issues associated with vaccinations highlights the need to assure safety because exposure may not be entirely voluntary.

3.1.3 Synthetic Biology Projects: Chassis Design

One of the major pushes in safe design for synthetic biology is chassis design. Since they are the bodies in which programmed pathways shall be implemented, they will determine the overall survival skills of the organism. Three laboratories were interviewed. Each of their approaches to chassis design is different as the design is very closely linked to the intended purposes of the chassis.

Knight Laboratory

Thomas (Tom) Knight's choice of organism for chassis design is a mesoplasma florum, a weak, non-pathogenic, non-motile bacterium that resides in the gut of insects. [Knight 2008] They don't have biosynthetic abilities and are extremely dependent on their host environments for nutrients. In addition, they need a 30 to 32C environment, which makes warm-blooded animals a poor home, and they lack DNA repair proteins needed to

heal environmental damage. Aside from their low survivability outside the laboratory environment, they also have low biocompatibility with other organisms, containing genes that code differently from other organisms. Given the natural survival handicaps that characterize this organism, its choice inherently employs a number of layered safety principles such as weak choice, nutrient requirements, and genetic compatibility. As inherent attributes, low survivability and low threat, allow for early work with this organism to occur in BSL1 laboratories and reinforces the safety aspects of the chassis.

Mesoplasma florum is a lesser-understood organism, Tom Knight's choice to invest more time in improving understanding of the bacteria's functions and behavior. His choice of a weaker organism with inherent safety properties reduces the difficulty in creating an organism that will not survive or interact with unintended environments. Additionally, the fact that the safety is naturally inherent might provide for a more stable and reliable safety system. The choice of mesoplasma florum highlights an intention to stress safety above functionality. In other words, much of the work Tom will have to engage in will be converting his safe organisms to safe and functional chassis.

To that end, his work on biological chassis is still in its initial stages. Current work involves developing tools to alter mesoplasma genetic code and conducting studies to develop a minimal mesoplasma genome. Developing a minimal genome is an endeavor at optimizing the organism for its specific purpose as a chassis. With the removal of redundant or unnecessary systems, there is less chance that these additional elements will interfere with mechanisms that are engineered into the organism.

The choice of *Mesoplasma florum* as the “foundation” of a synthetic biological chassis reduces the risk of escape, survival and genetic interaction with an unintended environment, while raising the susceptibility of the chassis to lethal culture contamination within the laboratory. Another challenge posed by *Mesoplasma florum* is its potential high mutability, which may inactivate existing or added safety mechanisms. Part of the safe design might involve managing this attribute. Perhaps, in creating a minimal genome, genetic sequences that promote mutability will be removed. However, this optimization may create additional challenges if systems that appeared redundant or unnecessary have remained in the genome because they play a role in unknown and unexpected circumstances. This possibility will call for testing geared to make sure that the chassis and the chassis plus additional systems will function as intended and in unexpected conditions.

Church Laboratory

Unlike Tom Knight, George Church’s choice for chassis design is *Escherichia Coli* (*E. coli*), a significantly robust organism. [Church 2008] *E. coli* is a familiar organism with a long history of research and study behind it. Though motile, it is usually considered harmless as it resides in the lower intestine of warm-blooded animals. They can survive in multiple environments that includes outside the body and in laboratory settings. In fact, they can be grown easily in a variety of mediums and are less vulnerable to contaminants.

While Knight chose *Mesoplasma florum* for its weaknesses, George Church chose *E. coli* for its robustness and functionality, considering it the “microbial powerhouse”. [Church 2008] In addition, the ability to work with it is highly enhanced by the body of research conducted over the years and facilitates a faster realization of the chassis concept.

Since his intended purpose for the chassis include producing biofuels, medical therapy delivery, and protein generation, it will need to have high survivability in order to support multiple purposes. The focus on functionality means that much of the engineering must go towards establishing safety in the design.

At the time of the interview, his laboratory had designed the chassis and was very close to producing a prototype and entering a testing phase. As common in biological practices, experimentation has accompanied the process of design realization. The Church chassis has been designed with specific genetic code and metabolic changes that make them multi-viral-resistant and unable to survive in the wild.

Since *E. coli* are robust enough to survive in multiple environments, including the human body, they pose a serious concern in the case of mutation or acquisition of virulent DNA, both of which are possibilities given the existence of pathogenic *E. coli*. In addition *E. coli* is capable of transferring its DNA through methods such as conjugation or transduction. If these escape into an unintended environment, their motility and increased chance of survival would make them a genuine health or environmental risk. A possible solution would be to design chassis that are specialized to a very particular environment and reduce their ability to survive or interact anywhere else. Some of the methods that could help address these concerns and enable solutions include incorporating specific nutrient requirements, genetic incompatibility, kill switches or any other safety principles. Church's design has incorporated nutrient requirements and genetic incompatibility by adjusting the organism's metabolism and by changing its genetic code. He has also proposed work on left-handed DNA as a method for incorporating genetic incompatibility.

Given the survivability and possible pathogenicity, the Church laboratory will definitely have to incorporate studies that report on the organism's ability to escape and interact with unintended environments. They will also need to show that the organism has a low chance of becoming a pathogen, especially in the case of medical applications. Demonstrations of safety will be especially necessary in this case since the intention is to use the chassis outside the laboratory environment.

Arkin Laboratory

Unlike the previous two laboratories, the research on chassis design conducted at Adam Arkin's laboratory is geared towards a very specific purpose. They are attempting to create tumor-seeking bacteria, for the purpose of hunting down tumors within the body and delivering a therapy that would serve to eliminate it. [Arkin 2009] Like George Church, the Arkin Laboratory has chosen to utilize *E. coli* as the foundation for their chassis design. Since the previous section discusses the general concerns faced when utilizing *E. coli*, this section will focus purely on the additional safety concerns elicited by the intention to use this particular chassis to perform a specific function within the human body.

In order to explore the safety considerations related to in vivo implementations of chassis design, it is important to understand how the tumor-seeking mechanism will work. Bacteria are programmed to express proteins called invasins which allow target tumors and infiltrate the necrotic region. The organism is encapsulated in a lipid coating that allows it to evade the immune system. Once at the necrotic site, the bacteria is programmed to recognize environmental signals and release phages that attack and enter the cancer cells.

These phages express a toxin that kills the cancer cells. They also express a signaling protein that allows the bacteria to regulate the phage expression. In this way, the bacteria can deliver just enough therapy as needed.

As of March 2009, the Arkin laboratory had engineered the tumor-seeking bacteria and was working on the tumor killing part. According to Adam Arkin, they had engineered the initial virus and coding, conducted initial delivery experiments and were working on increasing the efficiency of the process and producing the signal that would regulate phage production in the bacterium.

The most pressing safety concern was the ability to control the bacteria so they could survive only as long as needed to perform their function. By deleting the gene that allows it to extract iron, they were able to confine the bacteria to simple survival without additional growth. Too much bacteria in the blood could be toxic to the patient. Similar to above, they are working on removing or producing factors that would reduce DNA exchange, and in addition to reducing the ability to mutate, they are trying to make their mechanism robust enough that it would take five or more mutations to interfere with the process.

In addition to the concerns directly associated with *E. coli*, the Arkin laboratory will also have to provide additional assurance of the safety of their design because they are engineering an entity to survive in a human host. In this case FDA clinical requirements for biologics will guide a share of the testing, addressing questions of bacteria dosage, as well as predicting how much phage must be produced or carried and what effects the human biological environment will have.

3.2 Organizing Cases

The following table organizes the various methods utilized in these cases above, with the addition of the synthetic biology cases. The safe designs listed below (Table 3.2-A) are not exhaustive, however, they provide evidence of design and testing and testing and demonstration, and can be labeled with the principles identified in the previous chapter.

Table 3.2-A

	Safe Design Used	Design & Testing	Testing & Demonstration
Crystal Palace	Choice of materials	Choice of materials	
	Design for inclement weather	Hazard Assessment & Mitigation	
	Use standardized parts	User-Friendliness	
	Multiple load paths	Redundancies	
	Testing parts	Testing Reliability of Parts	
	Testing the platforms	Testing Reliability of Parts/ In vitro experiments	Testing Reliability of Parts/ In vitro experiments
	Testing dynamic forces	Testing Reliability of Parts/ In vitro experiments	Testing Reliability of Parts/ In vitro experiments
	Demonstrated safety in front of the Queen		Demonstrating function
	Utilized Queen's endorsement		Choosing an audience
Brooklyn Bridge	Used steel in cables instead of iron	Substitution	
	Reinforced with cable stays	Redundancies	
	Used stone masonry on the tower	Choice of materials	
	Built for future loads and advances	Robustness	
	Riding suspended across river to test cable strength	Field testing	Field testing/ Demonstrating Function/ Choosing a venue
	First crossing by Emily Roebling		Demonstrating Function/ Choosing a venue/ Demonstrating designer's faith
	The President & Governor opened the bridge		Choosing an audience
New I-35W Bridge	High performance concrete	Choice of Materials/Substitution	

	Safe Design Used	Design & Testing	Testing & Demonstration
	Multiple load pathways	Redundancies	
	No failure critical pathways	Redundancies	
	Designed for future loads	Robustness	
	Anti-icing sprayers	Hazard Assessment/ Mitigation	
	Used prior studies to assess Concrete	In vitro experiments/ Testing reliability of parts	
	Conducted interim tests of materials during implementation	Testing reliability of parts	
	Tested sections before adding to bridge	Testing reliability of parts/ In vitro tests	
	Installed sensors for continuous monitoring		Natural experiments
	Held community meetings and incorporated input		Choosing audience/ Choosing venue
	Representative held weekly talks with community		Choosing audience/ Choosing venue
	Packed the bridge with traffic on the first open crossing		Controlled Field Test
Elevator Brakes	Utilized brakes on freight		Tested before demonstrated
	Utilized World's Fair as a venue		Choosing venue
	World's Fair visitors as audience		Choosing audience
	Dramatic risk to own life		Demonstrating the designers faith
Pacemakers	Requires biosafety and biostability in materials	Choice of Materials/ Substitution	
	Years of safety adjustments and advancements	Iteration	
	Use steroid eluting tips to prevent infection	Hazard Assessment/ Mitigation	
	Designed to adapt to multiple heart problems	Hazard Assessment/ Mitigation	
	Electronics completely encased	Shielding?	
	Lithium iodine battery	Simple failures	
	Pre-clinical testing	In vitro/ Animal models	
	Clinical testing		In vivo
	Experience used as demonstration		Natural experiments/ Demonstrating function
	Marketing & teaching physicians		Choosing venue/ Choosing audience
	Gaining physician input		Choosing audience
Probiotics	Choice of probiotics strain	Substitution/ Choice of material	
	Eliminating antibiotic resistance genes	Limitation of effects	

	Safe Design Used	Design & Testing	Testing & Demonstration
	Studies on probiotics properties	In vitro testing	
	Pre-clinical studies	In vitro testing/ animal models	
	Observance of behavior in the body	In vivo testing	
	Clinical studies		In Vivo testing/ Randomization & Matched controls
	Surveillance studies		Natural Experiments
	History of use		Natural Experiments/ Demonstration of function
	Beneficial presence in intestines		Creating associations
Vaccines	Use weakened or killed strain	Limitation of Effects	
	Use weaker relation of disease	Substitution/ Choice of material	
	Pre-clinical studies	In vitro testing/ Animal models	
	Clinical testing		In vivo testing/ Randomization & Matched Controls
	Testing batches of the vaccine		In vitro test
	Testing the growth substance for contaminants	Testing reliability of parts	
	Administered to own family and self		Demonstrating function/ demonstrating designer's faith
Knight Chassis	Choice of mesoplasma florum	Weak choice	
	Nutrient specificity	Nutrient requirements	
	Genetic incompatibility	Biological isolation	
Church Chassis	Choice of E. coli	Robust choice	
	Metabolic dependencies	Nutrient requirements	
	Multi-virus resistance	Biological isolation	
	Genetic incompatibility	Biological isolation	
Arkin Chassis	Choice of E. coli	Robust choice	
	Regulating phage production	Limitation of effects	
	Deleting ability to acquire iron	Nutrient requirements	
	Reducing DNA exchange	Biological isolation	
	Reducing susceptibility to mutation	Redundancy - no- failure critical pathways	
	Reducing the ability to mutate	Genetic stability	

The various principles identified are representative of only a sampling of the many design specifications made for safety as well as the testing and demonstrating practices to ensure and assure of that safety. Given these identified principles, the cases can also be categorized in a style similar to the principles (Figure 3.2-1). The Crystal Palace and Brooklyn Bridge provide clearer examples for design and testing. The elevator brake story on the other hand is a short but effective example of testing and demonstration. The New I-35W Bridge, as well as the pacemaker and vaccine examples all seem to balance the necessity of both design and testing and testing and demonstration. Probiotics, as they are developed currently tend to be existing bacteria, though newer efforts have been directed towards genetically modified probiotics. Still, this fact pushes it into the testing and demonstration domain. The pacemakers, vaccines and probiotics all have a certain amount of standard they need to meet in order to be acceptable, courtesy of the FDA. Though many probiotics are on the GRAS list, some do require a small bit of clinical study. As for the synthetic biology cases, all three designs fall within the realm of design and testing however the Arkin design and the Church design are moving very quickly towards a phase of testing to validate. All three are placed near the vertical center since the methods of safe design do involve layering and limitation of effects.

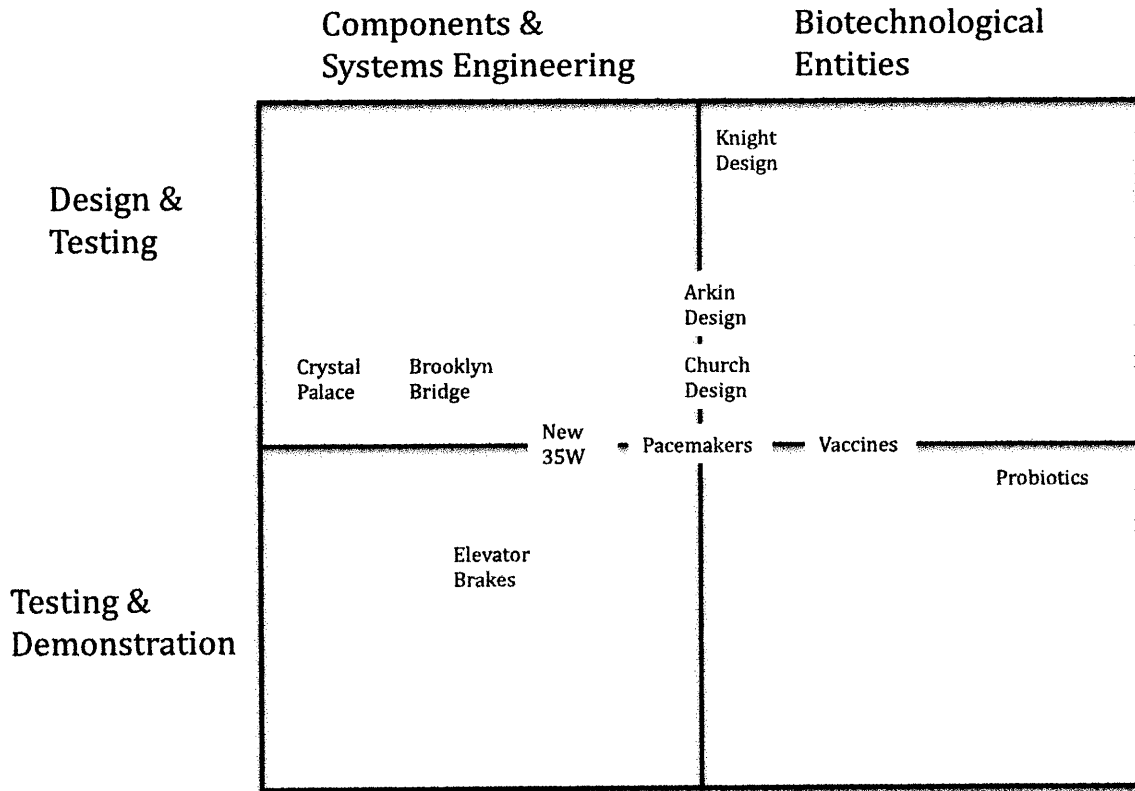


Figure 3.2-I

3.3 Implications for Synthetic Biology

The retrospective cases such as the Crystal Palace, the Brooklyn Bridge, and the New I-35W Bridge, as well as the pacemakers, verify that where design and testing are of high priority, successful safety is possible. This endorses the approach of the three laboratories in engineering safe design, especially the Knight laboratory, where safety is prioritized above functionality and speed. Furthermore, where testing and demonstration have been effective, the progress of the technology has been supported. The elevator brakes is the best example of this, however, the fact that the Crystal Palace and Brooklyn Bridge succeeded in standing despite cynical assertions to the contrary also acted as a demonstration of safety. The novelty of the I-35W Bridge's success in standing may need a little more time before that aspect gives it credibility, however, the interaction with

stakeholders provided a different method of gaining the public trust. All of these can provide examples of demonstration methods that can assure successful progress.

While design and testing is the current phase occupied by the Knight, Church and Arkin designs, all three will eventually enter the testing and demonstration phase. The individual projects of each of these laboratories will determine the extent to which they will need to provide safety validation for their designs. The Knight design has been shown not to survive outside the laboratory through natural design, therefore it may be easier to establish the safety of the design. Though minimal, the Knight lab has the benefit of established research to back claims that the organism is safe, however it will need to demonstrate that changes to incorporate chassis functionality will not have changed the inherent safety of the organism.

The Church design will have a greater challenge validating the safety of the organism because that safety has been added by genetic adjustments. In order to convince stakeholders that it can be used safely as an all-purpose chassis, the Church lab will need to show reliable control over the behavior of the organism in addition to assurances that future added pathways will not alter that safety.

Finally, the Arkin design, might have the greatest challenge in that this design is deliberately placed in contact with human tissue for the purpose of delivering a medical therapy. Out of the three, this design will definitely have to undergo some type of clinical testing before it can be used and adopted. In addition, it must not only prove itself safe, but there must be proof of effectiveness. It was mentioned above that pacemakers, vaccines and probiotics are subject to FDA approval, therefore, they have established methods of

testing and demonstration since gaining approval requires substantial validation of safety. It may be that the fact that the Arkin design already has a set of regulated standards it must meet, might mean that it gains overall approval, acceptance and adoption earlier than the other two designs.

Chapter 4: Conclusion

4.1 Revisiting Research Questions

In Chapter 1, it was asked: what methods are used to implement safety and increase public confidence, both currently and in the past, and how can those methods be applied in synthetic biology to help advance the field safely. This study took a look at past cases and at synthetic biology cases and explored the types of safe design, testing, and demonstration being used.

4.2 Thoughts on Current Synthetic Biology Research

The three laboratories investigated have different approaches to chassis design and therefore different safety challenges to manage. Tom Knight's safety challenges will most probably focus on preventing functional design to interfere with the natural safety of the organism. While he has traded immediate functionality for immediate safety in his design, the time he invests in developing tools and learning about the organism serve to his benefit in proving his organism is safe. George Church has taken the less obscure route with *E. coli* that promises more immediate advances and results, however he has a tougher safety challenge in that his organism is robust and will need to be endowed with a number of safety mechanisms. Furthermore, since these mechanisms are not natural to the organism, he will need to assure the stability of his altered organism. Adam Arkin has the greatest challenge. In addition to the ones that Church faces with *E. coli*, he has to ensure safety within a human host because he intends his chassis to be injected into the human bloodstream.

In all three cases testing will need to show that these designs can participate reliably in a system that considers external forces as functional components. When changes in the environment occur, these designs must show that they will not be changed nor will they affect the environment. In looking at these three cases, it is clear that they share methodologies similar to both traditional engineering and biotechnologies. It is unclear yet whether the testing and demonstration methodologies will fall follow the same pattern. Testing and demonstration as yet seems to be a less emphasized area in the safe design process, however, in all cases, when there was a necessity to prove to the public or other stakeholders that the design was safe, there was specific effort expended. With the amount of cynicism and perceived uncertainty associated with biotechnologies, it seems that greater attention testing and demonstrating safety will have to be paid.

4.3 Concluding Observations and Further Research

The following are additional conclusions or observations arrived at through this research. They are essentially thoughts proposed with ideas for future investigations that may further explore their validity.

It has been said from the outset that one of the main reasons for this research has been the interest in the synthetic biology community to not only design safety, but also to establish that safety in a credible way that avoids the complications that GMOs created, especially in the European reaction. To that end, it is worth separating the above cases along the lines of reactive and pre-emptive. In attempting to do that, the only case that clearly stands out as pre-emptive is probiotics. In this case, safety has already been established, whether reliable or not, by a history of natural consumption. Yet there are

scientists questioning the wisdom of the GRAS list and calling for increased testing and demonstration given the advent of newer or genetically engineered probiotics microbes. It is also interesting in that the probiotics have already gained public approval and it is the scientific approval that needs attention. While it is a similar case of pre-emptively highlighting and establishing safety, the fact that they have already gained acceptance may provide the luxury of being able to call attention to safety issues. The other cases have some measure of reactive motivation. In exploring the cases, it seems that there is a fine line between reactive and pre-emptive motivation. In reactive cases, things have gone wrong that have prompted a safer design. In pre-emptive cases, one would assume that the concern has not happened before. However in the cases, there seems to be a mix, because each engineered design incorporates novel methods of handling possible failures where the concern for possible failures is motivated by some past occurrence. In this way, technologies tend to take on an interactive process of safe design where past failures may prompt thoughtful recognition for other failures that have not occurred. However, in the case of synthetic biology the failure of safe design is not an option. The apparent thin line between reactive and preemptive safe design, might ultimately support the claim that nothing can be 100% safe nor is it possible to be preemptive in all cases. It is yet early and time may show some other challenges faced by synthetic biologists where future endeavors to design safety might become a reactive response.

The cases presented above reveal stories of safe design, testing and demonstration. In exploring these cases, details were presented discussing the efforts on the part of the designers to ensure the safety of their designs. In reviewing the research currently being

conducted on chassis development in synthetic biology, it is worth exploring what synthetic biologists are doing that is similar and what they are doing that is different. Synthetic biologists are clearly looking for ways to engineer safety into their designs. This matches the actions of Joseph Paxton and John Roebling. However these designers did not need to consider public fears in the same way as Synthetic Biologists. In that way, proactive efforts to discuss safety and determine expectations may single synthetic biology out from previous genetic engineering fields. Further research might return to the cases, adding parallel failures for comparison and exploring the main principles of failures in those cases. Then follow-up on the chassis cases would provide more evidence from the additional progress, allow for a more rigorous comparison and discussion either confirming or denying the whether current chassis design seems to be following practices common to past success or past failures.

Given the categories of design and testing and testing and demonstration, mapping the principles according the four quadrants allowed a better understanding of where the subsequent cases fell. It seems that the Crystal Palace and the Brooklyn Bridge had immense focus on design and testing while the elevator and the new I35W had significant focus on testing and demonstrating. When exploring what practices go into design and testing, and testing and demonstration, it could be that design and testing may have greater influence over establishing safety, while testing and demonstration may have greater influence over progress in the field. If these associations are correct, it could be inferred that the Crystal Palace and Brooklyn Bridge cases might have had more focus on establishing safety, while the Elevator case may have had more focus on promoting progress

associated with their technology. This draws interesting attention to the cases in the middle that manage a balance. Further research would also recommend a broadening of the cases to include failures and more detailed study in all of them. A categorizing of these cases would hold more rigor if empirical measures of success were determined and applied. Perhaps a systems dynamics approach modeling the retrospective cases might provide a tool for empirically assessing the relationship between principles in those categories and success.

Looking at which quadrants the cases occupy, it seems interesting that with the exception of the Elevator Brakes, the New I-35W bridge, pacemakers, vaccines, and even probiotics are all areas where the final product must meet a type of standard set by the government. State governments typically set building codes, but the builders of that bridge had the additional pressure of answering the public's expectations, especially because they chose to interact with the public on a regular basis. They implemented an extensive outreach campaign in order to show the public that they were truly building a better, safer bridge. Designs that tend to have medical applications seem to gain credibility through practice and clinical trials, however it isn't the clinical trials that convince patients, it's their doctors who may have greater trust in medications or devices that have been FDA approved. Which leads to an interesting question for synthetic biology. They are currently working on safe designs, yet no matter how safe an organism might be, prior knowledge on the part of the public or legislators may diminish hopes of being accepted or adopted. In the discussion of self-regulation and government involvement, this observation seems to support government involvement and the setting of safety standards. This is a question that would need further follow-up and analysis, perhaps exploring the influence of government

standards on the safety of designs. This might involve exploring the regulatory structures that may have been present or may have imposed requirements on both the success and failed cases and truly analyze whether that involvement increased safety, or non-involvement decreased it. Cases would have to extend to those with no government involvement.

4.4 Advice For...

4.4.1 Synthetic Biologists

While attention to safe design and testing is critical, there is a need for appropriate validation that credibly demonstrates that safety. Given the physical nature of synthetic biology, short of licking the petri dish, it may be difficult to present something as dramatic as the elevator brake demonstration. Taking cues from the new I-35W Bridge where true demonstration of successful safety may need time, interacting with stakeholders has seemed to create exceptional community support and faith for the project. Exercises such as red teaming designs may be a similar step that brings the same type of rewards.

Given the ongoing debate between self-regulation and government involvement, it seems useful to suggest that government involvement may eliminate arbitrary establishments of safe design. Standardized practices for design, testing and demonstration that are regulated may provide the best method for ensuring safe design across the board as well as gaining safe acceptance and adoption in the field. However the challenge then lies in who will determine those standards for safety and what type of evidence would be considered convincing and substantial?

4.4.2 Regulators

Synthetic biology is not a field relegated to the elite, university scientist crowd. With the adoption of standardized parts and protocols, the ability to tinker with genetic elements will become extremely accessible. Recognizing that scientists are looking for a way to manage safety before something goes wrong, the goal should not be to regulate what synthetic biology is creating, but what types of safety standards the creations should meet. This could involve interacting with experts in the field to establish measures and expectations of safety as a result of safe design.

Once the chassis become functional, the open-source nature of synthetic biology may make it difficult to ensure the safety of the chassis, as they will enter the hands of other scientists or tinkerers. Perhaps a good way of managing this might be establishing rating systems for these designs, where certain designs may not be able to survive anywhere but a BSL1 laboratory and therefore access to these is easier, while designs that allow for more complicated engineering might have higher safety requirements, requiring the individual to satisfy those. This may allow for regulating safe design without interfering with scientific progress.

References

- Arkin, A. (2009). Interview with Adam Arkin. N. Yeddanapudi and K. Oye. Berkeley.
- Banuri, S. "Development and Economic Impacts of Elevators." utdallas.edu.
- Birdsall, B. (1983). "The Brooklyn Bridge at 100; centennial of John Roebling's Brooklyn Bridge." MIT Alumni Association Technology Review 86(April, 1983).
- Branchflower, S. and J. Petit. (1995). "The Crystal Palace of Hyde Park." 2009, from http://darkwing.uoregon.edu/~struct/resources/case_studies/case_studies_simplebeams/paxton_palace/paxton_palace.html.
- Burns, K. (1982). The Brooklyn Bridge. American Stories. A. Stechler, PBS.
- Chen, R. T. and B. Hibbs (1998). "Vaccine safety: current and future challenges." Pediatric annals.
- Church, G. (2008). Interview with George Church. N. Yeddanapudi and K. A. Oye. Cambridge.
- Church, G. M. (2005). "Let us go forth and safely multiply." Nature 438(24).
- Ellenberg, S. S. and R. T. Chen (1997). "The complicated task of monitoring vaccine safety." Public Health Reports (1974).
- Ellenberg, S. S., M. A. Foulkes, et al. (2005). "Evaluating the safety of new vaccines: summary of a workshop." American Journal of Public Health.
- F. Lodish, H., A. Berk, et al. (2007). "Molecular Cell Biology;" 1150.
- Fenner, F., D. Henderson, et al. (1988). "Smallpox and its eradication." orton.catie.ac.cr.
- Finder, T. G. I. "Elevator." The Great Idea Finder, from <http://www.ideafinder.com/history/inventions/elevator.htm>.
- Frey, D. and C. Dym (2006). "Validation of design methods: lessons from medicine." Res Eng Design 17(1): 45-57.
- Group, J. F. W. W. (2002). Guidelines for the Evaluation of Probiotics in Food. London, Ontario, Canada, Food and Agriculture Organization of the United Nations World Health Organization.
- Infoplease. "Elisha Graves Otis." [infoplease](http://www.infoplease.com/biography/var/elishaotis.html), from <http://www.infoplease.com/biography/var/elishaotis.html>.
- Ishibashi, N. and S. Yamazaki (2001). "Probiotics and safety." American Journal of Clinical Nutrition.
- J. Dyson, F. (1981). "Disturbing the universe;" 304.

- Jackson, R. J., A. J. Ramsay, et al. (2001). "Expression of mouse interleukin-4 by a recombinant ectromelia virus suppresses cytolytic" Journal of Virology.
- Jeffrey, K. and V. Parsonnet (1998). "Cardiac pacing, 1960-1985: a quarter century of medical and industrial innovation." Circulation 97(19): 1978-91.
- K. Long, T. (2006). "Forty Letters to Carson Long, 1904-10." 196.
- Käppeli, O. and L. Auberson (1997). "The science and intricacy of environmental safety evaluations." Trends in Biotechnology 15(9): 342-349.
- Khan, F. and P. Amyotte (2003). "How to make inherent safety practice a reality." The Canadian Journal of Chemical Engineering 81(1).
- Knight, T. (2008). Interview with Thomas Knight. N. Yeddanapudi. Cambridge.
- Krimsky, S., R. P. Wrubel, et al. (2007). "Standardized Microcosms in Microbial Risk Assessment." jstor.org.
- Lawler, A. (2005). "BIODEFENSE LABS: Boston University Under Fire for Pathogen Mishap." Science.
- Leveson, N. (2003). "White paper on Approaches to Safety Engineering."
- M. Rogers, E. (1995). "Diffusion of innovations." 519.
- Magee, C. and D. Frey (2006). "Experimentation and its Role in Engineering Design: Linking a Student Design Exercise to New Results from Cognitive Psychology." International Journal of Engineering Education 22(3): 478-488.
- Mallela, V. S., V. Ilankumaran, et al. (2004). "Trends in Cardiac Pacemaker Batteries." Indian Pacing and Electrophysiology Journal.
- McCarthy, E. (2008). New Minnesota Bridge Plans Arise as Bad Plates Fingering in Collapse. Popular Mechanics.
- Mcdermott, R. (2002). "EXPERIMENTAL METHODS IN POLITICAL SCIENCE." Annu. Rev. Polit. Sci. 5(1): 31-61.
- MOND, H. G., J. G. SLOMAN, et al. (1982). "The first pacemaker." Pacing and Clinical Electrophysiology.
- Mukunda, G., K. A. Oye, et al. (2009). What Rough Beast?: Synthetic Biology, Uncertainty, and the Future of Biosecurity, Massachusetts Institute of Technology; Boston University.
- Noireaux, V. and A. Libchaber (2004). "A vesicle bioreactor as a step toward an artificial cell assembly." Proc Natl Acad Sci USA 101(51): 17669-74.
- NSABB (2007). "Roundtable on Synthetic Biology." 59.
- NYCRoads.com. "Brooklyn Bridge: Historic Overview." from <http://www.nycroads.com/crossings/brooklyn/>.
- Offit, P. A. (2007). "Thimerosal and Vaccines--A Cautionary Tale." The New England Journal of Medicine.

Peter D. Hart Research Associates, I. (2008). Awareness of and Attitudes Toward Nanotechnology and Synthetic Biology: A Report of Findings, A National Survey Conducted on Behalf of the Woodrow Wilson International Center for Scholars.

Petroski, H. (1992). "To engineer is human: the role of failure in successful design." 251.

Rogers, M. (1975). "The Pandora's Box Congress." Rolling Stone: 9.

Russel, H. (2008) Reaching Closure. Bridge Design & Engineering,

Salminen, S., A. von Wright, et al. (1998). "Demonstration of safety of probiotics—a review." International Journal of Food Microbiology.

Schmidt, M., H. Torgersen, et al. (2008). "SYNBIOSAFE e-conference: online community discussion on the societal aspects of synthetic biology." Systems and Synthetic Biology.

Sofge, E. (2008). 5 Engineering Lessons From the New, Reopened Minnesota Bridge. Popular Mechanics: 2.

Stever, H. G. and J. C. Fletcher (1988). "Collected Reports of the Panel on Technical Evaluation of NASA's Redesign of the Space Shuttle Solid" books.google.com.

Stolberg, S. G. (1999). "The biotech death of Jesse Gelsinger." New York Times Magazine.

Sun, M. (1986). "Local opposition halts biotechnology test." Science.

Transportation, M. D. o. "I-35W St. Anthony Falls Bridge." from <http://projects.dot.state.mn.us/35wbridge/overview.html>.

Unknown (1883). Dead On The New Bridge. The New York Times. New York. **The New York Times**.

Unknown (1986). "Biotechnology: Review needed for intergeneric organisms, pathogens under OSTP, agencies' statements." Chemical Regulation Reporter: 355 - 356.

Unknown (2004). "Some lessons learned from the redesign of the Space Shuttle solid rocket booster." 1-6.

Unknown (2009). Merriam-Webster Online Dictionary. Merriam-Webster Online Dictionary, Merriam-Webster Inc.

Virginia, R. a. V. o. t. U. o. (2001). "Great Buildings Online: The Crystal Palace." Great Buildings Online, 2009, from <http://www.greatbuildings.com/cgi-bin/glk?http://jefferson.village.virginia.edu/london/model/>.

Warren, K. S. (1986). "New scientific opportunities and old obstacles in vaccine development." Proceedings of the National Academy of Sciences.

Wikipedia. (3 August 2009). "Elisha Otis." Wikipedia, from http://en.wikipedia.org/wiki/Elisha_Otis.