BIOFAB HUMAN PRACTICES REPORT 1.0

What is a Part?

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> ***Part*** *(Latin part-, pars) one of the portions into which a thing may be divided, each of a number of equal portions into which a thing may be divided, element, component, ingredient in a situation, part of speech, part of the body, aspect, respect, portion, share, share in an activity, function, role, part in a play, direction in space, one of two or more contrasted sides or aspects of a situation, standpoint.*
>
> —Oxford English Dictionary (abbreviated)

The proposal and prospect of establishing a "parts-based" approach to biological engineering has become a prominent (and, at times, contested and controversial) feature of contemporary synthetic biology (Baker, *et al*. 2006; Andrianantoandro, *et al.*, 2006). For almost a decade, a core set of practitioners have defined synthetic biology as the effort to conceive and render biological systems as integrated sets of components, components that could be fashioned, refined, and assembled in a relatively easy, predictable, and, above all, standardized manner (Endy, 2005; Rabinow and Bennett, 2009).

What a biological part actually is, and why such an object may or may not be significant is not as obvious as either proponents or critics might suggest. So, in this report we ask: what is a part in biology? Or, more precisely, what is a *standard* biological part in *synthetic* biology? More precisely still, what *could* a standard biological part *be*? The italics make all the difference, as we hope to show.

Standard biological parts don't exist, or, at least they don't yet exist in anything like a satisfactory form. Which is another way of saying that standard biological parts are artifacts; they are made and not simply discovered. And unlike many other engineering disciplines, biology doesn't yet possess shared criteria for what a useful standardized part might be (Arkin, 2008; Lucks *et al.* 2008; Purnick and Weiss, 2008). In short, what a standard biological part might (or might not) turn out to be, and what significance this might (or might not) have for biological engineering, remains an open question, a question to which the BIOFAB is addressing itself.

Making Biology Engineerable: From a Problem Toward a Solution

In 1999 Adam Arkin and Drew Endy submitted a proposal to DARPA to develop "a set of well-characterized and systematized biological components that can be generically assembled" (Endy and Arkin, 1999). The justification for such work turned on the assertion that standard interoperable parts constitute a basic feature of other engineering fields, a feature central to managing the economics of scientific practice. If biotechnology aspires to have anything like the same capacity for predictable, reliable, and scalable design and construction characteristic of other fields of engineering, such parts, they argued, would be crucial.

Arkin and Endy made comparisons to the design of integrated circuits to underscore what a central problem confronting would-be bioengineers. Unlike designed circuits, natural biological "circuits" have idiosyncratic mechanisms, rates, reactions, and effects. Hence, "rational design of biological systems by humans," they concluded, has "remained restricted to rather small or hit-or-miss efforts and has often relied on the ability to 'select' for biochemical parts that fulfill some criteria." The project wasn't funded—a fact as politically as it is scientific salient.

Parts as BioBricks

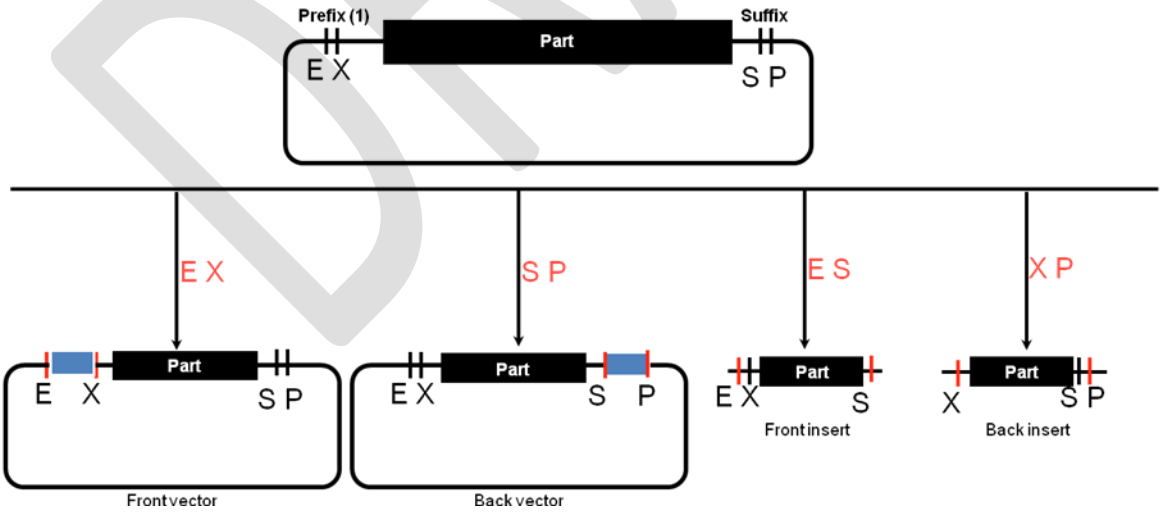
Three years later, Tom Knight *et al*. offered a first specification for what a standardized part might look like. Knight *et al.* asserted that, "The lack of standardization in assembly techniques for DNA sequences forces each DNA assembly reaction to be both an experimental tool for addressing the current research topic, and an experiment in and of itself" (Knight, 2003). Resonances with Arkin's and Endy's diagnosis are evident.

As one means of overcoming the *ad hoc* and tedious character of DNA assembly Knight *et al*. proposed what he called a "BioBricks" assembly standard. In its simplicity the proposal was potentially quite powerful. Its power turned on the natural capacity of enzymes to cut DNA at highly specific locations in a DNA sequence—i.e. restriction sites—using a specific class of proteins—i.e. restriction enzymes. When a restriction enzyme makes a cut at a restriction site it leaves an "overhang" which can subsequently be made to anneal to a complimentary overhang, effectively suturing the cut.

Knight *et al*. ventured that this natural cut-and-paste mechanism could be better leveraged for DNA assembly and thereby for rational design of genetic "devices." He proposed that engineered sequences of interest to be flanked on both the "upstream" and "downstream" ends with specific *double* restriction sites. These paired restriction sites could effectively function as sets of biological parentheses. A would-be engineer could cut the DNA between the parentheses (either upstream or downstream), and insert a new "part." Moreover, and more importantly, all of this is designed in such a way that the insertion of any new part leaves the outside restriction sites unchanged, making it possible to continue chaining together more and more of Knight *et al.*'s BioBricks (Figure 1).

Figure 1: Diagram of Knight *et al.* BioBricks Assembly Standard (sites: E, EcoRI; X, XhoI; S, SpeI; P, PstI)

The standard part vector can be cut in four different ways. The prefix and suffix can be opened, allowing insertion of a another part up or downstream. The part can also be cut out of the vector for insertion into a second vector.



On a basic level, the first BioBricks standard worked as advertised. It did indeed facilitate physical composition in an "idempotent" manner. That is, "each reaction leaves the key structural elements of the component the same" and therefore (in principle) can become part of a

"library" of reusable components that can (it is hoped) be compiled into components of ever-increasing physical and functional complexity. The trouble was the BioBricks standard had its limitations, biological, technical, and political (Lentzos *et al*., 2008; Rabinow and Bennett, 2009).

On the biological level, the main limitation was that although the BioBrick assembly standard provided a simple t solution to the problem of *physical composition* (i.e. getting segments of engineered DNA to physically connect), it did not yet sufficiently account for challenges of *functional composition* (i.e. getting segments of engineered DNA to work together in a predictable manner) (Andrianantoandro *et al.*, 2006; Ross and Arkin, 2009; Silver, 2009). To paraphrase one synthetic biologist: part 1 + part 2 ≠ function 3.

Physical boundaries, in other words, do not yet make a functional part. A given sequence of DNA may encode for a function. But it only encodes for the successful performance of that function given a particular set of other relationships. What one would need to know about the behavior of a given BioBrick part *in context*—i.e. how it is characterized—turns out to make all of the difference. None of this, of course, came as a surprise to the Knight lab. One step at a time, as the saying goes.

Initial Ramifications

Eight years following on the circulation of Knight *et al.*'s initial report pressing questions remain. Is a part a self-contained object? Is it a function? Is it a relationship? What do we need to know about the relation between the BioBrick part and the constructs of which it is a portion? And what needs to be specified about the ways in which a part occupies and therefore is partially determined by a biological situation?

Technical challenges aside, the real-world significance of Knight *et al.*'s proposal bears noting. Within a few years of Knight's proposal, an initial infrastructure for the cataloguing and sharing BioBricks had been established as the MIT Registry of Standard Biological Parts, and the groundwork had been laid for the generation of BioBrick users through the undergraduate International Genetically Engineered Machine competition, iGEM. Since their inception both the Registry and participation in the competition have grown by a factor of 100 (Smolke, 2009).

Like most first generation efforts, the critical limitations of this early work have shown themselves. In hallway conversations and, increasingly, in publications, practitioners remind each other that the original BioBricks format only works for some kinds of projects and under only limited conditions (Anderson *et al.*, 2010); that most of the parts in the MIT registry are not sufficiently well characterized to be immediately useful (Peccoud *et al.*, 2008); and that, despite the enthusiasm of the iGEM youth, few projects work as designed (Smolke, 2009).

Lest such limitations be cited as a warrant for stopping course, however, it's also worth remembering that like other first generation efforts, remediatory work is underway. An increasing number of iGEM projects do in fact work as designed (Ginsberg, 2009). More registry parts are being characterized and integrated into high-level engineering projects (Tabor *et al*., 2009). And serious players have proposed new generations of BioBricks standards (Anderson *et al.*, 2010).

In short, a simple lesson to be learned from these initial efforts is that the notion of fabricating standard biological parts has opened up one possible solution to the trenchant problem of rational design and construction in bioengineering.

A Screw is Not a Circuit is Not a Cell: Of Measurements & Standards

Another way of putting this is to say that despite limits, initial efforts to specify and fabricate standard biological parts have helped orient and intensify efforts at making *biology engineerable*. A key strategy throughout has been to ask whether or not, and to what extent, techniques and technologies in other fields of engineering might be useful for engineering biological systems. Hence, the notion of a part. This strategy shows itself in the frequent appeal to analogies among synthetic biologists (Keller, 2009; Rabinow and Bennett, 2009). Paying attention to how standardized engineering works with screws, computers, integrated circuits, or programming languages, we are told, might take us on the way to figuring out how standardized engineering practices might be established for biology (Baker *et al.* 2006; Andrianantoandro *et al*. 2006).

Retooling Analogies: Parts Datasheets

In this light, a paper published in 2008 by Canton, Labno, and Endy can be read as a key step in the push toward standardization (Canton *et al*., 2008). In that paper, Canton *et al.* tackle the problem of standardization by providing a candidate version of a "datasheet" for a device made up of standard biological parts—i.e. a sheet of details about the parts that make up their composite device, how they work and don't work under a given set of conditions, what kinds of "inputs" one might need and what kind of "outputs" one might expect, and so on. The datasheet, it is hoped, provides the kind of conceptual technology that, if successful would "enable engineers to rapidly select from a vast list the parts that will meet their design requirements" (Arkin, 2008).

Two aspects of the Canton *et al.* paper warrant note in this context. First, it recapitulates the challenges identified in the Arkin-Endy and Knight proposals and elsewhere (Endy, 2005; Baker *et al*. 2006; Andrianantoandro *et al*., 2006). Acknowledging the limits of any strict analogy, Canton *et al.* nevertheless assert that "despite the differences in materials and mechanisms, biological devices may often be defined with functions that are identical to the functions of electrical, mechanical and other types of existing engineered devices…Consequently, many of the characteristics found on existing device datasheets might also be useful for biological device datasheets." How useful, and useful in what ways becomes the question.

Second, despite this recapitulation, Canton *et al.* introduce a subtle maturity in the definition of what might constitute a standard biological part. The authors' write: "We define a standard biological part to be a genetically encoded object that performs a biological function and that has been engineered to meet specified design or performance requirements." The *maturity* lies in the fact that Canton *et al.* offer specific categories and candidate criteria for "specified requirements" might entail: "first, a definition of the function and interfaces of the device (inputs and outputs); second, the operating context of the device; third, measured characteristics describing the quantitative behavior of the device."

The *subtlety* lies in the fact that the authors take seriously that a "genetically encoded object" may not, per se, be a part. Such an encoded object may only be what it is under certain biological conditions. Said differently, they understand that their device is, in some way, a function of the context in which the device is operating. The question, then, is how much can such a device and its constitutive elements be abstracted out of a given context and how much of

that context needs to be embodied in the conceptual abstraction which is the datasheet? And which connections and interactions need to be accounted for in something like a datasheet (or a library, or a parts list, or a registry, etc.) in order for a would-be engineer to know what to expect from that part across a range of experimental circumstances? The answer to such questions is by no means trivial.

From Standard Biology toward Biological Standards

Such questions press home the point, as Canton and company recognize, that standardization is matter of relationships and measurements (Arkin, 2008; Lucks *et al.*, 2008; McArthur and Fong, 2010). That is, standards capture a range of relational variables and embody them in a set of measurements, which can be used to anticipate behavior. In this light, the answer to the question of what a standardized part might be turns to a large extent on what one chooses or does not choose to measure. Such choices admit to what an engineer thinks and does not think is significant.

This means, in short, that although measurements of "parts" and datasheets of measurements are necessary for achieving standardization, the question is: measurements of what? A golden rule of engineering is that measurements are the key to transforming a qualitatively complicated situation into a quantitatively regular and manageable one. It follows that synthetic biology, taken on the analogy of other engineering disciplines, will need to figure out what needs to be measured, in what way, and to what end. Perhaps it goes without saying, but in order for measurements to be significant, they need to be measurements of the things we know we need to know. Not all measurements are equal, as it were.

An analogy, it has been said, is not an identity (Burke, 1965). We may want biological engineering to be like other forms of engineering, but presumably the answer to the question of which refinements and which standards are needed for the design of screws or integrated circuits will not be identical to those needed for the design of genetic activity (Rabinow and Bennett, 2009).

So, what in biological engineering, taken up with an eye toward the eventual production of standardized parts, do we need to know? And if it's difficult to give a simple answer to such a broad question, this is in no small part because it's also not yet clear what we don't need to know. How one decides to slice this difference between what needs to be measured and what can be safely ignored would seem to make all the difference (Arkin and Fletcher, 2006; Purnick and Weiss, 2009). A turning point for advocates and critics alike.

Complexity, Boundaries, and Evolution: Can Biology Be Standardized?

In the abstract to Canton *et al.* the authors offer a now-familiar diagnostic claim: "The ability to quickly and reliably engineer many-component systems from libraries of standard interchangeable parts is one hallmark of modern technologies." To this claim, however, they add a nod to synthetic biology's critics and critical challenges: "Whether the apparent complexity of living systems will permit biological engineers to develop similar capabilities is a pressing research question."

Two parts of this nod warrant attention. The first, obviously, is the reference to complexity. References to complexity have become a hallmark of critical evaluations of synthetic biology (Galbadon *et al.*, 2008; Kwok, 2010). What is meant by complexity, however,

is not always clear. Is it number of components, number of interconnections, the nonlinear and stochastic physics of these interconnections?

The second, which might easily be passed by without much consideration, is no less important. Given such complexity, the question is not whether or not standardization is possible, i.e. whether or not biology can be made easier to engineer. Rather, the question is a matter of scope and degree. The extent to which synthetic biology may catalyze new capabilities in biological engineering is not only an open question, but an experimental one (Endy, 2005; Purnick and Weiss, 2009; Skerker *et al*., 2009; Lu *et al*., 2009).

Complexity and Context-Dependence

Perhaps it goes without saying, but the mode of analytic and material reductionism characteristic of synthetic biology is not, per se, new to biology or bioengineering. Nor, strictly speaking, is the attempt in biology to conceptualize and render complex systems as a set of composable parts. Throughout the 1990s, after all, it many argued that the genome sequencing projects—the projects to determine the order and the location of all of the genes in the human and other organisms—were, in fact, creating the basic "parts lists" for molecular biology (cf. Bains, 2001). Although it should be clear that a mapped sequence of protein coding regions in the DNA is not really what synthetic biologists are imagining when they talk about refinement and standardization.

Such refinement and standardization is predicated on the idea that, such as they are, natural systems are not amenable to the kind of rational design sought after by bioengineers. Engineers are thus not only oriented toward deconstruction and analysis but also to reconstruction and synthesis. In this light, it is not surprising that synthetic biologists are running up against a problem long faced in other scientific and engineering domains: It is one thing to break down living systems into finer and finer analytical, theoretical, or material units. It is quite another thing to work back from those units to a macro account of things.

It is precisely at this reconstructive juncture between the part and the whole that the criticism that synthetic biologists "aren't really doing anything qualitatively new" runs up against the criticism that "the overwhelming complexity of living systems will prevent biological engineers from fully achieving" the same capacity for control and standardization that has been achieved in other fields (Skerker *et al*., 2009; Kelly *et al.*, 2009).

Neither criticism is exactly right, however. The challenge faced by synthetic biology, we would argue, may in fact be more daunting than the appeal to "the sheer complexity of living systems" might suggest. The challenge may not complexity, per se, if what is meant by complexity is the quantitative fact that there are too many interconnections doing too many different things. The problem, rather, is *context dependence*.

Connecting Three Problems

It is the case that organisms have evolved a number of strategies (some spatial, some temporal, some topographic) to ensure that, despite the number of possible interactions within even a single cell or a single cell and its environment, only a smaller subset of actual interactions are only relevant at any one time. Presumably engineers could leverage some of these naturally given strategies. But here's the problem: these strategies may allow living organisms to manage complexity, but they themselves are characterized by a kind of contextual fine-tuning (Haseltine and Arnold, 2007; Arkin and Fletcher, 2006; Skerker *et al*., 2009).

What this means is that a parts-based approach to biological engineering must face the difficult fact that any given element in a living system may have been fine-tuned to perform in just the right way under just the right conditions; any engineered element inserted into that system may need to be similarly refined (Haseltine and Arnold, 2007). At a minimal level, and as an orienting first step, facing this fact will consist in conceptually connecting, and designing parts in view of, three problems: the problem of genetic context, of host context, and of environmental context.

First, *genetic context*. How will an engineered construct interact with, relate to, and be determined by the DNA in a given cell (Haseltine and Arnold, 2007)? Once a targeted sequence of DNA has been refined or designed, it has to be inserted into the genetic context of a cell. Where in the cell's DNA should it be placed? Should it be inserted directly into the chromosomal DNA of a given cell? If so, where in that genome should it be placed, upstream and downstream of which other genes? Or should it be inserted into the DNA of a plasmid (i.e. any one of the extra chromosomal DNA, which are capable of replicating independently from the chromosomal DNA)? If so, should it be a plasmid that makes lots of copies of itself and therefore lots of copies of the designed construct (Sprinzak and Elowitz, 2005; Arkin 2008)? The difficulty is that even carefully designed parts and devices—attention to the refinement of interfaces, characterization of inputs and outputs, analysis of key properties—can nevertheless result in unanticipated and perhaps even unpredictable interactions.

Second, *host context*. It is one thing to design a genetic construct and even to have some sense of what kind of genetic architecture would be best for its functioning. It's quite another thing to get this construct to run in a living organism. Which organism would be best for a given construct? It is estimated that there are 10^{33} microbial cells on earth? The question of how designed DNA can be made to function in a given cell type—even a cell type as thoroughly studied as *E. coli*—is a daunting question. But how about across and between various cell types? Presumably if its standardization we're after, some kind of predictability of parts performance across various hosts will be crucial (Forster and Church, 2006; Arkin, 2008; Carr and Church, 2009).

Said another way, if we picture the famous phylogenic tree of relatedness, what kinds of relatedness matter for synthetic biology? The bacteria on one branch of the tree might be very similar to another one right next to it. But perhaps they evolve at very different rates? Or perhaps one is more "promiscuous" with its DNA than another? From one host organism to another the sets of genes are quite different. From case to case, how will those differences matter? At what point do mutations become problematic? On even the finest branches of the tree, where variation is most slight, we still find myriad little differences. Will these differences matter? Sometimes (Haseltine and Arnold, 2007; Skerker et al., 2009).

All of this is compounded by the fact that the host cell isn't operating in a vacuum. The cell lives in an environment, an environment which includes other cells, nutrients, structures, temperature, signals, pressures, and so on. Hence, the problem of *environmental context*.

Environmental context is, of course, potentially the most variable and difficult part of the challenge. Once we've designed our genetic construct, placed it in a specified plasmid, and inserted that plasmid into a well-characterized host cell, what medium will it be given? What kind of container will the media and host cells be placed in? Even within the relatively managed

space of laboratory technologies and protocols, environmental context is a complicated state of affairs, what happens when we "get outside of the bioreactor" (Arkin and Fletcher, 2006).

In light of context dependence where should we make the conceptual and material cut that would constitute the boundaries of an individual part? And even if we determine some workable answers to that question, how might we deal with all of the other challenges association with evolutionary dynamics, with all that entails in terms of mutation and stabilization. The question must be asked: to what extent will biological organisms prove to be the kinds of things which admit to being conceptualized as a series of interoperable parts? Are they somehow too functionally integrated? And if functional integration is the result of long evolutionary time and refinement, maybe it can't be easily reworked, or even, for that matter, imitated?

Refactoring Context: Or, Can a Part Embody a Relationship?

The problem of context-dependence, however formidable in scope, does not yet present an overwhelming conceptual or practical obstacle for a parts-based approach to biological engineering. Indeed, there are multiple experimental warrants continued efforts. As early as 1981 the Rosenberg lab demonstrated that the inclusion of "insulators" (i.e. "stop codons") upstream of an inserted gene helped facilitate relative predictability of function within varying genetic contexts (McKenney *et al*., 1981). And with an eye to the problems of host and environmental context dependence very much in mind, the Bujard lab developed a system to quantify genetic activity in relation to an internal standard. They showed that despite contextual challenges certain aspects of gene activity can be made to function in a largely independent fashion (Deuschle *et al.*, 1986). More recently Kelley *et al.* proposed a standard for achieving relative measurements across differing contexts, a standard which cut reported variations in genetic activity by more than half (Kelly *et al*., 2009).

A challenge then is to design *projects* which are points of entry into this broader *problem* as a means of opening up new points of view and thereby expanding *capacities* (Weber, 1949). In this light, a published review of the Canton *et al.* paper is instructive (Arkin, 2008). The review poses a long series of questions that might be read as a rejection of Canton *et al.'s* proposal; indeed, the series of questions itself runs a page longer than the original article. Strikingly, the author of the review does not, in the end, propose abandoning the project. Rather, he asks how problems might be better framed, experimental practices refined, and goals suitably tempered.

The review's conclusion that parts-based work warrants continued pursuit not only turns on past engineering successes, but also on a simple premise of evolutionary biology: "the number of different types of elementary functions is finite." Evolutionary biology, for all its complexity and context dependence, is characterized by a curious fact: "it has been assembled in a limited number of ways to create the variety of organisms we see today. There are modules of function and evolvable structures of proteins and circuits that are shared, tuned and rewired across and within organisms to create new behaviors."

Hence evolutionary biology offers a kind of "existence proof" of modularity-in-context. The question is: to what extent, then, might engineers be able to transform lessons learned into elements for an engineering tool-kit?

Modular Evolution

In 2003 biologists from the Lim Lab at UCSF published a letter in Nature detailing research on the question of how proteins interact in yeast, with an eye to how specific these interactions might be (Zarrinpar *et al.*, 2003). The paper's findings occasioned a minor behind-the-scenes disagreement about what lessons might be drawn from *natural* systems for work in *synthetic* biology (Endy and Yaffe, 2003; Keller, 2009). The disagreement turned on whether these lessons could be marshaled in support of a parts-based approach to biological engineering, or if, to the contrary, they demonstrated that the task is riddled with too many unknowns, and hence, different strategies are called for.

The Lim letter begins and is oriented by a well-founded supposition about protein behavior in cells. "Most proteins that participate in cellular signaling networks," they write, "contain modular protein-interaction domains." A "protein-interaction domain" is a part of a protein that can function and develop independently from the rest of the protein; hence, these domains can be module in the sense that multiple versions of that same domain might form part of multiple proteins in the cell. So, the point and supposition that orients the Lim study is simply that those proteins that form part of signaling networks do so by way of domains which, because they are modular, might be found throughout the cell.

This basic insight into the functioning of cells might not, on the surface of things, seem particularly noteworthy. For those interested in biological engineering, however, it raises a curious and perhaps significant question. If a cell contains multiple copies of the same "modular protein-interaction domain," how does it ensure that the right domain gets connected at the right place and right time within the cell signaling network despite the fact that there are multiple "modules" of the same or similar type around?

The simple answer is that, despite modularity and similarity, each protein-interaction domains is genetically encoded in just the right way to "recognize" and bind to just the right place in the signaling pathway at just the time. No doubt there are subtle variations among similar domains. But, as the Lim study tells us, "It is generally thought that isolated domain–ligand pairs"—the interaction-interface between two domains—"lack sufficient information to encode biologically unique interactions." In other words, fine-tuned specificity of interaction is not simply encoded in the "part" of the cell itself. Rather, and quite significantly, "that specificity is instead encoded by the context in which the interaction pairs are presented." In other words, something about the context ensures that, despite similarity among multiple protein-interaction domains in the cell, just the right "parts" connect to form just the right relationship.

Positive and Negative Selection

Possible lessons for a parts-based approach to biological engineering begin to come into focus. Evolutionarily speaking, a question is: within a natural signaling pathway, how much of the specificity of interactions has been "positively selected" based on the genetic information encoded in a given domain, and how much has been "negatively selected" by way of the context "weeding out" those versions of the domain which are not quite right for the interactions that the cell needs to adjust to its environment at any given moment?

On one level, the Lim study is a fresh retelling of an old lesson in evolutionary genetics: the fine-tuning of an organism is as much about "positive selection" for a genetically encoded element or function as it is about "negative selection" against mutants of that element that doesn't achieve a given function. Yeast, it seems, fine-tunes its parts not only by getting the code

for a part right, but by eliminating the versions of that part that are not functionally sound. After all, a given part of a cell is not worth much if, in carrying out its function, it screws up the cell's other operations.

If this is an old lesson for evolutionary biology, it nevertheless provides pertinent insight for a parts-based approach to genetic engineering. As Drew Endy and Michael Yaffe put it in a response to the Lim letter: "if an integrated system is to function correctly, its components must be wired together accurately" (Endy and Yaffe, 2003). Such "wiring" is achieved not only through having the right interaction domains (think parts), but "by eliminating nonspecific interactions through evolution." Endy and Yaffe take this to mean that something like a parts-based approach to genetic engineering is not at all contrary to what's already going on in biological systems as long as the background refinement mechanisms can be managed, or even leveraged. The Lim study, in the end, "reveals an elegant example of how biology has solved the problem of wiring dynamic systems at the molecular scale."

A more cautious line, however, might be taken. Whatever else the study teaches us, it reinforces two major unknowns for synthetic biology. First, can we really draw general inferences from a study of this one pathway in yeast? It may be the case that, despite the myriad of possible interactions, cells use tricks of timing and spatial segregation to achieve high specificity and hence functional integrity. But do the mechanics of specificity in one case really inform what's going on in other cases? The problem of generality, and hence of standardization across multiple contexts, looms large.

A second unknown follows. If it is the case that the high specificity of parts in relation to their cellular context is achieved through a play of positive and negative selection, how might biological engineers interested in creating standardized parts reproduce this kind of fine-tuning? Evolution has had millions of years to combine and recombine interaction domains and binding motifs in order to achieve a kind of "polishing" effect on the various components of signaling networks. How could such polishing be engineered in the lab? More to the point here, how might the contextual mechanisms and relationships of evolutionary polishing be accounted for and embodied in a part or datasheet?

Geneticists, of course, have extensive experience in producing variations of a gene or of gene combinations and selecting out only those versions that seem to fit-the-bill (Haseltine and Arnold, 2007). But in synthetic biology we are talking about forward engineering in which the challenge is to design parts that predictably and reliably perform a specific function within designed constructs. If such specificity is not only context dependent in the sense that it needs a particular genetic, host, or environmental context in order to work properly, but context dependent also in the sense that it needs to be optimized by negative selection, how should we proceed?

From a Solution to a Problem: Making Engineering Biological

The extent to which lessons learned from the Lim study, or from the myriad other studies of functional specificity in cells, will prove to be generalizable and thereby useful for synthetic biology is, of course, not known. But one thing is clear: the tensions between those who draw positive and negative lessons for a parts-based approach will likely only be resolved experimentally.

It is also clear that questions concerning a part's *ontology*—i.e. what it's made to be and how it's understood—constitute a key threshold that will have to be crossed if synthetic biology is to prove worthwhile. We know that evolution has a combinatorial logic to it—there are only finite number of biological motifs and moves. This combinatorial logic, however, is such that the function and significance of any one part of a system depends, to a greater or lesser extent, on its relation to other contextual variables. Whether or not those contextual variables are of a similar combinatorial ilk, and therefore whether or not a sufficient number of contextual parameters can be "brought on board" and accounted for in the design of a standardized part is another question altogether.

That being said, the ontology of a part need not, and indeed by definition will not be identical with the ontology of any given natural system (and certainly not identical with all natural systems). A natural motif or repeating functional unit is not, after all, the same thing as an engineered part. What does a part need to be in order for it to work reliably ("interoperably") across a range of contexts? Minimally, if the Lim lesson is taken seriously, a part would need to be not simply a functional sequence of DNA. Additionally, it would need to be the embodiment of a specified (i.e. limited) series of relationships.

The point is not as subtle or mysterious as it might sound. Keeping in mind that context-dependence entails not only the positive functioning of a given part, but also its relative harmony with other operations in the cell, a part would thus be a sequence that functioned in view of a specified series of interactive variables. The fact that a part must be constituted and characterized in view of a number of key relationships is really just another way of saying that a part needs to be able to operate predictably in context. And this, of course, is just another way of saying that a part needs to be standardized. A part will be an object that conceptually and materially captures and blocks a range of specified relationships and embeds them in an object that can subsequently (it is hoped) be used without constant reference to these relations and their dynamics.

The terms "refactoring" and "abstraction hierarchy" are sometimes loosely used by would-be biological engineers; understood with some precision, however, the two terms introduce both the right concept and the right objective (Chan *et al*., 2005; Arkin and Fletcher, 2006). In computer programming refactoring means to simplify a unit of code while retaining a designed function. Abstraction hierarchy refers to a strategy of conceptualizing interconnected and complex problems and domains as integrated and integral units. A part may, in colloquial terms, be a piece of a whole. A standardized part, however, consists of a more complicated set of scales and relations, both conceptually and materially. A part is a designed object which, it is hoped, will embody and thereby refactor context dependence, opening up the possibility of conceptualizing, connecting, and reworking key engineering problems and vital biological domains.

From Parts to Systems

It has been remarked that, today, most researchers in bioengineering move forward by way of by way of projects which are "one-offs"—projects that feed the ever-increasing specialization and fragmentation of the biological sciences, while failing to simultaneously turn an experimental eye toward better integration. In such cases, a designed pathway or set of interactions is engineered and tested against a limited set of contextual variables (Martin *et al.* 2009).

It turns out that these kinds of experiments are not one-offs, strictly speaking. A successful experiment provides the basis for other engineering undertakings (Ro *et al.*, 2006; Yoshikuni and Keasling, 2007;). That said, it is nonetheless clear that the goal of much work is not the formulation of generalizable practices and standards. The goal is to make an object or mechanism operate under specified contextual constraints in the name of some (eventual) application or outcome.

Operating with a greater degree of general relevance other researchers are trying to determine or regularize context itself. In several highly publicized cases, the problem of context dependence is framed in terms of genomic context (Gibson et al. 2008; Gabaldón et al., 2008; Forster and Church, 2006). These researchers have argued that the use of standardized genetic components won't made quotidian until genetic context itself is standardized in the form of whole engineered genomes. To much acclaim, and to some (significant) success, researchers at the J. Craig Venter Institute, for example, have recapitulated natural genomes in a minimized form (Gibson *et al.* 2008; Endy, 2008).

Context dependence in these cases, however, is usually framed in terms of complexity, and complexity framed in terms of number of components and thereby number of interactions. If complexity is reduced, then context dependence might likewise be reduced. A key outstanding question is determining the extent to which reducing complexity, per se, will make genetic context more engineerable. Simplifying and thereby standardizing the genetic context into which a designed set of constructs will be inserted is clearly a laudable and ambitious goal.

Finally, and not insignificantly, there are also synthetic biologists who are tackling the uncertainty of context dependence by working directly at the level of intra- and intercellular systems (Purnick and Weiss, 2009; Haseltine and Arnold, 2007; Anderson et al., 2006; Lu *et al*., 2009). The strength of these approaches is that their unit of concern—the system—is clearly an object at the horizon of everyone's interest. Why else engage in biological engineering if not to eventually be able to design and build systems of various scale, function, and complexity?

The bet for these systems engineers is that there is something integral to systems that require working—conceptually and materially—at that level and not at the level of more discrete units. Working from basic parts up is less the goal than identifying the sub-systemic domains that might be made to serve as key pressure points in existing systems. Once identified these domains might be inflected—through the insertion of parts, through directed evolution, etc.—in order to produce a desired outcome. A potential disadvantage here is that a level of modularity and control in design and composition is lost—at least the kind of design and control that today is taken for granted in other fields of engineering.

Much current work will be limited in its scope and impact without the development of supportive computational tools. For better or worse, a crucial lesson learned from the genome projects is that biology can be technology driven. An equally important lesson, however, is that data, numbers on a spreadsheet, is not yet understanding. Hence, computational technologies need to not only generate and manage the right kinds of data, but, more importantly, need to be designed and calibrated to the right problems—particularly if we hope to use the accumulated data to discern the rules for forward engineering. (Cai *et al.*, 2009; Purnick and Weiss, 2009; Densmore, *et al.*, 2010). Software tools focused on data/information/knowledge management are actively under development (MIT Registry, OpenWetware, JBEIR) as are an emerging cohort of

design tools (Invitrogen/VectorNTI, DNA 2.0/Gene Designer, Densmore/ Clotho, Ham/Vector Editor, Peccoud/genoCAD).

If context dependence and the standardization of parts do not prove to be intractable problems (either for technical or political reasons), each of these strategies—case-based work, context engineering, work on systems, and the development of software tools—is likely to play a vital role in working out an answer to the question: to what extent can biology be made engineerable on the analogy of other engineering practices? Moreover, once more is known about the critical limitations of using familiar engineering tactics within biological contexts (e.g. measuring inputs and outputs, building from the bottom up using off the shelf parts, and the like), these other modes will no doubt prove indispensible in the difficult work of making engineering biological.

The Scientific and Political Future of Biological Parts

One reviewer of this report pointed out that the dictionary definition of the word part provided as the heading to this report risks getting the reader off on the proverbial wrong foot. The definition begins with the notion that a part is a "portion into which a thing may be divided." Such a definition is reductionist in the simple sense of movement from whole to part. But a key feature of the notion of a standard biological part, the review insisted, is precisely that it is made and not discovered. In this light, a standard biological part (whatever such an object proves to be) is not a portion of a thing divided, but rather, an "element, component, ingredient in a situation," to cite the second part of the definition lifted from the Oxford English Dictionary.

Taking a cue from our reviewer, we hasten to add that the last part of the OED's definition—"a share in an activity, function, role, part in a play"—is equally telling and apt. A standard biological part may indeed prove significant as an element in a biological situation. It has already, however, proven significant as an element in the situation of biology, with all that entails organizationally, politically, and economically (Rabinow, 1999; Gibbons, 1999; Guston, 2000; Hayden, 2003; Wilsdon and Willis, 2004; Jasanoff, 2005). Indeed, whatever its significance as an object within engineered biological systems, it may be its role in these broader situations that are likely to be determinate.

Keep in mind that Arkin's and Endy's 1999 proposal for producing standardized parts was not funded. The fact that it was not funded turned as much on political and economic factors as it did the biotechnical state of play at the time. The notion of biological parts, after all, concerns the status, organization, and potential significance of biological *practice* as much as it does the *nature* of living systems.

In 1999 DARPA—the US agency responsible for the development of new military technology—wasn't interested in funding the creation of standardized parts. In 2010 the story is quite different. DARPA has included a significant line for research in synthetic biology in its latest budget. Moreover, in the intervening years the NSF—whose mandate, formal and informal, is to animate US industries—has become the major US funder for synthetic biology, and underwrites the work of the BIOFAB.

The difference in the last decade, to repeat the point, is thus not technical advance, per se, although such work by cannot be taken for granted. Rather, the security situation within which biology is developing is not what it was a decade ago. And the economic hopes for biotechnology, however elevated on the heels of the genome projects, have risen with a singular

intensity in the name of a "green economy." The role parts-based biological engineering may play in these "situations" must be included in any sufficient answer to the question posed in the title of this report (Zoloth, 2008; Rabinow and Bennett, 2008; Schmidt *et al.*, 2009).

Indeed, the lead author of this report would not be undertaking this work if it were not for the fact that political, security, and economic questions have been catalyzed by the prospect and project of parts-based bioengineering. Human Practices, as it has been called, was introduced as an integral component of synthetic biology precisely because whatever else a "part" is or may turn out to be, it constitutes a potential vector for developments in health, wealth, and security (Bedau and Parke, 2009).

In short, we must take stock of the fact that the challenge of fabricating standardized parts grew up in the post-9/11 security milieu, and (quite against the trend in so many other domains) has been nurtured by the failings of the US economy. This means that questions concerning the positive and negative outcomes of fabricating parts take on a particular urgency (Schmidt *et al*., 2009). What, for example, is to be made of the contradiction that organisms altered or composed using standard parts need to be functionally robust across multiple contexts and yet also need to be fragile enough not to negatively impact the environment? And what are we to do with the seeming quandary over double goal of wanting biology to be easy to engineer and yet not available to dangerous uses and users (Bugl *et al*., 2007; Garfinkel *et al.*, 2007; Bennett *et al.*, 2009)?

These questions, not unlike the biotechnical questions of context dependence, are not, per se, new. And, like past work in bioengineering, matters of security, economics, and the environment, not to say ethics and organizational politics, have been posed and reposed (Lash *et al.*, 1996; Nowotny *et al*., 2001; Rose, *et al*., 2006; Barben, *et al*., 2007; Khushf, 2007; Rabinow and Bennett, 2008; Ganguli-Mitra, 2009). Human Practices has been an active part of synthetic biology for three years in the US; consonant programs are active in the UK and the EU. Strategies, venues, and time, however, are new. The world of parts-based engineering is not the world of the genome projects, the cloning of Dolly, or the birth of recombinant DNA.

Recalling the first section of this report, it is fair to say that the problem of physical composition, addressed through early work with BioBricks, has so far proven a difficult enough challenge that the problem of functional composition, with all this entails in terms of context and refinement, has effectively been black-boxed. For many social scientists thinking about the security and economic ramifications of synthetic biology a similar point could be made; diagnostic work about the basic mechanics is underway, but strategic efforts to work through organizational and political contexts has not been fore-grounded (Rai and Boyle, 2007; Parens *et al.* 2008; O'Malley *et al*. 2008; Mauer, 2009).

They are, however, beginning to be fore-grounded in the work of the BIOFAB and elsewhere. The mandate of the BIOFAB, it is crucial to keep in mind, is to produce families of standardized parts, not to characterize and debug context dependence. It is a fabrication facility, after all. That being said, to the extent that we take seriously everything noted so far about the difficulties, significance, and possibilities presented by context dependence, then we certainly cannot proceed in anything like a naïve fashion about the challenges before us.

The challenge of specifying general rules of composition and thereby working toward the goal of predicting and designing genetic expression, will, in other words, require serious progress on characterizing and debugging context. Similarly, questions of risk, worth, fitness, failure, and

success are in play not only biologically, but politically and ethically as well. These constitute the strategic parameters for the work of the BIOFAB. The challenge of transforming these parameters into experimental and operational platforms which not only increase technical capacity, but which contribute to human flourishing is clearly the demand of the day.

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