The Living Commons

A Spatial Theory for Biological Design

by
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Submitted to the Department of Architecture in Partial Fulfillment of the Requirements
for the Degree of
Doctor of Philosophy in Architecture: Design and Computation

at the
Massachusetts Institute of Technology
September, 2013

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Abstract

Biological design is as ancient as human civilization. For thousands of years, living systems and natural processes have been manipulated by humans and their biological outcomes have been customized for different purposes. While the idea of biological design has always been prevalent throughout history, especially with the discovery of DNA, the ability to manipulate the form, function, and behavior of the living has significantly advanced. Today synthetic biology is pushing the frontiers of biological design even further. Now, living things can be completely abstracted from their original biological contexts, assembled like molecular constructs, and engineered like circuits or programmed like computational hardware. biological designers compose biological form and function by running modeling and simulation software; order standardized biological parts from online libraries and databases; utilize fabrication companies to synthesize gene products to prototype their designs; and build complex artifacts, applications and services that meet human wants, needs, fears, and desires on a daily basis.

In this dissertation, I examine different practices of biological design in life sciences and engineering based on different theoretical models. I trace the history of information-based, relational, synthesis-oriented methods and present a new design framework that offers a spatial and a context-driven approach to the design of living matter. Being rooted in a different interpretation of space and spatiality in design, the framework approaches biological design systematically, at three stages: 1) the design of the basic units of the living (Units), 2) how different units are arranged and composed for different functionalities and behavior (Logic), and 3) the design of the biological contexts where biological artifacts live and perform their objectives (Context). This new framework intends to bring together a multitude of approaches from different design fields such as engineering, architecture and product Design that have their unique histories with living matter. The goal here is to demonstrate the ways different design paradigms can potentially shape our relationship with biological design in new ways; altering the design process, the objectives, the outcomes, and the social, cultural, and ethical perception of synthetic living.

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Acknowledgements

It is needless to say that a dissertation owes most to its advisors. William J. Mitchell (1944-2010), both with his presence and absence, has always been the key figure who challenged me to look for new territories at the intersection of design, computation, and biology. After Bill, George Stiny's mentorship helped me continue with the difficult problems and bring the work to a completion. It was Terry Knight and Mark Goulian's careful readership that kept the scholarship on track.

Alexandra Daisy Ginsberg has always been a dear friend and advocate. She deserves the credit to be the person who introduced me to the world of synthetic biology. The Synthesis workshop organized by her and others at London was one of the most rewarding learning experiences I ever had.

Oron Catts, Ionat Zurr, Stuart Hodgetts, Chris Cobilis, and all other dear friends at SymbioticA are responsible for most of the development and the intellectual maturation of the work discussed in this dissertation. Only at a place like University of Western Australia (UWA), I could learn the intricate relationship among life sciences, critical thinking, and artistic inquiry to be able to realize how one makes sense only in the presence of the other. Thank you again SymbioticA.

I am also very grateful to Julie A. Plummer, Emilio L. Ghisalberti, Elizabeth L. Barbour, and Christopher G. Jones—The Sandalwood Team—at UWA and Andrew Brown from Mt. Romance Sandalwood factory at Albany, Western Australia. They all welcomed me to their research and have generously given their time and energy to support my inquiry towards the future of Sandalwood—the magical plant that has been haunting me for the past year two years. This work and its successors will always be indebted to the open minds and rigorous scholarship of the Sandalwood Team.

In the making of things, Guz Gutmann has not only been an avid supporter but also the fabrication guru who solved many of the challenges during the Sandalwood Well design. A special thanks for Guz.

A number of dear friends: Dietmar Offenhuber, Duks Koschitz, Sergio Araya, Alexandros Tsamis, Mahir M. Yavuz, Azra Aksamija, Tulay Atak, David Boardman, Simon Kim, Federico Casalegno, Asli Arpak, Arzu Ozkal, Ozlem Ozkal, Selda Banci, Veronika Valk, Maya Catts, and Alison Copley have been always supportive with their big hearts and crazy minds like the rest of my family—those who had to go through another graduate degree from afar.

The intellectual environment created by the members of at MIT's Design and Computation group felt like the right intellectual basesto embark on this adventure. I am especially grateful to Cynthia Davidson and Renée Caso for their invaluable support during the administrative hurdles of this process.

The intellectual environment created by the members of the teaching labs at the University of Pennsylvania's Biology Department... Thank you Karen Hogan for providing invaluable insights about the world of the living and letting me use the facilities. And thank you Amanda Cotton for the technical support.

Last but not least, I would like to dedicate this dissertation to Bill. I will always aspire to your intellectual brevity and curiosity towards the unknown.

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Chapter 1 – Introduction

1.1 Preface

Biological design is as ancient as human civilization. For thousands of years, living systems and natural processes have been manipulated by humans and their biological outcomes have been customized for different purposes. Crops have been domesticated for different agricultural needs; animals have been crossbred to yield more productive races; yeast, fungi, and bacteria have been grown to become the staples of the food, beverage, and medical industries; forests, deserts, and coral reefs have been recovered, restored, and often tried to be saved from excessive human intervention. While the idea of biological design has always been prevalent throughout history, especially with the discovery of DNA, the ability to manipulate the form, function, and behavior of the living—from their molecular compositions to their living environments—has significantly advanced in the past fifty years. With the emergence of transgenic design, genetically modified organisms became the first living artifacts that started to challenge the biological integrity of the so-called 'natural' or 'nature-born living.'

Today, with the advances in biochemical technologies and the confluence of research among disciplines such as Bioengineering, chemistry, molecular biology, and synthetic biology, the frontiers of biological design are being pushed even further. Now, living things can be completely abstracted from their original biological contexts, assembled like molecular constructs, engineered like circuits or programmed like computational hardware. The convergence among these fields not only enables the design of new kinds of living artifacts but also identifies new functions for biological products such that they circulate outside laboratories, factories, or distant farm lands. From medical applications to fashion, product, graphic design, and architecture, products of biological design have the potential to shape social norms and influence the aesthetic, economic, ethical, and moral values of the everyday.

One of the most recent areas of investigation, synthetic biological design, is rooted in Electrical engineering and computer science. It applies rational design principles and shares many tools, techniques, methods, and formalisms with most modern design disciplines. Designers compose biological form and function by running modeling and simulation software; order standardized biological parts from online libraries and databases; use fabrication companies to synthesize gene products to prototype their designs; and build complex artifacts, applications, and services that engage with human wants, needs, fears, and desires on a daily basis.

As new kinds of synthetic biological products begin to interface with people, the current status of biological design is not only discussed as a technical framework but also as a cultural project with its extended implications to society (Church and Regis 2012). However, when artists, product designers, fashion designers or architects are invited to work with synthetic biology, their biological imagination is usually driven towards finding new applications for existing design techniques and technologies. Thus, we witness a plethora of proposals for new kinds of algae-fueled night lamps, bio-degradable building materials, or clothing grown by genetically modified organisms (Myers 2012). While such products and applications—regardless of being realistic, speculative or imaginary—have a significant role in augmenting the perception, use, and public acceptance of synthetic biology, by only focusing on applications and end products, conversations around biological design often miss the potential to incorporate new intentions and alternative techniques and processes that have evolved through other design disciplines.

Design, in its long history, has evolved into many different discourses and practices in response to different needs and wants. Analytical techniques such as black boxing (hiding complexities from users), abstraction, standardization, and generalization are useful when designing 'control' or 'decision-making' systems, whereas processes that are interested in 'variation' or 'differentiation' use generative

processes, rule- or grammar- based, parametric and non-deterministic design approaches. No standard design process or method are universally applicable for all design challenges. And even when there is a clear objective—like building a bridge to be able to cross to the other side—there are many different alternatives that can solve the problem while differing in cost, efficiency, aesthetic appeal, and cultural significance. Thus the effectiveness of bridge design is always evaluated from many different criteria at the same time.

As most design processes are shaped by their initial design motivations, the results also have cascading social, cultural, and political implications. When a particular design principle—such as feedback control—allowed us to build self-regulating systems such as steam engines, it also give birth to the design of thermostats, cruise control systems, elevators, and many other innovations that applied the principle to different domains. 'Feedback' also created a new cultural and economic framework based on mass production and industrialization, which eventually determined the types of artifacts and objects that were turned into household items or everyday products (Mumford 2010). Consumerism, for example, is directly correlated with the design and fabrication techniques that define what can be manufactured in an affordable manner given the level of advancement in the automation technologies of the times; whereas 'supply and demand,' a different type of feedback loop, extends the design principle into business models and political agendas that apply the principle to higher level design decisions such as policy-making or urban planning.

Similarly, when design processes allow the living to be broken down into standardized elements or discrete building blocks such as molecules, genes, or cells, this not only allows designs to be black boxed so that they can be cascaded, scaled up, and generalized into complex assemblies, but it also turns individual units into intellectual property since they can be identified as human-made 'novel' constructs. When parts of living organisms can be synthesized and patented this not only privileges certain

economic agendas, but also disrupts the culture of innovation by prohibiting those who could imagine new applications and uses that might conflict with profit models imposed by medical and pharmaceutical establishment from working with these pieces of living organisms.

Today's biological design methods, driven by new tools, technologies and deeper understanding of living matter, are intimately linked with different discourses and practices of design that have evolved over the histories of most design-driven disciplines from life sciences to engineering, product design and architecture. Therefore, regardless of their disciplinary background, all design methods share similar social, cultural, ethical, economic and aesthetic responsibilities as all of them together transform our interaction with both living and non-living matter, i.e. life.

In this dissertation, my goal is to initially identify what has been referred to as 'design' within the practice of biological design, and to discuss what else the field can potentially be in light of a design framework that utilizes different spatial design and synthesis methods. The ultimate objective of this thesis is to contribute to the diversification of the design culture in biological design with new threads of investigation from other contemporary design methods. Here, my goal is not to discuss the confluences of different technical approaches or to offer alternative solutions for existing design or synthesis questions, but rather demonstrate the ways that a new methodology can shape our relationship with new biologies; by altering the design process, the objectives, outcomes, and the social, cultural, and ethical perception of synthetic living.

The thesis is organized around a design framework that approaches biological design in three main stages: 1) Abstraction: The design of the units, objects and building-blocks that constitute the living, 2) Synthesis: How these units are arranged, assembled, and composed for different functionalities and behavior, and 3) Context: The design of biological containment—spaces where biological artifacts 'live,' work, function, or perform their objectives.

In this chapter, I will introduce biological design, provide the reader with the context of what it means to design the living, and discuss the understanding of what is 'designable' in light of new kinds of biological artifacts that can be conceived, designed, synthesized and built from molecules and compounds without aspiring to existing organisms or their analogies. Chapter 2 traces key moments in the history of biological designs through a series of artifacts and discusses their cultural, social or economical significance. Chapter 3 discusses the history of design methods that inform the three-tier design framework of biological design with the evolution of different units of design, different logics of assembly and alternative types of biological contexts that host historical and contemporary organisms. Chapter 4 presents the spatial theory of biological design and discusses the partitioning of the liquid space for new design and synthesis methods using liposomes and other forms of encapsulation methods. A case study that demonstrates the application of the methods and a prototype will be presented in Chapter 5. Here, I will discuss the potential futures of the Sandalwood plant, in light of different abstraction, synthesis, re-contextualization, and re-spatialization strategies. By utilizing some of the methods discussed in the previous chapters, I will show how our perceptions of Sandalwood plant and its biological products can change under the influence of new biological design methods. I will present this work both within the context of new methods and technologies of biological design but I will also show how different design methods—shaped by different intentions, values, and anxieties—change our relationship with the newly created biological artifacts. Chapter 6 will discuss the findings, future research directions, and complete the dissertation with my final remarks.

1.2 Biological Design

Throughout history, from ancient horticultural practices to synthesis of advanced medical products, many different activities can be labeled as biological design. Here, to be able to present a clear and systematic approach to the reader, I will adopt a provisional definition of biological design as 'the set of

intention-driven activities that engage with the living—organisms, plants, animals, ecologies—and non-living—inert materials, chemical processes—to achieve desired biological outcomes.

This broad definition may entail one or more of the following:

i. Manipulating the sexual reproduction of two species to get an offspring with desired traits. E.g., cross-breeding or selective-breeding of plants to achieve better yielding, better tasting, or more resilient crops, etc.

ii. Joining parts from two plants (asexual reproduction) to achieve a hybrid that has augmented capabilities which are not possessed by either of them. E.g., grafting tissues between plants to give them fruit-making or color-blossoming capabilities.

iii. Removing living things from their original habitats and repurposing them for different needs. E.g., domesticating crops or wild animals for different needs such as food supply, labor, etc.

iv. Utilizing living parts of existing organisms so they can be incorporated into other living things to give them new traits and characteristics. E.g., transferring gene or gene sequences from one fish to another to change its growth rate, skin color, or capacity to deal with environmental stress.

v. Regulating and conditioning the lifecycle of large living systems, habitats, or ecologies to restore them to historically viable moments or newly desired states. E.g., Forest restoration, riverbed transformation.

vi. Creating novel artifacts that exhibit different degrees and kinds of life-like behavior. These could be chemical processes that are designed to exhibit features such as growth, self-organization, or metabolic activity that are usually attributed to living things. E.g., Protocells.

vii. Synthesizing living materials or biological functions using entirely chemical processes. E.g., The production of urea purely through chemical reactions, or the production of artificial ribosomes or DNA molecules. These would qualify as biological systems since purely chemical systems can

also be used as support systems for biological habitats, and they can replace the role or function of a process that is normally taken by another biological organism.

viii. Building hybrids that combine biological materials with non-biological (abiotic) chemical processes to produce biological outcomes. E.g., The use of cell-free, *in vivo,* protein synthesis kits that produce custom proteins and enzymes that can be used by living organisms.

ix. Designing new forms of biological containment. Building new types living spaces and biological contexts for synthetic organisms. E.g., The design of electromechanical enclosures, chemical cells, and synthetic host organisms where partially-living artifacts can pursue their activities.

x. Designing new forms of biological confinement. The design of physical, chemical or biological measures such as barriers to isolate GMOs from non-GMO in production plants, farmlands, or nature.

While in all of these cases of biological design there is explicit human intervention, the type and level of intervention is highly specific to the objectives of the design. Transgenic design practices, which require highly invasive and low-level manipulation techniques that challenge the biological integrity of the organisms, for example, exhibit a different kind of design process than ecological restoration, in which the motivation can be on a higher level and be about mitigating human influence or trying to render its effects invisible with subtler interventions. The design process here may focus on reconditioning the overall environment of the living rather than transforming the individual members of a habitat.

In this dissertation, as I investigate the types of interventions in the design of living things, my objective is to approach different theories and practices of biological design case-by-case. Instead of looking at how biological design can be defined from a particular disciplinary perspective, say for the natural sciences, engineering or architecture, I will try to present it on its own grounds. Rather than seeing it merely as a practice of making living things to solve problems or find solutions to address known issues,

my intention is to frame biological design as a design discourse: a mode of compiling and organizing knowledge about the known and unknown parts of what we call or may call "living" such that the discourse of making can change with what we may refer as the objects, products, and spaces of biological design.

1.3 Designing the Living

Biological design is a varying set of activities with different scopes of intention, but the outcomes of these activities implicitly rest on the definitions of what living is, what makes things called 'alive,' or how the living can be manipulated, augmented, or sometimes redefined; for example, when they are created from parts that are formally not referred as living.

The definitions of what the living 'is' and what it 'does' have always been contractual agreements among those who study them. Historically, there have been a quite number of publications in the literature with the title "What is life?" that offer partial views, list new sets of observations and propositions based on scientific, ethical or philosophical standpoints (Schroedinger 1992) (Lynn and Sagan 2000) (Regis 2011). However, most of these views—inevitably or intentionally—end the discussion with inconclusive remarks as exceptions can challenge most established criteria. For example, when self-reproduction is asserted as a quality to distinguish living from non-living, sterile animals—such as mules—do not meet the criteria, despite the fact that most people consider this animal to be alive. Instead of falling into the trap of generalizations, it often becomes more productive to asses the vitality of the living conditionally or on a case-to-case basis. Gánti , for example, uses two sets of criteria—"real (absolute)" and "potential"—to be able to locate different capacities of vitality in living organisms. While the abilities to possess an inherent unity, inherent stability, an information-carrying subsystem, a metabolism, and a program control system make up the real requirements for the living, Gánti reserves

growth and reproduction, capability of hereditary change, evolution, and mortality as the secondary, potential criteria (Gánti 2003).

When the criteria are negotiated, different disciplines impose their own perspectives. Hence an evolutionary biologist's, physicist's, chemist's, and electrical engineer's way of understanding the definition of life gets highly influenced by the specific methods, tools, techniques, instruments, and the disciplinary legacy that motivates and evaluates their studies in those fields.

As the so-called living rests on a negotiated set of criteria, most of the time they are identified based on observations of what is formerly known as living and where such living is commonly observed. In most studies, existing organisms, plants, animals, hybrids, exceptions, and unusual cases are the source material that are observed in the wild—in non-human made environments often referred as nature. However such observations may not necessarily be enough to discover entirely new or uncategorized species. If it cannot be cultured, animated, or grown in a laboratory environment with known procedures that are based on interactions with other microorganisms, it would be impossible to find previously unknown bacteria in a scoop of soil randomly collected from the garden.

While it is even hard to identify a single living organism in nature that can fully meet a general set of biological criteria to qualify as living, it becomes even more difficult to assess those that are not the offspring of other living organisms. Thus 'life-like' artifacts that are created through chemical processes in laboratories, with or without using parts from living organisms, demand their own criteria to assess in what capacity they can create living matter. While a 'fully alive' laboratory made organism that can exhibit identical properties of a nature-born equivalent has yet to be made, there are partial successes and speculative outcomes based on what is possible to achieve, and what is desired to be achieved based on the specific criteria of what is agreed upon as living.

Because disciplinary boundaries, value systems, and different social and cultural criteria shape the definitions and meanings of what living is, where it lives, what it does, and how it does what it does, in this dissertation I will use the term quite deliberately for a number of newly designed artifacts without subscribing to a set of previously determined criteria. My objective is to discuss each attempt case-by-case such that it does not necessarily limit the potential of what living may be based on what is already agreed upon.

As one of the main premises of this work is to extend the current views on biological design, throughout the dissertation I use a three tier design framework—abstraction (units), synthesis (logic), context—as a means to ask what else living can be, what else can it do, and where else it can eventually live—given different disciplinary perspectives and the social, cultural, ethical, and economic implications of the design work produced by them. As disciplinary discourse shapes the design work, it not only influences the answers to these questions but also embeds different ideals and ideologies into them. With a framework that is informed by a multitude of design fields, my hope is not only to contribute to repertoire of biological design methods but also shed light on how certain disciplinary objectives, biases, and assumptions shape the applications of existing design methods and show how they can be applied differently across different disciplines.

1.4 Space of Biological Design

What the living 'is' implicitly calls into question where it 'lives;' its biological context, embodiment, containment or confinement conditions. In this dissertation, biological design is seen as a practice that not only makes attempts to design new artifacts but also decides where these artifacts can exist; i.e., where they physically interact with each other and other living beings. The space of biological design is the place where the living interfaces with larger ethical, aesthetic, social and cultural values of humans.

As synthetic biological products claim new uses, make significant value propositions or suggest alternative remedies to an ecologically-challenged planet, it becomes important to assess the relationship between the manipulated, augmented, and designed with what is native, wild or nature-born. While nature may source all the chemical and biological ingredients of the living, the designed artifacts can hardly ever go back to where they come from. Once manipulated with a different gene, a thousand year old native maize can rarely be planted back in its original setting. And even if it is, this has to be done under very specific regulations using a process that abides by many rules of biological confinement so that the designed type does not contaminate the wild types.

Since the first genetically-modified organisms (GMO), "Flavr Savr" tomatoes, were lawfully admitted to nature in 1994, there have been serious concerns towards modified organisms. Unlike an oil spill, or wild fire that may cause a one-time, albeit short impact onto a specific ecosystem, a foreign gene can potentially cause uncontrollable outcomes and cascading effects for generations of species even if they leave or migrate away from their habitat.

Genetically modified and biochemically synthesized biological products on the other hand have already been willfully permitted into our lives for many years. "Humulin©," a type of artificial insulin—analogous to that produced by the human body—is synthesized by inserting human genes into yeast. Unlike GMOs that need to produce their work in open farmland or greenhouses, yeast can be grown in physically concealed fermenters. Also, once the yeast produces the insulin, it can be discarded like a byproduct and not allowed back into a setting where it can interact with other living organisms. Being a staple of Type II diabetes treatment, Humulin© sales are over a billion dollars as of 2010 and hardly cause a stir in the public consciousness (Lilly 2013).

As the fear and appreciation of biological products are almost always influenced by the biological, social and cultural context, it is important to assess their value and risk within a more complex set of relations.

In Chapter 2, I will present a number of biological products, their design processes, and their varying cultural perception and address more specific issues regarding the role of the design of biological containment and confinement in shaping public opinion.

1.5 Living Commons

As biological design relies on a complex set of embodiment conditions, navigates a diverse space of biological contexts, and utilizes both living and non-living matter as ingredients, here I will refer to the space of biological design as "Living Commons." While the understanding of Biology has always been contingent on a specific rendering of that space—micro or macro, a resolution of study that is shaped by size, scale, optical visibility, or the amount of information available—by making this deliberate abstraction my aim is rather to identify a common ground which can encompass the necessary physical, chemical, and biological design processes and define what they act on and how they transform the outcomes of the living in today's design practice.

Today, most design fields, from chemistry to engineering and architecture, adopt methods and techniques derived from rational design principles. With the emergence of computational design especially, these fields embraced similar abstraction and standardization techniques—design motives, patterns, objects, components—and synthesis, computation, and generation methods—combinatorial, rule-, or logic-based, parametric, unit-based or unit-free that may apply the same style of thinking to very different problems. While computation, here, can be treated as common framework, it does not necessarily dictate a single paradigm of design. Thus computation of architectural form, drug-discovery, or design of biological circuitry can utilize a diverse set of approaches that can use computational media differently in their specific design agendas.

For biological design, my intention is to present a series of approaches that evolved through different disciplinary trajectories and compare their outcomes. I will specifically show how different techniques

can influence alternative results for the same design problems—creating unexpected, more diverse sets of outcomes—and show how different techniques or logics shape new ways of thinking about function, utility and behavior for synthetic living artifacts and influence their value and perception within the society.

Design at the molecular level offers a different meaning for the living. At this low level, the boundaries between what is chemical and biological, what is living, semi-living, or not-living often get blurred and become open for interpretation. Chemical processes can be considered living or non-living and matter can behave temporarily as if it is living. Living commons not only provides a broader scope of ingredients and set of activities but also a larger context to rethink what it means to be living. Like the insulin molecule, which now can be fully synthesized through artificial means, molecular commons suggests the notion of 'molecular equivalency,' in which regardless of the origin, matter can be potentially produced or conditioned with biological or chemical processes 'for' or 'as' the living.

In this space, the design of molecular equivalents invites an understanding of conversion or translation that is beyond function or performance. When functional equivalence is reached, it becomes possible to address issues about value that are beyond an immediate understanding of utility. Similar to other products of design—like buildings, cars, or clothing—biological design can begin to claim a disciplinary autonomy and facilitate the intellectual, symbolic and aesthetic function of its artifacts that are independent of the values imposed by different disciplines such efficiency, cheapness, or abundance. Among the equivalents, the questions can be raised towards a design culture—preference, style and other forms of subjective criteria—that can include a different quality of human intervention and potentially shape the perception and use of future biological artifacts. If equivalence suggests that we think about implications and consequences, it becomes important to think about the values that will

make us prefer one way or the other when cost, efficiency, performance can be considered relative to socially, culturally, and ecologically responsible and accountable forms of design and production.

1.6 When Living Becomes Designable

In this dissertation, I refer to a very broad set of human activities as biological design. This inevitably conflates a design terminology that has evolved through the history of tools, devices, machines, systems, and environments with a vocabulary that is shaped by activities such as manipulation, transformation, or synthesis that can only be done on living things.

While we call activities performed both on living and non-living design, these activities greatly differ from each other. The way we conceive a camera, assign functions to its parts, and make it 'see' through its sensory apparatus is very different from the way Nature selects the relationship between the parts that make up an 'eye.' Tim Lewens, in "Organisms and Artifacts," already reminds us the dangers of this conflation and how designed artifact-centric thinking inevitably limits our understanding of the organisms that evolved through natural means (Lewens 2004). Here, adapting an established design framework to the design of new biologies bares a similar kind of limit. An engineering-inspired synthetic biology, for example, not only reduces design to engineering, but also yields applications that mimic the characteristics and capabilities of non-biological systems: bacteria become modeled like factories, insects are described as machines, and forests are described as systems, and so on.

On the other hand, engineered and industrially produced artifacts—buildings, clothing, cars, lamps—are all conceived based on a notion of 'work'—they transform energy into a particular function or utility. In other words, they are need- or goal-oriented creations. When design activities respond to identifiable needs and wants—solve problems, improve performance, "satisfice" human desires—the products of design are inherently evaluated based on their capacity to respond to those needs (Simon 1996). This

function and intention-driven framework provides a common language among all designed artifacts based on a common understanding of work for designed artifacts (Levin et al. 2011).

However, does this means that when living things are manipulated, transformed, and synthesized that they also need to be defined, designed, and evaluated based on how they work? And if we assume that all designed things need to work, do we also assume that designed living things would work similarly the way nature-born living things work?

In this dissertation, as I intend to lay the foundations for alternative motivations behind the design of the living, I center my framework on the term 'designable' which would refer to an experimental format that does not have to be defined based on an existing product of design. A biologically 'designable' would differ from a typical non-living design in the sense that it can take on a prototypical status, which would suspend its need to respond to an existing need, desire or want. Also, as the living components have to come from existing living things, vitality cannot start from scratch. A formerly non-designable living thing has to be abstracted and be prepared for being designable. The ability to design an experimental set of relations between different biological parts may precede the need for conceiving an outcome with a objective that is prescribed by the interests of a particular domain—such as making drugs, cheap fuel, better seeds, etc. Thus, it is argued that the biologically designable can become a series of experimental artifacts that can interrogate the very possibility to designing living artifacts without making the claim that they will behave as if they are the approximations of Nature-born living things or that they have to function like other designed artifacts such as machines or computers.

For example, today *Escherichia coli* is not designed like a machine but it is designed as if it is a machine. Our ability to design it does not necessarily make it an experimental research object whose biology may be reconsidered for alternative outcomes. Knowing that *Escherichia coli* can synthesize proteins, it is often manipulated to produce ones that is not originally evolved for. The design intervention on the

bacterial genome is inherently the application of a design methodology that is translated from the history of fabrication, manufacturing, and synthesis into domain of the living. As a 'living machine,' *Escherichia coli* is inherently designed to work and produce not like itself, but like a machine, which is conceived often within a resource-driven and restriction-based economic framework: how can *Escherichia coli* produce such and such with minimal amount of effort and expenditure.

As a designable, on the other hand, *Escherichia coli* offers a multifaceted biology. It is a genome that can receive new genes (instructions) to make new proteins that are not useful for its own well being; a vesicle that can move around the human gut and host other viruses; a sensory organ that can detect the physical and chemical changes in its environment; both a single organism and member of a colony where an individual's role can be suspended. Both conceptually, and materially, the organism offers itself both as a set of biological parts—RNAs, enzymes, membrane, and other sub cellular parts—and a series of relationships between the parts and the way they can be related to other chemistry and non-species centric biologies. As a designable, *Escherichia coli* can be decontextualized and recontextualized into alternative forms. Its ribosome, for example, can continue synthesizing proteins in test tubes or artificially created membranes. Alternative design methods can engage with the organism on different levels, suspend certain assumptions of what we think *Escherichia coli* is, to be able to design—imagine, see, and realize— what else it can be.

* * *

Today, as we began to negotiate new roles for biological design, it is important to acknowledge that every different conceptualization of design and the designable will present its own values. When biological design is treated as its own stand-alone framework that will alter existing definitions and offer new relationships among the living, it inevitably deviates from certain propositions, assumptions, expectations and ways to conserve them. Thus new biologies may have different ways to negotiate their

place in a 'general ecology'—claim a niche, survive or become extinct—among the living and the non-living; without necessarily being bound to certain economic, ecologic, and socio-political agendas imposed by existing disciplinary frameworks and their favorable design paradigms.

After all, the design framework offered in this dissertation is also an invitation to think about alternative modes of responsibility and accountability towards the living and the nonliving that may mend the relationship that has already been severely broken in many ways.

Chapter 2 – Products of Biological Design

In this chapter, I survey the interaction between biology and design over time. Given the broad nature of activities that can be grouped under biological design, I present a selection of biological products that reflect both the outcomes and the intentions behind manipulating living matter in different ways.

Like every designed product, biological artifacts are almost always responses to human needs and wants. Sometimes they appear as convenient solutions, sometimes they are driven by research explorations directed towards finding alternatives, and sometimes they can be geared towards commercial interests for maximizing profit. Here, with a few exceptions, my selection highlights designs that are already realized or commercialized to discuss different kinds of value propositions they make in response to social, cultural, political, moral ethical, and economic needs.

A survey that is only told through realized products inevitably portrays an incomplete history. It cannot capture all the efforts that are spend towards the designs that are prototypes, experimental research objects, or trial-and-errors. Without showing the attempts that could not be realized due lack of resources or flaws in methods, technologies or business plans, it would also be hard to capture the full history of biological design. Here, while my intention is to portray a diverse selection of artifacts to cover a broad spectrum of design activities, my goal is not to give a full historical account of biological design. It is rather to use this survey to trace a history of human intentions embedded in the design processes. I am interested in capturing the diverse history of design as organizing, controlling, regulating, domesticating, growing, exploring, optimizing, programming or tinkering with living matter with its fundamental reasons. And by focusing on intentions, my goal is to show how different intentions

inherently align with particular design methods and how they drive the design objective towards a specific disciplinary direction.

Genetically engineered fish, for example, can be made to grow faster, with less food and more resilience to its environmental changes; therefore its design has resemblance to the ways we engineer cars. Similarly, bacteria that synthesize plant proteins are designed to work like factories—where ingredients come in, processed by the genetic machinery, then the byproducts are removed by industrial processes. In drug discovery, on the other hand, when there is a need to deal with unknown compounds and chemical reactions with the aim to produce many different variations of a single design, the process resembles parametric or rule-based design processes used in Product Design or Architecture. Designs are computationally generated with software then evaluated based on certain selection criteria that can be prioritizing fitness, aesthetics, efficiency or effectiveness.

The cultural perception and use of biological products not only depend on the design methods, but also to the designs' capacity in addressing different needs. Some biological products are perceived more valuable than others and different designs inevitably have different levels of social acceptance. For example, while genetically modified—Frankenfish—is not immediately welcomed to the dinner tables, synthetic insulin produced by genetically engineered bacteria hits sales figures over billions of dollars.

Today, most design-aided-biology is tied to specific scientific methods, engineering-driven design frameworks, and value systems that ultimately shape the ways we interact with biological products. Through this survey, my goal is to present these artifacts holistically—with their design processes, values, functions, social acceptance rates, economical successes, and failures. Criteria commonly used to evaluate most designed products like food, industrial products, clothing, or buildings. The material

presented in this chapter will be the basis to discuss what is missing from today's design vocabulary and biological imagination, which I will expand in the coming chapters.

Figure 1. Greyhound bus design by Raymond Leowy. 1940.
Source: Designboom 2013 (Right)

2.1 Greyhound

Biomimicry—the ability to design things 'like' they appear in nature—is also as ancient as biological design. Since the earliest times, the form and function of artifacts, tools or mechanisms are designed based on animals, plant forms or equivalents.

If nature has 'solved' a problem, it is often considered that it would be easier to copy that solution. Progress—the term we often use to meter human achievement, is historically based on our design's capacity to observe, understand and mimic nature to be able to overcome its limits (Mumford 2010). Leonardo da Vinci's drawings of flying devices were driven primarily by his study of bird wings. Analogies with other animals began to define the models for primitive machinery. The ability to build digesting and defecating automata set important milestones in human design following the 'to be able to understand, one needs to build it' motto. Even today, entire design fields still work on building animal-like machines—"animats"—that can fly like birds or walk, and maneuver like caterpillars (Send et al. 2013).

This mindset has not only brought a formal translation of nature—by copying the look, features and behaviors of living things—but also established a system of values and ideals that are based on how

nature would—simply, accurately, efficiently, or optimally do things. Thus, proportions, ratios, patterns that are repeatedly observed in nature have become accepted as universal values; established measurements systems, and form the basis of social, cultural and aesthetic norms (Schummer et al. 2009).

In this dissertation, I primarily focus on biological design, which works directly with the materiality of living organisms instead of reviewing designs that take living matter as models for inspiration. However, it is important to include some examples of biomimetic, or bio-inspired design, to survey design inspiration of living matter as a comparison. When products start to look like animals, animals are bred like products to follow their styles and values too.

Greyhounds have been bred for thousands of years as sighthounds, a type of hunting dog that tracks through sight instead of smell and hunts prey by outrunning them. Being identified by speed and agility, Greyhounds naturally became an inspiration for the infamous bus line. They not only became part of their visual identity, but also Raymond Loewy's inspiration for the bus design.

Streamline is one of first design styles in the history of modern design, which explicitly takes science and technology as the primary reference for design of everyday artifacts. While it originated from an attempt to find "true form of least resistance" for machines that rely on hydrodynamic and aerodynamic efficiency, it quickly formulated a set of universal principles—such as optimality, reductionism, and elimination of extraneous details—that influenced the design of almost all artifacts from submarines to telephones, and vacuum cleaners (Bush 1975, 6).

The first drawings of streamlines are attributed to Sir George Cayley in 1804. By measuring the girth of a trout at regular integrals along its length, he calculated the dimensions of a wooded spindle to drive a boat with least resistance (Bush 1975, 6). Donald Bush calls his research, "an homage to teleology," a

philosophy that seeks evidence of intention, purpose, and intelligent design in nature—a view that still haunts the perception of living systems in certain circles.

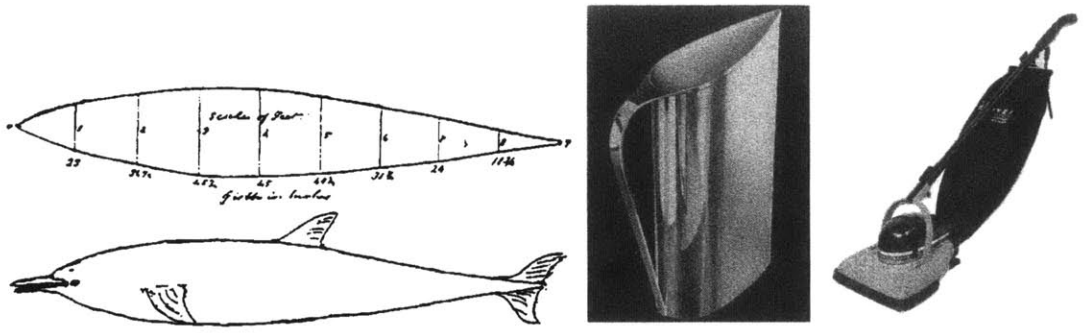

Figure 2.
True Forms of Least Resistance by Sir George Cayley. Source: Bush 1975, 6 (Left)
Normandie water pincher by Peter Mueller-Monk. 1937. Source: Bush 1975, 142 (Middle)
The Hoover Model 150 Vacuum. 1936. Source: Bush 1975, 142 (Right)

Animal form and locomotion became the natural sources of inspiration for curvilinear shapes, round corners, smooth surfaces, and organic forms. The style quickly transformed the identity of products that don't need streamlining features to deliver their primary functions.

While streamlining became analogous with the current understanding of nature, studied mostly within the context of industrialization and modernization, Christina Cogdell presents another perspective to unpack that period in her book "Eugenic Design (Cogdell 2003)."

Through a comparative study that demonstrates the relationship between genetics, eugenics, and Streamline design, Cogdell demonstrates how scientific developments, the current world-view, and design values comply, implicitly and explicitly, with the ideological agenda of 1930s America.

Here, nature is the basis of research, yet the primary intention is to replicate the features or the process of living systems with natural mechanisms. As Cogdell demonstrates in the work of Loewy and Norman Bel Geddes, the goal is to seek improvement, refinement and fitness, or practical perfection in design by cross-breeding, selection or elimination. The only methods available to designers of the time were these

principles—as they apply the laws of evolution both to their products and world-view. Thus, streamline designers were complacent with racism according to Cogdell not politically but ideologically. Stylistic evolution and progress clearly followed the demand for purification and the elimination of the hybrid, the inferior, and the unfit. Being informed by Mendel's laws of evolution, the modern aesthetic of a "civilized" nation was designed by breeders of both products and bodies (Cogdell 2003, 45).

For Cogdell, streamlining ideology also received public attention in United States during the same period, especially due to Egmond Arens's efforts. Being both an industrial designer and publisher, Arens toured widely and lectured on "Streamlining in Nature" in high schools and colleges. In his lectures, he used greyhound as a canonical example both for championing its visual form as well as celebrating the superiority of the breed over the others.. Comparing the work of industrial designers and breeders, Arens not only stated the visual similarities between the curves of the animal and stream lines but also emphasized the importance blood lines—the importance of being purebred—for a beautiful and functional design. After all, the form of the Greyhound is in its blood for Arens:. And " greyhounds were being bred for lines like these long before the engineers discovered the slipstream (Arens quoted in Cogdell 2003, 49)."

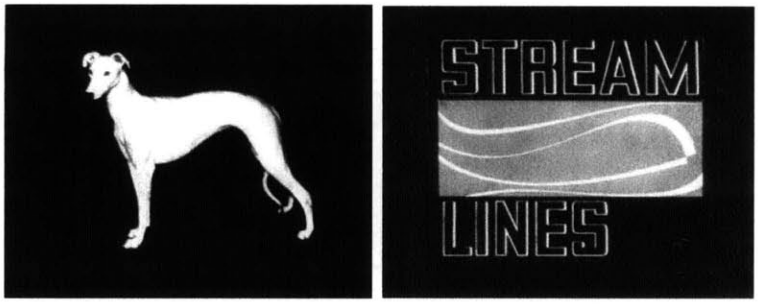

Figure 3.
Egmont Arens, laterns slide of a greyhound and 'Stream lines' that accompanied his lectures on "Streamlining in Nature." Source: Cogdell 2003 (Left)

Today, greyhound breeding and racing is a million-dollar industry. They are engineered high-performing products built by genetic design methods. The study of parameters that yields the fastest racing dog is ongoing research in animal genetics (Täubert et al. 2007) (Parker and Ostrander 2005). The genetic profiling of the dogs is correlated with their performance and therefore has a direct impact on their racial value. Once canine genomics reveal the components of the 'better' dog such as larger hearths, leaner muscles, and long thin legs, it becomes easier to design pedigrees with desired traits—first with selective breeding methods than transgenic design.

Biological design is commonly pursued in the racing dog world through negative screening (elimination) or selective mating. For example, in the search for a better racing dog, the aim would be to get the right amount of muscle composition in the animal's body for optimal performance.

Whippets, who have been known as the 'poor man's greyhound,' are a newer breed developed in the late 19th Century for high speed and small body size. Mosher et al. shows the effects of mutation on a specific gene in Whippets. Dogs with mutated genes have increased muscle mass and therefore yield better racing performance. As a contribution towards research on competitive athletics, the authors suggest that a screening of *myostatin* mutations in the dogs is beneficial in the selection of the elite athletes (Mosher et al. 2007). While they are careful about pointing out potential health issues regarding dogs with mutations, the research promotes the utility of the mutation and presents evidence that that it will give competitive advantage over non-mutated breeds. While the mutation is a natural process in living species, by selecting the mutated pedigrees in the name of better performance, breeders potentially direct the evolution of the dogs towards becoming products that serve more towards the owner's needs than the dog's themselves.

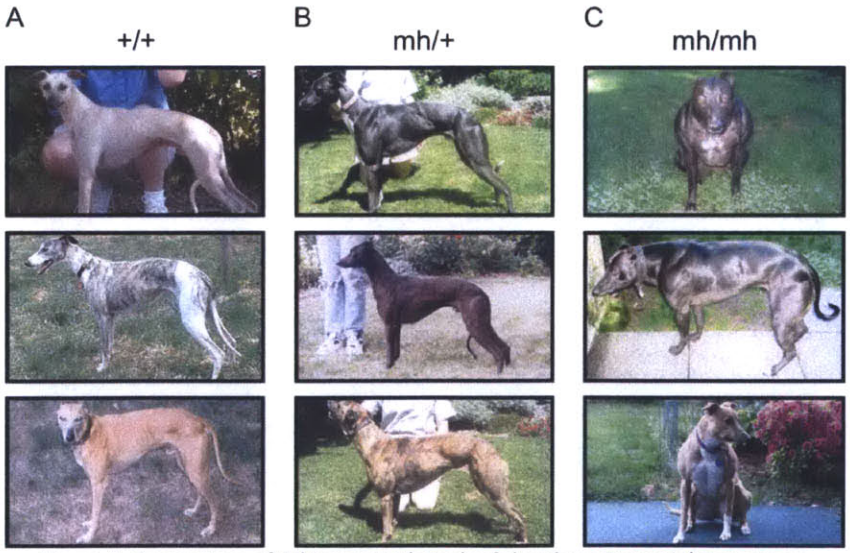

Figure 4. Comparison of Whippets with Each of the Three Potential Genotypes
(A) Dogs have two copies of the wild-type allele (+/+).
(B) Dogs are heterozygous with one wild-type allele and one mutant cys → stop allele *(mh/+)*.
(C) Dogs are homozygous for the mutant allele with two copies of the cys → stop mutation *(mh/mh)*.

All photos represent unique individuals except for the top and middle panels in the righthand column.
Source: Figure reproduced from Mosher et al. 2007

The preference or bias towards particular breeds also has consequences regarding the management of diversity among species as well as maintaining healthy individuals that would have to deal with the consequences of the unnatural changes in their bodies.

While today's Eugenic Design methods manifest themselves less in form and more in function, the reciprocity between modeling the design of fast, fuel-efficient buses and mutated bodies of the racing dogs still share common ground—the same desire for enhancement and betterment projected onto industrial designs or living artifacts.

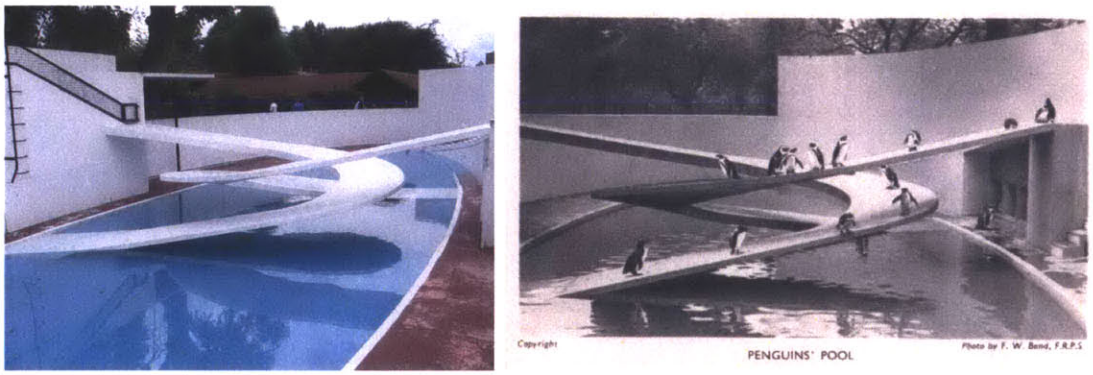

Figure 5.
Penguins' Pool at the London Zoo designed by Berthold Lubetkin's
Tecton Architectural Group in 1934. Source: Yale 2013 (Left)
Penguins' Pool postcard image by F.W. Bond, F.R.P.S. Source: Postcard 331 (Right)

2.2 The Penguin Pool

Building the right kind of environment to meet the needs and wants of the living has always been a premise of Architecture. While designing for the living is not the same as directly designing the living or designing with the living, Architecture has always been responsible for designing the conditions for the containment and confinement of the living. The built environment is not only the enclosure that

provides the necessary settings for the survival, but also the space for biological, social, cultural, and political interaction. In its most fundamental terms, Architecture defines boundary conditions—who has accesses to whom—whether it is for hygiene, comfort, privacy, security, economic, religious needs, or merely for pure pleasure. Not only gardens, zoos, hospitals, cemeteries, prisons, but all forms of architecture are responses to the inclusion and exclusion dynamics that are defined by different biological and social margins.

While Architecture has evolved through a long history, and many different styles of confinement, it also has different levels of interaction with the Natural Sciences that study the needs and wants of the living. Peder Anker, in his study of the origins of Ecological Architecture, highlights the interaction among the Bauhaus Designers and the influential Biologists of 1930's London. Equally concerned with social climate due to the upcoming war, Walter Gropius and Julian Huxley, looked at ways to reconcile the relationship between humans and nature, and rely on design that is fueled by advances in the sciences and technology. The London Zoo became a site of experimentation for new ideas, Anker notes a mutual turn towards mathematical and the mechanistic models both in modern design and biology (Anker 2010).

The penguin pool designed by Berthold Lubetkin in 1934 is one of the boldest manifestations of this thinking. It features a double-curved pathway—an entirely synthetic construct in form, function, and material designed to show how penguins thrive in an artificial environment. The concrete walkway not only imposes a challenge to the delicate feet of the animals, but also creates an unusual social setting as they would need to walk down the helix to reach water where they are always on display both in relation to each other and to their spectators.

Today, Lubetkin's pool is closed and modern penguins live in larger and 'arguably' more comfortable environments. However, as a space of highly aesthetic confinement, the penguin pool meets many objectives. It successfully mediates the modernist design vision and the model of healthy living for the

times. If the fragile animals can thrive in an unnatural setting once they are separated from their enemies, and given enough food and hygienic accommodations, workers—those who live in poor, criminal, and filthy settings—may thrive too once they are liberated from their adverse conditions as well (Anker 2006).

All forms of biological enclosure, from cell membranes, to the bodies of organisms, biotopes, and habitats that separate different living things from each other are necessary for the differentiation, development and evolution of living things.

As an artificial biological context, the penguin pool also emphasizes the fact that all designs for containment and confinement meet the needs of visibility; a gaze imposed by the designers, observers or spectators who interact with the exotic life form from a critical distance and without being threatened by their biological presence.

Figure 6. Humulin® N Human insulin (rDNA origin) Source: Humulin.com (Left)
Computer-generated image of insulin molecules in assembled form. Source: Wikipedia (Right)

2.3 Humulin©

A number of key developments in genetics and genomics from the 1940s to the 1970s marked an important era in the study and design of biological systems. The progress in understanding both the biological and chemical basis of deoxyribonucleic acid—DNA—opened further possibilities for the genetic manipulatibility of the living. Today it is not only possible to transfer genes coming from the DNA

of different organisms to achieve desired features, but also feasible to use one organism to produce the biological products of another.

Humulin© is a type of insulin analog available to Diabetes type II patients who cannot produce insulin in natural ways. It is produced by a recombinant DNA technique commonly referred as biosynthesis. Genes that are responsible for the insulin production in the human body are inserted to a host organism—such as *Saccharomyces cerevisiae* (baker's yeast) or *Escherichia coli*—which then synthesize the exact hormone using their own genetic mechanisms.

While porcine- and bovine- derived genes can also be used to produce insulin—and sold under different brand names—recombinant human DNA, reportedly, yields the most indistinguishable molecule from the naturally-occurring one, regardless of which organism is used for production.

According to WHO, as of March 2013, 347 million people worldwide have diabetes (WHO 2013). Given the increase in Diabetes patients in the past years, Humulin© and animal-driven insulin products are more than a billion-dollar market, which is not highly affected from the debates surrounding GMO or recombinant DNA technologies (Schmid 2003). However, unlike interacting directly with the GMO, here, the end-user consumes a product of the organism. Being treated almost like medicinal insulin analogs—and the growing need for them—surpass the social and ecological concerns. This demonstrates how a design process, by abstracting out the ingredients and the biological context of creation, can offer a successful business model for consuming synthetic artifacts.

Recombinant insulin has been in commercial use since 1985. Therefore patents that have secured its production to select companies are expiring, promising cheaper and generic insulin to become available for public use. Expiring patents and broadly available information also makes insulin biosynthesis a viable research area for do-it-yourself biologists, who are interested in engineering relatively simple and well-studied fermentation processes with recombinant design techniques. These studies occur with the

goal of either breaking away from dependency on pharmaceutical establishments or merely advancing research on personal biotechnologies (DIY Insulin 2013).

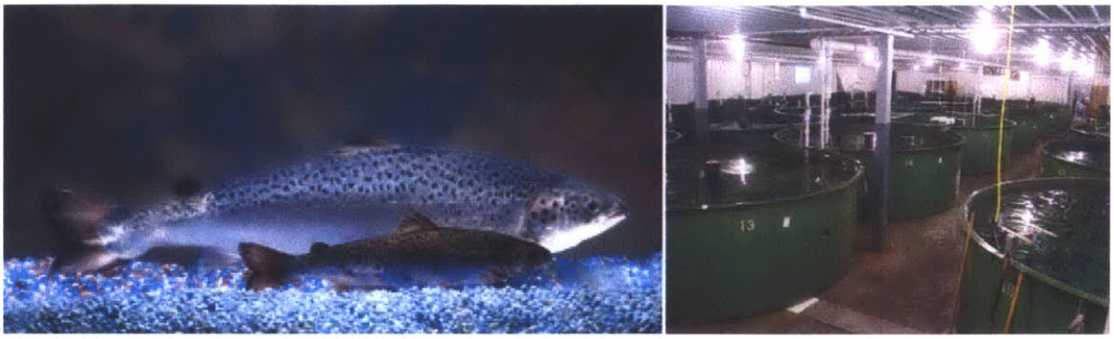

Figure 7. AquAdvantage® Salmon (Left) and Fish tanks (Right)
Source: Aquabounty.com

2.4 AquAdvantage©

AquAdvantage© is the latest product of AquaBounty, a US-based company that has been designing salmon since 1989. It is a transgenic Atlantic Salmon with an additional Chinook salmon gene that produces a growth hormone in the fish, making it grow twice as fast compared to those that live in the wild.

AquAdvantage© Salmon targets a $ 100 billion aquaculture market that tries to meet the growing demand while the seafood supply is increasingly in depletion (Aquabounty 2013). It is advertised as an ecologically friendly, sustainable solution to wild fish farming that have adverse effects to coastal areas. The fish are designed to live their short lives—approximately 700 days before they reach optimal weight of 6 kilograms—in land-based facilities. The population of all-females AquAdvantage© Salmon, in theory, can neither mate with any non-GM Salmon to make an offspring, nor transfer genetic material to other organisms living on land. As a result of their physical captivity, the breed is virtually outside the food chain and consumed only by human.

As of today, AquAdvantage© Salmon is approved by the U.S. Federal Food and Drug Administration (FDA) as it could not find any scientific reason to prevent its production as long as the fish are grown according to the "proposed conditions of use" (AquAdvantage 2013). While the product, by law, is regulated like an animal drug, the FDA is responsible to treat it as food additive for its nutritional composition and not for its environmental impact.

AquAdvantage© Salmon is the first GM animal product that is about to become an everyday commodity. While its design process in nature is similar and even less complicated than the biosynthesis of insulin, it has been widely received with contention. The GMO is the sole product of consumption and will be directly ingested by humans as opposed to the Humulin© synthesis in which the GMO organism gets separated from its product and not have direct contact with humans. Transgenic design and biosynthesis, therefore, demonstrate two different design methods that use similar principles yet arrive to two different levels of perception and acceptance by the public.

One main design concern in Aquabounty lies in the labeling of the product. As GMO products are nutritionally equivalent to non-GMOs, US regulations do not require them to be labeled specially. However, as different kinds of synthetic designs emerge, it will become increasingly important to communicate what type of biological design method has been utilized during the production of the artifacts, as different methods clearly imply different levels of risk and adverse implications to consumers.

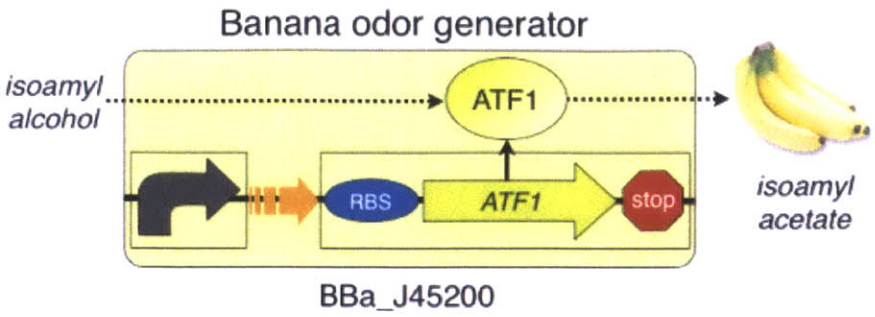

Figure 8. Schematic of the Eau d'coli banana odor generator designed by 2006 MIT iGEM Team.
Source: http://parts.igem.org

2.5 BioBrick® BBa_J45200

Eau d'coli is a project submitted by the MIT Team to the International Genetically Engineered Machinery (iGEM) competition in 2006. It is about engineering a special strain of odorless *Escherichia coli* so that it can exhibit wintergreen mint or banana smell during the different growth phases of the bacteria (Dixon and Kuldell 2011).

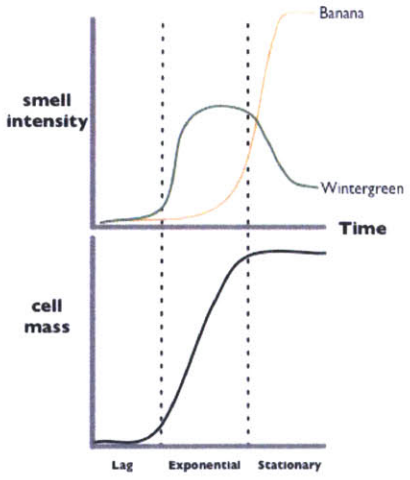

Figure 9.
Schematic overview of smell intensity and bacteria mass over time
Source: http://openwetware.org/wiki/IGEM:MIT/2006

The project is, in itself, not a traditional product available to the market; however it is a simple and effective demonstration of synthetic biological design using the BioBrick assembly method. BioBrick is an open-source design method, but the BioBrick assemble kits are commercially available to designers who want to produce genetically modified bacteria using a standardized design process (BioBrick Assembly Kit 2013). The kit allows designers to attach their desired gene sequences to bacterial genome without necessarily worrying about the underlying complexity. The assembly kit consists of a number of chemicals—reagents that are necessary to insert gene sequences to the bacteria, the protocols, instructions, and method of assembly.

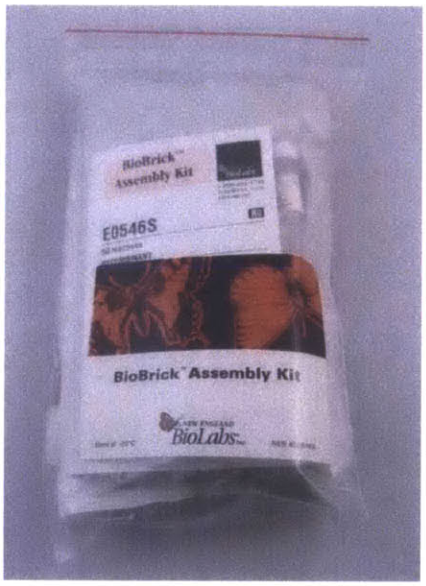

Figure 10. BioBrick® Assembly Kit Package sold by New England Biolabs (neb.com).
Source: http://d.hatena.ne.jp/bact/20110131/1296592931

BioBrick® BBa_j45200 is named as a "Banana odor generator device (Parts registry 2013)." It is a specific set of genes that will let the bacteria produce the proteins that can convert the *isoamyl* alcohol in the environment to *isomyl acetate*. This conversion causes the banana odor. BBa_j45200 includes the ATF1 gene from *Saccharomyces cerevisiae* that is responsible for producing the enzyme for the converting the alcohol and the other sequences such as ribosome binding sites, promoter or terminator genes that

make it an interchangeable part that can be plugged into any *Escherichia coli* that is compatible with the BioBrick® assembly method.

Today, designers can order BBa_j45200 directly from a gene synthesis company or produce it themselves by composing the different parts using polymerase chain reaction (PCR) or other forms of gene replication technologies.

In letterform, BBa_j45200 can be represented as the following:

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>BBa_J45200 Part-only sequence (1801 bp)
tccctatcagtgatagagattgacatccctatcagtgatagagatactgagcactactagagattaaagaggagaaatactagatgaatgaaatcgatga
gaaaaatcaggccccgtgcaacaagaatgcctgaaagagatgattcagaatgggcatgctcggcgtatgggatctgttgaagatctgtatgttgctctc
aacagacaaaacttatatcgaaacttctgcacatatggagaattgagtgattactgtactagggatcagctcacattagctttgagggaaatctgcctga
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gaatggcttacggatatttttatccccgcagattgccgctcacaactaccagatgatgatgaaatgagacagatgtacagatatggcgctaacgttggat
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tagtcaagaatctctcgaagagctttgctccatttacaaagctctccttttaggcccttaataatactagagccaggcatcaaataaaacgaaaggctca
gtcgaaagactgggcctttcgttttatctgttgtttgtcggtgaacgctctctactagagtcacactggctcaccttcgggtgggcctttctgcgtttat
a
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Figure 11. BBa_J45200 represented as nucleotide sequence (1801 bp).
Source: http://parts.igem.org

iGEM is one of the most popular venues that promote synthetic biological design. Here, teams of undergraduate students use existing BioBricks, design new ones and submit them to a registry of parts for further dissemination. The competition not only fosters a research community but also promotes the use of standardized parts like BBa_j45200 in different designs. Each year, as teams enroll, they receive a standard distribution from the registry of parts in a kit, where a selection of bricks is made available for them. BioBricks provide a common interface both as material and information for different biological designs. They encourage designs to incorporate features from each other and allow projects to scale up in complexity to demonstrate more advanced biological behaviors. While the majority of the designs are still pursued in an academic environment by science and engineering students, the competition is also

getting ready to invite amateur participants who can use the same BioBrick distributions to participate in iGEM without being affiliated with an institution.

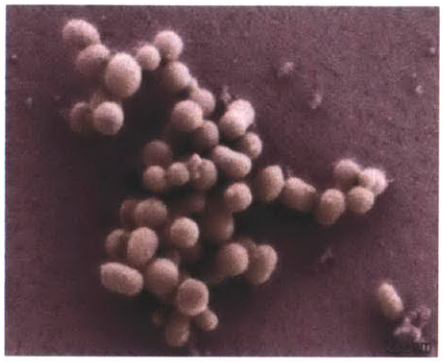


Figure 12. Scanning electron micrographs of *M. mycoides* JCVI-syn1.
Image by Tom Deerinck and Mark Ellisman.
Source: Sleator 2010

2.6 Synthia

Mycoplasma mycoides JCVI-syn1.0, popularly nicknamed as 'Synthia,' is an important step towards the first-self replicating synthetic bacterial cell (Gibson et al. 2010)." It is a new form of synthetic biological construct that carries a human-made copy of the Mycoplasma bacterium's genome that is started synthetically with an oligonucleotide printer, grown with the help of yeast cells, and finally inserted back into a host Mycoplasma to be part of its natural life. Being partially synthetic, partially wild, the new design can not only carry the capabilities of the original bacteria but also has the potential to have features introduced by its creators at the Craig Venter Institute (JCVI).

"It is the proof of principle that genomes can be designed in the computer, chemically made in the laboratory and transplanted into a recipient cell to produce a new self-replicating cell controlled only by the synthetic genome (JCVI 2013)."

And unlike partial gene transfers done by recombinant DNA methods, the bacterium now provides a full blueprint of its genome. It can function like a chassis organism, where designers can plug-in new functionalities and expect relatively more control over the outcomes.

The project is part of a larger initiative called the "Minimal Genome Project," which focuses on reducing the original 482-gene genome of *Mycoplasma* to 382 genes, to be able to minimize its complexity while still being able to keep it alive. The reduced *Mycoplasma* is not only a model organism for research but provides a business model, which can be customized for new margins of profit. Venter's institute has already filed a patent application for it (US patent application 20070122826).

Synthia is a fifteen years fourty million project (Sleator 2010). And the final product, as a novel life form is intended to be patented by the Institute. While historically there have been many disputes over the patenting of genes or processes of living things found in nature, Synthia, like the minimal genome project, starts a new discussion in intellectual property for industrial bacteria; whether existing organisms now can be patented once their genomes are completely replaced by synthetic versions or not.

Synthia currently does not do anything different than a natural *Mycoplasma mycoides*. But it has the genetic signature of JCVI imprinted in its genome—a watermark—that features the names of its creators. However, it has already generated 300 million dollars of external funding for research towards utilizing the bacteria to synthesize cheap biofuels.

Synthia is an important milestone in the history of biological design because of its artificial biological context that hosts synthetic DNA in a living organism, and attempts of protecting the use of organism with patents and other forms of intellectual confinement. Synthia inspires many questions regarding the future of biological containment and confinement. The project has also been severely criticized by ecological watchdog organizations such as the ETC group, which actually baptized JCVI's design as

'Synthia.' The group has actively engaged with social media campaigns to inform the public regarding the social, cultural and ecological implications of synthetic biology and genetic engineering in general.

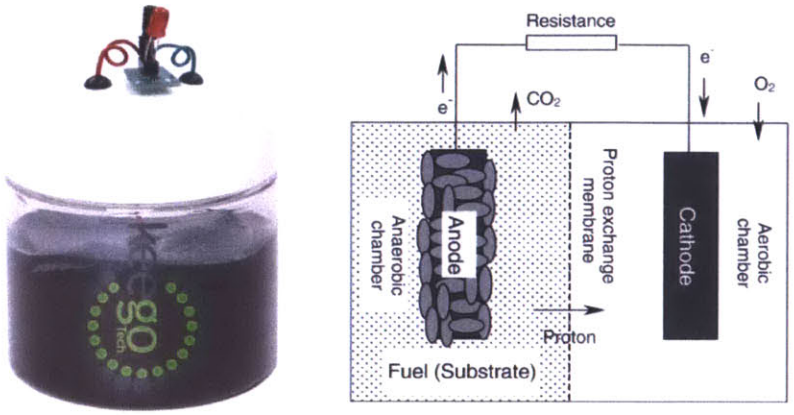


Figure 13. MudWatt™ Microbial fuel cell kit in assembled form.
Source: http://www.keegotech.com/ScienceKits/MudWatt (Left)
Schematic diagram of a two-chamber microbial fuel cell. Source: Zhuwei et al. 2007 (Right).

2.7 MudWatt™ Microbial Fuel Cell (MFC) Kit

Since the early 20th Century, it has been known that bacteria and yeast can be used to produce electricity. As microorganisms break down the organic compounds in the soil, they can convert the chemical energy into electrical power through an oxidization reaction by releasing the electrons in the organic material through their respiratory processes. When sandwiched between two layers of conductive materials that can function as positive and negative electrodes—and encapsulated in an oxygen-free environment such as a glass or plexiglass chamber, microorganisms can be made to become part of a circuit that can pass the electrons in the organic substrates in the soil from the positive electrode to the negative one and form. In principle, a battery or microbial fuel cell is created that produces electricity, carbon dioxide, and water.

MudWatt™ fuel cells demonstrate this simple principle as a commercial product. They work with two pieces of carbon felt electrodes, a container, and scoops of soil. Once set-up, the batteries start producing electricity (~0.5-07 Volts, 20-50 miliamps) in a matter of 2-3 days and run a s long as 2-3 months until there is no organic compound left in the soil.

This simple set-up is a complex biological design that involves many interactions. While being black boxed in the container, the soil hosts an entire ecology of microorganisms—geobacteria and others—that live together and produce the necessary means for mutual survival.

The electrical output of MudWatt™ batteries are certainly lower than fuel cells made in industrial settings. Microbial species available in nature are not always capable of passing the electrons outside their membranes, and therefore need chemical mediators that will carry the electrons outside the organism. Fuel cells highly benefit from a proton exchange membrane between the anodic and the cathodic compartments that increase the energy transfer.

The ability to design a system with an entire ecology available in an everyday environment rather than bringing together individual components—the optimized microorganisms, the best catalyzing agents that will increase electron mediation, and so on—marks an important conceptual shift in the design process. The MudWatts™ certainly lack the efficiency made of optimized components, but demonstrate a higher level design intervention which is fundamentally spatial and is nothing but bracketing an unknown set of parts between two conductive layers in an airtight container in principle. This shift also extends the view on ecological design, which is not limited to making large-scale changes in landscape, riverbeds, and deforestation, but also in a micro-scale where individual members of a system may not be identified or known. However, the effects of their interaction can have measurable outcomes.

Unlike MudWatt™ batteries, Carolina® Microbial Fuel Cells, are sold as a set of parts—from the yeasts to their food supplies, exchange membrane, and so on. These parts can be assembled for a similar

electrical performance. While pedagogy of the latter kit aims for a more scientific audience, it not only provides more details about the process and a description of what type of roles individual parts take in the circuit, but also provides a standardized set of components that can be repeatedly used to achieve the similar output. MudWatt™ batteries can include soil that can vary in terms of concentration of organic compounds. Included are types of bacteria and unknown elements that can catalyze or slow down the reactions; it would not be possible to fully anticipate the outcome and the types of byproducts that can emerge as a result of the biochemical interactions.

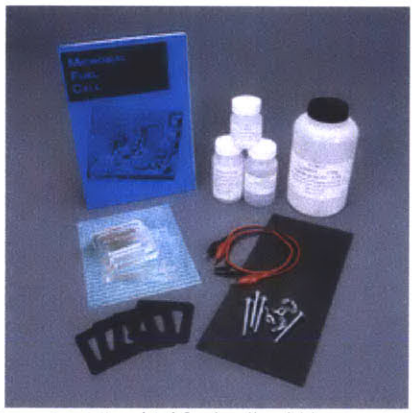


Figure 14. Microbial fuel cell sold by Carolina.
Source: Carolina.com

Both kits also point to different design affordances regarding spatial design practices. The ability to use an existing microbial ecology in a local context—in marine environment or landscape—points to additional benefits besides electrical production. While even the most efficient microbial fuel cells cannot compete with the energy density of combustible fuel resources, they can be set-up for large scale waste water treatment, ocean desalination, or hydrogen production—which focuses on the byproducts of the reactions instead of the energy production. Once embedded inside in a larger ecology, artificially manipulated microbial ecologies can be incorporated to other biological circuitry to degrade unwanted organic compounds such as removing sulfites from water streams (Zhuwei et al. 2007). A stack of microbial cells equipped with desalination chambers are also demonstrated to desalinate ocean

water and produce similar amounts of current to microbial fuel cells that are in equivalent form factors (Kim and Logan 2011).

Chapter 3 – Designing the Living

Biological design spans across a broad set of techniques, technologies, processes, and methods inherited from different disciplines. Like most other design fields, its scope and objectives are inherently tied to human intentions, which respond to old or new needs, values, and ideals that continuously evolve over time. In this chapter, I present an overview of different biological design frameworks that have emerged in the past eighty years and trace the evolution from information-based, relational, to synthesis-driven models of biological design in order to build a foundation for a spatial design framework. I will introduce this framework in the coming chapters. I will cover a period that begins with the formulation of first physicochemical understanding of the living based on molecular abstractions, expand it with the impact of the discovery of DNA, and end with today's synthetic biology. This field explores the design of the living by incorporating design methods from fields as diverse as chemistry, electrical engineering, computer science, product design and architecture.

Our capacity to design living matter relies very much on the analysis and synthesis techniques that have developed since 1930s. As Lily Kay argues, this is a time when the "molecular vision of life" dominates both the abstractions and logics for studying, designing and making the living and, inevitably, transforming our expectations from it (Kay 1996, 3). This vision is also the precursor for the "Information-based Biology" of the 1950s. By integrating techniques, ideas and ideologies from Information Theory, cybernetics, communication, and control engineering the molecular vision ultimately lays the foundations of the current understanding of synthetic biology. As I trace key processes, technologies, and techniques from Kay and others' critical reading of these periods, my goal is to provide a brief history of biology to the reader with highlights from major threads of research that constructed the molecular and information-based views of the living. These philosophies prefaced most of today's design methods. Here, my ultimate aim is to propose alternative ways to look at 'design-

driven' or 'intentional' biology to be able to uncover a more discursive design space than the approaches of rational design that drives the current practices in life sciences or engineering.

The analysis and synthesis methods used by most design fields are closely related to the abstraction paradigms and models that govern the field's imagination. From agricultural to industrial and information-based societies, these methods provided every period unique vocabulary and capacity to respond to the values and ideals of their times. Some methods are usually developed in response to the advances in production techniques. Some methods set the objectives that drive new technologies. All align with the social, cultural and political agendas of the time. Here, I will start by introducing a series of motivations behind the design of the living as part of an extended history of abstraction methods that render the living as functional units or discrete building blocks. I will use this as an underlying theme to be able to register the variations among different approaches in different periods. Then, I will focus on the kind of methods developed to assemble these units in different logics, and discuss how this line of thinking ultimately relates to the design of different embodiments and new biological contexts including: artifacts, products, and consumables that ultimately shape the way the society engages with synthesized life. I will emphasize the products and process-driven nature of biological design to discuss impact on the modern design of the living and what alternative ideologies they may serve in the future.

3.1 Life at the Molecular Level

The vision of life at the molecular level, for Kay, is simultaneously a scientific and an ideological project pursued both by the scientist and their patrons of post First World War America. The goal is to "direct the study of the animate along selected paths towards a shared vision of science and society" (Kay 1996, 3);. This vision integrates scientific ideals with the economic needs and political agendas of this period.

The molecular vision is primarily responsible for building a unifying framework to study life; it focuses on identifying the principles behind common biological processes such as reproduction, respiration, and

digestion. It follows a reductionist logic and intends to build generalizations and models explain phenomena across all organisms. Jacques Monod famously explained the transferable nature of this ideology: "anything found to be true of *Escherichia coli* must also be true of elephants (Friedmann 2004)."

At the molecular level, all living can be abstracted from their biological context and described as chemical processes. Molecular Biology investigates the interaction between the biologic and non-biological, or the inanimate basis of the living. This field relentlessly tries to locate the point at which the living begins to transition from being a merely chemical construct to autonomy that can reproduce and maintain its survival. As the molecular space requires a high-level of abstraction, it avoids considering interactions between organisms versus organisms and their environments. Once living organisms are reduced to their parts and observed as a set of physical and chemical reactions among macromolecules, it facilitates understanding of an 'ahistorical' view of life. This view discounts the emergent and evolutionary aspects of organisms. Often the living is observed in a short time domain, without considering the causal reasons predicating current embodiment. Instead, the focus shifts towards upward causation—what the current state of the molecular entity may do and how will it affect the next steps (Kay 1996).

According to Kay, the molecular vision is also defined by a number of technical advances of its times. The electron microscope, ultracentrifuge machines, electrophoresis, spectroscopy, and x-ray diffraction techniques are some common equipment used at that area to define the salient features of life through the language of molecules. Around 1930, they were considered to be proteins. As this is the time before the discovery of the DNA structure, and the molecular machinery of protein synthesis, proteins and amino acids themselves were considered to be the units of the living responsible for the transmission of hereditary traits.

Visualizing the living at a specific microscopic range, 10^{-6} to 10^{-8} cm, makes the molecular vision of the 1930-40 inevitably a myopic vision, as it would render life only within a limited visual domain compared to what we know today.

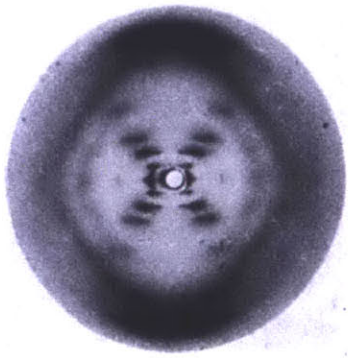


Figure 1. "Photo 51" also known as the X-Ray diffraction image of DNA taken by Rosalind Franklin's lab.
Source: Wikipedia

It took decades until the x-ray crystallography image of the DNA taken by Rosalind Franklin's laboratory changed that view and informed James Watson and Francis Crick about the possible structure of the DNA. They published "A Structure for Deoxyribose Nucleic Acid" in *Nature* on April 25, 1953 (Watson and Crick 1953). The discovery of the double-helix lead to a model that accurately described the compositional details of DNA with its constituent parts (e.g., the nucleobases Adenine, Guanine, Thymine, etc.) and its role in passing hereditary information: "It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material (Watson and Crick 1953, 737)."

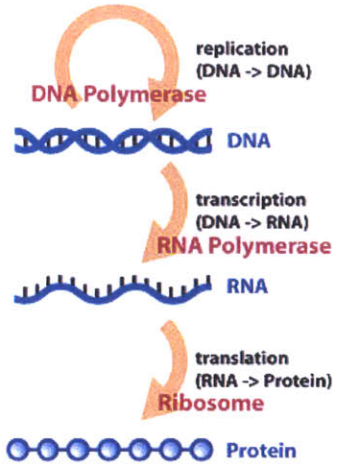


Figure 2. Central Dogma
Source: Wikipedia

DNA makes RNA, and RNA aligns the proteins in all living organisms. First stated in 1958 by Crick as the "Central Dogma of Molecular Biology," our understanding of protein synthesis became one of the most important abstractions in the history of biological design. It described a model of information transfer among molecules. The process originates when a DNA strand duplicates itself to be able to copy the specific ordering of its nucleobases onto a separate strand of nucleic acid molecule. This new strand called messenger RNA, then moves towards a subcellular part called 'ribosome,' which utilizes the nucleobase order like a template to bind a specific sequence of amino acid molecules with each other to make proteins.

When different orderings of the nucleobases are used to build amino acids not with their material presence but rather with their indirect influence on the ribosomal machinery, the mechanism immediately invites an information-centric interpretation of the process of molecular synthesis. Also known as the "replication-transcription- translation process" (See Figure 2) the Central Dogma today is contested, yet still widely accepted as the one-way information transfer model from DNA to proteins within a molecular framework. It is still referred to as one-way transfer because once the proteins are produced they will primarily work towards building things up for more complex organizations such as

cells, tissues, and organs and not influence the information on the original DNA that caused it. However, as I will discuss later, once the molecular view broadened, significant progress in understanding the complexity of protein-DNA interactions has been achieved. This progress has influenced the protein synthesis process in a multitude of ways.

3.2 Life as Information (Scriptural Model)

The information-centric view of living has been established by a number of different agendas besides the need for supporting the molecular vision. According to Kay, it is also closely tied to the desire to see life in scriptural representations: as a series of codes, words and messages that form the hereditary basis of living matter. In letter form, life can be read and written. This has changed the discourse of Judeo-Christian religious order which states the "word" as the origin, but also suited the needs for the post-Industrial Second World War economy, which was building its financial infrastructure on the production, processing, control, and distribution of information (Kay 1998, 611).

The production of biological knowledge, since 1950s, is often informed by advances in information theory and cybernetics that provide mathematical abstractions and formalisms for quantifying and measuring information for domains as broad as autonomous biological processes, self-correcting anti-missile war machinery, and communication networks. Kay's analysis of this period through a critical review of Norbert Weiner, Claude Shannon, Von Neumann, and Warren Weaver foregrounds these scientists' attempts to introduce their theories to a broader scientific community to be able to make alliances between fields and build a unifying vision. Influenced by the biological discoveries of their times, Von Neumann was especially inclined to translate his model of computational automata to self-replicating systems and to find the digital equivalences of the gene copying processes. Likewise, by studying homeostasis and the self-preserving characteristics of the living, Weiner, a former physiologist,

generalized his concept of feedback to be applicable for the control and communication of both animals and machinery (Wiener 1948).

The "Mathematical Model of Communication," as formulated by Shannon in 1948 states that all communication be represented by units of ones or zeros regardless of the source of the information (Shannon 1948). By stripping meaning from the transmitted message, it also presents a semantic-free model. Thus "information is a measure of freedom of choice when one selects a message" (Kay 1998, 611). This presumes that biologically speaking, anything can be picked as a meaningful unit including a message in a sequence of nucleobases called 'genes.' Then taking genes from one organism and placing them to another one can be seen as a means of communication.

Communication and control are therefore the two sides of the same coin for Kay. The measurement of information is not only relevant to be able to quantify the redundancy or noise in a stream of communication but also is a process that identifies its elemental units—bits, codes, messages, and words—that can be controlled during the process. This new paradigm immediately attracted biologists such as Haldene make attempts to describe the information content including noise and signal ratio, of living cells and therefore quantify and qualify their role among the living:

"I suspect that a large amount of an animal or plant is redundant because it has to take some trouble to get accurately reproduced, and there is a lot of noise around. A mutation seems to be a bit of noise which gets incorporated into a message. If I could see heredity in terms of message and noise I could get somewhere (Haldene Quoted in Kay) (Kay 2000, 87)."

3.3. Life as Systems (Relational Model I)

Molecular biology can be identified as a counter movement towards the top-down approach of the study of the living, inherited from studies in zoology, physiology and medicine, which were interested in studying the form, function, behavior and purpose of the living starting from the perspective of the complex and already developed organism.

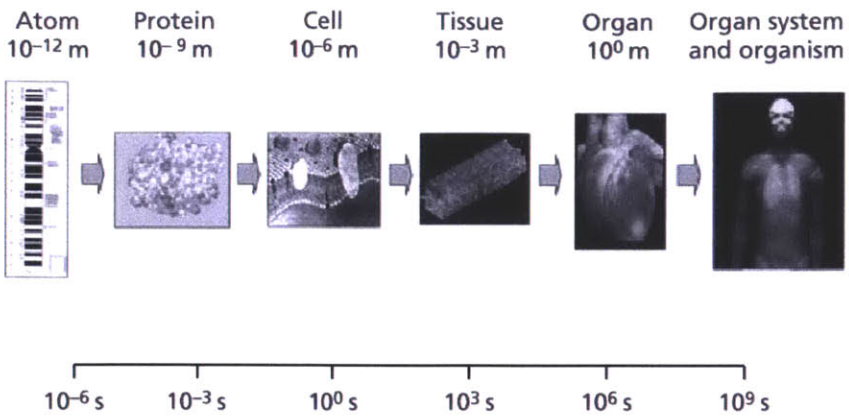


Figure 3. Molecular Resolution of Life
Source: Noble 2006

systems biology, historically, grew out of a need to reconcile the difference between top-down and bottom-up according to Denis Noble. It expands the scopic view of the molecular world both spatially and temporally in order to take into account complex interactions between the different organizations in high-level organisms.

The field expands the information-centric view of the body and utilizes mathematical and computational methods to explain biological processes among multiple parts, components, and elements. It uses different levels of feedback as the underlying motive to describe these interactions as systems and networks. Thus organisms can be broken into different resolutions (Figure 3) and parts can be observed both for their downward and upward causation. This dynamic view can utilize the same components of Molecular Biology but offer alternative modes of interaction between them.

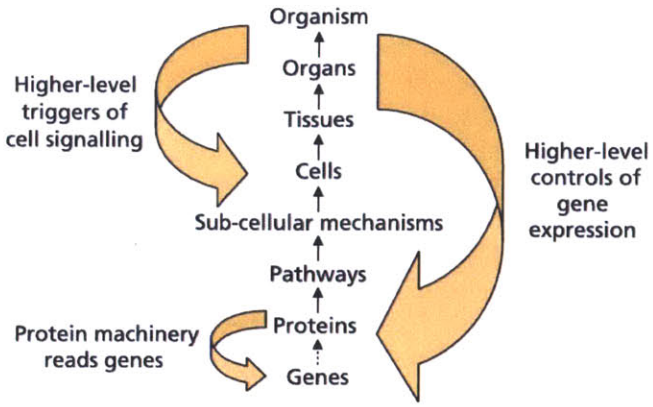


Figure 4. Top-down, bottom up
Source: Noble 2006

Life, at the higher level, needs many explanations to describe the functioning of complex processes. For example, pumping blood to the heart or the communication among the nerves in the brain. The pumping of the heart may require a coordinated activity of hundreds of genes, and proteins. Here, the Central Dogma would only be capable of describing the production of the proteins, but would not be sufficient enough to describe how unique hearth cells are generated from a genome that is common to every cell in the body. Nor can it explain how and why the cells coordinate an emergent activity such as oscillation behavior, which cannot be prescribed by a sequence of nucleobases at the DNA. Genes are blind to the activities their products perform (Noble 2006, 34).

For Noble, a computer simulation of the virtual hearth is the better approach. A life-like mathematical construct can in fact provide more information regarding the feedback loops and chemical exchanges among the cells to coordinate a seamlessly precise single beat and be able repeat this over 60 times a minute till the entire lifespan of the organism. As he elegantly compares it with Magritte's painting "This is not a pipe," Noble's work is not a hearth, but according to him a "convincing" reconstruction of it through representation of the biochemical pathways, gene expressions, neural pathways, etc (Noble 2006, 84-86).

Systems Biology is interested in finding a middle ground, while well aware that it would not be able to provide the full picture. The goal is to accumulate enough information for each level of the desired activity, not only to be able to study what it is, but also reactions to different interventions. The view of the lower level will always be partial, but can be selected by the filter of the high level (Noble 2006, 81).

The field, like most design fields that use simulations, is interested in predicting or modeling how a component would behave under different conditions with respect to different constraints or interventions introduced over time. When it knows the function and behavior of certain cells *a priori*, it can go back and try to reconstruct what it's seen to be emerging. Noble's research is consulted among a number of pharmaceutical companies as reference on the effects of new medicine on cardiovascular activity.

The information-based model aspires to be integrative, but as also admitted by Noble, still has to remain reductionist in nature. It has to comply with the reductionist principles of Molecular Biology to be regarded equally as 'Hard Science;' and also has to work with the inherent limits of the logic imposed by combinatorial view of computation. When the goal is to study gene interactions to be able to model the function and behavior of proteins, for example, the work quickly runs into a "combinatorial explosion:"

> "[...] if just two genes were required to co-operate to generate a biological function, how many possible functions would there be for a genome of 30 000 [genes]. The answer is 30 000 x 29 000 / 2 which is 449 985 000. So even with this minimal number of genes per function, there could be nearly 500 million different biological functions (Noble 2006, 28).

Genes also have multiple functions, besides a single gene can be used in the production of multiple proteins the complexity scales even further. As multiple proteins are involved in studying biological functions, there is no clear way to draw a line between how many genes has to be incorporated into a study model. Bottom up construction using computational methods simply is not feasible to describe the heart (Noble 2006, 75). However, Noble demonstrates the possibility of a "successful" simulation of

a heart with 100 biological components, which would be the 2% of 5000 genes that may actually be involved in a real heart (Noble 2006, 86). He calls this middle-ground reductionism "quantitative computational physiology;" a name that integrates three disciplines in one field (Noble 2006, 90).

Compared to the molecular view, this way of reasoning certainly offers a relatively more scaled up and more dynamic view to study the interaction between different components of a living organism. However, it is important to note that both the selection of the objects and the framing of the clusters, groups or organizations are highly influenced by the popular scientific paradigms, motives or patterns of the times.

The modeling of interactions starts as single then multiple functions; seeing multi-part organizations as systems, networks or circuits; Modeling change first as discrete, then in continues domains; reducing the errors of deterministic calculations with probabilistic measures, certainly reflect an interdisciplinary spirit both in methods and objectives. It also shows how shared metaphors, mannerisms, and styles of reasoning influence each discipline.

Noble's extended view also integrates a number of limits and bottlenecks. It can avoid combinatorial explosion problems in simulations at the expense of adopting a representation technique that will write, define, and visualize an abstraction that can only provide more elaborate stand-ins or approximations of the living.

The complexity of the level of abstraction is almost always partial—or good enough—for the intended purpose. However, as long as there is a clear intention—such as inventing a new scientific field and providing explanation for its need in relation to certain economic agenda—there are certainly ways to justify and evaluate the successes of a model accordingly.

As a simulated approach, it would be hard to argue that Systems Biology can discover anything that it cannot account for already in the beginning. As a closed system, simulation-driven approaches cannot predict the emergence of new functions from results of processes that were not established before. While simulations are known to be very good in dealing with the complexity of too much information—making combinatorial trials to find the logic within the complexity—they can only be successful in a limited scope. In fact, in Rodney Brooks' words: computer simulations are 'doomed to succeed' as they almost always find a solution from a solution space where all possibilities are perhaps not enumerated but expected within certain margins of possibility (Brooks and Mataric 1993).

I will reflect more on the intentions behind alternative approaches and their implications in the coming sections.

3.4 Life as Synthesis (Relational Model II)

Uri Alon, in his book "Introduction to Systems Biology," uses computational motifs and patterns to describe the functioning of genetic circuits that can in principle be used to explain the basis of adaptive behaviors, system level self-regulation, or neural networks (Alon 2006).

This approach takes Weiner, von Neumann, and Shannon's control and communication paradigms to a next level, yet Alon calls his work a study of "design principles." This subtle detail has numerous consequences. Once it is articulated as a design language, while complementary in nature, the project deviates from the typical scientific intention. It not only imposes a design-centric view of explaining the interactions among living phenomena but also shows ways to diverge from the existing ways to study the living. Regardless of their claim—whether they provide partial or full views for the workings of complex systems—these abstractions use living matter for different purposes. They may build on existing units of the living, but work with them using different logics. Alon's designs for biological

oscillators, gates, and switching circuits are based on a design vocabulary borrowed from other disciplines. They are not biologically evolved through the mechanisms of Evolution to function as such.

As we move towards synthetic biology, today it is possible to trace some of the earlier historical models inherited from molecular and information-centric biology used to govern the study, control, and design of living systems. However, while there are significant similarities among engineering-driven approaches to Biology, it would be important to emphasize the difference between the scriptural representations of the living inherited from earlier models of information theory (those that utilize non-linear, network-driven representations).

The 1960s to the 1990s is an important period. At this time, abstractions inherited from the reductionist molecular view of the world were contested by Systems Theory, which began to incorporate broader interactions between biological processes. This was done to explain both upward and downward causations and model processes as mathematical models and functions with varying inputs and outputs.

Being conceived in similar grounds, design paradigms from computer science and engineering draws parallels with a systems-oriented modern biological design. Biological products are designed as abstract chemical or fluidic automata. Biological functions are grouped in modules and assembled like electronic circuits or networks of signaling pathways. The kinetics of living systems are modeled with differential equations and controlled by numerical methods (Purnick and Weiss 2009).

Today, synthetic biology intends to build the biological equivalents of electronic parts such as transistors, oscillators, logic gates, and chips that can be assembled together to build complex biological machinery. This approach is often compared to Mead and Conway's methods for designing large scale integrated circuits. Their work "Introduction to VLSI Design" (1979) is a cornerstone in electrical engineering that bridged the gap between the design and fabrication of very large integrated hardware systems (Mead and Conway 1979). The premise of VLSI design is quite simple: It allows system designers

to compose circuits, hierarchical abstractions, by hiding the inner-workings of the components or their fabrication methods. The design method is based on the ability to 'black box' the function of unknown parts, as each part is expected to operate like a module within a predictable margin of error.

Synthetic biology embraces the VLSI logic to abstract the biological design process from unit-based and modular design to computation-driven synthesis techniques. The level of abstraction follows the same logic: sequences of DNA molecules are 'packed' as discrete parts. Parts with different functions can then be combined with each other to build devices. Devices are eventually ordered based on computational logic for the design of biological circuitry.

The field aspires to build biological systems like computational hardware in which networks of components can work with each other in a predictable manner to deliver complex tasks (Endy 2005). Synthetic biology models chemical processes among organisms to compose complex interactions, utilizes rational design principles to standardize biological parts to be able to design scalable biological circuits, and programs new functions, features, and behaviors for existing organisms that cannot evolve through the evolutionary mechanisms of nature.

Synthetic biology is rooted in engineering. Its objectives are based on the regulation and control of living systems to be able to generate new ones. The field is one of the first in applying design methods that are not driven directly from nature but rather inherited from control theory, information theory, electronics, and computation. Synthetic biology shifts its frame of reference away from the driving force of evolution—natural selection—and intends to conceive organisms with features that are targeted for specific purposes.

K12, a laboratory strain of *Escherichia coli,* for example, can be engineered to give out different wavelengths of light when it is equipped with genes from different organisms such as fireflies or glowing bacteria. Unlike before, K12's genome can be designed like a computer circuit. The *Escherichia coli* can

be made to produce the genes that allow it to emit light only when it is triggered to do so. For example, it responds to the concentration of a particular type of pollutant in the environment. The organism, then, can be part of a larger decision-making system, a biosensor, where it can be used like an 'AND' gate. If the output of two different biosensors matches, then the light output can trigger response in another organism such that it can start breaking down the chemicals of the polluting matter. Thus, the existing negative and positive feedback loops among single-cell organisms can be manipulated differently to create an environmental remediation system that can eventually run within alternative biological contexts such as inside animal and human bodies for different medicinal uses.

3.5 Life Before the Living (Spatial Model)

Synthetic biology as an emerging field has many different voices in it. While rational design methods embrace a top-down approach that is geared towards simplifying complexity to understandable parts; another side, which focuses on the molecular basis of the living drives the field towards chemical synthetic biology, which intends to explain the biological basis of the living using the principles of Chemistry and Physics and then build new forms of living by assembling them from the most basic chemical components (Luisi and Chiarabelli et al. 2012, 157).

Synthesis does not mean the same in every field. While the engineering approach can be primarily concerned with using 'natural' biological parts like gene sequences, proteins, or biomolecules found in nature to build systems by re-contextualizing these parts for new functions that are not evolved by nature. Chemical synthetic biologists explore the synthesis "unnatural" parts to design new biologies. Driven by the desire to uncover the chemical-basis of biological behavior such as self-assembly, replication, adaptation and evolution, chemists build molecular constructs that may provide explanations for the origin of life.

Thus, at the intersection of biotic, pre-biotic, and abiotic lies a design space for artificially created DNA molecules, synthetic genetic codes, and designer proteins made of chemically synthesized amino acids that are not at Nature's disposal. Chemical synthetic biology assumes even a lower level view than Molecular Biology. However, rather than the resolution or size of the components, what matters here is the mixing of the new and old principles regarding what we used to know as living. Thus, instead of abstracting out the known parts of the living to be able to compose complex, yet calculable and predictable relationships, the chemical designers, seek for emergence, in which a set of parts can show a different behavior than what they would normally do in isolation.

In this respect, chemical cells—also referred as chemical cells (chells) or protocells—use lipid-based membranes that are designed to create artificial encapsulations. As the cells encapsulate different chemicals, they can mimic the design of simple prokaryotic cells and can be used to study the types of chemical events that may have caused life emerge from non-living matter. This is a phenomena referred as abiogenesis (Luisi et al. 2004; Luisi et al. 2006). While the artificial cells have been used as research objects to study the biochemical origins, they also have begin to embrace different roles next to, even sometimes within, living cells as special types of containers or transporters. For example, they are designed to run parallel metabolisms within real cells, to deliver chemicals within different parts of the body, and to execute site-specific tasks such as gene therapy (Filipovska and Rackham 2008).

While protocells do not yet arrive to the complexity of single cell organisms, they promise different affordances. They provide a much more constrained environment that can be engineered with rather predictable outcomes. The fear of 'bioerror': unintentional creation and release of inorganic organisms that can cause harm in the outside ecosystem, applies less to biochemical artifacts. Biochemical artifacts are expected to have less potential to interface with the rest of the organisms in the nature. Thus, not being 'truly' alive in the cellular sense, they cannot reproduce or maintain a continuous metabolism that

would allow them to survive independently like other cell-based organisms in nature. In short, protocells are chemically and biologically confined with their designer's intentions and capabilities.

While still very new, protocells already found a small role with the language of contemporary architecture, namely in the works of Rachel Armstrong and Philip Beesley. While in Armstrong's work aspires to use protocells to design "metabolic" materials that would allow buildings to respond to the changes in the environment, and participate in more sustainable architectural practices, Beesley explores the potential of using them to generate energy in his immersive installations (Spiller and Armstrong 2011).

Figure 5. Light averse protocells by Rachel Armstrong's Living Architecture. Source: Architectsjournal.co.uk (Left) Protocell Mesh by Philip Beesley. Source: Philipbeesleyarchitect.com (Right)

3.6. Abstractions and Units of Life

As it can be seen from the different motivations that shape techniques and methods of intervention—whether they are based on informational, relational, or spatial models—biological design relies on different types and levels of abstraction. However, abstractions are not always driven by the need to find the smallest, most predictable or controllable elements of the living. They can be selective encapsulations based on a number of different arbitrary or informed criteria. Thus, in a more general

form, abstractions can be described as the desired level of control for the amount of information the designer would like to work with in a design process.

The design of the living is motivated by a diverse set of needs and wants—better food, more effective medicine, or cheap and abundant energy. It is also driven by the curiosity towards explaining the unknown. Manipulating, regulating, or transforming living processes were always complementary to observation, experimentation and analysis. They not only shaped the ways of knowing the living but also made attempts to transcend the limits of that knowledge. Richard Feynman's famously stated, "What I cannot create, I cannot understand" still lies at the hearth of many of the design-driven biologies.

Sciences, engineering and most design-driven disciplines have always intervened with the living through tinkering, trial-and-error, and experimental methods—without necessarily knowing many of the underlying details. Seeds collected from different places were tried in new environments without a complete understanding of the nature of vegetation; animals with different traits were cross-bred for different characteristics long before the discovery of hereditary mechanisms; different flavors of cheese, beer and bread were in human culinary tracts much before it was known that they are produced by yeast, fungi, or bacteria. In other words, living artifacts, like other objects of design, have always been abstracted and black boxed from their regular contexts and opportunistically utilized without a full understanding of their inner workings or even before it was able to visualize them. Fermentation and cheese-making, for example, were practiced for thousands of years before the invention of the microscope.

As knowledge of living matter advances over time so does the intentions and capacity to design. The analytic inquiry towards the living always yields new abstractions including: elements, objects, parts, fragments, and components. These abstractions constitute the living at different levels or resolutions of knowledge. Since the earliest attempts of classification, nature is broken into a series of habitats,

environments into plants and animals, animals into species, organisms into organs, cells, organelles, molecules and so forth. From high to low, top to bottom, precise to the vague, arbitrary to informed, this type of inquiry defines the living at different levels and isolates what is not known from what cannot be known at a given moment.

biological design that stems from the analytic approach almost always works in reverse. It makes attempts to assemble, re-arrange, the living from these abstractions. It also works with these units, sometimes as building blocks which originate from the living and sometimes as ingredients that mix together the living and non-living parts of nature.

The study of the most essential units of living systems—from the molecular composition of cells to the transportation of the information of hereditary characteristics—informs the units of design that can be used to build or rebuild the living (Jacob 1993). For example, while in transgenic design the goal is to abstract the units of the hereditary information (genes) from their current organisms to be able to mobilize them into new biological contexts, synthetic biology intends to build new units. These bricks or standardized building blocks can encapsulate both nature-born or human-made biological functions into standardized components that can then be used to compose complex biological systems that cannot be evolved through evolutionary dynamics.

Thus, the ways to produce a desired offspring through selective breeding has evolved over many abstractions from animal husbandry to *in vitro* fertilization, genetic manipulation, and now to synthetic biology. Chemical cells or artificial compartments can abstract out the entire genetic machinery outside a living body as a process referred as cell-free protein synthesis.

The analytic inquiry is certainly not the only way to study the living. Breaking down into observable or identifiable parts inherently poses a limit to the form of understanding of the living. Thus, as mentioned before, since the second half of 20th Century, in relation to General Systems Theory, Information Theory,

and cybernetics especially, the study of the living devised alternative methods to approach living matter as holistic, indivisible systems, signaling pathways or interrelated networks. It became clear that once abstracted out of from the complex whole, certain parts may no longer behave the same way as they would in their original context. Regardless of how inclusive an abstraction can be, it would always have to leave out some important detail that may explain the core function and behavior.

Today, the field of metagenomics, intends to offer even a broader view on the understanding of hereditary information by looking at the role of genes across entire populations. Instead of focusing the individual genetic signatures of species, it uses methods to sequence genomes of communities of bacteria and work towards building a more diverse view of the living that is based on sampling DNA from environments, habitats, and ecologies, where living is studied at its own context instead of an artificial environment. metagenomics, inherently tries to find ways to eliminate the shortcomings of laboratory protocols that are developed to grow previously known species of microorganisms. "What I cannot culture, I cannot know" is a bottleneck for new discoveries as formerly established culturing protocols can often be ineffective for growing unknown species that prefer radically different environmental conditions such as extreme temperatures, pressure, pH levels, and so forth. The field also aims to reduce the bias caused by abstractions that are based on what is already known. Polymerase chain reaction (PCR) is a process that is commonly used to amplify and duplicate gene sequences. For example short DNA sequences called 'primers' are used to allow new fragments to bind and replicate. Hence, when an unknown piece of DNA is processed, the choice of primers used during the process would impose a certain preference towards what can be duplicated or not.

Throughout history, biological design has not only evolved in terms of new abstractions but also in its ways to apply different logics to bring together parts and building blocks. For example different design-driven methods utilize the black box approach differently—sometimes even with the same parts—when

the objective is to build regulation and control systems, efficient synthesis methods, or generative processes that need to maximize variation and differentiation. Black boxing, has also acquired multiple meanings and purposes over time. It is first and foremost used for standardization, generalization, and orthogonality. In principle, once a biological component can be isolated from its environment and treated as a discrete, self contained element, it can interface with different contexts regardless of its origin. Standard parts can be grouped into modules or libraries, which can then be combined for multiple features. Encapsulation here not only allows each unit to embody a single feature or function but also make it a single point of failure if it cannot deliver that function. Combinatorial growth then provides the basis of scalability and ultimately promises higher level, hierarchical and complex organizations that can be composed of more parts and more relationships. In theory, if a system can be built out of predictable building blocks, the overall organization would yield predictable results, as the entire system will run within the margins set by the individual units.

As the management of complexity requires different levels of regulation and control of the black boxing, it is a useful strategy for building systems that would operate within a predictable margin of error. When systems need to be composed as input output, cause and effect relationships, encapsulation allows them to utilize orthogonal parts that would produce not only reliable but also deterministic systems. So one can design a module X, whose function would be to receive type A input, to be able to output type B. So, if B is found in the environment, it is very likely that A is also present in the environment.

Similarly, biological processes A and B can be designed such that the presence or absence of their outcomes can trigger cascading effects. If A is present and B is not, C will happen. Ultimately, this form of abstraction can be useful to be able to transfer the compositional language or logic of standardized formal systems to each other. Algebra, Boolean logic—or any symbolic system, which can lend itself to a

formal representation—can easily be implemented with the combinatorial arrangement of computational building blocks known as gates.

Such standardization also introduces the ability of quantification and qualification—in the form of more precise management of input, output relationships, or calculating difference or change over time. This naturally introduces the concept of performance, and yield with respect to values such as efficacy, efficiency, and optimality.

Designing for repeatability also differs depending on how much ambiguity is allowed into the process. In this regard, rational design process not only differs based on the amount of black boxing but also the type of black boxing and how that type of black boxing is maintained through the lifetime of the design.

When the objective is to guarantee an outcome within predictable margins, one can use black boxing either to eliminate complexity by reducing the set of unknown interactions to a calculable approximation (e.g., a transistor), or use it to create more complexity by grouping many black boxed parts together by assuming that the final outcome will be mostly determined by the known properties (e.g., building chips with many transistors). Thus, black boxing what is known is inherently be different from black boxing what is unknown, and the same strategy can be used to arrive completely opposite results.

It is often also assumed that abstractions would preserve their identity and status both throughout the design process and during the actual lifetime of the design. Unless there is failure or accident that is external to the design, black boxed components are assumed to fulfill the same function over time. What is known or unknown is expected to remain the same within the margin of error. The parts that make the engine of a car operate within the same abstraction scheme throughout the lifetime of the car. The amount of predictable fatigue or failure is often built into to the function and calculable performance of that function. This allows parts to become interchangeable with each other. Thus,

components with different lifespans can be substituted over time by different manufacturers, who do not need to know whole car system, but instead have an understanding of specifics of its parts.

Different black boxing methods are useful for different design problems. If the goal is to engineer a bridge that need to span across tens of miles, build complex aircraft that is made of hundreds of thousands of components, or design electronic or computational hardware that need to process signals in gigahertz-frequency, standardization and generalization yield predictable outcomes.

However, even the design of a control or decision-making may serve for different purposes. An airport traffic system requires a different type of rational control than a design that tries to control the maximum variability of a process. In Chemistry, computational methods can be used for discovering new functions for existing molecules, such as drug discovery. In biology, on the other hand, 'targeted' mutations or directed evolution of organisms are processes that are designed to generate emerging outcomes that cannot be evolved through natural means.

However, when living organisms, such as bacteria are used as building blocks, the abstraction process inherently is different than the design of electro-mechanical systems. The continuously changing materiality of the living, the infinitely complex set of interactions they have among themselves and with their environment require different strategies of abstraction, unit-making, and logics of assembly both for implementing the existing strategies of rational design and also for opening up the design process to new methods.

The following chart maps a vocabulary of abstractions utilized in rational design, their intended objectives and assessment methods (Figure 7). In the next section, I will provide examples, how different methods of abstractions are utilized for during the design of different biological contexts.

BIOLOGICAL ABSTRACTION

UNITS	ASSEMBLY	MULTIPLE UNITS
Fragment	Boolean	Module
Part	Algebraic	Library
Component	Rule-based	Whole
Element	Parametric	Message
Object	Grammar-driven	Structure/Form
Unit	Combinatorial	Composition
	Probabilistic	Organism
	Object-oriented	Systems
	Geometry	Networks
		Circuits

INTENTIONS	APPLICATIONS	
Standardization	Modeling	**USES of MODELING**
Generalization	Control & Regulation	
Repeatability	Decision-making	Representation
Unification	Generation	Visualization
Identification		Simulation
Simplification		Measurement
Isolation		Quantifying
Decomposition		Qualifying
Spatialization		
Scaleability	Assesment	**USES of ASSESMENT**
Integration	Synthesis	(Qualitative & Quantitative)
	Assembly	
Hierarchy	Emergence	Performance
Complexity	Interchangeability	Fitness
		Optimality
	Orthogonality	Efficiency
	Specificity	Efficacy
	Explaining Causality	Maximum
	Change	Minimum
	Input-output	Value (Worst <-> Best)
	Function & Behaviors	
	Predictability	**USES of PREDICTABILITY**
		Overcome ambiguity
		Explain difference/change
		Randomness
		Overcome unpredictable
	Spatialization	**USES of SPATIALIZATION**
		Encapsulation
		Containment
		Confinement
		Mobility
		Compartmentalization
		Re-contextualization
	Variance	**USES of VARIABILITY**
		Supervised / Unsupervised change
		Directed / Specified /Underspecified generation
		Computationally / Chemically random process

Figure 7. Biological Abstraction

3.7 From Standardization to Biological Intentionalism

Building with components is the primary means in rational design and engineering. William Sellers, the director of Philadelphia's Franklin Institute in 1864, is frequently cited as the exemplar whom initiated the standardization mindset in United States towards the end of the Civil War (Surowiecki 2002). Sellers' focus was the importance of unifying the threads of a screw and to agree on a national standard that would allow all manufacturers build their hardware for screws, nuts, and bolts that have standardized dimensions, thread counts, and so on. Sellers own proposal, the flat head 60-degree thread screw, got quickly adopted as one of the standards as it was easier to make, required less fabrication tools, and easier also easier to measure and qualify compared to the competing alternatives. According to Surowiecki, Sellers demonstrated such a compelling case for unification that soon all machine shops and government agencies were expected to follow his standards. His became an important measure of progress as it accelerated the adoption of Industrial revolution in North America. His work directed the standardization of many elements from mechanical parts, to software and now synthetic biology.

Today, different degrees and kinds of component- or unit- based functionalism are at the core of almost all modern design disciplines that use mechanistic, combinatorial, or object-oriented design methods. Circuits are built with standardized electrical components such as transistors, gates, integrated chips, construction design details for most architectural design for windows, and doors. Lighting design is picked from building information models. Computers and cars are manufactured by networks of suppliers that use interchangeable OEM parts, which can be used in different designs for different brands. Software is built from code that is encapsulated within objects, libraries, and frameworks such that it can run reliably run on different hardware platform and be developed by different designers.

As biological design is increasingly adopting rational design and engineering methods, it is important to assess this influence on designed objects. While biological designers use similar design, synthesis, and

fabrication methods with engineering, biological artifacts are inherently different. Living matter inherently is not a single medium that can be fully characterized and translated to existing design practices. Basic logic still applies: organisms grow, change, adapt, evolve, and mutate. They have their own means and ways to exchange information with each other. Biological systems cannot be fully controlled and predicted like machinery; at most, they can be regulated to execute the desired functions within tight margins of predictability. Synthetic biological designers can choose parts and processes from databases and compose functions, circuits and decision-making systems using standardized laboratory protocols, but there is high degree of variation in the outcomes. Biological components function both in the time and space domain and often have a finite lifespan. They are often not reusable; they may alter behavior while executing their functions and eventually change such that they cannot be utilized for the same process again. Parts also often perform within host organisms that can range from microorganisms to humans, which would impose their own logic and means of existence.

Both the analytic drive of the Life Sciences—breaking up the living into understandable parts—and the synthetic drive inherited from synthetic chemistry and biological engineering—building up new designs from standardized components—inherently tied to functionalism; a desire to create intentional wholes that are based on the various functions of the units. Functionalism, historically, has been a highly debated concept in Biology. Assigning retrospective functions to systems that have not evolved for a predictable purpose can be misleading and often promote a teleological view of life as intentionalism and functionalism are often found to be correlated (Krohs and Kroes 2009).

Attributing functions to existing biological parts, such as making statements like the "heart is an organ that has the function to pump blood like a motor," inherently relies on many assumptions. As Perlman suggests, it applies language developed for artifacts to naturally biological. A heart assumes its functions within the context of a living body. The system it operates within provides the context for interpreting

the meaning of the actions it performs. While as an object it can be isolated from its original body so that it can be fixed, transported, or substituted with another one, its function is embedded in a framework of other biological parts, as it will cease to operate without their presence. It is functional or dysfunctional always in relation to its context. As an evolved part, the organ cannot be considered outside its history within this context. While it would not be possible to attribute a goal-directed intentionality to the evolutionary framework, the current heart is the result of a selection of many other attempts that either could not survive certain selective pressures due to its own characteristics that are not compatible with the remaining body or due to the body itself that could not survive because of other reasons that is related to the larger context—environment, habitat, ecology—that defines its capacity to survive without a heart. Thus within nature, function of a biological part is almost always tied to the larger framework that attributes the meaning, value, and function to it in relation to other parts. In some cases, it may even be impossible to pick a specific part and evaluate it outside its context such as tissues, cell formations, and so on that would not allow any clear demarcation between themselves and the context where they exist. As mentioned before, this 'inseparability' has become an important research area in different fields—such as microbial ecology, where the living being cannot even be observed living as it cannot be cultured or made survive long enough in an isolated laboratory setting.

When living 'parts' meet human intentionality on the other hand, functionality gets attributed, described or defined mostly within the part itself. Their selection can be based on specific criteria—such as availability, size, cost, and aesthetic choices. While it may not be possible to grow hearts for different characteristics, it is possible to breed cows or dogs with hearts with specific traits. At different resolutions, such as a distinct organisms, plants or animals, or a piece of DNA, it is possible to attribute functionality beyond the original context of what the living things has historically evolved for. In a way biological design relies on the definition and redefinition of functionality such that they can be mobilized, recontextualized, and respatialized among other living beings. Here, standardization is seen

as one possible interface that allows the exchange of parts among different organisms, which can naturally or artificially incorporate each other into their own contexts. While on the DNA level the basis of exchange can be discussed on the molecular resolution and before the organ or organism is formed, the more complex and evolved the part gets, it becomes harder to interface them with each other. Thus, once grown and differentiated exchange gets restricted to species level interchange or to external chemical signaling and communication that operate on different types of standardization.

When living things are created entirely by synthetic means, they can result from entirely goal-oriented functional attributes. Synthetic cells, ribosomes, artificial DNA and other molecular constructs are made to mimic, replicate, or become analogs of Natural parts resemble more designed artifacts—such as motors, pumps, or transistors—which can be fully characterized for standardization, exchangeability, and interfacing options. Then, the current practice of biochemical design can be seen as an attempt to translate what is possible in artifact design to the biological domain, build on its abilities, and look for what else is possible, once certain functionalities are suspended or reconfigured in different contexts.

Living artifacts, regardless of being synthetic or nature-born, preserve a certain agency. They adapt, evolve, survive, change through mutation and differentiation, which is based both from the internal organization (the activity within a membrane) and their relation to an exterior—other parts, systems, networks and context—that incorporates this internal organization. Thus biological design, regardless of whether the agency is created artificially or incorporated from an existing living system, is also a practice for administering different degrees and kinds of vitality. Regulating, reprogramming, and resituating in relation to its capacities provided by nature and the design context, which frames it for a particular utility, task, or function.

In the next sections, I will explain the use of different units and parts used in biological design, how different aspects of intentionalism influence not only their logics and methods of assembly but also their conceivable functions.

3.8 Units and Logics of Assembly of Modern Biological Design

3.8.1 Genes and Recombinant Design

Whether they are discovered, decomposed from other living organisms or synthesized from chemical origins, the units of biological design start at the molecular level. Some of the underlying foundations of contemporary biology lie in the interpretation of the interactions among a series of molecules that are common to all living things.

Most fertile living organisms encode their hereditary information in their genome and pass it to their offspring. Genomes consist of a combination of deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules. DNA and RNA are single or double stranded large molecules that are made of sequences of nucleic acids (Pearson 2006).

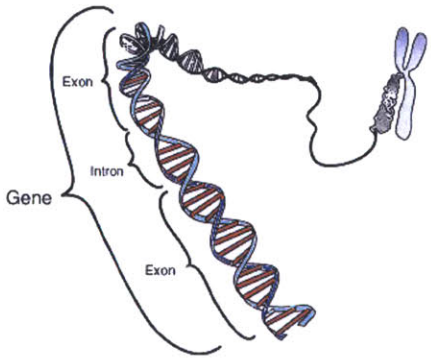


Figure 6. Gene
Source: Wikipedia

Genes are regions located on genomes. They refer to areas with different functional attributes—regulatory regions, and encoding regions where information is encapsulated within molecular sequences. In other words, genes are the units that are responsible for providing the instructions for the synthesis of particular biochemical products such as proteins, or sequences of RNA that are used during the lifetime of the organism. By activating or silencing genes, organisms can differentiate their cells and gain different structures and functions that are necessary for adapting to different biological contexts.

Gene transfer not only gives birth to genetically modified organisms but also provides some of the most fundamental tools for today's biological design. For example, genes that make jellyfish *Aequorea victoria* synthesize the green fluorescent protein (GFP) for bioflorescence can be transferred orthogonally to almost all living organisms from bacteria to mammals. As genomes that incorporate GFP genes become traceable under light microscopy, they function as biomarkers that allow researchers to trace the transfer of genetic materials from organism to organism and to their offspring.

While genes can be isolated to encapsulate orthogonal functionalities, organisms carry genomes that vary in size and organization, thus genetic abstractions that can cross between single and multi-cellular organisms require a special unit-making process. Eukaryotes—higher-level organisms that have cells with a nucleus, and prokaryotes whose cells do not carry one—have different kinds of genomes. While, Eukaryote genes are made of a mix of regions of nucleobases called 'introns' that may not have any direct affect for the genetic functionality and 'exons' which encode the core information of the gene, Prokaryote genes only consist of exons. When Eukaryote genes, such as those used to synthesize insulin in humans, they need to be transferred to *Escherichia coli*, and the DNA sequences have to be converted into a special form called 'complimentary DNAs' (cDNAs). As the ribosomes of the bacteria will not be able to interpret the introns within the human gene, the original DNA sequence of the gene needs to be cleaned from the extra information. The cDNAs are made once the gene information is transcribed to

RNA. Once the area of interest is on the RNA, it can be copied back to a DNA strand with an enzyme called reverse transcriptase without the introns. Reverse transcriptase can utilize the single stranded RNA as a template, and form a double stranded DNA out of it. The newly formed DNA can carry a functionally equivalent yet not an identical version of the gene and be used in for expressing the product of the gene in a new host organism (Hartl and Jones 1999, 350).

Today, genes can be artificially synthesized as fragments of nucleobases with an oligonucleotide synthesis printer, cloned from an existing DNA fragment using the polymerase chain reaction machine, or extracted from DNAs of existing organisms using manual techniques. The practice of genetic engineering, also known as transgenic or recombinant DNA design involves assembling genes to compose genomes with desired functionalities such as the synthesis of a specific protein.

One of the earliest techniques of genetic assembly requires the physical removal of a DNA fragment from a source so that it can be incorporated to another. Custom DNA sequences are produced using special proteins called "restriction enzymes," which can selectively cut a DNA strand from specific places—known as 'restriction sites' or 'recognition sites.' Restriction sites are four to six nucleotide long sequences. They are palindromic sequences, which are the symmetrical inverse on the 5' and 3' direction of the DNA strands. Different restriction sites make cuts with different patterns, EcoRI, a restriction enzyme isolated from *Escherichia coli* can cleave the DNA with the GAATTC / CTTAAG pattern where as XBaI would recognize a TCTAGA/AGATCT pattern to cleave.

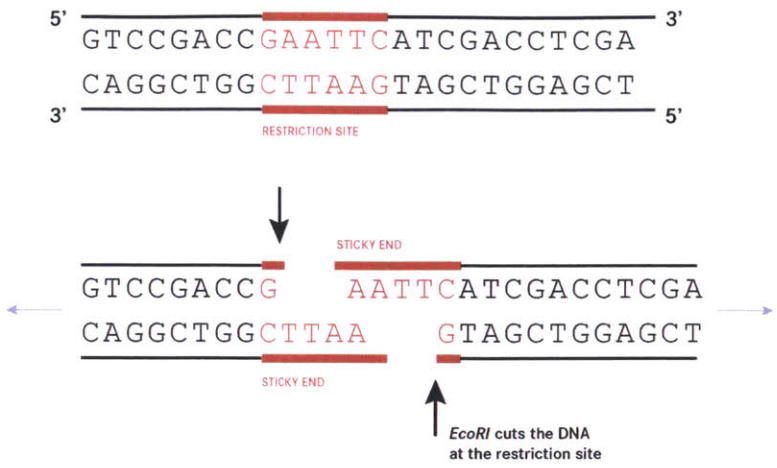


Figure 7. Illustration of EcoRI restriction enzyme.
Source: Figure drawn based on
http://www.emunix.emich.edu/~rwinning/genetics/tech.htm

Different genes of interest are transferred to their destination through a carrier molecule called a vector or plasmid. Plasmids are typically self-replicating DNA molecules that are found in most organisms. While they differ from the chromosomal DNAs that carry the hereditary information, they are used to transfer genetic information from one host organism to another.

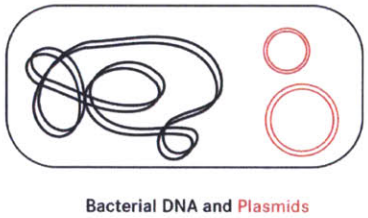


Figure 8. Bacterial DNA and Plasmid.
Source: Figure based on Wikipedia

A plasmid with the EcoRI restriction site can deliver a DNA sequence to a genome that has the same restriction site. Here, the CTTAA sides (also referred as the 'sticky ends') can fuse with another DNA fragment coming from another organism that is also cut by EcoRI or synthesized using a PCR or an oligonucleotide machine with the right ends.

Figure 9

Two fragments are joined together with another enzyme called DNA ligase. The final outcome, the recombinant DNA molecule then can be transferred to another host organism where it can replicate itself.

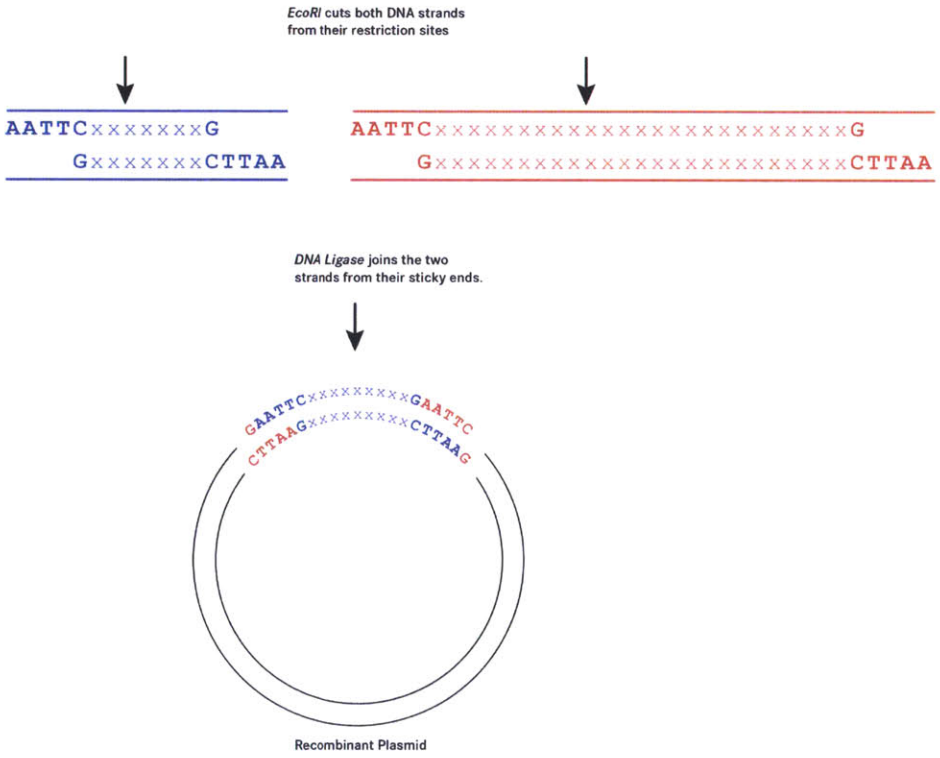
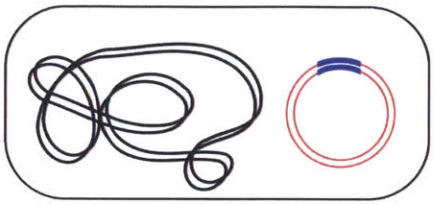


Figure 9-10. Illustration of genes on nucleic acid molecule

Source: Figure drawn based on
http://www.emunix.emich.edu/~rwinning/genetics/tech.htm

Plasmid-containing bacterium can be selected from other bacteria with the introduction of antibiotics. By introducing antibiotics, resistance genes to the plasmid of the desired bacteria, the non-intended bacteria can be filtered out from the environment.



Bacterial DNA and Designed Plasmid

Figure 11.

3.8.2 BioBricks and Their Assembly

BioBricks are a new type of abstraction and design method introduced at MIT in 2003 (Knight 2003). Being rooted in methods of transgenic design, the methods intend to provide both new units and a standardization of assembly of DNA sequences that can generalize Recombinant DNA techniques for broad usage. The bricks are biological parts designed as custom nucleotide sequences. They are refined versions of natural nucleotide sequences and that follow a specific composition suitable for the process of assembly and vector-based delivery. By using the brick scheme, designers can simply "insert" their desired sequences and expect the bricks be compatible with each other (Figure 3). When two bricks are combined each other the composite is another brick with an identifiable function. The compatibility among the bricks assures the repeatability of design processes and offers ways to optimize and automate assembly (Shetty et al. 2008).

```
5' --gca GAATTC GCGGCCGC T TCTAGA G --insert-- T ACTAGT A GCGGCCG CTGCAG gct--
   --cgt CTTAAG CGCCGGCG A ACATCT C ---------- A TGATCA T CGCCGGC GACGTC cga--
         EcoRI  NotI        XbaI               SpeI        NotI     PstI
```

Figure 11. Standard Biobrick Sequence Interface

Source: Knight 2003

BioBricks is an open-source project. It is organized as a registry of parts, and a knowledge repository that is published on a website: Biobricks.org. As the website catalogs all submitted functional sequences that make different parts of the parts—such as starting sequences (promoters), ending sequences (terminators). A physical archive of all parts is also hosted at MIT. As of July 2012, there are more than three thousand biological parts submitted to the archive. Since 2004, the International Genetically Engineered Machinery (iGEM) competition has fostered an international design community that invents new applications using the bricks. As new functions are needed, new bricks are produced and contributed to the registry with the proper documentations.

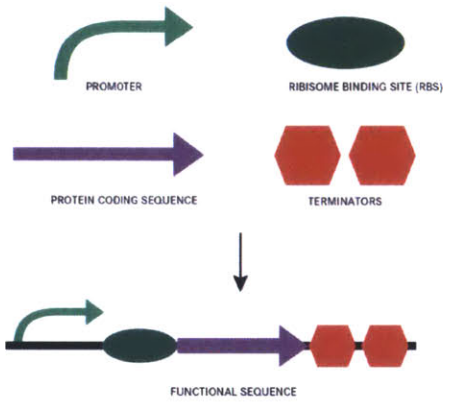


Figure 12. Biobrick design by composing different functional units.
Source: Telhan

BioBricks can be fabricated using oligonucluotide synthesis machines. These machines can chemically deposit and assemble fragments of molecular sequences similar to a printing process that uses nucleobases—adenine, guanine, cytosine, and thymine instead of inks. This method offers a significant advantage over physical cloning methods as it allows multiple steps of gene assembly be made at once. As the cost of synthesis per kilobase pair goes down, oligonucluotide synthesis is becoming increasingly available to designers for prototyping their biological designs.

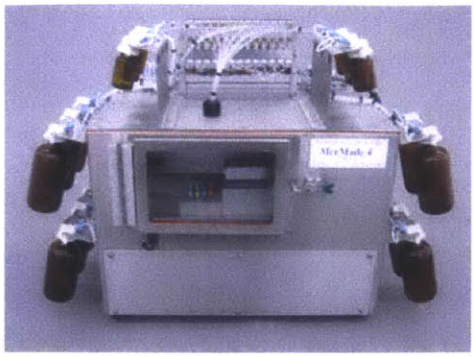


Figure 13. MerMade-4 Oligonucleotide synthesizer
Source: Bioautomation.com

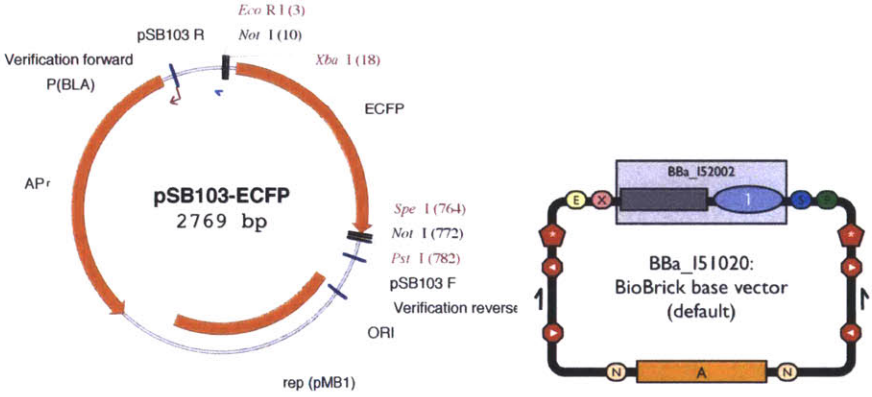


Figure 14. Depiction of Biobrick that can encode
Green florescent protein (GFP) on a non-standard plasmid (Left)
and a BioBrick on a standard base vector
Source: Knight 2003 (left) and Shetty 2008 (right)

Since 2003, BioBrick method has also evolved to standardize the plasmid-based carrier vectors (Shetty et al. 2008). BioBrick vectors provide templates for adding bricks to plasmids and form the basis of an assembly system that abstracts the design process one step further. Bricks ordered from a synthesis company can easily be plugged into ready-made plasmids, which can be combined into a destination plasmid. Here, the assembly method standardizes the use of a particular plasmid that would be compatible with the use of a particular microorganism (e.g., pSB103 for *Escherichia coli*), the restriction sites needed to cleave the plasmid (e.g., sites marked as E, X, S, P in Figure 15). In this design, plasmids

would also include a positive selection marker such as a resistance gene towards an antibiotic, so that the antibiotic can kill the other bacteria in the environment when it is mixed to the growth media.

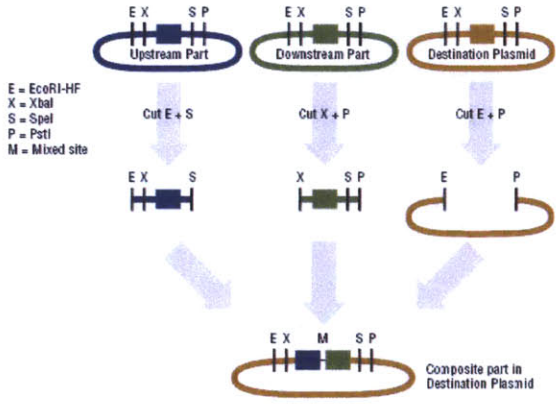


Figure 15. Biobrick Assembly Diagram
Source: New England Bioworks

At the heart of the Biobrick method is the desire to scale up biological design. By combining compatible bricks, the goal is to design interchangeable parts that can be used with each other for combined functionalities. Bricks are assembled together to build devices, modules, libraries, which would allow the design of systems that can exhibit complex behaviors. The common analogy is to see them as LEGO bricks, which are also made of standardized modules that can be combined in numerous ways to be able to design complex objects.

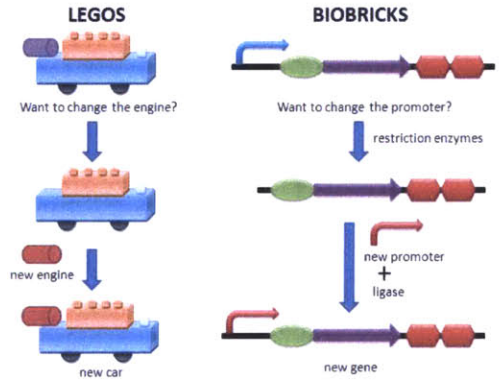


Figure 16. Biobricks compare to LEGO blocks.
Source: Raymond Yip and Nelson Chu

The entire workflow from brick design, plasmid composition and synthesis now can be carried through software. Gene Designer, for example, allows designers to compose their bricks on the plasmids visually, letter-by-letter to optimize their sequences for the desired organism, initiate random mutations to add variability to the output, receive quotes for their final designs, and finally have their sequences synthesized and delivered.

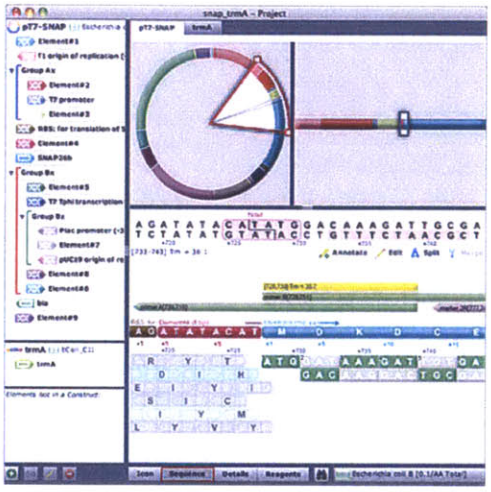


Figure17. Gene Designer 2.0 Software Interface
Source: Dna20.com

The need for assembling multiple DNA sequences at once had also driven research for alternative methods of assembly. In 2009, The Gibson Assembly emerged as part of the work done for the synthesis of the artificial genome by the J. Craig Venters Institute (Gibson et al. 2009). These methods allow more than ten fragments with blunt, non-sticky ends to join together in parallel within a single reaction. Instead of the restriction sites, the technique relies on overlapping DNA fragments that can be digested and rejoined by enzymes. As the method eliminates the need for restriction sites and uses an enzyme (DNA ligase) that repairs the joining areas, it is known as a scar-free assembly method that can eliminate the problems caused by the impurities in assembled DNA sequence.

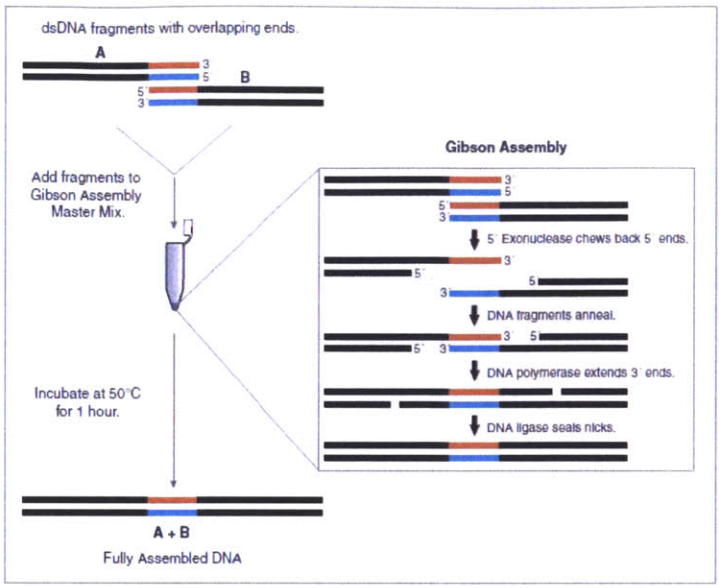


Figure 18. Illustration of Gibson Assembly™ Master Mix sold by New England Biolabs.
Source: Neb.com

3.8.3 Synthetic Biological Compounds and their Assembly

The systematic and comparative study of the structures and functions of molecules also allow chemists to find synthetic substitutes that are analog to the molecules found in nature (Eschenmoser 2005). Xeno nucleic acids (XNAs) for example, are experimental molecules that use alternative sugar bases that are not used in DNA or RNAs. The research not only pursues questions regarding the evolution of DNA, but also investigates the design of alternative gene regulation systems that can yield more robust and versatile synthetic circuits that can be customized for new applications. Inside artificial biological artifacts, XNAs are expected to function like DNAs store and transfer genetic information to other artifacts and eliminate the risk of interacting with a Natural organism as the chemical incompatibility between the hereditary systems would prevent the possibility of any biological exchange (Luisi et al. 2011, 62).

RNAs are single-stranded molecules. While they are expressed by the genes of the DNA, they can also make copies of themselves. They can store both genetic information and also catalyze chemical reactions. By applying either randomized or directed mutation techniques, De Lucrezia and co-workers create a library of RNA alternatives—synthetic versions also referred as "never-born" RNAs—which can be selected for specific purposes. This process, also known as directed evolution, today is one of the main methods for generating variations. By rationalizing nature's mutation-selection-amplification process, directed evolution can fast forward molecular evolution in thousand folds. It can expand the molecular vocabulary with arbitrary and subjective criteria and yield units of design that do not need to survive the evolutionary stress of survival. Newly generated molecules can be set aside and utilized for functions that they are not evolved for.

A combinatorial approach to creating RNA libraries, on the other hand, is often preferred for having orthogonal and modular, self-contained libraries. Variation is sacrificed for standardization, predictability, and repeatability. Jaeger and co-workers referred to the creation of artificial modular RNA architectures with preferred shapes and properties as "RNA tectonics (Westhof et al. 1996)". When modular RNA units are brought together as segments, they can self-assemble and form new structures with new functionalities.

In the language of rational chemical synthetic biology, RNA's modularity is considered an intrinsic property, which is naturally exploited for its designers (De Lucrezia et al. 2011, 60). However, like most combinatorial methods, this approach inherently has its limits. In theory, the structures cannot yield any novel functions or shapes that cannot be predicted *a priori* by their designers as the structures will inevitably be based on the combinatorial arrangements of previously known parts. The overall success of the designs will also be based on evaluation criteria that are determined by the success and failure of previous designs or natural analogs. While this method promises the convenience of exhausting the

space of possible combinations in short time, the process cannot be compared to the random molecular interactions that happen in nature, in which the logic of combination, the number and type of components, and the selection criteria can change in unforeseeable ways.

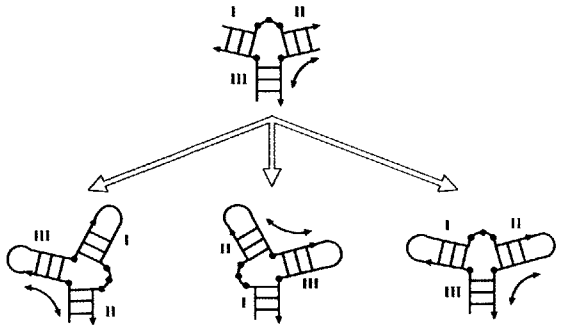


Figure 19. The mosaic structure of the RNA molecule allows it to be folded in different configurations.
Source: Westhof et al. 1996

3.8.4 Chemotons: Chemical Units and their Assembly

Tibor Gánti's work marks another important milestone in Theoretical Biology in identifying units of life that could meet most criteria shared among the living. After distinguishing the real (absolute) and potential criteria for life, which I mentioned in Chapter 1, Gánti refers to another distinction, which is between two different kinds of life exercised by the living. "Life itself is always the property of an entity (Gánti 2003, 8)." "Primary life" is the organization at the level of biochemical processes; they make up entities, which can encapsulate self-reproducing and sustaining reactions. In Biological terms, Gánti refers to life starting at the prokaryotic level where the organisms feature a membrane that separate them from the environment but lack a nucleus. "Secondary life," on the other hand is the higher level organization unique to certain eukaryotic cells which can assemble into multiple units to form hierarchies, structures and geometries differentiated into specific forms such as tissues, skin, muscles, bones and so on based on coordinated actions. An animal, while alive, would experience both kinds of life together and at the same time as an integrated body and as cells. However, when the animal dies, it

can only continue experiencing the primary life as its cells, tissues, and organs can be transplanted into other bodies and continue to survive.

To be able to identify the most minimal living entity, as early as 1971, Gánti puts forth an abstract model—a fluid automaton that involves three coupled subsystems which can work together inside a membrane under the direction of a program and exercise the capacity produce macromolecules through a process of polymerization or assembly based on a template of information (Figure 20). This model, also known as the "Chemoton," is capable of describing a minimal organization that can maintain a metabolic process—a chemical motor through the flow of materials, chemical energy and autocatalytic reactions.

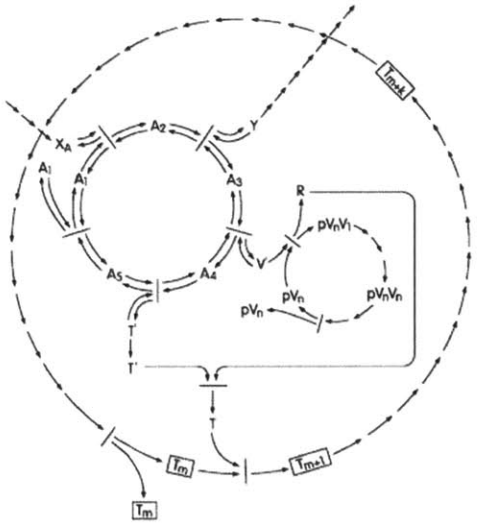


Figure 20. Chemoton model that shows three coupled systems working together.
A_1-An: Self-reproducing chemical motor
T_m - T_{m+k}: Chemical Boundary System (Spatial Separation)
pV_nV_1- pV_nV_n: Chemical Information System
Source: Gánti 2003

In contrast to electromechanical automata, fluid automata cannot be described by solid components as they feature the most minimal, indivisible, encapsulation that is required to keep three systems together. They are also independent of any geometric structure as within the flow of the liquid space the relations between the systems are described stoichiometrically—as relative quantities and ratios of

reactants and products of chemical reactions. Chemotons, instead foreground the role of organization within liquid space, as a network of chemical reactions that can simultaneously take place at different speeds and in different cycles where they produce the necessary compounds for each other.

The model not only intends to provide scientific explanation, and measurable criteria to define the units of life, but also informs what it may mean to think about new units and their assemblies if they can be decoupled from the necessity to explain what living already is, what it does, under what conditions. The theory also highlights the importance of a spatial domain that brings together substances and enables interactions through the flow of matter and energy.

Gánti, stresses the fact that the fundamental units of theoretical biology are not analogous to other theoretical unitary abstractions such as points in geometry, mass-points in mechanics, molecules in chemistry or charge in the theory of electricity. Yet being an abstract model, the Chemoton theory can be realized with different substances and different types of reactions as long as one can identify a self-reproducing cycle, a membrane, and an information system that are compatible with each other. Thus, any set of chemical reaction that can auto-catalyze itself and produce what it needs to consume in later steps of the cycle can be the driving program of a metabolism. The spatial boundaries can be artificially created from lipid molecules as long as they can permeate the flow of materials and energy from the outside. Such chemical constructs can be made to self-reproduce, (multiply by division) and made to polymerize larger molecules that can be based on a template molecule that can autoctalyze itself like RNA or DNA.

Chemoton theory can also be decoupled from existing biologies and be implemented for other types of intentional artifacts. Gánti's living units are measured against a set of criteria that intends to generalize and unify the foundations to explain what it takes to be considered living. If Biology can be formulated as a spatial domain—a context of interaction between liquid units and their assemblies—such chemical

groupings can be organized to produce alternative biologies. The stoichiometric design space suggests a different level of abstraction to organize, encapsulate, and synthesize matter outside the familiar form and molecular structures that have evolved for specific biological contexts. It formulates a notion of fluid automata as a set of chemical interactions that can be programmed to maintain activity at specific speeds for different intervals of time based on the inherent chemistry of the reagents. Such media inherently would respond to different selective pressures, intentions, and ideologies that will shape not only how an artificial biology lives but also why it lives.

3.8.5 Cells and Tissue Culturing

Cells of plants and animals have already been subject to unit-based design methods since 1930s. The growing of cells outside of their original bodies has been actively practiced since the beginning of 20th Century, since Alexis Carrel introduced the techniques for making chicken cells live inside a Pyrex container for more than twenty years (Carrel 1912). Carrel was interested in proving that cells can live permanently outside their bodies. While his assumption had been proven to be wrong, his work has been quite influential in developing the field called tissue culturing.

The abstraction of the cells from their original bodies offers a multitude of ways to think about the units of the living. Compared to artificial molecules, genes, bricks, or chemical reactions, cells are highly developed complex biological organizations. They exhibit most of the characteristics of the living; grow, differentiate, exchange materials with their outside world, reproduce a number of times and die or divide endlessly and pursue an immortal life like cancer cells.

Cells can be addressed individually or in large quantities. They can be identified discreetly in certain times but then lose their identity as they form groups and become tissues. As they can physically,

chemically and biologically change over the course of their lifetime, they have to be treated as different kind of units in rational design. Again, here the unit-based treatment can be about introducing a level of abstraction to be able to engage with higher-level design activities such as growing them in particular forms or structures instead of identifying them as countable discreet parts.

Stem cells are known to be pluripotent cells that can differentiate into other cell types to form tissues and organs. Once differentiated into new cells, Stem cells lose their original physical form and biological functions. Once they become a particular type of cell, they can continue differentiation and achieve emergent properties that are unique to their newly defined type. C2C12 mouse myoplast cell line, for example, can merge with each other over time and form multi nuclei carrying myotubes, which can eventually turn into myofibers where cells align and form fibrous muscle tissues that can act in coordinated manner and turn into muscles (Figures 21).

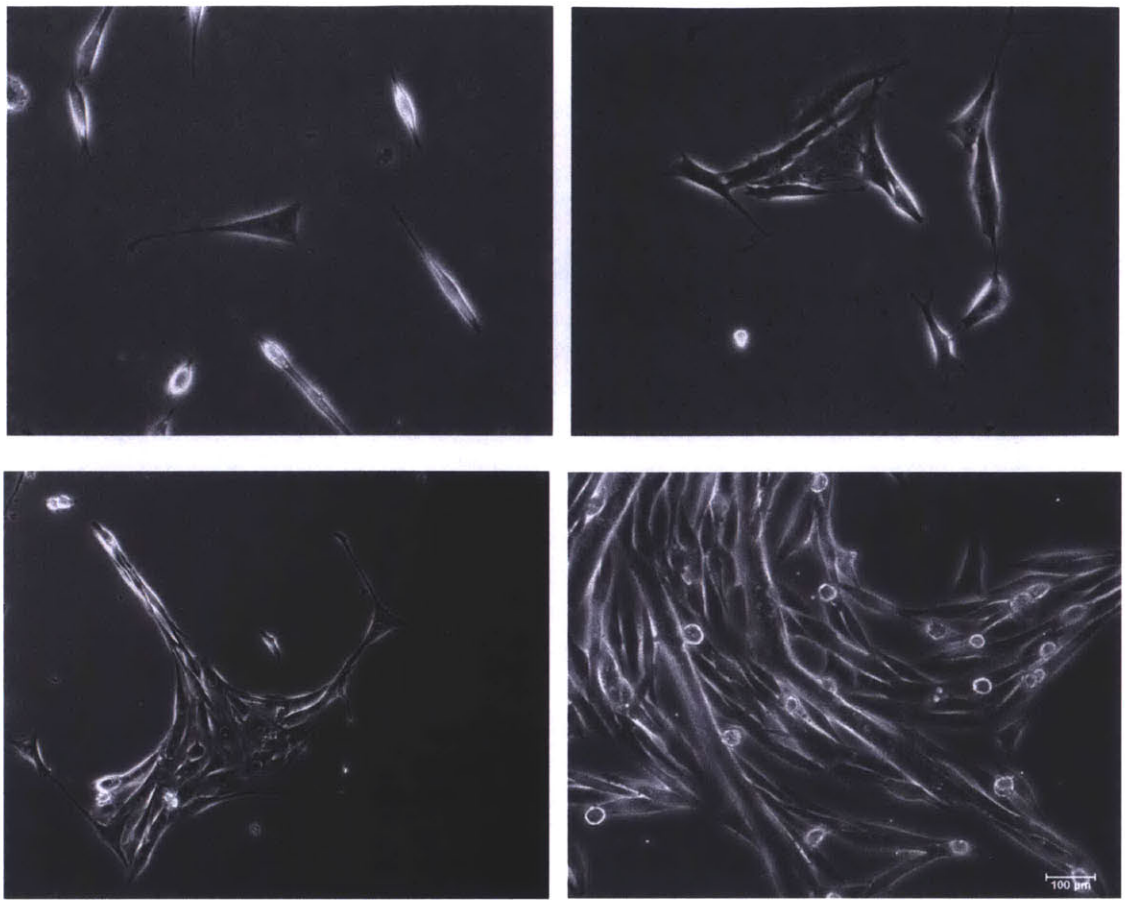


Figure 21. Individual muscle cells grown from C2C12 Myoblast cell line are forming tissues
Source: Telhan

Tissue culturing has been an active area of research for biological sciences, engineering, as well as product design. Cell lines and cultures have been developed to study effects of drugs without the need to try them on animals or humans. They are important research areas in cancer treatment and skin repair, aging, and even biomaterials. 3D tissue printing, artificial organ synthesis have been active areas of research in issue culturing, which still have not fulfill the promise despite growing demands. More recently, Kevin Kit Parker from Harvard University demonstrated the design of an artificial tissue-construct that can morphologically look and function like jellyfish. The "Medusoid" consists of cells that are grown on a thin layer of elastomer made of PDMS. Being patterned with microscopic grooves, this elastomer allows the cells grow and align in a specific manner. As mouse cardiac cells began to contract

and expand once they grown into a certain stage, the coordinated pulsing behavior can be guided with an electrical field like a propulsion that would make the elastomer layer swim like a jelly fish (Figure 24).

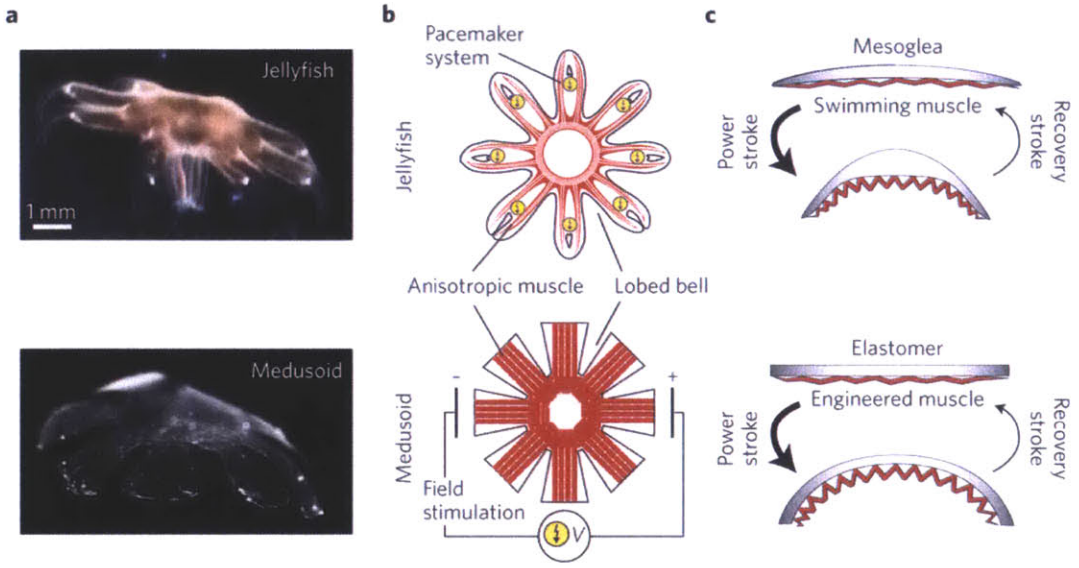


Figure 22. Structural features of jellyfish and synthetic medusoid mimic.
Source: Vogel 2012

Despite the recent advances, once units and abstractions of the living are based on higher-level biological organizations, it becomes really hard to apply the same kind of rational design principles that would work for less complex biochemical units. The number of unknowns and the ways to deal with them increasingly become exponential. The living, after a certain level of complexity, becomes very dependent on their original biological contexts. Human cells outside the body behave very different under different lighting conditions, become very vulnerable to contamination without the support of an immune system and have the tendency to show spontaneous mutations. While cancerous cell lines, such as HeLa, can be cultured outside the human body and made to survive for long periods of time, normal cells cannot sustain their existing behaviors outside their host body. Once out of their context, healthy cells also cannot be considered analogs to those that are in the human body as external

conditions would make them exhibit unpredictable behaviors, imposing many limits on how much they can be tested as their *in vitro* equivalents for research purposes,.

3.9 Designing the Space/Context of the Living

In biological design, it becomes crucial to think about the inner workings of the units, their spatial organization and the biological context that shape this organization in relation to each other. Whether it is fragments of genes on a DNA molecule, or a bacteria uptaking a plasmid, or cells grown on extracellular scaffolds, most design activities discussed in this chapter happen within a biological context. The sites for these activities imply a certain form of containment that provides the necessary conditions for them to thrive. Reactions that happen within bacteria, for example, require specific temperatures, pH levels, nutrition, and even toxic proteins that could eliminate other organisms that would compete for the same resources.

In this section, I will discuss the design of the space of the living as the third component of the framework. As biological design moves towards synthetic units and assembly methods, the vocabulary of biological contexts that host new forms of living are also changing. Thus, designs become inherently related to where they are initially developed and where they deliver their functions. I will discuss the design of synthetic biological contexts regarding their roles in providing both containment and confinement for the living. From the design of biochemical compartments, artificial cells, to electromechanical capillary spaces, I will present a variety of synthetic habitats where the living is designed to both interact and also prevented to interact with other living.

3.9.1 Biological Containment and Confinement

Life depends on a territorial organization. Every living thing can only survive within a very specific set of conditions that are tied to physical, chemical, and biological interactions with their environment. Even the smallest single-cell organism needs a membrane—a cell wall to separate itself from the environment. Higher level organisms, like Eukaryotes, not only need an outer cell wall but also a nucleus—a central core—that can isolate itself from the rest of the cell with its own membrane. The need for separation and creation of a core within a core has attracted many theories since the earliest days of biological research (Margulin 1970). According to a thread of research that explains the "origins of life", cellular encapsulation is one of the main requirements that triggered the differentiation between living and non-living.

The biological complexity of the living draws parallels with the evolution of multi-cellular organisms. The symbiotic interaction among different cells allowed organisms to develop higher-level capabilities that relied on coordination among different groups of cells. Each nature-born living thing can be registered with its capacity to contain its biological complexity through its integral organization and how it enables it to exchange materials with its environment. A membrane defines both the physical scope and the type of the metabolic activity that is allowed to be in and out of the organism, thus making it almost always part of its environment. On the other hand, while such membrane can draw a distinction between human and non-human cells, every human body approximately needs ten times more foreign cells to be able to maintain its functions. It becomes increasingly evident that those microbes not only take basic responsibilities such as helping the digestion system by breaking down complex molecules, but also do very complex functions such as training our immune system against attacking itself by monitoring our psyche for obsessive-compulsive disorder and suicidal tendencies (Ackerman 2012). As the real boundaries of biological containment blurs, it becomes more important to evaluate the

definitions and functions of the living in different biological contexts—in their networks of interactions that extend much beyond their physical boundaries.

When it comes to the design of the synthetic living, the conditions that determine what is allowed to exist with other living is regulated with many factors. Certain genetically modified organisms are not allowed to exist within certain habitats due to their known or potential threats to the other living. Thus, when created inside the laboratory, synthetic biological designs cannot opportunistically roam around and claim a niche area to grow, evolve, and continue its hereditary line.

When biological design becomes a design space—both literally and metaphorically—it faces with social and cultural resistance. The acceptance of the living is often measured against its value proposition. While GMO seeds are not welcomed, oil-cleaning bacteria and insulin-secreting yeast can become billion-dollar business models. The cultural acceptance of these biological products heavily relies on our ability to isolate them from the wild and lock inside industrial production mechanisms where they are only allowed to live to deliver their functions.

The evolution of biological design can also be traced in the ability to decontextualize the living from its original contexts, such that it can be incorporated into new molecules, cells, organisms, animals, plants, environments, and habitats. As discussed in the process of creating abstractions and units of living, biological design involves shifting contexts and creating compatible interactions between different parts of the living.

3.9.2 Cell-free biology

One of the most significant accomplishments for biological design is, for example, the challenging the existing membrane and cell structures of organisms so that their parts can be reutilized for different

intentions. With the invention of cell-free protein synthesis in 2001 by Shimizu and co-workers demonstrated that it is possible to synthesize proteins with custom gene sequences outside a living host organism (Shimizu et al. 2001). By mixing custom DNA fragments, RNA, and ribosomal machinery, and reagents extracted from the living organisms, proteins can be fully synthesized in test tubes and then used as ingredients for other biological reactions (PURExpress 2013). Cell-free protein expression even lets the creation of novel proteins that cannot be hosted by an existing organism due toxicity or extreme environmental conditions. They also form the basis of new biological confinement conditions in which the intention is to utilize the parts or products of the living without the living—for economic, ethical or ecological reasons. Thus, when the living organisms are not involved in their entirety, the process of modifying their genes, genomes, can be described as a novel process that can overcome the current restrictions on GMO products or intellectual property laws.

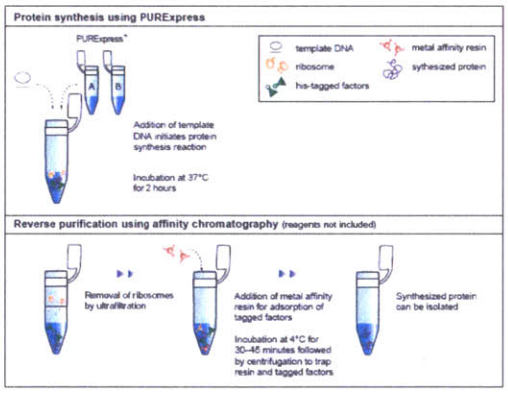


Figure 23. Protein synthesis demonstrated using PURExpress
Source: https://www.neb.com/products/e6800-purexpress-invitro-protein-synthesis-kit

3.9.3 Synthetic Chemical Compartments

While they can take place outside living organisms, biochemical reactions almost always happen in spatially confined environments. In nature, most organisms have membranes that are made of different types of lipid molecules. By mimicking the physical and chemical compositions of cell membranes, today,

it is also possible to design artificial compartments that can host new biological contexts. Lipid encapsulations, known as liposomes, can be used to package both biological and chemical content and interface naturally with living cells in their own contexts.

Being ultimately abstracted from nature-born organisms, this chemistry-oriented biology promises new directions to design (Rasmussen 2009). Here, while molecular composition, logic of self-organization, growth, and adaptation is learned, molecule by molecule, cell by cell, unit by unit. From living things, the ultimate products can be fully synthetic and do not necessarily have to carry living material from living organisms. This type of biological design has the ability to extend beyond our current understanding of biology or bio-mimicry and ultimately give birth to new kinds of biologies that operate with their own logic, carry program, instructions, or a drive for survival beyond their existential needs. Eventually, such biologies may need to considered different kinds of living, and not necessarily in their capacity to show how much they vitality resembles to those that originate from nature.

3.9.4 Chassis Organisms

Instead of building cells artificially from bottom-up, another direction in biological design research investigates the top-down simplification of living organisms so that their genomes can be synthesized in laboratory conditions ideally without any living parts from nature. For example, a number of strains in bacteria today are used as 'model' organisms in laboratories worldwide. Their genomes are reduced in size and mapped out in great detail. For designers, these organisms are simplified biological contexts almost like chassis where synthetic parts can easily and predictably be incorporated to genomes with using plasmids. As mentioned in Chapter II, in 2008, researchers at J. Craig Venter institute reduced the *Mycoplasma genitalium* genome into 582,970 base pairs. *Mycoplasma* are known as the smallest self-replicating life forms in terms of size of their genome. JCVI's researchers managed to make it even smaller so that they can fabricate it segment by segment using DNA synthesis then assemble them

together using different single-cell organisms, and ultimately copied the finalized artificial genome into a living cell so that the synthetic genome can start living in its host (Gibson et al. 2008). The newly-generated artifact, *Mycoplasma laboratorium* is an important step towards making semi-synthetic organisms. The research also marks an important moment in unit-based design, both for its advancement in the synthesis of genomic material and for the way in which simplified chassis organisms now pave the way for hybrid artificial biological contexts that can be piecemealed with units of function and behavior that can originate both from living and non-living origins.

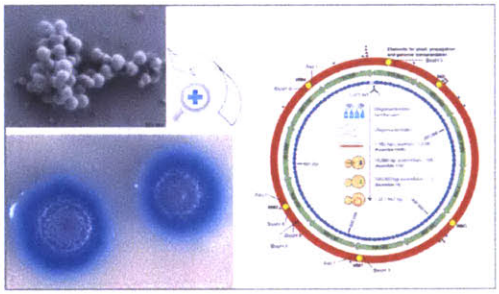


Figure 24. Different representations of *Mycoplasma laboratorium*
Source: Gibson et al. 2008

3.9.5 Packages, Enclosures, and Biotopes

The term 'biotope' is a German word that literally means: where life lives. Synthetic cells, model, chassis, or natural organisms all provide different contexts for biological design. However, as these designs are intended to be part of everyday life, one important design challenge is to find alternative places for this new biology to exist safely outside the laboratory conditions. The fear of ecological catastrophes and the unforeseeable impact of genetically-modified organisms in nature push designers to think about new biotopes where biological products can still meet with their users without becoming threats to existing habitats. In other words, synthetic organisms, protocells, biological circuits (biology outside a living body) are faced with a packaging problem.

Here, new directions in microfluidic device research promise the design of new kinds of biological Microsystems that can combine electrical, computational, and biological functionalities within the same enclosure (Wagler et al. 2003). Unlike petri dishes, which traditionally host cell cultures or single-cell organisms, microfluidic environments are spatially articulated environments, which control the flow, movement, synthesis, and progression of biological contexts over time and in space and become the basis of a new kind of architecture for living matter. As the spatial configuration of microfluidic hardware can be computationally regulated via pumps, valves, and other control devices, these systems can be integrated into the logic of operation of the biology that inhabits that space.

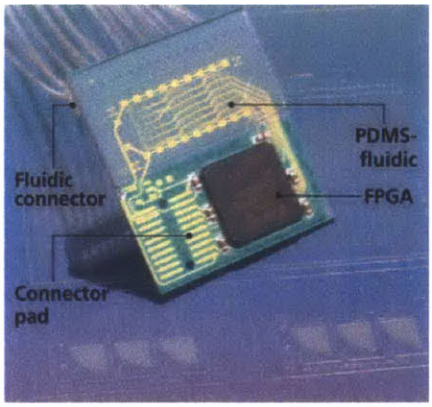


Figure 25. Chemical microprocessor (ChμP)
Source: Wagler et al. 2003

While the common use of microfluidic systems today is similar to embedded electronic systems such as field programmable gate arrays, which utilize combinatorial or sequential logic. There are also different research directions that investigate alternative computational paradigms. While such systems inherently reinforce the representation schemes of hardware and logic design and programming with biology, they open up new directions for unitary design. Being compartmentalized, both through cellular and computational means, these designs are locked within units. However, their logic of operation can still be governed by the rules of fluid dynamics. They can follow alternative representations and visual

diagramming techniques for the construction of metabolic pathways and use new design rules and grammars that can shape their synthesis, growth, and differentiation of biologic products.

Microfluidic systems, for example, compress the space between visual systems, physical hardware, and biologic behavior. The biological context can be both visually informative and a tool for diagnosis. "Diagnosis for All," for example, uses biological agents encapsulated on the surface of a patterned paper to detect chemicals in blood or urine. Primarily targeted for point-of-care diagnosis in low-income countries, the design is also a good example of how biological design that can be explored as mass product out of traditional laboratory settings (Sia et al. *2004).*

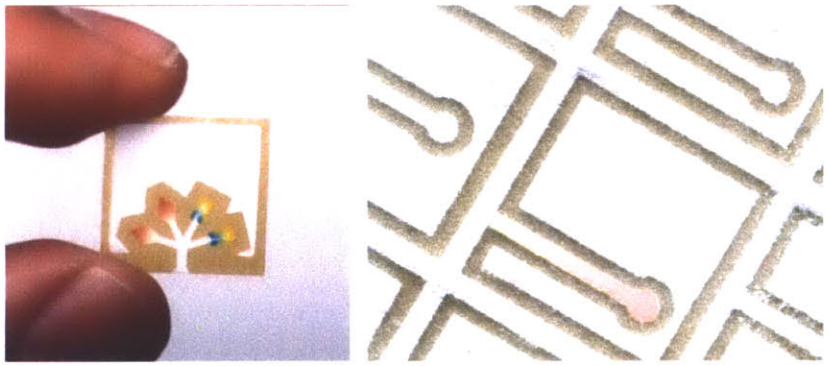


Figure 26. Diagnosis kits made of paper microfluidics from
Source: "Diagnosis for All" Media Kit. Dfa.org

Chapter 4 – A Spatial Theory for Biological Design

In Chapter 3, I surveyed various design methods that shaped biological design in the past decades. I used a three-tier framework—unit-logic-context—to be able to provide a common vocabulary to discuss the similarities and differences between alternative methods of biological design and the types of abstractions they utilize while they engage with the manipulation of the living on different levels.

In this chapter, I will articulate on ways to extend this thinking and specifically look at a design space that emerges between chemistry, chemical engineering, synthetic biology, and chemical synthetic biology. This domain studies the physicality of living and inert material on the molecular level and typically seeks answers regarding the origins of the life and the boundary and transition conditions where inert matter can become living. From a design and engineering perspective this area also promises various novel products, applications and services: self-healing, bio-degradable materials, artificial replacements for biological parts—such as synthetic ribosomes, alternative DNA molecules, artificial cells and tissues—and new forms of molecular factories that can assemble proteins, enzymes and biological compounds by chemical means. Here, my aim is to look at the methods and techniques used in some of these applications and try to theorize the design affordances of these biochemical methods with respect to different understandings of space, spatiality, containment and confinement conditions and how they can expand the current practice of biological design.

At the junction of the living and non-living lies a space of 'molecular commons.' This is a domain where the molecules interface with each other and share a common vocabulary of parts, relationships between parts and different contexts that construct their meaning physically, chemically, or biologically—as animate or inanimate matter.

Cellulose, with the formula ($C_6H_{10}O_5$) n, for example, is an organic polymer that can be produced by plants, algae, or even some type of mammals. Being essentially a type of sugar—polysaccharide—it is a biological product that has responded to different needs in the evolution of many different species and therefore found a place in their genomes and metabolic pathways. As cellulose fibers are part of a commons, they can also be artificially synthesized by microorganisms that have not been evolved to synthesize them. Thus artificially synthesized cellulose can be brought to new contexts where it can function as new materials or designed artifacts with different features (Monteiro et al. 2009).

The space of molecular commons offers alternative ways to think about form, structure, instruction, methods of synthesis, containment and confinement conditions for the living. It provides conditions to design new types of biological and chemical contexts for different units, assemblies of synthetic-, semi- or partially-living, and life-like artifacts, which can follow different motivations and modes of being.

Unlike manipulating the genes of existing living organisms or altering the living conditions of plants and animals for desired outcomes, this space engages with design methods that work with "approximations" of the living (Stano and Luisi 2010) (Stano et a. 2011). Today, one can design semi-permeable enclosures that can contain liquid environments to host metabolic activities—such as letting ingredients of chemical reactions in and out, releasing energy, etc.—and exercise, albeit limited, a capacity to grow and divide like a natural cell. These chemical constructs cannot be given a piece of DNA and expected to maintain their life without any external support but they can be designed such that they use ribozomes, mRNA, and catalyzing proteins harvested from real cells to synthesize compounds that can be used to maintain their membranes, etc.

These constructs currently cannot fully attain the properties of living artifacts, but they do help us investigate what 'life-like' may mean at a very primitive level when certain ontological limits of what we know of biology are suspended. For example, while almost all natural cells are driven by DNA-instructed

metabolisms which determine when cells replicate, perform activities, or die, designed cells open up the possibility to explore alternative modes of self-guided and maintained living. Instead of evolving through Darwinian mechanics of evolution, they can be directed to differentiate in a guided manner, be selected based on different kinds of preferences, and made to last longer or shorter depending on the intent behind their creation. On the other hand, unlike computers or industrial machinery that is created to execute specific tasks, biochemical cells offer an extended view on agency, automation, self-instruction and regulation, which can combine principles from electromechanical and computational design and work with chemical and biological contexts that can be formed within liquid media. Here, I will discuss design in molecular commons as a rational design framework and present an assembly-driven, constructivist, and generative approach to biological design. My intention is to outline a theory which can potentially overcome some of the limits of other forms of unit-based, assembly-driven computational methods of design, and demonstrate how aspects of information representation, physical, chemical, and biological functions of design can be addressed with a common vocabulary in static or moving liquid environments.

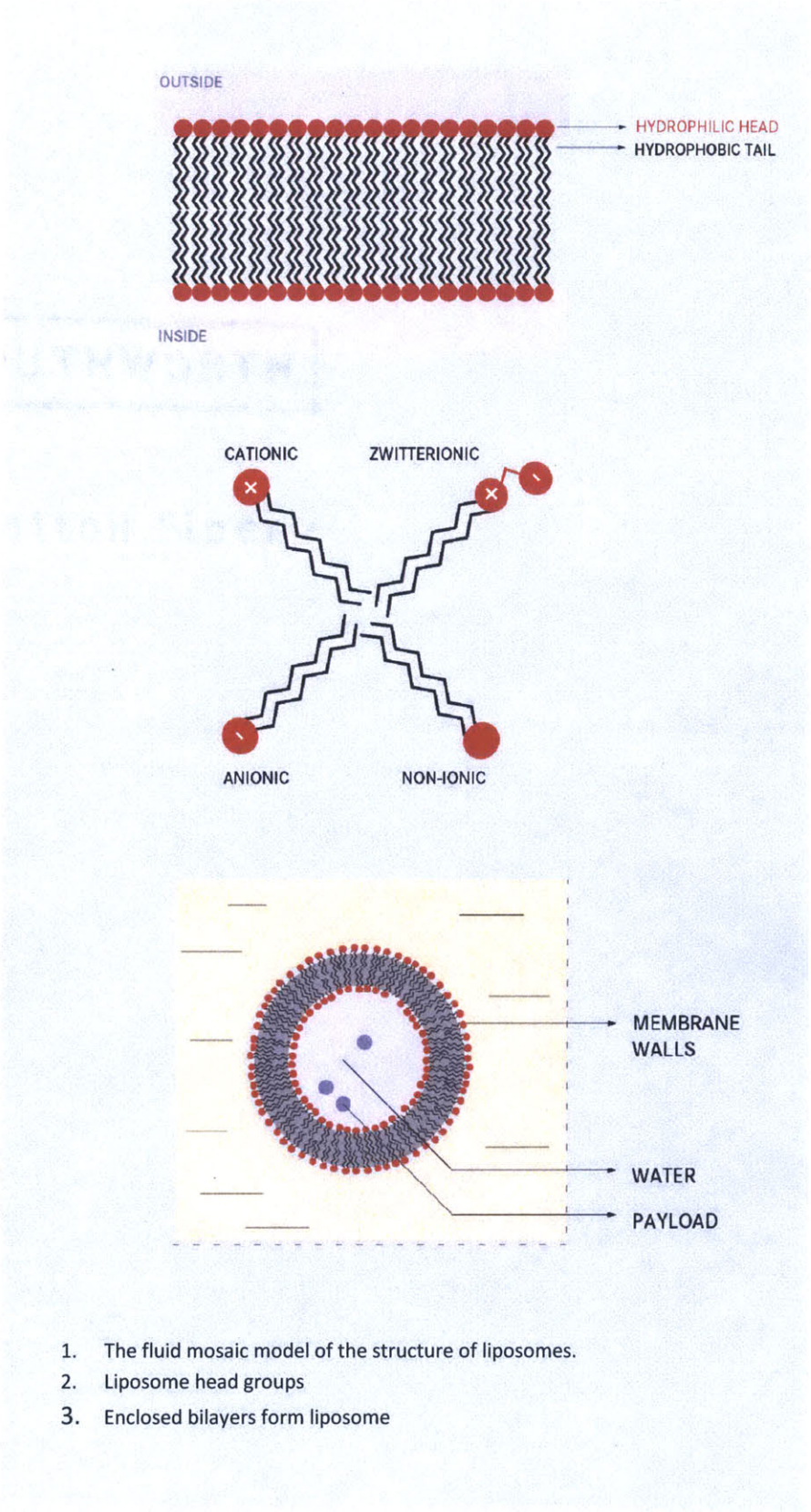
As a design thesis, this section of the dissertation features work that is a combination of my own studies and experiments with scientific research carried by others. While the theory is formulated based on my own interpretations, interpolations, and speculations; the work intends to outline a comprehensive grouping of different methods and principles extracted from Biology, chemical engineering and Soft Matter Studies. Here, I deliberately refrained from making any design claims that cannot be verified scientifically. I tried to be sure that when the scope of the work can be set broader with more time and resources, the feasibility of each design proposition can be verified within the current level of science. When necessary, I will point the reader to references that show a potential use of a scientific principle in a design application or consider the scientific proof of an existing principle enough to be considered for another experimental design setup without further verification. I deemed these gestures necessary to

provide a rich ground for future discussion without sacrificing the scientific soundness of the concepts. At the end of this chapter, I will provide a discussion section where I explain the specific details of my own experiments which are intended to provide proof of concepts for the theory.

In this section a different layout is used to illustrate principles visually, next to their accompanying explanations. I will also make use of a graphical and schematic language to maintain the consistency and simplicity of the visual explanations throughout the section. Here, liposomes, for example, are illustrated in a rather simplified form and most of the time in two-dimensions. Their photographic depictions are only presented when it is important to demonstrate their possible physical appearances.

4.1 A Unit of Design: Liposome

Liposomes are artificially created spherical vesicles that that are made of lipid molecules. These molecules align and form semi-permeable membranes that can physically and chemically separate an interior space from its outside in liquid media and allow selective exchange between the two environments. Known as the fluid mosaic model, the structure of the membranes are usually made of two layers of natural lipid molecules which consist of a hydrophilic head and a hydrophobic tail. Lipid molecules in the membranes can have different head groups (Singer & Nicolson 1972). They can be positively- or negatively-charged, be non-ionic (non-charged) or zwitterionic (hybrid or neutral)—including both charges. Different head groups allow the membranes to exhibit different electrostatic properties and therefore influence the interactions between different liposomes so that they can attach, repel, or fuse with each other. Due to having both hydrophilic and hydrophobic parts in their composition, the bilayer lipids enclose onto themselves and form spherical cavities when introduced to aqueous environments. During this auto-encapsulation process, other molecules in the environment can be trapped inside. Once formed, various compounds, proteins and ions can flow in and out through the membrane's environment due to the internal and external osmotic pressure difference between the liposome and its environment or the charge difference between the molecules and the membrane. Units can also be loaded after they are formed with ingredients using micro-injection techniques.



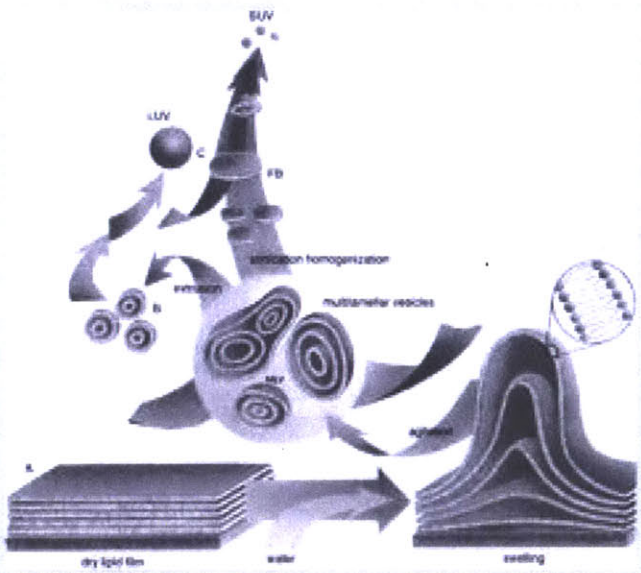
1. The fluid mosaic model of the structure of liposomes.
2. Liposome head groups
3. Enclosed bilayers form liposome

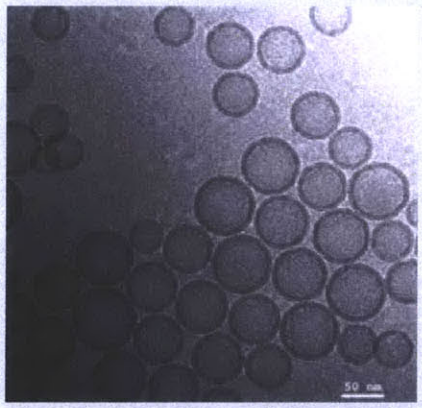
Liposome sizes can vary from tens of nanometers to tens of micrometers. They can be produced with a number of methods such as reverse evaporation or sonication and filtered to be made fixed size or as vesicles with identical properties.

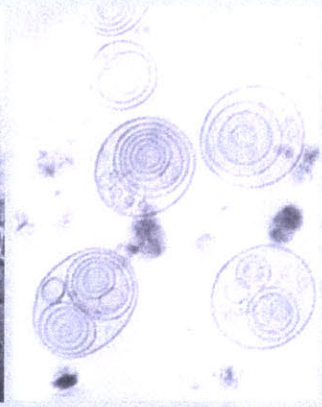
Based on their molecular composition, liposome membranes exhibit different phases and change their form depending on their transition temperatures. They stay in rigid form between lower temperatures and their phase temperature and turn into gel form in warmer temperatures. The integrity of liposome membranes can be disrupted with external stimuli—such as changing the PH-level, introducing charged molecules, or applying acoustic pressure from the environment.

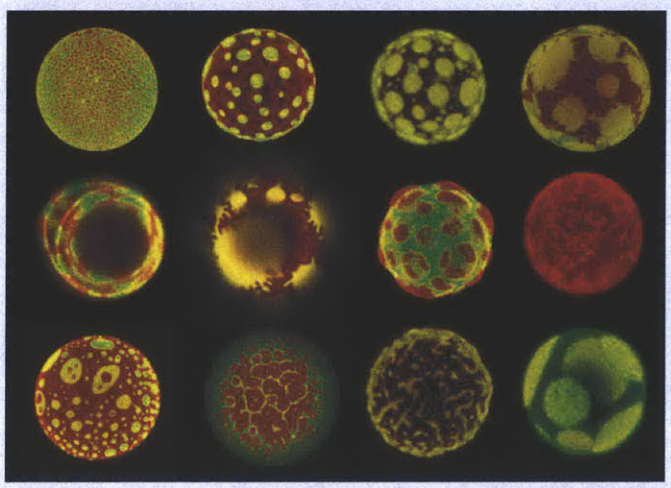
The ability to control the size, physical shape, payload, membrane polarity, coating, and durability has made liposomes a very attractive research area since their discovery in 1960s. They have found industrial applications in cosmetics, food and cleaning industries, and pharmacology, in which they are used as carriers for drug delivery or compartments that can host alternative chemical reactions.

Smaldon and co-workers also theorized a liposome-based logic, in which liposomes can become the units for information processing circuits for various types of computation. (Smaldon et al. 2010).









1. Preparation of Liposomes (Vanniasinghe et al. 2008) 2. Cationic Liposomes (Battersby et al. 1998) and Multilamellar liposomes (image taken from http://www.dr-baumann-turkiye.com) 3. Giant unilamellar and multilamellar vesicles (liposomes) imaged by Dr. Jorge Bernardino de la Serna (from http://www.nikonsmallworld.com/gallery/search/all/liposome/2)

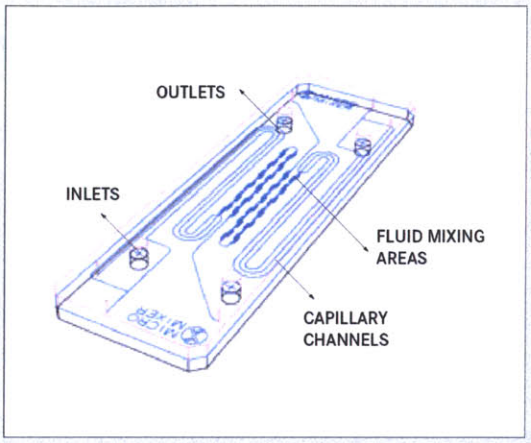
4.2 A Design Context for Molecular Commons

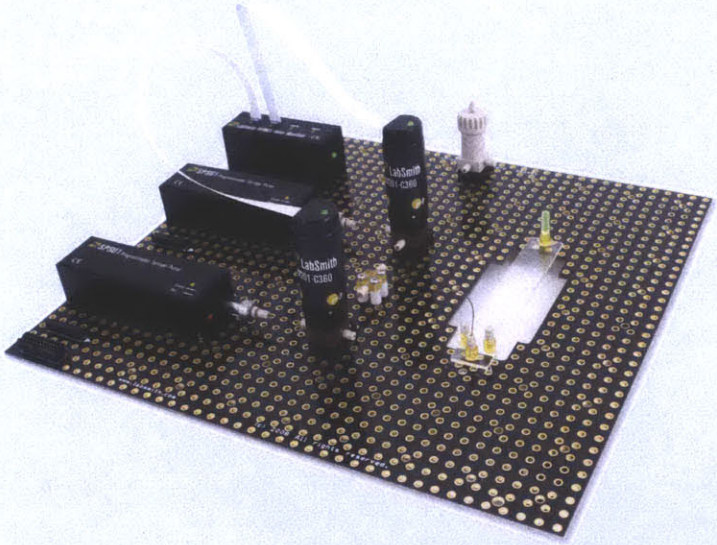
Microfluidic systems are a general name given for single or multi-layer circuits that are built to move around picoliter or microliter amounts of liquids within capillary channels.

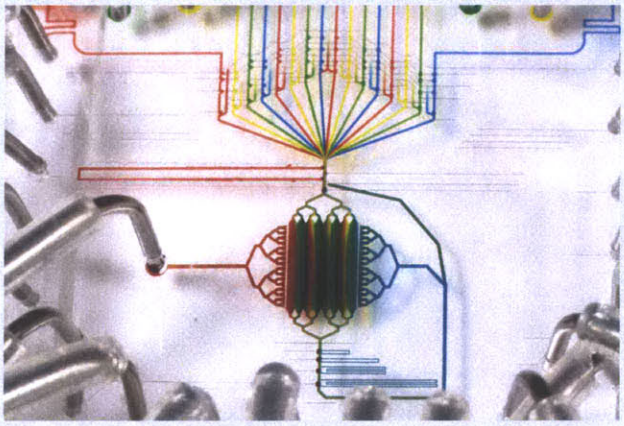
They are made of biocompatible transparent materials such as glass, quartz, Polydimethylsiloxane (PDMS), Poly(methyl methacrylate) PMMA or other kinds of thermoplasts, where different geometries are etched or molded using photolithography techniques.

Microfluidic systems are usually equipped with digitally controlled pumps, valves, reservoirs, heating and cooling elements, which can precisely control the flow, mixing, circulation, and storage of multiple liquid streams at different temperatures.

Among their many uses for conducting biochemical experiments, it has also been demonstrated that such systems can be built as bioreactors for growing and monitoring microorganisms and cell-cultures (Balagaddé et al. 2005) (Pasirayi et al. 2011), used as PCR machines for synthesizing genes (Kong 2008), function like biochips to design proteins (Buxboim et al. 2007), and become interfaces that can separate liquid mixtures—such as milk or blood—into their constituents (Grenvall et al. 2009) (Yang et al. 2005).







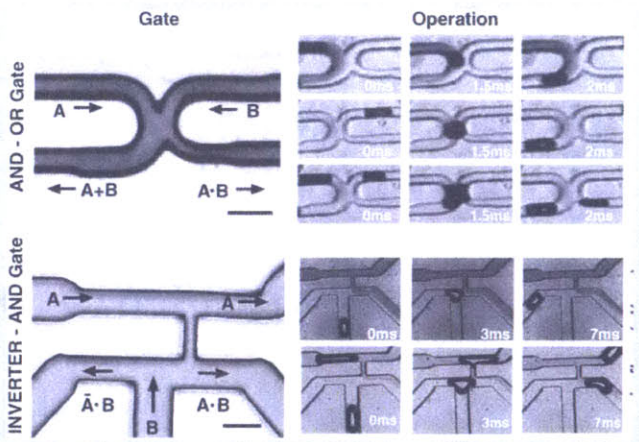
1. Sample Microfluidic chip design from Dolomite Co.
2. Micfrofluidic workbench with integrated pumping and automated valves from Labsmith Co.
3. Multi-channel and multi-layer mIcrofluidic chip design from Albert Folch Laboratoy.

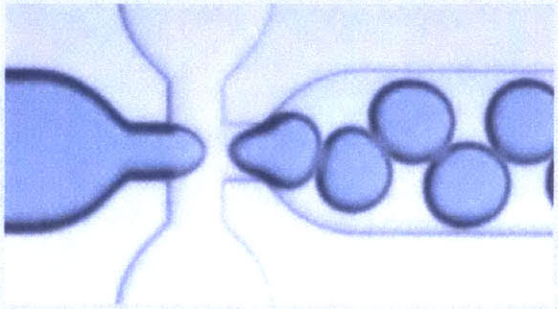
Prakash and Gershenfeld demonstrated that it is possible to do computation within liquid circuits by implementing a bubble-based Boolean logic that relies on the precise timing and placement of bubbles within capillary junctions (Prakash & Gershenfeld 2007).

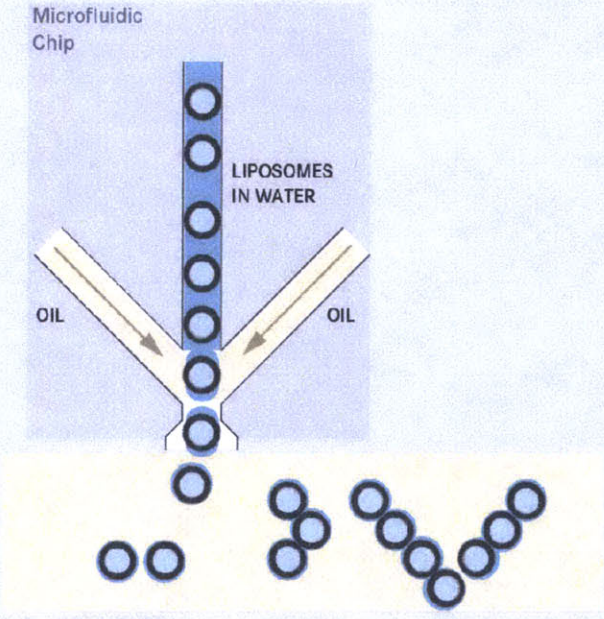
The use of liposomes has been investigated in numerous spatial contexts. While most emphasis is on their *in vitro* use—in which vesicles freely move about within an animal or human body—microfluidics are extensively used during the precise manufacturing of liposomes (Tan et al. 2004). Inside the capillary environment, it is easier to control their transportation, structuring, and interaction with each other. Microfluidics also provide spatial contexts where chemical, biological, physical, electromechanical and computational design principles can be combined with each other.

Liposomes can be encapsulated within liquid droplets using microfluidic droplet generators that have X, Y or T type capillary junctions.

Once captured inside water droplets liposomes can be moved around using different flow strategies. They can be treated both in static and moving design contexts. A liposome vesicle inside a droplet is both a discrete, addressable, and movable unit within the design space, and also an individual element that can be equipped with chemical or biological agency which can determine its own interaction with other units.







LIPOSOMES INSIDE WATER DROPLETS DISPENSED INTO OIL

1. Bubble logic (Prakash & Gershenfeld 2007).
2. Dolomite Microfluidic chip with X-junction.
3. In theory, bubble generators with a moving head can disperse different droplet geometries and spatially group them in continuous media.

4.3 Theory of Liposome-based Design

IDENTITY: A self-enclosing liposome can be identified as a unit.

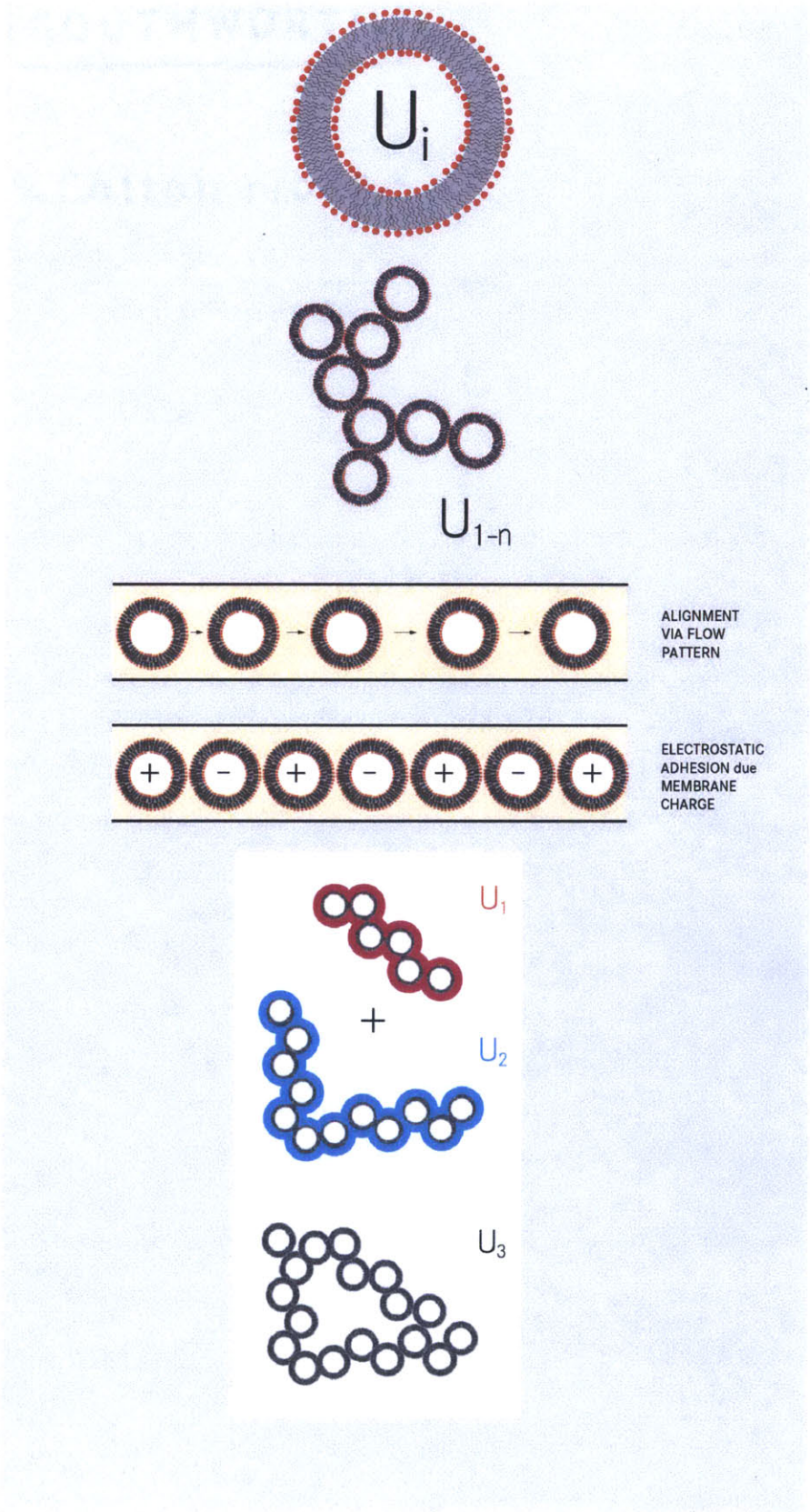
It can be visually marked by staining its membrane with a dye or incorporating a light-emitting biochemical agent such as the green fluorescing proteins as its payload. Magnetic particles or radioactive dyes also offer non-visible tracing options if their use does not interfere with the objectives of the application.

ASSEMBLY: Different liposome units can be grouped together within capillary tubes. Groupings can be non-adhesive and based on accumulation in compartments or alignment caused by flow and circulation conditions when units are forced to float and follow each other in the direction of the flow.

By interleaving vesicles made of membranes with cationic (+) and anionic (-) lipid head groups one after the other, liposomes can be made to adhere to each other electrostatically and form "string-like" structures.

Once they became identifiable, discreet units can still be traced optically or through sensing devices that track their markings.

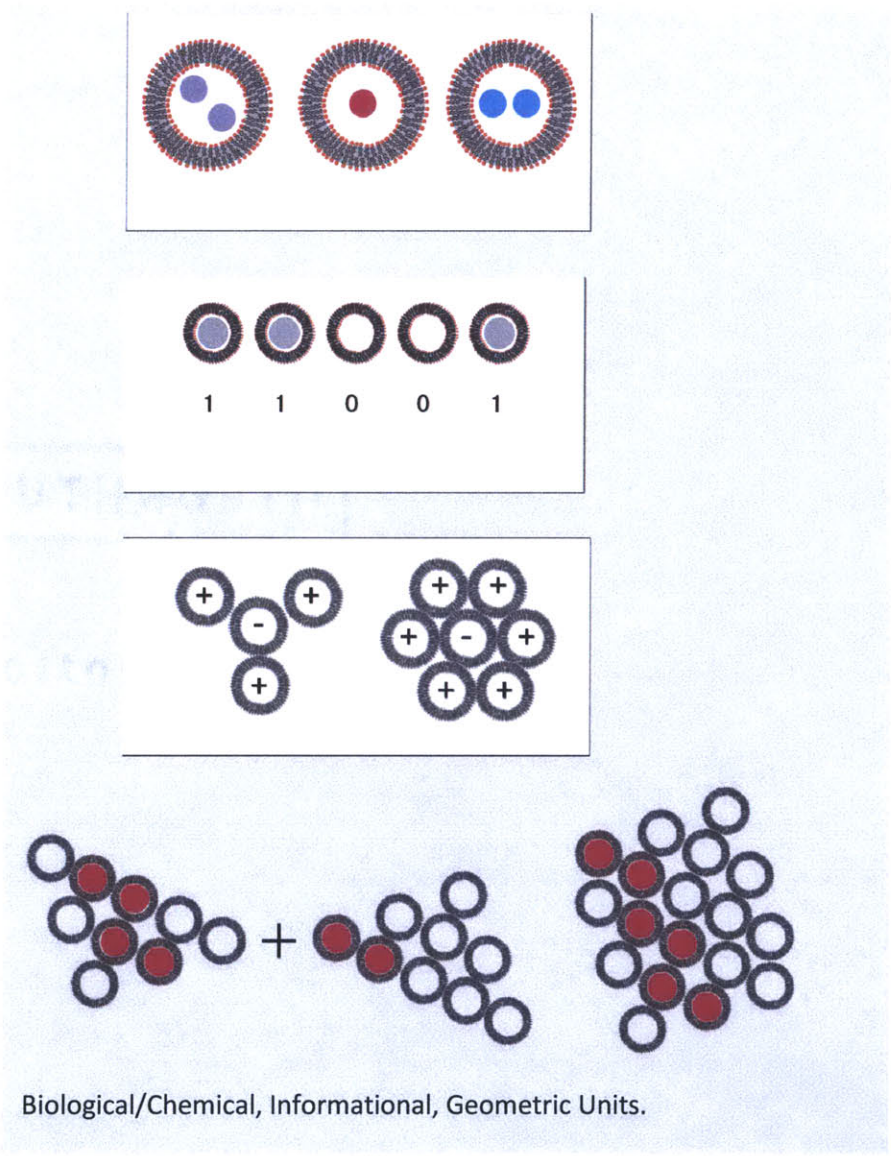
Different groupings can be assembled into larger structures and become new units. By pumping free floating structures back into the capillaries, units can be reconfigured into different organizations.



TYPES OF UNITS: Liposomes can be characterized into different types of units based on their physical characteristics, contents, and how the units can be used to represent other phenomena.

Biological/Chemical Units: Liposomes are foremost created for encapsulating content and carrying that content to a different location where the ingredients can be part of a reaction. They can also be used as the site where reactions and chemical or biological events such as *in vitro* DNA replication or protein synthesis can take place.

Informational Units: Like bits which represent information by sequential ordering of states (e.g., 101011), liposome streams can be lined up to represent digital information. By loading the vesicles with a traceable marker (e.g., optical dye, electrical charge, magnetic particles, etc.), it is possible differentiate between visible/non-visible, negative/positive states as the liposomes flow through capillary channels.



Biological/Chemical, Informational, Geometric Units.

Geometric Units: By utilizing different membrane adhesion methods liposomes can form geometrically meaningful formations. They can be lined up to string structures or more advanced constellations. Multiple units can form primitive geometries which can also be grouped to create complex shapes.

Liposomes can exhibit multiple typologies at the same time. They can be assembled into traceable geometries which can be represented with symbols or numbers. By adding unit groups to each other, one can also create higher level abstractions and compose more complex organizations. Units can not only change features over time (e.g., change color) due to their internal reactions or by incorporating other liposomes, but also change their type. A chemical unit can switch to an information carrying unit over time, or from application to application.

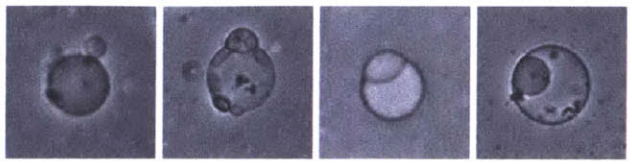
The polymorphic nature of the units can be utilized during different assembly operations. For example, visual operations can have chemical consequences; biological operations can be visualized by different optical compositions; and strings of liposomes can be composed like machine- or human-readable words and form text-like assemblies as they are prepared for biological or chemical processes.

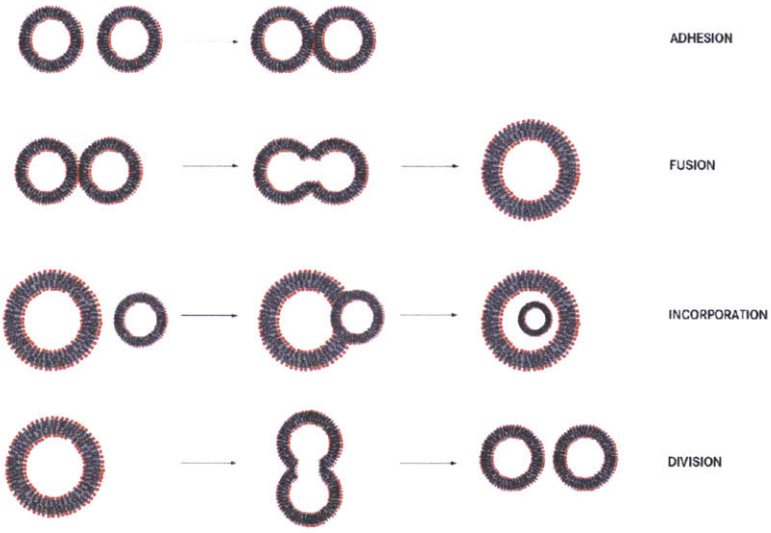
INTERACTION: The interaction between different types of liposomes is extensively studied and four types of interaction have been commonly reported (Paleos et al. 2011) (Stano & Luisi 2009).

While adhesion can be used for combinatorial structuring of the units (by maintaining their identity), with fusion, incorporation, and division it is possible to design structures with irreversible transformations, which can extend the use of liposomes beyond combinatorial applications.

The interactions among the liposomes are primarily due to their membrane chemistry. However fusion and adhesion operations can be modulated with changes in temperature, PH levels, by incorporating extrinsic molecules such as Ca^{+2} to the membranes (Bailey & Cullis 1997) or by applying external forces such as high-intensity sound waves or magnetism. For example, magnetite cationic liposomes are often used for interacting with DNA molecules that are negatively charged (Shinkai & Ito 2004). They can be used not only to guide vesicle to vesicle interaction, but also to pass different ingredients to the membranes in controlled ways.

Once liposomes arrive to a certain size they can divide into smaller and more stable units. Thus, growth and self-division have been among the most attractive features of liposomes. These features have motivated researchers to build simplified, proof-of-principle cell-models that can mimic single cell organisms (Luisi & Stano 2011).





$$U_1 + U_2 \longrightarrow U_3 \longrightarrow U_1 + U_2$$

ADHESION or INCORPORATION

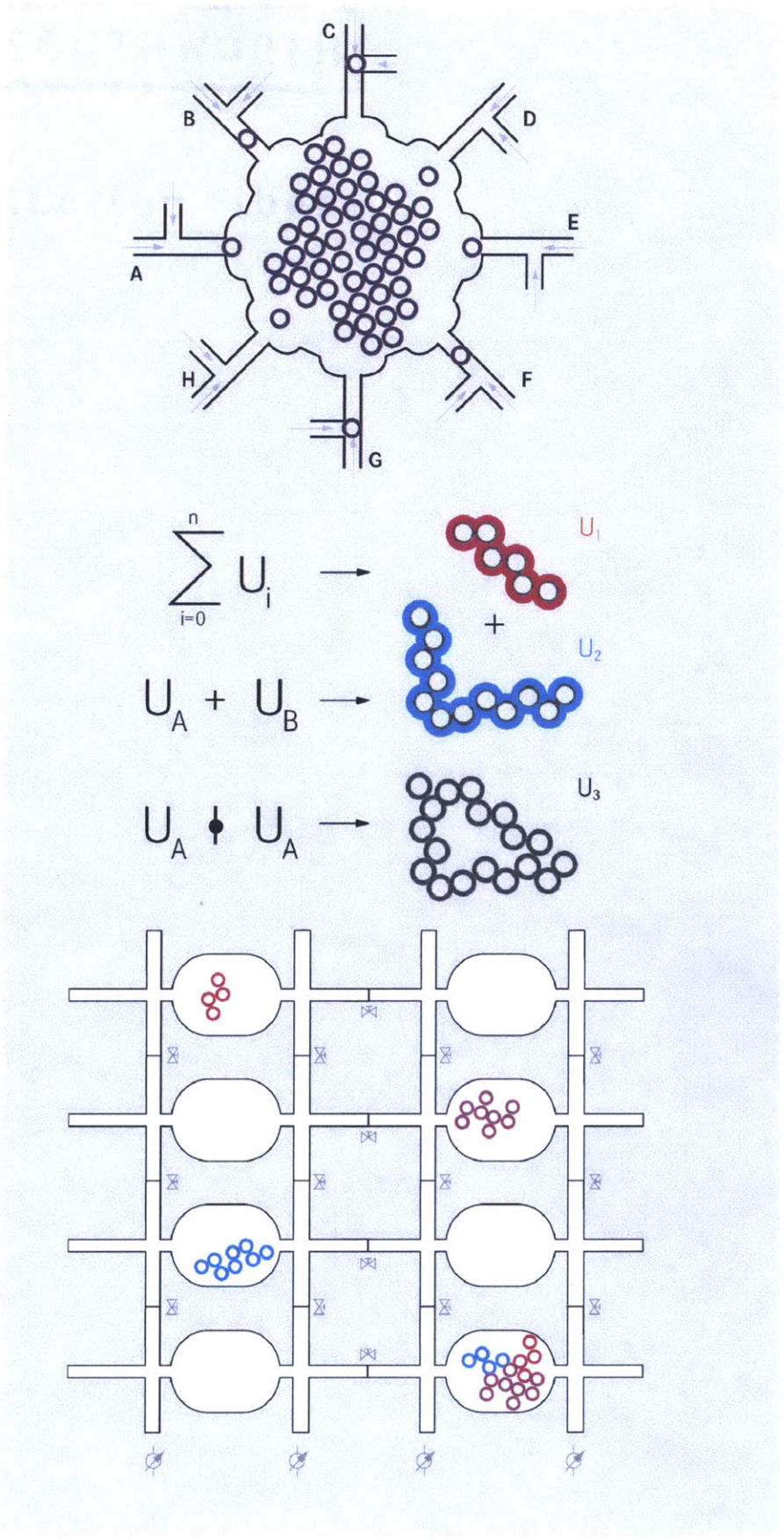
$$U_1 + U_2 \longrightarrow U_3 \not\longrightarrow U_1 + U_2$$

FUSION or DIVISION

1. Image from Paleos et al. 2011.
2. Based on Paleos et al. 2011, Stano 2009), and Stano & Luisi 2012.

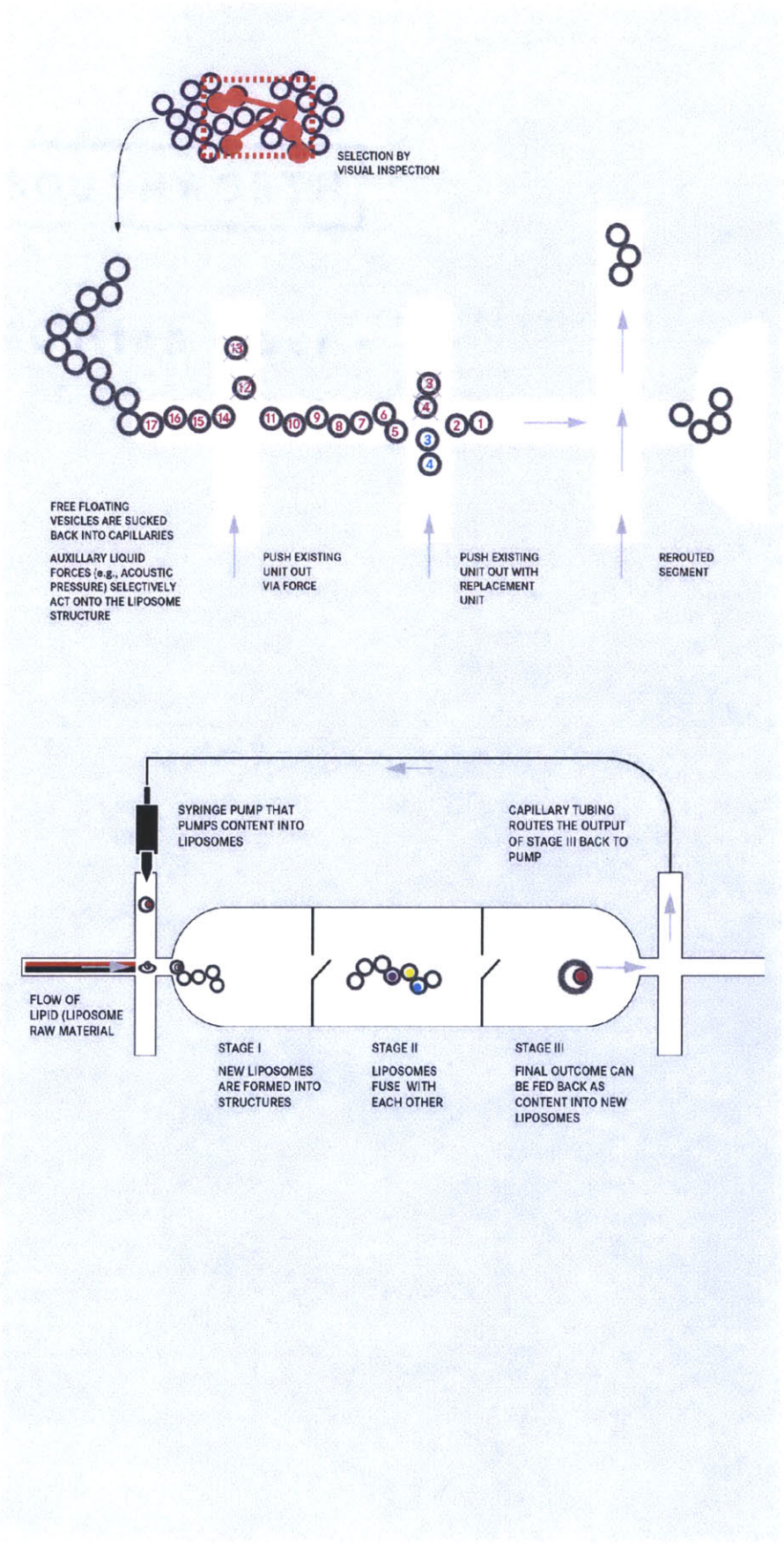
FORM OF FUNCTION: Liposome assemblies can be made from single or multilamellar—vesicle-in-vesicle—compartments. Their formal arrangement can be made differently for static or moving structures that can be based on a variety of functions. Here, I will present three different design methods which can be used to compose different liposome formations based on spatial, temporal, and biochemical functionalities.

Spatial Design: The spatial design operations can be visual, combinatorial, reversible or irreversible. Both the units and the ways to combine them in microfluidic environments can be specified as high-level rules in software and implemented via flow control mechanisms. Micado, a programming language developed by Thies and co-workers offers, for example, different types of abstraction layers to design computational behavior in microfluidic environments (Ananthanarayanan & Thies 2009) (Thies et al. 2008).By modulating the flow patterns in the capillary channels, units can be organized in different geometries. The flow patterns can be designed using rules which can be translated into pump actuation and speed during the liposome creation process. By also specifying the ingredients or membrane properties, liposome formations can be designed such that they alter their structure by themselves over time, based on their internal reactions (bursting after a while) or being incorporated by others.



Liposomes can be inspected with a microscope, and units can be visually selected and incorporated into new designs. Liposomes can be tracked via software and be rearranged based on visual preferences. The designs can be translated into new formations which can be restructured using different design methods. For example, existing droplet structures can be sucked back into microfluidic systems. Once the liposomes are linearized, individual units can be selectively added or removed from the group. Computationally controlled liquid streams or piezo transducers can knock in & out individual units and introduce new features to the formation. The final design can be collected or rerouted to a different location. This type of restructuring can preserve the identity of existing units. However, liposome structuring and restructuring can also be designed as a recursive process. Liposome units can be embedded inside other units and thus form multi-compartmental formations. Existing units can either incorporate other units' identities or lose their identities altogether as the new unit takes over the identity of an existing unit or annuls its own identity by dissolving itself and mixing the contents with the host vesicle.

In this design scheme, new structures can be designed with generative rules, grammars, or constraint-based formalisms. These instructions can organize both the spatial and temporal flow of liposome streams and direct structures into different formations.

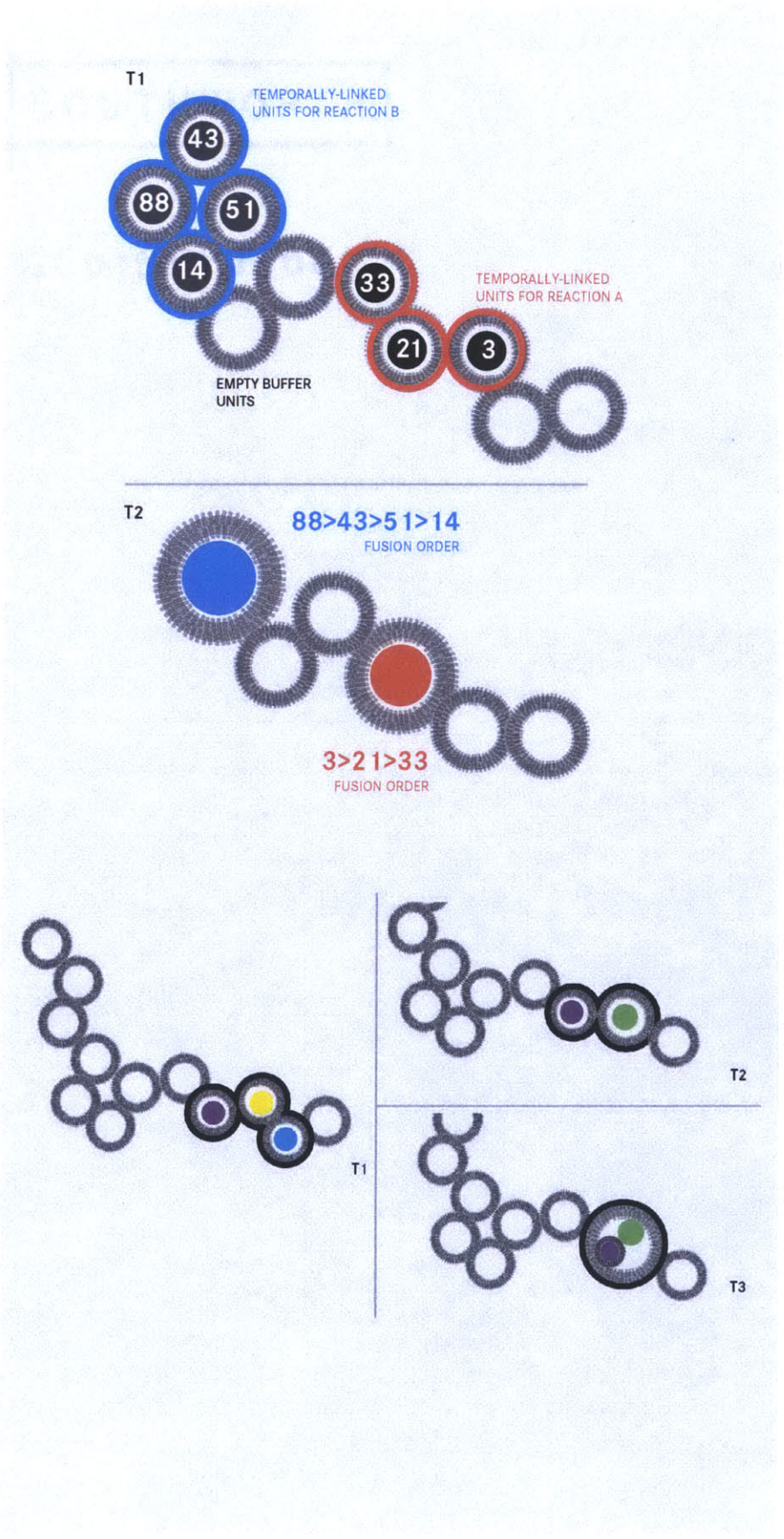


Temporal Design: Droplet structures can be ordered such that liposomes with fusing and self-dividing capabilities are next to each other. Fusing allows the exchange of materials or the incorporation of one liposome into another. This also allows the formation of multi-compartmental liposomes.

After these operations, existing units become new ones and therefore lose their identity. After the self-elimination of certain units, liposome groups can structurally self-organize and form unpredictable formations.

This level of uncertainty allows the design of underspecified liposome formations which can change their physical shape and function over time.

By bringing together liposomes with different chemical histories, it is also possible to construct structures with different timelines. Certain compounds such as enzymes function like molecular chaperons. By fusing enzyme-containing vesicles into those that carry the ingredients of other reactions, it is possible to chemically accelerate or decelerate reactions and use liposomes with different histories to change each others' behavior over the course of the lifetime of the whole group.

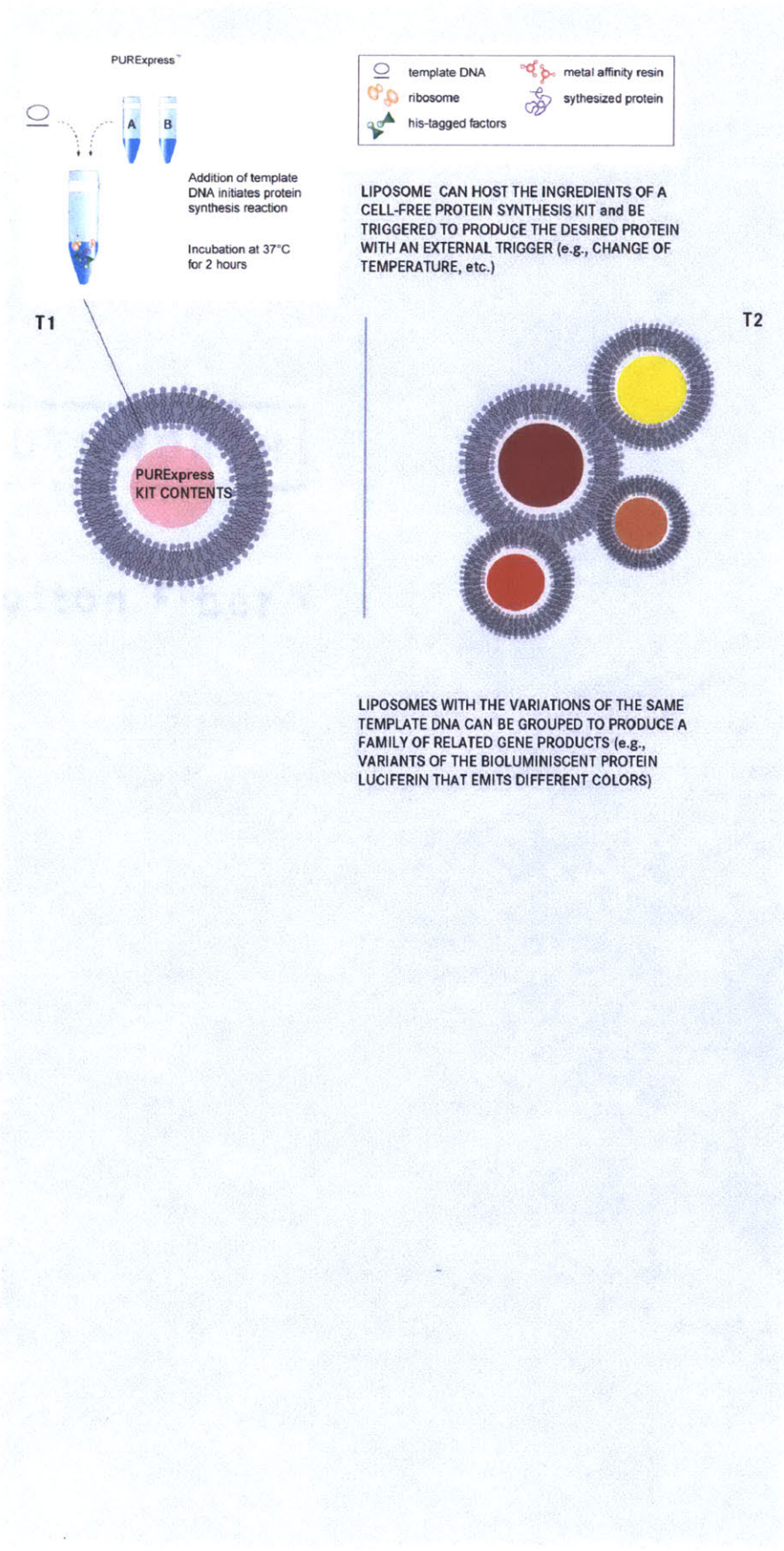


Biological/Chemical Design: Liposome units can have ingredients that become meaningful at a specific stage of a chemical reaction. During the assembly of the structure, units that need to be utilized at similar times can be placed next to each other, so that they can fuse and mix their contents at the same time.

The temporal fusion of the units can be triggered by the internal reaction of the units. Domain interactions can also be externally timed and activated using optical, electrical, or sonic input. For example, bacteria encapsulated within vesicles can be triggered to express certain proteins by the selective activation of the genes in their genomes using optogenetic methods (Schroeder et al. 2012).

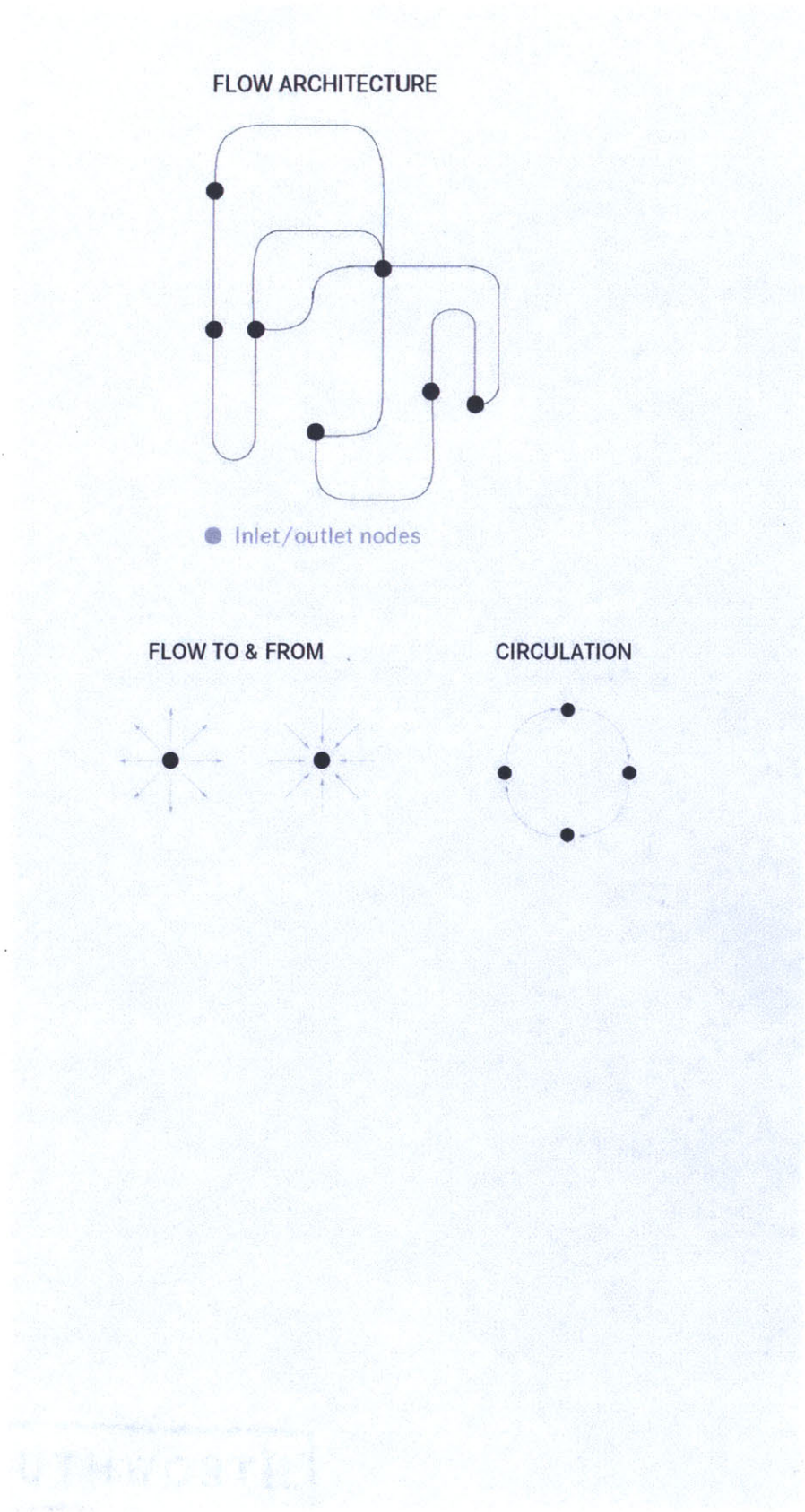
Selective removal can also be timed by biochemical reactions. Vesicles can be loaded with contents of reactions and produce enzymes that can destabilize or break down the lipid structure and cause the vesicle to burst.

As it can be seen from the examples, the elements of spatial, temporal, biological and chemical design can often be used together and create different biological contexts. Such compartmentalization allows a different level of control that usually does not exist in an open body environment, where all chemicals float among each other and interact at the same time. The discretization of this liquid space allows the use of a different design language to compose new biochemical events.



4.4 Designing With and For the Flow

Besides their uses in encapsulation and compartmentalization, one of the main advantages of liposomes is the ability to design structures and interactions while they are in flow. Liposomes within moving liquid streams can be continuously manipulated within the capillaries and re-routed to different locations through flow-control mechanisms. The liquid around the vesicles creates an external environment, which not only shapes the function and behavior of the contents of the vesicles but also makes them part of a larger liquid framework, a medium, which can be regulated with a different design scheme.



Flow To and From: By designing the flow architecture of liquid streams, it is possible to design where to and from liposome formations can flow. While the routing of liquids can be single-step operations, they can also be designed in constant circulation in which units are selectively given to or taken from structures in order to replenish resources or take away by-products.

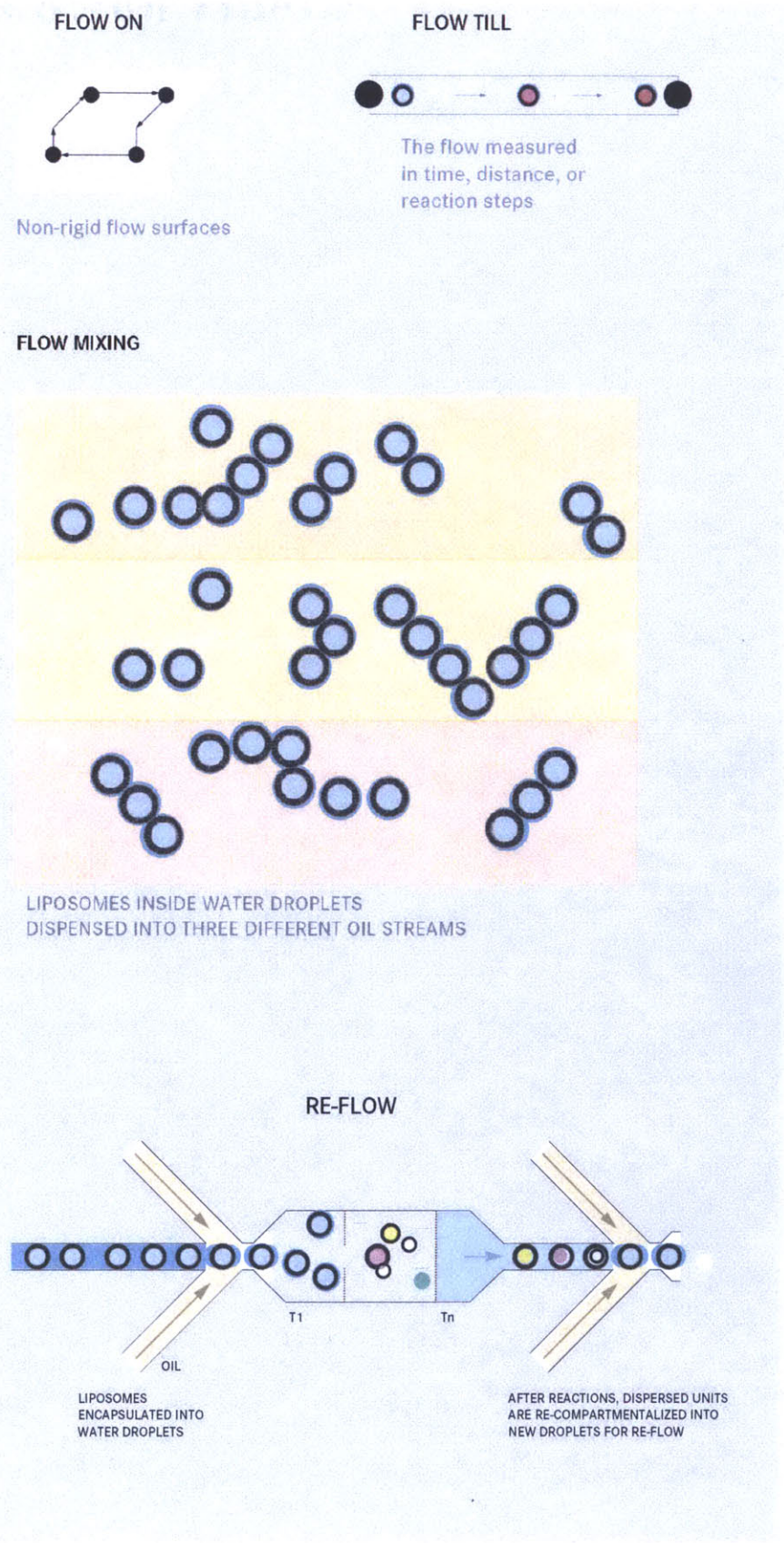
Flow Speed: Lipid vesicles can be programmed to exhibit different levels of stability and therefore be designed to last for fixed durations. By regulating the speed of the carrier environment, liposomes with different time spans can be moved to and from target locations. Speed can be a parameter for designing functional events, such as just-in-time delivery, or be a control element for the selective insertion or removal of units during restructuring operations.

Flow On: Most of the microfluidic environments I discussed so far referenced rigid surfaces such as glass or PDMS. However, it has been demonstrated that capillary channels can also be engraved on flexible surfaces such as foils (Focke et al. 2010) or printed on paper using masking tapes (Martinez et al.. 2008) or photoresists. Flexible surfaces allow the mixing of microfludics with different materials or let them be used in three-dimensions such as packaging materials.

Flow Till: The controlled flow of liquids from one inlet to an outlet provides another important design parameter. As the movements of liquids can be broken into capillary segments, the length of these segments can be utilized to make design decisions for programming liposome interactions, or to build designs that rely on internal reaction times based on mixing time, distance or reaction steps. Thus it would be possible to design an 'n-step liposome' knowing that the internal reactions within a liposome can be stopped at a given distance or time once it arrives at a control point.

Flow Mixing & Separation: Liquid streams with different densities can also be mixed with each other to create liquid-in-liquid suspensions that can co-locate different groups of liposomes with each other.

Re-Flow: Free-floating liposomes—those who lost their carrier droplets—can be re-compartmentalized into new droplets with new biochemical compounds. As units get delivered into new contexts, they can be equipped with new features and made to execute new tasks.

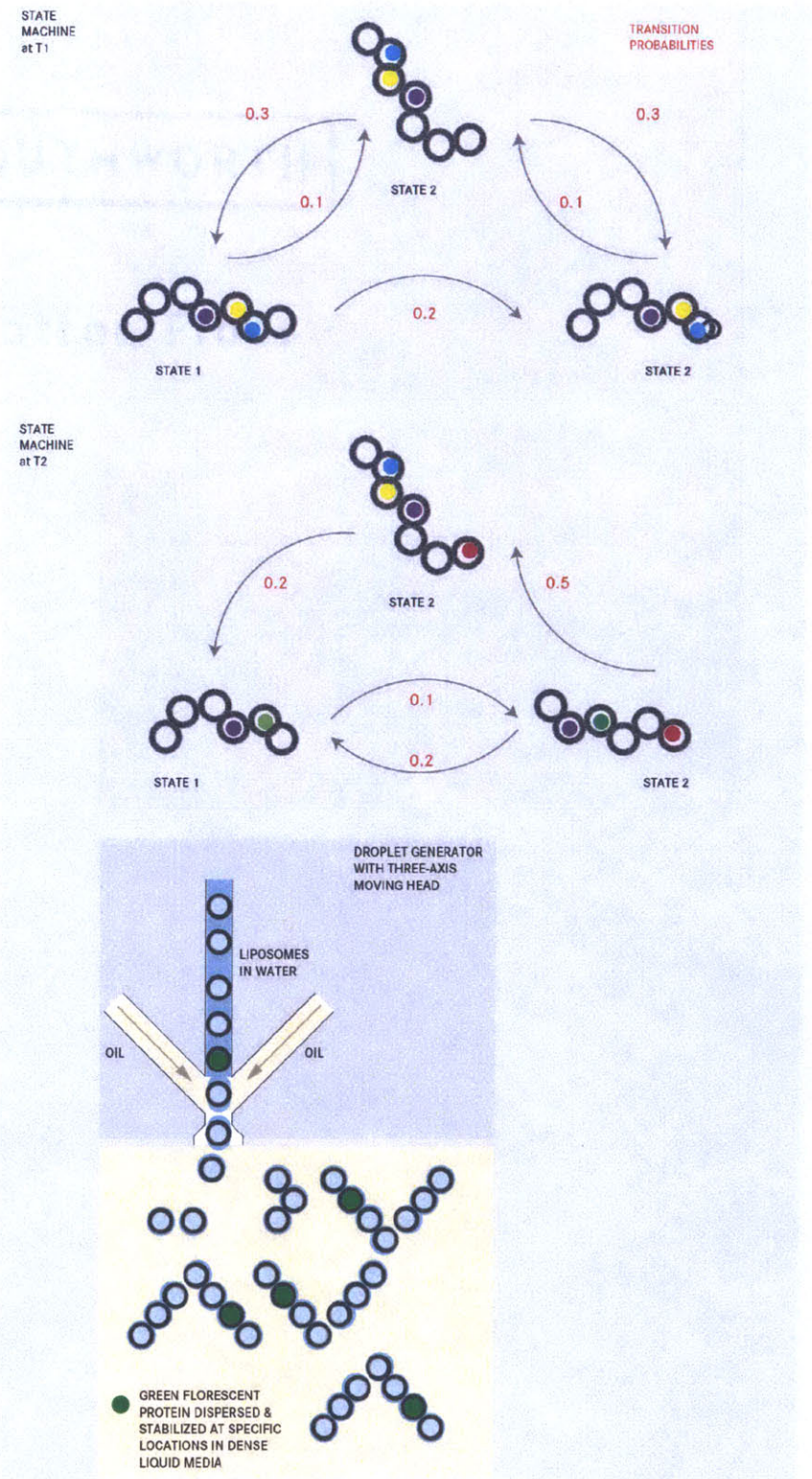


4.5 DNA and Non-DNA Instructed Biochemical Contexts

Liposomes can be designed to make use of both genetic information (e.g., via genes or randomizing nucleotide segments) and non-DNA based instructions. Individual units can be selectively instructed by introducing external stimuli (e.g., fusing units) that can trigger internal reactions with catalyzing enzymes and molecular chaperons. Similar to horizontal gene transfer, liposomes can be made to uptake plasmids, or be packed with RNA, ribosomal machinery, and reagents that can deliver the necessary payload to synthesize proteins when and where they are necessary.

Groups of liposomes can perform tasks while they are externally programmed within a closed-loop microfluidic framework. An assembly can be driven in liquid and made to switch from one state to another based on a computational model such as a state machine or automata. Unlike machine implemented computational models which work with persistent objects, units, and representations, individual liposome units can cease to exist—dissolve or be incorporated by others—due to their physical nature. However, a model-based instruction scheme can still be implemented by updating the status of the model over time or using probabilistic estimation techniques (Hofbaur & Williams 2002).

Liposome units can also be visually and geometrically programmed with a moving droplet generator head that can disperse them in particular geometries within continuous liquid media.



1. A sketch for a liposomic state machine
2. Computational arrangement of vesicles with an automated droplet generator

4.6 Vesicle interaction in Continuous Media

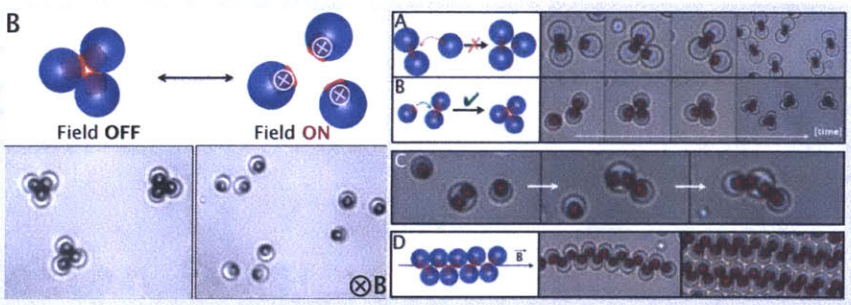
In addition to composing interactions based on the electrostatic properties of their membranes, vesicles or vesicle-in-droplet formations can be manipulated with other programmatically controlled sources such as electromagnetism and acoustics.

With such external control mechanisms, magnetized vesicles can be manipulated outside capillary channels and form reversible interactions in continuous media.

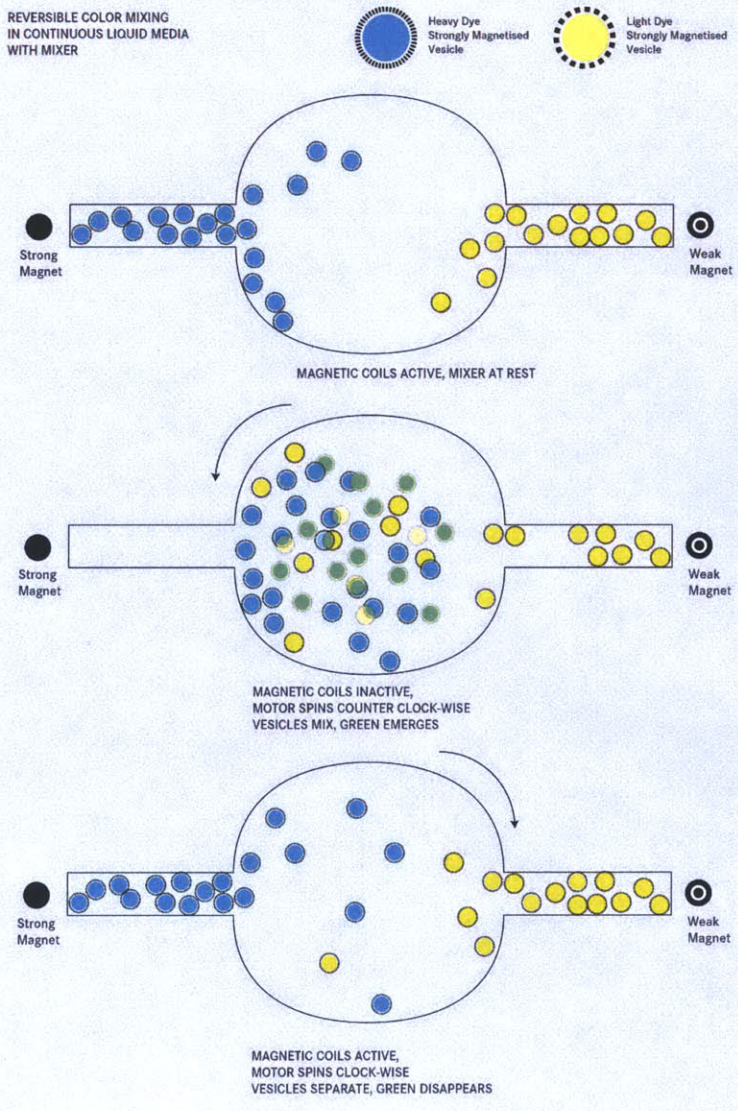
By incorporating iron oxide into the membrane chemistry, or hematite micromagnets into the payload, arrays of vesicles can be assembled into different organizations and form colloid structures (Franke et al. 2009) (Sacanna et al. 2011).

Membrane magnetism and the density of the vesicles can be used to differentiate vesicles from each other, and thus allow individual or groups of vesicles to be identified, separated, sorted, or mixed for different purposes.

From a spatial design perspective, reversible and calculable interactions in continuous media provide many affordances over microfluidic-only interactions. The possibility of juxtaposing different materials next to each other not only allows the implementation of the spatial and temporal design strategies discussed before, but also extends these methods to be applied to geometrically unbound settings.



(Left) Clusters formed at high salt concentration in zero-field can be disassembled by imposing an external magnetic field. (Right) Various structures made with magnetic colloids: (A) Large particles with a single magnetic patch form dimers, (B) smaller particles with a single magnetic patch form trimers, (C) two particles with a single magnetic patch join with one particle with two magnetic patches, and (D) under an external magnetic field, particles form long structures. Image credit: Sacanna, et al. ©2012 American Chemical Society



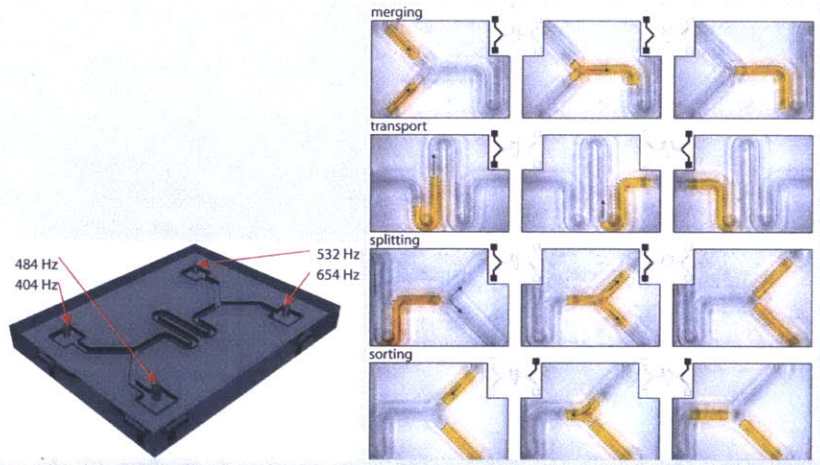
In addition to liquid streams caused by pumps, vesicles can be moved—pushed or carried—by acoustic pressure. Langelier et al. 2009 discusses a number of acoustically-driven liquid operations in capillary channels using transducers set to different frequencies.

Similarly, standing waves caused by acoustic sources can be used together to move or carry vesicles with different densities. Transducers with different frequencies can be used together to tune movement to a particular group of vesicles such that they can be mixed or separated based on the period of the standing wave.

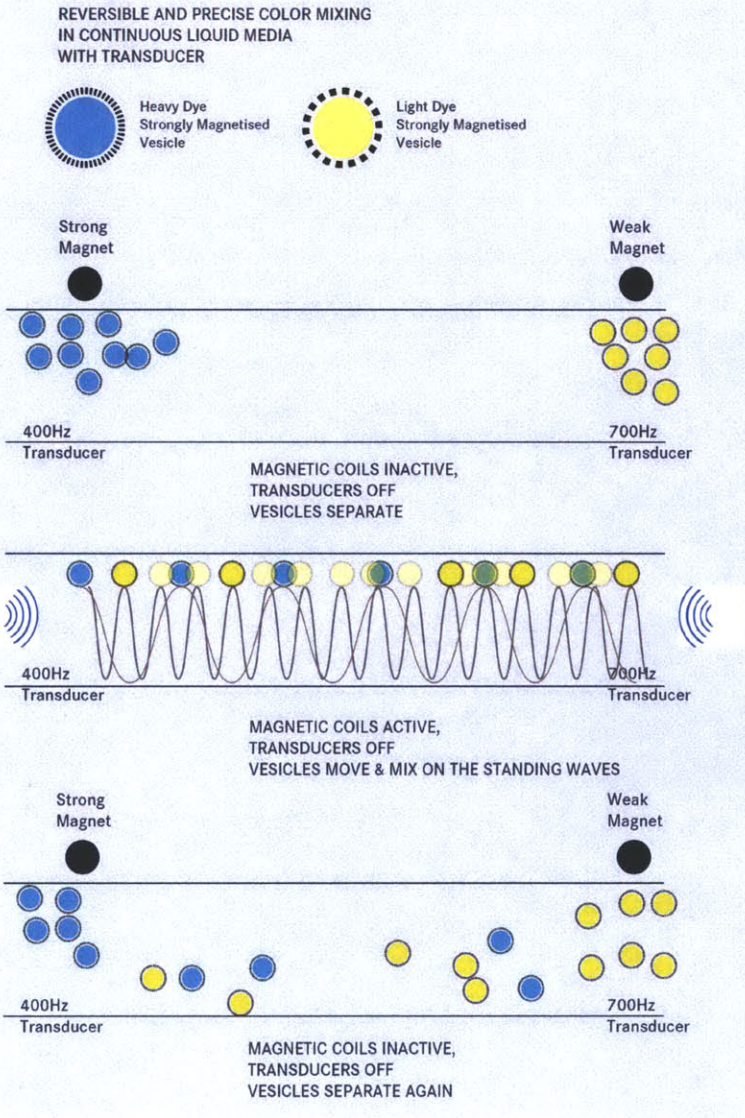
In both acoustic and magnetic mixing, as the source of movement is external to the vesicles, it can be programmatically switched on/off to create time-based, dynamic or responsive interactions. Here, the figure demonstrates the selective mixing of cyan and yellow dyes to create the impression of green without the presence of the green dye in the environment.

As the physical proximity, content, and the number of vesicles can be controlled precisely with sound waves, the mixing of different colors would allow the vesicles to create color patterns with their structural organizations and movement.

Acoustic energy can also be used to burst the membranes of vesicles such that they can release their contents once they are in their desired locations.



Microfluidic unit operations using acoustically controlled droplet motion. (Langelier et al 2009)



4.7 Discussion

As a summary, here I list some of the key of elements of the theory using images taken during my own design experiments. Liposomes within a microfluidic manipulation framework offer new ways to think about addressability, encapsulation, compartmentalization, transportation and differentiation for designing new applications in molecular commons. Some of the key findings of the theory can summarized as follows:

i) Droplets provide a form of articulation to the otherwise continuous space of liquids. They partition the aqueous design space into different geometries and make it quantifiable, addressable, and controllable offering different applications torational design. They can provide both a static, grid-like partitioning as well as a moving space, in which liquid streams can carry units that join, split or fuse, and interact with each other at different speeds.

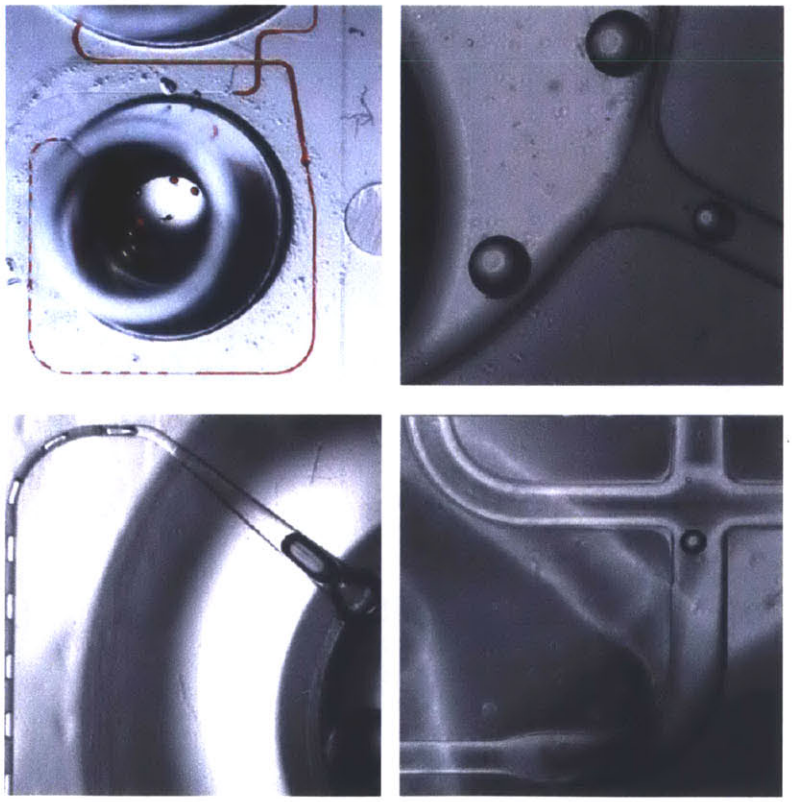


Figure 1. Microfluidic droplet generation using X-juction chip obtained from Biorad (Bio-rad.com).

Source: Telhan

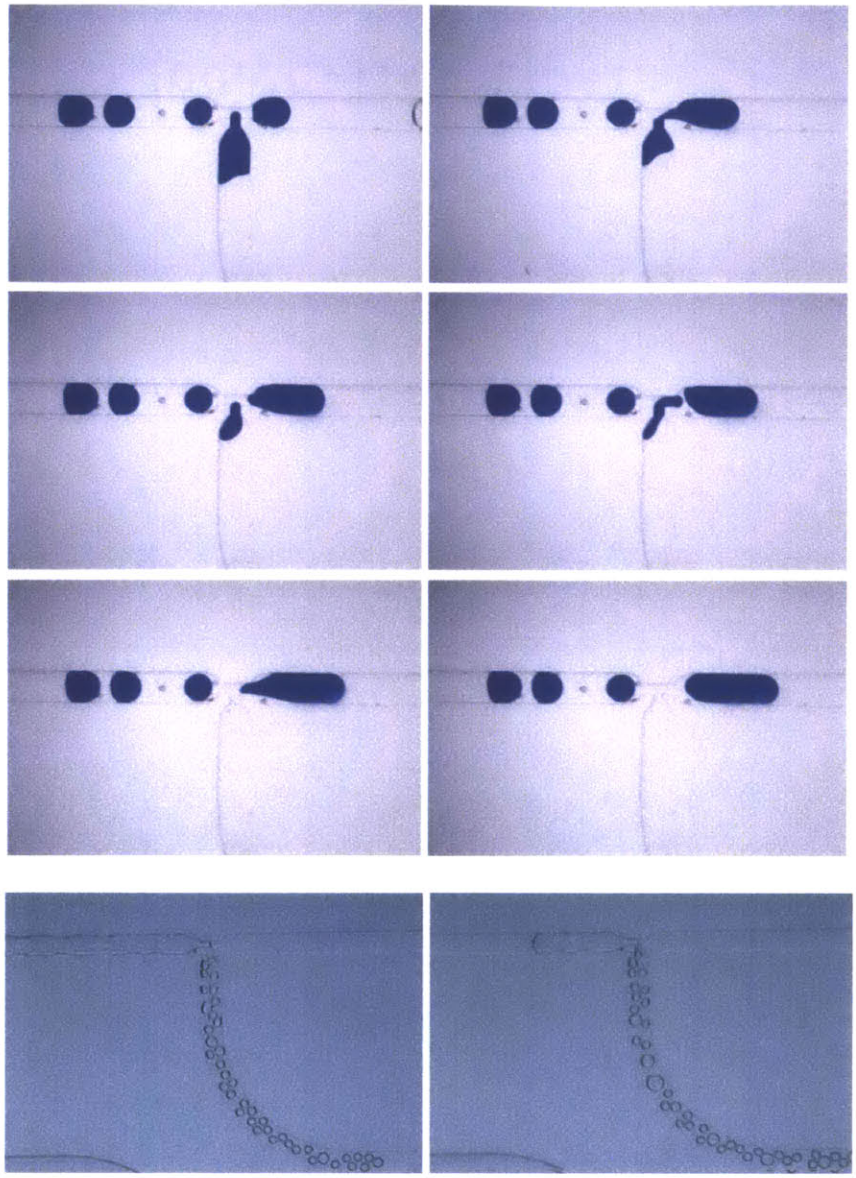


Figure 2. Dye-in-oil droplet formation in emulsion using a -junction chip (6-image sequence). Water-in-oil continuous droplet formation using a T-junction chip (2-image sequence)
Source: Telhan

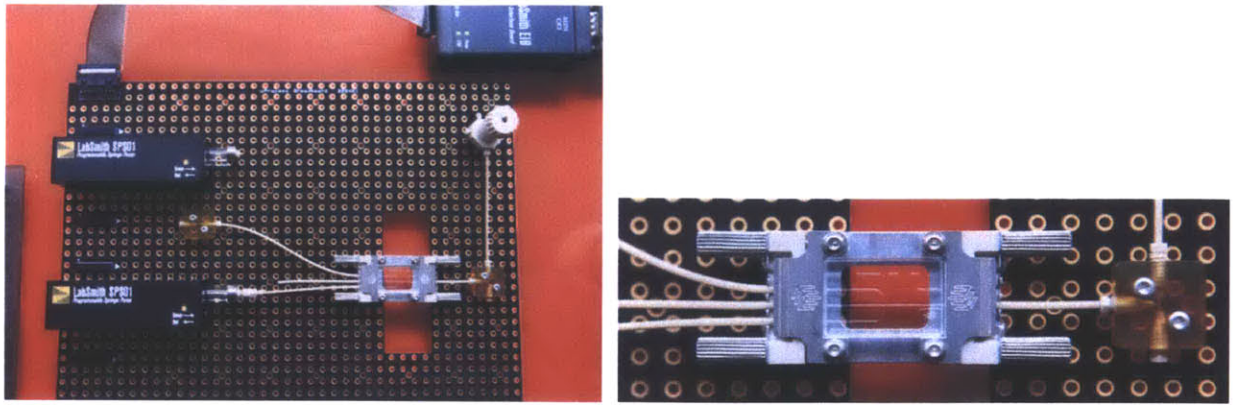


Figure 3. Programmatical droplet formation using liquid syringe pumps at a prototyping workbench. Pumps, control interface, and workbench obtained from Labsmith, the microfluidic chip is from Dolomite).
Source: Telhan

ii) Liposomes-in-droplet formations combine the features of the liquid typology, its animated character—such as flow or circulation—with biochemical capabilities. As liposomes can encapsulate different types of living and non-living agents, they can be utilized to assemble new forms of biological artifacts that can partially exhibit life-like behaviors. Liposome formations can incorporate parts of living elements and host reactions that produce chemical compounds.

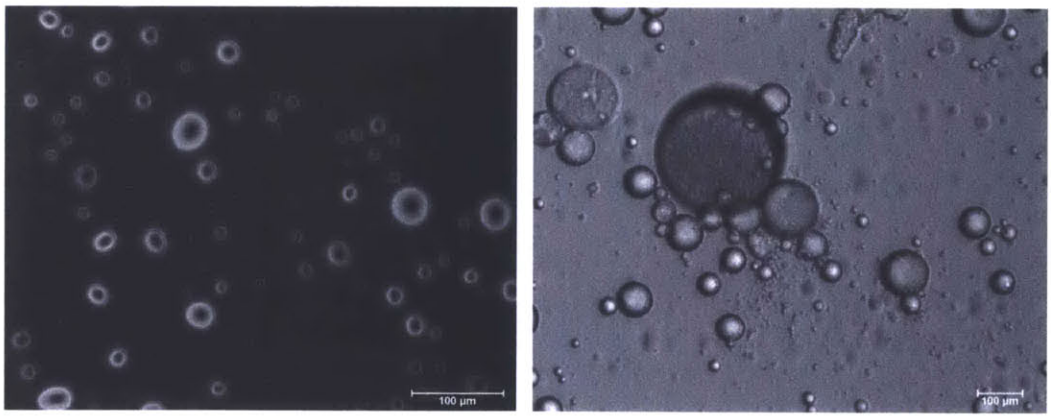
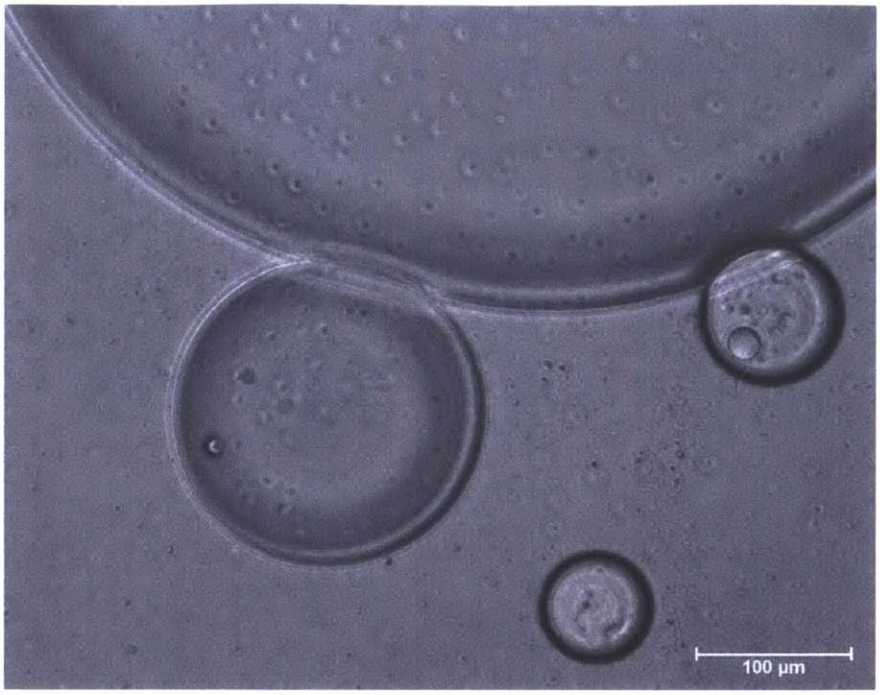


Figure 4. Liposomes.
Source: Telhan



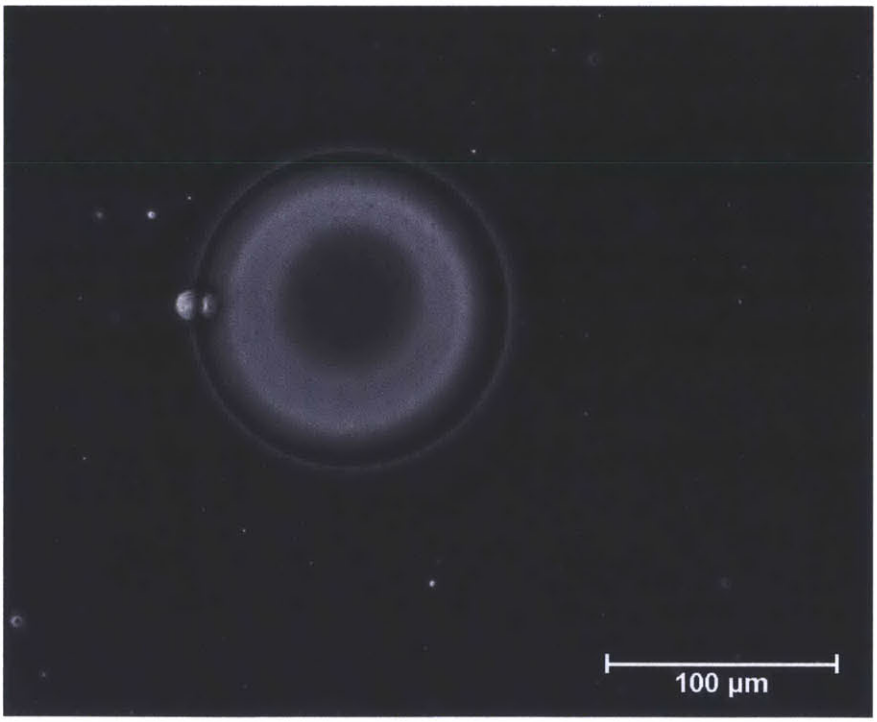


Figure 5. Adhesion and incorporation experiments with liposomes.
Source: Telhan

iii) Liposomes with different membrane properties can be assembled in bead like formations, which then can be used to form assemblies that can have different biochemical, chemical, visual, geometric, and informative functions.

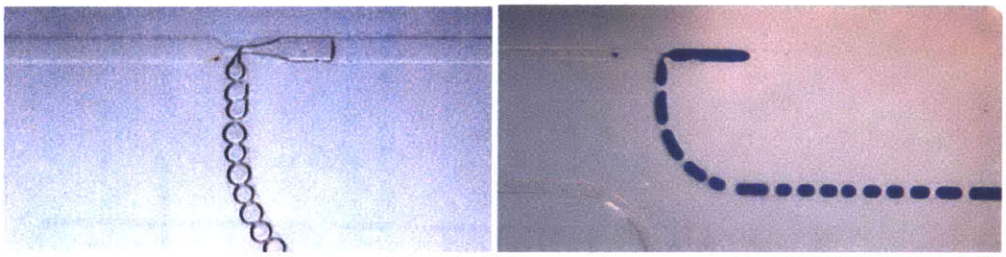


Figure 5. Two droplets merging and combining contents
Source: Telhan

I don't provide any images of droplet assemblies here as I did not do any experiments to build such formations. However, during the experiments I assumed that droplet assemblies could be considered analogs to liposome assemblies as a proof of concept to demonstrate how different ingredients can be interleaved one after another during the droplet formation process. For example, the temporal design is demonstrated by producing droplets with different contents and placing them next to each other such that as the droplets merge they can share content and start new reactions (Figure 6).

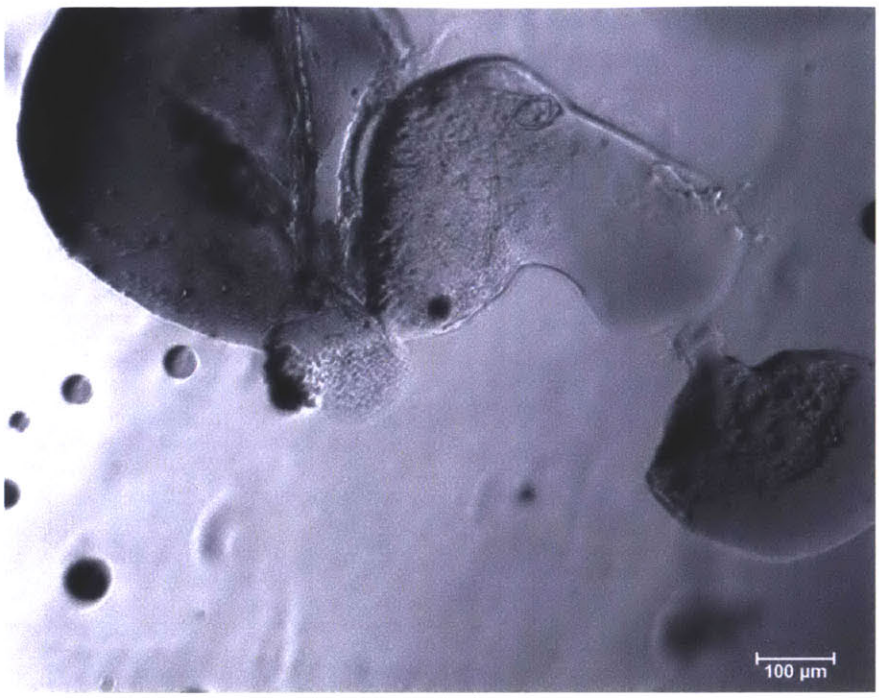


Figure 6. Two droplets merging and combining contents.
Source: Telhan

Figure 7 shows images from a cell-free protein synthesis experiment that shows the use of water-in-oil droplets to encapsulate the ingredients of a commercially available PURExpress cell-free protein synthesis kit to synthesize the green florescence protein with the presence of a plasmid that carries the genetic information. Here, other than the ingredients of the kit—such as RNA, ribosomal machinery—and the plasmids which are obtained from living organisms, no living host is used during the creation process of the natural protein GFP that causes green florescence. After an incubation period of 2 hrs at 37 degrees Celcius, GFP is observed under the microscope.

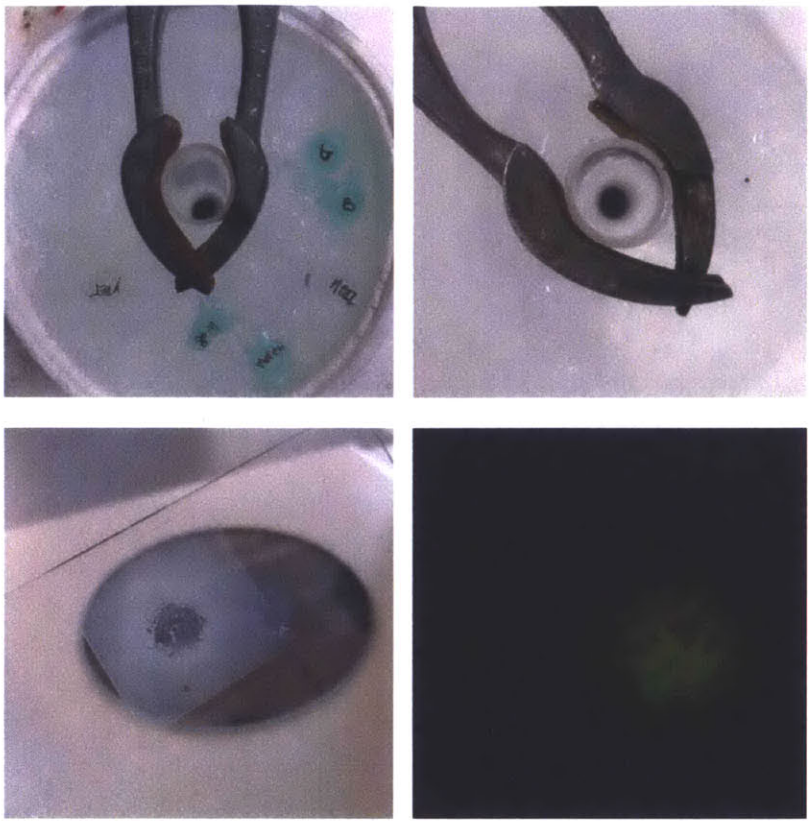


Figure 7. Cell-free green florescent synthesis (GFP) done inside liposomes
Source: Telhan

iv) The representational and physical meanings of liposome units introduce a different way of thinking about abstraction in design. If one uses a semiotic context, liposome constructs—or 'words'— can follow different rules of assembly (syntax), where their interaction would have visual (e.g., color), geometric (both visual and structural), chemical, biological, informational or computational meanings (semantics). Here, the physical function of the units (themselves) and what they may mean in relation to each other (their mapped meaning, or what they can stand for beside their physical function) provide a different way to compose design interactions. Thus a biological arrangement can have visual meaning; a visual arrangement can have different chemical consequences; a biological event can be

recorded as digital information and so on.

v) Microfluidic channels not only add additional articulation and control over the liquid design space but also provide computational control and automation for liposome-based design. The ability to control the confinement of newly created biological constructs—both physically and chemically—makes them quite important in their role of managing the containment and confinement of biological design in relation to everyday products. As microfluidic channels can be designed with different soft and hard materials, they can interface liposome-based designs with different forms of products such as medical or food packaging, liquid bottles containers, etc.

In the next chapter, I will present a case study that details how some of the elements of this framework can be used within biological design.

Chapter 5 – Biosynthesis and the Futures of Sandalwood

In this chapter I discuss the role of biological design within a specific area of research referred to as biosynthesis. Like the "Humulin©" example discussed in Chapter 2, today a significant portion of synthetic biological design activities are geared towards synthesizing the biochemical equivalents of organic compounds produced by plants, animals, and humans using single-cell organisms such as bacteria, yeast or fungi. While most compounds found in Nature can be derived from each other using chemical synthesis methods, the number of steps in reactions, scarcity of ingredients, low yields and toxic byproducts make chemical synthesis a not always viable solution. On the other hand, utilizing genetically modified microbial organisms to do the synthesis, when possible, can be highly profitable. Today, *Escherichia coli* and yeast, for example, are grown in massive industrial fermenters to produce biofuels (Fortman et al. 2008), medicine (Ro et al. 2006), and perfume (Alifano et al. 2009).

In addition to making significant economic value propositions, the conversion of biomass into desired products has many different ecological, social, and cultural implications. Here, I will specifically discuss a case study around the Sandalwood tree and how the futures of the plant may change given the new directions in biological synthesis and design. The case study will detail a biological abstraction and design process that synthesizes some of the ingredients of Sandalwood oil (e.g., Farnesol) using synthetic biology and liposome-driven design methods. It will feature both theoretical and partially implemented aspects. The implementations will be presented as proofs of concept that are discussed in Chapter 3 and be discussed within the context of a prototype machine—a microbial perfumery—that synthesizes gene products that can be mixed with natural products.

5.1 Biosynthesis

Biosynthesis refers to the type of metabolic processes—chemical reactions, molecular couplings-transformations, conversions—living organisms do to produce the biological compounds needed for their survival. Life processes often involve the transformation of basic inorganic substrates (e.g., CO_2, H_2O, NH_3) taken from the environment into organic molecules such as amino acids, nucleotides, or monosaccharides, which then get synthesized into macromolecules such as lipids, proteins, DNA, and vitamins in the presence of solar energy or its chemically stored form ATP. Biosynthesis is an anabolic activity in which complex compounds are made from simple ones by self-assembly (Smith and Wood 1992). It involves the synthesis of different units of molecules based on different intramolecular forces. While reactions can often be traced in steps, they also happen in parallel and within the same location, making it difficult to pinpoint the exact ingredients and outputs, or the exact causes and effects.

The study of metabolic pathways that lead to different products has been an important research area for biological design. Living organisms come from common ancestors, so one organism's ability to produce a particular product, such as a protein, can be transferred to another. While species from a common genus may have similar genes at their disposal, due to their response to different evolutionary forces and selection mechanisms they may not develop the same type of metabolic pathways that yield the same products. Genetic manipulation may reactivate otherwise silenced genes. The expression of a particular gene product can also be stimulated by introducing genes that can produce the catalyzing enzymes that will speed up the synthesis of that product.

With the increase of biosynthesis applications, it also becomes important to evaluate the perception of biological products from different perspectives as there could be many implicit trade-offs. The bias towards Nature-made products synthesized by non-modified organisms, for example, is challenged when a plant or animal has become endangered; such as in the case of bear bile, elephant tusks or

orchids. Chemical substitutes, for example, when proven to be safe, not only offer low cost alternatives to biological products, but also have the potential to reduce the demand for the natural products and help protect the future of endangered or threatened species. When genetically modified organisms are utilized to produce Nature-equivalent products in highly confined environments, they may offer healthier and safer products than natural organisms grown with pesticides. Different synthesis methods have different consequences, and Figure 1 is presented as a summary of different biological design methods and their implications:

	SYSTEMATIC PRODUCTION OF NATURAL PRODUCT OR ORGANISM (e.g., Plantation, farmland)	NATURAL PRODUCT BY NATURAL YET SUBSTITUTE ORGANISM (e.g., Grafted trees.)	NATURE-IDENTICAL PRODUCT BY GENETICALLY MODIFIED ORGANISM (e.g., Humilin.)	GENETCIALLY MODIFIED ORGANISM AS PRODUCT (e.g., AquAdvantage Fish.)	NATURAL PRODUCT WITH CHEMICAL MANIPULATION (e.g., Pesticides.)	NON-NATURAL (CHEMICALLY SYNTHESIZED) PRODUCT (e.g., Saccarin.)
HEALTH CONCERNS (e.g., Carcinogenic effects.)			O	O	O	O
ENVIRONMENTAL CONCERNS (e.g., Impact on habitats & natural resources.	O	O		O	O	
BIOLOGICAL CONCERNS (e.g., Impact on biodiversity.)	O	O		O		

Figure 1. Impact of various biosynthesis methods.

In addition to its role in replacing the source of production of existing biological products with better, cheaper, abundant, or faster growing analogs, biosynthesis also offers a number of perspectives as a design method. It provides new ways to think about control, decision-making, customization, personalization, and variation in biological design. It allows biological processes to be designed for different types of non-scientific or commercial interests. Biological knowledge and manipulation methods are becoming increasingly available to amateur audiences, and there has already been a shift from scientific research investigations towards creative explorations that prioritize aesthetic, personal, social, cultural or critical interests. While a number of groups have been working on developing do-it-

yourself versions of glowing fish and plants (Kickstarter 2013), there has also been work examining the democratization of the tools used for the analysis, design, and synthesis of designed artifacts.

These alternative research objectives combined with low-cost equipment allow amateurs to explore different methods such as controlling the variability of the outcomes in biological processes. Deliberately giving up some amount of control can become an element of style where both synthesis methods and their limited-edition products indicate a sign of authorship or novelty. For example, when bioluminescence genes are randomized by site-directed mutagenesis methods, they can yield a wider spectrum of light-emitting compounds that differ from those that are evolved through Nature. While different tones of purple would not make a scientific value proposition when compared to a fixed shade in a scientific context, within the hands of their designers these randomized gene sequences mark the basis of a new kind of biological signature, similar to the generations of artists and designers who invented their own color schemes as a form of personal expression (IGEM Cambridge 2010).

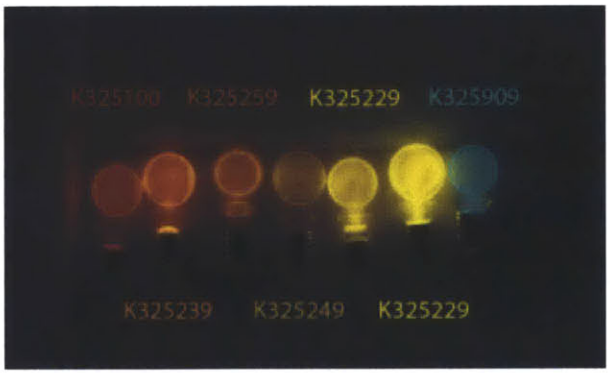


Figure 2. Different colors created by randomized Luciferine genes.
Source: IGEM Cambridge team (2010).

With synthetic biology, it has been frequently demonstrated that biosynthesis applications can be tied to different kinds of human-made pathways that consist of alternative feedback loops, network motifs, or decision-making mechanisms. By manipulating the metabolic pathways, the timing, sequence, quantity, and location of biosynthesis, the process can be oriented towards alternative applications.

Proteins can be produced on demand; reactions can be mixed and catalyzed as responses to sensing and decision-making schemes.

When human-augmented production methods allow temporal and spatial advantages over the traditional forms of biosynthesis, the outcomes also change the perception and demand for biological products. When the use of Earth's corn biomass is shifted towards ethanol production, it inevitably creates significant shortages in food supply, as fuel cannot be used in exchange of food in certain parts of the world. When Artemisinin—the main ingredient used to fight *Plasmodium falciparum* malaria—is synthesized through bacteria, the low cost medicine not only saves lives but also causes poverty in the regions that rely on harvesting the plant which had been the traditional source of the drug (Dietrich et al. 2009) (ETC 2013).

When biological products are synthesized outside their original contexts, they navigate through a complex set of values. As designed products, they may not only offer cheaper, better, standardized and improved solutions to existing products, but can also suggest alternative ways to think about bioproduction and its implications. When it becomes increasingly fashionable to grow algae as a source of fuel or food, it shifts the perception of the organism from merely being a living thing to a machine that produces something for humans—whether it is for utility or aesthetic reasons. If colored light-emitting artifacts start to run on sugar instead of electricity, the designed products will not only interface with taste or personal preferences of people, but also with their anxieties and fears. An increased demand for a fashionable algae lamp can easily drive a particular species of algae towards extinction or over population such that its shifts the natural habitat it has been evolved for.

In the following sections, I will expand on these remarks and present a case study on how the advances in biosynthesis may affect both the value and perception of the Sandalwood plant and what the futures of the tree may be when its products turn into design objects.

5.2 Sandalwood Tree and its Products

Sandalwood, the *genus* known as *Santalum*, is one of the most valuable plants in the world. The plant and its products have been part of the economic, social, cultural and religious history of a broad geography for thousands of years.

Figure 3. *Santalum Spicatum* tree
Source: Jesse Moniodis

Sandalwood kernels and nuts are known to Australian Aboriginals as medical remedies; the leaves and twigs are burned in Zoroastrian temples; sandalwood paste and incense sticks are used in many rituals in Buddhism, Hinduism, Chinese and Japanese Religions; and the heartwood, the inner core of the tree, is highly valued for woodwork due to its distinctive fragrance and is used in making accessories, sculptures, and souvenirs in most Eastern Asian countries. The exotic sweet Sandalwood oil, which is the most precious part of the plant, has numerous applications in the fragrance, cosmetics, and pharmaceutical industries.

Figure4. Sandalwood wood and nut oil are used in a range of products
from cosmetics to medicine
Source: Mt. Romance Sandalwood Factory Product line.

Figure 5. Sandalwood logs are easily carved due to the fine grain
and oily texture of the wood.
Source: Telhan

The cost of pure wild Indian Sandalwood oil can cost up to US$ 1,850-1,900/Kilogram, making the plant a highly valuable commodity in international trade (Alibaba 2013). This high demand inevitably makes Sandalwood an overharvested plant. In Western Australia between the 1840s and 1920s, three hundred thousand tons of sandalwood—nearly ten million trees—were exported to Asia (Statham 1990).

Figure 6. Sandalwood logs are often smuggled from East Timor to Bali.
Source: Telhan

Today, the conservation of certain wild species of Sandalwood is highly regulated around the world and most commercial production has shifted towards domesticated plants grown on farmlands and plantations. Sandalwood cannot grow everywhere though. It requires yearlong sunshine and tropical climate to flourish. Being a type of parasitic plant, it also needs a specific habitat where it can feed off nutrients from different types of host plants. While different species of *Santalum* grow in locations as diverse as India, Indonesia, Vietnam, Western Australia, the Pacific Islands and Hawaii, plant phenotypes show great variety in different geographies and have different values in the global market. The heartwood of Indian *Santalum album*, which is richer in oil than Australian *Santalum spicatum*, is higher in demand, a situation that has brought wild Indian *Santalum* to the brink of extinction. Having almost completely exhausted their wild flora, both countries have heavily invested in naturalized or cross-bred Sandalwood plantations using incentivized farming initiatives to be able to meet the world-wide demand for the oil.

Sandalwood is a slow-growing tree and the amount of oil it produces is related to its age. The older the tree gets the more oil it stores in its heartwood and roots. *Santalum album* can live about a hundred

years and becomes ready to harvest in forty. Because the plantation-grown trees need to be harvested in seven to ten years to become financially viable, they often produce less oil than the wilder species, making them a lower quality yet more sustainable commodity.

5.3 Biosynthesis of Sandalwood Oil

The extraction of Sandalwood oil from the plant is a terminal process. The tree is cut into billets and separated in to heartwood, sapwood, roots and branches. The heartwood and the roots are grinded into power and then steam distilled to extract the oil, whereas the sapwood is separated for carving and woodwork (Plant Cultures 2004). Given its precious and precarious nature, Sandalwood oil has been under scrutiny for some time as a candidate for biosynthesis. The synthesis of the ingredients of the oil promises high quality and cheap products from highly controllable manufacturing processes. If biosynthetic oil can supply a significant amount of demand, this would also reduce the stress on the wild and protected Sandalwood species.

The essential Sandalwood oil has a complex composition. While different species' oil shows high variation, there are more than hundred identifiable compounds that are responsible for the oil's aroma, fragrance, anti-microbial, and anti-inflammatory characteristics. *Santalenes*, the major compounds responsible for the characterization of the smell, belong to a group of organic molecules known as *Sesquiterpenes*, which are synthesized by a variety of organisms such as plants, bacteria, and fungi for their semiochemical functions. These chemicals are used for communication, sensing, or as defense agents, and form the basis of plant hormones and pheromones (Crock et al. 1997) (Nickerson et al. 2006). *Sesquiterpenes* are also the building blocks of many different biosynthesis applications, and therefore are heavily researched beyond their uses as aromas or fragrances. Being a class of hydrocarbons knows as *Terpenes*, they are also investigated for their potential uses as biofuels,

anticancer drugs, and antimalarial medicines. Given their high value, different stages of *Sesquiterpene* biosynthesis pathways have been extensively researched in many different organisms over the past few years. The necessary precursors for *Santalene* synthesis, such as the enzyme *Farnesyl diphosphate synthase (FPP)* which creates a number of different *Santalenes* depending on their different binding patterns, have been already mapped out by different research groups for *in vitro* synthesis using bacteria (Sallaud et al. 2009).

More recently, researchers from University of Western Australia have identified the metabolic pathways of *Santalene* synthesis in different species of Sandalwood (Jones et al. 2008) (Jones et al. 2011). Based on previously characterized Terpene Synthesis Genes (TPS) in *Santalum album*, Jones and co-workers have cloned the genes from the RNAs of *Santalum austrocaledonicum* and *Santalum spicatum* and identified four genes responsible for different types of *Santalenes* found in Sandalwood oil. By reverse transcribing the RNAs, the researchers have built cDNA libraries that can express the plant genes in bacteria. More information regarding the cDNA making process can be found in Chapter 3.

Here, Figure 7 is provided as a summary of Jones's paper. This research is not only important because it identifies the steps of biosynthesis for the six types of *Santalenes* found in the Sandalwood oil, but also because it offers an inspiring framework to think about different ways of augmenting the biosynthesis process for different design outcomes.

SANTALENE BIOSYNTHESIS IN SANDALWOOD

1 Extraction of RNAs from trees.

The RNAs of Sandalwood species *S. album*, *S. austrocaledonicum*, and *S. spicatum* are extracted from their lower stems for cloning.

2 Complimentary DNA (cDNA) Library Construction

Based on the previously identified genes known for Terpene Synthesis Genes (TPS) in *S. album* the other three sandalwood species' RNAs are cloned and then reverse transcribed to be able to build the DNA libraries that can express the plant genes in bacteria.

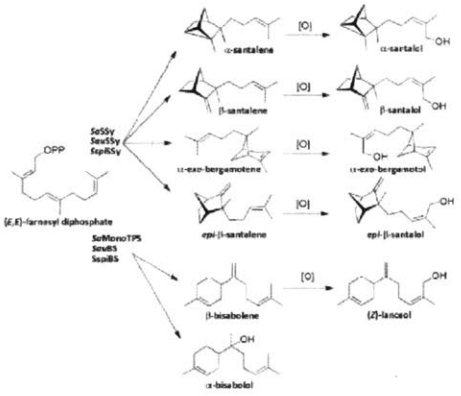
Characterization of Terpene Synthesis Genes in three different Sandalwood Species

S. album DNA is used as a template to identifty the corresponding genes in the other species. Once the genes are cloned, they are sequenced for identification. The newly characterized genes *Sa*SSy, *Sau*SSy, and *Sspi*SSy are deposited into the Gene Bank.

3 Bacterial Expression & In Vivo Santalene Synthesis

TPS genes are cloned into a plasmid and introduced to *E.coli*. The synthesis of Santalenes require the presence of ezymes FPP (Farnesyl diphosphate), its isomer GPP, and Mg2+ in the environment.

Once the substrated are introduced the bacteria expresses four of the main santalenes and two additional compounds that characterize the fragrance.



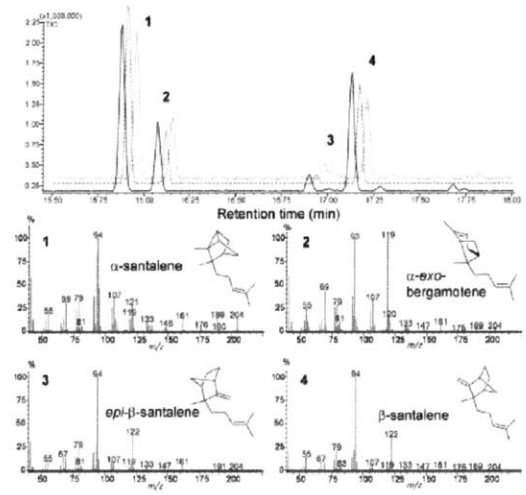
Biosynthesis of sesquiterpenes in sandalwood commences with the TPS-catalyzed rearrangements of farnesyl diphosphate. Specific oxidation at C12 is proposed to occur via a cytochrome P450 enzyme.

4 Santanol Synthesis

The santalenes need to get converted into santanols. With the presence of another enzyme, P450, in the environment, the santalenes oxidize and turn into santanols

5 Product Identification using Gas chromatography–mass spectrometry (GC-MS)

The product mixes resulted from in vivo production are then analyzed using GC-MS where different molecules can be isolated, identified, and quantified.



GC-MS chromatogram of in vitro assays with recombinant santalene synthases; SaSSy (solid trace), SauSSy (dashed trace), and SspiSSy (dotted trace) using (E,E)-FPP as substrate. Peaks: 1) α-santalene, 2) α-exo-bergamotene, 3) epi-β-santalene, 4) β-santalene. Traces of α- and β-farnesene isomers are also found in the mixture. Mass spectral data of the main compounds are shown.

Figure 7. *Santalene* Synthesis
Source: Figure drawn based on Jones et al. *2011*

5.4 Futures of Sandalwood

The research on the biosynthesis of Sandalwood oil has introduced new ways of thinking about what the plant may mean to us in the coming years when it becomes a designable artifact. If Sandalwood can be abstracted into different parts, the relations between these parts and alternative contexts would let us imagine new designs beyond what the existing tree can offer. Here, I offer a series of research questions to motivate this thinking and use the design framework offered in the previous chapters to provide some possible responses to them.

What happens to the biological, social, cultural and economic futures of the Sandalwood tree once its products can be partially or fully synthesized by other organisms? How does this change the perception of the cultural, social, and symbolic history of the tree given its thousands of years of interaction with people? How can we think about biosynthesis beyond this substitution mindset and utilize the process to design qualitatively different value propositions and experiences that go beyond faster, better, and cheaper versions of the same products? What do different types of design mean when users are confronted with the real (e.g., oil from wild sandalwood), fake (e.g. chemically-simulated version of the oil), and nature-identical (e.g., oil synthesized by bacteria) versions at the same time? If we shift the inquiry towards the microbial workhorses that produce on behalf of Sandalwood and ask when a living organism, such as the *Escherichia coli*, turns into a product, what role can design play in conceptualizing the organism as more than a machine that serves the functions and features of another organism? And finally, what does it mean to be a plant, organism, and a living thing if eventually biosynthesis can be pursued with an all-purpose, universal organism that can synthesize any of the desired ingredients for human use (Church and Regis 2012)?

If the Sandalwood plant can be imagined as a set of parts and relationships between parts, it opens up a series of possibilities that can potentially recontextualize the plant—ecologically, culturally, symbolically and finally economically—as a different species. Depending on the kinds of strategies that can decompose and compose the plant—sometimes as a set of predefined parts, sometimes as units that are determined on the fly, or sometimes as parts that can change their behavior over time—the plant can offer a range of modes of existence from being living to non-living at different time-scales. The design framework discussed in the Chapter 4 allows the plant to be abstracted into molecules, genes, proteins, and cells formations. Once decomposed, these parts can be animated through artificial vesicles such as liposomes or polymersomes and be instructed with different objectives which the plant is not evolved for. Here, I will propose a possible abstraction of the plant and a case study that explores the design potential of decomposed and disembodied sandalwood and discuss the potential implications with a series of biological design propositions.

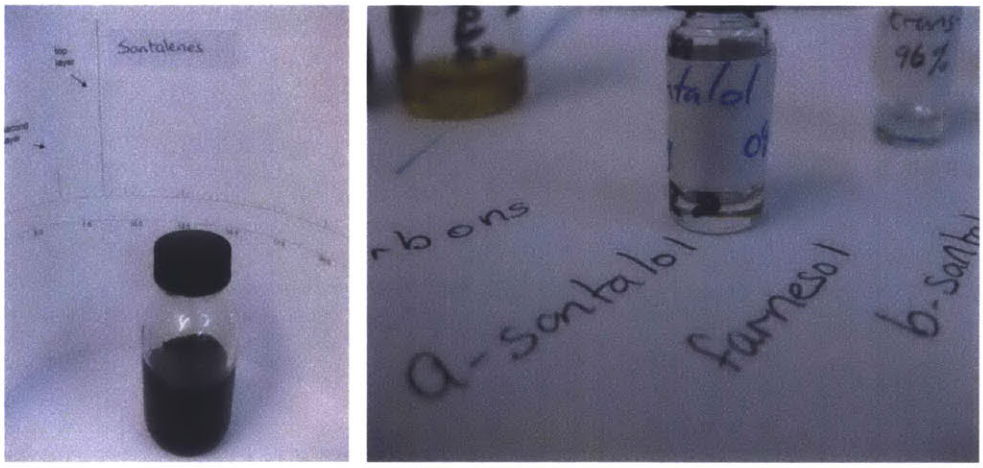


Figure 8. Compounds extracted from *Santalum Spicatum* oil.
Source: Andrew Brown, Mt. Romance Sandalwood Factory

5.4.1 Sandalwood oil in Parts

Alpha- and *Beta-Santalenes* are among the most common compounds that make up the unique smell of Sandalwood oil. The genes SaSSy, SauSSy, and SspiSSy that encode the *Santalenes* in *Santalum album*

and *Santalum spicatum* can already be used as design units during the *in vitro* synthesis of these compounds using yeast or bacteria. While the genes that synthesize the other constituents of the smell are still missing, those that are identified can be selectively mutated to produce new variations of the *Santalenes*. Once brought into the original context with the unidentified elements from the oil, novel *Santalenes* can potentially lead to new smell configurations. Unlike simulated or combinatorially generated design methods, by utilizing the rather unpredictable nature of molecular interaction it is theoretically possible to combine an accelerated form of directed evolution and have unpredictable results.

Farnesol (FOH), another important compound found in Sandalwood oil, offers a different set of possibilities that are capable of changing the nature of Sandalwood products. *Farnesol*, with its sweet-oily odor, is an important component of Western Australian Sandalwood, *Santalum spicatum's*, smell. However, *Farnesol* is often artificially removed from the *Santalum spicatum* oil as it is not favored by the fragrance industry. This industry prefers the Indian Sandalwood, *Santalum album,* which has little or no *Farnesol* in its oil.

While not desired in the oil, *Farnesol* is a highly valuable organic compound that is used as raw material in cosmetics, perfumes like "Chanel No. 5," food flavoring, and pharmaceutical applications (Wang et al. 2011). In Nature, it is mainly found in the essential oils of plants such as citronella, neroli, lemon grass, rose, and sandalwood but also exists in trace amounts in most animals, especially mammals. *Farnesol* is also found in the hormones that are related to the immune system of the plants (Nickerson et al. 2006). The compound takes on various roles in chemical signaling such as quorum sensing and apoptosis—the programming of cell death.

Farnesol is a hydrocarbon. Therefore its biosynthesis has been extensively studied as a substitute to alcohol-based biofuels such as ethanol, methanol, or butanol (Fortman et al. 2008). Wang and colleagues demonstrated that significant amounts of *Farnesol* can be synthesized by overexpressing the ispA using *Escherichia coli.* The newly synthesized *Farnesol* can be selectively added to Sandalwood oil using vesicles and a computational microfluidic environment that structures the organization of the compounds. *Farnesol* that is synthesized in isolated liposomes can be procedurally added to the oil to control the quality of the smell or its energy content. It is possible to design high energy density oil with different amounts of the authentic smell. Or, to run the biosynthesis process of two different gene products from *Santalum spicatum* and *Santalum album* in parallel inside different encapsulations. The biosynthesis of *Farnesol* is based on the commonly-explored Mevalonate pathway that is used in the biosynthesis of many other commercially viable *Terpenes*-based products. This pathway is used by all eukaryotic species and some prokaryotic species for synthesizing compounds—such as IPP and DMAPP—that are the building blocks of other organic molecules, such as *limonene*—the active agent found in the oils of citrus fruits—or *isoprenoid amorphadiene*, which can be used in the production of antimalarial drug artemisinin (iGEM Wisconsin-Madison 2012).

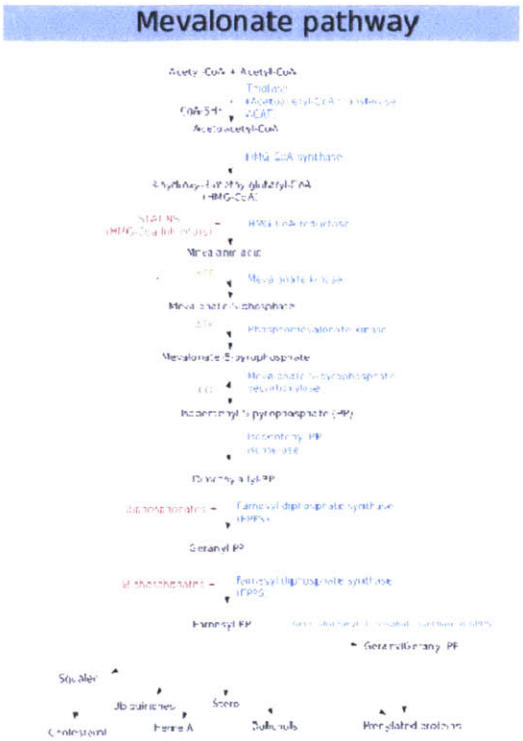


Figure 9. Mevalonate Pathway showing Farnesyl-PP, the precursor for Farnesol, synthesis
Source: Wikipedia

By selectively intervening in the different stages of the Mevalonate pathway, such as spatially isolating the outcomes of different stages and re-compartmentalizing different active agents with each other using vesicles, it becomes possible to create different compounds—such as other types of essential oils, aromas or flavors—that are historically evolved through different species. Thus, during the production of *Farnesol*, it is also possible to simultaneously produce *limonene*—another terpene that functions as the active agent in citrus fruits—which can introduce a different array of functions to the oil. Such additional compounds not only would change the color, viscosity and medical nature of the oil but also spatially co-locate different economic, social, and cultural uses next to each other.

5.5 Case Study: The Sandalwood Well

'The Sandalwood Well' is the hybrid of a microbial perfumery and a bio fuel refinery. It demonstrates the ways that different products of the Sandalwood plant can be synthesized in different configurations

in a disembodied context: outside the plant body. The study intends to show a possible implementation of the design framework by showing the selection process of different units of design, the assembly logic for the design of color, smell, and fuel production capabilities in a new physical environment (i.e., a new spatial context) where the unit assembly can utilize bacteria to express Sandalwood compounds.

'The Sandalwood Well' is a prototype of a biosynthesis machine that is set up to design new forms of molecular interactions between different parts of living organisms—Sandalwood genes, carrier plasmids, *Escherichia coli* etc.—with the help of liposomic encapsulations. The system uses computational methods to produce artificial vesicles that carry bioluminescence proteins and smell compounds synthesized from genetic information based on the North American firefly (*Photinus pyralis)*, Japanese firefly (*Luciola cruciata*) and Western Australian Sandalwood (*Santalum spicatum*). The microbial well incubates two strains of recombinant bacteria that are designed to produce *Luciferase* and *Farnesol* (FOH). Once synthesized, the compounds are encapsulated within liposome vesicles, mixed with each other in variable ratios, and pumped up to an ignition system that burns them and releases the smell. The outcome is a new biological assembly that is grown, synthesized, activated, and consumed within the liquid media. The use of organisms to synthesize inks and dyes, bio-renewable energy, and compounds of smell and medicine exemplify a broad spectrum of needs that have motivated biological design since its early days. Here, it is important to note that by using well researched molecules like *Luciferase* and *Farnesol,* the objective is not to offer new ways of producing the synthetic equivalents of these natural proteins; rather, it is to explore avenues that can expand the repertoire of biological design with multi-species, non-repeatable, underspecified, and just-in-time biological products that can change both the perception and the use of Sandalwood as a designable.

5.5.1 Designing new Biological Abstractions for Sandalwood

The design of this system relies on multiple levels of biological abstraction. These abstractions not only influence what can be encapsulated into new units to create new assemblies, they also define the amount of control in the design process. In this study, abstractions allow both the standardization of certain type of interactions by assuring that only certain parts can be interfaced with each other and ensure that a certain amount of variation and unpredictability can be maintained during the process.

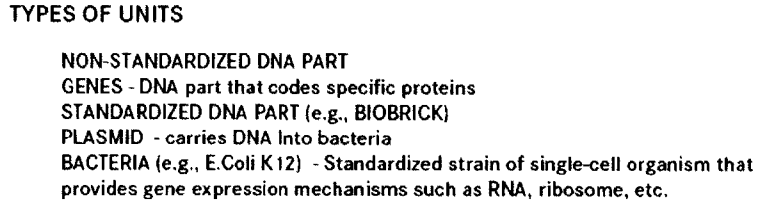
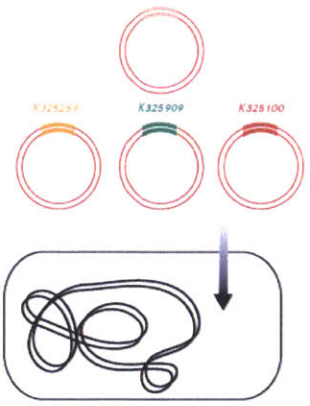


Figure 10. Summary of different biochemical units that can be encapsulated with vesicles.

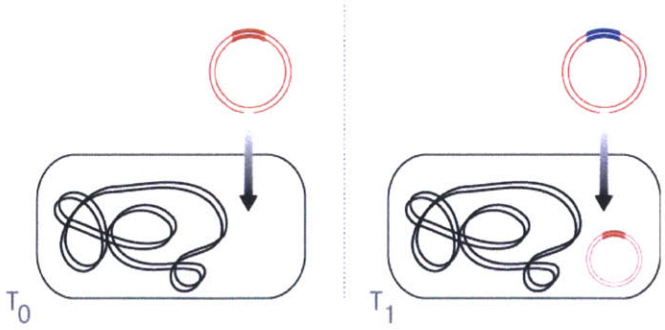
To be able to arrive to the desired proteins, specific nucleotide sequences have to be introduced to the ribosomal machinery of the bacteria. Therefore the first level of abstraction begins by choosing biological parts at the genetic level. The genes that encode *Luciferase* are already identified and can be commercially obtained either by copying from an existing DNA sequence using Polymerase Chain Reaction (PCR) or by having them synthesized through an oligonucleotide synthesis machine. BioBricks provide a higher level abstraction in which the *Luciferase* encoding gene sequence is optimized and standardized with specific beginning and termination patterns so that it can be inserted to bacteria with predefined gene carrier vectors. When different BioBricks are carried by different plasmids, even when equal concentrations are introduced, it is still impossible to know exactly which bioluminescence gene on which plasmid will be taken by the bacteria. The process yields inherently unpredictable results regarding the exact amount of bioluminescence and the color of the emitted light.



Bacterial Transformation with Multiple Plasmids

Figure 11.

Standardized plasmids also allow a second type of abstraction at this level. For the synthesis of *Farnesol*, for example, instead of working on the gene or BioBrick level, it is possible to purchase a ready-made plasmid "Plasmid 17817: pMBIS" from the plasmid repository addgene.org and incorporate it to the bacteria's cell wall through a transformation process (Add Gene 2013). The standardization of plasmids allows them to be used in the same design flow as a standardized delivery method. They can be used to add different type of genes into the same bacteria at different stages.



Bacterial Transformation at Multiple Stages with Different Standardized Plasmids

Figure 12

Both these BioBricks and the plasmids are constructed for a specific strain of *Escherichia coli*, YYC912. This strain carries a mutated version of the *tnaA* gene, which is known to be responsible for the foul smell of the bacteria. YYC912 is commonly used as a chassis organism to design custom odors, such as winter green or banana, using BioBricks (CGSC 2013). By using this strain under specific growth conditions, under specific growth conditions, it becomes possible to eliminate most odor causing proteins that would repress the smell of *Farnesol*.

This third-level of abstraction—the need to work with a specific type of organism—is another attempt to standardize the process and allow it to be repeatable. When different strains of Bacteria are present in the same environment, they compete for resources and one strain may outgrow the others. To assure the success of the design process, *Ampicilin* resistance gene *ampR* is included in the plasmids so only YYC912 can survive within the environment in the presence of the antibiotic.

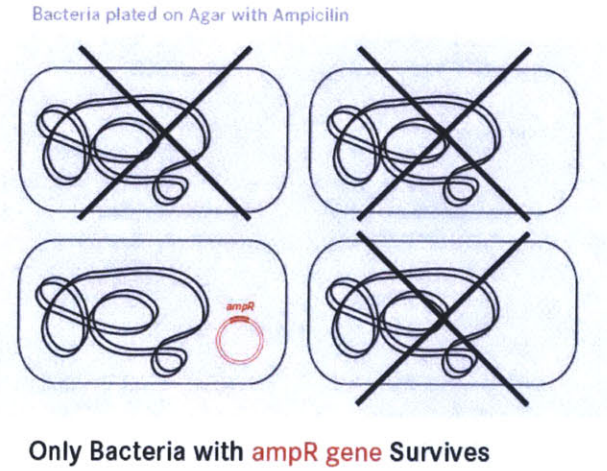


Figure 13

5.5.2 Unit Design

The packaging of these biological parts into vesicles introduces a forth-level of abstraction. Unlike a free-form and continuous liquid space, vesicles allow different units to be deliberately juxtaposed. Vesicle interactions not only spatially and temporally configure alternative assemblies, but also allow

incompatible products to come together. Liposomic units within these assemblies both physically and chemically animate; interacting with each other and also combining. These provisional formations suggest a temporary form of living where the vesicular interactions have underspecified biological outcomes. These arrangements can be based on quantitative criteria such that it would be possible to count precisely how many units are used in the process or be used to make qualitative arrangements so the designs can be customized based on preferences such as desired color, bitterness of smell, and so forth.

While programming interactions between vesicles are primarily set by DNA-based instructions—to regulate the synthesis of the desired gene products using plasmids—spatial organization and temporal planning also allow a different type of regulation by coordinating chemical events. Different reactions can be timed such that their outcomes supply ingredients to subsequent reactions. Spatial compartmentalization can be used to juxtapose reactions and allow parallel processes to run together.

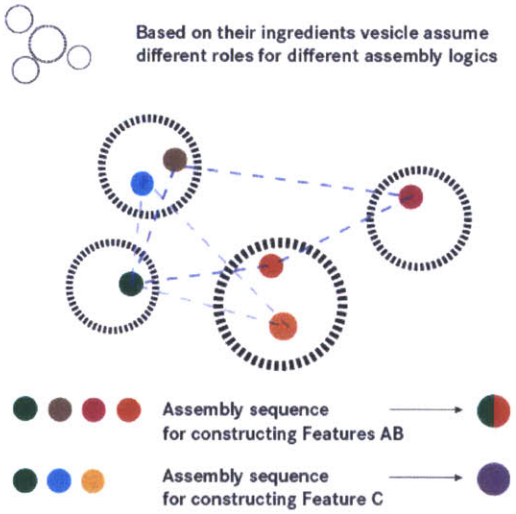


Figure 14. Different units encapsulated in vesicles can be used selectively in different contexts and be used for different assembly logics.

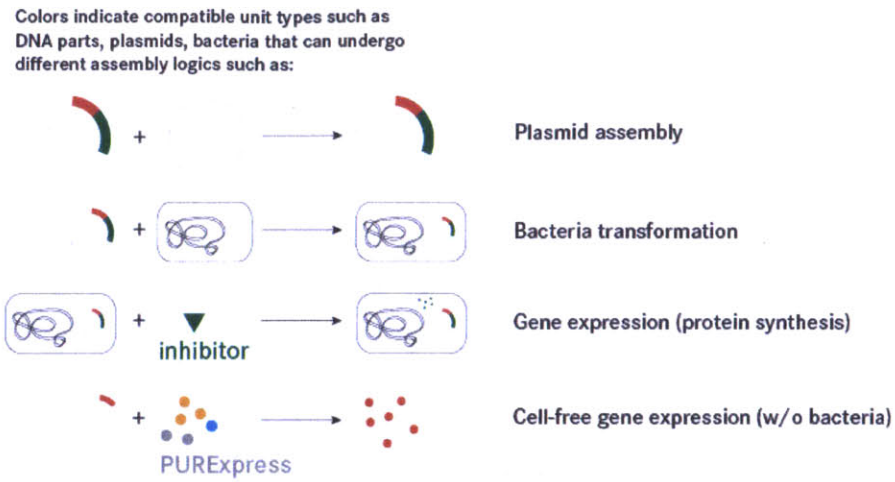


Figure 14. (Continued)

Re-arrangements of previously defined abstractions inevitably create the impression that this is a combinatorial design process. For example, once the units of design are identified on the gene, BioBrick, plasmid or organism level, the outcome appears to be the mere combinations of these units. However, here I would argue that combinatorial design in the biochemical domain has different qualities than units that are described in abstractions in representational domains such as computation, algebra, or computation. These units cannot only combine both physical and representational abstractions but also 1) provide ways to compose volatile and transitional vesicles that can create underspecified and timely varying interactions (Differentiation), 2) create new encapsulations for biological units by single or multi-lamellar layouts such that they can have 1-to-1 or 1-to-Many interactions (Compartmentalization), and 3) go through re-encapsulation such that once vesicles merge and their contents combine, the new mix can be re-encapsulated into new vesicles (Recompartmentalization).

Vesicular abstractions provide the possibility of creating new interactions between biochemical parts that cannot otherwise come together due physical, spatial, temporal, or contextual limits (Figure 14). Vesicular interactions provide new interfaces between units that allow them to take on new roles, functions, and meanings based on the context of assembly or interaction. Features can also be based on

selections that use compatible different units and assembly contexts, which can be utilized across multiple domains such as color, light intensity, smell or energy content. Thus, the same unit can determine either light intensity or color based on different structures. (Figure 15). This relational, multi-domain assembly would also extend the combinatorial juxtaposition to new types of assembly in which different ingredients could be mixed together. Such a multi-domain design process can incorporate units based on their different selection markers—such as color, electrical, or magnetic charge—and let them form constructs that can have parallel uses at different stages. This spatial and context-driven design framework integrates the previous methods such from transgenic design, synthetic biology, and liposomic encapsulations, and suggests a more integrative approach to biological design.

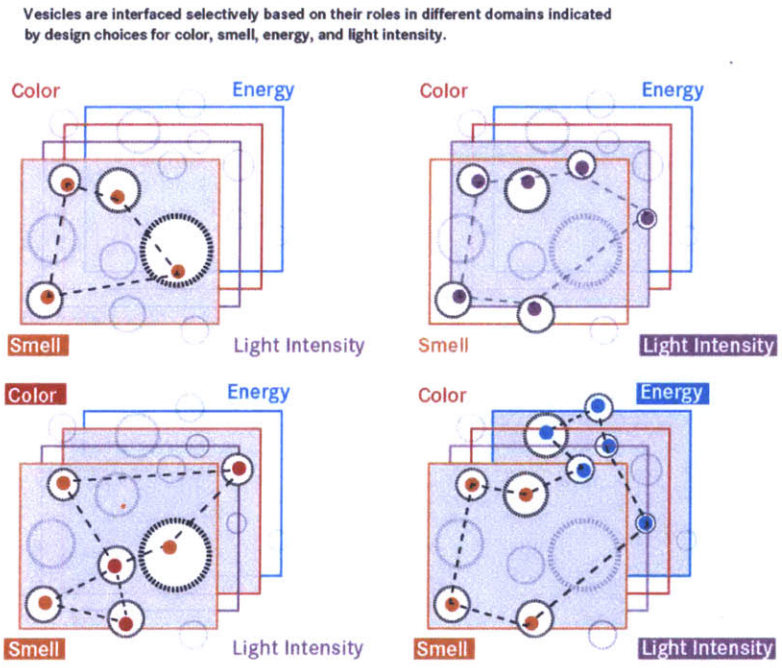


Figure 15

5.5.3 Selective Mixing

Breaking down functionalities or features into units such as nucleotide sequences, genes, reagents, plasmids, and bacteria encapsulated into vesicles allows a generalized approach that can black box

implementation details and allow the end user to specify outcomes or goals without necessarily prescribing instructions at the biochemical level.

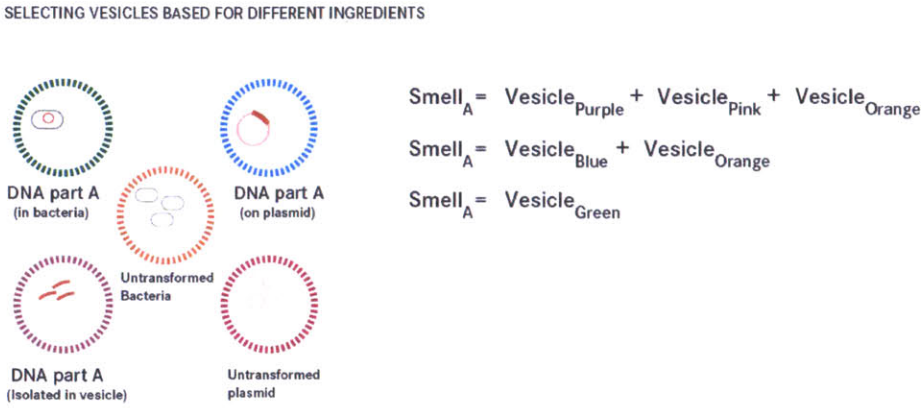


Figure 16

Instead of deciding which BioBrick to use to transform the target *Escherichia coli* to achieve the desired visual effects, the users can simply describe tasks on a higher level and let a program break the tasks down into specifics. Then, the mixing and the flow of chemicals can be driven by the microfluidic environment such that the vesicles can be assembled in different quantities for different results. The bitterness—the ratio of *Farnesol* to *Santalenes*—and the character of smell—the ratio of Limonone to Sandalwood ingredients—can be parametricized based on personal preferences. Similarly, the color, the energy (fuel) content, and the light intensity of the outcome can be tweaked according to aesthetic or cultural tastes, along with the type of bacteria used to produce the results. In this case, as each strain will not only introduce different yield factors and authenticity (lab-grown vs. natural-born), but also introduce different economic and ethical factors that are associated with them.

Selective mixing allows the concurrent production and assembly of multi-species biological products that can be located together while being physically isolated from each other. As units may change their identity over time—either by being incorporated by others, by breaking down, or through recompartmentalizations that would encapsulate them into other vesicles—they can yield unpredictable

assemblies. For example, when different BioBricks that encode different bioluminescent compounds are introduced to the *Farnesol* synthesis process, they change the naturally yellowish oil into different colors and emit light. A randomized interaction between different vesicles that incorporates different plasmids can create unpredictable variations in color.

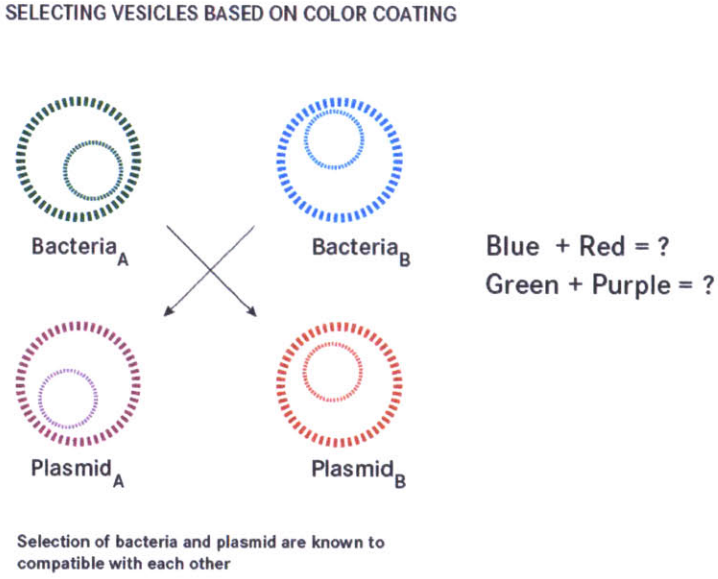


Figure 17

For the synthesis of bioluminescent compound *Luciferase,* I have experimented with three different BioBricks— BBa_K325259 (Orange, 2013), BBa_K325229 (Yellow, 2012), and BBa_K325219 (Cyan, 2013)—designed by the University of Cambridge in 2010. The bricks can be obtained from the iGEM repository from Spring 2012 and 2013 distributions. The bricks came separately inserted on pSB1C3 plasmids that are designed to be taken up by competent *Escherichia coli.* In the presence of *L-Arabinose*—a form of sugar—*Escherichia coli* can express the BioBricks and synthesize *Luciferase.*

Unlike their analogs found in Nature, *Luciferase* in the BioBricks are optimized nucleotide sequences, meaning their expression rates are increased. These *Luciferase* genes are artificially mutated during the oligo nucleotide synthesis process where randomized nucleotides are attached to specific sites of the

gene to differentiate it from the typical ordering found in the Nature. These new assemblies make proteins that can emit 'brighter' and different colors of light that fluoresces at different wavelengths. By encapsulating BioBrick-carrying plasmid vesicles, the color options can be randomized. Bacteria carrying plasmids can be circulated using vesicles and the plasmid uptake during the bacteria transformation process can be left to chance (similar to Figure 8).

For randomizing gene sequences on the fly, selective mixing can also be utilized at the nucleotide synthesis level as well. This time, by incorporating a thermal cycler into the microfluidic chip hardware, new nucleotides or longer standardized fragments can be selectively incorporated into existing genes to alter the coding sequences before they turn into plasmids. The assembly of the plasmid DNA can also be parametricized by letting standardized fragments be assembled via vesicles. Figure 17 demonstrates an example assembly which consists of DNA segments that include genes that encode enzyme *β-galactosidase* and monomeric red fluorescent proteins. When the assembly is completed using the anchoring, capping sequences, and the required sequences that initiate the gene expression at the ribosomal machinery, the construct becomes a pUC19 compatible plasmid ready for bacterial transformation. As demonstrated in the diagram, the assembly sequence can follow different configurations and incorporate different genes that encode different coloring or light emitting proteins. Once incorporated into the bacteria, this plasmid enables the fluorescing of red under UV light. If X-gal, an analogue of Lactose, is found in the environment, this turns the bacteria blue and therefore dyes the liquid such that it can be traced under normal lighting conditions.

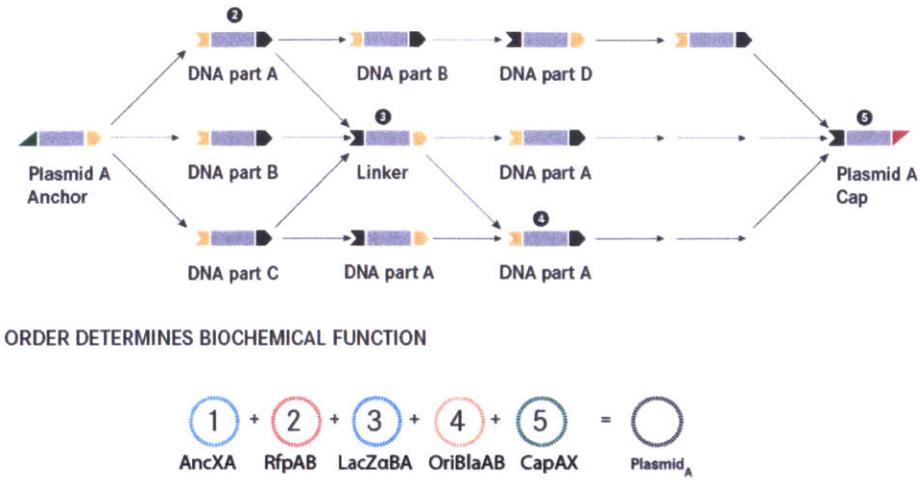


Figure 18. pUC19 assembly based on Genomikon DNA assembly prototype kit (Genomikon 2013)

5.5.4 Selective Inhibiting

When vesicles are assembled with features that are functional across multiple domains, it becomes important to selectively start and end processes and therefore control not only the mixing and assembly but also to select the conditions that activate/inhibit and terminate the processes that determine the outcomes of biochemical interactions. One form of achieving this level of control is, again, to use vesicles that can carry the reagents, catalyzers and terminators that influence the processes at different levels. As different abstractions would require different types of inhabiting and termination agents, these need to be both specific to the domains and also be provided at the right level of abstraction such as DNA parts or plasmids so that they can interface with each other. *L-arabinose*, the inhibitor that initiates the synthesis of *Luciferin* for bioluminescence, can either be provided readily to the environment or be synthesized at an earlier stage of the process. Thus, its production can be made contingent on other initiation conditions or the presence of other inhabiting agents. By layering the activation process, it would be possible to time reactions externally even after the vesicles are selectively mixed and formed into assemblies. When vesicles feature multiple options, the final outcome

can be decided at a much later stage than the assembly, where reaction triggering agents can be delivered to different parts of the assembly at different times. Please see Chapter 3 for more information on selective structuring and temporal design techniques.

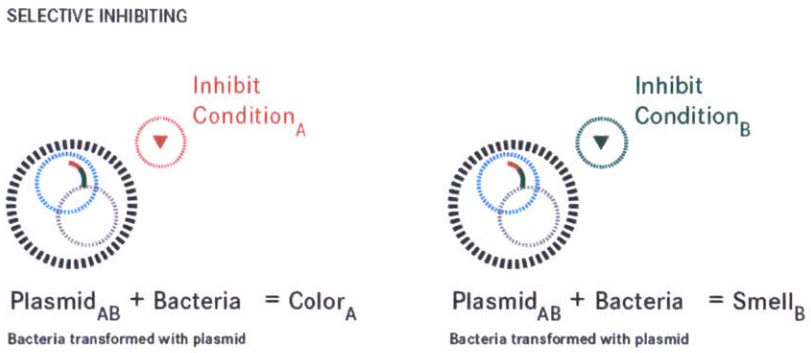


Figure 19

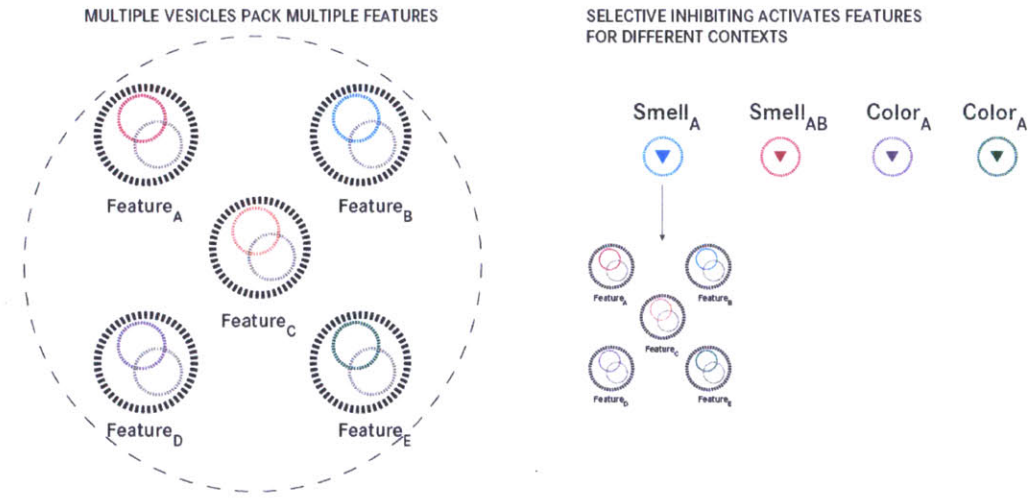


Figure 20

Combinatorial design with biochemical units offer another perspective related to their multiple functions and organization. As units in the biological design may have concurrent uses based on their biological, informational, aesthetic, and structural characteristics, by controlling their structure, spatial and temporal ordering it is possible design inter-domain relations that would not only yield variations but also yield entirely new and unforeseeable configurations. As abstraction and standardization in one

domain would not preclude a different role for the same unit in another domain, units can simultaneously interact with each other at multiple levels. Similar to selective mixing, units defined based on DNA parts, genes, BioBricks, plasmids or organisms can be utilized for different purposes in different contexts. Their logic of assembly—visual, structural, informational ordering—therefore allows them to be considered for different purposes at the same time. When different contexts are overlaid with each other, units can form qualitatively different organizations that can still be made of quantifiable and identifiable parts.

When units become standardized re-usable parts they can be also be removed from one context and embedded into another over time. At different resolutions, units can be part of different contexts and also continuously mobilized in the liquid environment for different purposes. Also, when disintegrated units are re-encapsulated into vesicles, the recursive nature of the process requires a different understanding of biological signs, form and function. In semiotic terms, vesicle assemblies yield functions or biochemical meaning through temporary nature units that do not hold onto their identity for a long time. Here, biochemical units can transform into others and constantly assume single or multiple new roles as they circulate in liquid media.

5.5.5 The Well Hardware, Wetware, and Software

The current implementation of the Sandalwood Well consists of a liquid control environment with syringe pumps, computationally controlled valves, and resource and collector reservoirs allocated for different chemicals (Figures 21 & 22). A microfluidic t-junction chip is used for encapsulating vesicles into droplets to be able to navigate them through different stages of the design. A heat controlled reservoir functions as a dual-stage heat shock and incubation space for bacterial transformation. Collector reservoirs are connected to piezo pumps that push the liquid into a custom silicon-cast housing inlaid with glass tubing. Once the liquid flows through the end of the housing, it meets a

computationally-controlled ignition system that heats the *Farnesol* content in the vesicles, causing it to evaporate and release its smell like an oil lamp (Figure 23).

The current system is designed to source materials for *Farnesol* and *Luciferase* synthesis. It uses two different competent strains of Escherichia coli (XL10-Gold® Ultracompetent Cells and YYC912), three BioBricks—BBa_K325259, BBa_K325229, BBa_K325219 (for *Luciferases*)—and two types of pUC19 compatible plasmids that are capable of expressing *ispA* gene (for *Farnesol*). The liposomes are created as oil in water emulsions. The *Santalenes* are provided in the form of diluted Sandalwood oil. The details of the biosynthesis protocols and the complete list of reagents can be found in the Appendix.

The different stages of the flow, encapsulation, mixing, bacterial transformation, incubation, and ignition sequences are controlled by a computer program that allows different automated and manual control settings for parameterising the processes.

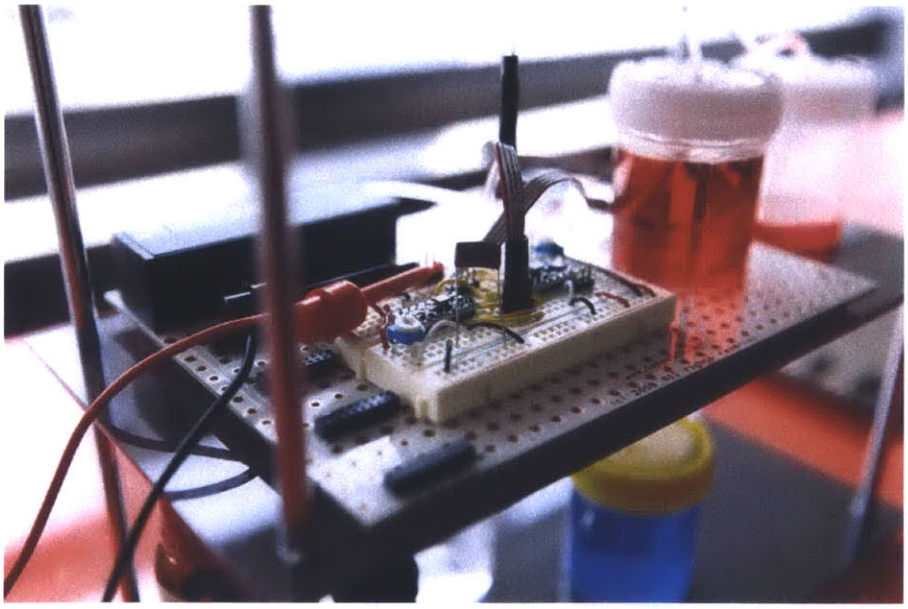


Figure 21. The oil well hardware set-up.

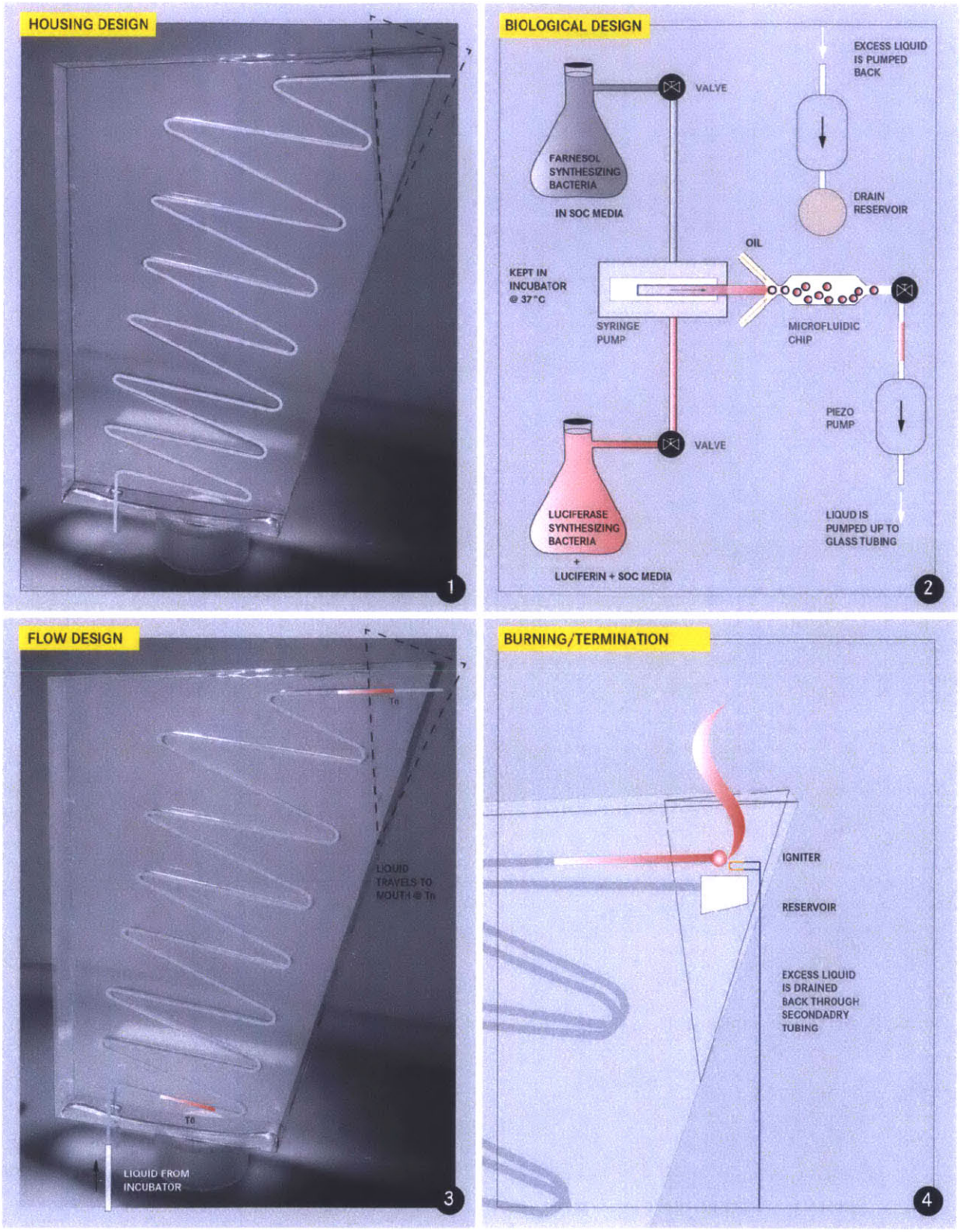


Figure 22. Schematic Description of the biosynthesis process in the well.

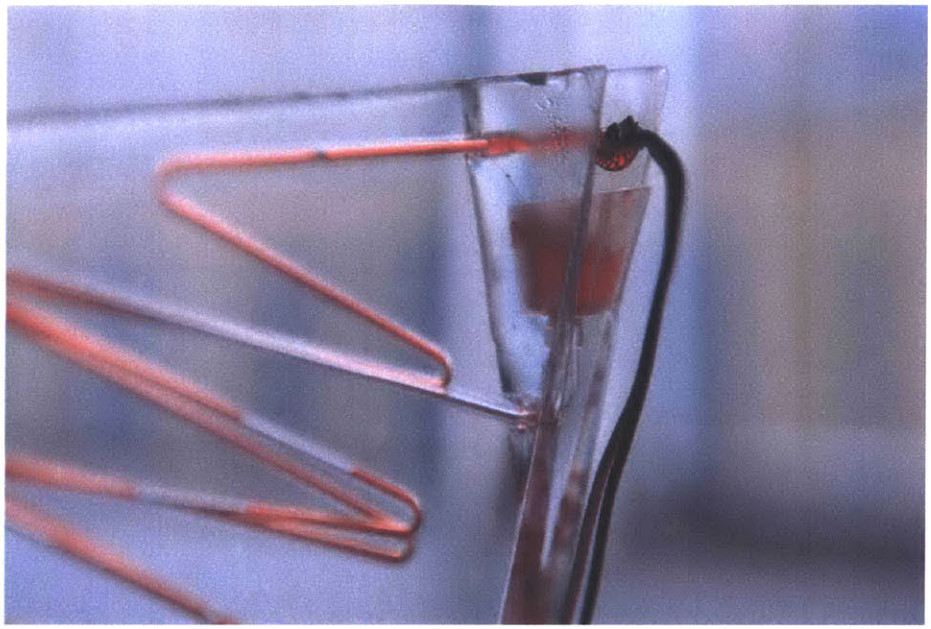


Figure 23. The heater mechanism that burns the outcome of the well.

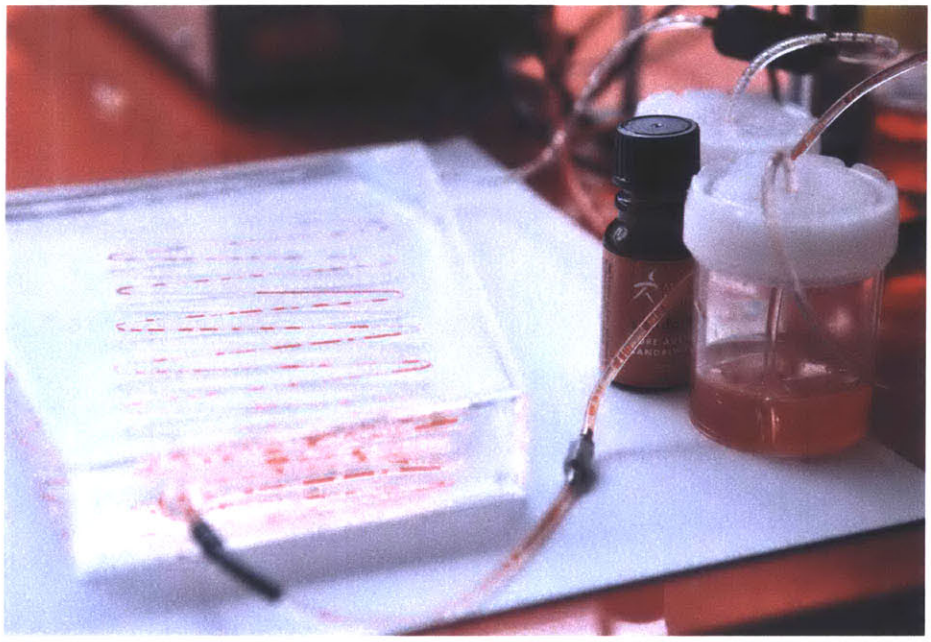


Figure 24. An earlier well prototype mixing Farnesol synthesizing bacteria into *Santalum Spicatum* oil.

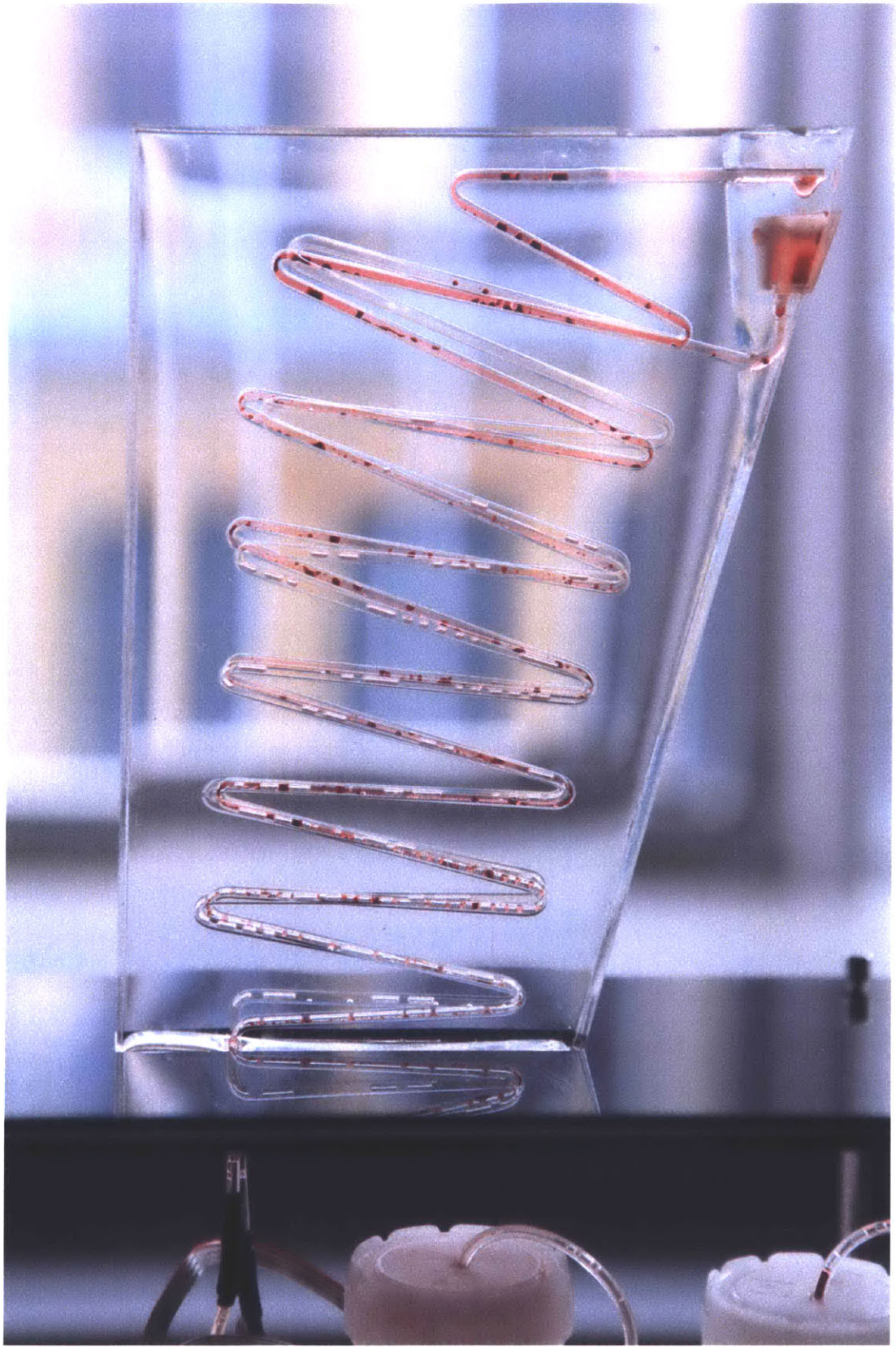


Figure 25. The final version of the well dprototype.

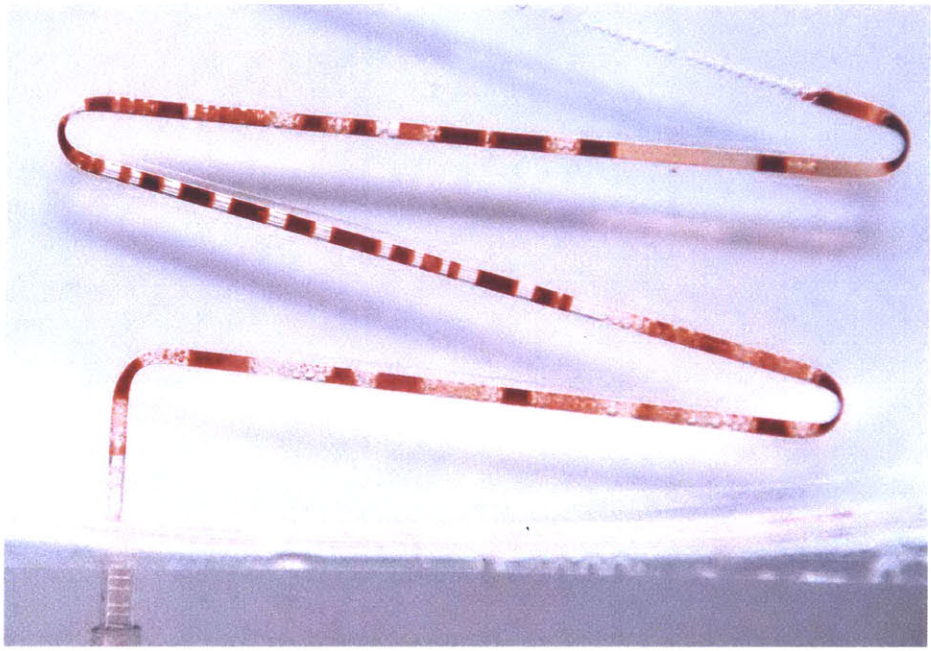


Figure 26. Detail from Farnesol synthesizing bacteria mixed with *Santalum Spicatum* oil.

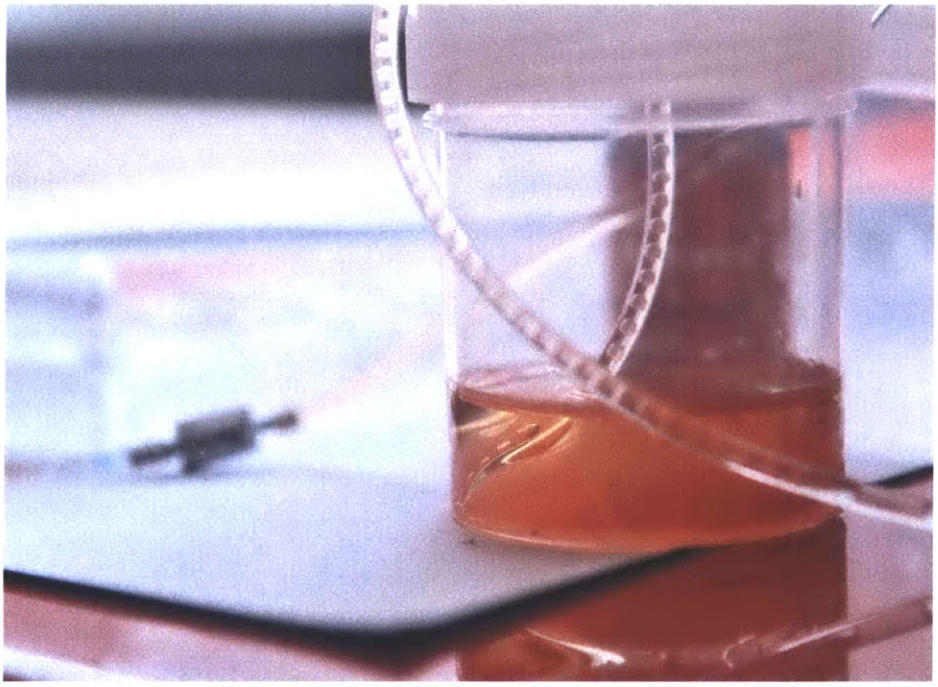


Figure 27. Synthesize Farnesol and oil before entering the well.

5.6 Discussion: Sandalwood after the Well

This well is a rudimentary example of a biosynthesis machine that can approximate Sandalwoood smell using complementary compounds that are synthesized and incubated in a microfluidic environment. As the entire metabolic pathway of Sandalwood oil synthesis remains partially unknown, I have focused mainly on ways to synthesize specific compounds in the oil and use the synthesis process to exemplify the potential of vesicle-based biological design. Here, it is important to discuss what implications this technology brings to the domain of biological design and also to discuss its products which can be synthesized, customized, and used in disembodied contexts. When it is possible to produce every ingredient of the oil in bacteria, what will our expectations from *Santalum Album* or *Santalum Spicatum* be? More importantly, if we can incorporate a DNA synthesis fabricator into the well and allow it to produce any genome on demand based on a database of standardized gene parts, does this mean that most of the smell-inducing proteins coming from the *Mevalonate* Pathway can be synthesized at home? And can we therefore replace the need for every plant—such as Bergamote or limonene—the produces smells from this pathway?

The futuristic possibility of having an all-in-one meta genome also highlights the possibility of a universal biosynthesizer that can not only fabricate what exists in the entirety of evolutionary vocabulary and bypass millions of years of evolution, but also produce numerous variations through directed mutagenetic processes that arrive at unforeseeable configurations—configurations that may never have been possible through evolutionary mechanisms.

On the other hand, with no physical connection to an original organism that produces this genetic information, can we still argue that what we produce in the machine using bacteria will be the same quality as if it were produced by Nature? Can the products of molecular commons be considered equals at the level of authenticity? Will they diminish demand for threatened and endangered species, allowing

them to survive? Or will they make Nature-born products even more authentic and precious and ultimately accelerate their consumption?

Today's biosynthesis technology is still far from making an all-in-one genome that is capable of reproducing most biological products. It is also incapable of synthesizing biochemical analogs that can substitute Nature's products. However, with this design framework, it is possible to decouple the questions regarding the substitution of Nature's Biology with Chemistry with a new set of intentions that can take the designs to a different level. Instead of mimicking or approximating the products of Nature, it is important to frame the question from the perspective of the 'designable,' and look for the experimental outcomes such a framework can provide in terms of its potential to carve out a new design space in liquid media. This way, it can be possible to mobilize different biochemical units into new and unforeseeable relationships that can extend the limits of combinatorial design methods—either as the generation of new colors or new smells as different types of aesthetic outcomes.

The physical design of the well also intends to introduce a cultural connection with the historical manifestation of ways we have packaged liquid such as tubes, containers, bottles and so on. The well, after all, is a perfume bottle that synthesizes its own content. While it still utilizes resources from outside, as long as it is replenished it is capable of making new and unpredictable aromas. At the end of the bottle, where all vesicles are burned, all genetically-modified components—DNA fragments, plasmids, or bacteria—are prevented from entering into the domain of Nature: the open world. With such confinement measures vesicles still have the ability to regroup, mix, and turn into new forms and functions based on the preferences of their designer. They do not mimic cells or single-membrane organisms but claim an in-between space among the strict and lose definitions of living things and intentional artifacts yet contribute both to natural and cultural means of evolution.

Sandalwood products, in the domain of molecular commons, will negotiate different cultural evolutions. When consumers are able to choose among products sourced from Sandalwood equivalents made by genetically modified bacteria, entirely chemical analogs, biosimilars, cheap fake versions or Nature-born organic, authentic, and highly-priced versions that are made of forty to hundred year old illegally traded Sandalwood trees, the future of the species will be determined not only by taste but also by personal morals and ethics. Like every design challenge, the communication of the social, cultural, and environmental implications of the product will partially be the accountability and responsibility of the designer.

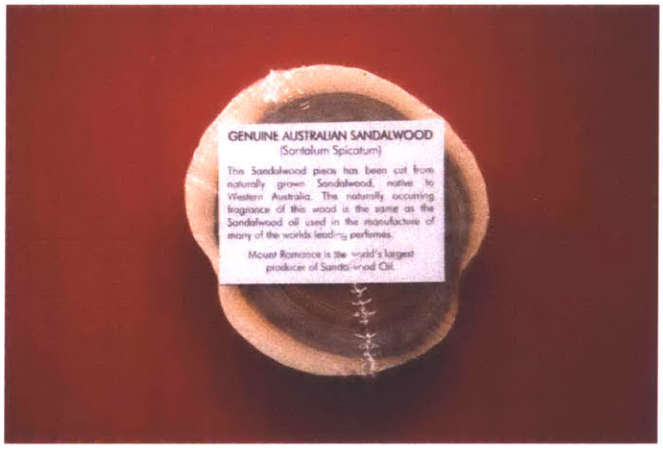


Figure 25. A piece of *Santalum Spicatum*

5.6.1 Design for the Biologically Designable

Herbert Simon in "Sciences of the Artificial" lays the foundations of a scientific understanding of the "artificial" and suggests a formal way to think about the means and ends of a design process (Simon 1996). The artificial by definition is teleological; it serves as an end conceived by human intentions. Simon uses the example of a watch. It is made of many components that are put together to build small mechanisms that can be organized to achieve a higher level goal: to keep track of time in a precise manner.

As an object, product or service, the artificial is often meant to solve a problem, to find satisfactory or optimal solutions. While there are many different ways to build time keeping devices using many different parts, the watch design demonstrates an imperative logic, following a set of procedures to arrive to the best outcome.

Simon tells us the parable of two watchmakers to explain the artificial within the context of evolution, which for him is one of the driving forces that gives birth to most complex systems (Simon, 1996, 188). He compares the ways that Hora and Tempus assemble their equally complex watches made from 1,000 parts. Hora puts his pieces together one-by-one in such a way that if he needs to interrupt his assembly for a phone call, he would have to start all over again. Tempus assembles his pieces in groups of 10 and then brings the subassemblies together to construct the larger whole. So, if a phone call interrupts him, he would only lose the last 10. Tempus also assembles the watch much faster than Hora as he can assemble things in a modular way.

While it is almost always modeled or foreseen in light of what something ought to be and tied to specific functions, goals, and utilities, the design of the artificial can also be about finding alternatives—extending the possible repertoire of solutions where it is difficult or infeasible to find the best solution. The artificial, in this case, is part of a declarative logic; instead of focusing on fixed solutions, it looks for the most satisfying outcome from a set of possibilities. In computation, for example, declarative logic is about defining a goal—what to achieve—without necessarily specifying the exact steps needed to achieve that goal. The 'artificial' then is the outcome of a process that operates within a set of constraints and satisfies a set of criteria determined apriori by the designer. A watch may still be built using a declarative logic, however, the result may not necessarily be better than an imperative design.

In the previous sections, I already mentioned literature that warns about the implications of confusing the language used to address biological and human-made artifacts, especially when human-intended

and biological evolutions are treated in similar ways. However, here Simon's definition of the artificial provides insights about how the designable can differentiate itself from the artificial as a biochemical artifact. It helps us to formulate questions such as: Until what point can a human-intervened artifact be considered Natural? At what point it becomes considered artificial? And can we talk about a hybrid nature for a bio-cultural artifact?

Sandalwood oil is not a watch. The oil is a mix of many more known and unknown compounds, which evolved together due to different selective pressures exercised either by Natural or cultural evolution shaped by human needs and preferences.

Also the oil of each species and almost every plant differs. Even the biosynthesis of a limited set of compounds in the oil is an approximate process that is far from being a standardized, repeatable, or optimized process. The process is not only spatial but also temporal as many different chemical reactions need to take place sequentially or in parallel.

The design method of Sandalwood oil discussed in this chapter intends to address another form of the artificial in the biochemical design space. In order to consider Sandalwood oil as a designable biological product, here I describe it as a series of abstractions—genes, BioBricks, plasmids, bacteria, reagents—that can be encapsulated together in the same space. Being encapsulated in vesicles, these abstractions form units that can spatially and temporally interact with each other. Here, these abstractions do not attempt to standardize a design process but rather to catalyze its interactions such that domain-domain (BioBrick-plasmid) or inter-domain (plasmid-bacteria) exchanges can be made between the vesicles.

A fundamental difference between the components of a watch and the units used in Sandalwood is that parts in the biochemical space have the ability to change and differentiate over time due to their material. Bacteria encapsulated in vesicles still evolve, mutate, and grow. Differentiation not only introduces instability as units can dissolve, disappear, or merge with each other but also introduces

variation as demonstrated earlier by the changes in color or smell that happen during the design process.

Biologically designable embodies different types of abstractions and multiple logics of assembly that can happen at different levels. Unlike a spatially fixed hierarchical system, the flow in a liquid environment enables vesicles to encounter each other at different times.

Units can carry multiple representations based on color, electrical charge, magnetism and so on. Thus the designer can interact with them at different stages of the process and form geometric, topological, or stoichiometric interventions, which can change the aesthetic or biochemical outcome of the synthesis process.

The Sandalwood oil well is a declarative system. The components are provided a priori with certain objectives in mind. However, instead of targeting for a fixed set of outcomes, it aims at maximizing personalization. Thus, the outcome of the well can be a set of new ingredients— additional features— that are incorporated the carrier base oil. Here, the notion of equivalency also brings an important dimension to evaluate the outcome of biological design process. If the well can synthesize a smell that is analog or functionally equivalent to Nature-made Sandalwood, the additional ingredients produced during the synthesis can be used to define secondary functions—such as cultural, ecological, personal or social uses—of the product.

When the outcome of the process cannot be tied to an optimal solution, such as the best smell or color, subjectivity plays an implicit yet important role in defining what the satisfactory is. Simon gives the example of the way two architects would design very different buildings if one starts from within and the other one from outside even if they agree on a set of features a satisfactory building should have but apply alternative emphases on achieving them. (Simon 1996, 130). According to him, such variety, within acceptable limits, can be an equally viable criteria and a desired outcome of the process.

Designing for maximum variability is a challenge in systems with a priori components. Even in Nature, the amount of genetic variation among species is highly variable and is determined by the context of where the organisms live. Thus it is possible to have animals that have remained essentially unchanged for millions of years next to insects that span across thousands of different species in Nature.

When a living organism becomes biologically designable, it is inevitably broken into a set of known abstractions. However, since the biochemical design space speaks a common molecular language, if the spatial conditions and the assembly conditions are arranged properly, molecular interactions can potentially cause a level of variation that can be of a higher degree than what the organism would evolve for in its given environmental context.

According to Simon, when design is not geared towards optimal solutions and rather aims at "alternative emphases" defined by the designer, the process becomes a determinant of style (Simon 1996, p. 130). biological design either in the form of direct intervention or as a process that shifts—negatively or positively—the natural evolvability of a living organism almost always includes an element of subjectivity—preference, taste, aesthetic judgment—exercised by the designer. Unlike Nature where the ultimate fitness function for the living is the ability to survive and resist disadvantageous selective measures, in biological design the existence of living organisms gets shaped by the will of the designer. Thus the subjectivity of the designer is always negotiated with the inherent agency of the living and the existential criteria that would drive its survival.

Albeit limited, the well's biological abstractions provide a set of affordances for the designer to be able to customize the aesthetic appearance and the function of the oil by changing its color, smell, and light intensity. All of these measures can be subjectively adjusted based on personal preferences and change over time. As the design decisions are repeatable and reversible, it is possible to arbitrarily go back to a previous choice and follow a non-linear preference scheme for the product. Also, biological units can

change their properties over time and interact in unforeseen ways. Thus, the customizability of the oil as a designed artifact and as biological agency allows the designable to incorporate features from both models and experience them in parallel.

Chapter 6 – Conclusion

All living things naturally affect each other. Single cell organisms, insects, plants, animals, bacteria colonies, flocks of bird, forests, coral reefs, and more complex ecological systems have all evolved through different kinds of intentional and non-intentional interaction with each other. Thus, from the predator-prey relationship to reproduction, parasitic and commensal relations to large-scale communal interactions that increase chances of coping with environmental stress, most interactions define species' capacity to survive in relation to one another.

Within the spectrum of these activities, humans have a particular place; their means of interaction extend far beyond mere survival. We intervene in the course of other living beings and directly manipulate the outcome of biological processes—both on the micro or macro level—for additional reasons. Aesthetic, cultural, or symbolic needs; taste, preference, style, intellectual curiosity or means to produce knowledge are among a number of activities that are specific to us.

When we define these activities in relation to the non-living, say when we make artifacts from inanimate matter, physically or chemically transform or combine them; plan, program, instruct their use and function; and find new uses and applications for them beyond their original contexts, we refer to these processes as 'design.' However, when we manipulate the living, or work on reconstructing life-like artifacts using parts of the living, we do not commonly refer to it as biological design.

6.1 Biochemical Design Space

In this dissertation, my aim was to formulate a specific framework that can be used to articulate on a design space called 'biological design" and bring it to the attention of non-scientific audiences. By looking at a number of products and tracing key moments in the recent history of biological intervention, I proposed a framework that engages with the living based on advances in manipulating

organisms on micro and macro levels, building molecular constructs that form artificial environments for hosting new types of biological activities. More importantly, I intended to integrate different design methods used by different disciplines such as synthetic biology, chemistry, bioengineering, computer Science, as well as product design and architecture to treat such biology as an integrated design space, where it is not only possible to experiment with new forms of living designs, raise questions beyond scientific means, or find applications for immediate needs, but also to explore what it means to be accountable for what it is created when the design interfaces with the larger values of the society.

As we are becoming more familiar with the possibility of designing plant-like biologies that can approximate some of the roles, functions and look and feel of plants, the question of what a plant will be exposes different intentions about what is possible when the evolution of natural species is directed or 'plantness' can be abstracted to an architecture of compounds that can be artificially synthesized. Different lines of thinking demand new kinds of ethical, moral, and designerly responsibilities.

6.2 Synthesis

As 'design' demands different interpretations under different disciplinary frameworks, instead of claiming an overarching framework that could cover all aspects of biological design, I framed the scope of the work within a limited set of activities that defines design as a constructivist effort, one which primarily utilizes the logic of 'synthesis.'

Synthesis assumes that parts can be combined to form different kinds of wholes. Here, it is conceived as a reductionist framework that explicitly makes the assumption that living things can be abstracted into units, additive and subtractive interactions can be formed between units through different assembly methods, and living things, partially, fully, individually or communally, can be encapsulated within new biological contexts and interact with each other. By tracing the history of analytical and rational methods in biological design, I intended to point out how similar abstraction techniques can be utilized

for very different types of control, regulation, decision-making schemes, and generative processes. My aim was not to celebrate synthesis as the hallmark model of biological design, but rather use its vocabulary, with its advantages and limits, as a common language to find the overlapping interests between different fields which utilize synthesis through rational design and computation.

6.3. Designable

Design not only implies a broad area of investigation that extends beyond efforts of solving problems, meeting needs, finding better, cheaper, efficient, and faster solutions to existing challenges, but also raises questions about what is 'designable' at a given moment regarding interests, affordances, and, more importantly, what else can be imagined as 'designable' if one suspends certain expectations and prescriptive outcomes. After identifying this framework, I proposed a vesicle-based design method using liposomes to extend the vocabulary of biological design beyond its mainstream applications in biochemical delivery. The liposome-theory, which has more than fifty years of scientific research to back its foundations, is presented here for the first time as a design framework that can extend chemical and biological applications and offer ways to build life-like constructs—structures that can perform biological and chemical reactions when assembled, programmed and interfaced with each other. This framework allowed me to propose a space- and context-oriented paradigm for biological design that utilizes encapsulation, spatial and temporal compartmentalization for different types of biochemical events and activities to facilitate different type of synthesis across multiple domains.

For my current formulation of the biologically designable, I primarily focused on the following features: 1) Liposomes with designable membranes that can provide different forms of containment conditions; 2) Microfluidic environments that can encapsulate liposomes into droplets so that they can be mobilized into different contexts and designed in flow; and 3) Different assemblage logics—such as geometric,

visual, computational, informational, application-driven—that can be used to organize liposome or liposome-droplet units into different assemblies and architectures.

The designable, at its current stage, inevitably and perhaps intentionally, engages with approximations about what we know as living. The contemporary definition of living is still open, but it is highly motivated towards research that attempts to explain the current state of Nature by building models of life-like artifacts that explore how life began, evolved, and differentiated itself.

Chemical design experiments are still primarily geared towards building bio-inspired experimental constructs, such as designing nucleotide sequences made of foreign chemicals, building synthetic equivalents of natural ribosomes, and replacing the natural DNA of organisms with artificial genomes. Such thinking can also lend itself to a goal-oriented biology where organisms are modeled as raw materials, production schemes or machinery that work, perform, and yield profitable interactions.

My conception of the designable, on the other hand, intends to resist such motivations and rather focus on theorizing the design of alternative biological contexts in which biological design—from molecular interactions to high level organizations—can be discussed the way Architecture or Product Design construct the physical, social, and cultural environments for the living. With the design framework, I presented alternative ways to think about units, assemblies, and contexts to discuss how they can be instructed differently to form cellular formations that align and bind together differently than cells would when forming tissues. Organisms are instructed to synthesize proteins that are not necessarily useful for their own well-being, but serve common goals that cannot be anticipated by individual units. These metabolisms and the agencies that drive them can be simple and approximate in nature, but can become important steps to understanding what new of biological interactions can emerge when we challenge our assumptions of the designable, and when we decouple it from our references to Nature-oriented biology.

6.4 Applications

In this work, I did not propose a case study that could become the hallmark application of liposome-based design theory. There are already many different commercial applications of liposomes that use one or more of the techniques discussed in the framework. My intention was to rather present a design provocation that can demonstrate the ways that methods like selective grouping, on-demand mixing, counting, decision-making, and generative making can be implemented in the biochemical domain.

My goal was to provide the ground for imagining alternative futures for the Sandalwood plant; to imagine what the plant may eventually be when parts of it can be abstracted from their original context, such that it can interface with alternative social, cultural, and ecological realities. The sandalwood oil well is a small case study that builds on the existing research done in University of Western Australia to imagine further what will happen to the futures of the sandalwood plant as the global socio-economic and ecological system is driving it towards extinction. The work is formulated around a key question: If our existing demands from the plant can be replaced by a designable object—one which is ultimately driven by a cultural values—what kind of a future will the Nature born plant have if it can be left to its biological evolution?

Currently, *Santalum Spicatum*, the Sandalwood native to Western Australia, is grown as an oil-sourcing product in large plantations. With advances in biosynthesis technologies, some of the ingredients of the oil will eventually be replaced by products grown at microbial factories that can produce approximations of the existing products. Here, my intention is to extend that thinking and investigate how our perception of the plant may shift if it becomes part of a biosynthesis fabrication machine which can grow its products in a customizable manner.

6.5 Liquid Space

Throughout the dissertation, I discussed the benefits of the compartmentalization of the liquid space. As

I presented the materiality of the liquid space in terms of droplets or droplet assemblies, I wanted to raise questions about the notion of designing for and within the internal corporeality of the living. As living organisms mostly consist of water, the biochemical design space offers a rich potential to think of containment and confinement conditions that can take place within the living. For a human context, it would be possible to imagine symbolic, aesthetic and cultural functions of assemblages, constructs, and flow architecture that takes place within the body.

While most designed products and interactions take place outside the human body, the space that lies within the living body is quite undertheorized whether in sciences, engineering, or architecture. While the body is always discussed in relation to other bodies—from the role of the immune system and the microbial networks that function within the body to the external perception of the body regarding beauty, race, ethnicity, kinship—very little has been said on what the role of designed biology can be when it becomes possible to design and build within.

As the living space of the body offers a multi-scale, static and flowing liquid environment, the space of design would inherently need alternative approaches from different disciplines. For example, with limited interaction with the outside world, the 37 degrees Celsius, mostly dark and wet human body offers a complex and contested biochemical design space that is open for new biochemical exchanges that can extend the vocabulary of commensal, parasitic, or symbiotic interactions experienced among its current inhabitants.

An immediate follow-up of this dissertation would be on discussing the potential of design within such conceptualization of the living body and to seek a design methodology and framework that looks beyond medical applications.

6.6 Liquid Media

Living things utilize many forms of signaling, feedback, and exchange mechanisms that take place at

different scales and at different complexities. Intracellular interaction can be the basis of material, energy and information exchanges within the same membrane, whereas certain compounds such as hormones can reach out beyond the immediate environment and regulate complex interactions in a different part of the organism. Pheromones, on the other hand, can function outside the body and be the basis of advanced chemical attraction (*chemotaxis*) or repulsion and defense mechanisms.

Liposomes in liquid space extend the repertoire of signaling, mediation, and exchange of biochemical events. Due to their permeable nature, they can constantly traffic small compounds and liquids in and out through their membranes. Inside microfluidic capillary channels they can be guided to different destinations and deliver their payload.

When liposome units or droplet assemblies are designed for specific biochemical interactions, not only the structural meaning—the syntax of the assembly—but also the semantics—what the payload stands for or mediates—becomes important. Unlike a natural living system that evolves both the means and techniques of it communication, a synthetic assembly can bear meaning both for its internal and external needs as well as the designer's intentions. As discussed in previous chapters, the construction of liquid unit assemblies—like words—can bear a multitude of meanings at the same time for different contexts. They can be abstract and representational, quantitative—computational and numerical—and qualitative—symbolic, visual, and geometric—as well physical, based on the type of payload.

Within the context of the Sandalwood plant, for example, a droplet sequence may mean a composite which incorporates material attributes that define the smell and essential oil characteristics, the genetic instructions that can randomize the color appearance of the proteins, or an identification scheme (e.g., a magnetic dye) that would help enumerate or locate it within a visual or spatial arrangement.

The logic of "reading" or "interpreting" such sequences certainly demands further investigation. As liquid space also lends itself to be a new form of media, there is a possibility to think of new types of

abstraction and representation schemes that can extend the typical semiotic, sign-centric syntactic and semantic operations that are inherited from verbal and visual communication.

The 'use' of biochemical words next to other words will also demand a further expansion in the lexicon and indicates the potential for a broader understanding of abstraction and mediation mechanisms. Biochemical words bear physical uncertainty and this feature makes them quite different from closed formal systems, such as geometry or computation. The liposomes can break down and be incorporated into each other. They can unpredictably vary and irreversibly change over time. Decisions regarding the stability and instability can be deliberate design decisions and not necessarily considered as error.

6.7 Synthetic Agency and Ideology

An important challenge the designers of living systems face is the difficulty of building self-sufficient systems. Currently no biological design can persist over time and 'survive' on its own like an evolved organism. The contemporary formulations of synthetic living, such as protocells or organisms with reduced or substituted genomes, partially fulfill that objective and carry over motivations that are built into them. Looking forward to the future of the field, it will become important to decide the role of autonomy and self-directed behavior in the designed artifacts: Which purposes will biological designs serve when they move beyond delivering molecular tasks; synthesizing desired proteins or manipulating other living systems? Synthetic organisms will demand a different articulation of agency that is beyond the mechanistic conceptions of function, behavior, or autonomy. For example, if a microbial or liposomic approximation of the Sandalwood plant can be designed to evolve over time, what environmental conditions can be constructed to stress the new organization and force it to adjust, diversify, mutate and change over time, giving it an independent life-cycle beyond the built-in intentions. Or if biological design will always be tied to human desire, need, preference, and want, what would be the ways to let

such artifacts produce indeterminate or underspecified products that can extend our immediate expectations.

* * *

I discussed a number of different outcomes on how space-oriented design theory can be used to reframe our current and future understanding of what is biologically designable. Looking ahead, it will become important to integrate different theories of space and encapsulation to extend the definition of what we call living and to examine what the new types of living may entail to the existing ones once new spatial boundaries, membranes, and architectures can construct new forms, functions, and interactions among each other. There is certainly a need to introduce more complex theories of spatial articulation and tectonics for liquid media.

As biological design will negotiate its place in the design repertoire, it will not only incorporate different understandings of design from different fields, but also introduce new ways to think about design and the designable from its own perspective, whether it is for living or non-living matter. With this dissertation, my hope is to contribute to this conversation and offer the space of biological design to new audiences.

Space, wherever it is imagined, theorized, or realized, always comes with historical, social, cultural, and biological implications. Architecture has always been concerned not only with 'building' enclosures for the living but also with contributing to a broader understanding of space that establishes our interactions with one another. It is also my hope that with this dissertation, Architecture, which has extensively investigated the space that lies outside and above the corporeality of the living, would identify new reasons to look for the potential of space-making in what lies within. The liquid medium inside the living poses new challenges regarding the meaning of interiority, encapsulation, containment, and confinement. These challenges shape the interactions of all living things; not only for what they

already are but also for what else they can be as we advance in our ways to alter or administer vitality in more creative, critical, and responsible ways.

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