Constructing Life

Early social reflections on the emerging field of synthetic biology

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Preface

As the parliamentary technology assessment organization in the Netherlands, the Rathenau Institute is always on the lookout for new scientific developments and technological trends that might have an important impact on society. In recent years, the institute has developed a special interest in topics related to nanotechnology and / or converging technologies.

Synthetic biology is a new emerging scientific field where ICT, biotechnology and nanotechnology meet and strengthen each other. The increasing number of people attracted to this field, the growing number of publications in which scientists publish new results and are seeking publicity to advertise their views and ideas, as well as the appearance of review articles in science journals, all indicate that 'something is happening'. In contrast to the 'classical approach' in molecular biology, synthetic biology appears to be an approach that enables the design of new biological systems. In a similar vein to genetic engineering or nanotechnology, synthetic biology raises important social and ethical questions about the possible impact on human health and the environment or possible abuses for biological warfare or terrorist attacks. The introduction of new biological systems can even force us to redefine 'life'.

The Rathenau Institute believed that the development of synthetic biology needed to be examined for its scientific and technological significance; and its potential impact on society. When research started in the spring of 2006, some publications that discussed the role of risk assessment were already available. However, a broader picture of the dynamics in this field, and an investigation of the social and political agenda were still missing. The study *Constructing life: early social reflections on the emerging field of synthetic biology*, written by Huib de Vriend, is an attempt to fill that gap. It should be seen as a starting point for further international research and debate, for example on the role of the government. The study was written in English as a contribution to a coordinated international approach, including the development of a balanced and effective EU policy.

Recently synthetic biology has been gaining prominence on the Dutch political agenda. In August 2006, the Ministry of Education, Culture and Science asked the Health Council of the Netherlands, the Advisory Council on Health Research and the Royal Netherlands Academy of Arts and Sciences to further investigate the technological developments of synthetic biology. A draft version of this report is being used

within the current debate. The Rathenau Institute will contribute to this debate by publishing a popular version of this study in Dutch.

Mr.drs. Jan Staman
Director of the Rathenau Institute

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1 Introduction

"This is the step we have all been talking about.
We are moving from reading the genetic code to writing it."
Craig Venter, founder of Synthetic Genomics Inc.,
Wall Street Journal, June 29, 2005.

The Rathenau Institute is the national technology assessment organization in the Netherlands and encourages social debate and the development of political opinion on technological and scientific development. It is therefore interested in the identification of new technological trends with a potential impact on society. A current field of interest is the convergence of nanotechnology, biotechnology, information technology and cognitive sciences ('converging technologies', or NBIC). This is increasing our understanding of the fundamental building blocks and mechanisms underlying the characteristics and properties of artifacts and living organisms (Nordmann, 2004). The increasing number of publications on synthetic biology in (popular) scientific journals in 2005 and the first months of 2006 was an indication of a certain level of 'maturation': the publication of new scientific results, scientists seeking publicity to advertise their views and ideas, and the appearance of review articles in science journals indicate that 'something is happening'.

At the same time, research into views on synthetic biology is progressing in Europe. Under the 6th Framework Program, the European Commission has financed a small number of research projects on synthetic biology (European Commission, 2005a; European Commission, 2005b: New and Emerging Sciences and Technologies (NEST)). The Swiss Center for Technology Assessment and the Austrian Organization for International Dialogue and Conflict Management (IDC, 2006) have shown an interest in the societal impact of synthetic biology.

Synthetic biology is a new trend in science and technology and a clear example of converging technologies. It can potentially trigger social and political debate. Therefore despite its 'immature stage', the Rathenau Institute has identified synthetic biology as a development that needs to be examined for its scientific and technological significance and its potential impact on society. So far, the vast majority of articles and papers on synthetic biology have focused on how the research should be organized and how risk assessment should be dealt with (such as COGEM, 2006). An analysis from a Technology Assessment perspective, drawing a broader picture of the dynamics in this field, and an investigation of the social and political agenda was

still missing. The Rathenau Institute therefore commissioned LIS Consult, an independent consultancy on social issues in innovations in the life sciences, to write an exploratory paper on synthetic biology. This paper was written in English so as to contribute to a coordinated international approach, including the development of balanced and effective EU policy and the 3rd international conference on synthetic biology to be held in Zurich in 2007.

In May 2006, the 2nd International Synthetic Biology Conference was held in Berkeley, California. This was an excellent opportunity to gain an impression of the latest developments in synthetic biology research, scientist's views on future expectations and relevant social and political issues, as well as the structure and dynamics of the institutions and people involved in this area of research. During the first two days, a wide range of research results and small business initiatives were presented. All of the presentations concerned *the design and construction of new biological parts, devices and systems, and the redesign of existing natural biological systems for useful purposes.* Together the presentations gave an impression of the latest scientific and technological developments in a field that takes the engineering approach to biological systems much further than 'conventional' genetic modification. It was absolutely fascinating to see how the biomolecular basis of growth of sponge skeleton is mimicked in the laboratory, or how cells that contain a predesigned and engineered artificial cell-to-cell signal system move in patterns. The design of two-dimensional structures with DNA molecules (DNA origami) and the idea of bacterial cells that perform basic functions based on a minimal set of genes ('the cell as a chassis') were explained. Some promising results with potential commercial applications were highlighted, such as the production of the anti-malaria drug artemisinin, and options for biobased energy production. Yet the majority of the projects focus on fairly basic elements and mechanisms, which demonstrates the 'immature' stage this relatively new area of biological engineering is still in. So far genetic modification has, to a certain extent, operated in the context of, and was limited by, what 'nature' has to offer in terms of genetic material, cells and organisms. By contrast, synthetic biology applies engineering principles to biology, which allows for the *intended design* of fully new biomolecular systems. Or, as Craig Venter stated in the Wall Street Journal, it is the step from reading the genetic code to writing it. Synthetic biologists prefer to draw the parallel with the design of electronic circuits. In Berkeley scientists were talking about the bacterial cell as a 'chassis', that can be stripped of unnecessary elements and used as a piece of electronic circuitry board to plug-in biological devices that can perform specific, well-defined functions. Based on a detailed understanding of the complex genetic, biochemical and biophysical mechanisms in cells, efforts are made to develop DNA-based hardware, which they call 'switches', 'toggles' and 'inverters'.

Although it seems too early to make specific claims – and the distinction between synthetic biology, genetic modification and nanobiotechnology is not always very clear – synthetic biology appears to be an approach that enables the design of new biological systems and can be used for a wide range of purposes. For the time being, it is still too early to predict what synthetic biology will deliver to society, for better or worse. However the scientists involved sincerely believe they can make new things, things that work, things that will enable us to produce better and cheaper medicine or energy. Synthetic biology represents a new way of looking at biology, or even a new paradigm that seems to contrast with the rapidly developing more holistic approach to biology, even though both approaches stem from recent developments in systems biology. Nevertheless, it is the intention of the engineers to make things that work, to make life as it could be, no matter how complex life is.

At the same time, however, synthetic biology raises questions about the possible impact on human health and the environment, and about possible abuses for biological warfare or terrorist attacks. Questions that do not differ fundamentally from the questions raised by genetic engineering or nanotechnology, and which most members of the synthetic biology community are well aware of. Fearing regulations that might limit the further development of synthetic biology, they propose a system of self-regulation. Yet several NGOs that focus on the social, economic, cultural and ecological consequences of new technologies consider this to be unacceptable.

Additionally, synthetic biology forces us to redefine 'life'. Is life in fact a cascade of biochemical events, regulated by the heritable code that is in (and around) the DNA and enabled by biological machinery? Is the cell a bag of biological components that can be redesigned in a rational sense? Or is life a holistic entity that has metaphysical dimensions, rendering it more than a piece of rational machinery?

Content

This study considers whether developments in synthetic biology require specific attention from a Technology Assessment perspective, and which scientific, social and political aspects should be further examined and discussed. This key question is further examined in a number of subsidiary questions or issues that are dealt with in the following four chapters.

To start with, a clear definition of synthetic biology was needed: how does it differ from other existing technologies and what are its unique characteristics? This was done by exploring definitions and examples mentioned in the literature and by attending the Berkeley Conference in May 2006. The outcomes of this are presented in Chapter 2. Special attention was paid to the position of synthetic biology within the continuum of developments in biology and engineering (such as genetic engineering, nano(bio)technology and information and communication technologies) as well as its relationship with several disciplines in science and technology. Finally, the 'newness' of the approach of synthetic biology is explored, including the question as to whether this approach will lead to fundamental changes in how biology and life are viewed, that is a paradigm shift.

The description of applications and products presented in Chapter 3 provides a window on current developments in synthetic biology. An analysis of future expectations, including an estimate of their probability, gives an impression of what the assumed capabilities of the technology are.

Like any other technology, synthetic biology develops in a social context, where decisions are taken about research programs, funding, legislation and acceptance by end users. Chapter 4 therefore presents a brief analysis of the driving forces, the structure and dynamics of the synthetic biology community – scientists, institutions, companies, authorities, and NGOs.

Chapter 5 lists the social, ethical, and legal issues related to synthetic biology that may trigger social and political debate and which the synthetic biology community will have to deal with.

Finally Chapter 6 states the main conclusions, and emphasizes the urgent need for societal and political reflection on specific issues. Thirty years of experience with technological, social and legal developments in genetic engineering is used to formulate suggestions for the next steps, which could be translated into future European activities.

2 The characteristics of synthetic biology

Like most other technologies, synthetic biology is not an isolated scientific and engineering discipline. It is rooted in experience and knowledge of molecular biology: understanding the interactions between the various systems of a cell, including the interrelationship of DNA, RNA and protein synthesis and the regulation of these interactions. Synthetic biology could not exist without the DNA sequence of an increasing number of organisms being available, the possibility to identify genes and their functions, and an understanding of the molecular mechanisms of cell behavior (genomics and systems biology). Synthetic biology codevelops with information technologies, robotics, and nanotechnology. Moreover, new technologies in this field will be applied in combination with other technologies, in the context of the convergence of nanotechnology, biotechnology, information technologies, and cognitive science. That makes it difficult to draw clear lines between synthetic biology and 'classical' genetic engineering, or nanotechnology. Accordingly this chapter not only deals with current definitions, but also uses characterizations from the scientific community and several institutions. The characterization presented will highlight two approaches in synthetic biology: deconstruction and construction of life. The question how new is synthetic biology will be explored by relating the development to similar fields of science and technology: genetic modification, nanotechnology and systems biology. As in synthetic biology newness is more or less equivalent to 'artificialness', the development will be ranked in terms of 'levels of artificialness'. Finally, whether the approach in synthetic biology implicates a paradigm shift – whether it leads to fundamental changes in views on biology and life, and might therefore have an impact on the scientific approach of living entities – will be explored.

2.1 Definitions

Today numerous researchers and engineers want to design and build biological systems, and call this work 'synthetic biology' (Brent, 2004). They distinguish their work from an older biological engineering canon, which encompasses fermentation and process engineering as well as biomedical engineering (prosthetic limbs, laser catheters guided through arteries and implants). They also distinguish it from a second, newer recombinant DNA canon, which encompasses engi-

neered organisms that produce proteins or simple chemicals, plants engineered to produce pesticides and phage vectors designed with attributes relevant to gene therapy. Instead, the synthetic biologists have defined their goal as the design and construction of systems that exhibit complex dynamical behavior, the ability to exist in a number of states or the ability to execute small numbers of programmed steps (for example, in complex chemical synthesis).
Several definitions of synthetic biology can be found in the literature, of which the following three definitions draw a more or less complete picture:

The Berkeley definition

In 2003 the Physical Biosciences Division at Lawrence Berkeley National Laboratory (LBNL or the Berkeley Lab) established a Synthetic Biology Department with the claim that this was the world's first research facility in synthetic biology (LBNL, 2006). Researchers from the Synthetic Biology Department and individuals from research laboratories at other institutions in the USA, among which the Massachusetts Institute of Technology (MIT) and Harvard University, have also set up a synthetic biology community (http://syntheticbiology.org). This community defines 'synthetic biology' as:

1. *the design and construction of biological parts, devices and systems, and;*
2. *the redesign of existing, natural biological systems for useful purposes.*

Examples used by the LBNL are:

- Production of the anti-malarial drug precursor artemisinic acid by the bacterium *E. coli* with genes from three separate organisms (Dae Kyun, 2006);
- Production of hydrogen by the bacterium *Bacillus subtilis* with genes for cellulose-converting enzymes;
- Genetically engineered microorganisms to remediate environmental contaminants, like heavy metals, actinides and nerve agents.

A Synthetic Society Working Group (SSWG) has been set up within this community to address societal issues, with a primary interest in considering the impact of new, engineered biological systems that are encoded via standard, that is naturally originated, four-base DNA, namely A(denosine), T(hymine), C(ytosine) and G(uanine). This definition leaves out certain aspects of synthetic biology research, for example understanding the origins of life by recreating a cell from raw chemicals in the lab. There are two pragmatic reasons for this. First of all, the SSWG believes that the direct engineering of living systems via standard DNA will have the widest societal impact over the near term. Secondly SSWG feels it is probably already trying to achieve too much.

The COGEM definition

According to the Committee on Genetic Modification (COGEM), the biosafety expert body to the Dutch Ministry of the Environment, the term synthetic biology was already used in 1980 for bacteria genetically altered through recombinant DNA technology (COGEM, 2006). But now synthetic biology constitutes a specialized field within genetic modification. In the view of COGEM,

> *synthetic biology focuses on the design and synthesis of artificial genes and complete biological systems, and on changing existing organisms, aimed at acquiring useful functions.*

Apart from the production of the anti-malarial drug precursor artemisinic acid by the bacterium *E. coli* with genes from three separate organisms, COGEM uses the following examples:

- Bacterial photography system based on *E. coli* with an inserted genetic network (Levskaya, 2005) and;
- Minimal genome bacteria or minimal cells for degradation of toxic compounds or production of energy.

Interestingly, in contrast to the SSWG, COGEM does not exclude attempts to create and use an alternative genetic alphabet based on unnatural DNA bases from its definition of synthetic biology.

An alternative genetic alphabet

Since 1990 attempts have been made to develop an alternative genetic alphabet to enable the production of peptides and proteins that do not occur naturally. Such peptides and proteins could lead to novel therapeutics.

One way of creating an alternative genetic alphabet is to extend the number of DNA bases by creating unnatural DNA bases, either through changing the sequence and combination of linkages of hydrogen bonds on the DNA bases, or through bases which form base pairs via hydrophobic interactions instead of hydrogen bonds (Szatmary, 2003; Wu, 2000). An alternative is to modify transfer (t)RNA to function as a carrier of an unnatural amino acid, enabling translation of an alternative genetic alphabet into an unnatural peptide (Bain, 1992). Moreover in 2004, researchers created a mutant polymerase for the replication of an unnatural synthetic DNA sequence with a codon of six instead of four bases (Sismour, 2004; Sismour, 2005). Now it is even possible to use unnatural amino acids for peptide synthesis without changing the number of base pairs. A modified tRNA is used that is able to read codons of four instead of three bases (Anderson, 2004).

The EU definition

A review published under the auspices of the European Commission Directorate-General Research in October 2005 noted that there are many different practical approaches to synthetic biology that emanate from different disciplines, all of which can claim to represent synthetic biology research and engineering (Synbiology, 2005). It was argued that any definition should reflect how synthetic biology differs from classical genetic engineering, while it should also be distinguished from the creation of total artificial proto-living particles (Rasmussen, 2004; Deamer, 2005). Eventually, the report puts more emphasis on the engineering component and suggested that:

> *synthetic biology is the engineering of biological components and systems that do not exist in nature and the re-engineering of existing biological elements; it is determined on the intentional design of artificial biological systems, rather than on the understanding of natural biology.*

Projects granted by DG Research illustrate how the European Commission defines synthetic biology (European Commission, 2005b):

- A cell-based system to produce dihydroxyacetone phosphate (DHAP)-derived monosaccharides for pharmaceutical applications based on the optimization of carbohydrate metabolism in *E. coli* (EuroBioSyn);
- Rearrangement of genes that code for antibody specificity, aimed at the creation of a 'library' of around one million hybridoma cells, each expressing a different antibody (HybLib);
- Synthetic cell nuclei analogues, capable of self-assembly in mixtures of DNA, macromolecules (or nanoparticles), and lipids for producing complex biomolecules (Neonuclei);
- Rationally designed gene networks ('artificial circuits') with the ability to sense particular conditions or signals within a cell, and to respond accordingly, in this case to detect and possibly correct aberrant cellular functions in cancer cells, which could be applied in gene therapy and diagnostics (Netsensor).

2.2 Top-down and bottom-up approaches

Synthetic biology can be viewed as the meeting point of two cultures in molecular biology (de Lorenzo, 2006): 'deconstructing life' and 'constructing life'.

Deconstructing life (top-down)

The first culture, represented by those in 'deconstructing life', dis-

sects biological systems in the search for simplified and minimal forms that will help to understand the adaptation and evolution of natural processes. By analogy, this culture could be compared with the top-down approach in nanotechnology. In top-down nanotechnology, the starting point is a larger block of material from which the desired nanostructure is carved out using physical methods. Top-down nanotechnology is a natural extension of current methods of microelectronics, in which structures of very limited dimensions are created by depositing thin layers of material and etching away the unwanted parts of each layer (Azonano, 2006).
Similarly, 'deconstructing life' is an extension of current methods in genetic modification. The 'deconstructing life' approach includes experiments to obtain information on isolated parts of biological systems, the simulation of these systems and then the prediction of associated properties followed by further experimental verification. Examples include work on metabolic pathways, like glycolysis, and the simulation of cell systems using stochastic approaches. Further, simplified systems, based on phospholipids or polymers, are used to explore possible prebiotic systems. Yet research into minimal life forms and minimal genomes (Csaba, 2006; Galperin, 2006), and the development of computer viruses to study properties of biological evolution can also be included in this approach. In short, the 'deconstructing life' approach focuses on the definition of material or virtual systems that contribute to understanding the properties of complex biological problems.

The minimal genome

While most microbes have hundreds or thousands of genes, some use only a fraction of these at any one time, depending on their surroundings. A minimal genome contains the smallest set of genes an organism needs to live in a particular environment. It can be compared to the design of software that performs a specific task in the least possible steps.
The first attempt to create a minimal genome organism focused on Mycoplasma genitalium, with 517 genes the smallest gene complement of any independently replicating cell so far identified. Global transposon mutagenesis was used to identify non-essential genes in an effort to learn whether the naturally-occurring gene complement is a true minimal genome under laboratory growth conditions. The analysis of the research results published in 1999, carried out by TIGR and Craig Venter from Celera Genomics, suggests that 265 to 350 of the 480 protein-coding genes of M. genitalium are essential under laboratory growth conditions. This includes about 100 genes of unknown function (Hutchinson, 1999). The J. Craig Venter Institute is still continuing its search for a minimal genome Mycoplasma genitalium (Glass, 2006).

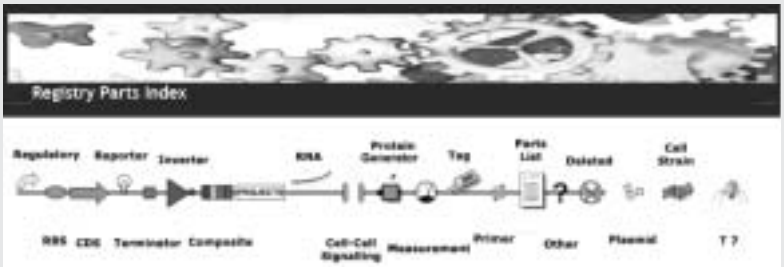
The bacterium Bacillus subtilis has about 4100 genes but needs just 271 genes to live in an experimental environment (Kobayashi, 2003).
Recently, European researchers published the results of an approach involving genome modeling which, given the organism's evolutionary history and knowledge of its surrounding environment, allows them to predict which genes a bacterium's genome should contain (Csaba, 2006). The results suggest systematic underestimation of the number of genes needed to create a minimal genome organism. Previous attempts to work out the minimal genome have relied on deleting individual genes in order to infer which genes are essential for maintaining life. The knock-out approach misses the fact that there are alternative genetic routes, or pathways, to the production of the same cellular product. If one gene is knocked out, the genome can compensate by using an alternative gene. Yet if the knock-out experiment is repeated by deleting the alternative, the genome can revert to the original gene instead. From the knock-out approach it could be inferred that both genes are expendable from the genome because there appears to be no deleterious effect in either experiment. Yet because there are alternative pathways to the same product, removing either of the genes makes the other essential for survival; each gene deletion reduces the available space for further reduction of the genome. Including these alternative pathways in the minimal genome almost doubles its size.

Constructing life (bottom-up)

The second culture is the 'constructing life' approach that aims at building systems inspired by general biological principles using biological or chemical components to reproduce the behavior of living systems. In this approach biological phenomena are often addressed with concepts from electrical engineering. The underlying notion is to combine autonomous, modular, robust and reusable input and output components in much the same way as electronic circuits. While input components sense a given environment – like interfacing with biological signals, internal components, processing of biological input information inside synthetic systems to minimize side effects – output components transmit the signal(s) processed by the synthetic setup back to the biological systems. Engineers believe it will be possible to design biological components and complex biological systems in a similar fashion to the design of chips, transistors and electronic circuits. Each part will need a standard interface and well-defined function, so that someone at a workstation can assemble working systems at the abstract level described in the catalogue and pass on the design to another group of biochemists for synthesis in a biological manufacturing system.

Biobricks

In an interview with EE Times, Tom Knight of MIT's Computer Science and Artificial Intelligence Laboratory explains how engineered segments of DNA that code for metabolic functions can transform themselves into functional components of bacteria.
The DNA codes are in a catalogue of so-called 'BioBricks' (Brown, 2004). BioBricks is a registry of Standard Biological Parts, which includes lists of formatted components, such as protein coding sequences, ribosome binding sites and cell strains. As they comply with international standards, these components can easily be distributed and shared (http://parts.mit.edu/registry/index.php/Main_Page). The subsequent step involves the combination of these components into well-specified working devices, which can then be applied in biological systems. Associated research seeks to develop containers for these devices, ranging from simple lipid vesicles to minimal genomes.

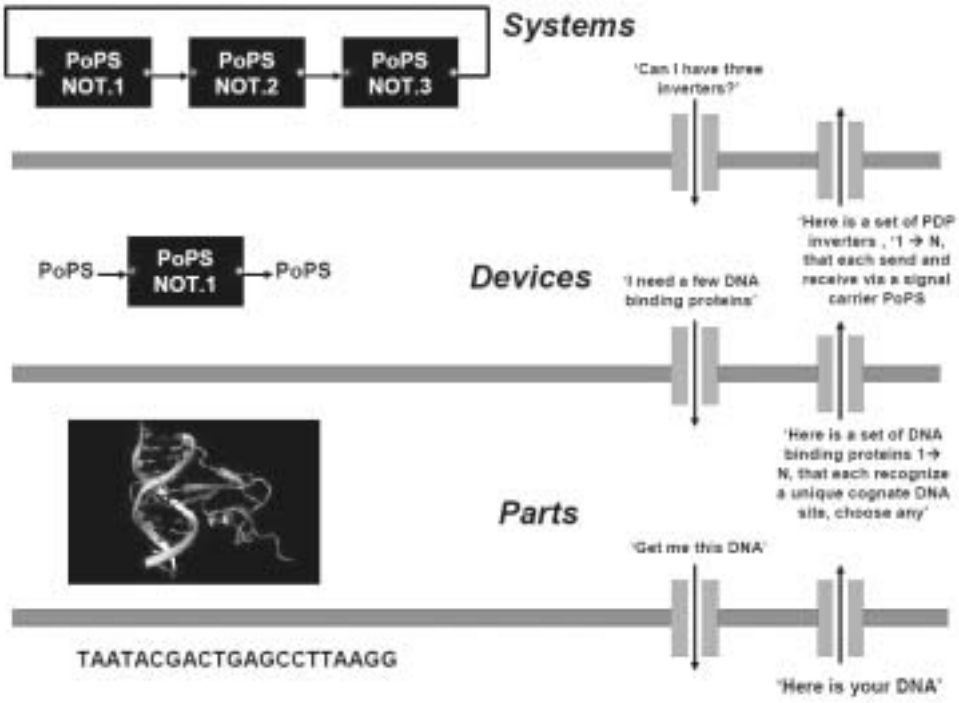


Basic Part Types		Composite Part Types	
Type	Description	Type	Description
R Regulatory	Operator region	E Reporter	Compound reporter devices
B RBS	Ribosome binding site	Q Inverter	Inverter and logic
C CDS	Protein coding sequence	Composite	Other composite parts
B Terminator	Transcriptional terminator	I Project	Student projects
RNA	RNA binding sites and coding	G Generator	TIPS-to-Protein converter
F Signalling	Cell-cell signalling	Measurement	Performance measurement constructs
E Reporter	Basic reporter CDS	T Temporary	Temporary and trial parts
M Tag	Tag or Modifier	S Intermediate	Generated during assembly
V Plasmid	Plasmids	Other	Parts not yet classified
V Cells	Cell strains		

The Registry of Standard Biological Parts (BioBricks)

By analogy, this culture could be compared with bottom-up approaches in nanotechnology, approaches that start with small components – nearly always individual molecules – which are assembled to make the desired structure (Azonano, 2006). In this approach, the term "self assembly" is often used. The self-assembling properties of biological systems, such as DNA molecules, can be used to control the organization of species such as carbon nanotubes, which may ultimately lead to the ability to 'grow' parts of an integrated circuit, rather than having to rely upon expensive top-down techniques.

In the summer of 2005, over 150 students and lecturers from 13 universities across the world made, shared, and used BioBricks as part of the International Genetically-Engineered Machine (iGEM) competition. Projects ranged from the design and *in vivo* implementation of a gene circuit that can count to 2, to engineering a biological wire capable of propagating a chemical signal down its length (iGEM, 2005).



Building systems with parts and devices (Endy, 2006)

There is a difference between the two cultures. The 'deconstruction' community seeks to understand biological systems and their evolution, whereas the 'construction' community searches for general design principles regardless of their relationship to actual biological systems. However both hope that the exploration and construction of these biological systems will expand scientific understanding of the organizational principles behind living molecular systems. Further the two approaches are linked by their dependence on very similar theoretical, experimental and computational techniques. Finally, both approaches address the key issue of how to synthesize genetic networks with well-defined functions under clear controllable replication, transcription and translation conditions, which requires skills in analyzing, predicting and designing genome elements.

2.3 Relationship with other scientific disciplines and technologies

Synthetic biology involves a wide range of scientific disciplines and technologies, systems biology and computational simulation and analysis being the major ones.

Synthetic biology uses the same type of knowledge as most of the ongoing developments in biology and engineering, such as genetic engineering and nanobiotechnology. In applications it will often be combined knowledge from other technologies, for instance information and communication technologies. Therefore the boundaries with other scientific disciplines and technologies are transient. Attempts to draw a clear line as to where synthetic biology starts and other technologies end could easily lead to endless discussions, and it seems more fruitful to consider synthetic biology in the context of several scientific and technological disciplines.

Scientific disciplines and technologies involved

While naturally-occurring genetic networks have been fine-tuned through evolution, synthetic genetic networks can be optimized using computational simulation (Bell, 2006) and computer software, like BioSPICE (http://biospice.lbl.gov; McDaniels, 2005) and BioJADE (Goler, 2004). Another key issue is the modulation of functional specificity. For both newly-designed components and those extracted from biological systems, a clear understanding is needed of how they adapt to specific working conditions and how the interactions that determine the properties of stability and adaptation of molecular systems could be engineered. Examples include the design of transcription factors, which are able to trigger the activation of specific genes, whether these are designed rationally (Bayer, 2005; Isaacs, 2004) or obtained by directed evolution (Yokobayashi, 2002). Computational analysis of protein families and of protein-DNA structures (genomics, proteomics) is clearly essential for the development of these components.

Synthetic biology also involves other scientific disciplines like nanotechnology, organic chemistry, immunology, biokinesis, and tissue engineering.

Synthetic biology, genetic modification and nanobiotechnology: fluid boundaries

The transient nature of the boundary between 'classical' genetic engineering, nanobiotechnology and synthetic biology is not apparent from the definitions provided in Chapter 2.1. Some of the examples mentioned in the preceding section contain clear elements, or could even be classified as (sophisticated) applications of genetic modification and nanobiotechnology.

Although genetic modification is sometimes presented as a technology that does not differ essentially from what has happened during many thousands of years in natural evolution, as a way to overcome hybridization barriers, the technology is usually considered a new, and artificial step in breeding organisms with new traits. In the past thirty years since the first genetically modified microorganism was created, recombinant DNA technologies have developed from rather basic trial-and-error methods to introduce naturally-occurring genes into organisms to sophisticated, and well-targeted methods to introduce desired new traits. Technologies such as protein engineering and DNA shuffling have been developed that enable scientists to alter the structure and functionality of proteins (enzymes) expressed in modified microorganisms and plants (Vlaggraduateschool, 2006; Castle, 2004). These technologies involve the creation of mutants – both well-specified and at random – starting from naturally existing organisms.

Moreover, the role of systems biology in genetic modification is increasing. In contrast to much of molecular biology, systems biology does not seek to break down a system into all of its constituent parts and study each process in turn. Instead it seeks to integrate different levels of information to understand how biological systems function, by studying the relationships and interactions between various parts of a biological system (e.g., gene and protein networks involved in cell signaling, metabolic pathways, organelles, cells, physiological systems, organisms, etc.). Increased understanding of the role and functions of DNA in a complex environment (cell, organism) will contribute to more predictable effects of genetic modification.

In this context it is worthwhile recalling the definition of biotechnology in the Convention on Biological Diversity of 1992: *any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products and processes for specific use* (CBD, 1992). This definition was adopted to ensure that virtually all applications of biotechnology that might impact biological diversity would be covered by the Convention. While it can be argued that synthetic biology falls within the Convention's definition of biotechnology, the US synthetic biology community, European policy

makers and COGEM believe there is a need to distinguish it from biotechnology. There is however no consensus as to whether the creation and use of unnatural DNA bases and total artificial proto-living particles should be viewed as applications of synthetic biology.

DNA molecules have a diameter of 2 nanometers. Thus, in terms of scale, nanobiotechnology includes any type of engineering at the DNA level, and synthetic biology could be considered a specific discipline of nanobiotechnology. Nanobiotechnology may be applied in the design of nanosensors or nanobots that perform specific functions in biological systems (such as the human body). It is generally believed that synthetic biology can provide the tools and understanding needed to develop nanobiotechnology in a more systematic manner (Ball, 2005). For MIT's Tom Knight, engineered organisms represent the quickest route for a true nanotechnology that could manufacture materials and systems on a molecular scale. His dictum: "Biology is the nanotechnology that works." (Brown, 2004).

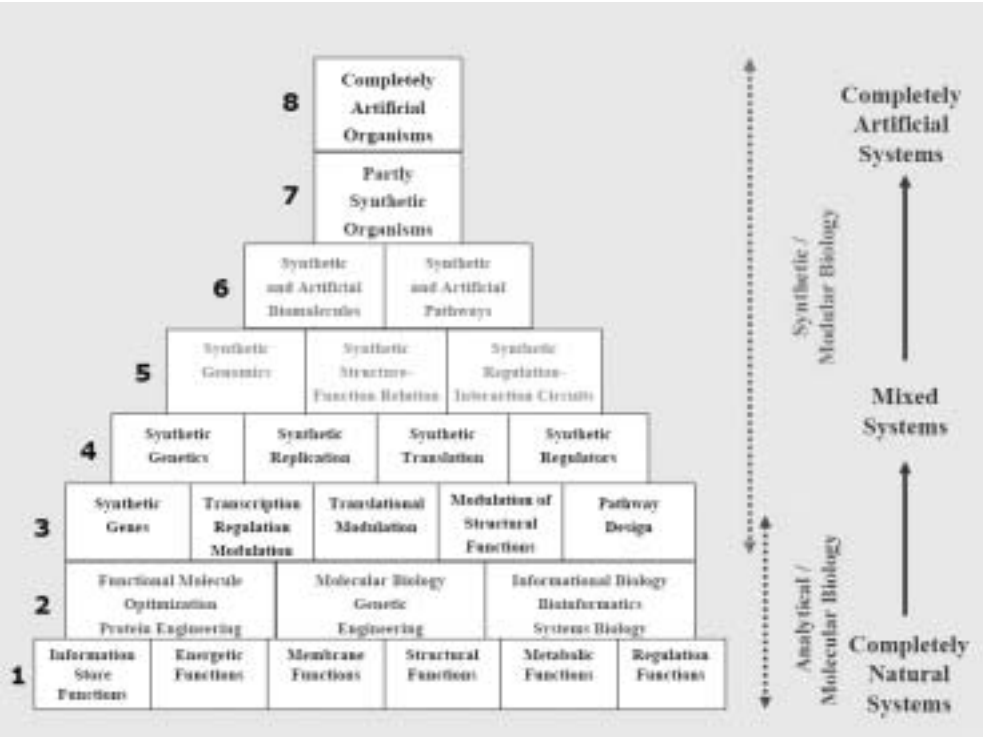
From natural to artificial systems

It seems helpful to create some structure in the chaos of scientific disciplines, technological tools and applications by arranging them in levels of 'artificialness', as has been done in a literature and statistical review by the European Commission (Synbiology, 2005). The results are presented in the diagram below, which has eight levels of artificialness, ranging from completely natural systems to completely artificial systems:

1. Systems biology is crucial to synthetic biology. It includes knowledge about the natural basic biological functions of RNA and DNA sequences in information storage, energy supply, membrane functions, cell structure, cell-to-cell signaling, gene regulation (gene expression), and metabolic functions in natural systems (the lowest level in the diagram).
   These functions can be influenced by modulating the molecules using different technical methods, leading to different levels of synthetic biology towards artificial carbon-based life.
2. Based on this knowledge, the function of molecules (proteins, enzymes) can be optimized, specific genes or (micro)RNAs can be added or suppressed (= genetic engineering) and molecular biology and bioinformatics developed. As these disciplines and technologies are based on natural systems, they are still on a relatively low level of artificialness in the diagram below.
3. At the next level, design principles are introduced, but still at a rather basic level, aimed at individual components and pathways. This includes the design of synthetic genes and metabolic pathways, the regulation of gene expression (transcription), and modulation of translation and regulation.

4. Synthetic approaches for regulation, translation and replication of synthetic genes are required to integrate these components and pathways in synthetic genetic systems, which are put at the fourth level. Information for self-replication in living systems is intrinsic – it is the central part.
5. The integration of genes, pathways, and mechanisms for regulation, translation and replication in complex biogenetic systems results in synthetic genomics, as well as synthetic cell structures, such as the endoskeleton that shapes the cell, and cell regulation systems that are responsible for interactions in and between cells.
6. The preceding steps should result in synthetic or artificial (not occurring in nature) biomolecules and pathways.

7/8. The highest level of integration is the organism, which can be partly natural, partly synthetic, or completely synthetic.



Typology of biological research and technologies as natural or artificial systems: From Molecular Biology to Modular Biology

Molecular biology approaches and genetic modification, which are still based on natural systems and synthesis of natural systems, are at levels 1 and 2.

Levels 3 to 5 represent different steps in the rational and evolutionary design of genes and their expression in the modulation of genes,

genomes, pathways, regulation systems and biomolecules. At these levels, combinations of synthetic and natural elements can be found. The top of the diagram represents the synthesis of artificial and partly artificial systems, such as artificial genes with an artificial function in combination with artificial regulation.

2.4 Synthetic Biology: A paradigm shift?

As the diagram in Chapter 2.3 shows, synthetic biology leads to a shift in focus from the understanding of naturally-occurring biological functions to using a minimalist approach to define and design them. In other words, a shift from analytic to synthetic, from 'molecular biology' to 'modular biology' (Synbiology, 2005). This implies fundamental changes in the type of questions that are supposed to be asked in biology, how these are structured and the answers probed for.

Views of synthetic biologists

This shift in focus is best illustrated by the views of scientists who consider themselves synthetic biologists, who are truly convinced they are involved in a totally new approach to biological systems. "Biology will never be the same", states Tom Knight in a commentary in Molecular Systems Biology, September 2005 (Knight, 2005). Just like electrical engineering grew from physics to become a separate discipline in the early part of the twentieth century, we are now witnessing the growth of a new engineering discipline: one oriented to the intentional design, modeling, construction, debugging, and testing of artificial living systems, Knight argues. According to Drew Endy, biology is going through a fundamental transition from preexisting, natural, and evolving systems, to synthetic, engineered, and disposable systems (Endy, 2005). In the second year of her electrical engineering degree, while testing antilock car brakes, Samantha Sutton felt there was something missing: "I felt the average engineer in my division wasn't really hacking and constructing as I wanted to. They were fine-tuning, refining. I just didn't find it terribly exciting." Sutton switched to biology after graduating, and is now building "circuits" from proteins rather than wires at the Massachusetts Institute of Technology. For Sutton and others involved in synthetic biology, a bacterial cell is regarded as a "chassis". The complexity of the cell can be stripped down and used as a circuit board and power supply. "Let's get rid of all the dangling wires that might short out our circuits", says George Church, referring to several attempts to create organisms containing the minimal amount of genetic information needed to perform basic functions (Aldhous, 2006). The next step is to plug in 'basic circuit elements' that make the cell predesigned, and precisely

define functions. A range of such 'basic circuit elements' is being developed, such as 'toggle switches', 'ring oscillators' and 'inverters'. Indeed, Tom Knight views the emerging field through the lens of electrical engineering and circuit design. "Why are engineers good at doing this, why are we the right people?" he asks. "I would argue that we have a set of tools and an intellectual approach suited to this task." (Brown, 2004). With such statements, synthetic biologists make a point of distinguishing themselves from 'genetic engineers'. "Genetic engineering doesn't look or feel like any form of engineering", Drew Endy says. He argues that the biotech industry rarely attempts anything much more sophisticated than getting *E. coli* to make large quantities of a single protein from another organism. Even then, it often takes extensive research to discover why a sequence borrowed in this way fails to work well out of its usual context.

Although it could be argued that synthetic biology is nothing more than a logical extension of the reductionist approach that dominated biology during the second half of the twentieth century, the use of engineering language, and the practical approach of creating standardized cells and components like in electrical circuitry suggests a paradigm shift. Biology is no longer considered 'nature at work', but becomes an engineering discipline. True engineers, notes Endy, build much more sophisticated systems, which slot together according to a predetermined design and work reliably. When considered as a piece of machinery, nature is imperfect and should and can be revised and improved.

The synthetic biology approach criticized

Meanwhile, new discoveries in genetics, epigenetics, evolutionary biology, genomics and other areas of '–omics' have resulted in an increasing number of scientific publications, challenging the reductionist approach that points towards DNA as being the stuff that generally determines an organism's characteristics. Nowadays, it is generally accepted that cell functions are not simply defined and controlled by genes. Gene expression, protein production, and intercellular interactions are instead controlled by a complex of (partly yet unknown) factors, including histon codes, various RNAs, as well as several environmental factors. Some scientists question the model of the double helix structure of DNA. A review of research on DNA's secondary structure in Current Science in 2003 shows that the double helix model, developed by Watson & Crick in 1953, is not the only type of secondary structure of DNA in living organisms (Delmonte, 2003). Recent research has demonstrated that protein-coding sequences do not always have a clear beginning or end, and that RNA is a key part of the information package. This makes the whole concept of the 'gene' fuzzy (Pearson, 2006).

"In biology details matter, and we do not understand the details", says Frances Arnold, a specialist in the engineering of cell-to-cell communication from the California University of Technology. She draws the parallel of writing a book: "Literature is not written by taking fragments from Internet and pasting them together. Now we have that funky code that looks more like the Google function of Moby Dick than Moby Dick" (Arnold, 2006a: webcast).

According to Carl Woese (expert in the field of microbial taxonomy, discoverer of the large-scale structure of the tree of life, with all living creatures descended from three primordial branches) the molecular vision had been realized by the end of the twentieth century, and is not capable of telling us how complex biological systems really work. He calls for a new, deeper, more invigorating representation of reality. "Knowing the parts of isolated entities is not enough... Machines are not made of parts that continually turn over, renew. The organism is... While machinery is a mere collection of parts, some sort of "sense of the whole" inheres in the organism." (Woese, 2004). Organisms contain different kinds of mechanisms to repair damage, or to overcome the consequences of damage. Woese chooses the metaphor of streaming water to explain how organisms function: "Imagine a child playing in a woodland stream, poking a stick into an eddy in the flowing current, thereby disrupting it. But the eddy quickly reforms, and the fascinating game goes on. Organisms are resilient patterns in turbulent flow patterns in an energy flow". To understand living organisms in any deep sense, we must come to see them not materialistically, as machines, but as (stable) complex, dynamic organizations, Woese concludes.

Both the reductionist approach in synthetic biology and the tendency to a more holistic approach of Woese and many other biologists stem from results in systems biology research. Once again it is Tom Knight who characterizes the difference between the two approaches in terms of the difference between a biologist and an engineer: "A biologist goes into the lab, studies a system and finds that it is far more complex than anyone suspected. He's delighted, he can spend a lot of time exploring that complexity and writing papers about it. An engineer goes into the lab and makes the same finding. His response is: 'How can I get rid of this?'" (Brown, 2004).

However, it would be a mistake to conclude that the engineers in synthetic biology do not acknowledge the complexity of biological systems; they are just convinced they can simplify it and make it work by design.

3 Current developments in synthetic biology: Applications, products and expectations

Theoretically, the possibilities of synthetic or artificial biological structures and systems seem endless. One could imagine the future design of artificial vesicles, containing a completely artificial genome designed at will, and completely artificial cellular machinery, which takes care of the translation of the genetic code into proteins and replication, that could actively communicate with other cells. For the social and political debate it is important to know whether this is a valid picture of the future of synthetic biology or merely science fiction. How real are the promises made, and how real are the threats? What can be expected from synthetic biology in the next five to ten years? This chapter gives an impression with the description of seven categories of applications, ranging from new therapeutics and drugs to biosensors and energy production. It demonstrates that there is more than the examples that recently drew a lot of media attention, such as photographic pictures produced with light-sensitive bacteria, and bacteria producing an anti-malarial drug. One example is the synthetic biology of stem cells. Combined with an analysis of future expectations, including an estimate of their probability, this gives an impression of what the assumed capabilities of the technology are.

3.1 Applications and products

So far, all applications of synthetic biology are in the Research & Development (R&D) phase. Specific press attention was attracted by gimmicks such as the construction of origami-like patterns with DNA, microbes blinking in coordinated rhythm, prototypes of synthesized biomolecular motility and light-sensitive bacteria that can capture a photographic image. The application of synthetic biology to design biosensors that can differentiate between espresso, coffee and decaf demonstrates the potential to construct ultrasensitive biosensors. Bacteria can be designed for use as remotely-controlled therapeutic agents in tumors or for the production of new drugs. Stem cells can be

programmed, for instance, to proliferate and differentiate into neural stem cells.

Live therapeutic agents

Pathogenic bacteria and viruses are experts at identifying specific cell types and organs, can evade immune responses and can manipulate individual cells. They can therefore be engineered for usage as live therapeutic agents. For example, it has been demonstrated that wild type *Salmonella typhimurium* localizes to tumors after intravenous injection and can impart a therapeutic effect. To improve efficacy, strains of the bacteria *S. typhimurium* have been engineered to require adenosine for growth, convert a pro-drug into an active form and ameliorate septic shock. In addition, the bacteria can be remotely controlled after being administered to a patient by using the antibiotics tetracycline and mitomycin C to elicit a response in engineered bacteria thriving in a tumor (Voigt, 2005). Programming bacteria to have therapeutic functions will require a toolbox of cellular sensors that respond to micro-environments in the body, genetic circuits to integrate this information and the ability to engineer the interaction between a bacterium and a mammalian cell.

Viruses can also have therapeutic properties. For example, the harmless human virus AAV2 kills a broad range of cancer cell lines but does not affect primary cells. Yet the wild type virus has drawbacks, like preexisting immunity, delivery efficiency, distribution in the body, production and cell-type targeting. Directed evolution has therefore been used to generate mutants that overcome some of these limitations (Koerber, 2006). Therapeutic viruses can also be engineered to interfere with an infection of harmful viruses. For instance, a lentivirus has been engineered that interacts with HIV-infected cells to reduce the HIV viral setpoint in blood, thereby preventing the transgression of the disease to AIDS.

Microbial and plant drug factories

Tools from synthetic biology already have an impact on metabolic engineering, in which the goal is to create a microorganism or organism that produces a maximum yield of a desired chemical. For example, by combining metabolic pathways from bacteria, yeast and the plant *Artemisia annua*, an *Escherichia coli* has been created that synthesizes large quantities of the precursor of the anti-malarial drug artemisin. The production of the precursor was maximized by codon optimization of the plant enzyme amorphadiene cyclase using DNA synthesis (142-fold improvement), incorporation of a heterologous yeast mevalonate pathway (30-fold), and optimization of the intragenic messenger RNA structures of the synthetic mevalonate operon (7-fold) (Ro, 2006; Pfleger, 2006). A similar engineering approach

could be used to optimize microbial production of other therapeutic terpenes, such as taxol, which is used for cancer treatment, and prostratin, which is used in clinical trials for HIV treatment (Voigt, 2005). Although more complicated, specific targeting of protein expression could allow for efficient production of drug components in plants too. Specific targeting has been applied in plants for elevated production of a synthetic analogue of spider dragline silk protein (DP1B) in *Arabidopsis*. Researchers used a synthetic DP1B gene for producing silk-like protein in genetically-modified plants at a level of less than 1.5 percent of total soluble protein in the plant cells. DP1B productivity in seeds was increased by 5.4 to 7.8 fold by using endoplasmic reticulum and vacuole targeting in seeds and apoplast, and endoplasmic targeting in leafs (Yang, 2005).

Programming stem cells

Stem cells are able to proliferate and differentiate into many different cell types and are a natural repair mechanism. Understanding the mechanisms of this process will enable the control and programming of stem cells and their use as a therapy to replace cells destroyed by disease or to grow new tissues.

Stem cells can be programmed in several ways. First, their behavior can be tuned by controlling the properties of a well-characterized external micro-environment. For example, an external matrix, consisting of gel polymers modified to display a small domain of the large protein laminen, can elicit stem cells to proliferate and differentiate into neural stem cells. Second, synthetic circuits can be introduced that control the activity of central signaling proteins. For example, the sonic hedgehog protein controls whether neural stem cells undergo proliferation or differentiation (Lai, 2004). And the enzyme Rho GTPase can be engineered to control the differentiation of stem cells into fat or bone.

Biosensors

RNA has the capability to bind small molecules and regulate gene expression, making it an ideal substrate for designing biosensors that mechanically link the input with an output response. For example, a cell-based biosensor has been built by linking a RNA aptamer that binds a small molecule ligand with a piece of RNA that inhibits or activates translation, which could differentiate between espresso, coffee and decaf (Bayer, 2005). Synthetic sensors can also be constructed by fusing an extracellular input domain of a protein to an intracellular signal transducer domain. This strategy was used to design an *Escherichia coli* that can record a pattern of light that is shown on a bacterial lawn, that is 'bacterial photography' (Voigt, 2005).

Genetic circuits

Programming cells will require the integration of signals from multiple (bio)sensors. Receiving information from multiple sources provides greater specificity when linking gene expression to an environmental niche. A so-called AND gate, in which the output is only activated when all inputs are on, is a particularly useful circuit. Several AND gates have been developed, using DNA, RNA and protein components that function in vitro, within bacteria and in eukaryotic cells. One example is an AND gate that identifies four 'symptoms' signals (abnormal up- or down-regulation of specific mRNA levels) and releases a nucleotide drug in response. But OR and antagonistic logical gates have also been created.

Further, obtaining programmable multicellular organization will require the use of genetic circuits that control cell-to-cell communication. A genetic circuit has for instance been created that regulates bacterial population density using a sensor linked to the expression of a toxic protein. Such genetic circuits could be used to program temporal-spatial patterns into cells, which has applications in the design of biofilms and the growth of synthetic tissue (Ball, 2004).

Rational biomolecular design

The ability to design the sequence of a biomolecule to fold into a target structure has been increasing rapidly. For example, through using a computational algorithm that generates pairs of proteins that bind to each other, protein circuits have been created and signal pathways have been rewired. Moreover, algorithms have been developed for the design of nucleotide sequences to program synthetic mechanical functions into RNA and DNA. This has been used to create a system of DNA fragments that are able to self-assemble into a motor analogous to kinesin and take the first steps along a piece of DNA. This opens up the possibility of programming mechanical functions into cells using nucleotide sequences. A possible application is the design of a new organism to produce hydrogen from renewable cellulose resources with high efficiency (controlled growth, elimination of side reactions that might reduce efficiency). Another possible application is the alternate design of promoters (Voigt, 2005).

Directed evolution as a tool

According to Frances Arnold (2006b), directed evolution is a rapid method for engineering a biological system. In natural evolution the complexity of living things are attributed to a 'Darwinian' algorithm of mutation and natural selection. The products of such an algorithm are apparent at all levels, from the diversity of life all the way down to individual protein molecules. Scientists and engineers who wish to

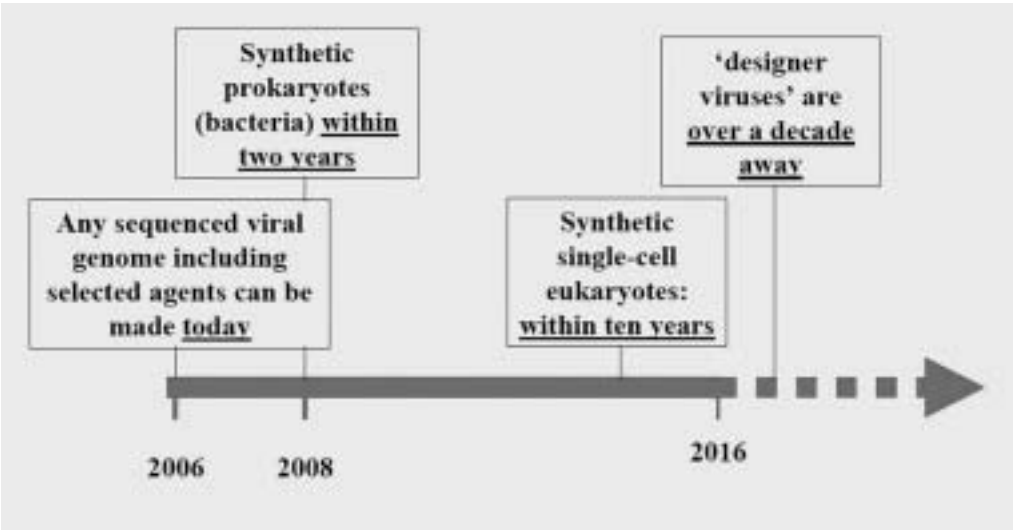
redesign these same molecules are now implementing their own versions of the algorithm. Directed evolution allows us to explore functions of biological molecules never required in the natural environment and for which the molecular basis is poorly understood. This bottom-up design approach contrasts with the more conventional, top-down one in which proteins are tamed 'rationally' using computers and site-directed mutagenesis. It can be used to improve metabolic pathways, mRNA switches, viruses and genetic circuits. Several strategies have now become available, to search through large libraries and to identify functional circuits.

3.2 Expectations: The pace and proliferation of biological technologies

Analyzing expectations in this field is difficult. The hype about synthetic biology needs to be separated from the hard results. It reminds Ari Patrinos, chief of genome research at the US Department of Energy, of the very early days of the Human Genome Project. "This is the frontier" of biology, he notes (Pennisi, 2005). Indeed, cellular systems are complex, yet not fully understood, and therefore difficult to mimic. The most overwhelming and certain use of DNA synthesis is, and will be its direct acceleration of ongoing, constructive experimental research. Thus, fully synthetic or artificial organisms are still science fiction. In terms of the level of artificialness, most examples are still in the range of level 3 (see Chapter 2.3.), where design principles are introduced, but still at a rather basic level, aimed at individual components and pathways. It includes the design of synthetic genes and metabolic pathways, the regulation of gene expression (transcription), and modulation of translation and regulation. Nevertheless, at this level several 'modest' goals are achievable and have already been demonstrated, and seven fields of application can be distinguished. Usually, it is difficult to predict the development of a technology when it is still at an early stage of development. Often, researchers are overenthusiastic, the need for more research funding is too dominant, journalists are too eager to write a story about 'revolutionary technologies', and promises are overestimated. Many researchers involved in synthetic biology seem well aware of this, which is why most of them are careful in making predictions. Several scientists stress that there are many technological, legal and commercial obstacles that must be overcome before the practical applications of the technology can be realized. And when such predictions are made, it is often added that similar predictions were made several years ago. Nonetheless, the ever-increasing speed of gene analysis and gene synthesis will decrease their costs and availability, and this will speed

up the recently started learning curve. Biological technologies will be applied to develop new, (partly) synthetic biological systems that can operate in contained environments within the next ten years.

Some predict that the advent of the home molecular or synthetic biology laboratory is not far off (Carlson, 2003). The use of mail-order oligonucleotides to build a functional poliovirus from constituent molecules in 2002, followed by the recreation of the Spanish flu virus by US Army scientists in 2005 are generally considered the first successful attempts to reconstruct a living entity (Cello, 2002; Tumpey, 2005). In 2006, the synthesis of most viral genomes are within the range of today's technologies. At this point, however, the field is more talk than reality, says Craig Venter. "There's not a lot of data yet." (Pennisi, 2005). Craig Venter confirms that any sequenced viral genome including select agents can be made today. He thinks that the construction of 'designer viruses', which are considered a potential threat, is over a decade away. His guess is that the synthesis of



Predictions for application of synthetic biology (Venter, 2006)

prokaryotes (bacteria) will be possible within two years, and synthesis of single-cell eukaryotes will be possible within ten years. Furthermore, by developing cassettes of 5–10 genes that are then assembled together with homologous recombination, Venter's team is trying to assemble synthetic chromosomes, which can replace the existing chromosome. Venter expects that in the near future robots will be able to assemble thousands to millions of chromosomes per day, which will be a big step forward in synthetic genomics. Meanwhile, Venter admits that he made the same predictions several years ago (Venter, 2006: webcast). Others, like COGEM, expect that many tech-

nological hurdles still need to be overcome, especially in the case of total artificial synthetic organisms (COGEM, 2006). Venter admits that the assembly of synthetic chromosomes is a totally new field, where the technology is not fully developed. No cell genome has yet been synthesized and genome replacement has not yet been demonstrated. Others expect synthesis of bacterial genomes will be possible within one to two years. Attempts are being made to design a synthetic *E. coli* genome and Jeff Boeke, Stanford University, plans to construct a complete synthetic *Saccharomyces cerevisiae* genome.

As a result of developments in bioinformatics, high-throughput analysis systems etcetera, the time required to design, construct and test a synthetic biological system or organism is declining due to several enabling technologies (Voigt, 2005). Large-scale genome sequencing projects have provided a toolbox of (microbial) genetic components that can be exchanged between organisms and combined. In addition, decreasing DNA synthesis costs are rendering the relatively slow process of cloning and classical molecular biology obsolete. Nowadays, automated commercial instrumentation handles an increasing fraction of laboratory tasks that were once solely conducted by doctoral level researchers, thereby reducing labor costs and increasing productivity. Moreover, apart from information about writing DNA from scratch, extensive instructions on standard chemistry and molecular biology techniques are available on the Internet, including detailed descriptions of PCR and other important DNA engineering procedures. While at the start of the 1990s the sequencing of DNA and the creation of genetically modified organisms were the province of PhD scientists, today they can be created by people with little formal education, as there are now commercially available kits that include relatively simple recipes for moving genes between organisms. And there are efforts, like the BioBricks project, to standardize genetic parts to improve the predictability of designs and facilitate the exchange of materials between research groups. These technologies will drive new R&D and enable practical application of synthetic biology. Other examples are the class taught at MIT in which students ranging from undergraduates to post-docs design and test new genetic circuits, and the International Genetically Engineered Machine (iGEM) competition; successful designs are included in the registry of Standard Biological Parts (BioBricks).

Commercial DNA synthesis capabilities can be used to illustrate the rapid proliferation of biological technologies. At present, there about 50 commercial DNA synthesis companies worldwide, the majority being very small (there are about four slightly larger companies: Blue Heron, Coda Genomics, DNA2.0, Codon Devices and GeneArt based in Germany) most in the USA and Europe and several in Asia (Morton, 2005). In July 2006, Codon Devices announced the successful synthesis of a sequence of DNA more than 35,000 base pairs long. Codon

hopes to create DNA fragments of more than 100,000 base pairs in 2008 (Herper, 2006). While gene synthesis is now conducted by PhDs, the processes will be largely automated within a few years. It is expected that commodity prices will drop by 30 percent to 50 percent per year. Moreover, the ability to produce bigger genes (containing more base pairs) is increasing rapidly. Any government or organization can now set up a gene synthesis facility for an investment of about 500,000 US dollars per year and three to six PhDs. It is not expected that it will become a 'garage technology' for lone hackers with few resources.

Drew Endy, assistant professor of biological engineering at MIT, is convinced that the most overwhelming and certain use of DNA synthesis will be its direct acceleration of ongoing, constructive experimental research. Today, for example, a practising experimental biologist or biological engineer can easily spend around 50 percent of their effort manipulating DNA just to produce the genetic material needed for an experiment; 'instant' DNA synthesis would provide a general twofold increase in research productivity (Endy, 2005).

It should be noted that in a changing environment, life needs to be able to continually adapt and evolve. Artificial environments, like those of technical processes of biotransformation or bioproduction by microorganisms, are much less intricate than nature, thus reducing the complexity needed in regulatory function (Synbiology, 2005). Creation of synthetic or artificial organisms with the ability to survive in natural environments will therefore be far more difficult, and will require a longer development time than the creation of synthetic or artificial organisms that can survive in artificial environments. Nevertheless, Venter states that (partly) synthetic organisms designed for bioremediation will need to survive in a (specific) open environment.

Synthetic biologists have accomplished a great deal in a short time, which creates an atmosphere of considerable and sincere enthusiasm, where anything seems possible. At this stage, synthetic biology is at a similar level of development to genetic modification in the late 1970s. Although the scientific environment has changed dramatically in the past 25 years, it is tempting to draw a parallel with the promises and realities of genetic engineering, and it may be assumed that the predictions made by the scientists involved in synthetic biology research overestimate the capabilities and speed of development. First of all, major technological and organizational obstacles must be overcome before the practical applications of the technology can be realized. One problem is that the behavior of bioengineered systems remains "noisy" and unpredictable. Genetic circuits also tend to mutate rapidly and become nonfunctional. Drew Endy of MIT believes that synthetic biology will not achieve its potential until scientists can accurately predict how a new genetic circuit will behave inside a living cell. He

argues that the engineering of biological systems remains expensive, unreliable, and ad hoc because scientists do not understand the molecular processes of cells well enough to manipulate them reliably (Tucker, 2006). Intellectual property rights may limit researchers' access to methods and technologies, and may hinder disclosure of materials in scientific publications and the open exchange of knowledge. Existing and eventually new regulations may require extensive documentation of health and environmental risk analysis and lengthy legal procedures. And finally, the limited number of end users at the Berkeley Conference in May 2006 illustrates the uncertainty of business prospects.

Meanwhile, several initiatives have taken to facilitate future R&D in synthetic biology. The BioBricks initiative has been set up as an effort to facilitate research in synthetic biology by standardizing synthetic DNA parts (standardized coding sequences for protein production and gene regulation) and devices that contain several parts, designed for specific functions (switches, logic operators, tags, detectors). The idea is that such well defined and characterized parts and devices can be combined to construct and test different systems. Drew Endy, one of the initiators of BioBricks, considers this to be a suitable engineering solution for avoiding or managing biological complexity and the apparent spontaneous physical variation of biological system behavior (Endy, 2005).

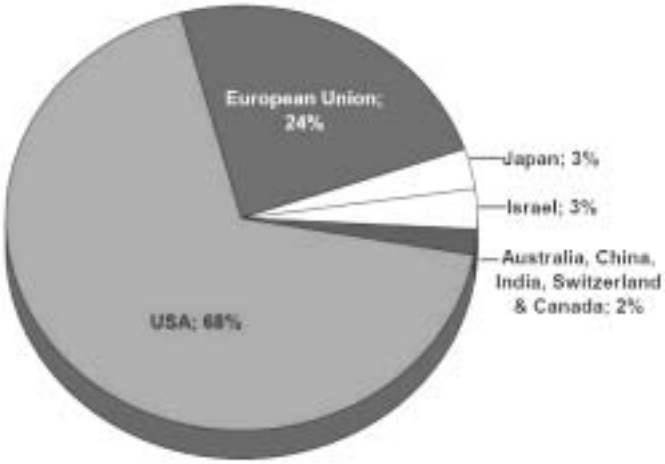
Within the next ten years these biological technologies will undoubtedly be applied to develop new, (partly) synthetic biological systems that can operate in contained environments. Although technological, environmental, and possibly also legal and commercial constraints may hinder the creation of completely synthetic organisms and applications in open environments, developed countries have entered a period in which the understanding of biological systems is resulting in new biologically based technologies. These in turn lead to new insights and new technologies, further enhancing the ability to understand and engineer biological systems. In addition, the demand for more capable technologies is both broad and deep. It seems very likely that the trend to increasingly sophisticated yet less expensive instrumentation will continue. Like genetic modification technologies in the 1980s and 1990s, biological technologies will become increasingly commoditized. Consequently, their wide distribution will further accelerate discovery and invention.

4 The synthetic biology community and its dynamics

Like any other technology, synthetic biology develops in a social context, where decisions are taken about research programs, funding, legislation and acceptance by end users. Therefore, a brief analysis of the driving forces, the structure and dynamics of the synthetic biology community – scientists, institutions, companies, authorities, and NGOs – is presented**.** Estimates of journal publications, the number of scientists involved and research funding are used to characterize the field of synthetic biology.

4.1 US domination in research

Estimates of journal publications, the number of scientists involved and research funding indicate strong US dominance in the field of synthetic biology.



Synthetic Biology Publications by Country

As of September 2005, the EU-funded Synbiology project estimated that 68 percent of all journal publications in this area have been pro-

vided by organizations in the USA and 24 percent by organizations in the EU, whereas 3 percent have come from Japan, 3 percent from Israel and the remaining 2 percent from Australia, China, India, Switzerland and Canada. Within the USA more than 30 percent of the US journal publications have been provided by organizations in California (Stanford, Berkley, Caltech, Scribbs, UCLA) and nearly 20 percent by those based in Massachusetts (MIT, Harvard, Howard Hughes).

The Synbiology project has also developed a search mechanism that identifies synthetic biology journal publications, funding sources, research and engineering activities, scientists and institutions (Synbiology, 2005; see Appendix 2). So far, more than 250 synthetic biologists have been identified worldwide, 66 percent of them based in the US and 25 percent based in Europe, operating in seven different fields. On the one hand, it should be noted that seven of the twenty-five individuals active in the field 'Concepts and political strategy' are science journalists rather than scientists – freelance or affiliated with scientific journals, like *Nature* and *Nature Biotechnology*, or popular magazines, like *Scientific American*, *Popular Science* and *EE Times*; one individual is a policy maker at the European Commission DG Research. On the other hand it should be noted that the "brand" synthetic biology was introduced in US, and many Europeans work in related fields without declaring themselves synthetic biologists. Therefore, the results include some degree of uncertainty.

From the funding perspective, the US dominance is even stronger. The main sources of funding of synthetic biology research and engineering have been the US National Institutes of Health (18 percent), US Defense Funds (14 percent), the US National Science Foundation (9 percent), the US Energy Department (6 percent), US Universities and Institutes (4 percent) and other US organizations and funds (34 percent). In 2004, the University of California at Berkeley, the Institute for OneWorld Health, Albany, and California-based upstart Amyris Biotechnologies received a $ 42.5 million grant from the Bill & Melinda Gates Foundation to refine a process for producing the antimalarial drug artemisinin in the lab with genetically engineered microbes (Kanellos, 2004). More recently, in 2006, the National Science Foundation decided to fund a Synthetic Biology Engineering Research Center (SynBERC) with a grant of $ 17 million dollar. SynBERC involves researchers from MIT, the University of California, Berkeley, the University of California, San Francisco, Harvard University, and Prairie View A&M University. SynBERC aims to build biological components and assemble them into integrated systems to accomplish specific tasks (SynBERC, 2006; Medgadget, 2006). About 15 percent of the funds spent on synthetic biology have been provided by sources in Canada, Japan, Israel, India, the EU, France, Austria, Denmark, Germany, the United Kingdom and Switzerland.

In the context of the 6th Framework program, the European Commission has developed the PATHFINDER initiative to stimulate forward-looking, cross-disciplinary research to demonstrate key principles and generate tools and parts for synthetic biology (European Commission, 2005b). In the first two calls a total of 8 projects were funded (see Appendix 1). A third call has already been evaluated as well; there will be 1 Coordination Action, 2 Specific Support Actions and 6 Specific Targeted Research Projects.

In terms of funding sources, the present situation in synthetic biology is quite similar to the situation in genetic engineering in the 1970s. To date, public institutions have been the main sources of funding for research activities in synthetic biology. Private enterprises are primarily represented by small companies, usually university spin-offs, delivering enabling technologies, such as gene synthesis and automated high-throughput systems. As the use of synthetic genes in any type of genetic research is time saving, natural genes are increasingly substituted by synthetic genes. The world market for gene synthesis is roughly divided among five companies: Blue Heron, Codon Devices, Coda Genomics, DNA2.0, and Germany based Geneart. Most of them are university spin-offs and work in close cooperation with universities. Software and electronic hardware producers such as Oracle, Intel, and IBM deliver powerful systems that can do the computational work.

Pharmaceutical, energy, biochemical and eventually agbiotech companies such as Merck, Pfizer, Chevron, Genencor, DSM, Cargill, and Syngenta are the (potential) end users of synthetic biology. So far, these end users do not seem to play a significant role in synthetic biology development, which is probably due to the early stage of development and the uncertainties of applicability.

From a business perspective, synthetic biology is typically in a stage where development of new technologies and business initiatives attracts mainly high-risk capital investment. Therefore it is not surprising that the Berkeley Conference in May 2006 was sponsored by several venture capital firms that are active in the field of life sciences (biopharmaceuticals), energy and materials.

4.2 Key players in the scientific community

Although the 'synthetic biology community' is still rather small, and knowledge is highly scattered, the key players represent prestigious universities and research institutes. Judging by the list of participants of the 2nd International Synthetic Biology Conference in Berkeley, the

community consists of about 500 people (including students), mainly scientists from US-based universities and public research institutes. Their scientific background is primarily in molecular biology, chemistry, computing & informatics and engineering. Most of the US-based work is in the Boston area (where the Massachusetts Institute of Technology is located), around Berkeley in California and at Craig Venter's Institute for Genomic Research in Maryland. Other major players are the California Institute of Technology, Stanford University, Harvard Medical School, the John Hopkins University, and the US Department of Energy. The BioBricks Foundation and the Lawrence Berkeley National Laboratory play a key role in getting the community organized on issues such as standardization and self-regulation.

Another key player is the J. Craig Venter Institute, home to approximately 200 staff and scientists with expertise in human and evolutionary biology, genetics, bioinformatics/informatics, high-throughput DNA sequencing, information technology, and genomic and environmental policy research. Hamilton Smith, Nobel Laureate, leads a synthetic biology group that includes National Academy of Sciences members Craig Venter and Clyde Hutchison, one of the pioneers of molecular biology who developed site-directed mutagenesis techniques. The Institute's areas of scientific focus include: genomic medicine with an emphasis on cancer genomics and human genome resequencing and analysis; environmental genomic analysis with an emphasis on microbial biodiversity, ecology, and evolution; use of molecular and genomic methods to develop biological sources of clean energy; synthetic genome development; and policy research on the ethical, legal, and economic issues associated with genomic science and technology. The team at the Venter Institute is concentrating on new methodologies to synthesize large segments of DNA that will eventually enable the construction of whole artificial chromosomes. The Synthetic Biology Group is also interested in understanding and thus engineering new pathways that could lead to new methods for carbon sequestration purposes. By synthesizing minimal genomes the team believes it is possible to construct simple cellular life with desirable synthetic properties (J. Craig Venter Institute, 2006).

The BIO FAB Group, a group of people from eight US universities (Baker, 2006), is also playing an important role, especially in furthering the idea of Biobricks. Members are:

- David Baker, professor of biochemistry at the University of Washington, specialized in protein folding mechanisms and protein-protein interactions
- George Church, professor of genetics at Harvard Medical School and Director of the Lipper Center for Computational Genetics
- Jim Collins, professor of biomedical engineering at Boston University

- Drew Endy, biological engineering assistant professor at MIT, and founder of the BioBricks initiative
- Joseph Jacobson, head of the Media Lab's Molecular Machines research group at MIT
- Jay Keasling, professor of chemical engineering and bioengineering at the University of California, Berkeley, and founder of Amyris Biotechnologies with whom he developed the artemisinin-producing *E. coli*
- Paul Modrich, professor of biochemistry at Duke University, specialized in mechanisms of DNA repair and recombination
- Christina Smolke, assistant professor in the Chemical Engineering Department at Caltech
- Ron Weiss, assistant professor of electrical engineering at Princeton University

All members are also scientific advisers to Codon Devices in Cambridge, Massachusetts, a company specialized in building biological devices.

A European Synbio community – in the sense of researchers branding themselves as 'synthetic biologists' and feeling connected to this field – is still very small. At present, most European scientists and research institutes that are involved in synthetic biology research consider their activities an extension of systems biology, of applied research such as cancer research, or of technologies such as nano(bio)technology, and genetic engineering. A statistical review of synthetic biology research in Europe and North America shows that most fields in synthetic biology are dominated by US-based researchers. European research is particularly strong in the area of analysis and modeling of molecular networks (Synbiology, 2005).

In October 2005, the European Science Foundation organized an exploratory workshop on synthetic biology in biocatalysis and biodegradation. It was aimed at setting up a transnational, European-wide framework for future cooperative projects that bundle the various expertises and concertedly work towards the translation of this joint know-how into concrete technological and economical developments (European Science Foundation, 2005).

Key players in the scientific synthetic biology community in Europe include:
- Prof. George Attard, professor of biophysical chemistry at the University of Southampton, coordinator of NEONUCLEI
- Prof. Frank Breitling, member of the Research Group Chip-based Peptide Libraries at the German Cancer Research Center, coordinator of HYBLIB
- Dr Victor de Lorenzo, Centro Nacional de Biotecnología, CSIC
- Dr Vítor A.P. Martins dos Santos, Helmholtz Center for Infection Research, coordinator of PROBACTYS

- Prof. Martin Fussenegger, Swiss Federal Institute of Technology (ETH), Institute for Chemistry and Bioengineering, Specialized in synthetic mammalian gene networks
- Prof. Helmut Grubmüller, Head of the Theoretical and Computational Biophysics Department of the Max Planck Institute for Biophysical Chemistry, coordinator of NANOMOT
- Prof. Piet Herdewijn, Laboratory of Medicinal Chemistry, Catholic University Leuven, coordinator of ORTHOSOME
- Prof. Phil Holliger, specialized in nucleotide chemistry, directed evolution and synthetic biology of DNA polymerases, MRC Laboratory of Molecular Biology, Cambridge
- Prof. Sven Panke, Swiss Federal Institute of Technology (ETH), Institute of Process Engineering, coordinator of EUROBIOSYN, SynBioComm, and EMERGENCE
- Prof. Luis Serrano, head of the Serrano Group of the European Molecular Biology Laboratory, Heidelberg, coordinator of NETSENSOR
- Prof. Jörg Stelling, assistant professor for bioinformatics of the Swiss Federal Institute of Technology (ETH), Institute for Computational Science
- Prof. Eckart Zitzler, assistant professor of Systems Optimization of the Swiss Federal Institute of Technology (ETH), Institute for Technical Informatics and Communications Networks
- Dr Hubert Bernauer, ATG: Biosynthetics (gene synthesis), partner in SYNBIOLOGY and coordinator of future TESSY producing a roadmap for synthetic biology
- Prof. Nikola Biller-Andorno, professor of biomedical ethics, Ethics Center of the University Zurich, partner in SYNBIOSAFE
- Prof. Augusto Medina, president of Sociedade Portuguesa de Inovação**,** coordinator of SYNBIOLOY
- Dr Adrian Rueggsegger, Head of the field Life Sciences and Health of TA-Swiss
- Prof. Peter Schaber (University Zurich, Ethics; contributed to the first ETH symposium on synthetic biology)
- Dr Markus Schmidt, International Dialogue and Conflict Management IDC, coordinator of SYNBIOSAFE

There may be a potentially huge community of synthetic biologists in Asian countries, such as India, Korea and China, that are highly involved in modern biotechnological research. However this has yet to be explored systematically.

4.3 Other key players

Other important key players include government institutions and non-govermental organizations (NGOs).

As the funding details in Chapter 4.1. show, the main players are the

US National Institute of Health, the US Department of Defense, the US Department of Environment, and the European Commission. The US Department of Agriculture was represented at the Berkeley 2006 conference, mainly because of the market potentials for agricultural commodities. During a Synbiology seminar on May 30, 2006, in Brussels, recommendations for stronger synthetic biology research were discussed. Six major challenges for the next 5 years were identified:

- Ensure security and safety
- Establish and achieve goal for DNA synthesis: low cost, very fast synthesis, no errors
- Develop robust set of well characterized "standard" parts
- Develop tests for quality control of "standard" parts
- Set funding priorities in context of long term/high cost research
- Identify achievable goals resulting in tangible products

Foundational technologies, health care, materials, environmental applications, energy, chemistry, and agricultural biotechnology were considered priority areas. Apart from funding priorities for the European Commission (core facilities, equipment), the question about how to attract private funding was raised (big business, new technology-based firms, equity funding). Training and education were discussed, as well as intellectual property rights and legal issues. Building cooperation with North America and Asia was given special attention. This requires identification of the barriers and ways to overcome them, of parties interested in cooperation versus competition for a future growth market, and of common priorities with buy-in from senior officials of funding agencies in both regions. Funding of joint symposia and workshops, of collaborative research projects, and for EU post-docs to train/teach in North America was suggested (Synbiology, 2006).

So far, NGOs have not been represented at any of the international conferences and seminars. Nevertheless, it was in the context of a critical analysis of nanotechnology and converging technologies in 2004 that the action Group on Erosion, Technology and Concentration (ETC) put synthetic biology on the agenda (ETC, 2004). ETC supports socially responsible developments of technologies useful to the poor and marginalized and addresses international governance issues and corporate power. In the field of life sciences, the organization campaigns against patents on life and the use of terminator technologies that create plants producing non-reproducible seeds. The ETC Group was probably one of the initiators of a letter sent to the organizers of the Berkeley Conference, in which 35 NGOs protested against a self-regulation initiative of the scientific community (see Chapter 5.3). The NGO protest is a clear elaboration of their protest against genetic engineering.

5 Social, ethical, and legal aspects

At present, the scientific community and research programs are placing a strong emphasis on the risk and security aspects of synthetic biology. The risk debate seems to focus on the differences between genetic engineering and synthetic biology. The context of the security debate is typical post 9/11, and appears to be more of an issue in the United States and the UK than in continental Europe (Schmidt, 2006).

For both risk and security, the scientific community is seeking a system of self-regulation analogous to the Asilomar Conference in 1975. However, several analysts challenge the effectiveness of the Asilomar approach under the present conditions. Some of them point at significant changes in the power structure of Western society during the past 30 years. Others argue that the present situation concerning the distribution of knowledge and materials is totally different from 1975, which makes a moratorium virtually impossible. Moreover, intellectual property rights were not yet an issue in 1975, and the 'September 11 effect' in the United States means that people are far more aware of biosecurity issues nowadays. This chapter analyses these similarities and differences between Asilomar (1975) and the present debate (2006) in more detail.

5.1 Parallel with Asilomar, 1975

Prior to the 2nd International Synthetic Biology Conference in May 2006 several members from the scientific community were interviewed about biosafety and biosecurity issues. A paper was drafted, which was meant to be the starting point for a debate about improvements in safety and security in synthetic biology by means of self-regulation (Maurer, 2006). The present emphasis on risks, security, and self-governance by the scientific community is reminiscent of the Asilomar Conference in 1975. In the early 1970s, the molecular biology community responded to the controversial development of genetic engineering by making a series of decisions that resulted in an international scientific conference at the Asilomar Conference Center, California, in February 1975. This conference established the pattern of American policy for controlling the field and served as an influential precedent for policy making abroad.

The Asilomar Conference has been lauded as an exceptional event in

which scientists voluntarily sacrificed immediate progress in their research to ensure that the field would develop safely. Yet according to Suzan Wright, who wrote a critical analysis of Asilomar, many, perhaps most, of the participants resisted questions raised about the implications of their work and simply wanted to proceed. Self-interest, not altruism, was most evident at Asilomar. Eyewitness accounts (and the conference tapes) make it clear that all moves to address the social problems posed by this field in advance of its development were firmly suppressed (Wright, 2001).

The culture in science, stressing individual autonomy, freedom and creativity of research as a free speech act, makes the scientific community allergic for government interference. The initiative presented at the Berkeley Conference reflected a strong mistrust of politicians and government regulators among scientists, especially in the US. The view that "an attempt to ban or limit access to DNA synthesis technology, and the sequence information that defines what to synthesize, would only be guaranteed to cripple biological engineering research and hinder biomedical research" is widely shared in the synthetic biology community. Therefore, the community's answer to the problem of dual use is self-regulation, which includes the development of general, agile capabilities for detecting, understanding and responding to biological risks (Maurer, 2006). David Baltimore, virologist and Nobel prize winner, president of the California Institute of Technology, and one of the organizers of the Asilomar Conference, explains why it was felt so important not to have regulation on genetically engineered organisms in 1975: "After conclusions from the conference had been drawn, the results were handed over to the National Institute of Health (NIH), who then established a Recombinant DNA Advisory Committee. This was important because the NIH could continually judge scientific evidence, which is something that regulators could not do. Legislation would have been fixed, and you would have had to go back to the legislators to unfix, which is very difficult to achieve" (Baltimore, 2006: webcast). In a review article in Nature, November 2005, Drew Endy writes: "Because technologies for engineering biology will be openly developed and widely distributed, political leadership is needed to encourage all members of society to help actively foster a worldwide community that celebrates the science of biology, and lead the overwhelmingly constructive development and application of future biological technologies" (Endy, 2005).

According to Paul Rabinow, social-cultural anthropologist at the University of California, Berkeley, Asilomar took place at a time that elite was ranked around status, not around money (Rabinow, 2005). The funding came from the federal government and it was mainly the American University system that was involved. Dissemination of scientific results took place through commercial, but high-status media,

such as *Science*, *Nature* and *The New York Times*. It was the elite scientists that were convinced they should regulate science. The trick of Asilomar was to turn 'danger' into 'safety'. By giving the assessment to the National Institute of Health, the matter was no longer a scientist problem and became a political problem, according to Rabinow. In 2006, the hierarchy of the system is no longer based on status, but on power, and the autonomy of science is limited by financial, legal and ethical constraints, Rabinow argues. That makes a scientist-only gathering, even if it were only to discuss the technology end, unacceptable. Also Baltimore indicates that our society is a different place compared to 1975, and the scientific community could no longer make decisions without involving other parties (Baltimore, 2006: webcast).

Rabinow's remarks were almost instantly illustrated by a joint letter of 35 NGOs (including Greenpeace, Friends of the Earth, the Canadian Farmers Union, ETC Group, the International Center for Technology Assessment, the Indigenous People's Biodiversity Network and the Third World Network) that criticized the attempt for self-regulation by the scientific community at the 2nd Synthetic Biology Conference in May 2006. The letter was sent to the organizers and the press the day before the start of the conference, and emphasized that (ETC, 2006):

1. Society – especially social movements and marginalized peoples – must be fully engaged in designing and directing societal dialogue on every aspect of synthetic biology research and products. Because of the extraordinary power and scope of synthetic biology technologies, this discussion must take place globally, nationally and locally.
2. Scientific self-governance does not work and is antidemocratic. It is not for scientists to have the determinant voice in regulating their research or their products.
3. The development of synthetic biology technologies must be evaluated for their broader socioeconomic, cultural, health and environmental implications and not simply for their misuse in the hands of 'evildoers.'

One of the intentions of the organizers of the Berkeley Conference was to vote on a common declaration at the last day of the conference. However, without an explanation from the organizers, the decision was taken not to have such a vote and to place the text on the website for further written comments. This may be considered a sign that the organizers are reasonably receptive to critical comments, both from inside and from outside the scientific community.

Other similarities and differences will be discussed in the following sections.

5.2 Biosafety: health and environment

Like genetic engineering, synthetic biology could pose a risk to other organisms, especially when new organisms are released to the environment or used in feed, food or medical/veterinary products. An analysis of risk definition and the first two conferences on synthetic biology (2004 and 2006) show that there is a lot of similarity between discussions in 1975 and the present debate on synthetic biology. However, there is one important difference: in 1975 the access to the relevant knowledge and technologies was limited, whereas nowadays the knowledge and technologies are widely disseminated, which makes it virtually impossible to implement a moratorium.

One of the main questions is how these potential risks should be managed. Because of similarities with the risks of genetic engineering, risk management is focusing on how synthetic biology changes preexisting risks for better or for worse. At present, opinions on the adequacy of the system designed for the risk analysis of GMOs differ.

Definition of risks

In his analysis of the challenges for synthetic biology, Arjun Bhutkar outlines the risks of synthetic biology as follows (Bhutkar, 2005):

- Risk of negative environmental impact: This includes scenarios in which a synthetically created microorganism designed for a particular task (e.g., environmental cleanup) could have a side effect of interacting with another environmental substance and impact the overall environment negatively.
- Risk of natural genome pool contamination: Any genetic exchange between a synthetic biological entity and a naturally-occurring biological entity would result in natural genome contamination. This is similar to the problem of "gene flow" in the context of transgenic plants.
- Run-off risk ("Grey goo" and "green goo" problem): This is similar to the problem often discussed in the context of nanotechnology. Synthetic biology products released into the environment to accomplish a specific task should have a controlled lifespan outside the lab. If this in not the case, one can envision unintended consequences of a system run amuck.

According to Michele Garfinkel, biosafety expert of the J. Craig Venter Institute, this list is incomplete – for instance, it does not explicitly include worker safety – and does not distinguish effectively between natural, bioengineered and synthetic organisms.

Similar to the Asilomar Conference in 1975, most scientists involved in synthetic biology nowadays consider the creation and accidental release of new human pathogens the biggest threat. During the Berke-

ley 2006 conference the possibility of unintended effects caused by the deliberate release of synthetic biology products to the environment (Bhutkar's first category of risks) was not discussed, probably because it is not considered an issue at this stage of the technology's development. The present focus on 'accidents' and references to work that is already needed given existing and emerging infectious diseases, reflects the strong focus on human health risks caused by experiments in more or less contained environments.

MIT, the Venter Institute, and the Center for Strategic and International Studies in Washington, D.C., have teamed up to examine issues such as how to keep any new life forms created under control. This effort is funded by a $570,000, 15-month grant from the Alfred P. Sloan Foundation and will be completed in autumn 2006 (Pennisi, 2005). Nonetheless, the near absence of ecologists who might raise a few questions on this issue in the present debate is also quite similar to the situation in 1975. In the Netherlands, for instance, it was only in the late 1980s that ecologists' opinions on the deliberate or accidental release of genetically modified organisms were first heard.

In 2006 an EU-funded project will start aimed at addressing bioethical and biosafety concerns, and potential or perceived risks and benefits of synthetic biology from the very beginning. This Synbiosafe project will start with a fact-finding exercise, including a series of interviews with synthetic biologists and biosafety experts, and an exploration of upcoming biosafety challenges by two task groups. Secondly, and based on this fact-finding exercise, a reference framework will be prepared by a number of task groups that will serve as the major input for a contribution to the "inaugural" International Conference on Synthetic Biology in Zurich, an open e-forum and an international meeting on safety and ethics in synthetic biology (Synbiosafe project objectives).

Risk management

Assuming that preexisting risks are known and understood, the authors of the self-regulation paper discussed in Berkeley argue that policy should be formulated by focusing on how synthetic biology impacts preexisting risks in a positive or negative sense. This means focusing on areas where synthetic biology potentially introduces qualitatively new pathways for accidents (Maurer, 2006).

The definition of risks related to synthetic biology and, consequently, the need for specific risk management measures, depends on the definition of synthetic biology. According to the Forum Genforschung of the Swiss Academy of Sciences, synthetic biology is considered a new research field, which usually involves more genes and results in changes that are more radical than those resulting from genetic modi-

fication. In spite of this difference, with respect to safety, the Forum argues that synthetic biology is similar to gene technology and can be understood as a subdiscipline of the latter. From this rather inconsistent argumentation the Forum draws the conclusion that in general, the creation of synthetic organisms is no more risky than the introduction of new species into an ecosystem, dealing with natural pathogens or gene technology as practiced to date. Thus, the criteria that apply for the risk assessment of genetic modification also apply to synthetic biology. The following questions should therefore be explored on a *case-by-case* basis:

- What is the function of the new biological systems?
- What can these do?
- Could they reproduce independently in the natural environment?
- Could they infect the cells of other living organisms and reproduce there?
- Does the new combination of the gene trigger any unexpected effects?

The Forum Genforschung continues by observing that current developments in synthetic biology are all intended for use in laboratories or chemical-pharmaceutical plants. If, despite rigorous safety precautions, organisms modified in this way were to be introduced into the environment, new pathogens could in principle arise from them. However, the more a new organism differs from natural life forms, the less likely it is to reproduce outside of the laboratory. For example, in order to increase safety, self-reproducing biological systems can be constructed such that they require a nutrient that does not occur naturally or that the gene cannot function in natural organisms.

Future developments of synthetic organisms for use in patients or in the environment must be tested thoroughly for safety. As is the case with natural pathogens or existing genetically modified organisms, experimental tests and well-controlled clinical tests or release tests are essential for ensuring that the relevant detailed safety information is provided (Forum Genforschung, 2005).

Tucker and Zilinskas do not share the view of the Forum Genforschung. They agree that for the near future at least, the vast majority of synthetic biological systems will be engineered by transferring small genetic circuits into a well-understood bacterial host, and therefore the familiarity principle will apply. The experience with the microorganisms used for genetic engineering, such as the bacterium *E. coli* and the yeast *S. cerevisiae*, which are well understood by scientists, proves that the transfer of one or two foreign genes is unlikely to change the characteristics of the host in a dramatic, unpredictable way. The use of familiar organisms, which are largely identical to the original organism after being slightly altered, limits the level of risk. Yet a decade from now, they argue, synthetic genomes may be assem-

bled from BioBricks that have been redesigned or are entirely artificial, having been created *de novo*. If a synthetic microorganism is built by combining these genetic elements in a new way, it will lack a clear genetic pedigree and could have "emergent properties" arising from the complex interactions of its constituent genes. Accordingly, the risks attending the accidental release of such an organism from the laboratory would be extremely difficult to assess in advance, including its possible spread into new ecological niches and the evolution of novel and potentially harmful characteristics (Tucker, 2006).

This is probably what makes the Dutch Advisory Committee on Genetic Modification (COGEM) less certain about the adequacy of the present system designed for the risk analysis of GMOs for dealing with risks related to synthetic biology. It has therefore formulated several questions that require more study, such as:

- If the present system for risk analysis for GMOs is not adequate, can it be adapted? If so, in what sense should it be adapted?
- What information is needed about the traits of an organism to make a proper risk analysis?
- Should risk analysis distinguish between totally synthetic organisms and new organisms based on existing organisms?
- How can risks of synthetic genes and organisms be analyzed without having a natural reference? (COGEM, 2006)

Some researchers are already exploring strategies to incorporate safeguards. For example, Church and Endy are developing ways to prevent synthetic genes from escaping and possibly wreaking havoc. One solution: Alter synthetic genetic codes such that they are incompatible with natural ones because there is a mismatch in the genes coding for amino acids (Pennisi, 2005).

Nevertheless, Tucker and Zilinskas think there are still too many uncertainties about the effectiveness of such built-in control mechanisms and the testing of pathogenicity of synthetic organisms in animal models. Therefore, the "precautionary principle" should be adopted, which means that synthetic microorganisms are treated as dangerous until proven harmless. According to this approach, all organisms containing assemblies of BioBricks would have to be studied under a high level of biocontainment (Biosafety Level 3 or even 4) until their safety could be demonstrated in a definitive manner Tucker, 2006). Biosafety Level 4 is required for work with dangerous and exotic agents that pose a high individual risk of aerosol-transmitted laboratory infections and life-threatening disease. Agents with a close or identical antigenic relationship to Biosafety Level 4 agents are handled at this level until sufficient data are obtained either to confirm continued work at this level, or to work with them at a lower level. Biosafety Level 3 is applicable to clinical, diagnostic, teaching, research, or production facilities in which work is done with indige-

nous or exotic agents that may cause serious or potentially lethal disease as a result of exposure by the inhalation route (CDC, 1999).

5.3 Biosecurity

Apart from the risks mentioned above, the risk of the creation of deadly pathogens for the purposes of bioterrorism is widely recognized by the scientific community. From this perspective, David Baltimore argues, the present situation is obviously different from Asilomar, 1975. In 1975 the scientists believed that there was a treaty not to use biology for making weapons to which everybody was adhering. Now we know that the former USSR had a large program for developing and testing biological weapons. But more important is the change of the source of threat: instead of states the world has to deal with terrorist organizations that cross boundaries, and who are not held up by treaties (Baltimore, 2006: webcast). Therefore, it is the contention of the community of self-identified synbiologists that the potential for harm, even for the development of weapons capable of "mass destruction," is not trivial. The creation of the complete genome of the polio virus in the lab, which was proven to be infectious, has demonstrated the potential of synthetic biology to engineer harmful pathogens (Cello, 2002). This technology, in rogue hands, could be used to engineer the genomes of deadly pathogens. Nevertheless, specialists seem to disagree about the new threats synthetic biology is posing in terms of bioterrorism or biological warfare. In general, and probably due to the devastating and shocking events on 9/11, the risk of bioterrorist attacks seems to be experienced differently in the US than in Europe (Schmidt, 2006).

The Ethics of Genomics Group notes that the combination of large-scale sequencing of human pathogens, determination of function of disease-associated gene products, and development of technologies to assemble large pieces of DNA could lead to the creation or release of organisms that could be used as biological weapons. The dangers of knowing the sequences of extremely deadly pathogens could pose threats to public health and safety that might outweigh the benefits (Cho, 1999).

In a paper that was distributed a few weeks before the 2nd Synthetic Biology Conference in May 2006, Raymond Zilinskas, head of the Chemical and Biological Weapons Nonproliferation Program, shows the technological difficulties in creating an organism that is pathogenic (Zilinskas, 2006). Pathogenicity requires that an organism is sufficiently infective, virulent, can survive before and after release, is resistant towards drugs and vaccines, and is specific in its preference to a specific host. The formulation (agents protecting the organism while it is in the environment), containers for storage and transport

(munition), and methods for dispersion (by aerosol, by injection, by explosion, or by food and beverages) have to be carefully chosen. And very important: the behavior of a synthetic organism in the open environment has to be measured and observed and the dispersal system has to be tested, which requires field-testing, again not an easy task.

Notwithstanding these limitations, it needs only one successful attempt to create a threat. This may be why the Swiss Forum Genforschung has adopted an ambivalent position on the issue of biosecurity. The Forum argues that the possibility of the abusive and criminal application of synthetic biology, for example for bioterrorism, is negligible. One of the arguments is that many potential biological weapons can already be found among the natural pathogens which require little effort to produce and use. Further to date, few attempts to produce and use biological weapons have been successful. Despite this, the Swiss Forum Genforschung continues, it may make sense to consider what kind of mechanisms exist or should be created to cover such activities.

The Ethics of Genomics Group advises ensuring the responsible use of knowledge that could be applied to the construction of biological weapons, by giving serious thought to monitoring and regulation at the level of national and international public policy. Should we regulate the science, and if so, at the level of specifying which genomes will be sequenced or at the level of access to the sequence information? Or will we regulate the application of the science?

According to Laurie Zoloth of the Center for Bioethics, Science and Society, oversight of 'problematical dual use' in the US has been inconsistent (Zoloth, 2006). She refers to Milton Leitenberg, who noted in 2005 that: The entire area of oversight and problematic "dual use" research in molecular genetics and its applications in the United States appears to range from inadequate at the local levels to virtually nonexistent at the national level and in terms of BWC treaty compliance." (Leitenberg, 2005). Nevertheless, the National Academy of Sciences has recommended that seven categories of "experiments of concern" be added to the NIH oversight process. In response to the recommendations of the NAS committee report, the administration announced the establishment of a National Science Advisory Board for BioSecurity (NSABB) on March 4, 2004. Its mandate was to last for 2 years. The NSABB has been established to provide advice to federal departments and agencies on ways to minimize the possibility that knowledge and technologies emanating from vitally important biological research will be misused to threaten public health or national security. The NSABB (2006) is a critical component of a set of federal initiatives to promote biosecurity in life sciences research, and is charged specifically with guiding the development of:

- A system of institutional and federal research review that allows for fulfillment of important research objectives while addressing national security concerns;
- Guidelines for the identification and conduct of research that may require special attention and security surveillance;
- Professional codes of conduct for scientists and laboratory workers that can be adopted by professional organizations and institutions engaged in life sciences research;
- Materials and resources to educate the research community about effective biosecurity; and
- Strategies for fostering international collaboration for the effective oversight of dual use biological research.

Proposed actions that are further discussed on the Internet site of the US synthetic biology community are (Maurer, 2006):

1. Insist That All Commercial Gene Synthesis Houses Adopt Current Best Practice Screening Procedures. While most gene synthesis companies screen orders for dangerous sequences, a few do not. This gives both community members and outsiders access to feedstocks for both wild type and genetically-engineered bioweapons. Community members should stop doing business with any gene synthesis company that fails to implement current best-practice screening methods by January 1, 2007.
2. Create and Endorse New Watch-Lists To Improve Industry Screening Programs. Improved watch-lists and software tools can make industry screening more accurate and efficient. Members should prepare the necessary lists and tools in time for Synthetic Biology 3.0.
3. Create a Confidential Hotline For Biosafety and Biosecurity Issues. All experimenters contemplating "experiments of concern" should obtain independent expert advice before proceeding. The community should make such advice freely available to all experimenters, including non-members (e.g., hackers) who cannot otherwise obtain such advice from formal university, company, or NIH safety committees.
4. Affirm Members' Ethical Obligation to Investigate and Report Dangerous Behavior. Members have an obligation to investigate and, if necessary, report dangerous behavior. Members should affirm this obligation by formal resolution at Synthetic Biology 2.0.
5. Create a Community-Wide Clearinghouse for Identifying and Tracking Potential Biosafety/Biosecurity Issues. Members who notice potential biosecurity issues have an obligation to share them with the broader community. A central clearinghouse will help the community to identify, track, and if necessary respond to the biosafety/biosecurity implications of a changing technology.
6. Endorse Biosecurity/Biosafety R&D Priorities. New technologies can potentially reduce current biosafety/biosecurity risks even further. Members should identify, endorse, and urge funding agencies to invest in priority technologies such as safe chasses and bar codes.

5.4 Intellectual Property Rights (IPR)

The building of new organisms also raises intellectual property and commercialization issues that will affect the conduct of research and the ability of both industry and academia to continue developing the technology for public good. Intellectual Property Rights (IPR) is another area where the situation has changed drastically during the past 30 years. In 1975 intellectual property rights was not yet on the agenda of the scientists involved in genetic engineering. Nowadays, most technologies used in genetic engineering and applications are patented by the companies and research institutes that invented them. Patents ensure the holder control over the technology and, eventually, legal protection that ensures return on investment. Therefore, patents are considered an important instrument for encouraging investment in technological innovation. On the other hand, the legal work that is involved in ensuring that all patents involving the use of specific technologies and materials in research are properly dealt with – in terms of permissions and fees – can be so enormous, that it is practically impossible for research institutes to use them. In addition, patent holders may ask for information about materials to remain confidential, resulting in scientific publications that lack essential information.

In the United States, the precedent for patents for altered organisms was set by the Chakrabarty case, which ruled that a genetically altered bacterium was not a product of nature and thus was patentable (Diamond versus Chakrabarty, 1980). Prior to this case, the US Congress had authorized limited protection for cultivated plant varieties, and in 1988 the US Patent and Trade Office granted the first patent for an animal; the Harvard Onco-mouse (Shorett, 2002). Although it took a long time for the European Union to create harmonized patent legislation, the European Patent Office has granted a large number of patents on genetically modified organisms.

In order to avoid limitations in research and education, experimental use exemption from patent infringement exists for university research. But in a recent case in the United States, the Federal Circuit found that major research universities often sanction and fund research projects with arguably no commercial application whatsoever. Yet these projects also further the institution's business objectives, including educating and enlightening students and faculty participating in these projects. These projects also serve, for example, to increase the status of the institution and lure lucrative research grants, students and faculty staff. In short, the profit or non-profit status of the user is not determinative. Only when the act of the alleged infringer is for amusement, to satisfy idle curiosity, or for strictly philosophical inquiry, does the act qualify for the very narrow and strictly limited experimental use defense. (Stephens, 2003).

However, it is not clear how far reaching patents on any newer methods to create genetically altered organisms will be, or how patents on individual genes will be reconciled if many of the genes are used in a newly assembled genome. Current patenting practices may already be restricting development of and access to clinical applications of genomics, as well as academic and industrial researchers' access to genetic information and reagents. Large-scale gene identification efforts such as those involved in minimal genome research, as well as other technologies that require use of large numbers of genes simultaneously (such as gene arrays) have great potential to exacerbate these problems.

Both the US and the EU patent system use criteria for patents such as utility, moral utility doctrine, and licensing. Utility requires that a claimed invention either has a well-established utility or asserts a specific, substantial, and credible utility (European Patent Law requires that to be patentable an invention must have industrial applicability) (Biojudiciary.org, 2006; European Patent Office, 2003). In terms of the BioBricks stratification of parts-devices-systems (see the figure "Building systems with parts and devices" in Chapter 2.2.), it could be argued that higher levels of integration (devices and systems) can easily be conceived to serve some aggregate complex function, such as environmental cleanup of a specific pollutant (Bhutkar, 2005). Bhutkar suggests that the lowest level of parts should be engineered for well-defined functions and a clear beneficial purpose should be articulated wherever possible, in order to easily meet utility requirements at higher levels of integration.

The US moral utility doctrine requires that the invention should not be frivolous or injurious to the well being, good policy, or sound morals of society. It is a rarely invoked aspect of patent law in the US. One of the few exceptions was the rejection of Dr Newman and Jeremy Rifkin's human-chimera patent application in 1998 on the basis that it embraced a human being and thus was not patentable (Newman, 2002). Article 53(a) of the European Patent Convention notes that: "The EPO will not grant patents against public order or morality." (European Patent Office, 1991) It is expected that in Europe, creations of synthetic biology will face tougher scrutiny from a patentability perspective.

Given the legal possibilities for patent protection and the potentially far reaching consequences for furthering the research in synthetic biology, it is not surprising that access to technologies and materials is high on the agenda of the research community. The question then, is how to design a regulatory framework that protects intellectual property in a way that stimulates innovation whilst not hindering basic research. As Bhutkar has pointed out (Bhutkar, 2005), patent policy is not a barrier to research efforts and regulatory frameworks

are a primary tool for ensuring that proper safeguards are instituted and researcher conduct adheres to established guidelines.

Several initiatives have started and proposals have been put forward for a new regulatory framework for intellectual property pertaining to genes and organisms, to ensure that public and commercial interests are protected. One of the goals of BioBricks is to develop and implement legal strategies to ensure that BioBricks remain freely available to the public (BioBricks Foundation, 2006). It seems appropriate to investigate alternatives to the patent system that are being developed, such as Public-Sector Intellectual Property Resource for Agriculture (PIPRA) and BIOS: 'Biological Innovation for Open Society'. PIPRA is an initiative of US-based public research institutes involved in agrobiotech research that wants to map the patenting and licensing practices of the public sector and create a common patent database. PIPRA also wants to develop 'shared technology packages' of key technologies for agbiotech research (Atkinson, 2003). Richard Jefferson, a geneticist from the US, is the driving force behind BIOS. BIOS wants to promote the use of agbiotech patents as a kind of freeware, comparable to what has been done with Linux in the field of software development. One of the first steps was the development of an alternative for the use of the commercially patented *Agrobacterium thumefaciens* method for plant transformation (Broothaerts, 2005). However, a Syngenta plant scientist has made some critical comments on the efficiency of the use of bacterium strains other than *Agrobacterium* in Nature Biotechnology (Chilton, 2005). She refers to the publication of the experiments with non-*Agrobacterium* transformation methods, which demonstrate a frequency of successful transformations in *Arabidopsis thaliana* and rice of 10–100 times lower than has been achieved with *Agrobacterium* methods.

Meanwhile, with some funding of the Rockefeller Foundation and IBM, CAMBIA, the organization charged with applying the idea of BIOS, got started in 2005 (Carina, 2004). A gift of 2.5 million US dollars from the Norwegian government enabled CAMBIA to cooperate with the International Rice Research Institute in December 2005 in creating a freely accessible database of rice-related patents in Korea, China and India (CAMBIA, 2005).

5.5 Ethical concerns

Thinking about the philosophical aspect of synthetic biology, one of the first questions that arises is: "What is life?". The distinction between an engineered machine and a living organism is clearly one of the main ethical concerns. In 1975, the organizers of the Asilomar Conference did not consider the ethical aspects because they felt unqualified to do so. David Baltimore, one of the organizers of Asilo-

mar explains: “We did not have ethicists or philosophers (or maybe only a few) at the conference, and we were not pretending to speak for the public” (Baltimore, 2006: webcast). Although ethical concerns were put on the agenda of the Berkeley Conference in 2006, little specific analysis has been made of the ethical implications of synthetic biology. Papers that mention the ethical aspects usually do not elaborate on them, and are restricted to raising questions in a rather unsystematic sense, or focus on the ethical behavior of the research community with respect to biosecurity and biosafety.

One of the few ethical reviews of minimal genome synthesis is from the Ethics of Genomics Group, and was published in *Science* in 1999 (Cho, 1999). This group articulated questions such as: “How does work on minimal genomes and the creation of new free-living organisms change how we frame ideas of life and our relationship to it?” and “How can the technology be used for the benefit of all, and what can be done in law and social policy to ensure that outcome?”.

According to this group, the attempt to model and create a minimal genome represents the culmination of a reductionist research agenda about the meaning and origin of life that has spanned the twentieth century. There are important concerns raised by this reductionist approach to understanding life. First, a reductionist approach can limit our scientific understanding of living organisms. Focusing on a reductionist approach has had some historical value in helping scientists produce a better understanding of cellular function: it provides information on some of the crucial functions necessary for cellular function, as well as insight into the evolutionary process. In spite of its usefulness, however, reductionism has also led to erroneous thinking, for example, that viruses were the phylogenetic precursors to cellular life. Similarly, by devoting far greater effort to understanding the role of the nucleus in the functioning of the cell compared with other cellular elements, which have their own causal roles to play, we can bias our understanding of how cells operate. Second, a reductionist understanding of life, especially human life, is not satisfying to those who believe that dimensions of the human experience cannot be explained by an exclusively physiological analysis. What are the ultimate implications of defining life in terms of DNA? Should we allow the definition of life to be treated as a narrow scientific issue, one that assumes that there is nothing in the world that is not physical? Can or should those in the natural sciences decide the meaning of life without input from theologians, philosophers, social scientists, and the general public? There is a serious danger that the identification and synthesis of minimal genomes will be presented by scientists, depicted in the press, or perceived by the public as proving that life is reducible to or nothing more than DNA. But life need not be understood solely in terms of what technology permits natural scientists to discover. This may threaten the view that life is special, that there is

interconnectedness of all living things, and the sense that living things are, in some important way, more than organized matter.

Reducing life to genes has profound implications for several critical societal debates, including what constitutes human life and when life begins. It is important that scientists and the general public understand the implications and limits of the claims being made by the scientific community about minimal genomes in order to participate effectively in the debates. As an example, scientists have suggested application of the minimal genome approach to higher organisms. If we extend the reductionism implicit in minimal genome research to a definition of human life, this has implications for the debate about whether stem cells, early embryos, or hybrid embryos combining human DNA with the cellular components of other species are human. Likewise, a genetic definition of when life begins would have implications for the abortion debate. We would argue that the complex metaphysical issues about the status of human beings cannot be discussed in terms of the presence or absence of a particular set of genes.

However, the type of ethical concerns described above did not yet seem to have an important place on the agenda of the synthetic biology community. Entering into a dialogue without being aware of fundamental differences in ethics is rather pointless. Unless the deeper background and significance of statements is well understood, including one's own statements, there is a risk of total non-communication. Therefore, a more structured analysis of ethical concerns will be helpful in stimulating this debate within the research community and between scientists and representatives of the broader public (NGOs), and politicians.

With respect to understanding the ethics of synthetic biology, it is useful to mention (at least) two types of ethics: consequentionalism and deontology.

Consequentialism (the ethics of consequences) concerns the assignment of instrumental values. From a consequentialist perspective, a morally right action is one that produces good consequences. From a consequentialistic point of view, synthetic biology will not really differ from genetic engineering or any other technology, except that synthetic biology offers new opportunities, and applications will be considered on a case-by-case basis. In science, consequentialist arguments often prevail.

Deontology (the ethics of principles) concerns the assignment of intrinsic values. Decisions should be made solely or primarily by considering one's duties and the rights of others. Deontology posits the existence of a priori moral obligations, further suggesting that people ought to live by a set of permanently defined principles that do not

change merely as a result of a change in circumstances. One of the most important implications of deontology is that praiseworthy goals can never justify immoral actions; the ends do not justify the means. The question is not which type of ethics is correct. Both exist and both are valid.

One of the difficulties in applying the deontology approach to synthetic biology is the fluidity of the boundaries between conventional breeding, 'classical' mutation technologies (chemical, radiation), genetic engineering and synthetic biology. Consequently, the boundary between 'natural' and 'artificial', between 'life' and 'machine' is fluid too. Computer algorithms and programs that process and maintain their informational heritage on the material basis of silicon, fulfill the criteria of the key features of life, and are termed "artificial life". However, it should be noted that in a more stringent definition, these programs are simulating life but do not actually live. This is due to the information being structural and the limited or missing congruency of matter. The limitation on the capability of creating higher orders of life lies in the matter itself. Silicon, an element, having a crystalline structure is not able to resemble information in its own structure. Only carbon provides sufficient molecular diversity so that structural information and matter can exist in an intrinsic marriage that is self sufficient for forming life. Carbon has the greatest flexibility and highest number of possible combinatorial molecular self-arrangements as well as a great variety of arrangements with hetero-atoms in forming hetero-molecules at temperatures where the solvent water is in its liquid form.

However, what would the biological definition of carbon-based life be if the information maintaining molecule, DNA, could be substituted with a different molecular species? The possibility of a totally artificial carbon based life without any resemblance to existing natural organisms arises from different assumptions.

Bhutkar proposes not only a clear articulation of the instrumental and intrinsic value, but also to expand the universe one step at a time (Bhutkar, 2005). This means being mindful of the fact that at higher levels of integration (moving up from biological parts to biological systems), there are additional unknowns. Researchers should be careful in proceeding to the next level of classification until the component parts at each level of classification are well characterized and their impact is known.

6 Conclusions and points of interest

The key objective of this exploratory paper was to investigate whether synthetic biology is a development that needs further examination for its scientific and technological significance and its potential impact on society. This was done by presenting a picture of the characteristics, key players, (potential) applications and future expectations, as well as the possible ethical, legal and social implications of synthetic biology. An impression of the characteristics of synthetic biology was obtained from a review of the scientific literature and participation in the 2nd International Synthetic Biology Conference in Berkeley, May 2006. The question focused on the 'newness' of the technology, and fundamental changes in views of biology and life, that is a paradigm shift. An impression of the assumed capabilities of the technology was created by presenting current developments in synthetic biology, including an overview of applications and products and an analysis of future expectations, including an estimate of their probability. A brief analysis of the driving forces, the structure and dynamics of the synthetic biology community – scientists, institutions, companies, authorities, and NGOs – explored where decisions are taken about research programs, funding, legislation and acceptance by end users. Social, ethical, and legal issues that may play a role in furthering the technology and supporting science, or that may trigger social and political debate were also investigated. Finally, thirty years of experience with technological, social and legal developments in genetic engineering was used to formulate suggestions for next steps, which could be translated in future European activities.

Characterization of synthetic biology

There is no doubt that the involvement of several new, non-biological scientific and engineering disciplines is what clearly distinguishes synthetic biology from genetic engineering and 'classical biology'. So far genetic modification has, to a certain extent, operated in the context of, and was limited by, what 'nature' has to offer in terms of genetic material, cells and organisms. By contrast, synthetic biology applies engineering principles to biology, which allows for the *intended design* of completely new biomolecular systems by *modulation*. In other words: synthetic biology represents a shift from *genetic modification* to *genetic* modulation, or from *molecular biology* to *modular* biology. The introduction of the modular approach in biology implies fundamental changes in the type of questions that are sup-

posed to be asked in biology, how these are structured and the answers probed for. Therefore, we can speak of a paradigm shift.

Although the outlines of the field have yet to become distinct, synthetic biology could be best described as a relatively new field of technology, which aims for the engineering of biological components and systems that do not exist in nature and the re-engineering of existing biological elements. The intentional design of artificial biological systems is key to this technology, which includes the design and synthesis of genes, of mechanisms for genetic regulation, translation, and replication. The objective is to design and synthesize synthetic and artificial (metabolic) pathways and biomolecules, which can be produced by partly synthetic or completely artificial organisms.

Synthetic biology can be viewed as the meeting point of two cultures in molecular biology: 'deconstructing life' and 'constructing life'. Or, drawing a parallel with nanotechnology: top-down and bottom-up approaches. The minimal genome, that is the smallest set of genes an organism needs to live in a particular environment, is an example of the 'deconstructing life' approach, using genomics tools to find out how biological systems function. The Biobricks initiative, which distinguishes three levels of building blocks for constructing synthetic parts, devices and systems, is an example of the 'constructing life' approach. It is an approach that is typical for electrical engineering, that is the well-defined design of electric circuits. The two approaches are complementary. In other words, synthetic biology is the merging of approaches in genetic engineering and electrical engineering.

Synthetic biology evolves with the development of many other technologies and scientific disciplines, such as systems biology, nanobiotechnology, and biocomputing. Moreover, in terms of application, synthetic biology will converge with other technologies (NBIC). Therefore, boundaries are fluid.

Current developments in synthetic biology

Synthetic biology is at an early stage of development, which makes it hard to predict how and to what extent the technology will be applied in the (near) future. At present, most work is being done on applications that contain synthetic genes or pathways, which are still close to natural systems and quite similar to genetic modification. Several scientists stress that there are many technological, legal and commercial obstacles that must be overcome before the practical applications of the technology can be realized. Completely synthetic or artificial organisms are still science fiction. Nevertheless, the ever-increasing speed of gene analysis and gene synthesis will decrease their costs and availability, which will speed up the recently started learning

curve. With reasonable certainty, it can be assumed that within the next ten years, biological technologies will be applied to develop new, (partly) synthetic biological systems that can operate in contained environments.

The synthetic biology community and its dynamics

The synthetic biology community consists predominantly of natural scientists and technologists from different disciplines, most of them based in the USA. Prestigious universities and institutes such as the Massachusetts Institute of Technology (MIT), the California Institute of Technology (CalTech), the Lawrence Berkeley National Laboratory, the J. Craig Venter Institute, and the Harvard Medical School are leading lights. At the heart of the Synbio community is the BIO FAB Group, a group of people from eight US universities. A European Synbio community does not seem to exist yet. At present, most European scientists and research institutes that are involved in synthetic biology research consider their activities an extension of systems biology, of applied research such as cancer research, or of technologies such as nano(bio)technology, and genetic engineering.

The vast majority of research in the US and in Europe is financed by public funding. At this stage, commercial involvement concentrates on the development of supporting technologies, such as DNA synthesis and biocomputing. Potential end users of the technology have yet to see any tangible results.

The action Group on Erosion, Technology and Concentration (ETC), a Canada based international group that is dedicated to technology and human rights issues, seems one of the few activist NGOs that is critically watching what is going on in synthetic biology. The ETC Group was probably one of the initiators of a letter sent to the organizers of the Berkeley Conference, in which 35 NGOs protested against a self-regulation initiative of the scientific community. The NGOs' protest clearly elaborates on their protest against genetic engineering.

Social, ethical, and legal aspects

The present emphasis put on risks and self-governance by the scientific community is reminiscent of the Asilomar Conference in 1975. It is the same strong liberty-based, rights theory approach within the field, stressing individual autonomy, freedom and creativity of research as a free speech act, which makes the scientific community allergic for government interference. Nevertheless, the organizers of the *Synthetic Biology 2.0* conference in Berkeley were sensitive to critical comments of various participants and the NGO letter. This has most likely contributed to the decision not to vote on a common statement on the third day of the conference in May 2006.

As in 1975, the scientific debate on risks related to synthetic biology is focused on the question as to whether the current approaches to the risk assessment of genetically modified organisms are adequate. For the near future, risk analysis and risk management systems developed for bioengineered organisms may still apply. Gradually, as the organisms will contain more synthetic components, the familiarity principle may no longer apply.

At this stage, the risk debate is also focused on health risks. Ecologists do not yet play a role in the present debate.

There are also two distinct differences with the situation in 1975. Thirty years after Asilomar, in the age of terrorist threats, the scientific community seems far more aware of the necessity to include biosecurity issues. Biosecurity issues related to synthetic biology are perceived differently in the US and Europe. In the US, worries about the abuse of synthetic biology have led to far reaching proposals for biosecurity management, like the establishment of a National Science Advisory Board for Biosecurity. Moreover, there is an undefined awareness of ethical and social aspects, which focuses on the accountability of synthetic biologists. The scientific community tends to translate this accountability into internal codes of conduct.

Points of interest

Synthetic biology implies a paradigm shift from molecular to modular biology. It is important that the government monitors and guides this development in an appropriate way. The gradual societal embedding of synthetic biology demands that a set of activities is timely organised. Below, various elements are listed that need attention.

1. **Monitor scientific and technological developments**
   To guide the new terrain of synthetic biology from a societal perspective it is necessary to monitor developments in the field of science and technology, as well as its related social debate. Pay attention to claims, promises and perceived threats, and try to do a reality check.

2. **Study scientific and social effects**
   The introduction of the modular approach in biology implies fundamental changes in the type of questions that are supposed to be asked in biology, how these are structured and the answers probed for. The impact that such changes may have on science and society needs further scientific, social and political attention.

3. **Study risks**
   The nature of health and environmental risks associated with (partly) synthetic organisms to which the familiarity principle no

longer applies and the strategies for managing those risks, clearly needs more study. Those studies should provide the information that is needed to define a) what type of organism (or replicable entities) is still familiar and can be considered 'bioengineered' in terms of regulation, b) how the risks of non-familiar synthetic organisms should be assessed, and c) what effective management strategies are, including technological options, to minimize risks.

4. **Consider social and ethical aspects**
   Social and ethical issues will play an important role in the public and political acceptance of the technology. The present scientific debate is strongly dominated by natural scientists. In this setting, there is a risk of the social and ethical issues being neglected or insufficiently understood. Integration of social scientists (ethicists, philosophers, sociologists, economists, legal experts, etcetera) in this debate could improve mutual understanding of technological and sociopolitical phenomena, and thereby improve internal decision making in the scientific community and communication with non-science stakeholders. In such a setting, the ethical and social impact of synthetic biology can be given the serious consideration required for a balanced approach and societal success.

5. **Involve social organizations and politicians**
   At the same time, in modern society stakeholders other than scientists are demanding to be involved in the R&D decision-making process. It is important to give NGOs and politicians a constructive role in the debate by providing them with adequate information and by supporting capacity building that enables them to reflect on the pros and cons of developments in synthetic biology in a systematic way. This debate should not only focus on the consequences of the technology, but should also include the research agenda.

6. **Involve ecologists**
   Such as in genetic modification, ecological aspects also play an important role in the case of synthetic biology. This will certainly be one of the major issues societal organizations will focus on. It is therefore important to involve ecologists actively in the debate and research programs on synthetic biology.

7. **Public communication**
   Although it seems too early to seek public participation, the process of communication with the public has to be started at this stage. The public (i.e., the media) has to be provided with balanced information and public attitudes need to be carefully monitored, preferably by using qualitative methods, such as panel discussions.

8. Regulation

Regulatory issues need to be identified and probably improved in view of three main criteria: do regulations a) allow for scientific development and technological innovation, b) cover real risks sufficiently, and c) create sufficient trust among public and politicians.

Self-regulation is a flexible mechanism of regulation that seems most appropriate for new technologies in an early and uncertain stage of development, when there are still many unknowns. Meanwhile, the same unknowns can be linked to 'potential risks', which is why others will argue in favor of government regulation. Yet, it remains to be seen whether and how existing regulation will apply to synthetic biology. Further reflection is needed on a smart combination of self-regulation and government regulation.

9. Discuss how to protect intellectual property

The present patent systems risks hindering scientific development in synthetic biology, and it therefore needs to be discussed in terms of a) the applicability of patents at different levels of integration (parts-devices-systems) and b) alternatives that guarantee open access for research.

10. International network

The developing international network of scientists and other stakeholders interested in the socioeconomic, ethical, ecological and political consequences of the developments in synthetic biology needs support from public authorities.

11. Bring knowledge and people together

For several reasons not all research activities in the field of synthetic biology are actually considered synthetic biology. This seems to apply to European research activities in particular. In contrast to the United States, there is no European synthetic biology community yet. The existence of a synthetic biology community may create the critical mass that is a prerequisite for a) furthering the technology, b) the societal reflection on the technological development and c) developing specific policies for synthetic biology. Creating opportunities for the emergence of such a community in Europe is a prerequisite for the development of effective policies. A first advisable step is to organize a meeting for relevant stakeholders in an exploratory setting.

References

Aldhous, P. (2006). Redesigning Life: Meet the Bio-hackers. In: *New Scientist*, May 20, 2006.

Anderson, J.C., *et al.* (2004). An Expanded Genetic Code with a Quadruplet Codon. In: *Proceedings National Academy of Sciences USA*, May 11, 2004, Vol. 101, No. 20, pp. 7566–7571.

Arnold, F.H. (2006a). 2nd International Synthetic Biology Conference, 20&21 May 2006, Berkeley: http://webcast.berkeley.edu:8080/ramgen/events/rssp/SynthBio_Arnold.rm

Arnold, F.H. (2006b). http://www.che.caltech.edu/groups/fha/Enzyme/directed.html; website visited August 2006.

Atkinson, Richard C., *et. al.* (2003). Intellectual Property Rights: Public Sector Collaboration for Agricultural IP Management. In: *Science*, July 11, 2003, Vol. 301, pp. 174–175.

Azonano (2006). www.azonano.com/details.asp?ArticleID=1207; website consulted on July 19, 2006.

Bain, J.D., *et al.* (1992). Ribosome-mediated Incorporation of a Non-standard Amino Acid into a Peptide through Expansion of the Genetic Code. In: *Nature*, Vol. 356, pp. 537–539.

Baltimore, D. (2006). 2nd International Synthetic Biology Conference, 20&21 May 2006, Berkeley: http://webcast.berkeley.edu:8080/ramgen/events/rssp/SynthBio_Baltimore_perspective.rm

Baker, D. *et al.* (2006). Engineering Life. Building a FAB for Biology. In: *Scientific American*, June 2006, pp. 44–51.

Ball, P. (2004). Starting from Scratch. In: *Nature*, Vol. 431, 624–626.

Ball, P. (2005). Synthetic Biology for Nanotechnology. In: *Nanotechnology*, January 1, 2005, Vol. 16, pp. 1–8.

Bayer, T.S., *et al.* (2005). Programmable Ligand-controlled Riboregulators of Eukaryotic Gene Expressions. In: *Nature Biotechnology*, Vol. 23. pp. 337–343.

Bell, D.G. (2006). Metabolomics, Modeling and Machine Learning in

Systems Biology – towards an Understanding of the Languages of Cells. In: *FEBS Journal*, Vol. 273, pp. 873–894.

Bhutkar, A. (2005). Synthetic Biology. Navigating the Challenges Ahead. In: *Journal of Biolaw & Business*, Vol. 8, No. 2.

BioBricks Foundation (2006). BioBricks Foundation. Our Goals. http://openwetware.org/wiki/The_BioBricks_Foundation:Our_Goals

Biojudiciary.org (2006). http://biojudiciary.org/subpage.asp?tid=125; website visited August 2006.

Brent, R. (2004). A Partnership between Biology and Engineering. In: *Nature Biotechnology*, Vol. 22, No. 10, pp. 1211–1214.

Broothaerts, W. *et al.* (2005). Gene Transfer to Plants by Diverse Species of Bacteria. In: *Nature*, February 10, 2005, Vol. 433, pp.629–633.

Brown, C. (2004). BioBricks to Help Reverse-engineer Life. In: *EE Times*, November 6, 2004, http://www.eetimes.com/news/latest/showArticle.jhtml;jsessionid=N5JMVBXM10VLSQSNDBC-CKHSCJUMEKJVN?articleID=21700333&_requestid=342683

CAMBIA & IRRI (2005). *Open Source Biotechnology Alliance for International Agriculture. Mapping the Patent Maze to Forge a Shared Research Toolkit*. December 7, 2005, http://www.bios.net/daisy/bios/1374/version/live/part/4/data

Carina, D. (2004). Biologists Launch 'Open-source movement'. In: *Nature*, September 30, 2004, Vol. 431, pp. 494–494.

Carlson, R. (2003). The Pace and Proliferation of Biological Technologies. Biosecurity and bioterrorism. In: *Biodefense Strategy, Practice and Science*, Vol. 1, No. 3, pp. 1–12.

Castle, L.A, and M.W. Lassner (2004). A New Strategy for Tolerant Crop Plants. In: *ISB News Report*, September 2004, pp. 6–8, http://www.isb.vt.edu/news/2004/news04.sep.html#sep0403

CBD (1992). *Convention on Biological Diversity, Convention Text, Article 2: Use of terms*. http://www.biodiv.org/convention/articles.shtml?lg=0&a=cbd-02

CDC (1999). *Biosafety in Microbiological and Biomedical Laboratories*, U.S. Department of Human Health and Human Services, Public Health Service, Centers for Disease Control and Prevention and National Institutes of Health, Fourth Edition, April 1999, http://www.cdc.gov/od/ohs/pdffiles/4th%20BMBL.pdf

Cello, J. *et al.* (2002). Generation of Infectious Virus in the Absence of Natural Template. In: *Science*, Vol. 297, pp. 1016–1018.

Chilton, M. (2005). Adding Diversity to Plant Transformation**.** In: *Nature Biotechnology*, March 1, 2005, Vol. 23, pp. 309–310.

Cho M.K. *et al.* (1999). Ethical Considerations in Synthesizing a Minimal Genome. In: *Science*, 10 December 10, 1999, pp. 2087–2090.

COGEM (2006). *Synthetische biologie. Een onderzoeksveld met voortschrijdende gevolgen*. COGEM signalering CGM/060228-03, February 28, 2006. http://www.cogem.net/pdfdb/advies/CGM060228-03.pdf

Csaba, P., *et al.* (2006). Chances and Necessity in the Evolution of Minimal Metabolic Networks. In: *Nature*, Vol. 440, pp. 667–670.

Dae-Kyun, R., *et al.* (2006). Production of the Antimalarial Drug Precursor Artemesinic Acid in Engineered Yeast. In: *Nature*, April 13, 2006, Vol. 440, pp. 940–943.

Deamer, D. (2005). A Giant Step towards Artificial Life. In: *Trends in Biotechnology*, July 7, 2005, Vol. 23, pp. 336–338.

Delmonte, C.S. and L.R.B. Mann, (2003). Variety in DNA Tertiary Structure. In: *Current Science*, December 10, 2003, Vol. 85 (11), pp. 1564–1570, http://www.ias.ac.in/currsci/dec102003/1564.pdf

Diamond v. Chakrabarty, 447 U.S. 303 (1980), US Supreme Court, http://caselaw.lp.findlaw.com/scripts/getcase.pl?court=US&vol=447&invol=303

Endy, D. (2005). Foundations for Engineering Biology. In: *Nature*, November 24, 2005, Vol. 438, pp. 449–453.

ETC (2004). *Nanotech News in Living Colour. An Update on White Papers, Red Flags, Green Goo (and Red Herrings)*. ETC Group Communiqué May/June 2004 Issue # 85, http://www.etcgroup.org/article.asp?newsid=470

ETC (2006). *Global Coalition Sounds the Alarm on Synthetic Biology, Demands Oversight and Societal Debate*. News Release, May 19, 2006, http://www.etcgroup.org/documents/NR%20Synthetic%20Bio%2019th%20May%202006.pdf

European Commission (2005a). *Synthetic Biology. Appling Engineering to Biology, Report of a NEST High-Level Expert Group*. EUR 21796, http://www.univ-poitiers.fr/recherche/documents/pcrdt7/syntheticbiology.pdf

European Commission (2005b). *Reference Document on Synthetic Biology, 2005/2006-NEST-PATHFINDER Initiatives*. October 2005, http://www.eurosfaire.prd.fr/nest/documents/pdf/refdoc_synbio_oct2005.pdf

European Patent Office (1991). *Convention on the Grant of European Patents of October 1973, Text as Amended by the Act Revising Article 63 EPC of December 17, 1991*. http://www.european-patent-office.org/legal/epc/e/ar53.html

European Patent Office (2003). *The Industrial Applicability Requirement*. March 11, 2003, http://listbox.wipo.int/wilma/scp-eforum/2003/msg00014/INDAPP2.doc

European Science Foundation (2005). *ESF LESC Exploratory Workshop – Deconstructing Life: Synthetic Biology in Biocatalysis and Biodegradation*. Avila, Spain, October 13–16, 2005, http://www.esf.org/articles/477/04068Programme0.pdf

Forum Genforschung (2005). *Synthetic biology, Fact Sheet*. Swiss Academy of Sciences, http://www.geneticresearch.ch/d/themen/documents/FactSheet_def_e.pdf

Galperin, M.Y. (2006). Genomics Update. The Minimal Genome Keep Growing. In: *Environmental Microbiology*, Vol. 8, No. 4, pp. 569–573.

Glass J.I. *et al.* (2006). Essential Genes of a Minimal Bacterium. In: *Proceedings National Academy of Sciences USA*, January 10, 2006, Vol. 103, No. 2, pp. 425–430.

Goler, J.A. (2004). *BioJADE: A Design and Simulation Tool for Synthetic Biological Systems*, T.F.K. Jr. Editor, Massachusetts Institute of Technology, Cambridge, Massachusetts.

Herper, M. (2006). The Biggest DNA Ever Made. In: *Forbes*, 07.13.06. http://www.forbes.com/2006/07/12/dna-artificial-genes-codon-cz_mh_0713codon.html?partner=biotech_newsletter

Hutchison III, C.A., *et al.* (1999). Global Transposon Mutagenesis and a Minimal Mycoplasma Genome. In: *Science*, December 10, 1999, Vol. 286, pp. 2165–2169, http://www.syntheticgenomics.com/pdf/MinimalMycoplasma.pdf

IDC (2006) Organization for International Dialogue and Conflict Management: http://www.idialog.eu/Projects/Unterverzeichnis/ProjectsSBE.htm; website visited August 2006.

iGEM (2005). *Project summaries*. http://parts.mit.edu/wiki/index.php/Igem_2005

Isaacs, F.J., *et al.* (2004). Engineered Riboregulators Enable Post-transcriptional Control of Gene Expression. In: *Nature Biotechnology*, Vol. 22, pp. 841–847.

Isaacs, F.J., *et al.* (2006). RNA Synthetic Biology. In: *Nature Biotechnology*, Vol. 24, pp. 545–554.

J. Craig Venter Institute (2006). *Synthetic Biology*. http://www.venterinstitute.org/research/

Kanellos, M. (2004). Gates Foundation to Promote Synthetic Biology. In: *CNET News*, December 13, 2004, http://news.com.com/Gates+foundation+to+promote+synthetic+biology/2100-1008_3-5489245.html

Knight T.F. (2005). Engineering Novel Life. In: *Molecular Systems Biology,* Vol. 1, September 13, 2005, http://www.nature.com/msb/journal/v1/n1/full/msb4100028.html

Kobayashi, K. (2003). Essential Bacillus Subtilis Genes. In: *Proceedings National Academy of Sciences USA,* April 15, 2003, Vol. 100(8), pp. 4678–4683.

Koerber, J.T. *et. al.* (2006). Construction of Diverse Adeno-associated Viral Libraries for Directed Evolution of Enhanced Gene Delivery Vehicles. In: *Nature Protocols,* Vol. 1, pp. 701–706.

Kuhn, T.S. (1962). *The Structure of Scientific Revolutions*, University of Chicago Press, 1962.

Lai, K. *et. al.* (2004). The Sonic Hedgehog Signaling System as a Bistable Genetic Switch. In: *Biophysical Journal*, Vol. 86, pp. 2748–2757.

Leitenberg, M. (2005). *Assessing the Biological Weapons and Bioterrorism Threat*. December 2005, http://www.strategicstudiesinstitute.army.mil/pdffiles/PUB639.pdf

Levskaya, A., *et al.* (2005). Engineering *Escherichia coli* to See Light. In: *Nature* Vol. 438, pp. 441–442.

LBNL, Lawrence Berkeley National Laboratory (2006). http://www.lbl.gov/pbd/synthbio/default.htm; website visited August 2006.

Lorenzo de, V. *et al.,* (2006). Synthetic Biology. Challenges Ahead. In: *Bioinformatics*, Vol. 22, No.2, pp. 127–128.

Maurer, S.M., K.V. Lucas & S. Terrell (2006). *From Understanding to Action. Community-Based Options for Improving Safety and Security in Synthetic Biology*. University of California, Berkeley, April 10, 2006.

MedGadget (2006). The internet journal of emerging medical technologies, August 4, 2006: www.medgadget.com/archives/2006/08/the_synthetic_b.html; website visited August 2006.

Morton, O. (2005). Life, Reinvented. In: *Wired Magazine*, Issue 13.01, January 2005, http://www.wired.com/wired/archive/13.01/mit_pr.html

Newman, S.A. (2002). The Human Chimera Patent Initiative. In: *Lahey Clinic Medical Ethics Journal,* Vol. 9, Issue 1, p. 4, Winter 2002, http://www.lahey.org/Pdf/Ethics/Winter_2002.pdf

Nordmann, A. (2004). *Converging Technologies. Shaping the Future of European Societies*. HLEG, Foresighting the New Technological Wave, European Commission, http://ec.europa.eu/research/conferences/2004/ntw/pdf/final_report_en.pdf

NSABB, (2006): http://www.biosecurityboard.gov/; website visited August 2006.

Pearson, H. (2006). Genetics: What is a Gene? In: *Nature*, May 2, 2006, Vol. 441, pp. 398–401.

Pennisi, E. (2005). Synthetic Biology Remakes Small Genomes. In: *Science*, November 4, 2005, Vol. 310. no. 5749, pp. 769–770.

Pfleger, B.F. *et. al.* (2006). Combinatorial Engineering of Intergenic Regions in Operons Tunes Expression of Multiple Genes. In: *Nature Biotechnology*, July 26, 2006, Vol. 24, pp. 1027-1032.

Rabinow, P. (2005). *Regulating Biology. From the Cold War to Vigilance*. August 19–20, 2005, University of Califormia, San Francisco, http://63.251.167.36/nakfi/progressive/Life%20Engineering/rabinow/rabinow.html

Rasmussen, S., *et al.* (2004). Transitions from Nonliving to Living Matter. In: *Science*, February 13, 2004, Vol. 303, pp. 963–965.

Ro, D., *et. al.* (2006). Production of the Antimalarial Drug Precursor Artemisinic Acid in Engineered Yeast. In: *Nature*, April 13, 2006, Vol. 440, pp. 940–943.

Schmidt, M. (2006). Public will Fear Biological Accidents, Not Just Attacks. In: *Nature*, June 29, 2006, Vol. 441, p. 1048.

Shorett, P. (2002). *Of Transgenic Mice and Men*. In: *Genewatch* Vol. 15, No. 5, September 2003, Council for Responsible Genetics, http://www.gene-watch.org/genewatch/articles/15-5mice.html

Sismour, A.M., *et al.* (2004). PCR Amplification of DNA Containing Non-standard Base Pairs by Variants of Reverse Transcriptase from Human Immunodeficiency Virus-1. In: *Nucleic Acid Research*, Vol. 32, No. 2, pp. 728–735.

Sismour, A.M., *et al.* (2005). The Use of Thymidine Analogs to Improve the Replication of an Extra DNA Base Pair. A Synthetic Biological System. In: *Nucleic Acid Research*, Vol. 33, No. 17, pp. 5640–5646.

Stephens, C. (2003). Madey v. Duke University. Federal Circuit Sets Limitations on the Common Law Experimental Use Exemption. In: *Baker Botts Intellectual Property Report*, July 7, 2003, http://www.imakenews.com/bakerbotts/e_article000166656.cfm

Synbiology (2005). *SYNBIOLOGY. An Analysis of Synthetic Biology Research in Europe and North America*. European Commission Framework Programme 6 Reference Contract 15357 (NEST), October 2005, http://www2.spi.pt/synbiology/documents/SYNBIOLOGY_Literature_And_Statistical_Review.pdf

Synbiology (2006). *Recommendations for Stronger Synthetic Biology Research, Synbiology*. Brussels, May 30, 2006, http://www2.spi.pt/synbiology/meetings.asp

Synthetic Biology Engineering Research Center: www.synberc.org.

Szatmary, E. (2003). Why are there Four Letters in the Genetic Alphabet? In: *Nature Reviews*, Vol. 4, pp. 995–1001.

Tucker, J.B., and R.A. Zilinskas (2006). The Promise and Perils of Synthetic Biology. In: *The New Atlantis*, Number 12, Spring 2006, pp. 25–45, http://www.thenewatlantis.com/archive/12/tuckerzilinskas.htm

Tumpey, T.M., *et al.* (2005). Characterization of the Reconstructed 1918 Spanish Influenza Pandemic Virus. In: *Science*, October 7, 2005, Vol. 310, pp. 77–80.

Venter, G.J. (2006). 2nd International Synthetic Biology Conference, 20&21 May 2006, Berkeley: http://webcast.berkeley.edu:8080/ramgen/events/rssp/SynthBio_Venter.rm

Vlaggraduateschool (2006). http://www.vlaggraduateschool.nl/courses/prot-engi.htm; website visited August 2006.

Voigt, C.A., *et al.* (2005). Programming Cellular Function. In: *Nature Chemical Biology*, Vol. 1, pp. 304–307.

Woese, C.R. (2004). A New Biology for a New Century. In: *Microbiology and Molecular Biology Reviews*, June 2004, Vol. 68, No. 2, p. 173–186.

Wright, S. (2001): http://www.biotech-info.net/legitimating.html; website visited August 2006.

Wu, Y., *et al.* (2000). Efforts toward Expansion of the Genetic Alphabet. Optimization of Interbase Hydrophobic Interactions. In: *Journal of the American Chemical Society*, Vol. 122, No. 32, pp. 7621–7632.

Yang J, *et. al.* (2005). High Yield Recombinant Silk-like Protein Production in Transgenic Plants through Protein Targeting. In: *Transgenic Research*, 14, pp. 313–324.

Yokobayashi, Y., *et al.* (2002), Directed Evolution of a Genetic Circuit. In: *Proceedings National Academy of Sciences USA*, Vol. 99, No. 26, pp. 16516–16518.

Zilinskas, R.A. (2006). 'Technical Barriers to Successful Biological Attacks with Synthetic Organisms'. *Security and Regulation of Experiments of Concern. A White Paper on the Ethics of Self-Governance in New Scientific Community*, Appendices pp. 8–24. Berkeley, Spring 2006, http://gspp.berkeley.edu/iths/UC%20White%20Paper.pdf

Zoloth, L. (2006). 'Ethical Issues in Synthetic Biology'. *Security and Regulation of Experiments of Concern. A White Paper on the Ethics of Self-Governance in New Scientific Community*, Appendices pp. 25–37, Berkeley, Spring 2006, http://gspp.berkeley.edu/iths/UC%20White%20Paper.pdf

Appendices

Appendix 1:
EU funded projects in synthetic biology

(European Commission, 2005b)

Project	*Participants*	*Characteristics*
Specific Targeted Research Projects (STREP)		
EUROBIOSYN	▪ Institute of Process Engineering, Bioprocess Lab, ETH Zurich ▪ Institute of Technical Biochemistry, Bioinformatics, Stuttgart University ▪ Department of Chemical Engineering, Technical University of Denmark ▪ Centro Nacional de Biotechnologia, Madrid	A modular platform for biosynthesis of complex molecules, engineering metabolic pathway – glycome
HYBLIB	▪ German Cancer Research Center ▪ Eucodis, Austria ▪ Academy of Sciences of the Czech Republic ▪ French Institute of Health and Medical Research ▪ Göttingen University	Human monoclonal antibodies from a library of hybridomas
NANOMOT	▪ Max-Planck-Institut für biophysikalische Chemie ▪ Universities of Osnabrück, Dresden, Oxford, Basle, ▪ ETH Zurich ▪ Consejo Superior de Investigaciones Científicas, Madrid	Synthetic Biomimetic Nanoengines: A Modular Platform for Engineering of Nanomechanical Actuator Building Blocks
NEONUCLEI	▪ University of Southampton ▪ Lund University ▪ Chalmers University of Technology, Sweden ▪ University of Coimbra ▪ Ludwig-Maximilians University	Self-assembly of synthetic nuclei: key modules for semibiotic chemosynthetic systems
NETSENSOR	▪ European Molecular Biology Laboratory, Germany ▪ Cellectis, France ▪ Medical School Hannover ▪ Centro Nacional de Investigaciones Oncológicas, Spain	Design and engineering of gene networks to respond to and correct alterations in signal transduction
ORTHOSOME	▪ Laboratory for Medicinal Chemistry, Catholic University Leuven ▪ Unknown partners	An orthogonal episome: An artificial genetic system based on a novel type of nucleic acids
PROBACTYS	▪ Helmholtz Center for Infection Research, Germany ▪ Unknown partners	Programmable bacterial catalysts
BIOMODULARH2	*	H2 production pathway

Project	*Participants*	*Characteristics*
Specific Targeted Research Projects (STREP)		
BIONANO-SWITCH	*	Molecular switch for biosensing
CELLCOMPU	*	Cell communication network
COBIOS	*	Synthetic oscillator network, insulin level
FUSYMEM	*	Synthetic membrane coupled biosensor
SYNTHCELLS	*	Synthetic minimal cells
Specific Support Actions		
SynBioComm	▪ ETH Zurich	Towards a European Synthetic Biology Community Organization of the 3rd International SynBio Conference in Zurich, 2007
SYNBIOLOGY	▪ Sociedade Portuguesa de Inovação (SPI), Portugal ▪ ATG: Biosynthetics, Germany ▪ Center for Economic Research and Environmental Strategy, Greece ▪ University of Maryland Baltimore County (UMBC), US	Analysis of the field, stakeholders, activities, funding and support services
SYNBIOSAFE	▪ IDC, Austria ▪ Austrian Academy of Sciences, Institute of Technology Assessment ▪ University of Zurich, Ethics Center, Chair of Biomedical Ethics ▪ Isthmus SARL, France	Fact-finding mission on safety and ethics in synthetic biology, contribution to the "inaugural" Conference on Synthetic Biology in Zurich, an open e-forum and an international workshop
TESSY	▪ ATG: Biosynthetics, Germany*	Synthetic biology roadmap,
Coordination Actions		community strategy
EMERGENCE	*	Setting the basis for a synthetic biology community (networking, education and training, infrastructure)

* These projects were evaluated by the European Commission shortly before this paper was drafted. No detailed information about the participants could be obtained

Appendix 2:
Overview of the international synthetic biology community

(Synbiology, 2005)

Field	*Area*	*Sub area*	*Number of scientists*		
			US	*Europe*	*other*
Concepts and political strategy			17	7	1
Theoretical bio-engineering in vivo	Modeling natural systems	Genome, proteome, metabolome analysis	5	-	-
		Analysis and modeling molecular networks	2	13	3
		Metabolic profiling	10	7	2
	Modeling synthetic systems	Design principles systems and networks	7	2	4
Biological inspired nanotechnology in vitro	Biomimetics, nanobionics, evolutionary nanotechnology	Computation using biological components and principles	6	2	4
		Self-assembling, biomimetic, biomaterials, bioelectronics	10	3	1
		Single molecule manipulation, measurement	2	1	-
		Reporters, sensors	4	2	1
		Molecular machines, actuators, devices	7	-	-
		Artificial life	8	3	-
Practical engineering in vivo	Engineering structural function	Molecular engineering (rational design)	29	7	3
		Artificial evolution (irrational design)	12	6	2
		Semisynthetic design (rational and irrational design)	1	1	-
	Engineering regulatory function	Biochemical or genetic network design	13	5	2
		Riboswitches	5	-	-
	Parts fabrication, characterization, assembly	BioBricks	9	2	-
		Programmable organisms or systems	10	1	-
Applications synthetic biology	Molecular biology analytical methods	Diagnostics	2	-	-
		Microarrays, biochips, microfluidic devices, nanotech analytics	6	1	-
		Computing	1	-	-
	Biomaterials	Biopolymers, colloids, nanoparticles	3	2	-
	Cell technology	Cell culture, transfection	1	-	-
	Bioenergy	Biofuels, biogas, bioelectricity	1	-	-
Products	Small molecular drugs/agents, flavors, biomaterials, (stem) cell cultures, tissues/organs, bioenergy, biomimetics, biocomputer		-	-	-
Other			2	1	-
Total			***173***	***66***	***23***

Appendix 3: Glossary

(RNA) aptamer	An oligonucleic acid or peptide molecule selected from a large random sequence pool to bind to a specific target molecule. Aptamers can be used for both basic research and clinical purposes as macromolecular drugs.
Apoplast (in plants)	The free diffusional space outside the plasma membrane. The apoplast is important for all the plant's communication to its environment.
Artemisinic acid	Anti-malarial drug precursor
BioBricks	Standard biological parts that can be used and assembled to engineer devices that perform specific tasks and which can be used to engineer specific, predesigned biological systems.
Biocatalysis	The utilization of natural catalysts, called enzymes, to perform chemical transformations on organic compounds.
Bioinformatics (computational biology)	The use of techniques from applied mathematics, informatics, statistics, and computer science to solve biological problems.
BioJADE	A graphical design tool built in Java that provides a comprehensive, extensible design and simulation platform for synthetic biology.
Biomimetics (also known as bionics, biognosis, biomimicry, or bionical creativity engineering)	The application of methods and systems found in nature to study and design engineering systems and modern technology.
BioSPICE	An open source framework and software toolset for systems biology, which is intended to assist biological researchers in the modeling and simulation of spatial-temporal processes in living cells.
Biotechnology (CBD)	Any technological application that uses biological systems, living organisms, or derivatives thereof, to make or modify products and processes for specific use.
Bioremediation	The use of microorganisms, fungi, plants or their enzymes to return the environment altered by contaminants to its original condition.
Cell culture	The process by which either prokaryotic or eukaryotic cells are grown under controlled conditions. In practice the term "cell culture" has come to refer to the culturing of cells derived from multicellular eukaryotes, especially animal cells. The historical development and methods of cell culture are closely interrelated to those of tissue culture and organ culture.

Codon	Trinucleotide unit coding for a single amino acid.
Directed evolution	A method used in protein engineering to harness the power of Darwinian selection to evolve proteins with desirable properties not found in nature. A typical directed evolution experiment involves two steps: 1. Library creation: The gene encoding the protein of interest is mutated and/or recombined at random to create a large library of gene variants. Techniques commonly used in this step are error-prone PCR and DNA shuffling. 2. Library screening: The library is screened by the researcher using a high-throughput screen to identify mutants or variants that possess the desired properties. Winner mutants identified in this way then have their DNA sequenced to understand what mutations have occurred. The evolved protein is then characterized using biochemical methods. The Frances H. Arnold Research Group is one of the most important directed-evolution laboratories.
DNA shuffling	A method for *in vitro* recombination, developed as a technique to generate mutant genes that would encode proteins with improved or unique functionality. It consists of a three-step process: 1. the enzymatic digestion of genes, yielding smaller fragments of DNA; 2. the small fragments are then allowed to randomly hybridize and are filled in to create longer fragments; 3. any full-length, recombined genes that are recreated are amplified via the polymerase chain reaction. If a series of alleles or mutated genes is used as a starting point for DNA shuffling, the result is a library of recombined genes that can be translated into novel proteins, which can in turn be screened for novel functions.
DNA base	An essential building block. DNA contains four complementary bases: adenine, which pairs with thymine, and cytosine, which pairs with guanine.
Endoplasmic reticulum	An organelle found in all eukaryotic cells. It is part of the endomembrane system. The endoplasmic reticulum modifies proteins, makes macromolecules, and transfers substances throughout the cell.
Eukaryote	An organism with a complex cell or cells, in which the genetic material is organized into a membrane-bound nucleus or nuclei. Eukaryotes comprise animals, plants, and fungi – which are mostly multicellular – as well as various other groups that are collectively classified as protists (many of which are unicellular).
Gene	The units of heredity in living organisms, encoded in the organism's genetic material (usually DNA or RNA), and controlling the physical development and behavior of the organism.

Genomics	The study of an organism's genome and the functions of the genes.
Glycolysis	A series of biochemical reactions by which a molecule of glucose (Glc) is oxidized to two molecules of pyruvic acid, which serves two principal functions: generation of high-energy molecules (ATP and NADH), and production of a variety of six- or three-carbon intermediate metabolites, which may be removed at various steps in the process for other intracellular purposes (such as nucleotide biosynthesis). Glycolysis is one of the most universal metabolic processes known, and occurs (with variations) in many types of cells in nearly all types of organisms.
High-throughput screening	A combination of modern robotics, data processing and control software, liquid handling devices, and sensitive detectors, which allows researchers to effectively conduct millions of biochemical, genetic or pharmacological tests in a short period of time.
Hydrogen bond	A type of attractive intermolecular force that exists between two partial electric charges of opposite polarity, which involves a hydrogen atom. Hydrogen bonding plays an important role in determining the three-dimensional structures adopted by proteins and nucleic bases. The double helical structure of DNA is largely due to hydrogen bonding between the base pairs, which link one complementary strand to the other and enable replication.
Kinesin	A class of motor protein dimer found in biological cells. A kinesin attaches to microtubules, and moves along the tubule in order to transport cellular cargo, such as vesicles.
Ligand	An atom, ion, or molecule that generally donates one or more of its electrons through a coordinate covalent bond to, or shares its electrons through a covalent bond with, one or more central atoms or ions. Most commonly the central atom is a metal or metalloid in inorganic chemistry, but ligands are also used in organic chemistry, for example, to protect functional groups (e.g., BH3 as ligand for the protection of phosphines), or to stabilize reactive compounds.
m(essenger) RNA	RNA that encodes and carries information from DNA during transcription to sites of protein synthesis to undergo translation in order to yield a gene product.
Metabolomics	The systematic study of the unique chemical fingerprints that specific cellular processes leave behind. The metabolome represents the collection of all metabolites in a biological organism, which are the end products of its gene expression.

(DNA) microarray (also commonly known as gene chip, DNA chip, or biochip)	A collection of microscopic DNA spots attached to a solid surface, such as glass, plastic or silicon chip forming an array for the purpose of expression profiling, monitoring expression levels for thousands of genes simultaneously.
Minimal genome	The smallest set of genes an organism needs to live in a particular environment.
Nanotechnology	The design, characterization, production and application of structures, devices and systems by controlling shape and size at the nanoscale. Eight to ten atoms span one nanometer (nm).
Nucleotide	The structural units of RNA and DNA: adenine, thymine, cytosine, guanine
Oligonucleotide	A short sequence of nucleotides (RNA or DNA), typically with twenty or fewer bases. Automated synthesizers allow the synthesis of oligonucleotides up to 160 to 200 bases. Oligonucleotides are often used as probes for detecting complementary DNA or RNA because they bind readily to their complements.
PCR (Polymerase Chain Reaction)	A molecular biology technique for enzymatically replicating DNA without using a living organism, such as E. coli or yeast. PCR is commonly used in medical and biological research labs for a variety of tasks, such as the detection of hereditary diseases, the identification of genetic fingerprints, the diagnosis of infectious diseases, the cloning of genes, paternity testing, and DNA computing.
Phospholipids	A class of lipids that are a major component of all biological membranes, along with glycolipids and cholesterol.
Phylogenetics	The study of evolutionary relatedness among various groups of organisms. Phylogeny (or phylogenesis) is the origin and evolution of a set of organisms, usually a set of species.
Polymerase	An enzyme whose central function is associated with polymers of nucleic acids such as RNA and DNA. The most well-known function of a polymerase is the catalysis of the production of new DNA or RNA from an existing DNA or RNA template, a process known as polymerization.
Precursor	A precursor is a substance from which another, usually more active or mature, substance is formed (in biological processes especially metabolism). For instance, certain liver enzymes are precursors to insulin.
Prokaryote	An organism without a cell nucleus (= karyon), or indeed any other membrane-bound organelles, in most cases unicellular (in rare cases, multicellular). Most of the prokaryotes are bacteria.

Protein engineering	The application of science, mathematics, and economics to the process of developing useful or valuable proteins.
Riboswitch	A part of a messenger RNA molecule that can directly bind a small target molecule, and whose binding of the target affects the gene's activity. Thus, an mRNA that contains a riboswitch is directly involved in regulating its own activity, depending on the presence or absence of its target molecule.
RNA (Ribonucleic acid)	A nucleic acid polymer consisting of nucleotide monomers. It is transcribed from DNA by enzymes called RNA polymerases and further processed by other enzymes. RNA serves as the template for translation of genes into proteins, transferring amino acids to the ribosome to form proteins, and also translating the transcript into proteins.
(DNA or genome) sequencing	The process of determining the nucleotide order of a given DNA fragment, called the DNA sequence.
Stem cells	In animals these are primal undifferentiated cells that retain the ability to divide and differentiate into other cell types. Stem cells have the ability to act as a repair system for the body, because they can divide and differentiate, replenishing other cells as long as the host organism is alive.
Synthetic biology (COGEM)	Synthetic biology focuses on the design and synthesis of artificial genes and complete biological systems, while it also focuses on changing existing organisms, with the aim of acquiring useful functions.
Synthetic biology (European Commission)	The engineering of biological components and systems that do not exist in nature and the re-engineering of existing biological elements; it is determined on the intentional design of artificial biological systems, rather than on the understanding of natural biology.
Synthetic biology (Synbio)	1) the design and construction of biological parts, devices and systems, and; 2) the redesign of existing, natural biological systems for useful purposes.
Systems biology	An academic field that seeks to integrate different levels of information to understand how biological systems function. By studying the relationships and interactions between various parts of a biological system (e.g., gene and protein networks involved in cell signaling, metabolic pathways, organelles, cells, physiological systems, organisms, etc.) it is hoped that a comprehensible model of the whole system can eventually be developed.

Transcription	The process through which a DNA sequence is enzymatically copied by an RNA polymerase to produce a complementary RNA. Or, in other words, the transfer of genetic information from DNA to RNA.
transfer RNA	Small RNA chains (74–93 nucleotides) that transfer specific amino acids to a growing polypeptide chain (protein synthesis) at the ribosomal site of the cell.
Vacuole	A membrane-bounded compartment within some eukaryotic cells that can serve a variety of secretory, excretory, and storage functions. Vacuoles are especially conspicuous in most plant cells.

About the author

Huib de Vriend (1958) is an independent consultant on societal issues in Life sciences and innovation. LIS (Life sciences, Innovation & Society) Consult wants to contribute to a constructive dialogue on responsible innovation in Life Sciences between relevant stakeholders. This contribution is made by a balanced analysis of technological developments, societal impacts and policy options for innovation strategies and stakeholder involvement. Huib de Vriend has conducted several studies on technological and regulatory developments, the socioeconomic impacts and public attitudes and public perceptions of biotechnology and genomics and has been involved in several initiatives aiming at debate and constructive dialogue between scientists, public authorities, industry representatives and NGOs.