NASA-NSF Joint Workshop on Synthetic Biology

A two day invitation-only workshop titled "Toward a Synthetic Biology", sponsored by NASA, NSF, and the Howard Hughes Medical Institute was held at the HHMI Janelia Farm campus in Sterling, VA on April 3–4, 2008. Among the attendees at the workshop were 24 individuals from academia, two from industry, four from NASA, and 15 from NSF. These included both senior and junior investigators, with a diversity of backgrounds and research interests. The co-organizers of the workshop were Jef Boeke (John Hopkins University) and Gerald Joyce (The Scripps Research Institute), who were responsible for selecting the invitees and formulating the agenda. Nearly every person who was invited to attend the workshop agreed to do so, and the feedback from the attendees was overwhelmingly positive, with the consensus that this was a unique, stimulating, and productive event.

On the first day of the workshop, 22 individuals made presentations of ~15 minutes each, followed by 5 minutes of discussion. These presentations covered topics pertaining to the origin of biological systems, synthetic genomes, engineered biological circuits, and the re-engineering of biology. The second day was devoted entirely to discussion, focusing on eight key questions in synthetic biology, and considering future research opportunities for NASA and NSF in the area of synthetic biology.

The workshop began with opening remarks by Jef Boeke, who admonished the participants to focus on the real promise, not the hype, of synthetic biology. In pursuing that promise he asked that we strive to anticipate potential deleterious consequences of our research so that appropriate protections can be put in place at the outset. He also asked that we focus on current technologic bottlenecks in the field that might be overcome by strategic investment from NASA and NSF.

What is Life?

The session on "Origins of Biological Systems" inevitably raised the question: "What is life?", which Andrew Ellington (University of Texas at Austin) took on with gusto. He pointed to the so-called NASA working definition of life: "life is a self-sustained chemical system capable of undergoing Darwinian evolution". He claimed that this statement, which appears in a NASA internal document, has unfortunately come to be regarded as definitive, and therefore has placed NASA on record, rightly or wrongly, as defining life. This position, Ellington argued, is hazardous for a variety of reasons, both scientific and social.

Ellington's position is that there is no such thing as "life", and that the working statement within a NASA document does science a disservice by attempting to pretend the contrary. "Life" is a term better suited for poets (or philosophers) than scientists, and continuing attempts to

determine whether a given system is alive or not hearken back to ancient philosophers, with a similar level of resolution. He asserted the following existence proof: if we haven't figured out what life is by now, there is little hope that we will settle upon a definitive definition in the near term, and there is no research program one can imagine, at any price, that will provide such a definition.

Ellington felt it obvious that there are a variety of systems that have properties that are generally grouped under the scientifically inaccurate term "life". He did not attempt to enumerate those properties, nor argue whether a given set of properties are definitive or not. Perhaps, he suggested, it might be better to look at the systems that scientists naturally tend to group together and decide on a probabilistic basis to what extent they meet any of a number of these criteria — to have a probabilistic view of "life". However, even this compromise has no scientific or practical merit, and the most honest and useful approach would be to do away with both the term and attempts to define it, at least within the scientific community.

Ellington further argued that the reason a rather pointless philosophical issue has become an important issue for NASA is because of its near-term political ramifications. While he does not believe we need to define the term "life" to conduct research that is of use to society, we nonetheless inherit the baggage of this term, as it is commonly used by the lay public and by many philosophers.

Ellington envisions that, within a year, the field will see the publication of a paper from The Venter Institute describing how a fully synthetic genome has been transplanted into an organism so as to cause the organism to adopt the features encoded in the fully synthetic genome. He believes this will be a watershed event, at least as far as the public's perception of scientific progress is concerned. The watershed event, of course, will be that scientists have supposedly created life. The lay public is already well-indoctrinated with the view that DNA encodes "life", and that if one transplants DNA, this amounts to transplanting "life". Therefore, by defining DNA, one is defining "life".

In Ellington's view, this watershed event will come with a full examination of the supposed ethical issues surrounding it. If scientists can create life, should we regulate what they are doing? This is a reasonable question from the lay public's point of view because if we do have the ability to control the form and function of organisms, it is in the legitimate interest of the society to regulate the potential harm that comes from such organisms. While Ellington admits to and encourages such regulation, he does not see this happening. Whether and how research will proceed will not be a measure of whether the research and its products are beneficial or harmful, but rather of whether scientists are treading upon a domain that should have long ago been reserved for religion, philosophy, and other disciplines that delight in creating purely intellectual problems for consideration.

If scientists can create life, then what does this mean regarding the origin of life and its likelihood? What The Venter Institute will accomplish will say nothing about origins, but everything about intelligent design. Irrespective of what the creators of "life" do or don't say, the event can and will be interpreted as an example of how life must be designed because it is too complex to have evolved. By buying into the notion that there is a definable "life", NASA is also co-opted into this unfortunate misinterpretation of what synthesizing "life" implies about our evolutionary history.

Both of these considerations suggest to Ellington that NASA should not be in the business of defining "life". Having made an ill-advised foray into this area, it is not too late to make an exit. His own preference would be for NASA to convene a blue ribbon committee to consider the issue, and then dump it, ultimately issuing a statement along the lines: "The definition of 'life' is a controversial position, even amongst scientists, and can only be taken on as a collaborative effort with leaders in religious, philosophical, and social thought. Because of this, NASA does not at this time have a working definition of 'life'". To the extent that NASA opts for this approach, it should still be possible to have interminable and unresolvable debates about the properties of "living systems", and the milestones inherent in making it can remain intact.

What is Synthetic Biology?

Prompted by the sessions on "Synthetic Genomes" and "Engineered Biological Circuits", Roger Brent (Molecular Sciences Institute) offered some cogent comments. When challenged to define synthetic biology, as happens seemingly once a day, he suggested that the simplest response, at least in the U.S., is to say that the term "synthetic biology" fills the linguistic hole that would have been filled by the term "biological engineering", by analogy with software engineering. Synthetic biology is that branch of engineering devoted to the design and construction of new self-replicating, living systems. Unfortunately, in the U.S., biological engineering means other things too, from propellers in fermentation tanks to neural human interface to artificial hip joints to surgeons blasting away at blood clots by firing lasers through optical fibers. Thus the term synthetic biology indeed fills a semantic hole.

Brent regards one of the foundational moments of synthetic biology as having occurred at a meeting in Boston, when Randy Rettberg, a coworker of Drew Endy's, recommended not defining it but letting people self identify. Brent doesn't know whether this was a good idea or a bad one, but feels it reflects some of the present condition. Thus it has come to pass in the U.S. today that the boundaries of synthetic biology are weirdly shaped. It encompasses much of the design and construction of digital logic circuits, even though the utility of these is questionable. It encompasses in the U.S. much of the design and construction and optimization of microbial

organisms used to produce useful products, fuels, and materiel, much of which in Europe is called “microbial chemical engineering”, the utility of which is not questionable.

To a large extent this problem of weird boundaries still exists. It excludes a great deal of the design and construction of biological systems of economic importance. In terms of economic importance and complexity of design and construction, the prize for a designed-and-built entire system probably still goes to the yeast artificial chromosome, telomeres, origin of replication, cloning sites, selectable markers, and different pieces containing most of the human immunoglobulin gene repertoire (heavy chains, light chains, V regions, and J regions), all built and placed into transgenic mice made by Abgenix, a company sold outright in the late 1990s with a market value of ~$800M. This kind of work, recombinant pharmaceuticals, and all engineered crop plants, are all outside the liturgy of what one means by synthetic biology, although perhaps they should not be — they are genetic engineering on a large scale.

At the same time, DNA synthesis and construction methods are very much in the liturgy. The situation is much like if the field of software engineering made an important part of its definition the technology used to write alphabetic characters on paper, and devoted much of its internal attention to the technical details and relative advantages of mechanical pencils and felt tip pens. Still, even though the boundaries of synthetic biology are weird, what is inside represents a respectable set of developments. The emphasis of this workshop on connections between the engineering and the origin of life allows one to reluctantly embrace the term.

Brent emphasized three points about the field of synthetic biology as it now stands. First, and most importantly, an often cited goal of synthetic biology is to learn the “principles” or “design rules” for biology by building biological systems. Yet, for the people who now identify themselves as synthetic biologists, that is an unrealistic goal, although it may not be a necessary one. Second, one can adduce by counterexample the unlikelihood of synthetic biology contributing to either the deeper understanding of, or the re-engineering of a system, critical to the biology of metazoan organisms. If we want synthetic multicellular organisms, we certainly will need sophisticated cancer prevention systems. Any attempt to reprogram existing animals that involves messing with the p53 system, a rat’s nest that breathes nightmarish life into the term “legacy code”, will require knowing what is going on, quite likely finding and characterizing the quantitative consequences of each of these regulatory protein modification events, and understanding the key design features of the system’s quantitative regulation. This could only be enabled by the same sort of careful experimentation that scientists do over the long haul. Third, given the current *ad hoc* boundaries of the field and its participants, one *should* enter into the spirit of the field with enthusiasm, and offer as much contribution to sensible engineering goals as our coworkers’ science, widely ranging scientific knowledge, technical capabilities, and knowledge of relevant history can contribute.

Brent's Center for Quantitative Genome Function has learned a great deal about the quantitative function of a particular signaling system in yeast. The system senses concentration of a ligand outside the cell, and transmits that information more deeply inside the cell, which then operates on it to make decisions. In this work, one must pursue every genetic and chemical trick in the book to address questions that come under a very old name. The name is *physiology* — more specifically, "quantitative physiology". Brent and colleagues have learned much about the quantitative physiology of this signaling system that are pertinent to other signaling systems. Some of these findings appear fundamental, in the weak sense in which biologists mean the term, which is that it is commonly found, and hence is pertinent to many systems of this type throughout biology.

The Chemist's Tradition of Making Things

Steven Benner (Florida Foundation for Applied Molecular Evolution) emphasized that synthesis is a research strategy that complements analysis and observation. Available over the past century primarily to chemists, it is now possible to use synthesis to test hypotheses in biology by creating new forms of living matter. In doing so, synthesis now provides for biology the opportunity to set large goals that drag scientists across uncharted terrain where they must address unscripted questions, driving discovery and paradigm change in ways that analysis cannot. Examples from Benner's own work included the total synthesis of genes, unnatural nucleic acids used in medical diagnostics, prebiotic synthesis experiments leading to components of RNA, and the resurrection of inferred ancestral proteins.

Roger Brent amplified upon the points made by Benner by noting that just as much knowledge of basic chemistry came from learning to synthesize complex natural organic molecules, much knowledge of biology will come from learning to build biological systems. What one sees today, however, is rather the reverse. All too often, what one sees are attempts by engineers to impose concepts derived from human engineering upon living systems. Here, the important distinction is that biological systems were not designed by a sentient designer. Rather, the reverse is the case, even though there is no *a priori* reason to expect that design principles used by humans might be used by evolved organisms. Circuits are built by people to perform specific tasks. One calls a circuit a circuit because the electrons would flow around within it until they come back to where they started. How pertinent at all is this concept to engineering goals in biology?

Anthony Forster (Vanderbilt University) argued that understanding life will require its reconstitution from molecules of known function. Of the 151 genes he has postulated to be necessary for self-replication from small molecules, the protein synthesis machinery encompasses 96% of them. Once such a reconstitution is achieved, Forster envisions applications

in the *in vitro* evolution of protease-resistant, cell-permeable ligands and drug candidates. Towards this goal, he has redesigned the genetic code *in vitro* for the synthesis and pure translation display of polymers of unnatural amino acids.

Richard Roberts (University of Southern California) described the use of mRNA display, an engineering of the protein synthesis machinery, to evolve polypeptide ligands that alter biological systems. The selected linear peptides could be designed with protein-like specificity and could alter complex phenotypes such as fly lifespan. Cyclic peptides with antibody-like affinity toward protein surfaces also were obtained. Roberts is currently exploring the possibility of engineering these molecules to be sufficiently stable and cell permeable to be orally-available molecules for the modification of protein-based, signal-transduction networks.

Sydney Brenner (HHMI Janelia Farm) argued that the deluge of DNA sequence information from model organisms and patients now cries out for tests of hypotheses using synthesis in model systems. He called for less big science and more "bedside-to-bench" research. He also warned that terming the field "synthetic biology" may do more harm than good, and suggested an alternate term from the literature, "molecular engineering".

Laurie Zoloth (Northwestern University) discussed the major ethical issues in synthetic biology. She argued that, while many of these issues are similar to those covered in Frankenstein and at Asilomar, synthetic biology is more dangerous than recombinant DNA technology because it is uniquely capable of blurring the line between being and non-being. She concluded by charging the field with two tasks: (i) making the research safe — not only with regard to biosafety, but also with regard to potential bioweapons applications; (ii) giving the public an opportunity to reflect on the new powers of synthetic biology with both honesty and justice.

John Cumbers (Brown University) entertained with a vision for how synthetic biology could potentially be used in support of extraterrestrial missions, and proposed a government- (or government/private-) sponsored contest for the best payloads to be lofted to Mars. These would be evaluated on the basis of the best potential to deliver useful goods to a nascent Martian "colony", using as starting materials those resources available on the surface. This prompted Roger Brent to initiate a stimulating discussion about terraforming fact and fantasy as a more extreme version of Cumbers' presentation.

Discussion Topics

The second day of the workshop tackled several key questions in synthetic biology, as summarized below.

What are the minimum requirements for life? What complexity of materials from outside the system are permitted? What will break the barrier between non-life and life? The following

properties were offered as requirements for life: compartmentalization (or at least co-localization), genetics that provides the basis for heritable information, metabolism driven by genetically-encoded catalytic function. All of these properties must be unified within a system that is capable of self-propagation.

The Venter Institute is making a unique contribution in attacking the important goal of a re-engineered synthetic organisms. However, Hamilton Smith (The Venter Institute) pointed out that this is engineering, not making an entirely new type of life. Steven Benner suggested that a truly separate origins would involve a breakthrough such as developing an RNA enzyme with generalized RNA polymerase activity, something that has not yet been achieved, and in any case is distinct from synthetic biology. Laurie Zoloth added that the system must be capable not just of transferring information, but doing so in a manner that enables adaptation to a changing environment.

Can engineers do biology? Ronald Breaker (Yale University) argued that most engineers have a fundamental lack of knowledge of biochemistry, which is the underpinning of biology. Pam Silver (Harvard Medical School) agreed that the lack of biological knowledge among engineers is a problem, but she, as well as Andrew Ellington and Roger Brent, argued that it is too late for biologists to stop the engineers from driving synthetic biology because they are already doing it, and will pick up relevant biological knowledge as they need it. Joel Bader (Johns Hopkins University) took this one step further by arguing perhaps one does not even need to know the biology. Design goals might be achieved without regard to knowledge about how existing living system achieve the same goal. By analogy he noted that the engineering achievement of controlled flight was not realized by the careful study of birds.

John Cumbers (Brown University) asked whether the field of synthetic biology will separate into science and engineering disciplines. Laurie Zoloth noted that a key difference between science and engineering is that only the former has a requirement for falsifiability. She argued that if synthetic biology is to provide benefit for science, beyond engineering, then it will need to be linked to a larger theory about how the world operates and evolves. John Mulligan (Blue Heron Technologies) raised the example of protein engineering, for which, he noted, design has been easier than prediction. Steven Benner went so far as to claim that engineering hasn't given us functional molecules, although Joel Bader offered the counter-example of engineered zinc finger proteins.

What are the current limitations on synthetic gene technology? Anthony Forster argued that the biggest limitation of synthetic gene technology, other than for companies and large consortia, is the cost. He suggested making the technology open source so that progress could be made more quickly on reducing costs, rather than maximizing profits. Pamela Siver echoed that

synthetic DNA still is too expensive for the individual investigator. John Mulligan countered that the cost of DNA synthesis *per se* is quite modest, and the true costs are in developing the expertise to design genes that will express efficiently and perform their intended function. Roger Brent agreed that design is still extremely difficult, especially for making multiple genes that act in concert.

Jef Boeke challenged representatives of the synthetic gene companies to provide a cost breakdown, and offer specific suggestions of how costs might be reduced. Mulligan responded that two things could be done to reduce cost: i) reduce the scale of oligonucleotide synthesis to the sub-nanomole range so as to reduce the cost of reagents; ii) expand the market beyond its present size of ~$50M/year so as to achieve greater economies of scale. The key, he felt, is to expand the therapeutics market, which potentially could dwarf the research market. The intrinsic efficiency of synthesis, now quite robust, is not a significant cost limitation. Nor is the cost of the DNA amidites themselves, especially if the scale of synthesis can be reduced. Forster offered the prediction that the cost of synthetic genes will soon fall below one cent per base pair, and such low cost will further drive economies of scale and more investigators to synthesize, rather than clone, their genes of interest.

Many agreed that if errors in synthesis could be reduced, it would further reduce costs by reducing the amount of sequencing and re-synthesis that needs to be done. Mulligan stated that it is necessary that at least one clone in eight have the correct sequence in order for gene construction to be practical. This sets a limit on the length of oligonucleotides that are synthesized. Without divulging the details, both Mulligan and Jeremy Minshull (DNA2.0) said that gene synthesis companies employ various proprietary tricks to enhance the fidelity of synthesis and to perform error correction on the synthesized materials. The most common source of errors are cytosine deamination events and single-nucleotide deletions, both of which can be addressed by appropriate countermeasures.

Perhaps, Jef Boeke suggested, it is time for government funding agencies to step in and support research in improved technologies for gene synthesis. Whereas it may not be possible to mount a “Manhattan Project” scale initiative, it might make sense for government agencies to make a strategic investment in seed grants to explore radically lower cost approaches to gene synthesis. Alternatively a pubic/private partnership to fund an “X-prize” for gene synthesis could spur innovation in this arena that is so central to synthetic biology.

Conclusion

Jack Szostak (Harvard Medical School) summed up the workshop by saying that it was a highly worthwhile event, although in his opinion perhaps too much concerned with defining what synthetic biology is, rather than with how to use synthetic biology to get things done. Clearly

there remains a huge gap between bottom-up approaches that are concerned with how life arose and top-down approaches that aim to re-engineer existing organisms. That gap is likely to remain for the foreseeable future, although a unification of the two approaches will eventually occur.

Szostak suggest that, in the near term, joint efforts by NASA and NSF program might focus on exploring the range of environmental conditions that can support life. This would be relevant both to the search for life elsewhere in the universe (exobiology) and the possibility of engineered life under extreme conditions (applied microbial ecology). For the more conventional type of synthetic biology, with its attendant practical applications, a joint program would be a reasonable way to address common goals such as biofuels and low-overhead chemical synthesis of drugs and materials. The central question, for both earth and for space applications, is whether standard chemistry or synthetic biology provides a more efficient, lower cost, practical approach to specific challenges, given projected advances in both fields. That might be an interesting topic for a future NAS/NRC panel or second NASA-NSF joint workshop.

Appendix A. Meeting Agenda
"Toward a Synthetic Biology" — NASA-NSF Joint Workshop at HHMI Janelia Farm

Thursday April 3

7:30–8:30 Continental breakfast

8:30–8:40 Introduction by co-sponsors, John Rummel (NASA) and Patrick Dennis (NSF)

8:40–8:45 Introduction by co-organizers, Jef Boeke (Johns Hopkins) and Gerald Joyce (Scripps)

8:45–10:45 **Session 1: Origins of Biological Systems** (session chair Andrew Ellington)
Robert Hazen, Carnegie Institution of Washington
Andrew Ellington, University of Texas at Austin
Gerald Joyce, Scripps Research Institute
Jack Szostak, Harvard Medical School
Elena Rivas, HHMI Janelia Farm
Sean Eddy, HHMI Janelia Farm

10:45–11:00 Break

11:00–12:40 **Session 2: Synthetic Genomes** (session chair John Mulligan)
Hamilton Smith, Venter Institute
Jeremy Minshull, DNA2.0
John Mulligan, Blue Heron Technologies
Joel Bader, Johns Hopkins University
Jef Boeke, Johns Hopkins University

12:45–1:45 Lunch

2:00–4:00 **Session 3: Engineered Biological Circuits** (session chair Pamela Silver)
Ronald Breaker, Yale University
David Liu, Harvard University
Pamela Silver, Harvard Medical School
Sergio Peisajovich, UCSF
Ronald Weiss, Princeton University
Roger Brent, Molecular Sciences Institute

4:00–4:15 Break

4:15–6:00 **Session 4: Weird Life — The Reinvention of Biology** (session chair Anthony Forster)
Steven Benner, Foundation for Applied Molecular Evolution
Anthony Forster, Vanderbilt University
Richard Roberts, USC
Sydney Brenner, HHMI Janelia Farm
Laurie Zoloth, Northwestern University

6:15–7:00 Reception and informal discussion

7:00 Dinner

Friday April 4

8:00–9:00 Continental breakfast

9:00–9:15 **Eye-opener:** Synthetic Ecosystems on Mars
John Cumbers, Brown University

9:15–10:45 **Thematic Discussion** (facilitators David Liu and Joel Bader)
1. Does the origin of life require genetics? metabolism? compartments?
2. What use is there for artificial life that is unrelated to terrestrial biology?
3. Should genomes be synthesized from amidites or assembled from PCR amplicons?
4. How can error correction in gene synthesis be improved? automated? obviated?

10:45–11:00 Break

11:00–12:30 **Thematic Discussion** (facilitators Ronald Breaker and Laurie Zoloth)
5. Can biological circuits be made to plug and play?
6. What are the "killer apps" for synthetic biology?
7. What is the role for modeling? screening? trial-and-error?
8. When does weird life become dangerous?

12:30–1:30 Lunch

1:30–2:30 **Wrap-up Discussion**

Appendix B. List of Participants

"Toward a Synthetic Biology" — NASA-NSF Joint Workshop at HHMI Janelia Farm

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