Synthetic biology: creating opportunities

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Gezondheidsraad
Health Council of the Netherlands

To the Minister of Education, Culture and Science

Subject : Advice *Synthetic biology: creating opportunities*
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Dear minister,

In august 2006 the Health Council of the Netherlands, the Advisory Council on Health Research and the Royal Netherlands Academy of Arts and Sciences received a request from your predecessor to answer some questions on synthetic biology. Herewith we present to you the advice of our organisations.

The committee that prepared this advice concludes that synthetic biology offers opportunities for science and application thereof in biotechnology in the Netherlands. Universities and industry have by now invested in the further development of this scientific area and the technology that may emanate from it. If the Netherlands wishes for synthetic biology to contribute to its knowledge economy, an appropriate investment by the government would be expedient. Ideally, this investment connects with existing initiatives that relate to synthetic biology. Research into ethical, societal and legal issues deserves substantial attention.

The advice was checked by several bodies within our organisations. We endorse the conclusions and recommendations of the committee. Regarding the question on legislation and risk assessment we refer to the advice that the Netherlands Commission on Genetic Modification (COGEM) today presents to the minister of Housing, Spatial Planning and the Environment (VROM).We will also notify the minister of Health, Welfare and Sport, the minister of Economic Affairs and the minister of VROM of our findings.

Sincerely,

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Synthetic biology: creating opportunities

to:

the Minister of Education, Culture and Science

No. 2008/19E, The Hague, September 25, 2008

The Health Council of the Netherlands, established in 1902, is an independent scientific advisory body. Its remit is “to advise the government and Parliament on the current level of knowledge with respect to public health issues...” (Section 22, Health Act).

The Health Council receives most requests for advice from the Ministers of Health, Welfare & Sport, Housing, Spatial Planning & the Environment, Social Affairs & Employment, and Agriculture, Nature & Food Quality. The Council can publish advisory reports on its own initiative. It usually does this in order to ask attention for developments or trends that are thought to be relevant to government policy.

Most Health Council reports are prepared by multidisciplinary committees of Dutch or, sometimes, foreign experts, appointed in a personal capacity. The reports are available to the public.

The Health Council of the Netherlands is a member of the European Science Advisory Network for Health (EuSANH), a network of science advisory bodies in Europe.

The Health Council of the Netherlands is a member of the International Network of Agencies for Health Technology Assessment (INAHTA), an international collaboration of organisations engaged with *health technology assessment*.

The Advisory Council on Health Research (RGO) is part of the Health Council of the Netherlands. Its remit is to advise the Ministers of Health, Welfare and Sport (VWS), Education, Culture and Science (OCW), and Economic Affairs (EZ) on priorities in health research and health services research, and on the technology development in this sector, including the accompanying infrastructure. The basic principle of the RGO is always the societal perspective.

The Royal Netherlands Academy of Arts and Sciences (KNAW) is a scientific society that protects the quality and interests of science. Furthermore, it is an umbrella organisation for 17 scientific institutes.

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Executive summary

Synthetic biology

Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature. This engineering perspective may be added at all levels of the hierarchy of biological structures – from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology will enable the design of "biological systems" in a rational and systematic way. The Committee has used this European consensus definition of synthetic biology in this advisory report. The Committee considers synthetic biology an innovative approach in the life sciences with potential significance for science and society. The advisory report addresses the questions posed by the minister of Education, Culture and Science.

Current status in the Netherlands

Currently, internationally prominent initiatives in this field of research are being developed in the Netherlands. Dutch research focuses on two main directions, both of which have accumulated a large body of expertise over time. One involves metabolic reprogramming of biological systems (*in vivo*, top-down approach) and the other bio-nano-science (*in vitro* approach).

Developments in synthetic biology

Developments in synthetic biology can be classified both by the degree of complexity and by the degree of divergence from nature. Metabolic reprogramming involves experimental systems with a high level of complexity and low divergence from nature. The experimental systems used in bio-nano-science are less complex but are very different from what exists in nature. To date synthetic biology has not yet enabled the construction of fully artificial systems with a high degree of complexity. In fact, many researchers doubt whether it will ever be possible to construct a fully synthetic organism, representative of the highest degree both of complexity and divergence from nature.

Possible significance of synthetic biology

Despite the uncertainties surrounding future developments, synthetic biology is clearly a promising and innovative research area, with potential applications for society. Products arising from synthetic biology can benefit people's health and their quality of life, make medications cheaper and more accessible, and enhance the sustainability of society. In the field of health and quality of life, such products may include live therapeutic agents, biology-based drug delivery systems and sophisticated diagnostic agents. More efficient production platforms could make medicines cheaper and thus more accessible. In the field of sustainability, synthetic biology is focusing on sustainable bio-fuels. Apart from the above applications, which have a direct and tangible impact on people and society, synthetic biology can be applied in areas such as new materials and the establishment of production platforms for fine chemicals. All these potential applications are of interest to the biotechnology industry. For researchers investment in synthetic biology offers the opportunity to successfully compete with the international research community in this field.

Whether synthetic biology can live up to these promises depends on a number of factors. Some of these are external factors which are difficult to influence but which can boost or cut demand for specific products. One example is the combination of decreasing fossil fuel supplies, high oil prices, fears about climate change, and rising demand for food and agricultural land. This generates a need for sustainable production of bio-fuels that does not interfere with food supply. The second factor that will determine the success of synthetic biology is the extent to which society accepts this technology. It is essential to provide people with accurate and balanced information, in order to avoid disproportionate public concern and to curb unrealistic expectations. Similarly, it is important to take society’s concerns into account, in order to establish and maintain confidence in this technology.

Legislation and risk control

The Netherlands Commission on Genetic Modification (COGEM) will advise the minister of Housing, Spatial Planning and the Environment (VROM) on legislation and risk control concerning synthetic biology. Furthermore, the working group *biosecurity* of the KNAW has formulated general rules of conduct.

Recommendations

Synthetic biology offers opportunities to the Dutch knowledge economy, while universities are expanding their existing infrastructure in this area. Therefore, it would make sense for the government to invest in this area of research. Such investment in synthetic biology by the government could very well relate to existing initiatives or plans, such as the Netherlands Genomics Initiative, NanoNed, and the Systems Biology Programme to be launched by the Netherlands Organisation for Scientific Research (NWO). Accordingly, an obvious approach would be to incorporate a sub-programme for synthetic biology into each of these initiatives. Secondly, given the special nature of synthetic biology, it is important to invest in interdisciplinary research and to adapt relevant Master's degree programmes to these new developments. Thirdly, there should be a substantial focus on research into, and communication about, the societal aspects of synthetic biology. The Committee also recommends to, after a given period of time (e.g. five years), survey the Dutch research in the field of synthetic biology in order to assess the need for targeted incentives.

Chapter 1

Introduction

1.1 Background

In August 2006 the then Dutch Minister of Education, Culture and Science (OCW) sent the Royal Netherlands Academy of Arts and Sciences (KNAW), the Health Council of the Netherlands (*Gezondheidsraad*) and the Advisory Council on Health Research (*Raad voor Gezondheidsonderzoek*, RGO) a request for advice with regard to synthetic biology (Annex A). The minister had five questions on this field of scientific activity: (1) What is the current situation with regard to synthetic biology in the Netherlands? (2) In which direction are current developments in the field of synthetic biology headed? (3) What are the potential interests in synthetic biology? (4) What developments need to take place in the Netherlands in the field of synthetic biology? And (5) Are current legislative frameworks and risk management protocols for genetically modified organisms (GMOs) adequate for the practice of synthetic biology? The answers to these questions were expected to supplement recently published reports on the ethical and societal aspects, and the possible risks, of synthetic biology. In 2006 the Rathenau Institute had published a report on the societal consequences of synthetic biology which had given much attention to its risks and ethical boundaries[1], and in the same year the Netherlands Commission on Genetic Modification (COGEM) had indicated that the existing regulatory framework on genetically modified organisms would prossibly not suffice for future synthetic organisms.[2] In the meantime synthetic biology in the Netherlands has developed to the point where we can speak of an improved general overview of the current situation and of significant developments in the professional field.

1.2 Committee and structure of the advisory document

On 28 January 2008 the KNAW, the Health Council of the Netherlands and the RGO set up a joint committee (Annex B) to answer the minister's questions on synthetic biology. The committee thereby made use of responses to a questionnaire which it had presented to about 100 experts from the research and biotechnology industry worlds (the questionnaire, the respondent list and a summary of the results are all available on demand from the secretariat of the Health Council of the Netherlands, www.gr.nl).

In the present document, the committee looks first at the question of what synthetic biology actually is (Chapter 2). In the next chapter it discusses the current situation in the Netherlands, and in Chapter 4 it looks at current international developments and future prospects. Chapter 5 examines the specific interests of the various parties involved: researchers, companies, government and society. Chapter 6 describes how the Netherlands can exploit these opportunities, and in Chapter 7 the committee considers the ethical, societal and legal aspects of synthetic biology. In Chapter 8 the committee concludes this advisory document with explicit answers to the questions posed by the minister. The final chapter is followed by the reading references and a number of appendices, the last of which is an explanatory glossary of terms.

Chapter 2

What is synthetic biology?

2.1 Definition

No conclusive definition of synthetic biology exists. As a consensus definition, the committee therefore decided to adopt the definition of a European expert group[3]:

Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems, which display functions that do not exist in nature. This engineering perspective may be added at all levels of the hierarchy of biological structures – from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology will enable the design of 'biological systems' in a rational and systematic way.

The concept of '*engineering'* in this definition should be understood here in its wider sense of 'design and construction'.

2.2 Experimental approaches within synthetic biology

Within the field of synthetic biology, broadly speaking two experimental approaches may be distinguished: the '*in vivo*' approach, in which a cellular system is the subject of engineering, and the '*in vitro*' approach, in which a non-cellular biological system is the subject of engineering. Within the *in vivo* category, a distinction is also drawn between a *top-down* and a *bottom-up* approach.

The *in vivo* approach is currently principally occupied with engineering micro-organisms so as to develop large-scale production or conversion systems. To a lesser extent, efforts are also being devoted to reprogramming mammalian cells to produce complex proteins. The engineering of micro-organisms is expected to pose fewer problems when this involves cells whose principal components are essential and well-defined; 'minimal cells' is the evocative term used to describe such cells. Many research efforts are therefore being directed towards creating a minimal cell with an appropriately minimal synthetic genome. Two approaches are currently being employed. One involves simplifying a micro-organism by removing as many non-essential elements as possible (the *top-down* approach). The other involves the development and synthesis of minimal genomes, created by introducing individual (synthetic) components into a cell (the *bottom-up* approach). The great challenge of the *in vivo* approach is to make such constructed organisms robust enough to withstand the different circumstances that apply in industry.

Systems created using the *in vitro* approach are based on polymers of biological building blocks (including nucleotides, amino acids, and lipids) or on molecules resembling such biological building blocks. These systems are often self-assembling – that is to say, the natural properties of the various components are such that they will amalgamate spontaneously. *In vitro* synthetic biology is truly a *bottom-up* approach, because the systems are composed of individual molecules. The engineering of biologically-inspired non-cellular systems is extremely flexible, and the large number of possible building blocks offers innumerable combinations. However, for systems which do not replicate themselves, scaling these processes will present huge challenges.

2.3 Relationships with similar areas in biology

According to some researchers, the *in vivo* approach to synthetic biology is essentially no different to current practices in the genetic modification of organisms. Others note that synthetic biology goes much further than today's genetic modification in that it attempts to work with standardised constructs that code for complex and sometimes entirely new reaction chains, or with constructs that interfere with the characteristic networks of a biological system (Chapter 4). Moreover, synthetic biology makes frequent use of synthesised, optimised gene sequences and of new, human-designed metabolic pathways. In doing so, one uses modelling to create predictability.

There is a clear affinity between synthetic biology and systems biology. The committee regards systems biology as the study and mapping of cellular and intracellular networks, whereas synthetic biology manipulates these networks. While synthetic biology is strongly directed towards the development of new applications for biological knowledge, this development will depend on fundamental systems-biological knowledge. By the same token, systems biology stands to benefit from the insights being gained by synthetic biology. Clearly, synthetic biology and systems biology are each likely to influence the other.

In *in vitro* synthetic biology, biological components or structures are synthesised and assembled into a functioning whole. The *in vitro* approach is not a component of genetic modification or of systems biology, but it does have overlaps with nanoscience, nanotechnology and systems chemistry. In many cases, genetically modified systems and components produced *in vivo* (e.g. proteins, lipids) will indeed be part of a range of building materials for the *in vitro* assembly of new systems.

Clearly, synthetic biology is more than just genetic modification, systems biology, nanotechnology or systems chemistry. In many ways synthetic biology is a converging technology which brings together a variety of scientific disciplines and technologies.

2.4 The need for interdisciplinarity

Biological research in the 21st century has been characterised by collaboration between researchers from a variety of disciplines. The achievement of the ambitions held by synthetic biology will demand a particularly high degree of effective cooperation between researchers in a wide range of disciplines: biology, medical science, chemistry, physics, bio-informatics, nanotechnology, process technology and mathematics. For many researchers and other interested parties, the distinctly interdisciplinary character of synthetic biology is a quintessential feature.

2.5 An innovative successor

Although resistance has been offered to the idea that synthetic biology represents an entirely new research domain, there are more than enough reasons (see above) to appraise synthetic biology in its current form as an innovative research approach within the life sciences. The convergence of different technologies, and the increasing speed with which these technologies are developing, have made synthetic biology a research area of great potential importance to science and society, as will be clarified in the next chapters.

Chapter 3

The state of affairs in the Netherlands

3.1 The Dutch context

Until recently Dutch researchers working in synthetic biology had a rather low profile, as they seldom used the term ‘synthetic biology’ to describe their research and did not describe themselves as synthetic biologists. This has changed. In 2008, no fewer than three Dutch universities announced that over the next five to ten years they would invest a total of € 60 million in centres for synthetic biology research. Most of this money, € 35 million, would be new funding, while € 25 million would be derived from the redistribution of existing funding. These three universities are the Delft University of Technology (the Department of Bionanoscience; € 35 million over ten years, of which € 10 million from new funding), the University of Groningen (the Centre for Synthetic Biology; € 10 million of new funding over five years) and the Eindhoven University of Technology (the Institute for Complex Molecular Systems; € 15 million of new funding over ten years). Existing budgets for synthetic biology research, such as allocated project funding, will remain available alongside this new funding. These extra investments are creating a valuable infrastructure for synthetic biology in the Netherlands and are giving an emphatic stimulus to this research field in this country. Two branches of synthetic biology can be distinguished in the Netherlands. The first is also known as ‘metabolic engineering’ and works with the substantial genetic modification of micro-organisms (the *in vivo*, *top-down* approach). The second is bio-nano-science, which is directed not towards entire organisms but towards the modification and construction of biomolecules (the *in vitro* approach); this is an approach which is closely linked to nanoscience and nanotechnology. The Netherlands already has a good reputation in this field.

3.2 Research and development in the Netherlands

The Netherlands is home to a great deal of research that is related to synthetic biology, but which resides largely in a grey area lying between synthetic biology (as defined above) and genetic modification, metabolic pathway engineering and systems biology. A relatively small group of researchers has recently left this grey area and moved into the field of synthetic biology, using both an *in vitro* approach and a *top-down*, *in vivo* approach that is strongly based on systems biology. The *bottom-up*, *in vivo* approach appears not to be employed at all in the Netherlands. Dutch researchers are working closely with other research groups, both in the Netherlands and abroad. The committee is of the opinion that this collaboration is essential, certainly in the initial phase of development which presently characterises the field.

In the pages that follow we describe a number of interesting examples of synthetic biology research in the Netherlands; an exhaustive overview of the field of synthetic biology in the Netherlands is outside the scope of this document. Part of this research is still located in the grey area between synthetic biology and other research areas, but here, too, the prospects for future synthetic biology research are highly promising.

3.2.1 In vitro research (bottom-up)

To effect movement and transport at the nano level researchers have been making use of so-called *molecular motors*. At TU Delft, researchers have been inspired by cellular transportation proteins which move their cargos from one place in the cell to another via a specific ‘transport network’.[4] The challenges are to manipulate the ‘railroad’ needed to guide these transportation proteins, to control the direction of cargo transport movements and to regulate the locations of their collection and deposition. Important steps have already been made; for instance, they have succeeded in manipulating the direction of transport by means of an electrical tension.[5,6] At the University of Groningen, researchers have developed ‘biohybrid’ motors constructed of biological enzymes and carbon nanotubes.[7] The two enzymes effect the conversion of glucose into kinetic energy, which enables the nanotubes to move autonomously. Groningen researchers also succeeded in developing entirely synthetic motors which rotate in a single direction and which can move objects many times larger than themselves.[8] In the future, such systems might be combined with biomolecules to form hybrid materials. The future molecular motors here under development could have a variety of applications in nanosystems.

Artificial vesicles inspired by nature can be used to provide the controlled release of active substances such as medicines or cytostatics in the human body. The Netherlands now has considerable expertise in the area of membranes and the structures involved in active transport across them.[9-11] Research into *controllable drug delivery systems* is being carried out at several places, including the University of Groningen. In recent years membrane systems have been constructed which contain biological ducts which can be opened and closed using light or pH changes.[12-15] This principle makes it possible to release substances in a controlled way.

Philips has been developing *biosensors* for some time: handy devices for checking the presence of certain substances in biological fluids. The best-known of these is probably the blood glucose meter used by diabetes patients. Another example is a sensor for detecting drugs in the saliva of car drivers, currently at the experimental stage. So far, biosensor technology has made use of antibodies and synthetic biology has played no role; but other biological detection methods might well exist, in which, for instance, sensors work with specific proteins constructed using *in vitro* synthetic biology. This would enable the detection of extremely low target substance concentrations. The application of magnetic biosensors would also enable extremely rapid detection, so that even very complex assays could be carried out within a few minutes.[16,17] By placing a large number of detection proteins on a single microchip, the presence of different substances could also be measured simultaneously. Philips is currently working on this in collaboration with a number of universities and other companies.

DNA has the remarkable characteristic of being able, in an aqueous solution, to organise itself into compact structures, so-called *self-assembling structures*. Eight years ago, researchers in Eindhoven were inspired by this fact to develop polymers which assemble themselves into helical structures in the same way.[18] The exact underlying mechanism is still largely unknown, which makes governing the process a difficult task. Once again inspired by natural processes, this time those of protein aggregation (such as the formation of actin filaments or the pathological formation of protein plaques), a few years later researchers were able to characterise the processes of chemical synthesis of a supramolecular nanostructure.[19] The self-assembly of nucleic acids presents the possibility of constructing DNA-inspired materials for the location-specific release of medicines. Research into this is currently being carried out in several places including the University of Groningen.[20,21]

3.2.2 *In vivo research (top-down)*

In principle, the use of *bio-fuels* such as ethanol, biobutanol, biodiesel and hydrogen is more sustainable than that of fossil fuels; moreover, the raw material for their production, biomass, is available everywhere. The use of crops for fuel production purposes has, however, a serious disadvantage in that it competes with food crops and with the agricultural land which would otherwise be used to grow food crops. The challenge here is therefore the maximisation of the efficiency of the process by which sunlight is converted into bio-fuels via biomass, by using crops which are not used for food and by not taking fertile land out of food production. Synthetic biology could play an important role in this process.

An initiative has been launched to combine Dutch expertise in *photosynthesis* (the formation of biomass using the power of sunlight) in a new Centre for Photosynthesis Research, a joint venture bringing together Wageningen University, Leiden University, VU University Amsterdam, and the University of Groningen. € 10 million will be invested in this centre over the next five years*. At this centre, systems biology and synthetic biology will go hand in hand.

Dutch research is also being carried out into the conversion of biomass into bio-fuels. Current research into second-generation *bio-ethanol* in Delft lies on the border between synthetic biology and metabolic engineering. The researchers have introduced genes from a mould involved in the conversion of woody sugars (C5 sugars, principally xylose)[22,23] into baker's yeast, so that it can break down not only C6 sugars (mainly glucose) but also the much tougher C5 sugars.[24,25] Besides food crops, this new yeast can also break down residual products such as the pulp left over from corn, grain, sugar beet and sugar cane crops, as well as from woody crops such as straw. In principle this makes it possible to double bio-ethanol production per hectare. Dutch expertise on filamentous moulds (for instance, at the Universities of Utrecht and Leiden) has made an important contribution towards this development.

On 1 March 2007 the Dutch ethanol manufacturer Nedalco announced plans to construct a factory by late 2008 which would use this fermentation process to

* Brochure: Center for Photosynthesis Research, Towards BioSolar Cells. Mei 2008.

produce 200 million litres of second-generation bio-ethanol per year*. Since then, however, Nedalco has deemed the investment too risky and has suspended these plans indefinitely**.

Researchers in Nijmegen have succeeded in manipulating a plant virus such that it can serve as the catalyst for specific reactions.[26] The principal advantage of this *biocatalyst* is that it can reproduce itself. Attaching more than one catalyst to a single virus makes it possible to exert control at the nano level over the spatial organisation of catalytic reactions that take place in discrete steps. This idea is inspired by nature, in which catalytic reactions often take place in organised structures such as the mitochondria which provide cells with energy.

3.2.3 Tools

The development and sale of 'building blocks' and tools for synthetic biology, and particularly of standardised synthetic DNA sequences, is a commercial market *par excellence*. In the Netherlands there are just two commercial companies active in the market for synthesised DNA and oligonucleotides, namely Base-Clear in Leiden and Biolegio in Nijmegen. Another firm, Pepscan Presto in Lelystad, supplies synthetic peptides.

* http://www.nedalco.nl/index2.html

** *Provinciale Zeeuwse Courant*, Tuesday 19 August 2008, http://www.pzc.nl/regio/zeeland/3579909/Nedalco-zet-bioethanol-in-ijskast.ece

Chapter 4

International developments

4.1 The international context

Synthetic biology arose in the United States at the start of this century and has since grown into a competitive research field. Up to now, news of breakthroughs in synthetic biology has invariably come from the US. This has partly to do with the effective marketing strategies that are sometimes pursued, but it is chiefly the consequence of the enormous funds invested in this research field in the US. The country's public sector (for example, the Ministries of Defence and Energy) and its private sector (for instance the Bill and Melinda Gates Foundation, Microsoft and BP) invest tens, if not hundreds of millions of dollars in synthetic biology.

Until September 2005, 64 percent of research publications came from the US, compared with 24 percent from Europe. The overwhelming majority of articles in high-profile journals originated in the US. These figures need to be treated with a certain caution, as the term 'synthetic biology' was used in American publications before it was adopted in Europe.[27] Nonetheless, as has been agreed in its 'Lisbon agenda', Europe is pursuing a '*knowledge-based bio-economy*', and the European Commission is playing an active role in stimulating synthetic biology in Europe. The *New and Emerging Science and Technology* (NEST) *Pathfinder* initiative of 2005/2006, part of the EU's Sixth Framework Programme, included the theme of synthetic biology. This programme funded 18 projects in the area of research, policy and strategy development. The TESSY project (*Towards a European Strategy for Synthetic Biology*) has resulted in a European roadmap for synthetic biology, which was presented in Brussels in June 2008[*]. Within the SYNBIOSAFE project, research has been carried out into the safety aspects of synthetic biology, and its social and ethical discussion is being actively stimulated[**]. The EU's Seventh Framework Programme has set aside funds for synthetic biology under the heading of Nutrition, Agriculture and Biotechnology. The European stimulus seems, then, to be having effects. Member states with a pioneering role in synthetic biology are now actively seeking ways of shaping their synthetic biology research. A good example of this in the UK, where the *Biotechnology and Biological Sciences Research Council* (BBSRC) has made synthetic biology one of its 'strategic priorities' and, together with three other UK research councils, has made networking funds available. The BBSRC also commissioned the University of Nottingham to map the social and ethical aspects of synthetic biology.[28]

4.2 The state of affairs in research and development

4.2.1 Complexity and divergence from nature

Research into synthetic biology can be further categorised on the basis of the degree to which the biological components and systems being researched differ from naturally-occurring biological components and system, henceforth referred to as their divergence from nature, and the degree of complexity of these systems. The wide variety of experimental developments in synthetic biology can be plotted on a graph having divergence from nature on one axis and complexity on the other[29], and in order to give a general impression of the current state of affairs, a number of developments in synthetic biology research have been plotted in this way in Figure 4.1.

Five levels of complexity can be distinguished. From low to high, these are:

1 The fundamental biomolecular building blocks of genetic code (nucleotides), proteins (amino acids) and membranes (lipids).
2 Assemblages of these fundamental building blocks: oligonucleotides, single-strand DNA, RNA and foldamers (synthetic molecules that can fold, similarly to proteins and nucleic acids, and can take on, for instance, a helical form).

* http://www.tessy-europe.eu
** http://www.synbiosafe.eu

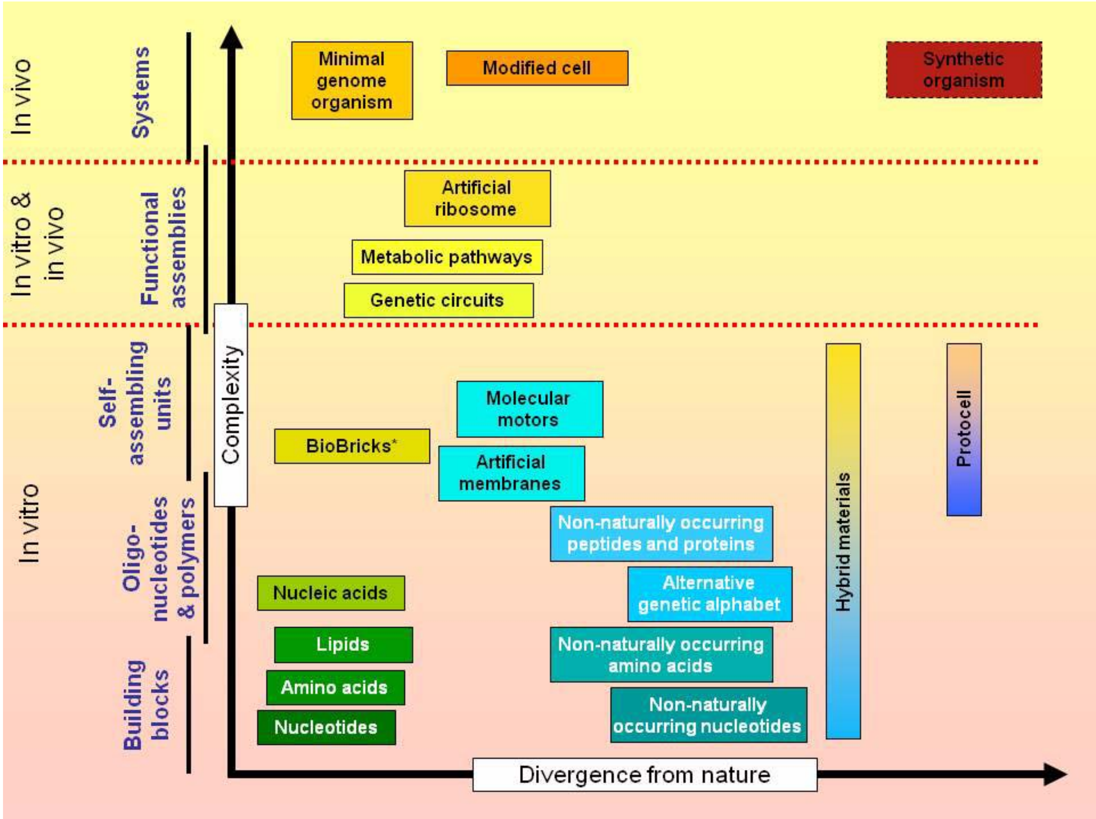

Figure 4.1 Developments in synthetic biology according to complexity and degree of divergence from nature. Adapted from Bromley *et al*[29]. The least complex systems are part of the *in vitro* approach and the most complex systems are part of the *in vivo* approach, but there is an overlap area in which systems can be constructed using either the *in vitro* or the *in vivo* approach.

3 Double helix structures formed by base pairing. Such structures form the basis of biochemical self-assembly. These include the so-called BioBricks, DNA modules having specified functions which can be used as building blocks in the construction or reconstruction of genetic circuits, and the liposomes and proteoliposomes, artificial membranes that arise from the self-assembly of lipids and proteins.
4 Functional units formed by combinations of self-assembling units, such as the coding units and cell components involved in protein synthesis. Examples of these include genetic circuits, metabolic pathways and artificial ribosomes. The design and construction of such units makes use of tools like BioBricks, directed evolution, and mathematical modelling.

5 Self-replicating biological systems, such as cells and cell systems.

In principle any complexity level can involve any level of divergence from nature – from natural amino acids to all kinds of hybrid materials, from natural DNA to entirely synthesised DNA containing artificial nucleotides, and from natural cells to entirely synthesised cells or protocells based on lipid membranes and other synthesised components.

Current research is grouped along the axes; in other words, it is largely directed either towards the increasing complexity of largely natural systems or towards the increasing divergence from nature of relatively simple systems. This includes both the development of protocells (organisms capable of carrying out basic functions to a level that we would associate with a simple form of life) and current efforts to develop cells having a minimal genome (the Craig J. Venter Institute). Hardly any research involves systems with a high degree of complexity in combination with a high divergence from nature. Many biologists doubt whether complex biological systems constructed from entirely artificial components (at top right in the diagram) will ever function properly; if this is ever achieved, it will be some time in the more distant future.

4.2.2 Tools

Important pillars of synthetic biology are the synthesis of macromolecules (especially synthetic DNA), the development of standardised DNA constructs (BioBricks), the simulation of evolutionary processes ('directed evolution'), bioinformatics/bio-engineering, and knowledge of systems biology.

Synthetic DNA

It has been possible for some time to obtain synthetic DNA in almost any desired sequence. Improvements in synthesis technology have increased the length of DNA sequences that can be synthesised impeccably, and have reduced the cost of synthetic DNA accordingly. In 2004 a synthesised base pair cost three to five dollar, but in 2007 this price had fallen to about a dollar per base pair. By mid-2008 the price of the single-strand oligonucleotides used for gene construction had fallen to about 20 dollar cent per base. The price of longer DNA fragments now lies between a half and one dollar per base pair, depending on the accuracy, the minimum length of the sequence, and the vector in which the fragment is placed*. The price also depends on the total amount of synthetic DNA required: bulk amounts for medical and industrial applications are relatively cheaper. Several companies market synthetic DNA at large discounts, although this applies only to a limited number of vectors and delivery times for these products are long. They do this by outsourcing the synthesis to Chinese firms. It is now possible to accurately synthesise DNA sequences tens of thousands of base pairs in length. A number of commercial suppliers offer internet access to software with which DNA constructs can be made to order.[30] For many public research institutes, however, fully synthesised genes are still too expensive; prices will have to fall still further before their use in these institutes becomes widespread. It is by no means certain whether this will happen. If it does, it will be the result of new developments in synthesis technology.

BioBricks

BioBricks is a universally accessible electronic catalogue containing a growing number of standardised genetic building blocks.[31] In the same way that standardised components are used in micro-electronics, BioBricks can be used to develop biological systems that are optimised for the production of specific biomolecules. The catalogue includes DNA components which code for proteins, components which function as on-off switches, and components for genes responsible for signal exchanges between cells. Because the BioBricks included in the catalogue meet given standards, bio-engineers anywhere in the world can make use of them. Moreover, anyone who so wishes can improve existing BioBricks or add new ones**.

Directed evolution

‘Directed evolution’ is the simulation of an evolutionary process. Using ‘DNA shuffling’ and ‘error-prone PCR’, it is possible to create a large library of gene variants having small differences in their DNA. This library can then be screened for the existence of variants (‘mutants’) having a useful property. For instance, those mutants which display improved enzyme activity can be selected. Knowledge of the protein domain (the three-dimensional folded structure of a protein) and its effect on, for instance, enzyme activity allows researchers to screen these

* Various websites, including http://www.geneart.com, http://www.biopioneerinc.com, http://www.epochbiolabs.com, http://www.exonbio.com and http://www.atg-biosynthetics.com.

** http://bbf.openwetware.org

databases more effectively.[32] The use of directed evolution has already resulted in improved enzymatic processes for the production of vitamin B_{12}, the semisynthesis of Taxol and cephalosporine antibiotics, and others.[33-35]

Bio-informatics and bio-engineering

Bio-informatics is an indispensable instrument for the storage, processing and interpretation of gene and genome data (genomics). It is also a prerequisite for the study of protein profiles and their influence on cellular processes (proteomics), and of changes in metabolite profiles and the processes underlying these changes (metabolomics). Bio-engineering provides support in biological modelling and simulation research. *Bio-SPICE* is a software toolset for modelling and simulating spatio-temporal processes in living cells[*]. Bio-JADE, developed by the MIT, is a design and simulation tool for synthetic biological systems that is linked to MIT's BioBricks system.[36] The Handel Laboratory at the University of California has developed software in the form of a genetic algorithm for protein design which makes it possible to predict the effects of mutations on various protein properties[**]. Such software can, for instance, be applied for the optimisation of enzymes (activity, stability) or for the production of large numbers of proteins and tests of their medical effects.[37]

Systems biology

Finally, knowledge of systems biology is essential to synthetic biology. In systems biology, too, which revolves around the iterative cycle of experiment, data integration, model and prediction, bio-informatics and bio-engineering are essential[***]. These models and predictions become the starting point for new (synthetic biology) research.

4.2.3 *Important developments*

To illustrate the current state of science, a few examples of important developments in synthetic biology are described below. The examples are also depicted in Figure 4.1.

* http://biospice.sourceforge.net
** http://egad.berkeley.edu/EGAD_manual/index.html
*** *Strategisch Actieplan Systeembiologie*, June 2008, NWO, NGI, STW, ZonMw, FOM

A good example of a *hybrid material* is the ‘bio-electronic interface’, employing nerve cells (neurons) placed on a semiconductor.[38,39] Neurons can conduct electrical signals, and because they are many times smaller than the electrical wires that have been used in chips thus far, they are particularly well suited to use in microchips. Research into bio-electronic interfaces also yields information on the dynamics of neural networks used with digital electronics, a subject of importance to the development of brain implants to treat a wide range of pathological disorders. Up to now, research has concentrated on the working of the bio-electronic interface. The next step will be the purposeful manipulation of neurons to optimise their function in the biochip.

To be able to ‘program’ cells, the signals generated by different receptors have to be integrated in so-called *genetic circuits*. This is comparable with the way a computer processes different information streams by performing incremental decisions using logic gates. By building logic gates in molecular biology, researchers can couple gene expression to a specific environmental signal. Various logic gates have already been developed on the basis of DNA, RNA and protein components which are applicable in both bacteria and eukaryotic cells. One example of this is a logic gate which reacts to four signals which are characteristic for a certain clinical picture; if all four signals are present, this starts the *in vivo* production of a certain medicine.[40] So far a set of building blocks has been developed containing genetic oscillators, inverters and toggle switches[*]. An example of a toggle switch is a genetic circuit comprising two genes which regulates the population density of bacteria. One gene is responsible for the production of signalling molecules. As the bacterial population grows, the number of signalling molecules increases. At a certain concentration, the expression of a ‘killer gene’ in the bacterium is activated, with fatal consequences for the bacterium. This reduces the density of the population and therefore the concentration of the signalling molecule, and the population can start to grow again[**].[41] The main challenges in this field are the integration of such components into more complex systems and the combination of synthetic-genetic circuits with natural reaction paths which make different demands on the input-output relationship.[42-44] Such circuits make it possible to develop biological systems which can carry out a variety of different tasks, according to circumstance and varying in time or space. An example of such a system is one enabling the growth of synthetic tis-

* http://partsregistry.org/Main_Page

** Arnold, F.H. (2006) 2nd International Synthetic Biology Conference, 20 & 21 May 2006, Berkeley. http://webcast.berkeley.edu:8080/ramgen/events/rssp/SynthBio_Arnold.rm sue.[45] Another example is the Swiss research in which genetic circuits are being used to influence the biological clocks of mammalian cells and mice.[46]

In an industrial fermentation process, it is important to manufacture the desired product as efficiently as possible. This means converting as much energy as possible into the desired product, and reducing the amount of waste products generated to a minimum. Optimising *metabolic pathways* in the cell, and their underlying genetic and regulatory mechanisms, involves some subtle genetic modification and a synthetic biology approach. These are often complex processes which comprise numerous successive enzymatic steps and involve more than one gene. An example of 'metabolic pathway engineering' is the introduction of a construct, of about forty synthetic genes and segments of regulatory DNA, into a bacterium (Genencor[*]). This enables the bacterium to produce 1,3 propanediol (PDO) from corn sugar in an extremely energy-efficient and economically feasible way. PDO is a raw material for the production of DuPont fibres and the biopolymer Sorona.[47] Another example concerns the production of the anti-malarial drug artemisinin. Obtaining natural artemisinin from the sweet wormwood plant (*Artemisia annua*) is perfectly possible, but it is also time-consuming and costly. By equipping a bacteria with three modified 'pathways' derived from baker's yeast, from the bacterium itself and from the wormwood plant, it has become possible to produce artemisinic acid, the precursor of artemisinin, microbially. This is expected to reduce the production costs of this anti-malarial drug by 90%.[48,49] A partnership comprising Amyris, the Institute for OneWorld Health, and Sanofi-Aventis expects to bring semi-synthetic artemisinin onto the market within three years[**].

In 2007 researchers at the J. Craig Venter Institute described in *Science* the first successful attempt to transplant an entire genome from one organism, *Mycoplasma capricolum*, to another, the related species *Mycoplasma mycoides*.[50] Genome transplantation is seen as being an essential step in the activation of chemically synthesised chromosomes in living cells. In January 2008, researchers at the same institute published details of how they had created an entirely synthetic copy of the *Mycoplasma genitalium* genome, which contains 582 970 base pairs.[51] Both these developments are at the heart of the development of *minimal genome organisms*: organisms which possess a genome capable of carrying

* European Patent Office: *Bioconversion of a Fermentable Carbon Source to 1,3-Propanediol by a Single Microorganism*. Publication number EP0826057, 4 March 1998.

** Press release dated 3 March 2008 (http://www.amyrisbiotech.com/pdf/Amyris_Press_Release_03-03-08.pdf) out a basic metabolism and a cell replication mechanism. In principle, these organisms can be used as a kind of chassis, with a minimum of genetic 'background noise', to plug in various genetic components. The resilience of such minimal cells – for instance, how they behave under stressful conditions or in an industrial setting – represents an important challenge in this research field.

Researchers at the Lucent Technologies Bell Labs have used algorithms to design a system of DNA fragments which can organise themselves into a *molecular DNA motor.*[52] This DNA motor can move along a DNA strand independently, with no external energy supply. This opens the possibility of using RNA and DNA to program mechanical functions into cells. Besides nanorobotics applications, the researchers are thinking about using the DNA motor for the design of new organisms able to efficiently produce hydrogen from cellulose. Cellulose is an abundant raw material also found in waste, and hydrogen is widely seen as the clean fuel of the future. Research into molecular motors is also being carried out in the Netherlands (Chapter 3).

Thanks to the development of solid-phase peptide synthesis (SPPS), a chemical synthesis process, it has been possible for decades to produce peptides and proteins which are difficult to express in micro-organisms. The technology also enables the incorporation of non-naturally occurring amino acids. From a technical standpoint the method is a simple one, but it is subject to limits with regard to yield, length and type of the peptides and proteins that can be synthesised.[53] A serious shortcoming of the technique is that complex proteins composed of more than one domain are often badly folded. An alternative strategy for the *synthesis of artificial peptides and proteins* is to modify the natural biosynthesis of polypeptides in the cell. This biosynthesis takes place in the ribosome, where a code defined by three successive bases (a 'codon') on the RNA is translated into a specific amino acid. There are more different codons than there are amino acids. By using mutants of the enzymes involved, it is possible to incorporate artificial amino acids into polypeptides, thereby giving them new properties.[54,55] The biopharmaceutical company Ambrx uses artificial amino acids in the production of therapeutic drugs[*]. An English research group has constructed bacterial cells containing an *artificial ribosome*, which can synthesise both naturally-occurring and non-naturally occurring proteins, independent of the synthesis of endogenous proteins by the endogenous ribosome.[56] The advantage of this paral-

* www.ambrx.com

lel synthesis is that non-natural amino acids are not incorporated into the cell's endogenous proteins, so that its metabolism is hardly disturbed.[42]

Current 3-base codons and a choice of four different nucleotides yield 64 possible different codons. However, using an *alternative genetic alphabet* having an expanded number of nucleotides and manipulating the codon system might mean being able to raise the number of possible codons. The Foundation for Applied Molecular Evolution (FAME) has been working for several years on an artificially extended genetic information system based on an expansion of the existing set of four different nucleotides from which DNA is constructed, and has succeeded in developing a non-naturally occurring base pair.[57] The EraGen Biosciences company is specialised in the development of diagnostic assays and uses its own MultiCode Technology, a patented system for the production of a new, non-naturally occurring base pair made up of isoC (5'-methyl-isocytosine) and isoG (isoguanine)[*].

Cell-free systems are used for the *in vitro* expression of genes for the production of proteins. A number of different avenues for cell-free systems are being researched. One example is the *protocell*, a simple self-assembling nanosystem consisting of three basic components: a metabolic system, a molecule able to store information, and a membrane that keeps the system together. A perfect protocell exhibits self-preservation and self-replication and is subject to evolutionary principles. For the cell membranes self-assembling lipid structures may be used. One of the challenges of this approach is to create selective permeability in these membranes without involving transport proteins.[58] Other possibilities, such as drops in emulsions and nanomaterial microcontainers are also under investigation.[59] For the biomolecular information component, it is in principle possible to use self-replicating RNA. The evolutionary component could be introduced by adding an RNA-encoded function which generates a selective advantage, growth or replication of the membrane.[60] Research into protocells is progressing slowly and the technology still has a long way to go before it has any practical applications. In the Los Alamos National Laboratory and the Santa Fe Institute, researchers have worked for many years on the development of a protocell. The Protocell Assembly project at Los Alamos is focused on the development of the scientific knowledge needed for the construction of self-reproducing molecular machines. Within this project researchers are collaborating closely with the EU-financed (€ 6.6 million) Programmable Artificial Cell Evolution (PACE) project,

* Eragen (2008) MultiCode Technology, http://eragen.senscia.com/contentPage.cfm?ID=428

aimed principally at the possibilities of building synthetic chemical cells for a new generation of ICT applications (self-repairing computer and robot technology) and for carrying out complex production and repair functions on the nano scale*.

4.3 Perspectives

The speed with which the sequencing and synthesis of DNA is now developing, combined with the integration of a variety of technologies and scientific disciplines including nanotechnology, bio-informatics, systems biology and metabolic reprogramming, is turning synthetic biology into a most promising research domain. Most short-term progress will be made in fundamental knowledge, particularly in the areas of biomolecular systems, genetic networks and regulatory systems. Apart from a small number of successful applications which will come to market in the next three years, the large majority of biotechnology applications is expected to become marketable only in the medium or long term, that is, in five years' time or more.

The potential application areas of synthetic biology are extremely diverse. In the health domain they include live therapeutic agents, drug delivery systems, and more efficient drug production platforms. They also include the development of sensitive diagnostic tools, for instance by employing biosensors for either external use or internal use in combination with imaging techniques (MRI and PET). In the field of sustainable energy production, they take the form of microbiological or plant-based production platforms for bio-ethanol and hydrogen. Such production platforms could also be used for the manufacture of fine chemicals. Finally, bio-nano-structures could also be applied in new materials.

Whether these applications actually succeed in practice will depend strongly on cost-benefit ratios. If oil prices continue to rise, demand for cheaper alternatives will grow, and that could make innovations in synthetic biology increasingly attractive. Growing interest in sustainable energy and food production may also stimulate such innovations. However, the absence of these stimuli might also delay innovation. A good example of this is the 'Single Cell Protein' project dating from the 1980s. The idea was to use oil or food wastes as a substrate for yeasts and moulds, harvesting the resulting protein as a foodstuff. Much was invested in this project but it did not lead to any great innovations, partly because oil prices fell. Other investments in the development of alternative energy

* http://www.istpace.org//index.html

sources, spurred by the oil crises of the past, have had few results. However, besides these economic factors, intangible costs and benefits, such as effects on health and welfare, will also play a role.

Chapter 5

Interests and interested parties

A variety of parties have an interest in synthetic biology. Civilians may in the future well make use of products that have arisen from synthetic biology, such as sustainable energy, products that improve their health and ones that enhance their quality of life. For researchers, investments in synthetic biology offer opportunities to take on the international competition. For biotechnology companies, synthetic biology offers the prospect of developing innovative products. And for the Dutch government, synthetic biology can contribute to the country's knowledge economy. The Netherlands has genuine opportunities in synthetic biology because the country already has a strong tradition of innovative research in related areas, a good potential in biotechnology, and universities which have decided to invest in synthetic biology research and infrastructure over the next five to ten years.

Innovative research tradition

Although synthetic biology in the Netherlands is still in its infancy, we may expect certain areas of this field to develop quickly, given the expertise available in this country. For instance, the Netherlands has a strong research tradition in the fields of molecular cell biology, structural chemistry, physical chemistry, biophysics, and macromolecular chemistry, the border area between biochemistry and synthetic chemistry. The field of metabolic pathway engineering on the basis of microbial physiology is strongly represented, and in systems biology, too, as an important foundation of synthetic biology, leading work can clearly be distinguished in the Netherlands. The country does not lack good bio-information scientists, but the question is whether there are enough to meet the anticipated demand for their skills. A lot of this expertise has already been grouped in large national programmes such as the Netherlands Genomics Initiative (NGI) and NanoNed. The anticipated Systems Biology programme would additionally contribute towards the needed grouping of expertise in synthetic biology.

Potential in biotechnology

In the Netherlands about 140 companies are actively engaged in biotechnology, and this number is growing[*]. In the early 1980s the Netherlands had a leading role in biotechnology, but since then the sector has developed more quickly in other countries, and the Netherlands has lost ground. Despite its good starting position, the Dutch biotechnology sector is now behind those in other countries.[61] The Dutch Patent Centre showed that around the year 2000 Dutch biotechnology was doing less well than the European average by looking at the number of patent applications received in the years 1995-2004.[62] It should be noted that these figures concern only ‘dedicated’ companies, which engage exclusively in biotechnology; so-called ‘diversified’ companies, often large organisations such as Unilever and DSM for whom biotechnology is just one of a broad range of commercial activities, have not been included. The report showed that growth in the number of patent applications in the biotechnology sector was higher than the average rate of growth in patent applications until the year 2000, but that this growth stagnated after 2000, and has fallen below the Dutch average.[62] According to Niaba (the Netherlands Biotech Industry Association), the causes for this stagnation are the country’s suboptimal investment climate, the persistent debate on biotechnology applications in agriculture, and the absence of an entrepreneurial culture at universities and knowledge institutes[*]. Considerable attention has been given to the latter in recent years; the government has actively stimulated the valorisation of scientific knowledge and encouraged entrepreneurialism at universities and knowledge institutes by means of initiatives such as the successful Biopartner project.[61] A number of public-private partnerships such as TI Pharma, TI Food & Nutrition, CTMM, BMM and the ‘Life Sciences and Health’ innovation programme were also set up to encourage innovation and valorisation. Nevertheless, questions are still being raised as to whether these measures will be enough to put the Netherlands back at the biotechnological top. For

* http://www.niaba.nl

instance, a restrictive legislative framework may also form an impedimentary element.[61]

Investments by universities

The top-down stimulation of a specific research field by means of government investments generally works well[63]. It is, however, a precondition of success that the research field has already organised itself. Current university investments in synthetic biological research and the accompanying infrastructure are meeting this need. A number of public-private partnerships have also been formed between research groups at these universities and Dutch companies, and this development, too, is an important condition of success for such investments.

In advance on Chapter 7, the committee notes that it is in the interests of society as a whole that not only the opportunities, but also the risks of synthetic biology are considered. It is vital that society debate the ethics of the technological possibilities that are going to arise in this field.

Chapter 6

What needs to be done in the Netherlands

6.1 Research

Synthetic biology is an innovative and ambitious research domain in which the Netherlands can play an important role. A good research base exists for the development of synthetic biology in this country, and the development of synthetic biology is being stimulated at several locations by means of extra funding.

In the long term, research into synthetic biology can contribute to the Dutch knowledge economy. With this in mind, the stimulation and valorisation of innovative synthetic biology research by the government is opportune. A timely incentive will prevent the Netherlands from lagging behind other European countries having a pioneering role in synthetic biology (particularly the UK but also Germany and Switzerland, and to a lesser degree France and Spain). In this connection the committee points to the Dutch systems biology programme, which was launched too late to prevent the Netherlands from falling at least five years behind the UK in this domain.

The Dutch government can stimulate synthetic biology research and its valorisation by promoting the development of a directed research programme. Research collaboration at national level will be crucial. Synthetic biology is a converging technology; in other words, it is a field which brings together different disciplines and research approaches. This is why it is important that researchers from all of these disciplines get together and communicate across their disciplinary borders. It would therefore be logical to effect the far-reaching integration of synthetic biology research in national systems biology, genomics, and nanotechnology research programmes. The committee therefore argues for the creation of a synthetic biology research theme within existing initiatives such as the upcoming systems biology programme, the NGI and NanoNed. The committee proposes that a synthetic biology sub-programme be instated within each of these three programmes. Because the field is still in development, programming should be done ‘bottom-up’ so as to profile the work of the best researchers and engineers. When a few years have passed and the positions of different players in the Dutch field has become clearer, programming can start to take place top-down, so that work in the field can be steered towards a strong, innovative and coherent nationwide research programme.

6.2 Education

Its markedly interdisciplinary character is not unique to synthetic biology, but it does make certain demands of the education of researchers in this field. There is no need for a new degree in synthetic biology; the challenge will be to set up Master’s courses such that first-rate synthetic biology researchers are produced. The University of Groningen and TU Delft have each made a start by putting interdisciplinary student teams together to take part in the international Genetically Engineered Machine (iGEM) competition*. The iGEM competition was launched in 2003 by the Massachusetts Institute of Technology (MIT) in Boston. Since then MIT has organised the competition every year, and this originally small-scale initiative (about 25 participants) has expanded into a large international event with about 1000 participants (in 2008) divided into 84 teams from five continents (19 American states and 20 countries outside the US). The international synthetic biology community is extremely enthusiastic about the initiative, particularly because of the educational aspect. Students have an intrinsic motivation in that they themselves determine what to do, as long as it impresses the jury. At the same time they learn to work and think in interdisciplinary ways. Moreover, a team cannot win if it pays no attention to the ethical and social aspects of the system designed. The committee believes that for certain components of a Master’s course the iGEM competition represents an excellent source of inspiration.

* http://2008.igem.org

Chapter 7

Ethical, social and legal aspects

7.1 Issues under discussion

Synthetic biology appears to be developing into an important new area of biological research and one which offers considerable opportunities for applied research. It is therefore logical that society has some critical questions to ask about the consequences of synthetic biology for people and society at large.[28,32,64] These normative issues are often placed in ethical, social or legal categories; however, many of the issues being debated do not belong to one of these categories alone, but touch two or even three of these aspects.

The ethical issues have to do with the ethical boundaries of medical and biological research. The principal discussion point is the question of whether *in vitro* synthetic biology might, in time, construct entirely new living organisms, 'life from scratch', which some would see as an irresponsible violation of nature. To be able to discuss this adequately we need, above all, an answer to the question "what is life?". Unfortunately, no conclusive definition of life yet exists, despite numerous attempts to draw one up.

The social issues have to do with the safety of the technology. As in recombinant DNA technology, the concern is for safety both within and outside the laboratory. Are researchers and production staff adequately protected against the micro-organisms they work with? Are effective and adequate protection measures in place if these micro-organisms unintentionally find their way outside the laboratory or factory? How controllable are these micro-organisms, if their application lies outside the laboratory or factory? And is the world adequately protected against biohackers and bioterrorism, now that standard biological components are so easy to obtain? There is also the question of whether our legislative frameworks are sufficiently specialised to deal with new developments in synthetic biology.[2]

Another social issue has to do with global justice. Most applied synthetic biology research has so far concerned the production of terpenoids, natural products generally derived from plants and used as aromatics, flavourings, and drugs against malaria and cancer. The cheap mass production of synthetic artemisinin (Chapter 4) is pushing wormwood farmers in East Asia and parts of Africa out of the market. This is just one example of how developments in synthetic biology can bring about shifts in global economic relations, with negative results for certain groups. It is important to give such matters due consideration.

An important legal issue concerns intellectual property rights. In the US in particular, large sums are invested in synthetic biology, which reflects the fact that there are high expectations of the commercial proceeds. There are, however, two problems with regard to intellectual property rights in this domain: broad patents, which hamper collaboration and inhibit development – and narrow patents, which make procedures overly complicated because to set up a production system using standard components means having to deal with hundreds of patents. Such patents also increase the likelihood of monopoly formation. There exists a fear that future bio-fuel production, for instance, could also become monopolised, just when it was hoped that bio-fuels would liberate us from the monopolies of the fossil fuel age. Craig Venter's own business strategy is a good example of the emergence of such monopoly positions; he not only holds a worldwide patent on Synthia, a minimal cell, but he has also patented the construction of synthetic genomes *and* their placement into cells. Not for nothing have his companies already been dubbed 'Microbesoft'. By analogy with the aims of open source software, the MIT in Boston launched the BioBricks Foundation[*] in order to keep standard biological parts accessible for everyone.

7.2 Research, legislation and debate

The more radical an innovative technology is, the greater are the concerns felt by society. It is best to take these concerns seriously, to take part in a constructive debate, and to address these concerns wherever possible. Good will and good

* http://openwetware.org/wiki/The_BioBricks_Foundation

faith must underlie all dialogues between interested parties; only then will broad, sustained societal support for synthetic biology arise.

Research into the ethical, social and legal aspects of synthetic biology can bring useful rational argument to the debate. The committee argues that this form of technology assessment should be a substantial part of the synthetic biology subprogramme proposed above. For instance, TESSY project members have argued that 5-10% of the funding for synthetic biology should be devoted to research into its ethical, social and legal aspects*. Within the research community opinions on the amount of attention that should be given to these aspects are divided (annex can be retrieved from the Health Council). It is the responsibility of government to provide active direction in this matter. Strong links between research into a technology (in this case synthetic biology) on the one hand, and research into its ethical, social and legal aspects on the other is desirable and is highly valued by research groups on both sides**.

Research into the social aspects of biotechnology and genomics is currently being carried out at a number of Dutch institutes. Within the NGI, the Centre for Society and Genomics (CSG) has been set up in order to stimulate researchers to see their work within a broad social context and to promote the quality of public debate. One of the NGI's 'centres of excellence', the Kluyver Centre in Delft, has a programme in *Industrial genomics for society* which devotes attention to synthetic biology. Two of the aims of the programme are to identify future areas of concern and to develop proactive communication strategies to address them. Within NanoNed, too, one of the programme lines is the 'technology assessment' of bio-nano-technology. As a last example of such initiatives, the Science & Society department of the University of Groningen is working together with the new Centre for Synthetic Biology to carry out independent research into the social aspects of synthetic biology. A number of other universities also have departments studying the relationship between science, technology and society.

Although the research community is very willing to self-regulate and to set up and adhere to shared codes of practice, government legislation is sometimes necessary to guarantee safety, to guard against the violation of ethical boundaries, and to counteract monopoly formation. Achieving these aims without needlessly hampering research and development is a formidable challenge. The Dutch Minister of Housing, Spatial Planning and the Environment (VROM) has therefore asked COGEM for appropriate advice. The COGEM will examine the

* Presentation of the *European Roadmap for Synthetic Biology* on 10 June 2008. See also www.tessy-europe.eu

** The first edition of *Ethiek, Onderzoek & Bestuur*, published by the NWO programme of the same name (2008) and the inaugural speech 'De Zwakste schakel. Over maatschappelijk verantwoorde genomics' ('The weakest link. About socially sound genomics'), on 15 March 2007 by prof. dr. H.G.J. Gremmen of the Wageningen University..

safety aspects of synthetic biology and advise on legislation that could contribute towards human and environmental protection. As has already been demonstrated for nanotechnology, risk governance* offers useful methods of handling risk.[66]

The successful introduction of a new technology into society strongly depends on obtaining and sustaining public legitimacy and support. The provision of accurate and balanced information is vital, both to avoid disproportionate public concern and to curb unrealistic expectations. Moreover, the research community has a duty to discuss the consequences to society of its research work and of the technologies that arise from it. The public debate has already been launched by the Rathenau Institute, with a number of publications on synthetic biology[1,32] and a message to the Dutch parliament.[67] Other Dutch researchers have also entered the debate**. The committee underlines the importance of constructive discussion, and is of the opinion that this can best be achieved by discussing the scientific and societal aspects together rather than separately.

* The term *governance* is intended to mean the structures and processes enabling collective decision-making, involving government, independent institutions and private parties.[65]

** On 23 March 2008 the Dutch weekly discussion programme *Buitenhof* was devoted to synthetic biology.

Chapter 8

Answers to the minister

1 What is the current situation with regard to synthetic biology in the Netherlands?

Synthetic biology is on the rise in the Netherlands. Two principal areas of interest within synthetic biology can be discerned in this country: (1) metabolic reprogramming, in which micro-organisms are genetically modified to the point that new functions arise (an *in vivo*, top-down approach), and (2) bio-nano-science, which sets out to alter biomolecules in such a way that new functions arise or so that they can serve as models for new chemically synthesised components (the *in vitro* approach). The Netherlands has a strong research tradition in both areas, and innovative research of international standing is taking place. Over the next five to ten years, three Dutch universities (TU Delft, the University of Groningen and TU Eindhoven) will invest a combined € 60 million of new funding in centres of research into synthetic biology. The Dutch biotechnology industry has not yet gained a strong profile in synthetic biology, although long-term joint ventures have been launched between a number of companies and universities, and DSM is the principal sponsor of a recently endowed chair in Synthetic Biology at Groningen. There are a small number of Dutch biotechnology companies which synthesise DNA for research and development purposes.

2 In which direction are current developments in the field of synthetic biology headed?

Until recently, almost all research into synthetic biology took place in the US. However, a number of European countries are now fast catching up. Most research is focussed on either simple systems having different degrees of divergence from nature or on largely natural systems having different degrees of complexity (Figure 4.1). Fundamental research and the development of tools play a pivotal role. This research could make important contributions to our understanding of complex biological systems. So far, only small steps have been made in the direction of complex systems having a high degree of divergence from nature (top right in Figure 4.1). It is impossible to predict the speed or success with which researchers will develop artificial biological systems. This depends not only on technological possibilities, but also on the societal need for certain innovations and their cost-effectiveness. Only time will tell whether an entirely synthesised organism will ever be created.

3 What are the potential interests in synthetic biology?

Several different parties in society have an interest in developments in synthetic biology: civilians (products that improve health, the quality of life, or the sustainability of society), researchers (international competition), the biotechnology industry (marketing innovations) and government (the knowledge economy). The committee is of the opinion that the strong research tradition that exists in the Netherlands in relevant parts of synthetic biology is a strength to be drawn on. Moreover, this innovative research climate is accompanied by an adequate level of commercial activity in the biotechnology sector. Although the Netherlands has a long history of not capitalising on its enormous production of knowledge, a change appears to be taking place. In the view of the committee, the growing attention now being given to the valorisation of knowledge in all sectors will also benefit the potential use of synthetic biology in Dutch society.

4 What developments need to take place in the Netherlands in the field of synthetic biology?

Sufficient initiatives have now been developed in the domain of synthetic biology to give additional government incentives a chance of being useful and productive. It is up to the government to decide whether to encourage innovative Dutch research in this area, thereby allowing the Dutch research community to compete successfully with the international synthetic biology research community, and – in the medium and long term – to simultaneously stimulate the Dutch knowledge economy. The committee is of the opinion that the country's excellent research tradition and well-equipped biotechnology sector warrant this encouragement. Government investment could take the form of additional financial contributions, provision of funds for synthetic biology within related programmes (NGI, NanoNed and systems biology), and the creation of favourable conditions for interdisciplinary research.

The committee considers that the programming and funding of synthetic biology research in the Netherlands ought to be closely linked to related initiatives, such as in systems biology (a new programme in preparation at the NWO), nanotechnology (NanoNed) and genomics (NGI). Synthetic biology research can be included at an early stage in programme development, whether this programme has yet to start (as in systems biology) or whether it concerns the extension of existing programmes (as for NanoNed and NGI). Like a number of existing initiatives, synthetic biology lends itself extremely well to public-private partnerships. Initial programming would be bottom-up in order to identify the best performing researchers and allow the research field to take shape. Investment would ideally be directed to those research centres where interdisciplinary collaboration is actively facilitated. Links can be sought with future systems biology research centres[*], with the NGI's centres of excellence[**] and nanotechnology research centres[***]. Initially synthetic biology will depend primarily on innovative research, however from the outset we should keep an eye on valorisation in order for the Netherlands' knowledge economy to profit optimally from the investments made. All parties involved have a responsibility to work to create broad social support for this new technology. This can be done by stimulating the discussion of normative issues and by doing (or commissioning) research into the ethical, social and legal aspects of the technology. The committee advises that a substantial proportion of initial investments be devoted to research of this kind. Cooperation between synthetic biology researchers and those researching into its ethical, social and legal aspects will be an important part of obtaining good linkage between them.

The nature of today's medical-technical-biological research means that it will become increasingly important to train interdisciplinary researchers. This applies not only to synthetic biology but also to systems biology and to a certain extent

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* *Strategisch Actieplan Systeembiologie*, June 2008, NWO, NGI, STW, ZonMw, FOM
** http://www.genomics.nl
*** http://www.nanoned.nl

also to bio-nanotechnology. Universities would be well advised to adapt their Master's programmes, where relevant and possible, to developments in synthetic biology.

Once the synthetic biology research field has taken shape in this way, the next step is to observe where Dutch strengths and weaknesses lie. For this reason the committee also recommends that after a few years (for instance, five years after the start of the programme) the Dutch systems biology research field be charted. If necessary, investments thereafter could be carried out partly or entirely top-down in order to steer research and innovation in the desired direction.

5 Are current legislative frameworks and risk management protocols for genetically modified organisms (GMOs) adequate for the practice of synthetic biology?

The Dutch minister of Housing, Spatial Planning and the Environment (VROM) has asked the COGEM, the country's pre-eminent forum of GMO expertise, to advise on safety and possible legislation.[68] The committee therefore confines itself here to a reference to this advice, which is to be published concurrently with the present document. With regard to safety, an obvious step would be adherence to the general code of conduct published by the biosecurity working group of the KNAW.[69] If necessary, an additional request for further advice to this working group can be made.

The committee wishes to emphasise, in line with professional opinions from the field, the importance of drawing conclusions from the public debate that took place on recombinant DNA technology. The main lesson of this debate for synthetic biology is that there is little value in discussing such radical technologies categorically, in the sense of being either 'for' them or 'against' them. The debate on recombinant DNA has made it clear that the advantages and disadvantages of such technologies have to be discussed on a case-by-case basis. The committee also believes that the expert field is correct to warn that any legislation drawn up to apply to a developing field such as synthetic biology must be flexible enough to quickly allow the incorporation of new insights.

References

1 de Vriend H. Constructing Life. Early social reflections on the emerging field of synthetic biology. Den Haag: 2006.

2 COGEM. Synthetische Biologie; een onderzoeksveld met voortschrijdende gevolgen. Bilthoven: 2006.

3 Europese Commissie. Synthetic Biology - Applying Engineering to Biology. Report of a NEST High-Level Expert Group. Luxemburg: 2005: EUR 21796. Internet: ftp://ftp.cordis.europa.eu/pub/nest/docs/syntheticbiology_b5_eur21796_en.pdf_.

4 van den Heuvel MGL, Dekker C. Motor Proteins at Work for Nanotechnology. Science 2007; 317(5836): 333-336.

5 van den Heuvel MGL, de Graaff MP, Dekker C. Op afstand bestuurbare eiwitbuisjes. Nederlands Tijdschrift voor Natuurkunde 2006;(September): 10-13.

6 van den Heuvel MGL, de Graaff MP, Dekker C. Molecular sorting by electrical steering of microtubules in kinesin-coated channels. Science 2006; 312(5775): 910-914.

7 Pantarotto D, Browne W, Feringa BL. Autonomous propulsion of carbon nanotubes powered by a multienzyme ensemble. Chem Commun 2008;(13): 1533-1535.

8 Eelkema R, Pollard MM, Vicario J, Katsonis N, Serrano Ramon B, Bastiaansen CWM *et al*. Rotation of a microscopic object by a light-driven molecular motor. Nature 2006; 440(7081): 163.

9 Bechtluft P, van Leeuwen RG, Tyreman M, Tomkiewicz D, Nouwen N, Tepper HL *et al*. Direct observation of chaperone-induced changes in a protein folding pathway. Science 2007; 318(5855): 1458-1461.

10 Biemans-Oldehinkel E, Mahmood NAB, Poolman B. A sensor for intracellular ionic strength. Proc Natl Acad Sci U S A 2006; 103(28): 10624-10629.

11 Geertsma ER, Mahmood NAB, Schuurman-Wolters GK, Poolman B. Membrane reconstitution of ABC transporters and assays of translocator function. Nat Protoc 2008; 3(2): 256-266.
12 Koçer A, Walko M, Feringa BL. Synthesis and utilization of reversible and irreversible light-activated nanovalves derived from the channel protein MscL. Nat Protocols 2007; 2(6): 1426-1437.
13 Folgering JHA, Kuiper JM, deVries AH, Engberts JBFN, Poolman B. Lipid-Mediated Light Activation of a Mechanosensitive Channel of Large Conductance. Langmuir 2004; 20(17): 6985-6987.
14 van den Bogaart G, Krasnikov V, Poolman B. Dual-Color Fluorescence-Burst Analysis to Probe Protein Efflux through the Mechanosensitive Channel MscL. Biophys J 2007; 92(4): 1233-1240.
15 Koçer A, Walko M, Meijberg W, Feringa BL. A Light-Actuated Nanovalve Derived from a Channel Protein. Science 2005; 309(5735): 755-758.
16 de Boer BM, Kahlman JA, Jansen TP, Duric H, Veen J. An integrated and sensitive detection platform for magneto-resistive biosensors. Biosens Bioelectron 2007; 22(9-10): 2366-2370.
17 Dittmer WU, de KP, Prins MW, Vissers JL, Mersch ME, Martens MF. Sensitive and rapid immunoassay for parathyroid hormone using magnetic particle labels and magnetic actuation. J Immunol Methods 2008;
18 Hirschberg J, Brunsveld L, Ramzi A, Vekemans J, Sijbesma R, Meijer E. Helical self-assembled polymers from cooperative stacking of hydrogen-bonded pairs. Nature 2000; 407: 167-170.
19 Jonkheijm P, van der Schoot P, Schenning A, Meijer E. Probing the solvent-assisted nucleation pathway in chemical self-assembly. Science 2006; 313(5783): 80-83.
20 Alemdaroglu FE, Wang J, Borsch M, Berger R, Herrmann A. Enzymatic control of the size of DNA block copolymer nanoparticles. Angew Chem Int Ed Engl 2008; 47(5): 974-976.
21 Ding K, Alemdaroglu FE, Borsch M, Berger R, Herrmann A. Engineering the structural properties of DNA block copolymer micelles by molecular recognition. Angew Chem Int Ed Engl 2007; 46(7): 1172-1175.
22 Harhangi HR, Akhmanova AS, Emmens R, van der Drift C, de Laat WTAM, van Dijken JP *et al*. Xylose metabolism in the anaerobic fungus Piromyces sp. strain E2 follows the bacterial pathway. Archives of Microbiology 2003; 180(2): 134-141.
23 Harhangi HR, Akhmanova AS, Steenbakkers P, Jetten MSM, van der Drift C, Op den Camp HJM. Genomic DNA analysis of genes encoding (hemi-)cellulolytic enzymes of the anaerobic fungus Piromyces sp. E2. Gene 2003; 314: 73-80.
24 Kuyper M, Harhangi HR, Stave AK, Winkler AA, Jetten MSM, Laat WTAM *et al*. High-level functional expression of a fungal xylose isomerase: the key to efficient ethanolic fermentation of xylose by Saccharomyces cerevisiae? FEMS Yeast Research 2003; 4(1): 69-78.
25 Kuyper M, Hartog MMP, Toirkens MJ, Almering MJH, Winkler AA, Dijken JP *et al*. Metabolic engineering of a xylose-isomerase-expressing Saccharomyces cerevisiae strain for rapid anaerobic xylose fermentation. FEMS Yeast Research 2005; 5(4-5): 399-409.
26 Carette N, Engelkamp H, Akpa E, Pierre S, Cameron N, Christianen P *et al*. A virus-based biocatalyst. Nature Nanotechnology 2007; 2: 226-229.

27 SYNBIOLOGY.European Commission Framework Programme 6 Reference Contract 15357 (NEST). An analysis of synthetic biology research in Europe and North America. Europe/North America comparative assessment. 2005. Internet: http://www2.spi.pt/synbiology/documents/SYNBIOLOGY_Literature_And_Statistical_Review.pdf.
28 Balmer A, Martin P. Synthetic Biology: social and ethical challenges. Nottingham: 2008. Internet: http://www.bbsrc.ac.uk/organisation/policies/reviews/scientific_areas/0806_synthetic_biology.pdf.
29 Bromley EHC, Channon K, Moutevelis E, Woolfson DN. Peptide and Protein Building Blocks for Synthetic Biology: From Programming Biomolecules to Self-Organized Biomolecular Systems. ACS Chem Biol 2008; 3(1): 38-50.
30 Villalobos A, Ness J, Gustafsson C, Minshull J, Govindarajan S. Gene Designer: a synthetic biology tool for constructing artificial DNA segments. BMC Bioinformatics 2006; 7(1): 285.
31 Canton B, Labno A, Endy D. Refinement and standardization of synthetic biological parts and devices. Nat Biotech 2008; 26(7): 787-793.
32 de Vriend H, van Est R, Walhout B. Leven maken: Maatschappelijke reflectie op de opkomst van synthetische biologie. Working document 98. Den Haag: 2007.
33 Dejong J, Liu Y, Bollon A, Long R, Jennewein S, Williams D *et al*. Genetic engineering of taxol biosynthetic genes in Saccharomyces cerevisiae. Biotechnol Bioeng 2006; 93(2): 212-224.
34 Otten L. Directed Evolution of a Cephalosporin Acylase: Changing the substrate specificity of the industrially relevant glutaryl acylase from Pseudomonas SY-77. [Proefschrift]. Groningen: Rijksuniversiteit Groningen; 2004.
35 Rohlin L, Oh M, Liao J. Microbial pathway engineering for industrial processes: evolution, combinatorial biosynthesis and rational design. Curr Opin Microbiol 2001; 4(3): 330-335.
36 Goler J. BioJADE: a design and simulation tool for synthetic biological systems. Boston: 2004. Internet: ftp://publications.ai.mit.edu/ai-publications/2004/AITR-2004-003.pdf.
37 Singer E. DNA Factories: Cheaply churning out made-to-order DNA could revolutionize molecular biology. Technology Review, MIT 2007;
38 Fromherz P. Three Levels of Neuroelectronic Interfacing: Silicon Chips with Ion Channels, Nerve Cells, and Brain Tissue. Ann NY Acad Sci 2006; 1093(1): 143-160.
39 Zeck G, Fromherz P. Noninvasive neuroelectronic interfacing with synaptically connected snail neurons immobilized on a semiconductor chip. Proceedings of the National Academy of Sciences of the United States of America 2001; 98(18): 10457-10462.
40 Voigt C, Keasling J. Programming cellular function. Nat Chem Biol 2005; 1(6): 304-307.
41 Haseltine EL, Arnold FH. Implications of Rewiring Bacterial Quorum Sensing. Appl Environ Microbiol 2008; 74(2): 437-445.
42 Chin J. Modular approaches to expanding the functions of living matter. Nature Chemical Biology 2006; 2(6): 304-311.
43 Kobayashi H, Kaern M, Araki M, Chung K, Gardner TS, Cantor CR *et al*. Programmable cells: Interfacing natural and engineered gene networks. Proceedings of the National Academy of Sciences 2004; 101(22): 8414-8419.

44 Simpson M. Rewiring the cell: synthetic biology moves towards higher functional complexity. Trends in Biotechnology 2004; 22(11): 555-557.
45 Ball P. Starting from scratch. Nature 2004; 431: 624-626.
46 Weber W, Stelling J, Rimann M, Keller B, oud-El Baba M, Weber CC *et al*. A synthetic time-delay circuit in mammalian cells and mice. Proceedings of the National Academy of Sciences 2007; 104(8): 2643-2648.
47 DuPont. DuPont Tate & Lyle Bio Products Begin Bio-PDO™ Production in Tennessee. DuPont News Release. 2006.
48 Hale V, Keasling J, Renninger N, Diagana T. Microbially derived artemisinin: a biotechnology solution to the global problem of access to affordable antimalarial drugs. Am J Trop Med Hyg 2007; 77(6 Suppl): 198-202.
49 Berkeley Lab. An age-old microbe may hold the key to curing an age-old affliction.Science@BerkeleyLab. 2006. Internet: http://www.lbl.gov/Science-Articles/Archive/sabl/2006/May/02-antimalarial.html.
50 Lartigue C, Glass JI, Alperovich N, Pieper R, Parmar PP, Hutchison III CA *et al*. Genome Transplantation in Bacteria: Changing One Species to Another. Science 2007; 317: 632-638.
51 Gibson DG, Benders GA, ndrews-Pfannkoch C, Denisova EA, Baden-Tillson H, Zaveri J *et al*. Complete Chemical Synthesis, Assembly, and Cloning of a Mycoplasma genitalium Genome. Science 2008; 319(5867): 1215-1220.
52 Turberfield AJ, Mitchell JC, Yurke B, Mills AP, Blakey MI, Simmel FC. DNA Fuel for Free-Running Nanomachines. Phys Rev Lett 2003; 90(11): 118102.
53 Guzman F, Barberis S, Illanes A. Peptide synthesis: chemical or enzymatic. Electronic Journal of Biotechnology [online] 2007; 10(2)
54 Carrico I. Unnatural amino acids in biomaterials and protein engineering. In: Protein engineering through in vivo incorporation of phenylalanine analogs, Caltech dissertation. 2003: Internet: http://etd.caltech.edu/etd/available/etd-09082003-110526/unrestricted/Chapter1.pdf.
55 Liu W, Brock A, Chen S, Chen S, Schultz PG. Genetic incorporation of unnatural amino acids into proteins in mammalian cells. Nat Meth 2007; 4(3): 239-244.
56 Wang K, Neumann H, Peak-Chew SY, Chin JW. Evolved orthogonal ribosomes enhance the efficiency of synthetic genetic code expansion. Nat Biotech 2007; 25(7): 770-777.
57 Benner SA, Sismour AM. Synthetic biology. Nat Rev Genet 2005; 6: 533-543.
58 Mansy SS, Schrum JP, Krishnamurthy M, Tobe S, Treco DA, Szostak JW. Template-directed synthesis of a genetic polymer in a model protocell. Nature 2008; 454(7200): 122-125.
59 Doktycz M, Simpson M. Nano-enabled synthetic biology. Molecular Systems Biology 2007; 3: 125.
60 Szostak J, Bartel D, Luisi P. Synthesizing life. Nature 2001; 409: 387-390.
61 Commissie voor Genetische Modificatie (COGEM), Commissie Biotechnologie bij Dieren (CBD), Gezondheidsraad. Trendanalyse Biotechnologie 2007 - Kansen en Keuzes. Bilthoven: COGEM; 2007.

62 Knecht J. Trendanalyse Biotechnologie. Informatie uit octrooiaanvragen van Nederlandse instellingen. 2006: KOB/2006/trendanalyse biotechnologie.

63 Raad voor Gezondheidsonderzoek. Onderzoek dat ertoe doet. Den Haag: Gezondheidsraad; 2007: 57. Internet: www.gr.nl.

64 ETC Group. Extreme Genetic Engineering: An Introduction to Synthetic Biology. Canada: 2007. Internet: http://www.etcgroup.org/.

65 Renn O. White paper on risk governance - towards an integrative approach. Genève: International Risk Governance Council; 2005.

66 Gezondheidsraad. Betekenis van nanotechnologieën voor de gezondheid. Den Haag: Gezondheidsraad; 2006: publicatie nr 2006/06.

67 Rathenau Instituut. Bericht aan het Parlement. Synthetische Biologie: Nieuw leven in het biodebat. Den Haag: 2007.

68 Ministerie van VROM. Verzoek om advies over synthetische biologie. Den Haag: 2007: SAS/2007124333.

69 Koninklijke Nederlandse Akademie van Wetenschappen. Een gedragscode voor Biosecurity. Amsterdam: 2007.

A Request for advice

B The committee

C Glossary of terms

Annexes

Annex **A**

Request for advice

In a letter dated August 8, 2006, ref OWB/WG/2006/29331, to the chairmen of the Health Council of the Netherlands and the RGO, and to the director of the KNAW, the Minister of Education, Culture and Science wrote:

Synthetic biology is a new research area originating from recent breakthroughs in the research areas of genomics and nanotechnology. The expected far-reaching future developments in this area will raise new questions.

Synthetic biology is defined as the designing and constructing of biological parts, constructions and systems (DNA) and the redesigning of existing, natural biological systems (e.g. a virus or bacterium) for specific purposes, such as the development of medicines.

On May 20-22 the second conference on synthetic biology took place in Berkely, USA*. At the conference there was a lot of attention for the societal aspects of synthetic biology. Biologists announced a voluntary code for self regulation of their work in synthetic biology. In reaction, an international network of societal organisations expressed their concerns and argued for sufficient societal debate regarding the regulation of the developments in this area. The Rathenau Institute sent a consultant to attend this conference and commissioned a report on the state of affairs concerning synthetic biology. The report will be ready September of this year.

* R.F. Service, Synthetic Biologists Debate Policing Themselves, Science, vol. 312, p. 1116 (2006).

In February of this year The Netherlands Commission on Genetic Modification (COGEM) formulated a monitoring report on the fast developments in synthetic biology for the Ministry of Housing, Spatial Planning and the Environment (VROM)*. In this report the COGEM concludes that the developments may eventually lead to a public debate, but that the discussion is currently limited to the scientific community. In the Netherlands little research in the area of synthetic biology seems to take place, research is mainly carried out in the US. The COGEM is of the opinion that current legislation for GMOs is not in all cases applicable to synthetic organisms. Framing an adequate risk analysis methodology in time will prevent surprises later on, warrant safety, and prevent unnecessary frustration of scientific developments.

Do you consider a joint exploration with regard to the content of synthetic biology, as a supplement to the monitoring report by the COGEM and the anticipated report by the Rathenau Institute, worthwhile?

Such exploration could address the following aspects:

- The state of affairs in the area of synthetic biology in the Netherlands.
- Where the developments are headed.
- What the possible interests in synthetic biology are.
- What should be done in the Netherlands in the area of synthetic biology.
- Whether the current legislation and risk assessment system for GMOs is adequate for synthetic biology.

The Minister of Education, Culture and Science
Signed by Maria J.A. van der Hoeven

* www.cogem.net/pdfdb/advies/CGM060228-03.pdf

Annex **B**

The committee

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- F.W.A. Brom, *observer*
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- S.G. van Keulen, *observer*
  COGEM, Bilthoven
- H. de Vriend, *scientific secretary*
  LIS Consult, Rijswijk
- S.H.M. Litjens, *scientific secretary*
  Health Council / Advisory Council on Health Research, Den Haag

The Health Council and interests

Members of Health Council Committees are appointed in a personal capacity because of their special expertise in the matters to be addressed. Nonetheless, it is precisely because of this expertise that they may also have interests. This in itself does not necessarily present an obstacle for membership of a Health Council Committee. Transparency regarding possible conflicts of interest is nonetheless important, both for the President and members of a Committee and for the President of the Health Council. On being invited to join a Committee, members are asked to submit a form detailing the functions they hold and any other material and immaterial interests which could be relevant for the Committee's work. It is the responsibility of the President of the Health Council to assess whether the interests indicated constitute grounds for non-appointment. An advisorship will then sometimes make it possible to exploit the expertise of the specialist involved. During the establishment meeting the declarations issued are discussed, so that all members of the Committee are aware of each other's possible interests.

The above procedure was also followed for this joint committee of the Health Council of the Netherlands, the RGO and the KNAW.

Annex **C**

Glossary of terms

Actin filaments
: Part of the cytoskeleton of eukaryotic cells. The filaments are polymers of a specific protein (actin) and are responsible for cell shape and motility.

Amino acids
: The building blocks of proteins, comprising a carboxyl group, an amine group, and a variable side chain.

Antibodies
: Proteins forming part of the immune system. Antibodies recognise substances that are foreign to their host, such as component parts of viruses and bacteria.

Base pair
: A complementary pair of nucleotides, connected by hydrogen bonds, which initiate the formation of a double helix in nucleic acid strands.

BioBricks
: Standardised ‘biological building blocks’ used for the construction of components that carry out specific tasks, which can in turn be used to construct more complex biological systems.

Codon
: A unit of three successive nucleotides on messenger RNA which codes on the ribosome for a single amino acid.

Directed evolution
: The purposeful creation of a large number of gene variants ('mutants'), which can then be tested for improved expression (products), e.g. improved enzyme activity.

DNA shuffling
: A method for the rapid propagation of beneficial mutations in an experiment using 'directed evolution' by recombining mutated genes.

Domain
: A part of a protein having a specific, 3-dimensional, folded structure and an accompanying function, e.g. calcium binding.

Enzymes
: Proteins able to speed up certain reactions; in other words, biological catalysts.

Error-prone PCR
: A PCR method which has a negative impact on the reliability of DNA polymerase. This increases the number of 'faults' and therefore genetic variance during PCR.

Genetic circuits
: Logical arrays of genetic elements, similar to electronic and micro-electronic circuits, which transform a given input signal into a given output signal.

Inverter
: A logic gate (in electronics) or a genetic circuit (in biology) whose output signal is the opposite of its input signal.

In vitro
: A cell-free system is the subject of research or engineering.

In vivo
: A cellular system (a few cells, unicellular organisms, or multicellular organisms) is the subject of research or engineering.

Lipids
: Fats and fatty substances composed of glycerol (hydrophilic) and three saturated or unsaturated fatty acids (hydrophobic). Phospholipids are the building blocks of cell membranes.

Liposome
: An artificial bubble consisting of a phospholipid membrane containing an aqueous solution.

Membrane
: A partition between two spaces. Cell membranes are composed chiefly of proteins and phospholipids.

Messenger RNA (mRNA)
: A single-strand copy of a gene that is responsible for carrying coding information from the DNA to the ribosome, where protein synthesis takes place.

Metabolic engineering
: The optimisation of genetic and regulation processes in the cell, aimed at raising the production of a certain substance.

Mitochondria
: Cell components responsible for its energy production.

Mutation
: An alteration in hereditable material. A mutation can, but need not, result in altered translation in amino acid production. If it does, we speak of a mutation in the resulting protein.

Nucleic acid
: A polymer of nucleotides. DNA and RNA are naturally-occurring nucleic acids.

Nucleotides
: The building blocks of DNA and RNA, consisting of three components: a phosphate group, a C5 sugar and a base (purine or pyrimidine).

Oscillator
: A circuit (in electronics) or genetic circuit (in biology) which generates a periodic signal.

PCR
: Polymerase Chain Reaction is a method of propagating certain parts of DNA from very small original amounts.

Polymers
: Molecules consisting of a linked sequence of one or more identical or similar parts.

Protein plaques
: Protein aggregates observed in disease, e.g. Alzheimer’s (in the brain) and type II diabetes (in the insulin-producing cells).

Ribosome
: A cell component that is responsible for protein synthesis. On the ribosomes, successive codons of mRNA are ‘translated’ into a chain of amino acids, forming a protein.

Toggle switch
: A circuit (in electronics) or genetic circuit (in biology) which reacts to an input signal by switching from one to the other of two states.

Valorisation

To make something useful. In the widest sense, valorising scientific knowledge and technology means making its use value available to society. This generally also implies its economic capitalisation.