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Lecture

Making Biology Easier to Engineer

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Abstract

In this lecture, Pam Silver describes some of the exciting work her laboratory has carried out in synthetic biology and illustrates how engineers are not only bringing new tools to traditional biology but also a new perspective. Traditional biology has emphasized understanding the causes and mechanisms of biological processes, but bioengineers put that knowledge to practical use. Our progress in understanding life, particularly the detailed molecular biology of cells, coupled with new technologies and computing power, is starting to transform biology from a 'soft' science focused on description to a 'hard' science focused on quantifying, predicting and controlling its properties. The lecture was given at the 12 May 2009 launch of the Imperial-based Centre for Synthetic Biology and Innovation—established in partnership with the London School of Economics and funded by the Engineering and Physical Sciences Research Council (EPSRC) as part of a science and innovation award.

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It is a privilege to be here to tell you about some of our attempts in synthetic biology to design organisms to do new things. I come to synthetic biology as one of the biologists, and biologists tend to think that if we can rebuild a system, it indicates that we actually understand it. But having worked in the field of synthetic biology for the last few years, my thinking has evolved somewhat from a biologist's perspective to an engineer's approach. Engineers tend to think that biology knows how to do a great many things that we do not yet know how to do. So the thinking is that we can learn a lot from biology and we can use it. In the next few minutes I will first spend a little time talking about biology and engineering; then, I will go on to discuss a couple of examples of how we can manipulate cells to store and compute information; and, finally, I will tell you about some of our newer efforts in studying photosynthetic microbes and their role in processing CO_2.

Pamela Silver is a Professor in the Department of Systems Biology at the Harvard Medical School. Her group focuses on systems and synthetic biology, and is trying to understand how sets of molecules and cells function together to generate spatial and temporal organization. At the same time, they are developing design principles to create complex biological systems. She is also the Director of the Harvard University Graduate Program in Systems Biology. For more about her lab see http://openwetware.org/wiki/Silver_Lab

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Biology and engineering

Let us think about biology for a moment and how there are similarities between biology and engineering. First, biology is exquisitely sensitive; for example, the olfaction system is capable of detecting a single molecule. So biology can send signals very specifically and very quickly just like we're able to do through engineering. Second, like engineering, biology is also highly modular; and it is this kind of modularity that underlies the concept of parts. The gene is the fundamental unit of biology, but we now know, from years of work in molecular biology, that the gene can be 'parted' into promoters, terminators and introns. We also know now that proteins are highly modular. It is this modularity that fascinates me, and it is also what synthetic biologists take advantage of. Finally, the idea from biology of self-replication is a crucial feature and what essentially distinguishes synthetic biology from regular engineering.

One of the other things that was posited at the outset in developing the field of synthetic biology (at least, among the group pioneering the field from MIT) was that one of the goals was to make biology easier to engineer. I want to mention some of the underpinning elements of this goal. First of all, if DNA is the fundamental unit of synthetic biology, one of the main challenges would be to come up with faster, cheaper DNA synthesis. There are already multiple companies engaged in that as well as academic research efforts in this area—though it is still not cheap enough and not fast enough. However, we have a lot of raw materials to work with. Every day there are new genomes decoded and deposited online, and we have access to those. This gives us raw material for new kinds of parts that we can begin to design to put into cells, giving them new functions.

Another important element of making biology easier to engineer is to ask the question, 'Can we take what we understand about engineering, say, computer chips, and combine that with this biological modularity so we may come up with a reliable way to build new biological systems?' Put another way, we must ask, 'What would it take to make biology more predictable?' That is really what we would like to do in synthetic biology. In this regard, it should also be highlighted that systems biology—studying the whole system of complex interactions in biology—provides some of the underpinnings for this as well. We have heard about standardized parts and how these different biological units that have standardized properties can be put together such that the researcher knows what she will get out at the other end. Importantly, this requires measurement. Just as it is important to an engineer, a synthetic biologist needs ways to measure how these components are behaving. Therefore, mathematical modelling will play a big role in synthetic biology, as it does in engineering. Finally, synthetic biologists hope to have the ability to synthesize anything we want. This will change how we do science.

Right now, it is not so bad: a student or a scientist in a laboratory can go onto the internet and find a sequence they like; if she can get hold of that organism, she can amplify that DNA, and use it. This ability comes from the PCR (polymerase chain reaction) revolution—sometimes called 'the liberation of the genome'—and it is certainly an advance from what used to happen 25 years ago, when there was no e-mail, there were actual letters, genes took five years to clone, etc. The technology has improved and, luckily, we do not have to do that any more! However, it is still a tedious process. But, we can imagine a time—for those of you younger people out there—when, instead, you could just sit at your

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computer and say, 'I want this sequence, I want that part, I know how they behave.' I then would type it into a computer; I would send it to a company; it would then come back to me 'good to go' into the organism; then, the experiment starts! Imagine that time! Forget all this pesky, 'Oh, the ligation didn't work', and stuff like that. Consider what this can do for biology.

Making cells that count

When my lab first started working on synthetic biology, it was my idea that I could make cells that could count how many times they had divided. In the case that I will illustrate, it is a yeast cell that is used and the idea was that every time it divided, it would turn colour. This takes advantage of naturally fluorescent proteins, for which the Nobel Prize was given last year. In broad strokes, if you can imagine a situation where every time a cell divided it turned colour, then at the end, you could register how old that cell was based on what colour it was—that was my vision.

If you look at my laboratory's imaging of our experiments in this area, you would see yeast cells dividing. You would also see two different kinds of cells: mother cells and daughter cells. You would see that, over time, a daughter will start out blue and then, as it generates a new daughter, its daughter will be blue, and as it becomes a mother, it then turns green. [A video was then played during the talk, illustrating this point.] If you watch this cell, here it starts out blue. Here is the new daughter—it turns blue. Then this one becomes the mother—it turns green. We made a set of cells count to two! This turned out to be hard. I do not know if it was hard because we did not know what we were doing, or because this represents that biology is actually hard to engineer. I think that in the near future we will begin to see examples of biological counters that can count higher, but remember that what we were trying to do was couple counting with cell division, which is something the cell already does. A cell can count how old it is, but we wanted to engineer this ourselves. It still may not be such a simple task.

In the course of carrying out this project, my lab decided to step back and think about things that counters do. One thing they do is they have to remember their previous state. Biology has ways of remembering, via bi-stable switches. There are two ways that biology does this, either by a double negative feedback loop or a double positive feedback loop. The basic idea is that, in response to some stimulus, the system is turned on, and in the absence of the stimulus, it stays on for some period of time. We decided to rebuild such a system, which we call a 'memory device'. We began with yeast cells, because they are very simple to engineer. Our idea was to make a cell that, when it was exposed to a signal, would turn colour. If you removed that signal, it would stay coloured because it remembered that it was exposed to the signal. Essentially, we have made a simple auto-feedback loop from synthetic parts. The basic strategy here is that when the cells are exposed to a signal, call it A, a transcription factor is turned on. It then binds to its cognate DNA binding site in a second factor, say B, which is then turned on, and then B is capable of sustaining itself, of keeping itself synthesized. When we did the analysis we could see the red and green cells respective to the stimulus provided.

(*Aside*: To briefly explain how we represent the synthetic biological parts, one can, for example, go online and look into the Registry for Standard Biological Parts to see how

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discrete boxes are used to represent a synthetic DNA sequence with a known function. One specific box represents a promoter sequence; another box represents a function that makes a cell turn colour; another box represents a DNA binding domain; another box represents a mechanism to target a protein to the nucleus; another box represents a gene activator, and so on.)

In my lab, we actually filmed the yeast cells with the 'memory device' as we exposed them to stimulus and saw how they would change colour and remember their exposure to the signal—you can watch this video from my lab on YouTube. What you can see is that these engineered cells, after many cell divisions, remember! This is a fairly robust system and the next step, for us, was to transfer this system into mammalian cells—we used CHEMO cells, which are a cancer cell line. It is a little more complicated, but it is the same idea and we used many of the same parts. So, there are several features in this system that make it a good illustration of synthetic biology in that it is made of parts and it can be transferred from one cell type to another.

Now, if we think about moving forward from this simple device, we need to ask, 'What can we do with it?' We imagined that we could build a system where a cell remembered that it had been exposed to DNA damage and then would register with some type of colour, or some other kind of signal. Since exposure to DNA damage is one of the fundamentals of cells becoming cancerous, this envisioned system could be very useful in perhaps helping us to know the history of a single cell within a tumour. This is a good example of how we could take a synthetic biology device and apply it for a practical purpose. Imagine further if, not only the device could tell us a history of that cell, but could also count how many times it has divided and then do something at a certain threshold, like secrete an anti-cancer drug.

So, so far I hope to have convinced you that we can design and characterize simple systems. I have illustrated with an example how a device is composed of interchangeable parts and can be transferred from one cell into another. In the long term, I think such a device could be very useful. Although I did not talk about predicting the behaviour of cells with synthetic biological systems, it should be noted that a lot of quantification and modelling went into the memory device in order to allow us to predict how it would behave.

Making bioenergy

I will now explore a newer research area for me, and one that is an example of how a small lab is trying to work on a big problem: how are we going to save the world through bioenergy? My lab thought it would focus on the role of sunlight. Sunlight is used to store energy in the form of carbon bonds and it is involved in the cycle of CO_2 as well. These two factors encompass a lot of fundamental issues with regard to all biology on earth. To us, using sunlight seems like something that we should be thinking about.

Others have been thinking about this for a long time. Several years ago, one of my colleagues at Harvard, Michael Brenner, led the writing of a government report, which I was particularly enthused about. It says that boosting the efficiency of fuel formation from microorganisms is the major technological application for synthetic biology! Some of the major conclusions from this report include the idea that engineering fuel production from

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microbes is essentially a systems and synthetic biology problem, which will require engineering and understanding of networks, feedback loops, etc.

In my laboratory, we began with a few things in mind. First, we knew that, as a small group, we were not going to compete with biodiesel or gasoline. I have worked in Brazil on the ethanol problem in the early 1990s and have had some experience in this area. Instead, we decided to focus on something with a scientific underpinning to it that seemed like it would be interesting for students, presenting them with a rather major challenge: we decided to try to engineer microorganisms that would make hydrogen. We took two approaches, which I will describe briefly. First, in a more traditional approach, we envisioned having cells that would harvest biomass—yeast—which, when working together with bacteria, in a dual-organism system, could produce hydrogen. Second, in a more ambitious effort, we wanted to engineer a photosynthetic organism that would directly convert energy from sunlight into hydrogen. Hydrogen is a high energy density fuel and it is clean; of course, there are a number of challenges involved in this project—such as getting a good production yield, storing hydrogen effectively and converting it into energy—but nonetheless we forged ahead.

My laboratory used three strategies. The first, as mentioned, is a co-culture system where we used yeast together with *E. coli* to produce hydrogen. In the second system, we actually tried to reconstitute an organelle within the yeast cell so that it produces hydrogen. The organelles that I am talking about are called hydrogenosomes, and they produce hydrogen; we are trying to transfer those into yeast. Lastly, as I said, we are trying to harness the photosystem and couple it directly with hydrogen production.

Let me tell you about the first approach briefly, which illustrates some of the basic metabolic engineering components of synthetic biology. We began with yeast because we know it well and we know how to engineer it. Our idea was to have yeast produce formate, which *E. coli* can very efficiently use to produce hydrogen. How can we engineer yeast cells to make a formate? The first challenge relates to making the appropriate chassis (a kind of encasing 'shell' for the synthetic biological system). Yeast is an excellent organism to use as a chassis in synthetic biology because we know its genome sequence and what the genes do, so we can easily eliminate genes we don't need. Now, one of the things that yeast—the type we used was *saccharomyces cerevisiae*—actually does naturally is degrade formate. In our experiments, this was obviously not a good thing because we were trying to get yeast to make it! There are two genes in yeast that encode formate dehydrogenases, so we made sure to knock out these two genes so that the cells we would work with could no longer degrade formate. That is pretty easy and illustrates how synthetic biologists must first choose a chassis and knock out genes such that one ends up with an appropriate 'shell' to build the desired system in.

How did my laboratory get the yeast to make formate? This is where the synthetic biology component really comes in. There is an enzyme, pyruvate formate-lyase or PFL, from *E. coli*, which is capable of taking pyruvate and converting it to formate. When we then looked at yeast metabolism, we found that, of course, yeast produces pyruvate; so, we decided to introduce the PFL gene from *E. coli* into our yeast cells. We had the PFL gene custom synthesized so that it was codon-optimized and would be expressed in yeast. Indeed, when we then put that custom synthesized, codon-optimized PFL into our yeast, the cells produced formate!

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But we wanted to see if we could do better. In metabolic engineering, one might say that the next step for my lab should have been to mess around with the flux in this pathway, or try to decrease the rate at which the system produces ethanol. Instead, what we decided to do was to look at the downstream part of the pathway and to introduce another enzyme that now will take Acetyl-CoA and move it back into ethanol. This is a slightly different kind of logic and by doing this we were able to increase the production of formate even more. So really we had used two enzymes from *E. coli* to increase formate production in yeast.

Lastly then, could we get these optimized formate-producing yeast cells to make hydrogen? We took the yeast cells and combined them with *E. coli*, which can very efficiently produce hydrogen, and indeed we could get hydrogen production. However, this was not a great system. It is not very efficient. It is not ready for any kind of industrial use. It is strictly a proof of concept.

I am now going to move on and talk about another way that my lab thought of making bioenergy—this is something more 'fantasmical'. I think the grand challenge is to engineer a microbe that can capture energy from the sun and convert it into a form that we can use. Plants of course already do this, but we would like to do this in a predictable way. There are lots of different kinds of photosynthetic organisms. My laboratory focused on the simple cyanobacteria, a photosynthetic bacterium. Remarkably, the work within this kind of bacteria accounts for about 50 percent of the photosynthesis on earth.

Let me tell you a story about these bacteria. When you look at them (using sophisticated microscopes and imaging techniques), they have red fluorescence and green in the photosystem; as well, they have these different dots that you can see and these represent compartments within the cell. These compartments are really a way that cells use engineering to compartmentalize chemical reactions—and this is something that we, as synthetic biologists, should be thinking about. Engineering organizes things into compartments; biology does the same. For example, biology will compartmentalize so that a certain set of enzymes will be coupled together for more efficient biosynthesis. This is just how cells organize their internal elements naturally.

Cyanobacteria fix CO_2 through the enzyme rubisco, which is one of the most abundant proteins on earth. One can look at an image of the organism, blown up as an electron micrograph, and observe these large, highly organized structures. These are what we call carboxysomes. It turns out that these structures are very common; many bacteria have them. In the case of cyanobacteria, they are almost like giant viruses—they have a shell, they are made wholly of protein and they package the rubisco inside them. These carboxysomes may be a way that the cell deals with a relative inefficiency of rubisco and carbonic anhydrates. So, the reactions to fix CO_2 are actually going on inside these carboxysomes; the idea being that, by packaging the enzymes together, there will be increased efficiency of what are otherwise not very efficient enzymes.

I want to tell you now about our efforts in trying to harness photosynthetic machinery to make hydrogen. There exist ideas out there to use yeast or bacteria to harness sunlight through a series of chemical reactions to make biodiesel, butanol, etc.; however, this would involve a number of different chemical steps and just seemed too onerous for my small laboratory. Instead, we imagined a situation where we would try to couple a collection of photons and electrons directly to the production of hydrogen. The photosystem captures

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electrons; it is composed of iron-sulphur clusters, which essentially act as nanowires, and the electrons are conducted through these iron-sulphur clusters so that they can be transferred to proteins such as ferodoxin. Taking a synthetic biology approach, we decided to black box the photosystem. In part, this choice was because, while one might think that photosynthesis is well understood, it really is not properly understood. Nonetheless, we imagined a situation where we would take a photosystem and we would fuse the ferodoxin and the enzymes that made hydrogen directly to the photosystem; in this way, electrons would move as if they were wired directly into the hydrogenases to produce hydrogen. This would give us more efficient and direct coupling of the production of hydrogen to the photosystem.

Conclusion: the 'bigger dream'

Let me conclude with the bigger dream. At the beginning of this project, we said, 'What can we do as a bunch of students in a small lab that is interesting?' I did not begin this project with the idea that we were ever going to try to make fuel to use in automobiles, although one of my first potential investors was GM! My idea was one of what I call 'personalized energy'. That is, I see no barrier to small-scale bioenergy production. Imagine that we could have energy-producing bacteria growing on roofs, maybe even locally; and, in principle, this could be as cheap as making beer. This was my vision.

My vision got a little bigger, because about a year ago Harvard was going through the planning stage of building a whole new campus. And, before Harvard decided to put a hold on the building of this new science complex, I was going to have a whole laboratory up on a roof. My plan would have included growing these kinds of energy-producing bacteria up there, where they would have actively been harnessing sunlight to do the job. Now, there is only a large, gaping hole where this campus would have been built, but my dream and vision remain and I'm confident that one day I will have energy-producing bacteria on my roof!

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