*BioSocieties* (2009), **4**, 275–282 © London School of Economics and Political Science
doi:10.1017/S1745855209990147

# Lecture

# Synthetic Biology in Pursuit of Inexpensive, Effective, Anti-Malarial Drugs

**Jay Keasling**
Department of Chemical Engineering and Bioengineering, 201 Gilman Hall, University of California, Berkeley, CA 94720-1462, USA
E-mail: keasling@berkeley.edu

**Abstract**
*One of the big success stories in synthetic biology is the re-engineering of an existing organism to produce the anti-malarial drug artemisinin. The drug is also found naturally in extracts of the plant* Artemisia annua *(Wormwood), but demand is far outstripping supply and extracting the drug from the plants is complex, time-consuming and expensive. In this lecture, Jay Keasling describes how he and his laboratory re-designed yeast microbes into living mini anti-malaria drug factories producing artemisinin more cheaply and efficiently. 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.*

**Keywords** Artemisinin, Malaria, Metabolic Engineering, Pathways, Synthetic Biology

Synthetic biology is the design and construction of new biological entities such as enzymes, genetic circuits and cells, or the redesign of existing biological systems. It builds on the advances in molecular, cell and systems biology and seeks to transform biology in the same way that synthesis transformed chemistry and integrated circuit design transformed computing. The element that distinguishes synthetic biology from traditional molecular and cellular biology is the focus on the design and construction of core components (parts of enzymes, genetic circuits, metabolic pathways, etc.) that

**Jay Keasling** is Hubbard Howe Jr Distinguished Professor of Biochemical Engineering in the Department of Chemical Engineering and Bioengineering at Berkeley, and Director of the Physical Biosciences Division, LBL and Synthetic Biology Engineering Research Center. His group focuses on the metabolic engineering of microorganisms for degradation of environmental contaminants or for environmentally friendly synthesis. To that end, his lab has developed a number of new genetic and mathematical tools to allow more precise and reproducible control of metabolism. These tools are being used in such applications as synthesis of biodegradable polymers, accumulation of phosphate and heavy metals, and degradation of chlorinated and aromatic hydrocarbons, biodesulfurization of fossil fuels, and complete mineralization of organophosphate nerve agents and pesticides. For more about his lab see https://keaslinglab.lbl.gov/wiki

can be modelled, understood, and tuned to meet specific performance criteria, and the assembly of these smaller parts and devices into larger integrated systems that solve specific problems. Just as engineers now design integrated circuits based on the known physical properties of materials and then fabricate functioning circuits and entire processors (with relatively high reliability), synthetic biologists will soon design and build engineered biological systems.

If we look at how computers, mobile phones and the other very low-cost, incredibly complex instruments that we use to communicate are designed, we see that they are systems made of *component parts* like hard drives, memory cards and so on. And software is, of course, built on top of that. Now, if one of those components fails, you can go out to a place like 'Radio Shack'—perhaps 'PC World' or 'Dixons' would be the UK equivalent—and you can buy a hard drive and a memory card, or whatever component part you want to replace. And component makers sell their components to makers of computers and phones. Apple, for example, doesn't make the components inside its powerbooks or iPhones, it buys them from component makers; the same applies for companies like Dell and Toshiba. This system of interchangeable components in computers and electronics works because we have something called *characterization*, whereby components and connections are well-characterized and *standardized*. If you think about the connection on a hard drive, it has a standard connection so you can swap in a new hard drive if the old hard drive fails. It is also crucial that *devices function independently*; hence, when a hard drive fails on a computer, it does not detract from the other components functioning inside the computer box. Then, if you are going to build software into a computer system, there are software components and standardization so that, again, we are able to diagnose any problems that may happen with the system. Finally, and obviously, we have the *ability to build all these components*.

Let us consider a different industry: the chemicals industry. I want to say something briefly about the chemical styrene and its various purposes. There is a great deal of Styrofoam used in tyres, in plastic bottles and toys, for example. How do we get styrene? Styrene is synthesized in a chemical factory that makes components—not necessarily off-the-shelf components—but in chemical engineering we call these 'unit operations'. These unit operations have many of the same qualities that we find in the components of a computer. Chemistry has been somewhat standardized, and we have a base of chemistries to build on. So, if you don't have a reactor and you would like to produce styrene, you can design one because there are models for unit operations and models to fabricate reactors so that you can build one to certain specifications. In addition, chemical engineers have got standard connections; this is just like how threads on a screw or threads on pipes have also been standardized, so that when you go to a hardware store you don't have to worry about getting the pipes and connectors and such from the same manufacturer; all you have to worry about is the size of those pipes and they will fit together. I have begun this talk by emphasizing that in electronics and computers, as well as in the chemical industry, there are *parts* and *devices* that can *function independently* and have been *standardized* and *characterized* in many ways, leading to *our ability to design and fabricate at will*. This is also the goal of synthetic biology. In the rest of this talk I want to tell you about how my lab has used synthetic biology to find the right components to develop a cheap, effective anti-malaria drug.

## Fighting malaria

First, let me say a little bit about the disease. Malaria is caused by a Plasmodium that infects red blood cells, rendering them unable to carry oxygen. This Plasmodium is transmitted by mosquitoes and exists in many parts of the world; primarily, it affects under-developed areas around the equator, where there is enough heat for mosquitoes to be around, and so Plasmodium is also around. At any one time about a quarter of the world's population is at risk of getting malaria. Between 300 million to 500 million people have malaria, and every year between 1 million and 3 million people die from malaria; 90 percent of malaria deaths are children under the age of five. Further, economists have noted that in countries seriously affected by malaria, it reduces their GDP by roughly half.

There are drugs that are widely available, which are based on quinine and have been around for about 400 years—at least, quinine has. There is also chloroquine, which has been around for about 60 years. However, these quinine-based treatments have been so widely used that they have lost effectiveness because the Plasmodium parasite which causes malaria has become resistant to them. So, even though the drug is inexpensive and widely available, it is ineffective in most parts of the world. There is, however, a drug that is effective: it is called *artemisinin*. Artemisinin comes from a plant, *Artemesia annua*, and it has a great history; it goes back to about 150 BC when there were some writings about the first uses of Artemesia for treating haemorrhoids and later for treating fevers (presumably, fever due to malaria). Artemesia then appeared to be forgotten until about the time of the Vietnam War when the Chinese were fighting in Vietnam, and the drugs that were then in use were no longer effective; the Chinese went back to their literature and purified the active ingredient from *Artemesia annua*. About five years ago, the World Health Organization recommended artemisinin as the drug of choice for treating malaria.

Now, let me talk about the issue of cost. If you, as travellers, go out and buy an artemisinin combination therapy you will pay between $20 and $40 for an adult treatment. While you will be happy to pay that, I'm almost certain that, for people in the developing world who live on around a dollar a day, this drug is unaffordable. Most people who need this drug cannot even afford the cost that was negotiated by the World Health Organization when it recommended that the drug cost about $2.50 for an adult treatment.

There is also a problem with supply. We get artemisinin from small farms that grow it in Southeast Asia, where they extract the drug with the only, relatively crude, mechanisms that they have available; this artemesia extraction is then sold to pharmaceutical manufacturers, who formulate it into combination therapy. The price on the open market will fluctuate anywhere from about $500/kilogram to $1,500/kilogram, making it very difficult for manufacturers to predict cost and how much they are going to need. In fact, you need about an 18 to 24 month lead-time in order to get enough drugs in the supply chain. It is actually predicted that in 2010 there will be a severe shortage of artemisinin going into the pipeline. What is more, when they were extracting the drug a few years ago they were using unsafe methods and there was a huge contamination problem. Consequently, there are real problems with the present source of artemisinin and the quality of the ingredient.

Finally, there is a very severe problem (that was documented on the front page of the *New York Times* a few months ago) where a number of pharmaceutical companies are producing monotherapies of artemisinin. As you may know, most organisms will

become resistant to monotherapy drugs, which is why we try to limit prescribing antibiotics and why our therapies for HIV are combination therapies with multiple drugs to fight the disease. Likewise, with treating malaria, it is recommended that combination therapies be used to help combat the development of resistance. Still, there are rogue manufacturers out there which are distributing a monotherapy, and this is only going to contribute to the building of resistance to this drug. They've already seen this in Vietnam, and it is coming to the rest of the world. We have to preserve artemisinin, because the only other drug that was in the pipeline and was about to launch failed in Phase II clinical trials. Artemisinin is really the only drug we have right now to fight malaria. We need some way to control supply so that it is only formulated in combination therapies. There is a chemical synthesis, but it is long, arduous, unyielding and too expensive to produce a low-cost drug. In fact, it couldn't even be used as a high-cost, high-value drug.

A number of years ago now, my laboratory decided to tackle this problem in a new way by engineering a microbe to produce artemisinin such that it would also hopefully reduce the cost by an order of magnitude. In the rest of my presentation I am going to talk about how synthetic biology helped us in thinking through this project.

## Engineering the production of artemisinin

To start the process, we used a microbe that produces a precursor to the drug called farnesyl diphosphate (which, as it happens, is also the precursor to cholesterol that you and I and every organism on the planet produce). We then transformed this precursor by using genes from the Artemisia plant to produce artemisinic acid, a precursor to artemisinin. Then, we purified the artemisinic acid from the organism, and used a series of chemical reactions to eventually turn the compound into artemisinin and the actual drug of choice that is currently used to combat malaria.

Let me now turn to how my lab planned to make this artemisinic acid *within an organism.* Almost invariably, in my lab, we start with *E. coli* as a chassis—and we do so because we know so much about this organism's biology that we can actually go straight on and do some real engineering without spending all of our time on biology. Helpfully, *E. coli* produces farnesyl pyrophosphate—our starting precursor—but only in small quantities (*E. coli* actually needs this compound for production of the cell wall and in electronic transport).

We also needed all the components to build the necessary metabolic pathways inside the cell—some of which are available and some of which are not. Where did we come up with some of these components? There is no 'Bioshack' shop where we can just go and buy the components that we need. Although genome sequencing did give us some of our components, we needed more. So, another way to get components is to write to colleagues who we know have worked on genome sequences of interest—and we did just that. We asked a number of colleagues something along the lines of, 'please, could you send us component *x* that you described in your paper?' This process, as you can imagine, almost invariably turns out to be more complicated than one might wish—components do not end up working the way they are described in the literature, there is a lot of back-and-forth and there is a lot of trial and error. Could you imagine this kind of circulation and trial and error going on for components in computer devices? This kind of thing just doesn't happen when the

components you are dealing with are hard drives or other standardized electronic parts; but this is what biology has given us!

Returning to my storyline, we had our *E. coli* producing farnesyl diphosphate, but we needed to get to artemisinic acid. The components that we needed for this are stuck in the *Artemisia annua* plant. As it turned out, the first enzyme in the pathway that we were interested in has been well described in the scientific literature as well as in the patent literature. However, when we wrote a letter asking a colleague to send us the gene he had described in a past paper, he explained that, 'No, your lab cannot have the gene—you are a competitor.' This all took place a few years before there were commercial DNA synthesis companies. At that point, we felt like we were almost at a dead end because the only way forward seemed to be growing the *Artemisia annua* plant ourselves and extracting the gene of interest; and we were not prepared to do that. So we ended up writing to a different colleague who had cloned a *similar gene* that capitalized on a *different reaction*. This colleague did send us the gene that was *similar* to the most desirable one for the job and we plugged it into *E. coli* to help us in trying to get artemisinic acid. When we did this, we had an absolutely miserable yield of the product. It was extremely far from the benchmark yield we wanted to be at in order to approach a biotech company with our work to commercialize production. To be more specific, at that stage, we were actually six orders of magnitude between where our yield was and where we wanted it to be. We had to deal with this problem. And, we still did not really have the ideal genes for the job at hand.

Skimming over the details of our next step, we decided to recode a plant gene into something that was more specific to optimal functioning in a microbe. When we synthesized this recoded gene and put it into our system, we saw about a 200-magnitude improvement in the production. (That step would now be much more efficient with the advent of DNA synthesis companies that will synthesize your ordered genes and send it to you in a couple of weeks, for a couple thousand dollars.) It then started to become clear that the gene was probably not our next limitation to overcome, but it was the upstream pathways that were the limitation. And working on this part of the problem was where my laboratory had the most fun!

In this next stage of research, my team had to do a lot of work in getting to grips with the metabolic pathways that were operating in the *Artemisia annua* plant, as well as in our engineered *E. coli*. It is worth knowing that, at the start of our project, many of the pathways that we were looking at were actually not very well known—even some of the pathways in *E. coli*, which is such a well-studied and thoroughly characterized organism. Because we did not really have well-characterized knowledge of all the genes we were working with and we did not completely understand what might disrupt the metabolic pathway, we decided to choose a different route. We chose to bring in a completely new pathway, the mevalonate pathway, which is responsible for cholesterol biosynthesis in you and me. Now, because cholesterol is associated with a multibillion-dollar business, there is a lot known about its metabolic pathway; the genes have been cloned from almost every carrier you could name and they have been characterized. This meant that, once again, we were in a stage of research where we had the advantage of working with a pathway that is well known and well studied. Another advantage for us, at that stage, was that there was a good chance that taking a pathway from one organism and putting it into another might mean that the regulation of the pathway would be left behind, not carried in the transformation. This means that we had a good chance of getting over the regulation hurdles and could

just, more simply, get on with working with the target pathway. Finally, another advantage for us was that we would be working with acyl-CoA, probably the most important metabolite in an organism, particularly in an organism like *E. coli*.

Now, if I skip over the details again, what ended up being beneficial in the next stage was to bring the use of yeast into our engineered system, creating a hybrid pathway. When we came to engineering this hybrid pathway, again, we ended up with another 200-fold magnitude increase in the production of the pathway. However, I do not want you to think that we were able just to put all these different genes together and get them to function; the process took a fair amount of optimization to get the genes to work as we wanted them to. But it is this optimization stage that I, as an engineer, really enjoy.

The next stages were quite technical as we struggled to understand discrete metabolic issues of toxicity, protein mis-folding, enzymatic dynamics, membrane and osmotic stress and the like. There was a lot of guess-work and experimentation with various elements in the chemical pathways that we were trying to engineer; there was also a lot of consulting what was available in the scientific literature, as well as a good deal of trying to fill in gaps along the way. But I want to tell you a little bit about how difficult it was to build the components needed to design the metabolic pathway to optimize the yield of the artemisinic acid product to give you a feel for what we achieved.

We tend to express the genes for the enzymes in a metabolic pathway; however, one challenge was that we started out having no connections between the enzymes themselves and their metabolites. We started exploring what was going on between these enzymes because it was clear that there needed to be high expression as they are scattered around the cell randomly. To think of an analogy, imagine that a plumber came into your house and, rather than connecting all the pipes, he just threw them in the basement and expected the water to get straight to your shower—you know what would happen. What we were doing was essentially like flooding the cells with metabolites, and some of these were potentially toxic pathways. Now, what would be far better is if we could connect these enzymes in some way that would make the pathway rather tighter and less chaotic. However, one problem was that we did not have the standards for how to put enzymes together.

So, how could we stick these enzymes together? Well, let's take a lesson from copper pipes, where a scaffold is used to hold the pipes together (and not from steel pipes, where threads are used to fit the pipes together). In these copper pipes, there is a ring that goes around them to hold two pieces together on a kind of scaffold. The question for us then was: 'How could we find a scaffold to attach these enzymes and hold them together so that the metabolites won't have to diffuse so far to find the next enzyme?' Well, a very smart post-doc in my laboratory at that time, John Dueber, had come from working on signalling proteins where he had used standard connections of a sort. Without going into the complexities, we worked out a way to build standard connections and a scaffold for the enzymes in our metabolic pathway. This development gave us another 50-fold improvement in amorphadiene production and that was close enough that we could declare victory and send our engineered microbe, with its optimal metabolic pathway, to our pharmaceutical partner, Amyris. At Amyris, they subsequently optimized the *E. coli* to produce over 25 grams of amorphadiene per litre, and that paper was actually published earlier this year.

But the story doesn't end there. We still needed to turn amorphadiene into artemisinic acid. Our colleagues proposed a metabolic pathway that used three enzymes to transform

amorphadiene into artemisinic acid; however, one of those enzymes worried my laboratory. This was because these enzymes are typically very difficult to express in an organism like *E. coli*. This was when we said, 'Darn, we might have the wrong host!' To make a long story short—which isn't so short for the PhD student who spent most of his career doing this—we were able to use yeast instead of *E. coli* to produce the optimal levels of artemisinic acid.

Now, the final miracle and surprise in all of this is where the product ended up. Typically, what we would do when we are producing a product on a small scale is we would look for it everywhere. We would take the whole batch of cells in the medium and we would break open the cells and look for the product. However, we couldn't find the product—there was no product inside the cells! As it turns out, artemisinic acid is toxic; it is not as toxic as artemisinin but it is still toxic enough. So what had actually happened turned out to be to our advantage. The yeast actually turned on pumps (the kind of cellular pumps that would normally be used to get rid of antibiotics, for instance) and pumped the artemisinic acid outside of the cells. Now, artemisinic acid is still pretty hydrophobic so it actually stuck to the outside of the cell. What this means is that the yeast we were working on had naturally invented a way to secrete relatively purified artemisinic acid (because it was this specifically that the cells found toxic) and stick it on the outside of cells. This was rather handy for us!

Could we get this same system to work in *E. coli*? You probably know that I have had funding from the Bill and Melinda Gates Foundation to do this work, and the nice thing about that kind of funding is that they give you enough so that you can pursue many avenues simultaneously. So, along the way we had also been looking at whether we could express this enzyme in *E. coli*. For quite a while, we really had two possible hosts in our hands, *E. coli* and yeast, and both of them produced artemisinic acid. The Gates Foundation was glad that we had met those goals, but they wanted to know which one was going to be the best host to produce the artemisinin drug. So, we built teams and had them compete against each other; it was two teams from my laboratory and two teams from Amyris Biotechnologies. The teams had all the resources they needed and also very high hurdles to get the candidate host organism to jump over. The deal was that whichever organism worked best would be the organism that we would take into the pipeline and actually through the process. Both organisms met all of their goals but yeast was chosen to produce the product. This was about a year ago when we announced that Sinopia Ventus was going to be our pharmaceutical partner. Sinopia is doing the scale-up of artemisinin right now; and hopefully, within a year to two years, this product will actually be out in Africa, saving lives and at a substantially reduced price. Our target is to get the price down tenfold.

## What's next? Biofuels . . .

Finally, I want to tell you that everything we did in my laboratory was patented. The University of California holds those patents. But the university said that, as this is a product for the developing world, it wouldn't take any royalties for the patents if we could get a company to take this product and produce it at cost. This was basically saying to a company that we would give it an exclusive license if it would not make any real substantial amounts

of money. Amyris licensed that technology and they're not making any profit on this. Amyris is turning it over to Sinopia to turn it into a pharmaceutical drug, and Sinopia won't make a profit on the drug either.

However, because we have developed a technological platform upon which it is possible to do many things, Amyris and my laboratory are now trying to produce biofuels from this platform. We are taking the host that we built to produce artemisinic acid, taking out the genes that specify artemisinic acid and putting in genes that now specify biofuels. The idea is that once you've got one host working well, you will be able to use it for multiple purposes—and that is really the hope for synthetic biology.

## Acknowledgements

I would first like to thank the people in my laboratory who did this work over a number of years and the many more people at Amyris who worked on this project. I would also like to thank the Joint Genome Institute for doing a great deal of gene sequencing for us; and our funding sources, in particular, the Bill and Melinda Gates Foundation, which funded the last three years of science and is still funding the final stages of development for this project. Finally, I would like to thank Caitlin Cockerton and Filippa Lentzos for their help in turning my presentation into this written manuscript.