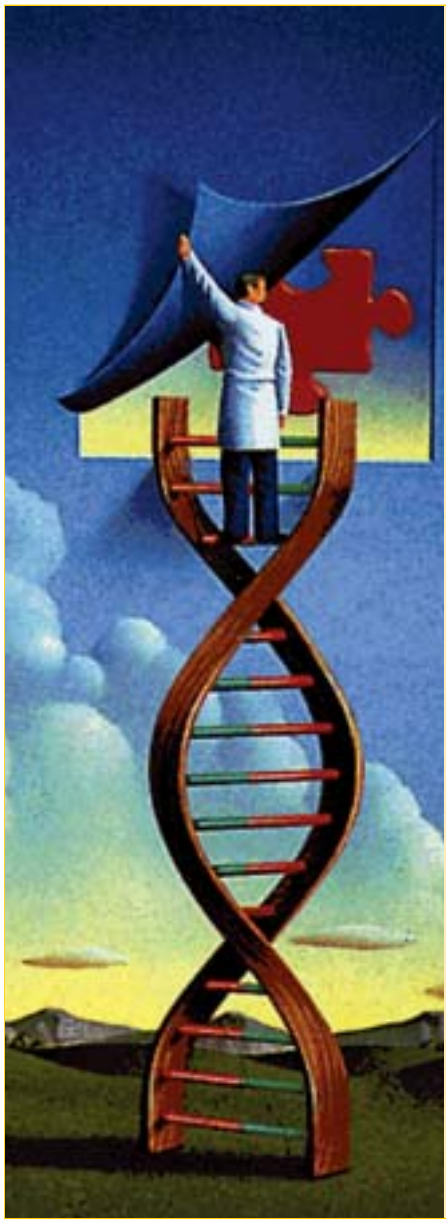

# Synthetic Biology...

... is a relatively new discipline in biology combining molecular biology, chemistry, engineering and information technology. At the beginning of this century, synthetic biology was still practically a wallflower. Today, however, this little plant has begun to flourish fervently. The idea of designing new organisms in order to produce bio-energy, drugs and new materials, as well as for applications in medicine has attracted great attention, from both the public and funding agencies.

The roots of synthetic biology lie in genetic engineering, which sprang up after the identification of restriction enzymes that allowed for the manipulation of small fractions of an organism's genome. "Work dealing with the modification of single genes using recombinant DNA was called 'Synthetic Biology 3' or 'SB3'", reports Hubert Bernauer, CEO of the gene design company ATG:biosynthetics GmbH in Merzhausen (Germany). "On the other hand, the use of naturally occurring DNAs for the generation of new combinations, with an underlying systemic idea based on functional genomics, is SB2. An example for SB2 is the genetic design of a whole signalling pathway."

While SB2 is still analytical, SB1 describes a paradigm shift that became possible with the help of synthetic gene production facilities, high throughput sequencing machines and bioinformatics. SB1 stands for writing 'new' DNA on the nucleotide level, thus enabling design and creation of completely artificial DNA sequences. And there is even an SB0, which describes the potential for true artificial biology, the design of totally new living systems based on synthetic DNA.

Though US American scientists were the first to work towards the creation of synthetic organisms and openly discuss chances and possible dangers emanating from such artificial life, Europe has not been outdistanced. The European Union (EU), for example, already began spending money on developing a network for synthetic biology during its last framework programme FP6 (2002-2006).

In particular, within the FP6 initiative 'New and emerging science and technology' (NEST) the project TESSY ('Towards a European Strategy for Synthetic Biology') was funded until the end of 2008, which, according to the TESSY website, aimed at setting up "an expert-based, investigative and participative process for the further development of Synthetic Biology in Europe". The programme was operated by people from the European Science Foundation in Strasbourg (France), from the Fraunhofer Institute for Systems and Innovation Research in Karlsruhe, ATG:biosynthetics GmbH and the University of Freiburg (all Germany).

Another EU NEST project, SYNBIOLOGY, is designed to identify key attributes of synthetic biology research in Europe and North America. Even more projects are currently supported by FP7, for example, PROBACTYS. This EU shortcut stands for 'Programmable Bacterial Catalysts', a project aimed at the construction of streamlined bacterial cells devoid of most of their own genomes and equipped with newly designed genetic circuits to transform chloroaromatics into other compounds.

Great ideas, noble goals. However, how far has synthetic biology really come to-date? Are authentic synthetic organisms already knocking at the door? Karin Hollricher talked to two experts from science and industry.

*Synthetic biology talk I: Victor de Lorenzo, Madrid*

## "Not Really New"

*LT: At present, synthetic biology is being heavily promoted. Conferences are being organised and more and more articles are being published. Are we witnessing the birth of a new discipline?*

**Victor de Lorenzo**: Already in 1912, the French biologist Stéphane Leduc published a book with the title *La Biologie Synthétique*. His ideas and theories were, of course, not identical to today's concept of synthetic biology. But the claim that synthetic biology is a completely new term is not accurate. The same holds true for the very concept, as it stems from previous work in different research subjects. That is at least my point of view.

*Indeed, the idea of genetically engineering bacteria dates from the 70s, when restriction enzymes were discovered.*

**Victor de Lorenzo**: By then, genetic engineering was a metaphor. Now it has become a reality, a *bona fide* technology. Under the umbrella of synthetic biology, we express the desire of taking genetic tools to an extreme and to apply string engineering concepts for designing new biological systems. We now have the knowledge to move the field into a new stage, to forward-design biological systems *à la carte* for certain applications. This promise is creating a tremendous hype, fostered by engineers who are becoming very interested in biology and are providing a new perspective on how to set up biological systems for a given purpose. The perception that there is a great potential in the field makes us feel we have to act now and not in 10 or 20 years. That's perhaps why one can observe such great interest in synthetic biology that materialises in meetings, international initiatives and a vibrant transatlantic debate between European and US scientists.

*What is that debate about?*

**Victor de Lorenzo**: A classical scientific concept is that what you cannot create, you do not understand. If you want to analyse

a complex problem you have to first dissect the problem into smaller parts for analysis. Then you synthesise the system. Synthetic biology is engineering but also fundamental science, and it is wrong to claim otherwise. In this respect, scientists in Europe and in the USA differ about the scope, the novelty and the territory of synthetic biology. Different communities of stakeholders claim complete ownership of the whole realm. One sector of US scientists and engineers goes on to declare that synthetic biology involves a complete refoundation of biology.

*Which sector is that?*

**Victor de Lorenzo**: The people who have been very active in this respect belong to what one may call the *BioBrick community*. The concept of BioBricks was developed by engineers from the MIT and later echoed through many other US and international universities by means of the iGEM competition (I'll talk about that competition later.) They've done great work to excite and involve young people but I am convinced, and I am not the only one, that the BioBrick approach, as it stands now, is very narrow and needs to share territory with other synthetic biology approaches.

Victor de Lorenzo works at the National Centre of Biotechnology in Madrid. His research focuses on the molecular biology and genetic engineering of microorganisms for environmental bioremediation.

*We have to explain the term BioBrick. According to the scientists' definition, BioBrick parts are standard biological parts. Each BioBrick part is a distinct nucleic acid-encoded molecular biological function that turns on or off gene expression, along with the associated descriptive information.*

**Victor de Lorenzo**: Such an assumption, interesting as it is, cannot address the whole synthetic biology agenda. I think the BioBrick standards shape a branch of synthetic biology, but not the whole because one cannot acritically translate engineering principles one by one into biology. Every biologist knows perfectly well that a biological system does not behave like a computer or a machine. Electrical switches work digitally: they are either on or off. In biology you have populations that are subject to statistical phenomena and kinetic constraints. Even though there are similarities between engineering concepts and biology, you cannot draw a straight line of equivalence between parts and pieces in biology and parts and pieces in engineering. It's wonderful to bring engineering into biology. But also it's a good idea to bring biology into engineering!

*What differences do you see?*

**Victor de Lorenzo**: First of all, every biological object is ultimately subject to Darwinian evolution. So, any engineered bio-

logical system will change and will stop working at some point if it is not compatible with selective pressure. Secondly, all biological functions are context-dependent. A switch or a transistor will generally work, regardless of all the other components of the whole system. However, in biology that's different. We know that it makes a difference whether a bacterial protein is located in the cytoplasm or in the periplasm; that a promoter close to the origin of replication works differently than at a distant location. Thirdly, we have the so-called emergence phenomenon. That means that the function of a system may be much more than the addition of the properties of the separate components. Emergence is, by definition, difficult to predict. We are facing a dramatic case of emergence right now with the new swine influenza virus that suddenly infects humans. This clearly shows that we cannot truly predict the behaviour of biological systems as you can predict the behaviour of electronic systems.

*So then, what's your definition of synthetic biology?*

**Victor de Lorenzo** I think synthetic biology is a conceptual frame that includes three aspects. The first is genetic engineering and molecular biology, mostly in connection to metabolic engineering and biotechnology. For a long time, people have been trying to take genes from different organisms and put them together for building new molecules or for degradation of toxic compounds. To give you an example: in the last few years Jay Keasling has worked out the production of anti-malaria drugs in engineered microorganisms. While this research is claimed to be done under the umbrella of synthetic biology, the work clearly stems from and follows the tradition of metabolic engineering. The second pillar is the whole body of research that has been done on the origin of life and minimal systems. The third pillar is computing, engineering and bioinformatics, now entering biology with a tremendous power. That's how I see it.

BioBricks?

*Engineering means design and implementation. As far as design is concerned, synthetic biology has evolved rapidly. Since 2003, students have created biological systems for a competition called International Genetically Engineered Machine (iGEM), which aims to establish whether biological systems are built from standard, interchangeable parts.*

**Victor de Lorenzo**: From the point of design, the iGEM-projects are absolutely beautiful, even brilliant. But, when it comes to implementing them as actual working systems, encoded by assemblies of BioBricks, most of them do not work.

*Not a single project?*

**Victor de Lorenzo**: Very few, maybe one to five percent of them, being optimistic.

*Did any of the iGEM-participators try to implement one of the project ideas?*

**Victor de Lorenzo**: Not many, as far as I know. Typically, such projects focus on the design but they are of no use to go further to verify the functioning of the systems engineered with BioBricks. I will give you an example. There is the iGEM project running in the University of Leuven called 'Dr. Coli'. Students wanted to construct an *E. coli* bacterium that produces a drug when and where it is needed in the human body. It does this in an intelligent way, such that the drug production meets the individual patient's needs. The design is excellent (note that this is a student project) but it was never realised. There are dozens of iGEM projects like this one. Generating a complex biological system is more than sticking parts together.

*"We now have the knowledge to move the field into a new stage, to forward-design biological systems à la carte for certain applications."*

*So what next?*

**Victor de Lorenzo**: It's clear that now the field is opening, we are developing new perspectives, So, sooner or later, we need an international agreement and consensus on standardisation of biological components.

*Why do you need standards?*

**Victor de Lorenzo**: Engineers are working with switches, transistors, magnets and many other components endowed with defined properties. Amperes, ohms, hertzes, teslas, etc. are their standard units. That sort of standardisation is needed in biology as well. For instance, we have to define how we measure transcription and translation, what kind of units we will work with. We have to discuss how we will do standardisation and who is going to lead that discussion. Actually, that discussion is one of the topics behind the international debate on synthetic biology, as it transcends a mere scientific discussion.

*We would also like to know more about your synthetic biology projects. What are you working on?*

**Victor de Lorenzo**: We are trying to anchor antibodies on the surface of bacteria to make communities.

*For what purpose?*

**Victor de Lorenzo**: Sometimes, you want bacterium A to be associated with bacterium B for running a catalytic reaction, in which A runs the first step of the reaction and B the second step. If they stick together, the efficiency of the reaction is much better. We developed a whole platform of genetic tools to take antibody sequences and express them in bacteria so that they produce antibodies on their surfaces. In this way, we can engineer artificial adhesins as anchor sites on the surface of bacteria to make them associate to other bacteria. In another, more advanced project we are developing bacteria that detect explosives in soil. We have engineered a transcriptional regulator that detects 2,4-dinitrotoluene, that is, DNT – one of the chemicals present in antipersonal mines. We're designing sensor circuits and implement them into the soil bacterium *Pseudomonas putida*.

*"Generating a complex biological system is more than sticking parts together."*

*You really want to detect mines with these engineered bacteria?*

**Victor de Lorenzo**: Yes. I want to make clear that this is not an academic exercise. We want to produce a working system, that can be released into the environment.

*That raises the question of biosecurity. When a biological system such as the above-mentioned influenza virus isn't predictable, how can one dare to release new organisms into the enviroment?*

**Victor de Lorenzo**: Biosafety or biosecurity are of course aspects of the synthetic biology debate that I see in a broader view. If you do not apply sound concepts to your engineered system, things will not work, regardless of the beauty of your design. So we have go a step back and ask, "Can we engineer predictable systems taking aboard what we know about biology?" Can we start with an existing system that is naturally context-dependent, evolvable and prone to develop emergent properties, and transform it into something truly predictable? That is what the challenge of synthetic biology is all about!

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*Synthetic biology talk II:*
*Peer Stähler, Febit Synbio GmbH, Heidelberg*

# "We Need Guidelines"

*LT: Synthetic biology is in its infancy. Right now, industrial synthetic biology is limited to DNA synthesis. Why did you think it was necessary to found a new association?*

**Peer Stähler**: Synthetic biology goes beyond genetic engineering in scope and scale. By making use of the genetic code, synthetic biology can reproduce gene sequences or gene products and even synthesise entirely new versions of microorganisms and viruses. These capabilities also mean additional risks accompanying the technology. So we have to show responsibility and create a framework of guidelines. To make the benefits of gene synthesis and synthetic biology in general happen, we need a positive stimulating environment. That's why we acquired several German members, the founding nucleus of the IASB.

Peer Stähler is President of Febit Synbio GmbH in Heidelberg (Germany ) and executive board member of the International Association of Synthetic Biology.

*Why only German members to begin with?*

**Stähler**: All over the world scientists are working on synthetic biology projects. However, companies doing gene synthesis, currently almost the only industrial application of synthetic biology, are located mainly in Germany and the USA. 80% of the worldwide gene production is controlled by companies in Germany and the USA, they need regulations and guidelines regarding producing and shipping genes and sequences. That's why we felt that we should become active.

*But now you are planning the internationalisation of the organisation?*

**Stähler**: That's right. We want to do that on two levels. Level one is building up relationships with international organisations or organisations in other countries. That could be authorities, academic and industrial organisations. On level two we want to invite additional academic and commercial members from other countries.

*What is the feedback?*

**Stähler**: Several organisations and companies are interested in becoming members, and we will be reporting on these new members as soon as we are ready.

*"Any gene synthesis company has to check whether an ordered gene sequence is potentially dangerous or pathogenic."*

*What is the IASB doing right now?*

**Stähler**: Any gene synthesis company has to check whether an ordered gene sequence is potentially dangerous or pathogenic. It is not clearly defined, which kind of sequences I am allowed to synthesise, which molecules I am allowed to ship to another country, whether the shipping of the pure sequence is allowed. So companies, as well as customers, need guidelines. Therefore, our first challenge is the development of a code of conduct and of best practice guidelines guarding us in our activities, guarding us in developing products and in interacting with customers.

*Who defines the hazardousness of a gene sequence or its product, apart from obviously dangerous substances and organisms?*

**Stähler**: There are neither general, international definitions for synthetic biology nor international valid guidelines. In Germany, we have guidelines for shipping organisms or DNA outside the country and the European Community. And we have the *Gentechnikgesetz*, a gene technology law that forbids the production and selling of toxins. Also, the EU and the USA, as well as a so-called Australia Group, have lists of organisms and agents they don't want you to handle. However, these lists overlap only partially. So how do I find out which sequence, which promoter I am allowed to ship to which country? Regulations about production and shipping of toxin genes or promoters of toxin genes are very diffuse. As a company it's frustrating to tell a potential customer, "Sorry, I don't know whether I am allowed to produce your order." So I cannot take it. And when the customer asks me where he can get approval, sometimes I might have to answer that I don't know.

*And who tells you whether a gene ordered by a customer produces a toxic product?*

**Stähler**: That's the key point. The majority of companies screen incoming orders against some gene databases of pathogenic organisms. But there's no unified, updated catalogue of potentially dangerous genes and gene sequences. Additionally, searching through a couple of databases is not economical. Hence, the IASB started creating a reference database in collaboration with the University of Berkeley that is already pretty active in that field.

As one of their additional activities they are working on a portal for experiments of concern, so that a company and any customer may easily qualify a project and identify whether an experiment planned is an experiment of concern.

*You will also need software for screening the database.*

**Stähler**: Right. Existing programmes like BLAST are not the ideal tools for this purpose. Robert Jones of Craig Computing has developed a tool called Blackwatch. That is faster, more efficient and more focused than BLAST in checking incoming orders against a list of selected sequences for potentially dangerous proteins. Using this programme and the new database, any company can systematically check its orders locally, so there will be no problems regarding confidentiality.

*How often do gene synthesis companies face problems with an order?*

**Stähler**: I know that from time to time companies have had to put orders on hold until they've checked the sequences. Sometimes they have stopped it. The customers can be highly regarded, honorable institutes. The Robert-Koch-Institute in Berlin, just as an example, asked for a couple of sequences for viral proteins because they're working on a vaccination programme. The assigned company didn't take the order because it could not judge on the final implications of synthesising the gene. That shows how urgently we need guidance.

*Do you think that any sequence available should be published, even those of very dangerous organisms or very toxic products?*

**Stähler**: IASB has no official opinion on that issue yet.

*Have policy makers and governments already noticed that there is a problem in legislation?*

**Stähler**: Yes, they have. There's a pretty active dialogue between the academic community, comprising of science, policy and ethics experts, and company experts and governmental authorities. In February 2009, the IASB was invited to an intergovernmental conference where representatives of the German and the US governments discussed that issue. There were people from both state departments, from the Robert-Koch-Institute, the FBI and the German intelligence service, BKA. We presented an industry-oriented perspective on how to deal with synthetic biology concerning regulation, security aspects and so on. Right now there are many groups actively discussing the current state of research, expected future developments and their potential impact.

*In 1975, the Asilomar meeting laid out the ground rules for future genetic scientific research. Do you think a second Asilomar-like conference for synthetic biology would be useful?*

**Stähler**: Yes. We have to talk about possibilities, developments and potential hazardousness of synthetic biology now to avoid a flop like green biotechnology is currently experiencing in Europe. And we have to emphasise the multitude of positive aspects of the technology, as well as how and why its application is beneficial.