Risk Engineering

Bernd Giese
Christian Pade
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Arnim von Gleich *Editors*

Synthetic Biology

Character and Impact

Springer

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Preface

The present contribution is mainly based on presentations of a lecture series held in winter term 2012/2013 at the University of Bremen. This work is part of the study "Technology Assessment of Synthetic Biology'' (SynBioTA) and has been funded by the German Federal Ministry of Education and Research (BMBF) (grant number: 16I1611). The scientific editors are responsible for the content of this volume.

We are grateful to the BMBF and our project manager VDI/VDE Innovation + Technik GmbH for the opportunity to conduct a research project on synthetic biology with particular focus on its scientific and technological character, achievements, and functionalities as well as consequences thereof. We also would like to express our gratitude to all contributors of this work, all speakers of the lecture series as well as all that were engaged in the organization and many fruitful discussions during the series. Finally, we highly appreciate the professional editing service of Ronald Naiditch and the substantial layout support of our student assistants Laura Hodrea and Jana Keller.

Bremen, May 2014

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Introduction

Synthetic Biology

At the beginning of this century, by establishing synthetic biology as a new field of applied science, increasing claims of comprehensive modification and recreation of biological entities became labeled. Through ambitious approaches of genetic engineering during past decades, they have already crossed the border from the nonliving sphere of traditional technology into the realm of the living. But synthetic biology should not be mistaken as just the next step of genetic engineering. It represents a rather young field of science and technology, which is supported by substantial progress in molecular biology, systems biology, bioinformatics, and biochemistry. Two main trends can be recognized within this field: on the one hand an increasing complexity of biological constructions, supported by systemic modeling, and on the other hand the more exterior influence of transferring engineering principles, concepts that have proven successful in dealing with nonliving objects, to living entities. Both tendencies affect the two major disciplines within synthetic biology: molecular genetics and systems biology (the latter is supported by bioinformatics). These disciplinary roots constitute a certain kind of stress between—in accordance with Westerhoff and Palsson—a more reductionist approach, in case of genetics, and a more holistic approach, represented by systemic modeling. Reductionist (mechanistic) and systemic (organic) approaches represent quite different attempts to master biological complexity. How successful they prove in domesticating complexity and what consequences they bear is of particular importance for the field of synthetic biology, as well as for the opportunities and risks combined with its applications. Besides these attempts to tackle complexity, synthesis in the form of preparing nonnatural constructs and compounds is a central element in synthetic biology. As in synthetic chemistry, synthesis is used as an opposing approach to a purely quantifying analysis. Beyond new and modified genetic circuits or metabolic pathways, synthesis on the molecular level is an especially important part of synthetic biology. And here, the influence of the third and often underestimated discipline within synthetic biology

comes into play: biochemistry, which is engaged in the integration of synthetic biomolecules into biological systems. Biochemistry supports constructions of the genetic as well as the systemic part of synthetic biology.

Synthetic biology in its self-concept is trying to replace previous, rather manipulative trial-and-error approaches by systematic and reliable design procedures derived from classical engineering. The aim is to tackle uncertainties, which up to now are accompanied by tinkering in complex biological environments. With the adoption of the term "synthetic biology," reassessment of biological processes as technical processes becomes a clear demand for a comprehensive redesign up to entirely new creations—far beyond the practice of classical biotechnology.

Due to the multifaceted character of the field, expectations and concerns differ widely. Great expectations are especially connected with medical applications (therapeutics, vaccines, and synthetic organs), energy supply (photosynthesis and biofuels), new biological materials, or environmental applications. But the claim turns out to be extremely ambitious. Envisioned paths were revealed to be more difficult to realize than originally expected. Biological matter, with its specific characteristics like noise, an adaptive and evolutionary behavior, as well as deeply interwoven molecular interactions seems to be more resistant to classical design approaches adapted from mechanical or electrical engineering than originally thought. Owing to a multitude of obstacles, synthetic biology still represents a field of mainly basic research up to now. Nevertheless, quite a number of promising applications have been presented and discussed, but the timescale for realization together with the prospects for a practical implementation are questionable, as we have learned from past innovation processes (e.g., in artificial intelligence). Accordingly, if opportunities and risks should be prospectively determined in the field's early stages of innovation, as well as corresponding future applications, analysis is confronted with a lack of detailed knowledge about products and processes and their application contexts.

Besides useful and promising functionalities, the prospect of using organisms created by synthetic biology evokes great apprehension. There are substantial concerns about the undesirable side effects and subsequent consequences for mankind and the environment, especially if these new creations could be released and are able to replicate. Beyond hazard potentials that are already known from synthetic chemistry or genetic engineering, risks may emerge from unexpected new properties and combinations of new qualities. Besides consequences of hazardous functionalities, especially for applications that are meant to be realized in an industrial scale, the debate on adverse effects is determined by indirect consequences like the excess consumption of feedstock from biomass for energy generation.

Organization of the Book

The present volume should provide insight into the character and the capabilities, as well as potential hazards and risks associated with synthetic biology. Thereby, it lays some ground to establish an appropriate and particularly early governance

approach for synthetic biology with the intention of tapping the full potential of synthetic biology with minimized risks, while complying with sustainable development in all affected application fields. Introduced by a discussion of definitions and the most affecting paradigms and methodologies, the edition provides an overview on the structure of this field of science and technology. It provides information about the stage of development and important application fields. But, the science itself is not the only focus. Ecological, socio-technical, and ethical implications are considered, which set the stage for a discussion of responsibilities in the context of this "field-in-transition" between basic and applied science. Finally, requirements for an appropriate governance and regulatory frame are discussed. Here, attempts for an early governance of innovation processes are confronted with the so-called Collingridge Dilemma, which means that in early stages of innovation the scope of action is still high and not reduced by increasing path dependencies, but knowledge of possible consequences is very low, as long as application contexts, products, processes, and intentions of use are not yet clear. As opportunities, as well as risks, are determined by technological functionalities and possibilities on the one hand, and application intentions and contexts on the other hand, two kinds of uncertainties are in focus: (a) uncertainties about specific applications as well as application contexts and (b) uncertainties about the effects and impacts of the applied functionality or technology. The present volume should assist in filling this gap by identifying facts—or better: evidence—which is already available. As application contexts are still unknown, the focus will be on functionalities of synthetic biology and their expected effects and impacts contributing to opportunities and risks.

This volume is meant to be a source of information and orientation for researchers in natural sciences, technology assessment, actors in governance and funding institutions, as well as for the public. For this reason, a broad range of important topics is addressed. Its thirteen chapters are dedicated to:

- the characterization of synthetic biology, an identification of its constitutive disciplines, its origin and the paradigms, which determine the theoretical and practical sphere of this discipline;
- an estimation of capabilities and competencies of synthetic biology by an analysis of methodological principles, objects of investigation and its technologically exploitable functionalities;
- the opportunity and risk potential of these functionalities;
- implications for health, safety, the environment and ethics;
- an analysis of legal requirements; and
- the question of responsibilities attached to the practice of synthetic biology as a field of basic and applied science.

With the first chapter *Jan C. Schmidt* explores major perceptions of synthetic biology and expands the analysis by investigating the core of this new technoscience: its dependency on instabilities as a basis for self-organization. Based upon the characterization as a self-productive technology, "late modern technologies" are introduced as a new wave of technoscience, with synthetic biology as one early exponent.

Synthetic biology as a nontraditional field of science—based on that notion *Alfred Nordmann* pleads for a broader perspective on the field's self-conception, its way of knowledge production, engineering, and design by taking into account alternative views beyond the traditional preconceptions of the philosophy of science. By this means, he develops a definition for synthetic biology that builds on the notion not of reducing but of systematically generating complexity.

Michael Bölker focuses on one of the most characteristic accompanying effects of biological self-organization: the inherent complexity of biological systems. Two major perspectives of synthetic biology are presented, promising to overcome the drawbacks emanating from the mere unpredictable nature of biological entities by reducing their complexity: on the one hand its famous demand, the application of engineering principles, and on the other hand orthogonalization—a strategy to construct freely combinable objects without interference—by modifications of the molecular basis of organisms. The latter option is strongly associated with aims of biological chemists like Eric Cool, who re-established the old term "synthetic biology" in 2000 for his approach of using synthetic molecules in biological systems.

The chapter by *Pade et al.* presents an overview of the field's methodologies, and introduces important functionalities that will be specifically enabled by synthetic biology. Relevant methods are introduced according to their relation to different organismic object levels (from the molecular to the cellular level). From the combination and integration of these approaches, in the second part of the chapter, enhanced and novel functionalities of synthetic biology will be derived.

By referring to some important product fields, *Gerd Klöck* gives an overview on the economic context of industrial biotechnology as well as a number of conflicts caused by boundary conditions or basic properties of these applied processes. He indicates that these problems could—at least partially—be overcome by approaches of synthetic biology.

French et al. present two application fields of synthetic biology which have attracted increasing attention in recent years: the use of whole cells with adapted or newly implemented genetic circuits and signal transduction cascades as sensors and, as one of the key aspects in energy generation, the stage of development in biomass conversion processes.

New functional dimensions in using unnatural amino acids for new cellular organelles and biohybrid-materials are explored in the chapter by *Stefan Schiller*. He explains how the beneficial combination of advanced methods in molecular biology with the potential of chemical biology enables the expansion of the variety of natural biomolecules, and creates entire "material libraries" of biological building blocks inside living cells.

Following the multifaceted applications introduced in previous chapters, *Antoine Danchin* focuses on important, but often overlooked requirements for a "cellular chassis"—the basis for most of these processes. Above all, he broaches the constraints of aging and rejuvenation, processes that continuously take place in cellular "factories" and thereby points towards the importance of thermodynamic phenomena in information recruitment of renewing cellular populations.

Bernd Giese and *Arnim von Gleich* trace potential risks of synthetic biology applications back to their roots in basic functionalities of newly designed or largely restructured entities. Further, they name critical application contexts for the risks to evolve. Based on this analysis the chapter continues with a survey of risk-reducing strategies, both those already applied and particularly new techniques, among which a safe and promising approach for critical application contexts could be identified.

A hierarchical risk assessment approach of the GeneRisk Research Consortium is presented by *Broder Breckling* and *Gunther Schmidt*. Despite the fact that it was developed for the cultivation of genetically modified organisms (GMOs), it is also adaptable to synthetic life forms when comparable risk dimensions have to be covered. The approach spans from the molecular level to landscapes and biomes, and should consider most of the relevant interactions on and between these levels.

The following chapters focus on regulatory and ethical aspects in the application of processes, products, and organisms of synthetic biology. Finally, in a concluding chapter the question of responsibility will be addressed.

Gerd Winter opens by asking to what extent the outcomes of synthetic biology are covered by the existing EU-regulation of GMOs. Accordingly, the applicability of the current risk assessment methodology for GMOs is questioned and the adequacy of liability schemes for eventual damage is addressed as well. This contribution indicates that there is an urgent need for adaption of the legal framework for synthetic biology.

Joachim Boldt makes a recourse to the theory of communicative action. He shows that synthetic biology up to now favors instrumentalization of nature by its claims for extensive redesign, instead of a more communicative approach in which beneficial effects would originate from an organism's inner tendencies and capabilities. As a consequence of corresponding future scenarios, he pleads for a careful step-by-step approach in synthetic biology's innovations.

Finally, in the last chapter *Armin Grunwald* closes the discussion with his investigation of how to attribute and distribute responsibility when—as in case of a technoscience like synthetic biology—the border between basic and applied science is rather vague. By applying an empirical, ethical as well as epistemological (EEE) model of responsibility for an analysis of the field, it turns out that further integration of all three dimensions is needed for a reflection of technological advances and an appropriate governance of the field.

Synthetic Biology as Late-Modern Technology

Inquiring into the Rhetoric and Reality of a New Technoscientific Wave

Jan C. Schmidt

Abstract The aim of this paper is to contribute to a *prospective science and technology assessment* (ProTA) of synthetic biology in order to enable an early societal shaping of this emerging wave of technoscience. To accomplish this goal, a philosophical approach towards the technoscientific core of synthetic biology—provided by philosophy of science and philosophy of technology—will be taken. The thesis is that if there is any *differentia specifica* giving substance to the umbrella term "synthetic biology", it is the idea(l) of harnessing self-organization for engineering purposes. To underline that we are likely experiencing an epochal break in the ontology of technoscientific systems, this new type of technology is called "late-modern technology." I start by analyzing the three most common paradigms and visions of synthetic biology (Sect. 2). Then I argue that one particular paradigm deserves more attention because it underlies the others: the paradigm of self-organization (Sect. 3). However, synthetic biology does not stand alone in making use of self-organization; it is a governing vision in robotics, ubiquitous computing, nano- and neuro-technologies (Sect. 4). Further, I show that instabilities constitute the conditions and, hence, the technoscientific core of self-organization (Sect. 5). Given the relevance of instabilities, I consider the inherent limits of late-modern (self-organization) technology in construction/design and control/monitoring, and in particular I elaborate why it is so difficult to control biosynthetic systems (Sect. 6). I end by drawing conclusions for the early-stage approach of ProTA and sum up the characteristics of late-modern technology as a challenging subject area of philosophy of technology (Sects. 7 and 8).

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1 Introduction: A New Technoscientific Wave?

Has yet another emerging technology—synthetic biology—rolled onto the shores of our late-modern society? Although the technology itself is in its infancy, the trendy buzzword is already heating up the debate over our society's future. It appears widely in science and innovation politics. Synthetic biology, it seems, is the crystallization point of late-modern technoscientific hypes and hopes. The research-entrepreneur Craig Venter is an outstanding player in this contested political and policy-driven arena. In 2010 Venter announced the forthcoming advent of an epochal break and envisioned a fundamental shift in our technical capabilities. Synthetic organisms "are going to potentially create a new industrial revolution if we can really get cells to do the production we want; […] they could help wean us off of oil, and reverse some of the damage to the environment like capturing back carbon dioxide" (Venter 2010). Venter's visionary claim was evidently induced by the success of his team in the *Creation of a Bacterial Cell Controlled by a Chemically Synthesized Genome*—as his article in the magazine Science was titled (Gibson et al. 2010). In fact, the hype Venter generated has actually started another huge wave: He has been accused of "playing God" or, at least, of advocating a dangerous type of "hubris" (Schummer 2011; Schmidt 2012a).

While such concerns and objections to Venter's (linear Baconian) optimism are central elements in the formation of public opinion and political deliberation, both extreme positions—Venter's and that of his critics—often lead to a deadlock. Maintaining them would mean missing opportunities to engage in a *prospective science and technology assessment* (ProTA) and to implement *anticipatory governance regimes* (Liebert and Schmidt 2010; von Gleich 2004; von Gleich et al. 2012; Grunwald 2012). To accomplish such an early assessment, our late-modern society needs methods to enable a detailed analysis and assessment of what "synthetic biology" is and what it encompasses. The interdisciplinary approach of ProTA (based on achievements in philosophy of science, technology, and technoscience) can help to provide well-balanced differentiations that substantiate early assessment procedures and allow us to shape the emerging technoscience. ProTA starts by addressing the technoscientific core and the common denominator within what is typically described as "synthetic biology." If there is any integrative essence and *differentia specifica* demarking "synthetic biology" from other recent technoscientific trends, it would appear that harnessing self-organization—including the ability to set off complex dynamical phenomena—could be considered as the central idea(l) giving substance to the trendy label. At the same time, it has to be conceded that framing "synthetic biology" from this angle addresses the visionary programs more than the current state-of-the-art. Synthetic biology is, indeed, still in its infancy; it is not clear whether an engineering approach of this kind is even feasible, particularly to the extent of control oriented "rational design" or "rational engineering," or whether it carries an inherent dialectic, conflict, or even self-contradiction (Giese et al. 2013). Should a technology based on self-organization ever be attained and implemented, we would enter a new era of technology in

which technical systems possessed high levels of autonomy and agency properties. The systems would "take on a life of their own such that we no longer appear to perceive, comprehend, or control them" (Nordmann 2008, 176). A shift, or perhaps even a turn, would take place in our understanding of what has to be considered as technology and, more fundamentally, of what technology actually *is*. The new type of technology could be called "late-modern," indicating that it is ontologically different to, and an extension of, the recent modern kind of technology.[1] In this paper I endeavor to give substance to the notion of "late-modern technology" as a critical-reflexive term from the perspective of *philosophy of technology* (Schmidt 2012a, 2014). Based on such a critical-reflexive analysis, the objective is to contribute to an early societal shaping of this new type of technology.

An interdisciplinary approach is essential to performing such an analysis: Insofar as synthetic biology is inherently interdisciplinary, it needs to be approached from an equally interdisciplinary angle in order to be able to analyze and assess this new technoscientific field.[2] ProTA follows such an interdisciplinary approach that brings together ("synthetically") various perspectives: political sciences, sociology, philosophy, ethics, history, and science and technology studies (STS), as well as physics, biology, informatics, mathematics, and systems sciences. In the following I will not present the rich framework of ProTA with its four different dimensions[3]; I will focus on one dimension: on the analysis and characterization of the technoscientific core. This dimension is particularly relevant when considering alternatives *to*, or *within*, the technoscientific core itself. In Sect. 2, I start by showing how "synthetic biology" is typically conceived. In light of its infancy and the minimal account of applications to date, I point out influential programs, paradigms, and visions. In Sect. 3, I argue that one particular program deserves more attention because it underlies and substantiates the other three: the program of self-organization, which is also interlaced with en vogue concepts of self-activity, self-assembly, self-replication, autonomy, and the like. My thesis is that synthetic biology can be regarded as an engineering approach intending to harness self-organization processes for technical purposes. As will be elaborated in Sect. 4, synthetic biology does not stand alone in making use of self-organization in engineering and technology. In fact, this is a very general trend in natural and engineering sciences, made possible by advancements in the systems and structural sciences: complexity theory, nonlinear dynamics, chaos theory,

[1] The main thesis presented in this paper is—to some extent—in line with what Nordmann calls "technology naturalized" (Nordmann 2008, 2005).

[2] The concept of critical-synthetic interdisciplinarity has been developed in Schmidt (2011/2014).

[3] Methodologically, ProTA encompasses four orientation perspectives that include four dimensions relevant to any innovation process: (1) early-stage orientation—the temporal dimension, (2) intention and potential orientation—the knowledge dimension, (3) shaping orientation—the power/actor dimension, and (4) orientation to the technoscientific core—the technoscientific dimension (Liebert and Schmidt 2010). The last dimension is closely linked to what von Gleich et al. (2012) refer to as "characterization of the system's type of a new innovation."

dissipative structures, synergetics, and others. These concepts convey a somewhat non-reductionist flavor, as they claim to facilitate holistic complex systems thinking. However, that is just one side of the coin. Two main trends converge in synthetic biology: the above mentioned general trend in systems and structural sciences, on the one hand; and a more traditional trend that could be called "technological reductionism," on the other. The convergence introduces a dialectical (or a contradictory) element into "synthetic biology." In Sect. 5, I dig deeper and examine the enabling conditions for self-organization. I show that instabilities constitute the conditions and, hence, the core of self-organization. They are therefore central to what can be called *late-modern* technology. This new ontology in the technoscientific core urges us to modify our understanding of technology and engineering. Section 6 considers the inherent limits of late-modern technology in construction/design and control/monitoring. On the basis of instabilities as the kernel of synthetic biology and, more generally, of late-modern technology, I elaborate upon why it is so difficult to engineer and construct biologically based systems, and why it is even harder to control them. Both issues question the idea(l) of rational design and traditional engineering that is widely favored by the proponents of synthetic biology. Late-modern technical systems have a life of their own; they seem to be autonomous and possess what could be called internal agency properties. In Sect. 7, I draw a number of conclusions for technology assessment (TA) and underline necessary requirements for an early-stage approach of prospective science and technology assessment (ProTA). I sum up the characteristics of late-modern technology as a challenging subject area of ProTA. If we foster the development of late-modern technology but neglect to contain it, we surrender our control over it.

2 Characterizing the Field: What Is "Synthetic Biology"?

Since synthetic biologists have not yet found a common voice, the exact meaning of the umbrella term "synthetic biology" is not clear at all. New labels and trendy watchwords generally play a key role in the construction of new technoscientific waves.[4] "Synthetic biology" is, indeed, an extremely successful buzzword, as was "nanotechnology" more than one decade ago. All TA scholars are aware of the fact that labels are strongly normative. Labels are not innocent or harmless, because they carry content and form the backbones of visions. They are roadmaps towards the future and can quickly turn into reality; they shape the technoscientific field and direct our thinking, perception, and judgment. Labels help to foster hopes and hypes, as well as concerns and fears; their implicit power to create or close new research trajectories and development roadmaps can hardly be overestimated.

[4] A new technological wave does not just occur or happen: it is also (probably even to a large extent) constructed (Liebert and Schmidt 2010).

Labels are part of what could be described as "term politics" that regulate and shape the field with a "gate keeper function" to decide who is *in* and who is *out;* whose research field can be considered as "synthetic biology" and whose is just part of traditional ("old-fashioned") biotechnology. Labels are relevant with respect to funding, publication opportunities, reputation, and career. Thus, they determine and direct our future, in one way or another. In brief: labels, buzzwords and visions matter strongly.

Although trendy labels have always posed a challenge for any kind of TA, this holds especially true with regard to any approach to an emerging (not yet established) technoscientific field. ProTA pursues an early-stage approach and therefore has to deal with labels, visions and claims, programs and promises (Liebert and Schmidt 2010; Grunwald 2012). ProTA starts by posing simple but fundamental questions: What does the umbrella term "synthetic biology" mean? Is there a unifying arc and a common denominator? What visions does synthetic biology have, and how likely are they to be achieved?[5] Three popular definitions[6] of "synthetic biology," and what it should be, stand out.

First, *the engineering definition* frames synthetic biology as being radically new since it is said to bring an engineering approach to the scientific discipline of biology. Such an understanding is advocated by a High Level Expert Group of the European Commission:

> Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems […]. This engineering perspective may be applied at all levels of the hierarchy of biological structures […]. In essence, synthetic biology will enable the design of 'biological systems' in a rational and systematic way. (European Commission 2005, 5)

This comes close to the definition given by the German Academy of Technical Sciences (acatech), which defines synthetic biology as "the birth of a new engineering science" (Pühler et al. 2011). Similarly, others view synthetic biology as "an assembly of different approaches unified by a similar goal, namely the construction of new forms of life." (Deplazes and Huppenbauer 2009, 58)

The engineering definition is generally based on the assumption that before synthetic biology arose, a clear line existed between biology as an academic discipline, on the one hand, and engineering/technical sciences, on the other. Biology

[5] Although it is sometimes helpful to consider the history of a notion in order to clarify what is meant, this is not the case with "synthetic biology." On the one hand, "synthetic biology" seems to be a fairly young term. It was (re-)introduced and presented by Eric Kool in 2000 at the annual meeting of the American Chemical Society. Since then, the term has gone on to enjoy a remarkable career and general circulation in the scientific communities as well as in science, technology and innovation politics. On the other hand, the notion of "synthetic biology" emerged about 100 years ago—though it was rarely mentioned until 2000. It seems more appropriate to consider the more recent understandings of "synthetic biology."

[6] The European Technology Assessment Group uses the term "paradigm" and states that synthetic biology can be considered a "new research paradigm" (van Est et al. 2010, 14).

is regarded as a pure science aiming at fundamental descriptions and explanations. In contrast, engineering sciences appear to be primarily interested in intervention, construction, and creation. Viewed from this angle, biology and engineering sciences have formerly been—in terms of their goals—like fire and ice. The proponents of the engineering definition of "synthetic biology" believe that the well-established divide between the two disciplines is becoming blurred. Today, engineering is transferring its goals to the new subdiscipline of biology. According to the advocates of this definition, these goals have never been characteristics of other subdisciplines of biology (*divergence from traditional biology*). The essential claim is that we are experiencing an epochal break or a qualitative shift within biology: the aim is not theory, but technology. Synthetic biology appears to be a perfect example of the "technoscientification", technicization, or engineering of biology.

Second, *the artificiality definition* of synthetic biology is related to the former definition but is more concerned with objects than with goals. According to the EU project TESSY ("Towards a European Strategy for Synthetic Biology") "synthetic biology" deals with "bio-systems […] that do not exist as such in nature" (TESSY 2008). In an equivalent sense it is stated that synthetic biology encompasses the synthesis and construction of "systems, which display functions that do not exist in nature" (European Commission 2005, 5). The German Academy of Technical Sciences similarly identifies the emergence of "new properties that have never been observed in natural organisms before" (DFG et al. 2009, 7). "Synthetic biology" is here defined by the non-naturalness, or unnaturalness, and artificiality of the constructed and created bio-objects. *Divergence from nature* appears to be the *differentia specifica* of "synthetic biology", with "nature" being seen as the central anchor and negative foil for this definition. Whereas bio-systems were formerly natural, i.e., they occurred exclusively *in,* and were created *by* nature alone, the claim here is that, from now on, they can also be artificial, i.e., created by humans. That is certainly a strong presupposition, and it is also linked to the idea of a dichotomy between nature and technical objects. The dichotomy traces back to the Greek philosopher Aristotle, who drew a demarcation line between *physis* (nature) and *techné* (arts, technical systems). In spite of Francis Bacon's endeavors at the very beginning of the modern epoch to eliminate the dichotomy and naturalize technology, the nature-technology divide broadly persists in the above definition.[7] In a certain sense, the artificiality definition of synthetic biology presupposes the ongoing plausibility of the Aristotelian concept of nature, neglects the Baconian one, and argues for an epochal break in the understanding of bio-objects and bio-nature: these are not given, they are made.

Third*, the extreme gene/biotechnology definition* leads either to a more relaxed perception of synthetic biology or, on the contrary, to it being condemned as a continuation of further trends already perceived as terrible and dangerous.

[7] Scholars argue that the split—inherently linked to the Aristotelian understanding of nature (and of technology)—is still present in the life-world (Schiemann 2006; Böhme 1992).

According to the proponents of this definition, we are experiencing a slight shift and mainly a continuation, not an epochal break; nothing is really new under the sun. Synthetic biology merely extends and complements biotechnology. Drew Endy, a key advocate of synthetic biology, perceives an "expansion of biotechnology" (Endy 2005, 449). Similarly, but from a more critical angle, the Action Group on Erosion, Technology and Concentration (ETC Group 2007) defines "synthetic biology" as an "extreme gene technology," mainly because synthetic biology is based on gene synthesis and cell techniques such as nucleotide synthesis, polymerase chain reaction (PCR) or recombinant cloning. The underlying methods, techniques, and procedures have been well established since the late 1970s. Although there have been tremendous advances from a quantitative standpoint, it is hard to discern any qualitative progress in the core methods. This definition rarely deals with goals or objects, but with methods and techniques. Its proponents claim (1) that methods constitute the core of synthetic biology, (2) that there has been no breakthrough in the synthetic/biotechnological methods and, moreover, (3) that a quantitative advancement cannot induce a qualitative one. Briefly, this position claims a *continuation in methods*—in contrast to a divergence from biology or nature as perceived in the above mentioned position.

Synthetic biologists, obviously, do not speak with a common voice. Consequently, we are faced with a plurality of different conceptions of what "synthetic biology" means or, put in normative terms, what it should mean. The three definitions—the engineering, the artificiality, and the extreme gene/biotechnology definition—tell three different stories. Each one exhibits some degree of plausibility and conclusiveness.[8] In spite of their apparent differences, all are concerned (a) with disciplinary biology or biological nature, and (b) with a rational design ideal in conjunction with a specific understanding of technology, technical systems, design and construction practice, and engineering action. But is such an approach justifiable and adequate, or does it need to be complemented with another perspective?

(ad a) The focus on biology (as a discipline) alone, including a discipline-oriented framing, prevents an exhaustive characterization of the new technoscientific wave. Since synthetic biology is *interdisciplinary* at its nucleus, this needs to be taken into account when looking for an adequate definition: Biologists, computer scientists, physicists, chemists, physicians, material scientists, and people from different engineering sciences are engaged in synthetic biology. Considering that various disciplinary approaches, methods and concepts coexist in synthetic biology, the term seems to be a label for a new and specific type of interdisciplinarity (Schmidt 2008d). Accordingly, a strong biology bias would surely be overly simplistic and entirely inadequate. To frame synthetic biology merely as a new subdiscipline of biology would represent a far too narrow approach. Thus, we need to ask ourselves whether we are facing a much more fundamental technoscientific wave than simply a change in one particular discipline or academic branch.

[8] In this paper, I do not address the question of evidence, i.e., whether each of the three definitions is sound and justified.

(ad b) In line with what has become known as bionano- or nanobio-research, the three definitions look at synthetic biology from the angle of technology and engineering. At first glance, this manner of approach appears viable in some respects: Synthetic biology extends and complements advancements in nanotechnology and hence spurs on a position that can be called "technological reductionism" (Schmidt 2004, 35f; cf. Grunwald 2008, 41f/190f). Technological reductionists aim at eliminating the patchwork of engineering sciences by developing a fundamental technology, or a root/core or enabling technology.[9] The slogan promoted by technological reductionism is: Shaping, constructing, and creating the world "atom-by-atom." Eric Drexler is a prominent advocate of technological reductionism. He argues that there are

> two styles of technology. […] The ancient style of technology that led from flint chips to silicon chips handles atoms and molecules in bulk; call it bulk technology. The new technology will handle individual atoms and molecules with control and precision; call it molecular technology. (Drexler 1990, 4)

The three definitions of synthetic biology given above concur strongly with technological reductionism. In fact, they can be characterized as different kinds of technological reductionism. It certainly seems plausible to put synthetic biology in the context of this new type of technology oriented reductionism. But whether that is all that can, or should, be said to characterize synthetic biology still needs to be clarified. Most clearly, synthetic biology differs from nanotechnology, which can be regarded as a paradigm of a technological reductionist approach. Synthetic biology claims to pursue an approach that is complementary to nanotechnology and has been called "systems approach" or, in a more visionary sense, "holistic." Given the widespread reference to "system," including the claim of successful application of "systems thinking," synthetic biology seems to involve a convergence, or dialectical relationship, of seemingly contradictory concepts: (system's) holism and (technological) reductionism (with its strong control ambitions and emphasis on rational engineering). This inherent dialectic is obviously central to an adequate and appropriate understanding of synthetic biology. The three definitions presented so far do not consider this point.

The latter two comments indicate that we should not adopt the three narrow definitions of synthetic biology given above; our characterization has to go further. Although it is in principle not erroneous or misguided to see synthetic biology (a) as a biological subdiscipline and (b) as a technological reductionist position, this view is one-sided, biased, and limited in depth and scope. It therefore needs to be supplemented with another approach that also considers fundamental tendencies in science and technoscience in general, and focuses in more detail on the technoscientific core of the emerging technoscientific wave. Briefly, a more (critical-synthetic) interdisciplinarily informed approach seems essential in order to address the interdisciplinary field of synthetic biology.

[9] This term was coined and developed in (Schmidt 2004, 42). A somewhat similar notion is "general purpose technology." Technological reductionism has not yet been recognized by philosophy of science and by STS. It is not to be confused with exploratory-oriented reductionism ("epistemological reductionism;" "representation").

3 Self-organization as a Common Denominator of Synthetic Biology

The three definitions presented above are, it seems, insufficient to grasp the technoscientific core of synthetic biology. For a more comprehensive characterization we should *not* restrict ourselves to goals (as in definition 1), objects ("ontology", as in definition 2), or methods ("methodology", as in definition 3), but also consider the underlying principles and concepts within the technoscientific field.[10] This is central to the method of *prospective science and technology assessment* (ProTA) (Liebert and Schmidt 2010).

We need to add a further definition—fourth, *the systems or self-organization definition*—that is prevalent in synthetic biology research programs. Synthetic biology harnesses, or at least aims to harness, the self-organization power of nature for technological purposes[11]: "Harnessing nature's toolbox" in order to "design biological systems," as David A. Drubin, Jeffrey Way and Pamela Silver state (Drubin et al. 2007). As early as 2002, Mihail Roco and William Bainbridge anticipated new frontiers in research and development by "learning from nature." They perceived the possibility of advancing technology by "exploiting the principles of automatic self-organization that are seen in nature" (Roco and Bainbridge 2002, 258).[12] According to Alain Pottage and Brad Sherman, the basic idea of synthetic biology is to "turn organisms into manufactures" and to make them "self-productive" (Pottage and Sherman 2007, 545).[13] The paradigm based on self-organization and self-productivity is implicitly present in many papers on synthetic biology. Pier Luigi Luisi and Pasquale Stano, the principle investigators in the *minimal cell mimicry* project, also advocate an understanding of synthetic biology based on self-organization:

> [S]ynthetic cells represent one of the most ambitious goals in synthetic biology. They are relevant for investigating the self-organizing abilities and emergent properties of chemical systems—for example, in origin-of-life studies and for the realization of chemical autopoietic systems that continuously self-replicate—and can also have biotechnological applications. (Luisi and Stano 2011, 775)

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[10] For example, the theories and concepts of the emerging technosciences (in a wider sense: "epistemology").

[11] There is a word family for "self-organization". Cognate terms encompass: self-assembling, self-optimizing, self-replicating, self-growing, self-(re-)producing, self-constructing, self-activity, self-moving, self-orientating ….

[12] In 1999 a working group of the National Science and Technology Council anticipated a new wave of technology based on self-organization: "With its own version of what scientists call nanoengineering, nature transforms these inexpensive, abundant, and inanimate ingredients into self-generating, self-perpetuating, self-repairing, self-aware creatures that walk, wiggle, swim, sniff, see, think, and even dream" (Amato 1999, 1; cf. Nordmann 2008, 174).

[13] A new phase of "instrumentaliz[ing …] animate nature" is just emerging (Pottage and Sherman 2007, 545).

They conclude:

> All this is very challenging, and places research on the bottom-up construction of synthetic cells at the cutting-edge of a multidisciplinary field that embraces […] systems chemistry and the concepts of self-organization and emergence. (ibid., 756)

Synthetic biology is, indeed, an interdisciplinary field. The philosopher and former engineer Jean-Pierre Dupuy discerns that "[t]he paradigm of complex, self-organizing systems is stepping ahead at an accelerated pace, both in science and in technology" (Dupuy 2004, 12/13). Biologists, physicists, chemists, computer and systems scientists from the *Brussels school of complexity* state that in their recent research they are aiming at

> designing and implementing artificial self-organizing systems in order to fulfill particular functions. Such systems have several advantages over more traditional systems: robustness, flexibility, capability to function autonomously. (Heylighen 2002, 23)

The computer scientist Jordan Pollack puts self-organization at the very center of his vision of designing advanced biomaterials. Pollack's goal is to "break […] the limits on design complexity," as his article is entitled.

> We think that in order to design products 'of biological complexity' that could make use of the fantastic fabrication abilities […], we must first liberate design by discovering and exploiting the principles of automatic self-organization that are seen in nature. (Pollack 2002: in Roco and Bainbridge 2002, 161)

In fact, the systems approach of putting the self-organization power of bioengineered entities at the very center of the new technoscientific wave has enjoyed an impressive history over the last three decades. It goes back to one of the most popular and highly controversial publications by K. Eric Drexler in the early 1980s. Drexler talks about "self-assembly," "engines of creation" and "molecular assemblers."[14]

> Order can emerge from chaos without anyone's giving orders [… and] enable[s] protein molecules to self-assemble into machines. […] Assemblers will be able to make anything from common materials without labor, replacing smoking factories with systems as clean as forest. (Drexler 1990, 22 f.; cf. Nolfi and Floreano 2000) [15]

Drexler goes even further and claims that emergent technologies "can help mind emerge in machine." Richard Jones takes up Drexler's ideas and perceives a trend towards "self-organizing […] soft machines" that will change our understanding of both nature and technology (Jones 2004). From a different angle but in a similar vein, the 2009 report "Making Perfect Life" of the European TA Group (ETAG) refers to advancements in synthetic biology:

> Synthetic biology […] present[s] visions of the future […]. Technologies are becoming more 'biological' in the sense that they are acquiring properties we used to associate with living organisms. Sophisticated 'smart' technological systems in the future are expected to

[14] His approach was very visionary—it probably overestimated the feasibility of manipulation.

[15] Ray Kurzweil argues from another perspective: "We already have a set of powerful tools that emerged from AI research and that have been refined and improved over several decades of development. The brain reverse engineering project will greatly augment this toolkit by also providing a panoply of new, biologically inspired, self-organizing techniques" (Kurzweil 2005, 265).

> have characteristics such as being self-organizing, self-optimizing, self-assembling, self-healing, and cognitive. (van Est et al. 2010, 4) [16]

And the philosopher and cultural scientist Alfred Nordmann sees a new kind of (understanding of) technology emerging in the field "where engineering seeks to exploit surprising properties that arise from natural processes of self-organization" (Nordmann 2008, 175; cf. Nordmann 2005). A "shift from" what Nordmann calls "nature technologized" to "technology naturalized" can be observed, which "is usually hailed as a new, more friendly as well as efficient, less alienated design paradigm." (ibid., 175)[17] These quotes underscore that a new technoscientific wave which is inherently linked to the label "synthetic biology" does indeed seem to be emerging.

The visions can hardly be expressed from the perspective of biology (as a discipline) alone. We are experiencing a widespread momentum towards interdisciplinarity in engineering and natural sciences and their two-way convergence which also gives substance to the frequently used notion of "technoscience" (cf. Liebert and Schmidt 2010; Nordmann 2006). According to the National Science Foundation, the concept of self-organization serves as a central element towards convergence, integration, and unification within our functionally differentiated, disciplinarily organized, heterogeneous science system: "Unifying Science and Engineering" seems to become possible by "using the concepts of self-organized systems, chaos, multi-length and time-scale organizations, and complex systems" (Roco and Bainbridge 2002, 84). This is certainly a strong claim.

4 A Visionary Promise: Towards a "Late-Modern Technology"?

If, for a moment, we take the visionary promises of a self-productive technology as serious claims, they herald the emergence of a new type of technology that is inherently linked to the concept of self-organization. We do not know whether the promises can be fully kept. However, if this were ever the case, we would encounter a different kind of technology: a *late-modern technology*, as this new type could be named (cf. Schmidt 2012a; Kastenhofer and Schmidt 2011).[18] The aim of this provocative notion is to anticipate a possible *late-modern turn* in the ontology of technology.[19]

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[16] And the ETAG goes on to stress: "Central in their ideas is the concept of self-regulation, self-organization and feedback as essential characteristics of cognitive systems since continuous adaption to the environment is the only way for living systems to survive" (van Est et al. 2010, 25).

[17] Nordmann continues: "Rather than force nature into the mold of crude machinery, biomimetic engineering learns from the intelligence and complexity of nature's own design solutions" (ibid., 175).

[18] In the same vein, Hubig talks about "trans-classical technology" (Hubig 2006) and Karafyllis about "biofacts" (Karafyllis 2003).

[19] The notion "late-modern technology" should not be mixed up with any kind of postmodernism.

Late-modern technology does not resemble our established perception and understanding of technology and technical systems. From a phenomenological perspective, it is a new type of technology which appears as (bio-)nature and displays nature-like characteristics; it appears "un-technical" or "non-artificial." Self-organization (late-modern) technology seems to possess an intrinsic momentum of rest and movement within itself—not an extrinsic one. Such characteristics come close to the Aristotelian and common life-world understanding of nature (cf. Schiemann 2004): Technology is alive or *appears* to be alive, as (bio-)nature always has been. The internal dynamics (i.e., acting, growing, and changing) of self-organization technology make it hard to draw a demarcation line between the artifactual and the natural in a phenomenological sense. Traditional technical and mechanical connotations have been peeled off. Nature and technology seem indistinguishable. Even where it is still possible to differentiate between the artificial and the natural, e.g., in robotics, we are confronted with more and more artifacts displaying certain forms of behavior that traditionally have been associated with living systems.[20]

Synthetic biology does not stand alone in this trend towards a late-modern turn in technology. Rather, synthetic biology can be considered the figurehead of a new type of interdisciplinary engineering that harnesses self-organization processes. Generally speaking, self-organization also plays a constitutive role in other kinds of emerging technologies such as (a) robotics, AI, pervasive computing, autonomous (software) agents/bots; (b) nano- and micro-systems technologies; and (c) cognitive, neuro- and pharmaco-technologies. Thus the paradigm of self-organization is broadly present in various kinds of sciences and engineering. Besides the notion of self-organization, several related concepts exist: self-assembling, nonlinearity, complexity, autopoiesis, emergence, instability, sensitivity, chaos, deterministic chance, interactivity, flexibility, adaptivity, evolutionary process.

Let us dig a bit deeper in order to characterize the root of this development. The *phenomenological convergence* of artifactual/technical systems with natural/living systems is induced by a much more fundamental convergence that could be called *nomological convergence*. Mathematical structures that describe self-organization in technical systems converge with those in nature. Although the disciplinary objects might differ, their behavior and dynamics show a surprising similarity on an abstract level. According to M.E. Csete and J.C. Doyle, "[a]dvanced technologies and biology […] are far more alike in systems-level organization than is widely appreciated" (Csete and Doyle 2002, 1664). The guiding idea(l) of *nomological convergence* can be traced back to the cyberneticist Norbert Wiener more than half a century ago.[21] Wiener defined structure-based convergence with regard

[20] Ancient philosophers would claim that it is physis/nature and not techné because of its internal capacity for self-organization.

[21] From a related but different angle—with reference to Kant—Nordmann talks about a "noumenal technology" to stress the "unknowability" of this kind of technology and "a limit to theoretical understanding" (Nordmann 2008, 180; see also: Nordmann 2005, 3ff).

to specific "structures that can be applied to and found in machines and, analogously, in living systems" (Wiener 1948/1968, 8).[22]

This recent development in sciences and engineering is made possible by advancements in interdisciplinary systems and structural sciences.[23]

> Structural sciences encompass systems analysis, information theory, cybernetics and game theory. These concepts consider structural properties and features of different objects regardless of their material realm or disciplinary origin. Time-dependent processes form a common umbrella that can be described by an adequate mathematical approach and by using the powerful tools of computer technology,

As the physicist, philosopher and programmatic thinker Carl Friedrich von Weizsäcker pointed out about 40 years ago (Weizsäcker 1974, 22f; cf. Schmidt 2008b).[24] Today, we can add self-organization theories which encompass nonlinear dynamics, complexity theory, chaos theory, catastrophe theory, synergetics, fractal geometry, dissipative structures, autopoiesis theory, and others. Following the first wave of structural and systems sciences such as information theory, game theory, and cybernetics (Bertalanffy, Wiener, Shannon, von Neumann) in the 1930s and 1940s, we are now experiencing a second wave (Prigogine, Haken, Maturana, Varela, Foerster, Thom, Ruelle) that began in the late 1960s. Self-organization, macroscopic pattern formation, emergent behavior, self-structuring, growth processes, the relevance of boundary conditions, and the Second Law of Thermodynamics (entropy law) with its irreversible arrow of time are regarded as conceptual approaches to disciplinarily different types of objects, based on evolutionary thinking in complex systems.[25] Assisted by the spread of computer technology, concepts of self-organization had a tremendous impact on scientific development in the second half of the 20th century.[26]

Structural and systems sciences address structural analogies of objects or material entities of various disciplines ("structural similarities"). Bernd-Olaf Küppers argues that we can observe a "convergence" of physics, chemistry, biology, computer and engineering sciences: "We are experiencing a process towards convergence of physics and biology, envision a new practice under the umbrella of structural sciences." (Küppers 2000; cf. Schmidt 2008c; Mitchell 2008) Central to structural sciences is dynamical systems thinking in conjunction with a context-based pragmatic differentiation between systems and their environment, as well as consideration of the interdependencies between the parts and the whole. Systems thinkers tend to regard reductionists' approaches, which are prominent in the grand unification project in physics, as misguided. According to systems

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[22] My translation from German (J.C.S.).

[23] In German "Strukturwissenschaften" (Weizsäcker 1974, 22). Weizsäcker coined the term "structural science".

[24] My translation from German (J.C.S.).

[25] In particular, thermodynamics with its open non-equilibrium systems and the exchange of matter, energy, and information plays a key role (cf. J. C. Schmidt 2010; Mainzer 1996).

[26] They have also induced the most recent developments in nanobiotechnology, robotics, artificial agents systems, and synthetic biology.

thinkers, reductionism—whatever that might mean in detail—is the wrong way to advance the sciences. For instance, Khushf (2004) alludes to systems thinking to support his thesis of a fundamental shift in sciences and technosciences towards a "more holistic approach to knowledge" (ibid.). During the last 40 years, structural and systems sciences have widened the methodology of various disciplines and initiated a fresh kind of interdisciplinary thinking to advance science. The same overall development can also be witnessed in the realm of biology: first as systems biology and, a bit later, as synthetic biology. The biologists Westerhoff and Palsson perceive a shift in biology from a molecular-biological approach towards a systems paradigm that includes fundamental questions relating to

> molecular self-organization. […] The problem of biological self-organization is to understand how structures, oscillations or waves arise in a steady and homogenous environment, a phenomenon called symmetry breaking. The Prigogine school and others developed the topic from the perspective of non-equilibrium thermodynamics in molecular contexts. […] Contemporary systems biology has a historical root outside mainstream molecular biology, ranging from basic principles of self-organization in non-equilibrium thermodynamics, through large-scale flux and kinetic models to 'genetic circuit' thinking. (Westerhoff and Palsson 2004, 1249)

Systems and synthetic biologists refer to classic works of structural and systems sciences, for example, Bertalanffy's *General System Theory* (1968) and Wiener's *Cybernetics* (Wiener 1948/1968; cf. Kitano 2002). Systems thinking, today, is not primarily directed at theoretical insight, but at technological progress. Advancements in synthetic biology seem to require systems thinking, as the synthetic biophysicist Petra Schwille underlines:

> [T]o be successful, synthetic biology of any kind will have to join forces with systems biology. (Schwille 2011, 1253)

The systems paradigm—interlaced with the ideal of non-reductionism[27]—appears to be the distinctive feature of synthetic biology and constitutes the main difference between synthetic biology and classical biotechnology, Schwille and Kitano both argue.

> A transition is occurring in biology from the molecular level to the system level that promises to revolutionize our understanding of complex biological regulatory systems and to provide major new opportunities for practical applications. (Kitano 2002, 1662)

Viewing nature through the eyeglass of systems and structural sciences entails a different conceptual understanding of nature—an understanding that obviously has many intersections with the technical sphere. Nature is regarded as active and productive: Nature is nature insofar as it is able to self-organize and to set off complex dynamic phenomena. This specific capability of nature is very appealing

[27] It should be noted that, although this is certainly a guiding ideal, it does not reflect the state-of-the-art. In synthetic biology we find a considerable internal dialectic between non-reductionism ("holism") and reductionism.

and promising to bio-engineers. Synthetic biologists aim to enhance and transgress nature by using nature's self-organization principles, in short: *transgressing nature by harnessing nature!* They conceptualize nature as a kind of technology or, more specifically: as a universal engineer (cf. Nordmann 2008, 173f). This specific framing of nature in the realm of technology is new. Although Francis Bacon naturalized technology by showing that it obeys the laws of nature, a strong, still somewhat Aristotelian nature-technology dichotomy has been the predominant view for a long time (cf. Schiemann 2004). Engineering did not commonly allude to an "active" nature, or regard nature as a model for developing and improving technology.[28] Although Leonardo da Vinci was a visionary pioneer of the program of using nature as a kind of blueprint for technological advancement, the first noteworthy engineering movement in this direction was *bionics* or *biomimicry* which emerged in the 1970s (cf. von Gleich 1998; Schmidt 2005b).[29] Bionics can, in some respects, be considered as a precursor of synthetic biology, although bionics was not broadly concerned with the principles and nomological structures of nature, but rather with concrete forms and material entities.[30]

Synthetic biology deepens the approach of bionics—and goes far beyond it. Nature's capacity to set off self-organization seems to appeal to synthetic biologists who see new technical opportunities for better (a) products, (b) processes, and (c) performance. The underlying understanding of nature comes close to the twofold concept of nature once advocated by Schelling and German idealism: "self-organization" as *natura naturata* (product) and as *natura naturans* (process). The idealistic concept of nature matches perfectly with a somewhat functional, technical, and economic perspective: as strategies of productivity, optimization, and adaptation. Nature is seen as the perfect problem solver; as being much more efficient and effective than any action of a classic engineer following the ideal of rational design or rational construction (cf. Giese et al. 2013). Late-modern technology appears to act by itself: It creates, designs and produces (a., productivity concept of self-organization); it selects means to ends and follows a means-ends rationality (b., optimization concept); and it takes decisions and acts according to its environmental requirements (c., adaptivity concept). These three interwoven characteristics of late-modern technology are highly appreciated by synthetic biologists. Technology evidently presents itself as an autonomous actor: "Autonomy"—a term that is central to our thought tradition and Enlightenment—seems to be ascribable to the late-modern technical systems. The words used by Schelling and Aristotle to characterize nature also seem to apply to the recent type of technology: Late-modern technical systems are "not to be regarded as primitive" (Schelling 1994) insofar as they have the momentum of rest and movement in themselves, not from humans (Aristotle, in his *physics*).

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[28] Nature is, in fact, viewed from a somewhat technical ("technomorphic") perspective.

[29] Bionics can be regarded as an "interdiscipline" between biology and engineering.

[30] In addition, it puts forward a somewhat static view of nature.

5 What Is Self-organization? Instability as the Core of Late-Modern Technology

Synthetic biology harnesses, or aims to harness, self-organization capability for technical purposes—that is the thesis put forward in this paper. However, it is not very precise to speak of "self-organization." Further clarification of this somewhat enigmatic notion is needed.

Immanuel Kant introduced and coined the term in the 18th century in his early works on the structure of the universe. Kant was in some respects a precursor to the evolutionary understanding of the entire physical cosmos when he spoke of the "self-organizing of the Universe" (Kant 1755). More than three decades later in his *Critique of Judgment*, 1790, Kant no longer focused on the physical, but on the biological sphere and considered "self-organizing beings" (§ 65) (Kant 2007/1790). His reservations regarding a mechanistic-physical explanation of the organismic world are well known: "There will never be a Newton of the blade of grass" (ibid.). Friedrich Wilhelm Joseph Schelling elaborated on the Kantian notion and further developed the idea of self-organization in his *Speculative Physics* (1801) as part of his work on transcendental idealism. Schelling, in the tradition of Aristotelian thinking, maintained a dichotomy between nature, on the one hand, and arts (technical systems, technology) on the other. Nature "organizes itself; it is not to be regarded as a work of art" (Schelling 1994, 94).[31]

Since Kant and Schelling, the concept of "self-organization" has been in flux, and the nature/technology border has, at least to some extent, become dissolved. In contrast to these changes, "self-organization" seems to have retained its central meaning, which is the intrinsic origination, creation, and construction of novelty; in other words, the emergence of novel entities, patterns, structures, functionalities and performance capacities, or—in a more far-reaching and far more contested sense—the "fulguration" or the very sudden appearance of consciousness, subjectivity, and intentionality (cf. Schmidt 2008a, 2010). Since the late 19th and beginning of the 20th century, the concept of emergence[32] has played a key role in formulating a semantic specification of "self-organization" (cp. Beckermann et al. 1992; Schmidt 2010). The main ideas date back to the ancient Greeks, in particular to Aristotle and, to some extent, to Plato. The emergence of novel systemic properties—which is crucial to the understanding of self-organization—has been an ongoing topic of philosophical inquiry. Until now, no consensus has been reached as regards how to characterize novelty and the dynamics towards novel entities, structures, properties, or functionalities. Besides ontological approaches, which perceive novelty as rooted in the deep structures of reality itself, others such as those of pragmatists, conventionalists, operationalists, and constructivists argue that "novelty" is nothing but a

[31] My translation (J.C.S.).

[32] Although the term and concept of "emergence" appeared late in the scientific-philosophical debate (it was coined by George H. Lewes in 1875 and popularized in the early 20th century by the scholars of British Emergentism, Conwy Lloyd Morgan, Samuel Alexander, Roy W. Sellars and William McDougall) its content matter has a fairly long history.

human ascription to certain classes of phenomena. Beyond the dispute on the characteristics of (a) novelty, further criteria to specify "self-organization" have been proposed. They include (b) *internal dynamics*, inherent processes and time-dependency ("ontology"), (c) *irreducibility* of knowledge, theories, models, or description length (epistemology), and (d) *unpredictability* of the self-organized or emergent phenomena (methodology) (cf. Schmidt 2005a, 2008a). Hence, (e) self-organization cannot be governed and controlled (by an external acting engineer) in all details—only initial and boundary conditions are accessible. In brief, the notion of self-organization is, from an engineering perspective, inherently linked to characteristics such as "productivity," "processuality," and "autonomy."

Such clarification can be regarded as a cornerstone requirement for an in-depth characterization of the new wave of emerging technology. The concept of *prospective science and technology assessment* (ProTA) additionally aims to achieve a deeper understanding of the technoscientific core of synthetic biology. It has been said that synthetic biology's core is its claim of harnessing self-organizing power for technological purposes. But what is the core or root of self-organization?—The basic answer that I propose is that *instabilities* are essential for self-organization phenomena; they are constitutive of all systems or structural theories (Schmidt 2005a, 2008a). The physicist J.S. Langer, for instance, underlines the role of "instabilities for any kind of pattern formation" (Langer 1980). According to Werner Ebeling and Reiner Feistel,

> self-organization is always induced by the instability of the 'old' structure through small fluctuations. […] This is why studying instability is of major importance. (Ebeling and Feistel 1994, 46)

Gregory Nicolis and Ilya Prigogine argue that "instabilities [are …] necessary conditions for self-organization" (Nicolis and Prigogine 1977, 3f). Wolfgang Krohn and Günter Küppers, in the same vein, emphasize that "instabilities are the driving force and the internal momentum for systems evolution and development" (Krohn and Küppers 1992, 3).[33] Instabilities can generally be regarded as situations in which a system is on a razor's edge: criticalities, flip or turning points, thresholds, watersheds. They generate sensitive dependencies, bifurcations, points of structural changes, and phase transitions. The prominent example used to illustrate instability is the "butterfly effect." The beating of a butterfly's wings in South America can have tremendous influence on the weather in the U.S. and cause a thunderstorm.[34] Instability is, therefore,

[33] Instabilities can be regarded as the source of self-organization, complexity, emergence, and noise. Nonlinearity is necessary, but not sufficient in this realm.

[34] The list of examples is extensive (cp. Schmidt 2011): the emergence and onset of a chemical oscillation, the role-dynamics of a fluid in heat transfer, an enzyme kinetic reaction, a gear chattering, or turbulence of a flow. A fluid becomes viscous, ice crystallization emerges, a phase transition from the fluid to a gas phase takes place, a solid state becomes super-fluid, a laser issues forth light, a water tap begins to drip, a bridge crashes down, an earthquake or tsunami arises, a thermal conduction process comes to rest, and a convection sets in, e.g., Bénard instability. New patterns and structures appear. These examples underscore the fact that instabilities are *the necessary* condition for *novelty*. The various definitions of complexity refer directly or indirectly to instabilities—even if there is no reference to the genesis and evolution of a new pattern as is the case with the more geometric definitions of complexity via "dimensions."

characterized by a relationship: similar causes, different effects. The classic-modern *strong* causation[35] does not govern these processes; rather, it is the *weak* type of causation that enables feedback procedures and amplification processes. Instabilities can induce random-like behavior, deterministic chance, and law-based noise.[36] Hence, instability should not be unfairly equated with the collapse of a system.

Contrary to the classic-modern view of technology, synthetic biology values instabilities. In the realm of late-modern technology, researchers welcome instabilities as positive—insofar as instabilities constitute the nucleus of self-organization and emergence. The positive view of cognate phenomena such as noise, randomness, stochasticity, and fluctuations comes close to the positive appreciation of instabilities. Instabilities can, in fact, be regarded as a source of law-based white noise (cf. Mainzer 1996). In their seminal review paper on "the functional roles for noise in genetic circuits," the biologists Avigdor Eldar and Michael B. Elowitz outline a research program on noise encompassing the recognition of noise as a positive factor inherent in biological matter and life (Eldar and Elowitz 2010). Like instability, noise is perceived as a relevant factor in development, symmetry breaking, self-organization, growth and becoming. Citing cutting-edge experiments and simulations on noise, Eldar and Elowitz pose an impressive set of general, but also very fundamental questions.

> Whereas it is clear how noise can disrupt otherwise precise genetic programs, it is less obvious whether it can, counter-intuitively, improve cellular regulation. (ibid., 168)

And they continue:

> The question of how cells and organisms use and control random variation in their own components to grow, develop and evolve goes right to the heart of many fundamental biological problems. We anticipate that future work will continue to reveal unexpected, and essential, roles for noise in diverse biological systems. (ibid., 172)

Eldar and Elowitz talk about "order from noise" and stress the positive role of noise in living structures in various respects. Noise is inherent to biological matter.[37]

Insofar as synthetic biology aims at harnessing self-organization power, it has to provoke and stimulate instabilities and instability-based noise: Self-organization requires that a system's dynamics pass through unstable situations.

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[35] Strong causation can be characterized as: similar causes, similar effects. In other words, small initial differences do not play a major role.

[36] These characteristics are not very precise. If we take a closer look, three kinds of instability can be distinguished: (a) Static instability (or watersheds), (b) dynamical instability (deterministic chaos), and (c) structural instability (bifurcations, thresholds, criticalities) (Schmidt 2011).

[37] They further state, "*first,* noise can enable certain useful physiological regulation mechanisms, such as coordinating the expression of a large set of genes. *Second,* at the population level, noise permits a wide range of probabilistic differentiation strategies from microbial to multicellular organisms. *Third,* noise can facilitate evolutionary adaptation and *developmental* evolution. […] Noise is not merely a quirk of biological systems, but a core part of how they function and evolve" (ibid).

Instabilities—in alliance with noise and random fluctuations—are the prerequisite for leaving stability behind and enabling emergence, novelty, change, and evolution, as well as adaptivity, flexibility, and sensitivity. Thus instabilities constitute the (techno-)ontological core of the activity and productivity that synthetic biologists hope to achieve and utilize. To put it metaphorically: Synthetic biology is the technoscientific attempt to stimulate a *productive dance on the razor's edge*; and, at the same time, its aim is to master the induced instabilities—which is by all means a technically tricky undertaking.

6 The Unknowable: The Inherent Dialectic of Late-Modern Technology

The instability-based type of technology is somewhat ambivalent as it obviously carries an internal conflict or considerable dialectic that cannot be overcome by minor modifications of the technical system itself. On the one hand, instabilities constitute the core of self-organization and, hence, of technologically relevant self-productivity. On the other hand, instabilities are intrinsically linked with obstacles and limitations not only with regard to the construction, creation, and design of the technical systems but also with regard to the possibility of subsequently controlling and monitoring them. The latter limitations can be inferred from the systems and structural sciences.[38] A closer examination of these limitations can help us to appreciate why it is so difficult to engineer and harness self-organization for technological purposes—in particular why it will forever remain a challenge to utilize bio-objects as technical systems. The source of the limitations can be found in the inherent instabilities that question the central ideas of both classic engineering and synthetic biology, i.e., "rational design" and "rational engineering". When instabilities are present, tiny details are of major relevance; minor changes in some circumstances can cause tremendous, unforeseeable effects. Unstable systems lack predictability and (re-)producibility. The tiny details are hard to control, due to empirical-practical and to fundamental-principle uncertainties. To put it in paradoxical terms: Although they are constructed by humans, the systems remain fundamentally inaccessible and elude comprehension and control (cf. Schmidt 2008b, 2012b; Köchy 2011; also: Nordmann 2008).

Their inaccessibility restricts various rational attempts (a) to intervene and manipulate (given) self-organizing systems, (b) to create and design such (new) systems, and further, (c) to control, monitor, and handle them.[39] Due to these limi-

[38] This is somewhat ironic because such technoscientific approaches form the very basis for emerging technologies such as synthetic biology.

[39] The limited availability (of the systems) becomes more apparent the deeper the technological approach goes. One could say in a more provocative manner that the *more* late-modern societies, facilitated by (the ideals of) synthetic biologists, seem to control the material world, the *more* they lose their ability to control it. A control dialectic is present, as Kastenhofer and Schmidt (2011) show.

tations, technology and instability were, traditionally, like fire and ice.[40] According to the classic-modern view of technology, instabilities existed in nature but ought to be excluded from technology. If instabilities occurred, the traditional objective was to eliminate them. Constructability and controllability only seemed feasible when stability was guaranteed. Technology was traditionally equated with and defined by stability. Today, synthetic biologists widen our concept of technology by considering both stability and instability to be part of technology: Instabilities are reaching the core of novel technical systems. At the same time, it is still an open question whether the late-modern type of technical system can be conclusively called "technology" or whether it is a "technically possible technology" at all—to paraphrase the sociologist and systems theorist Luhmann (2003, 100f). It can be convincingly argued that traditional "rational design" approaches in engineering and technology, which are typically based on assumptions of stability,[41] have their limitations in the late-modern field of technology (cf. Giese et al. 2013; Nordmann 2008).[42] Therefore, reasonable concerns can be raised as to whether "synthetic biology will enable the design of 'biological systems' in a rational and systematic way," as the EU-NEST High-Level Expert Group on emerging technologies claims (European Commission 2005, 5). Alfred Nordmann states from a critical angle:

> No longer a means of controlling nature in order to protect, shield, or empower humans, technology dissolves into nature and becomes uncanny, incomprehensible, beyond perceptual and conceptual control. (Nordmann 2008, 173)[43]

In brief, whenever instabilities are involved, non-knowledge, uncertainties, and ignorance also prevail and, in principle, cannot be eliminated; problems with regard to monitoring and controlling emerge (cf. Schmidt 2012b).

Late-modern technical systems have a life of their own. From a (traditional) modern concept of technology, instabilities render engineering (construction/design and monitoring/controlling) difficult or even impossible. As early as 2005 the pioneer of synthetic biology Drew Endy anticipated these obstacles to any traditional engineering approach:

> Today, four challenges that greatly limit the engineering of biology are (1) an inability to avoid or manage biological complexity, (2) the tedious and unreliable construction and characterization of synthetic biological systems, (3) the apparent spontaneous physical variation of biological system behavior, and (4) evolution. (Endy 2005, 450)

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[40] The central characteristics of traditional technology encompass predictability and reproducibility; these only partially hold in late-modern technology.

[41] This includes (stability-presuming) traditional action theories such as von Wright's approach.

[42] In fact, instability-based "tinkering", or the usage of random-based or non-rational processes, also constitutes the basis for the techniques of synthetic biology.

[43] Nordmann does not explicitly mention synthetic biology (he addresses nanobiotechnology, classic biotechnology, ubiquitous computing, and the like)—nor does he analyze the underlying nomological structure (e.g., instabilities) in detail (Nordmann 2008, 173ff). However, his thesis that we are facing a new trend towards "technology naturalized" generally concurs with the thesis of this paper.

Although he is well aware of the central characteristics of bio-systems on their various levels of complexity, Endy pursues a traditional engineering approach. Not only does he advocate mechanical metaphors such as “biobricks," he also believes the challenges to be simply “problems” that are solvable in principle. He suggests a strategy based on the traditional classic-modern view of engineering and therefore envisions a prototype of the paradigm of “rational engineering”: (a) “standardization,” (b) “decoupling,” and (c) “abstraction” (Endy 2005; cf. von Gleich et al. 2012; Giese et al. 2013). Endy’s view can probably be called anachronistic, since present-day systems and structural sciences underline the strong dependency of the new kind of technology on biomaterial and living matter: In synthetic biology there is no escape from self-organization, instability, and complexity. Any kind of “mechanization” is infeasible because this would destroy the essential characteristics of living matter. Had Endy succeeded in reducing or eliminating these essential characteristics, he would have simultaneously also eliminated the main advantages of biotech-objects: their self-organization, including self-productivity, self-optimization, and self-adaptivity (see above). Thus the limits of a traditional engineering approach arise through the basic structure and inherent properties of the bio-systems under consideration.

In some respects coming from a similar stance, the philosopher and ethicist Hans Jonas anticipated the characteristics and the limits of “engineering biology” even back in the mid-eighties (Jonas 1985, 163; cf. Köchy 2012). In contrast to Jonas’ notion of “engineering biology,” I use the term “late-modern technology” in order to underline that we are experiencing a qualitative change in what we now consider to be technology.[44] Jonas is well known for his seminal book “The Imperative of Responsibility” (1984); he also contributed to the philosophy of biology with his “Phenomenon of Life” (1966). He diagnosed a historically new technoscientific era and perceived a radical “newness of biologically based technology” (Jonas 1985, 163). Jonas draws a dividing line between the classic engineering type of technology—including what he calls “art of the engineer” and, synonymously, “engineering art”—and a biologically based type of technology. As Jonas argues, this new type of technology differs in a qualitative way from our common perception and understanding of what technology is or could be.

> In th[is latter …] case of dead substances, the constructor is the one and only actor with respect to a passive material [= classic-modern technology]. In contrast, [… in the case of the] biological organism, activity meets activity: biological technology is collaborative with the self-activity of an active [= animate] ‘material’. (Jonas 1985, 165)[45]

[44] The chapter (“Laßt uns einen Menschen klonieren: Von der Eugenik zur Gentechnologie,” 1987) and the book (“Technik, Medizin und Ethik”, 1987) have only been published in German.

[45] The new “collaborative kind of technology” seems to be closer to humans and to their actions and self-perception; it is not alien to humans like the mechanical type of technology of classic-modern engineering. From the same perspective, and a few decades earlier, the Marxist philosopher Ernst Bloch coined the term “alliance technology” to underline the difference between mechanical and biology-based technology (Bloch 1959). According to Bloch, we may call a technology based on self-organization “alliance technology” (von Gleich 1989).

Jonas lists several cognate characteristics of this new type of biologically based technology: (a) self-activity, autonomy, and collaborativeness; (b) complexity, time-dependency, evolution, and limits of predictability; (c) individuality, non-experimentability, and limitation of reproducibility; (d) irreversibility and historicity; and finally (e) a different kind of causality that Jonas terms "interactive causation" (ibid., 163ff). He argues that, since biologically based technology inevitably carries an internal activity, engineering

> means releasing the bio-object into the stream of becoming in which the engineer and constructor is also drifting. (ibid., 168)[46]

With regard to the present wave of synthetic biology, Jonas' anticipation, and in particular his differentiation between "engineering art" and "biologically based engineering" is certainly very fascinating. However, Jonas did not take his very convincing phenomenological description any further; he did not attempt to fathom and analyze the underlying structure and the onto-technological core of the two different types of technology. The recent wave of emerging technologies in the 21st century is gradually clarifying that it is not the organismic, animate or biological that constitutes the central difference but, more fundamentally, instability-based self-organization. Thus, further pursuit of Jonas' ideas beyond his restriction to the organismic world could contribute to deeper reflection on and critical revision of the research and development trajectories of synthetic biology.

7 Challenges to Procedures of a Prospective Science and Technology Assessment

To even associate "technology" with the sphere of instability is remarkable: Our notion and understanding of "technology" seems to be changing. We need to concede that there is a dialectic or conflict between the (late-modern) idea of an instability-based technology, on the one hand, and the (classic modern) ideal of rational design, predictability, and controllability, on the other. Synthetic biology is still in its infancy. It is still unclear whether an instability-based self-organization technology will—beyond some prototypes—ever be feasible at all. The central question seems to remain open: Will we ever experience a massive movement towards a late-modern type of technology—or will this merely remain a hype and hope, nothing other than the unrealistic promise of certain research and development entrepreneurs? The task of a *prospective science and technology assessment* (ProTA) here is to unmask unrealistic promises, speculative visions and unsound hopes from a critical-realist's and technoscientifically informed perspective.

[46] He had this in mind when he formulated his *precautionary principle.* He believed that we should stick to classic-modern technology. His conception of adequate technology is therefore, in some respects, similar to what Drew Endy (2005) advocates.

ProTA questions whether the promises and visions are technoscientifically feasible.[47] It addresses general technoscientific issues of possibly emerging science and technology fields in order to contribute to a second-order shaping of the actual technosciences under consideration: shaping science, technology, and innovation policy in order to shape sciences, technologies, and innovations themselves.

Even if reasonable doubts still remain, let us assume for a moment that a late-modern technology—in other words: one that features instability-based, self-organizing (bio-)technical systems—will become, in principle, technically feasible, applicable, and successful. We would then be faced with new challenges such as restrictions of predictability and limited control—the flip-side of self-organization. The fundamental properties of late-modern technology (evolution, growth, and autonomy) have the power to change the world we live in. Metaphorically speaking, those who dare to stimulate and induce instabilities are, at the same time, provoking a risky *dance on the razor's edge*. "Because engineered micro-organisms are self-replicating and capable of evolution," Jonathan B. Tucker and Raymond A. Zilinskas argue, "they belong in a different risk category than toxic chemicals or radioactive materials." (Tucker and Zilinskas 2006) Indeed, this objection already applies to classic substances of biotechnology. But the related challenges in the realm of synthetic biology go much deeper and are to be regarded as more pressing. In particular, the *principle of similarity (and resemblance)* that constitutes the backbone of any risk assessment cannot be applied to most substances and tissues of synthetic biology. This principle is based on the assumption that if a new (bio-)system has some similarity to one that is already known, the new system will behave similarly to the well-known one and exhibit essentially similar properties. Most self-organizing bio-systems are not similar, owing to their intrinsic instability, and therefore they cannot be compared to other bio-systems: The principle of similarity is not applicable, due to the onto-technological core of late-modern technology.

Non-knowledge, ignorance, and uncertainty are co-produced with the productiveness of the late-modern technical systems; they are by-products and do not simply emerge in the societal context of diffusion, use, and consumption. Instability-based technology has a life of its own. As Jean-Pierre Dupuy puts it:

> The novel kind of uncertainty that is brought about by those new technologies […] is intimately linked with their being able to set off complex phenomena in the Neumannian sense. (Dupuy 2004, 10)

It follows, as Dupuy and his co-author Alex Grinbaum argue, that

> [t]he engineers of the future will be the ones who know that they are successful when they are surprised by their own creations. (Dupuy and Grinbaum 2006, 289)[48]

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[47] ProTA shares some elements with vision assessment (cf. Grin and Grunwald 2000). For general elements of ProTA in this regard, see: Liebert and Schmidt (2010).

[48] Dupuy states: "The unpredictable behavior […] means that engineers will not know how to make [… these] machines until they actually start building them" (Dupuy 2004; cf. Nordmann 2006). The famous physicist Richard Feynman is quoted as saying: "What I cannot create, I do not understand" (cf. Schwille and Diez 2009; Schmidt 2009).

In a similar tenor, scholars from Prigogine's *Brussels School* have raised concerns regarding control options: We have

> focused on designing and implementing artificial self-organizing systems in order to fulfill particular functions. Such systems have several advantages. [… However,] [d]isadvantages are limited predictability and difficulty of control. (Heylighen 2002, 23)[49]

The disadvantages become obvious when we consider the instability-based new (unknown or unknowable) risks. These thoughts concur with what Alfred Nordmann (from a critical perspective) perceives as a

> limit [that] could […] be reached where engineering seeks to exploit surprising properties that arise from natural processes of self-organization. (Nordmann 2008, 175)

We are on the way to "surrender[ing] control to pervasive technical system." (ibid., 182) In other words, the late-modern "complexification" (cf. Dupuy 2004) of technical systems—generated through the implementation of instabilities—not only induces a loss in the ability to control and manage a certain technical system, but also limits the acquisition of knowledge about the opportunities and risks of a certain new technology in general. This might raise concerns as to whether classic TA concepts can access this type of technology—and, consequently, might also question whether options for a societal shaping of a new technoscientific wave even exist. According to Dupuy and Grinbaum, Inone of these [well-established TA] tools is appropriate for tackling the situation we are facing now. (Dupuy and Grinbaum 2006, 293)

What Dupuy and Grinbaum express, is certainly true of classic TA approaches. However, as the present paper indicates, more recent directions in TA such as ProTA—in alliance with cognate concepts like Technology Characterization (von Gleich 2004; von Gleich et al. 2012), Real Time TA (Guston and Sarewitz 2002), Vision Assessment (Grin and Grunwald 2000), or "hermeneutical TA" (Grunwald 2012)—offer certain prospects. These TA concepts analyze the technoscientific core and assess each specific type of technology in detail from a critical perspective.[50] Their approach is particularly relevant when it is a case of inquiring into alternatives (a) within or (b) to the technoscientific core itself, and, based on this, of searching for different directions in science, technology, and innovation policy.—(ad a) A central question is: Can we identify within synthetic biology research and development trajectories that target the design of bio-systems possessing internal safety features—for example, cell-free systems that share certain positive properties or desired functionalities with cell-based systems but are essentially less fraught with instability and, therefore, not capable of strong forms of self-organization (von Gleich et al. 2012; Marliere 2009, 77f; M. Schmidt and de Lorenzo 2012, 2201f)?[51]

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[49] In line with this, Bill Joy advocates the well-known and highly disputed dystopia: the "gray goo" (Joy 2000).

[50] The "systems type of synthetic biology" is analyzed and assessed in more detail in: von Gleich et al. (2012).

[51] In order to provide clear-cut criteria, a further inquiry into different types of instability and self-organization would be necessary. A proposal for such is given in Schmidt (2005a, 2011).

Other questions address a positive direction: Are there certain fields in synthetic biology that carry a realistic potential to meet the requirements for sustainable development?—(ad b) A key issue that arises on a much more fundamental, and certainly more pressing, level is whether our late-modern societies should really foster and facilitate a "late-modern technology"—that is, a technology that is inherently unstable and linked with the ability to set off self-organizing, complex, and autonomous dynamics. Can we cope with the co-produced non-knowledge, uncertainty, and ignorance?[52]

8 Summary

Inquiring into issues like these is a further task of ProTA. Let us summarize the main points discussed in this paper. Synthetic biology should not be regarded as merely a new trend in biology or in the life sciences. The promise of a novel kind of technology seems to be emerging on a more fundamental level; it is rooted in advancements in the systems and structural sciences, and fostered by the progress of computer resources. We have called this new type of technology "late-modern technology" to underline that an epochal break is occurring in our understanding of technology and also, should this promise ever become reality, in the ontology of technical systems.[53] Synthetic biology matches perfectly with the general trend towards a late-modern type of technology and, moreover, it is at the cutting-edge of this trend. If there is any *differentia specifica* giving substance to the umbrella term "synthetic biology" and demarking it from other developments, it is the idea(l) of harnessing self-organization for engineering purposes—an idea(l) that is central to late-modern technology.

What are the characteristics of late-modern technology in general and of synthetic biology in particular? *First* of all, synthetic biology does not stand alone within the trend towards a late-modern technology. Synthetic biology can be considered as the most recent tip of the iceberg of a new technoscientific agenda. Self-organization plays a constitutive role in other kinds of emerging technologies, too, such as (a) robotics, AI, pervasive computing, autonomous (software)

[52] The latter questions come close to Hans Jonas' approach in his seminal book *Imperative of Responsibility* (Jonas 1984); Jonas was always very concerned and hesitant towards new technology movements in which properties such as uncertainty and non-knowledge are co-produced. Based on his *heuristics of fear* Jonas would have raised objections to and, to some extent, have rejected an uncontrollable global project such as synthetic biology.—Nordmann remains skeptical as to whether we can cope with this kind of technology (cf. Nordmann 2008, 2005). His objections are far-reaching—and advocate a fundamental critique: "This is a critique no longer of what we do to nature in the name of social and economic control. Instead it is a critique of what we do to ourselves as we surrender control to pervasive technical systems." (Nordmann 2008, 182).

[53] Hence, it is rather narrow to argue that technology is becoming biological and at the same time that biology is becoming technological (van Est et al. 2010, 25).

agents; (b) nano- and microsystems technologies; and (c) cognitive, neuro- and pharmaco-technologies. Late-modern technology is inherently *interdisciplinary*.—*Second*, late-modern technology differs from the modern type of technology in terms of its *phenomenological* characteristics. Late-modern technology does not resemble our established perception and understanding of technology and technical systems. In a phenomenological sense, this new kind of technology appears to be (bio-)nature and displays nature-like characteristics; it looks "un-technical" or "non-artificial." Self-organization based (late-modern) technology seems to possess an intrinsic momentum of rest and movement within itself, not an extrinsic one as in the Aristotelian and common life-world understanding of nature: It *is* alive or *appears* alive and seems to have autonomy and actor characteristics. The internal dynamics (such as acting, growing, and changing) of late-modern technology make it hard, from a phenomenological perspective, to draw a dividing line between the artifact on the one hand and nature on the other. Traditional technical and mechanical connotations have been peeled off. Ancient philosophers such as Aristotle would claim that late-modern technology is more *physis/nature* than *techné*.—*Third*, the central *ontological* characteristic of late-modern technology is instability. The instability-based ontotechnological, or equivalently, nomological core includes: self-organization (self-activity, autonomy), complexity, and also often aspects of individuality and irreversibility.–*Fourth*, late-modern technology can, from an *epistemological* and *methodological* perspective, be distinguished from modern technology with regard to characteristics such as (a) limits to predictability, (b) restrictions on reproducibility, (c) obstacles to monitoring, controlling, and intentional shaping. These aspects characterize tendencies and trends. A proper and deeper analysis would certainly require further explication and elaboration of the above mentioned points. Hans Jonas was precursory in this respect, and we could easily adopt and adjust his argumentation with regard to the self-organization trend in technology.

From a societal perspective, we need to face this late-modern type of technology and undertake the task of developing procedures either to restrict and contain, or to shape and deal with it.[54] *Prospective science and technology assessment* (ProTA) offers an interdisciplinary, critical-reflexive approach that enables us to analyze the technoscientific core of this new wave of emerging technologies (cf. Liebert and Schmidt 2010). Alternatives *within*, or *to*, the core may become visible and assessable.[55] In this respect, ProTA can contribute to fostering and facilitating public opinion formation, political decision-making, and anticipatory governance in order to contain or shape late-modern technology at the very inception of research and development processes.

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[54] With reference to Dupuy's approach, Nordmann (2008, 184) argues that we should "carefully contain […] the implementation of these technical visions"—because, in principle, it is impossible to monitor, control, and shape these late-modern technical systems.

[55] Whether this is possible or not certainly remains an open question. Nordmann (2008), for instance, doubts that this kind of (late-modern) technology—or "naturalized technology"—can be shaped and controlled from a societal perspective.

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Synthetic Biology at the Limits of Science

Alfred Nordmann

Abstract What happens when some of the traditional questions and concerns of the philosophy of science are brought to the non-traditional field of synthetic biology? Given that synthetic biology is a very diverse field, this might serve to highlight the many ways in which it is business as usual. However, prominent concepts and research practices of synthetic biology can be seen to confound established ideas of how knowledge is produced and validated in the sciences. By highlighting and readying for discussion the tension between alternative images of knowledge production in synthetic biology, this paper seeks to open up debate among philosophers of science, and within the diverse community of synthetic biologists. With the advance of emerging technosciences like synthetic biology what is at stake is not primarily how they might or might not change the world. At stake, first of all, are epistemic values, the ethos and authority of science, and the relation of knowledge and power. Building on ongoing discussions, the paper begins by exhibiting contested notions of understanding, rational engineering, and design. In a second step, it turns to different conceptions of biological "systems" by presenting divergent accounts of the origin of synthetic biology and of how systems biology gave rise to synthetic biology. Finally, it seeks to focus the debate on a definition of synthetic biology, according to which it builds, for constructive purposes, on achievements of technical control of biological complexity, that is, that it uses these achievements to generate, rather than reduce, complexity.

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1 Introduction

As in many so-called technosciences, some of the research practices and epistemic ideals of synthetic biology confound traditional conceptions of scientific method, regarding questions such as "how is knowledge generated and validated," "what does it mean to understand or explain something," "how important is the development of new theories," or "what is the difference between explorative experimentation and experimental hypothesis testing?" Indeed, on some accounts of what synthetic biology is and how it works, it does not even appear to be interested in traditional *scientific* methods of reducing complexity by intellectual means. Instead, it promotes the controlled generation of complexity by technical means, that is, by drawing available theories and tools into a *technoscientific* design process.[1]

By inquiring how synthetic biology agrees with or confounds established notions of science, philosophers of science and of technoscience[2] contribute to a much larger process of delineating what synthetic biology *is* in comparison to other fields of biological research such as molecular biology, bioinformatics, systems biology, or genetic engineering. This is not a matter of classification or definition but of characterization: what are the basic assumptions, what are the routines and laboratory practices, what are the promises and ambitions, what is the special mind-set of synthetic biology? To the extent that it speaks with a distinctive voice at all, how does synthetic biology set itself apart from other endeavors? Given the fairly recent emergence of synthetic biology, there is already an impressive body of philosophical literature that

[1] Here and throughout, the default meaning of "complexity" is simply that a structure or system is "not simple" or "difficult to conceive as a sum of simple processes" or "complicated." Science and classical theories of knowledge conceive the task of the human intellect as making sense of a bewildering multitude of sensory impressions by isolating from them simple patterns or lawful causal relations. This "reduction of complexity" is considered a major achievement of the mind. Accordingly, the first "limit of complexity" arises when things get to be too complicated to be tractable by the human mind (though a computer might still be able to achieve predictive control or to isolate strict causal dependencies). In contrast, the challenge of synthetic biology is seen as building up or generating complexity (the first sessions at the 2013 SynBio 6.0 conference were dedicated to the question of "realizing biological complexity," see the program under http://sb6.biobricks.org/, accessed January 5, 2014; also (Mast et al. 2013). Only at two points in the following discussion (see Footnotes 10 and 16 below), does "complexity" assume a more exalted systems-theoretic status, thereby explicitly becoming a theoretical term. In the theoretical context of systems thinking, there is as second limit of complexity, namely irreducibility in principle. And only systems thinking thus conceived calls for an alternative, non-reductionist approach and thus a different kind of "reduction of complexity"—reduction not to aggregates of simple processes but to dynamic systems as integrated wholes.

[2] Many readers will not be familiar with the juxtaposition of science and technoscience as distinct modes of knowledge production. This is not necessary. The distinction will take shape over the course of this discussion as different ways of conceiving synthetic biology become aligned with the different epistemic values and ideals of science and of technoscience (Bensaude-Vincent 2009a; Forman 2007; Nordmann 2010b).

addresses these questions (Bensaude-Vincent 2013a, b; Delgado and Porcar 2013; Gelfert 2013; Gramelsberger 2013; Kastenhofer 2013a, b; Knuuttila and Loettgers 2013; O'Malley 2009, 2011; Schmidt 2015, this volume; Schyfter 2013).

2 Familiar Concepts, Divergent Meanings

By engaging claims of what synthetic biology can be and what it should be, philosophical scrutiny sharpens awareness, exhibits what is at stake, and thereby facilitates scientific controversy as well as public debate. Since synthetic biology is said to bring an engineering approach to biology, these debates concern how one needs to understand biological systems for the purpose of achieving technical control.

Three issues, in particular, stand out. They involve conflicting notions of understanding, rationality, and design. Though each of them deserves separate treatment, we will see that they are framed by different images of knowledge production, images that we will encounter also in the stories one tells about biological systems and the relation of systems biology and synthetic biology.

2.1 Creating Understanding

The first of these issues is contained in Richard Feynman's oft-quoted statement "What I cannot create, I do not understand."[3] This is a familiar issue in that it evokes philosophical positions that go back to philosophers as diverse as Thomas of Aquinas (Aquinas 1986), Francis Bacon, or Giambattista Vico (1979).[4] Strictly speaking, it articulates a necessary, but not sufficient condition for what it means to "understand" something. It can be paraphrased as follows:

> No matter how good our scientific models or our explanatory and predictive theories are, these are not sufficient for "understanding" as long as another condition has not been fulfilled. This necessary condition is the requirement that with the help of these models or theories, one can create in one's mind or in the laboratory the process or phenomenon in question.[5]

[3] A review article speaks of the "repetitively, almost dogmatically, cited Feynman quote" (Rollié et al. 2012). The source of the repetitively cited maxim is a photograph of "Feynman's last blackboard" which can be found, for example, at http://archives.caltech.edu/pictures/1.10-29.jpg (accessed January 3, 2014), compare e.g. (Schmidt 2009).

[4] Aquinas argues that only God truly knows the world because he created it and one can only know what one creates (Aquinas 1986); Bacon declared that the power to control or to make things is a criterion of knowledge (which is why the statement "knowledge is power" is often attributed to him, see e.g. Smith (2004, 238–241); Giambattista Vico distinguished mathematics and the sciences of human culture from the natural.

[5] It is not clear why Feynman formulated this strong requirement on his "last blackboard." This may have been his objection to string theory in physics, or informed perhaps by his recent experience of discovering and demonstrating the cause of the Challenger space shuttle accident, see O'Malley (2009, 385–386).

For philosophers of science, this formulation raises many problems,[6] but one thing is clear: According to this paraphrase, the ability to create appears as a crowning achievement, the final bit of evidence that proves all our previous thinking, our models and theories right. In the statement "what I cannot create, I do not understand", the notion of "understanding" remains first and foremost an intellectual notion that refers to science as an effort of gaining understanding through theories and models.

Now, when it comes to synthetic biologists, some adopt this strict and narrow interpretation of Feynman's statement. However, by embracing Feynman's statement to the point of treating it as a fundamental credo, synthetic biologists are expressing first and foremost that the seemingly opposing goals of human understanding and material construction can be jointly satisfied even as one brings an engineering approach to biology. If one wants to engineer a biological structure or process, one "cannot help" but gain understanding, also (Benner and Sismour 2005, 538–542). If this is the fundamental message, it is still an open question, what kind of understanding this is, and whether it serves as the capstone to theoretical knowledge production. Are synthetic biologists typically referring to the intellectually tractable, theoretical understanding that has been achieved, e.g. by systems biologists, and that is now ennobled and completed by efforts to actually create organismic structures? If only for the generally acknowledged large gaps in the explanation and prediction of biological phenomena, at least some synthetic biologists advance another way of paraphrasing Feynman's credo[7]:

> The ability to create or recreate biological entities or structures proves that we know enough to do just that, and the more dexterity we acquire the better we understand what makes these entities or structures work – even if what is known explicitly is only fragmentary and if it is complemented by much tacit and procedural knowledge, including technical know-how.

Unconcerned with Feynman's intention and unconcerned with the grammatical construction of "What I cannot create, I do not understand," this paraphrase inverts

[6] The paraphrase suggests, for example, that understanding is more than the ability to explain and predict something. This prompts the empiricist suspicion that it is in this case too much to ask for scientific understanding. Also, if the material (re)production of a process or phenomenon is not the only way of creating what one seeks to understand, does the creation in one's mind require intellectual tractability as in a thought-experiment, or would a highly complex computer simulation also fit the bill?

[7] Famously, Craig Venter and his collaborators encoded in 2010 the Feynman quote as an identifying watermark in the genetic code of the first chemically synthetized genome of a working bacterial cell. Notoriously, in so doing they misquoted Feynman ever so slightly.

the formula to read "What I can create, I do understand."[8] The ability to create now appears as a sufficient condition for understanding, suggesting that "understanding" can leap ahead of explanation and prediction, and that it derives from a more immediate relation of knowing and making. To be sure, it is easy to dismiss the second paraphrase as not being in line with Feynman's thinking, as being implausible from the start, or as being incompatible with the traditions and epistemic values of science (O'Malley 2009, 385–386). However, the philosophical analysis of synthetic biology cannot simply dismiss the second paraphrase but needs to reconstruct why it appears credible to those who maintain it. What is the "image of technoscience" that underwrites such an apparently "unscientific" conception of achieving understanding through making? What kind of learning takes place and what kind of knowledge is achieved if one submits to a program where one "cannot help but gain understanding" as one pursues a technical goal (Benner and Sismour 2005, 538–542)?

Here is one example, then, of questions for the philosophy of synthetic biology. After determining what is meant—in any given instance, but also in general terms—by appeals to Feynman's dictum, it will discover, undoubtedly, a diversity of interpretations and usages which pose the challenge of reconstructing their intelligibility. Here, it might discover that the more familiar and less problematic first reading of the dictum as offering a necessary "capstone"-condition for understanding exaggerates and, idealizes the availability, scope, and power of explanatory theories in biology. At the same time, it may well discover successful design strategies that lend credibility to the seemingly more problematic notion of understanding a biological system while black-boxing mechanistic detail and without requiring intellectual tractability (MacLeod and Nersessian 2013).

2.2 Engineering Principles

The second of the three issues concerns the familiar question of rationality, one that is typically understood by way of stark contrast. Either inquiry or engineering follow rational principles of construction and validation, or they are beholden

[8] Schmidt (2009) warns that the inversion of Feynman's dictum does not follow logically from the original formulation. For examples of authors who adopt the second reading, see Sect. 3 below. But see also the example of Alfonso Jaramillo who proved quite committed to the second reading during his oral presentation at the CAS Conference Synthetic Biology (July 23–25, 2012, at the Biocenter of the LMU, Munich). Arguing for automatic design, computational evolution, high throughput characterization he claimed for these methods that one does not need that much (theoretical) knowledge about structure to succeed, and that they allow quantitative testing with and in spite of limited knowledge. In published work this is expressed in a more muted fashion, more careful, in particular, to advertise this as a virtue of his approach: "As our automated methodology uses few specifications as inputs, it could also be used to test new mechanisms and hypotheses despite the lack of a complete molecular understanding of the living cell" (Rodrigo et al. 2012).

to mere empiricism, haphazard tinkering, and exploratory experimentation. This either-or reflects a particular point of view, namely one that ranks rational engineering or rational design higher than strategies of trial and error. Accordingly, the distinction is often used to probe how far our knowledge of the world has advanced. Those who are still working in the mode of trial and error have not ascended as yet to a level of intellectual and technical control that would allow them to invent new processes or devices simply from considerations of theory and principle. Thus, the idea of "rational drug design" was advanced in the 1970s by biomedical researchers who were scandalized by the notion that in this day and age drug discovery should proceed by way of randomized search procedures. And after trial and error—in the form of automated high-throughput methods—triumphed over rational drug design (Adam 2010), the scientific community came back with nanomedicine and visions of targeted drug-delivery ("this time we'll get it right"). Similarly, the aspirations of synthetic biology are often judged in these terms: Can the "synthesis" of biological structures or processes proceed in a planned, deliberate, theory- and evidence-based manner such that the intended outcome issues as if from a blueprint (Giese et al. 2013; O'Malley 2009; Gramelsberger 2013; Lewens 2013)? And, if this is not the case, is this only "not yet" the case, likely to become possible in just a few more years, or does it owe to a disciplinary style of doing things—with chemists seeking rational control while bioengineers are more comfortable with tinkering? Or does the failure of rational design owe to a limit of biological complexity that is irreducible and thus an insurmountable limit of control?

The hierarchical conception that places rational engineering above tinkering is blind to the possibility of rational tinkering. This is because scientific rationality is tied to calculability (*Berechenbarkeit*) and the ability to plan in advance, thereby tied also to the notion of natural law and the predictive abilities that flow from it. From an engineering point of view, however, rational engineering principles may well be opposed to blind groping but they are not necessarily opposed to search strategies that exploit random variations and thus trial and error. They are also not opposed to design strategies that involve iterative processes of adaptation and tuning. These, to be sure, are rational strategies by which to work around limits of knowledge, and to achieve technical solutions in the absence of information about mechanical detail. In other words, these are rational strategies to create robust black boxes[9] or modules.

Quite in line with this engineering point of view, appeals to the "design cycle" are at least as frequent in synthetic biology as those to Feynman's dictum

[9] The term "black box" refers to a technical unit of reliable functioning that is not and need not be scrutinized for the specific causal processes that would account for its functioning (Royal Academy of Engineering 2009, 19–20). Not all modules in a modularized architecture are black boxes, but black boxes can serve as modules (see, paradigmatically, Canton et al. 2008). Black-boxing is the decision or strategy to create black boxes. Tal (2013) offers a critique of the notion of black box and seeks to identify instead rational strategies that provide "ignorance affordances."

(Cheng and Lu 2012; Royal Academy of Engineering 2009, 18–23; Tabor 2012; UK Synthetic Biology Roadmap Coordination Group 2012, 13). Typically, the design cycle consists of three steps that are iterated until a desired technical performance is achieved. If the task is to create an informational technological expert system, or to create a climate model, the first step is to analyze the situation to be emulated, automated, or modeled. In a second step and on the basis of this analysis, a skeletal technical system is created. This first prototype is as far as the application of rational engineering principles will reach—it results from well-established procedures of mapping known features of the situation into a technical architecture. The third step consists in testing the prototype. Now, the performance of the prototype or model is observed and its ability assessed to emulate, automate, or model expert or climate behavior. At this point, any discrepancy between the actual and the desired performance of the prototypical system induces a second, third, and further iteration. Each new iteration begins with another analysis, but this is no longer an analysis primarily of the original situation but now of the technical system, and why it does not yet perform as desired. On the basis of this analysis (step one again), modifications are introduced, and an improved prototype is created that may serve to better tune the expert system or climate model to the target system (step two). Some of these modifications draw on specific scientific knowledge of features that may have been neglected and are now added in. Other modifications draw on familiar engineering strategies, such as adding noise in order to dampen sensitivity, and yet others are simply tried out to see whether this or that may do the trick. All of these modifications of the behavior of the designed system are compared against the target system (step three), and subsequently rejected or further modified. With each iteration new elements are introduced and the designed system as a whole gains complexity. In the limit, the designed system emulates the target system near-perfectly and does so because it is similarly complex. Thus one finds that a predictively successful simulation model can be nearly as complex and just as intractable as the "natural" system that is modeled by it (Lenhard and Winsberg 2010). This is a technical achievement by rational means. Though it does not consist in the application of "rational engineering principles," it is not "mere" tinkering either, but a strategy to systematically optimize the performance of a technical system.

For the philosophy of synthetic biology, the competing notions of rational engineering are of interest not only because they implicate the question of systemic limits of complexity: after all, ab initio rational engineering is possible only to the extent that calculability is even achievable. The different conceptions are of further interest because they speak to entirely different kinds of pursuit. On the one hand, there is science as an analytic enterprise which reduces complexity in order to arrive at principles which can be used to translate mechanistic accounts of biological processes into procedures of rational biological engineering. On the other hand, there is the technoscientific enterprise of synthetic biology which generates complexity by way of an iterative design process, that is, by way of a rational strategy to fine-tune engineered systems so that they can emulate biological systems. The philosophy of synthetic biology has thus to countenance at least the

possibility of a research enterprise which seeks to exceed limits of intellectual tractability, of human understanding, or calculability; and does so in pursuit of technical robustness at ever higher levels of biological complexity. In this pursuit, the modules from which synthetic biology builds up greater complexity can be black boxes that work together in reliable ways.[10] To be sure, when engineers stuff matters of detail and complexity into a black box and then compose larger technical systems out of input-output relations among these modular black boxes, they are not just building up but also managing or handling complexity—without claiming, however, that what formerly looked complex now appears to be merely an aggregate of so many simple relations.[11]

2.3 Intelligent Design

The third issue for a philosophy of synthetic biology arises from the previous two, and is a classic question also for the philosophy of biology: At first, everyone believed that species were designed by their creator. Then Darwin contradicted this. So, how is it possible even to speak of creation and design in biology? While anti-Darwinian theories of intelligent design are in ill-repute, how can synthetic biology speak of design processes and simultaneously take the insights of evolutionary biology into account?

Darwin showed that biological entities and processes are products of natural history and not of design. When synthetic biologists now get into the business of producing them by design, they are not thereby denying the ubiquitous and powerful action of evolution by natural selection upon anything that is subject to variation or less-than-perfect replication. On the assumption that they are interested in maintaining the continuity between the scientific naturalism of Darwinian biology and

[10] Note that for the analytic enterprise of science, the issue of calculability is central and, by the same token, the nature of complexity or of emergent properties. Science seeks to know whether biological structures and processes are irreducible in principle or subject, sooner or later, to an analytic reduction of complexity. In contrast, the technoscientific interest in generating complexity is quite indifferent to this question. Perhaps, new and irreducible systems qualities emerge over the course of iterating the design cycle, perhaps not. No matter how one conceives the "limits of complexity," the design process aims to overcome them (compare Footnote 1).

[11] This important qualification owes to comments by Maureen O'Malley. "Reduction of complexity" usually and in this text refers to an intellectual achievement: Complex phenomena can be reduced to simple processes and their aggregate effects. But some speak of a different kind of reduction of complexity: "synthetic biologists simplify and build" (Ferber 2004; Calvert 2010). Whereas systems biology seeks total information and thus incorporates into its representational models all the findings of Omics-research, synthetic biology wants to find out how far we can get with what little we know—it does not try to incorporate as much information as possible into the process of generating biological complexity. Synthetic biology attempts to find technical means which afford ignorance (Tal 2013), allowing it to succeed with less information rather than more. This might be considered synthetic biology's technical "reduction" of complexity.

their engineering-oriented enterprise, synthetic biologists are therefore engaged in a philosophically significant effort. Implicitly and explicitly, conceptually and practically, they establish the compatibility of evolutionary and synthetic biology. This effort consists firstly and primarily of isolating the design efforts from evolutionary processes—be it by studying all organisms as if they were humanly engineered,[12] be it by limiting the work of synthetic biology to closed industrial processes, be it by adopting design constraints that prevent replication, variation, or interaction with biological systems, or be it by downplaying the likelihood that synthetic biology might alter the course of evolution. Each of these approaches raises questions of its own. What holds true for all of them, is that by conceiving the same biological entity at one time as an object of design (as far as that will go), and at another time as an object of evolution (to the extent necessary), synthetic biologists are tending to the boundary between organism and artifact even as they appear to undermine or even reject it.

A second dimension of the relation between design and evolution comes in when the trial and error aspects of the design process are analogized to variation and selection. Variations are introduced more or less randomly into the design cycle and the resulting system behavior is then selected for, or selected against, in a process that adapts performance to expectations (Bujara and Panke 2010). Indeed, if the aim of synthetic biology is to generate complexity, it may well appear as if the goal was to reproduce the work of natural evolution, albeit in a more accelerated and more purposeful manner. At first sight, this would give license to saying that synthetic biology is biomimetic: that it merely seeks to emulate or imitate nature, and for that reason, that it is presumably more or less benign. This analogy is haunted, however, by the same problem that confronted Darwin's analogy between artificial and natural selection, namely that there is place for a benign purpose, for a breeder or creator only in artificial selection, and not in natural history. Accordingly, strategies of trial and error that select proposed variations by way of performance criteria should be likened to artificial selection and breeding, not to natural selection and evolutionary history.

With the ambition, however, to reproduce the work of natural evolution in a more purposeful manner, the problem of technological hubris begins to raise its head as the biological engineer is likened to the divine creator that was banished from the modern scientific worldview (Schummer 2011, 190–210). This ambition owes to a popular notion that preceded and accompanied the appearance of synthetic biology. This is the notion that in the development of human culture we are ("finally") reaching the stage where we can take evolution into our hands. This notion implies not only that humanity is now fully in command of its own destiny, it implies also that we are no longer subject to the haphazard, cumbersome, and often inefficient ways of evolution (Dyson 2007). This is different from worrying

[12] In the terms of Daniel Dennett, after rejecting that natural organisms are the product of design, one can adopt a design stance towards them and studying them as if they had been engineered.

that synthetic biologists are "playing God," for, how could they, if there is no God in a scientific account of nature? It is different also from looking at nature mechanistically in order to discover principles for the construction of mechanisms. Instead, this is a view that considers "nature" an engineer of sorts, one of us and one like us, who is in the business of designing biological artifacts and whose creations are considered to be wonderfully subtle and intricate but also as a bit roundabout, full of redundancy, and perhaps unnecessarily complicated. As an engineer, nature is constrained by evolutionary history, by the relative fixity of species, and a small range of variations. In contrast, synthetic biologists or genetic engineers are not limited by lineages and the restrictions they impose on the gene-pool. This gives them the significant advantage of not having to work as slowly and conservatively as evolution by natural selection.

Once one arrives at this image of technological hubris, a last and perhaps most remarkable fact about synthetic biology needs to be countenanced, namely the near-absence in the scientific literature and in review articles of explicit discussions of synthetic biology's possibly problematic relation to evolutionary biology.[13] But surely, there must be an explanation for this and perhaps the fault lies with those who see the need for this discussion. On the whole, perhaps, synthetic biologists need not worry about the consistency of the scientific world-view of evolutionary biology and their own technoscientific mind-set. Molecular biology has shown that one can engage in structural investigations without immediate reference to theories of evolution. Not unlike engineers in other fields, it is proving quite sufficient for molecular and synthetic biologists to work along pragmatic lines: "If there are laws of nature, we can't violate them anyhow, and in the meantime, it is our job to push the limits of technical possibility." In other word, attempts to probe what can be done by way of creating biological entities and processes do not advance ideas that need to be fitted into a larger biological world-view; instead, they merely find themselves more or less constrained by some general facts of nature (Nordmann 2010a).

The very questions of how biological engineering should be related to natural history, or of how evolution by natural selection differs from the design of biological artifacts thus depend on our conception of synthetic biology—is it an intellectual enterprise with at least some theoretical ambitions or should one judge its attempt to advance understanding of biological systems only in engineering terms? Only in the former case does the problem arise of having to reconcile the competing ideas

[13] The place where evolutionary considerations are most likely to appear is in "what if" scenarios that begin by valorizing synthetic biology and portraying its success at creating artificial organisms. Only then the question is asked what will happen once these are subject to evolution—either by way of "mutating" from benign to dangerous organisms, or by way of their ability to outcompete natural organisms, changing the make-up of biological diversity, and the like. The engagement with evolutionary concepts thus tends to begin only when synthetic biologists look at the potential impact of their work through the perspective of technology assessment. Arguably, though, it should enter in right at the beginning of their work, in reflections on the rhyme and reason of naturally evolved biological complexity.

of evolution and design, while in the latter case it is merely a practical challenge to insulate as far as possible the construction of biological entities and processes from the vagaries of evolutionary influence. This fault line between synthetic biology as somewhat theory-oriented and as exclusively engineering-oriented also separates the two ways of paraphrasing Feynman's dictum as well as the two conceptions of rational engineering. It is the fault line that runs between science and technoscience.

3 From Systems Biology to Synthetic Biology

So far, we have been considering only conceptual issues that resonate with familiar discussions in the philosophy of science and that receive another turn of the screw through the contemplation of synthetic biology. These have drawn our attention to the general scientific or technoscientific character of synthetic biology which, in turn, refers us to its history. Promoters and observers of synthetic biology position it on the one hand in respect to the history of the biological sciences, and on the other hand to the prospects of biological engineering, and to biology as a technoscience. In particular, they position it in respect to systems biology that may have laid the groundwork for the appearance of synthetic biology. But as to how, and to what extent, there are different stories that can be told. Of these, only two will be juxtaposed here.[14] The first treats the move from systems biology to synthetic as the consequence of a paradigm-shift or a whole new chapter in the history of the biological sciences, one that revolves around systems-thinking as the best way of coming to terms, intellectually, with complexity. The second treats synthetic biology as a technoscience that considers systems only as more or less efficient units of technical functioning, and that goes beyond our simple intellectual ways by seeking the means for generating or increasing complexity.[15]

3.1 Sublime Thinking

The first of these stories underwrites a comprehensive report that was commissioned by the German Ministry of Research BMBF and that gave rise to the present volume. It goes as follows. Biology has run up against the limits of complexity as it

[14] For a sketch of a third story, see Footnote 20 below.—Like all myths of origin, these three are idealized to the point of caricature, and they are told for reasons not of descriptive accuracy but of the moral they contain. Each in its own way has normative implications, suggesting what synthetic biology ought to be and what opportunities and risks it poses, what obligations and expectations come with it.

[15] Following upon and adding to the section on "familiar concepts, divergent meanings" the two stories might be said to expose the divergent meanings within synthetic biology of the notion of "system."

has tried to become a lawful and predictive natural science in the guise of molecular biology. Under the heading of "evo-devo" this was pointed out by a coalition of biological researchers and philosophers of biology. Processes of self-organization, laws of form, the reciprocal relations between a biological entity and its environment—all these were thought to elude the grasp of a physico-chemical methodology that needs to isolate and control specific causal processes as much as possible. There appeared to be only one way for biology to move forward and to become predictive or even constructive. It had to take biological complexity seriously, that is, to understand biological structures at least from the cellular level upwards as systems that exhibit the dynamics which are the subject of a general system science or a theory of non-linear complexity.[16] This way of "learning from nature" led to systems biology which, in turn, prepared the ground for synthetic biology which, on this account, can be understood as applied systems biology: The processes of self-organization that are the subject of systems biology are applied in synthetic biology to the task of engineering biological structures. Inversely, synthetic biology can be said to contribute to basic biological science in that it constructs and exhibits structures and systems for study.

A kind of paradigm-shift within science thus becomes a paradigm-shift for engineering, too (Schmidt 2015, this volume). Just as the science of biology has moved from causal analysis by physico-chemical means to the identification of dynamic patterns through systems thinking, so bioengineering is moving from the science-based construction of genetic blueprints to synthetic biology as a form of engineering that harnesses self-organized growth for the creation of novel artifacts. Accordingly, the most prominent risk of synthetic biology is the release of synthetic organisms, and the disruptions these technologically evolved structures might cause in naturally evolved systems. By the same token, our best protection is awareness of the sensitive dependencies of complex systems—if synthetic biologists avoid making their constructs too robust, or avoid making them independent of very specific environmental conditions, all might be well (Schmidt 2009, 96–97). Complexity demands respect and this respect, in turn, might assure the proper fragility of artificial organisms that will not be able to survive outside the very special contexts for which they were synthesized or grown.

This first story of the rationale for systems biology and its application in synthetic biology is normative in that it demands that the research by synthetic biologists properly applies systems biology.[17] This takes the form, for example, of maintaining that synthetic biologists ought to incorporate noise into the design process—not as something that needs to be minimized, corrected for, or excluded; but as something that in a proper understanding of biological systems is an essential feature of any self-regulatory biological system, natural or engineered. How

[16] Here, then, "complexity" becomes a theoretical term that differentiates complex systems from merely very complicated aggregates of simple processes (see Footnote 1 above).

[17] This normative insistence on proper systems thinking extends the debate within and about systems biology (Wolkenhauer and Mesarovic 2005; O'Malley and Dupre 2005).

one deals with noise (as a disturbing factor or as essential element) is thus said to betray whether one is or isn't truly engaged in systems thinking (Schmidt 2015, this volume; von Gleich et al. 2012).

3.2 Technical Opportunities

The first story ended on a note of suspicion. Despite its being called "systems biology," it appears unclear what is meant by "system" here. Does one mean a dynamic structure that requires general systems theory or a theory of non-linear complex dynamics to describe it, or does one mean a technical construct that consists of at least several interacting parts?

If it turns out that many or most systems and synthetic biologists do not aspire to a holistic way of thinking, a second story can be told. It is a story of technical opportunism according to which the concepts, theories, and methods of biology, biochemistry, and genetic engineering become absorbed into an engineering idiom. This second story does not begin with philosophical insights about a non-mechanistic type of causality, about the profound difference between organism and mechanism, about biological complexity and systematic limits of molecular biology. Instead, it begins with the lessons learned from the Human Genome Project. On the one hand, the project represented a triumph of analytic methods, having been completed sooner and more efficiently than anticipated—an achievement that continues as genomics produces cheaper and faster methods by the year, if not by the month or day. On the other hand, it delivered a blow to straightforward genetic determinism in the sense that only very few single genes can be correlated to single traits. This insight prompted neither retreat nor profound reorientation, however, but an attitude of "offense is the best defense." If the causal determinants of dispositions, traits and also of disease are far more complicated—and "complex" only in *this* sense—one needs to expand the tool-set developed for the Human Genome Project, and for that one requires the accumulation of yet more data, trusting that new insights and tools will be generated by the ever-improving technologies for the representation and processing of large data-sets. This data-fetishism and the many kinds of "omics" proved pervasive in the funding and organization of research, even without enjoying much intellectual prestige. The mere accumulation of data and the race to fully map genomes and proteomes appears rather pedestrian, and this is where systems biology comes in. It provides a kind of format and form, rationale and rationalization for the idea of "total information" and its accumulation. This rationale comes from the idea that one might model whole structures and organisms by integrating as many data as possible and by approximating a complete description of a biological "system" (which is nothing more on this account than an aggregate of very many components and causal pathways).

Since the computer served not only as the tool but also as the site of much systems biology research, it became apparent that computers are far more than

devices for the storage and organization, representation and modeling of data. Computers are physical systems in their own right that can instantiate dynamic processes such that the behaviors of data-systems can be created, modulated, and observed, such that input-output relations can be studied, such that control can be achieved and stabilized even as the particular causal pathways remain opaque. It is this fact that leads from systems biology to synthetic biology in the second story.[18] Here, systems biology comes first and takes priority only because it integrates a multitude of data in order to represent biological systems such that they can be studied and understood. Synthetic biology comes second and short-circuits the ambitions of systems biology: Where the latter produces representations, synthetic biology takes these as substitutions, that is, it regards model systems that are subject to modulation and control in silico as a prototype for the construction, modulation and control of biological systems. Thus, while systems biology begins by capturing complexity and rendering it for the purpose of reducing complexity through theoretical modeling, synthetic biology does not demand theoretical understanding and the reduction of complexity but has learned from systems biology that complexity can be generated in a controlled manner.[19]

Though synthetic biology shares many of its ambitions with genetic engineering and other fields of molecular and bioengineering, it is engineering not by way of creating a knowledge-based hypothesis-driven blueprint of how things should work, and then implementing it. As suggested above, it does not work in the mode of rational engineering. But it is also far more than mere tinkering, trial and error, and the development of automated high-throughput search strategies—though these have a role to play. Synthetic biology is opportunistic by asking strategically how much technological knowledge and control one can achieve with what little we know scientifically, finding that through an iterative design process one can achieve a great

[18] Computation for systems biology enabled better ways to "acquire, store, analyze, graphically display, model, and distribute" information. Without yet going there, the discussion of computer models in systems biology prepares the ground for the exploitation of what they afford in terms of performance, behavior, intervention and construction (Ideker et al. 2001). This holds also for that brand of systems biology that takes complexity seriously. Here the proposals by Kitano (2002, 2004), for example, mark the point of transition. He advocates engineering concepts and computing tools for the purposes of modeling, representation, and theoretical understanding of biological complexity. He thereby paves the way for modes of constructing and handling such systems without reference to complexity theory: his concepts and tools afford their employment towards constructive ends by synthetic biology (O'Malley et al. 2008, 62).—This point of transition is also discussed by Schmidt (2015, this volume). He sees bioengineers who adopt systems thinking. The story of technical opportunism sees systems thinking appropriated and vulgarized by the ordinary idiom of engineering (Nordmann 2010a).

[19] Gabriele Gramelsberger identifies the simulation approach as a common denominator of systems and synthetic biology and suggests that it provides rational design methods that support tinkering in the lab (Gramelsberger 2013). She thereby downplays that modelling in systems biology is said to be "for basic research (i.e. generating knowledge) whereas synthetic biology's modelling is for the design of constructs" (O'Malley et al. 2008, 62): "Ultimately, mathematical models developed for research purposes (e.g. in systems biology) will be employed as design models in synthetic biology" (Heinemann and Panke 2006, 2796).

deal. The IGEM competition pursues this strategy most overtly as a proof of concept that US undergraduate students can "do" synthetic biology (Check 2005; Dyson 2007; Delgado 2013; Frow and Calvert 2013). This type of engineering is inspired by software engineering, for example, by the creation of expert systems which tune models to performance parameters, enriching the models until they achieve the desired functionality. According to this story, the impact of synthetic biology is first and foremost on the culture of research and the way of doing science itself. And the "risks" associated with synthetic biology concern societal expectations of the kind of knowledge and experience that is needed for the adoption, assessment and regulation of technologies; they thus concern our tolerance for black-boxed processes. This has been discussed, for example, in respect to the "kludge" as a module in a large software program or in an engineered assembly of biological pathways which plays an unknown, yet apparently necessary, role for the correct functioning of the system (O'Malley 2011, 2009; Lenhard and Winsberg 2010).

3.3 (Techno)Scientific Biology

Having heard first the story of sublime ascendance to systems thinking in engineering and then the story of technical opportunism for building up un-theorized complexity, it is finally important to reflect on the juxtaposition of these stories or the starkness of their opposition.[20]

There is a strong temptation to believe that the two stories about the relation of systems biology and synthetic biology are easily reconciled, that they are but two sides of the same coin (Breithaupt 2006; Kastenhofer 2013a, b): Synthetic biology advances profound theoretical understanding of biological systems even as it opportunistically pursues an engineering approach to the design and creation of biological entities and processes. This would amount to denying that there are profoundly different ways of conceiving synthetic biology. And to the extent that there is a philosophical difference to speak of, it would appear to be one that has been rather familiar since the times of Kant, namely the tension between a holistic understanding of organisms and the mechanistic materialism of modern science. It might be sufficient—and this would be an argument for reconciliation and business as usual—that synthetic biology is dedicated to theoretical understanding as well as the constructive project of building up ever more complex biological

[20] There are other stories that could be told. One does not have to assume that synthetic biology is somehow derived from, or intimately related to, systems biology. Instead, one might foreground the relation between chemistry and biology as exemplified, for example, by the work of Steven Benner (Benner et al. 2011). Just as physicists were told, many years ago, that there wasn't much work to be done in physics anymore but that they might find interesting problems in biology, so chemists have been told a similar story in recent years (I owe this suggestion to H. Ulrich Göringer). On this account, it is the chemical approach that distinguishes synthetic biology and genetic engineering. The possibility that the "synthetic" in synthetic biology derives from synthetic chemistry was discussed by Bensaude-Vincent (2009b, c, 2013b).

structures. It sometimes does so in the name of an ambitiously holistic notion of "system" and sometimes in reference to a rather more mechanistic conception of a system as a complicated unit of technical functioning.

Against this proposed reconciliation and the notion that synthetic biology can have it all, the present analysis suggests that there is no easy way out. If there is anything different and new about synthetic biology, it may well consist in the way it challenges the traditional orientation of the biological sciences and even of biotechnological research. Indeed, it would be a misunderstanding of the juxtaposition of the two stories about systems and synthetic biology if one took it simply to rehash the contrast of irreducible holism *vs.* reductionist mechanism. Instead, the debate of holism vs. mechanism belongs altogether to the first story which is driven by theoretical concerns and debates. According to the first story, systems and synthetic biology constitute a paradigm-shift of sorts, and it is entirely within that story that anti-reductionist "systems thinking" prevails over attempts to reduce biological phenomena to deterministic causal relations that can be isolated in the laboratory or in the mind.

If the debate between different intellectual conceptions belongs to the first story, it is characteristic for the second that the clash between competing research paradigms and all its attendant questions fade away and become irrelevant. Questions of reductionism, of natural philosophy, or the fundamental difference of organism and artifact are of no concern to the technical opportunism of synthetic biology. These questions are neither answered nor dismissed, but merely absorbed into an engineering idiom (Nordmann 2010a). The engineering approach of synthetic biology is not holistic or engaged in systems thinking as it builds up complexity in a controlled manner through iterations of the design cycle (*pace* Schmidt 2015, this volume), but it is also not mechanistic. Likewise, it does not challenge in a profound or principled manner the difference between artefact and organisms as it constructs a robust black box which, in its opacity, is not at all unlike the biological organism as a black box with stable behavioral patterns.

To put it a bit metaphorically, then, in relation to systems biology, the two stories about synthetic biology do not attribute to the researchers different theories, opinions, or beliefs but an entirely different mind-set, a different way of living the laboratory life, of participating in history and relating to the tradition of science and the Enlightenment. On the one hand, there is the scientific mind-set of those who query the limits of reductionism and embrace systems thinking; on the other hand is the technoscientific mind-set of those who no longer seek the most appropriate way of reducing complexity and promoting intellectual understanding, but who proceed instead to generate biological complexity from available theories and techniques.

4 Scenes of Conflict

We first saw the fault lines between scientific and technoscientific orientations of research that separate different notions of understanding, rationality, and design. We then saw how these fault lines provide contour and organize the stark

juxtaposition of two powerfully coherent stories about the origin of synthetic biology and its relation to system biology. The easy way out would be to blur these boundaries and vaguely have it all. By blocking this easy way out, the terrain has been laid out. In a third step, we can now observe how researchers position themselves in this terrain. Though it is possible to wear different hats at different times—in one context advancing theory development and the reduction of complexity, in another context promoting the design of highly complex entities—this does not hold for publications that each belong to just one context. Any given publication expresses only one mind-set, exhibits one research agenda, establishes one kind of relation to its object. So, even if researchers might not position themselves unambiguously, every particular publication can be assigned a definite place on the map.

Different researchers engage in different research practices, and by looking at the published products of these research practices we can see how the same researchers can belong to very different epistemic communities in that their work is informed by different values, methods, standards of evidence and criteria of success.[21] Therefore, questions, issues, and hypotheses can be sharpened by treating the publications of synthetic biology as scenes of conflict between the values of the different epistemic communities. In conclusion then, we might cast a brief glance at three such scenes of conflict.

4.1 Accommodating Ignorance

One does not have to cast far and wide to find scenes where different epistemic and, indeed, generational communities clash in the field of synthetic biology. This occurs at any conference where senior researchers confront so-called iGEM teams that impatiently seek to achieve on extremely short time-scales what others

[21] Arguably, the initial promise and attractiveness of synthetic biology is much like that of nanotechnology. However, the clash between epistemic communities is far less pronounced in nanotechnology than in the case of synthetic biology. Nanotechnological research is "pure technoscience" because it is geared to the development of basic capabilities of control that generally expand the toolset of technology—it isn't dedicated to any one engineering agenda but seeks to recruit scientific theories, scientific expertise, scientific labor for the purpose of putting technological change on a new footing. Nanotechnology is thus an effort to retool the scientific enterprise by dedicating the accumulated knowledge, methods, and personnel for knowledge production to a different, perhaps complementary end. Synthetic biology is "pure technoscience" in a different way. It does not seek to retool or rededicate laboratories and academically trained researchers. Instead it seeks to produce new kinds of researchers even before it produces new kinds of biological entities. The creation of epistemic communities with non-traditional values is part of what synthetic biology is and, for some of its protagonists, what it ought to be. The promise and attractiveness of synthetic biology thus lies *also* in its appeal to a new generation of researchers. This is somewhat problematic, however, since the staging of a generational conflict over epistemic ideals does not go so well with the idea of drawing together a diverse group of researchers.

frame in terms of multidisciplinary, sometimes career-long research trajectories (compare Frow and Calvert 2013). This discrepancy of expectations cannot be ascribed simply to naïveté on one side and many years of experience on the other. The iGEM teams seek to find out through a strategic design process how much they can achieve with what little they know. They are not held back by seeking to learn all that would be needed for rationally engineering some biological structure or entity. Instead, they are invited and resolved to short-circuit the scruples of their teachers. If science is about the search for knowledge in order to reduce specific areas of ignorance, iGEM's technoscientific approach acquires a kind of working knowledge that can work around and accommodate ignorance.

Such scenes of conflict rarely take the form of overt disagreement, opposition, or antagonism. As with the encouragement of iGEM teams also within relatively conservative departments, they can involve something like the turning of a blind eye to the differences. An issue of *Nature* dated January 21, 2010 provides such a scene of conflict. Its featured article, Roberta Kwok's "Five Hard Truths for Synthetic Biology," offers a review of five major obstacles to the ambitions of synthetic biology and the prospects for overcoming them.

The text begins, predictably enough, by pointing to the "daunting knowledge gap when it comes to how life works" and, quoting Christina Agapakis, to the fact that "there's a lot of biology that gets in the way of the engineering." It goes on to show, however, that synthetic biologists are undaunted by the daunting knowledge gap and that they might have ways to meet the key challenges which they encounter all along the way. The first difficulty of defining standardized biological parts provokes efforts to substitute relative for absolute measures, thereby taking a first stab at evading Martin Fussenegger's verdict that "[t]his is the type of complexity that is very difficult to capture by standardized characterization." The second difficulty is the familiar predicament that predictable design procedures are not available, whereas trial and error is too arduous. Here, the synthetic biologist's answer is said to consist in a process of directed evolution, i.e., the design cycle and its iteration "until the system is optimized." The third difficulty arises as one moves to ever greater levels of system complexity which prompts the proposal to overcome the bottleneck by automating the process quasi-robotically, or by using bacteria as assemblers. In order to avoid unexpected interactions—the fourth difficulty—procedures need to be found to insulate the biological machinery to be designed as far away as possible from a cell's "natural machinery." And the final difficulty is to avoid variability and to increase stability, for example, by using noise to one's advantage rather than try to eliminate it. Accordingly, the review closes on a note of cautious optimism that synthetic biology can move forward without closing the daunting knowledge gap:

> As the cost of DNA synthesis continues to drop and more people begin to tinker with biological parts, the field could progress faster, says [Rob] Carlson. 'It's a question of whether the complexity of biology yields to that kind of an effort.' (Kwok 2010, 290)

In summary of Kwok's arguments, then, she argues on all five points of difficulty that the only chance for synthetic biology to succeed is by way of design processes that can accommodate or work around ignorance.

But how do the editors of *Nature* relate Kwok's assessment of the first decade of synthetic biology? On the tenth anniversary of the repressilator and a switchable regulatory network, they declare that "[c]ontributions to and from basic science are the part of synthetic biology that most deserves celebration":

> Both of those pioneering experiments transposed two great traditions of physics to biology: first, to understand something one must build it, and second, start from the simplest imaginable principles. These directives have set the basic-science agenda for synthetic biology: to design, and thus define, the minimal systems sufficient to produce a given function. […] Bringing these applications to reality has proved much harder than was originally hoped (see [Roberta Kwok's analysis]). But the difficulties have proved instructive. Indeed, the decade-old papers raised several new and fundamental issues in biology, for example by pointing to the crucial role of noise in gene expression, both as a nuisance and as a great computational opportunity. It is now an active area of research. […] It took endeavours in synthetic biology to illustrate what systems biology perhaps should mean: to enlist mathematical formalism in producing biological insights that are beyond the reach of mere intuition. (Nature Editorial 2010)

The editors thus try to assimilate Kwok's analysis into the traditional idiom of basic *versus* applied science. This is the vain attempt to accommodate the epistemic ideals of a technoscientific design community within that of traditional science. In which sense, for example, is the design of the repressilator an "experiment?" Is "to understand something one must build it" really a principle of physics as a basic science? How and when is "to design" the same thing as "to define"—even if one considers operational rules as definitions, what is defined by the complete "design" of a minimal system? What makes the synthetic bottom-up design of a minimal cell preferable to the classically analytic "knock-out" methodology if the aim is to discover the contribution of individual genes to the workings of a cell? And, finally, the editorial states that biology moves beyond the reach of mere intuition when aided by mathematical tools. Does this not imply that knowledge or understanding now reside in the ability to build a computer model, rather than in theories that are tractable by the human mind?[22]

Here, then, the scene of conflict appears as an unresolved tension within a pair of texts that does not wish to acknowledge, and turns a blind eye to the profound difference in the conception of a research field that contributes to basic biological science and one that pursues a knowing-by-building design agenda.

4.2 Discontinuous Continuities

In their 2010 paper on "Engineering in Complex Systems" Matthias Bujara and Sven Panke also produce an argument that explores the tension between different epistemic communities, those of knowledge-based rational design and those of

[22] The editors' text continues here in agreement with the story of technical opportunism which was told above and which contradicts the idea that synthetic biology advances basic science: "In that aspect [of using mathematical formalism to manage data beyond human intuition], synthetic and systems biology now seem indissociable."

"evolutionary" design that feeds variation and selection into the iterations of a design cycle.[23] They distinguish three types of optimization strategies, showing that designers can work more effectively if they know what they are looking for, that is, what to vary and what to select for:

> Random evolution based on mutagenesis and brute force screening only requires limited knowledge on [sic] the system but the cause for the beneficial effect frequently remains unclear. Directed evolution needs at least knowledge of the element that should be modified (e.g. gene, promoter, or ribosome binding site), while a detailed optimization strategy is not needed. Combinatorial design follows a semi-rational strategy based on characterized parts and compensates for the current lack of detailed instructions for a comprehensive blueprint for optimization. (Bujara and Panke 2010, 589)

The progression from "random evolution" to "combinatorial design" thus involves an increase of knowledge about the elements to be modified and the functions to be achieved which is illustrated by a very telling graph that extrapolates from the three "evolutionary" strategies all the way to rational engineering (cf. Fig. 1).

By suggesting a continuous progression from "evolutionary" to rational design, this graph stands in a peculiar relation to the main body of Bujara and Panke's paper. The paper speaks primarily to the discontinuous differences between the three modes of iterative design and it does not address at all the growth of knowledge beyond the "current status of biological system engineering." In particular, it does not suggest that the further development e.g. of combinatorial design strategies will produce the knowledge base that would be required for rational design. Indeed, the paper does not even suggest that rational design is more effective than evolutionary design—it appears to be more superior only in being more "rational," that is, in being knowledge-based.[24] In addition to supplying all these added considerations, the most interesting feature of this graph is that the demands of knowledge loom like a dark cloud above the scene, rendering the image highly ambivalent: On the one hand, it tells a story of progress and a sequence of steps towards the ultimate goal of rational design based on a comprehensive blueprint for optimization. On the other hand, it identifies a daunting demand for knowledge,

[23] See Sect. 3 in Schmidt (2015, this volume) as to why "evolutionary design" is an oxymoronic misnomer. Though they both work with variation and selection, Darwinian evolution by natural selection is different from breeding by artificial selection: what is selected for and against in natural selection does not depend on the specifications of a designer, but on adaptedness to the complex and changing conditions of life. What Bujara and Panke are referring to is more appropriately called "design by breeding."

[24] The graph suggests continuity and thus makes the implicit, albeit highly problematic, assumption that the knowledge required for better ways of running the design cycle is the kind of knowledge that could provide the basis for rational design. Indeed, in their paper Bujara and Panke question that "reducing the complexity of biological systems will facilitate its engineering," commenting that this is only "a hypothesis that still needs to be confirmed in the laboratory" (2010, 589). This cautionary remark applies to the reduction of complexity by an increase of knowledge of causal relations and also to its reduction by insulating engineered biological systems from natural ones. If it were possible to run such an experiment, the laboratory test proposed by Bujara and Panke would measure the scientific assignment of priority always to the improvement of causal knowledge against technoscientific requirements of what it takes to achieve effective control. And if the hypothesis would fail to be confirmed, discontinuity would be reestablished.

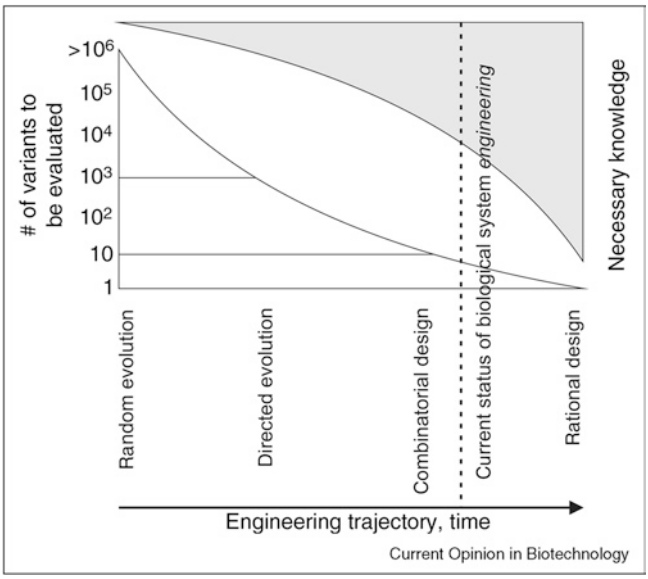

Fig. 1 The relationship between required knowledge and different manipulation strategies on the road to engineering design (reproduced from Bujara and Panke 2010, 588)

prompting the reader either to abandon this trajectory with a gloomy outlook on the future of synthetic biology or else to seek out an alternative trajectory that bypasses the need for all this knowledge but accommodates ignorance.

This moment of ambivalence leads to another scene of conflict. Here, anxiety about departing from the respectable path of science becomes transformed into a hopeful image of continuity and a belief in the fusion of opposites. At the beginning of a review of two methods for the more reliable construction of gene circuits one finds a double-pronged credo:

> Engineered organisms enable studies of the general organizing principles of life and have the potential to transform industries including medicine, agriculture and energy (Tabor 2012, 1061).

Noting that "[s]ynthetic biologists must often iterate through cycles of optimization when composing even well-understood parts," Jeffrey Tabor welcomes a method that accelerates this design process by offering a standardized "plug-and-play" modification scheme.[25] Aside from speeding up the achievement of the

[25] Tabor offers an epistemologically telling description of the design-cycle approach: "Here, the first design is based on the ligand-inhibited repressors LacI and TetR. Each is initially placed upstream of an associated fluorescent reporter on a polycistronic mRNA. The operons show poor reporter expression, which is then improved by 'plugging in' additional copies of the appropriate promoter upstream of each reporter. This increases reporter expression, but reveals that the circuit cannot reach the TetR-dominated state. The *tetR* promoter is then swapped for a stronger version, but this overcompensates for the problem making only the TetR state stable. A library of random *tetR* ribosome binding sites (RBSs) is then screened, and a variant that hits the bistable sweet spot is found" (Tabor 2012, 1063; compare Litcofsky et al. 2012).

desired system performance, this method is "also more scalable and amenable to future automation." In the conclusion of his review, Tabor returns to the initial credo not only with respect to the transformation of industries but also in regard to the study of the general organizing principles of life. He could easily make the point now that the design process is a kind of technical probing and as such in and of itself a way of studying these organizing principles of life. However, by "studying" Tabor means something akin to theoretical understanding. Accordingly he first notes a tension between the advance of design methocalcds and the search for true understanding, and then proceeds to dissolve this tension:

> As automated circuit design and assembly dovetails with iterative optimization, our ability to engineer circuits should extend beyond our ability to truly understand how they work. The tractability of modularly constructed synthetic circuits, however, should also feed back to accelerate the cycle of hypothesis generation and testing in systems biology. (Tabor 2012, 1063)

Along the lines of "what I can create, I can also understand" Tabor proposes that by synthesis engineers learn what it takes to get something to work. Systems biology can then take this up to generate and test hypotheses about the way in which nature gets analogous things to work.

He thus arrives at a fusion of design practice and hypothesis testing, but this should not be mistaken for a fusion of technoscientific synthetic biology and scientific notions of truly understanding how things work. After all, when Tabor speaks of the accelerated generation and testing of hypotheses that extend beyond our ability to understand them, he can only be referring to the iterative design process in systems biology that leads to the construction of computer models that step in where humans reach the limits of their ability to understand. Accordingly, Tabor's image of the fusion of gene-circuit construction with the study of organizing principles of life amounts to the construction in parallel of two technical systems, one in vitro or in vivo, the other in silico, each built through a strategic process of generating complexity, such that one can be said to model or instantiate the causal dynamics of the other: It is not the human mind but the computer simulation that "understands" organizing principles of life by learning to model the engineered structures of synthetic biology. Thus, the simulation "understands" these in virtue of resulting from a similar iterative design processes. Accordingly, Tabor's construction of continuity between circuit engineering and achievements of understanding leaves untouched the break with the epistemic values and traditional ideals of science.

4.3 The Matter of Definition

According to Jeffrey Tabor, synthetic and systems biology can exploit how engineered biological devices and engineered computer models inform each other in various ways. This fundamental notion had already found expression in Tabor's student days when he helped produce one of the founding moments of synthetic

biology in the popular imagination. He co-designed a roughly 10 cm^2 "lawn" of e-coli bacteria that served as a light-sensitive biofilm which produced the message "hello world." Not only is this friendly greeting the first thing that Tabor's bacteria say to us when we ask them to speak, "hello world" are also the first words that computers programmers learn to program and that verify the working of a programming language or computer system.[26]

Quite in the spirit of "hello world," when synthetic biologists aim to construct synthetic organisms, what they do is take biological knowledge, techniques, and parts in order to build up a complex artificial systems that can stand in for natural biological systems. As such, what they are doing with biological tools is what bioinformatics modelers do with computing tools and algorithms, namely build up a complex artificial system that for explorative purposes takes the place of natural biological systems.[27] Synthetic biology is thus "synthetic" firstly in the sense of not being analytic, of generating rather than reducing complexity, and secondly in the sense of being a non-natural, artificial biology, that is, in virtue of engineering not within the domain of the natural, but entirely within the sphere of the synthetic even as it utilizes knowledge about and materials from the material sphere of the biological. This is what sets it apart from molecular biology as well as genetic engineering.[28]

Classical science or the pursuit to reduce complexity for the purpose of explanation, calculation and mechanistic control assumes the standpoint of an antagonism between mind and world, theory and reality. How can the mind with its limited means and its peculiar demand for human intelligibility forge agreement between its formulae and the infinite variety of appearances? The technosciences in general, and synthetic biology in particular begin in the middle of things, they are right there and on friendly terms ("Hello World") with the world that they squarely inhabit as an extended laboratory which is overflowing with phenomena of their own making (Bensaude-Vincent and Simon 2008). These technosciences build on the achievements of science and technology to further enlarge technological and predictive control. Instead of presenting the external world to the human mind, they amalgamate the workings of the human mind with the workings of machinery and the workings of black-boxed biological nature in order to create highly complex, yet reasonably robust structures or processes.

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[26] Compare the Wikipedia entry "Hello world program."

[27] "[A]s opposed to simulation models transformed into a computational algorithm and run on a digital computer, here the theoretical model rendered as a synthetic model is of the same 'natural kind' as the native networks as well as being embedded in a simulation environment of the 'same materiality,' i.e., the host organism" (Knuuttila and Loettgers 2013, 168). Knuutila and Loettgers argue that this supports a "basic-science approach to synthetic biology." However, whether it actually does this or not depends on the question whether one can pick out "theoretical models" as traditionally conceived.

[28] Also, this perspective affords a way of distinguishing the simulation approach in synthetic biology from that in systems biology, and thus a way of re-interpreting the examples discussed in Gramelsberger (2013), compare Footnotes 18 and 19 above.

This, then, suggests a definition of synthetic biology that highlights its specific epistemic values and ideals: For constructive purposes synthetic biology builds on the achievement in silico, in vitro, and in vivo of technical control of biological complexity, that is, it is the endeavor of drawing together de facto achievements of technical control for the generation of technical systems with greater biological complexity.[29]

5 Conclusion

The philosophy of synthetic biology seeks to characterize an emerging, indeed contested field of inquiry. In this survey, it therefore began by showing that different epistemic communities might attach different meanings or interpretations to central concepts such as "understanding," "rational engineering," "evolution," and "design." It was then shown that these different interpretations give rise to different stories of how systems biology led to synthetic biology where each of these stories expresses different epistemic values and ideals. But from the tension between scientific and technoscientific epistemic commitments it was still possible to finally distill a definition of synthetic biology. That this was possible is due to the fact that the tension between an engineering approach and the quest for understanding biological processes cannot be resolved in any old way. And pointing this out is a valuable philosophical contribution to synthetic biology at this early stage in its development.

It is quite impossible to simply marry the epistemic ideals of technoscientific synthetic biology to those of biology as a theoretical science—they pull in opposite directions, after all: Here the reduction of complexity for the purposes of intellectual tractability, there the drawing together of scientific knowledge and technological capability for the generation of complexity beyond our ability to truly understand how our own creations work. Here the identification of bottlenecks and needs-to-know for rational engineering, along with the demand for more and better theoretical knowledge in order to diminish ignorance, and there the attempt primarily to discover how much one can achieve even with how little we know, with considerable tolerance for ignorance of everything that can be black-boxed. The tension, even antagonism, between these epistemic ideals cannot be dissolved—which does not preclude, of course, that the corresponding research findings can inform, even inspire one another.

It is not at all impossible, in contrast, to marry the notion of bringing an engineering approach to biology and the notion of knowledge production through

[29] To be sure, "de facto achievements of technical control of biological complexity" does not require an understanding of biological complexity, it refers only to the local and partial success stories where some biological process can be manipulated or replicated (in a biological system or in a simulation model).

synthetic biology—but in order to do so one might have to become dishabituated from the established scientific image of knowledge and of knowledge production. From the point of view of the engineering approach, knowledge and understanding need not be tied to the intellectual tractability of causal relations, nor does it consist in the truth or falsity, or empirical adequacy of linguistic statements such as theories or hypotheses. Instead, knowledge and understanding might reside in computer models and other technologically robust constructions, tied to the iterations of the design cycle as a learning process of sorts.

This allows us finally to appreciate the last sentences of the *Nature* editorial that appeared on the occasion of Roberta Kwok's analysis, and the tenth anniversary of synthetic biology:

> As it develops along this and other paths, synthetic biology itself will demand more by way of new fundamental biological knowledge—quantitative, systematic, computational and biophysical. And conversely, one of the deepest lessons from these first ten years is that biological knowledge will require synthetic approaches if it is to become a mature and reasonably predictive science. (Nature Editorial 2010, 270)

There is little to disagree with in these concluding remarks. To the extent that they gloss over the antagonism between epistemic ideals, these two sentences require only a bit of rephrasing and clarification: Of course, synthetic biology can only benefit from new fundamental biological knowledge—this is an argument for a pluralism of approaches, scientific and technoscientific, within biology. By the same token, synthetic biology will continue in its search for design solutions that do not depend on the availability of new fundamental biological knowledge. And as for the "deepest lesson" offered by synthetic biology, it leads to the question of how the very notion of "biological knowledge" will be transformed through the synthetic approach. This includes the question, for example, of the difference between predicting on the basis of explanatory theories, and predicting on the grounds of technological robustness. With this deepest lesson there is much to do for the philosophy of synthetic biology.[30]

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Complexity in Synthetic Biology: Unnecessary or Essential?

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Abstract Synthetic biology aims at the design or redesign of living systems for useful purposes. This aim requires a predictable and reliable behavior of synthetic cells in their environment. The inherent complexity of biological systems renders any strict calculations impossible and thus poses an enormous challenge to synthetic biology. Two alternative strategies have been adopted by synthetic biologists to deal with this problem: (1) Reduction of complexity by applying engineering principles to biology like standardization and modularization and (2) orthogonalization through chemical or biological modification of synthetic cells to prevent genetic interactions with other organisms. While the first strategy aims at a transformation of biology into an engineering science, the second reduces complexity at the ecological level but not at the individual level. I will discuss both strategies and show that they also follow different safety concepts. The engineering branch of synthetic biology builds on extensive control of synthetic cells via their predictive behavior. The safety of chemically modified organisms will be provided by a genetic firewall due to their chemical or genetical incompatibility with existing cells.

1 Introduction

Complexity appears to be a characteristic and inherent feature of all living beings. The high degree of functionality and the intricate organization of biological systems have even been regarded as proof for the existence of an ingenious creator, named God (Paley 1802). Since Darwin, however, we know that life on earth is not the creation of an intelligent designer but rather the product of chance mutation and selection. Even more: evolution as a trial and error process resembles more tinkering then rational design as François Jacob once insightfully remarked

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(Jacob 1977). Nevertheless, evolution has brought about all the astonishing phenomena of life that have both fascinated biologists and inspired engineers for technological inventions. But in a modern view biological complexity even reaches further. It does not only refer to the inner organization of organisms, but also encompasses their manifold interactions with other living beings and their common environment. This ecological complexity depends upon species diversity resulting from evolutionary adaptation and specialization. The complex structure of ecosystems has already been recognized by Darwin:

> It is interesting to contemplate an entangled bank, clothed with many plants of many kinds, with birds singing on the bushes, with various insects flitting about, and with worms crawling through the damp earth, and to reflect that these elaborately constructed forms, so different from each other, and dependent on each other in so complex a manner, have all been produced by laws acting around us (Darwin 1859).

Synthetic biology is an engineering technology based on living systems and aims at the design and construction of novel biological parts, devices and systems for useful purposes; alternatively redesign of existing, natural biological systems can be used for the same purpose (definition of synthetic biology at http://www.syntheticbiology.org; Knight 2005). The idea of engineering living substances is not completely new in biology (Campos 2009) and 'genetic engineering' emerged as a scientific enterprise immediately with the advent of recombinant DNA technology in the 1970s (Jackson et al. 1972). However, when contemporary engineers revisited the field 30 years later, gene technology appeared to them as "still an expensive, unreliable and ad hoc research process" (Endy 2005). As a reaction to this perception, a manifesto was published ("Foundations for Engineering Biology") to promote transformation of biology into an engineering discipline (Endy 2005). This was possible since in the meantime reading and writing of DNA became available on a large scale and at low cost (Pettersson et al. 2009; Tian et al. 2004). This progress was largely due to the human genome sequencing project that had pushed the development of new methods. This remarkable scientific and methodological progress not only provided hundreds of genome sequences but also paved the way to synthesize complete genomes from scratch. The technological breakthrough in DNA technology finally attracted scientists from outside biology. Especially scientists trained in the traditional fields of mechanical, chemical or electrical engineering were drawn into the new science of synthetic biology. It was these engineers who proposed to introduce into biology the principles of standardization, modularization and automatization which had made the great successes of classical engineering possible in the 20th century (Endy 2005).

2 Getting Rid of Complexity

However, classical engineers deal with energy or inanimate matter, while biological engineers have to deal with living systems that are characterized by their astounding complexity. This immediately posed a problem for these engineers turned synthetic biologists: while conventional biologists appear to be especially

attracted by the complexity of living systems, engineers try to avoid unnecessary complexity as far as possible (Breithaupt 2006). These different points of view are best characterized in a statement by one of the promoting figures of early synthetic biology and founder of the biobricks registry, Tom Knight:

> Here is the difference between a biologist and an engineer: A biologist goes into the lab, studies a system and finds that it is far more complex than anyone suspected. He's delighted; he can spend a lot of time exploring that complexity and writing papers about it. An engineer goes into the lab and makes the same finding. His response is: 'How can I get rid of this?' (Brown 2004).

Thus, the immense complexity of living systems appears to them more as a technical obstacle than as a scientific challenge. Engineering-oriented synthetic biologists want to streamline their synthetic creations and to get rid of the detritus of evolution. But can we actually eliminate the 'messiness' of biology? And what makes the biological substrate different from other substrates that we engineer? (O'Malley et al. 2008).

At least for some synthetic biologists the difference between biological substrates and those that are normally engineered is not so large. Some of them regard Nature itself as a technology:

> Biology is the oldest technology. Throughout the history of life on Earth, organisms have made use of each other in sophisticated ways. Early on in this history, the ancestors of both plants and animals co-opted free-living organisms that became the subcellular components now called chloroplasts and mitochondria. These bits of technology provide energy to their host cells and thereby underpin the majority of life on this planet. (Carlson 2010)

Thus, natural systems built by biological evolution can also be seen as technology-based in an emphatic sense. This view is further corroborated by the analogies between the modular and layered structure of technical systems and the comparable design of living cells (Andrianantoandro et al. 2006). The different layers of parts, devices and modules of a computer e.g. resistors, capacitors and transistors on the physical level, integrated circuits, logical gates and processors at higher levels etc. are compared with biological molecules, that are connected by biochemical reactions to form biological devices and modules. Thus, if biological cells are by themselves already organized as parts, devices and modules, then it appears rather natural to improve living systems further by implementing explicit technical standards.

3 Different Strategies to Reduce Complexity

Synthetic biology is often classified into different fields or branches according to certain criteria. Most popular is the distinction between top-down and bottom-up approaches. Top-down means the redesign of existing cells by downsizing and minimization, while bottom-up indicates all attempts to construct synthetic cells "from scratch." Here, I divide synthetic biology according to alternative strategies how to deal with biological complexity. These are (1) standardization and modularization or (2) orthogonalization via biochemical or genetic alterations. Both strategies aim at reducing complexity but operate at different levels. Modularization implies

the rigorous redesign of complex metabolic pathways and signalling networks to generate a highly integrated system, whose behaviour is computable and thus largely predictable. This reduces the complexity of the inner organization of living beings, but does not affect interactions with natural organisms in the ecosystem. As an alternative, it is proposed to isolate synthetic cells from interactions with other living systems by implementation of a genetic firewall. This can be achieved by different means: the most extreme would be the construction of cells in which the genetic information is stored not in DNA but in alternative molecules commonly termed XNA (for xeno-DNA). This will prevent any exchange of genetic information with natural biological systems be it by mating or horizontal gene transfer. Reduction of complexity occurs here at an ecological level, but does not necessarily require the reduction of functional complexity of these cells. These two approaches are somewhat complementary, not mutually exclusive; and intermediate solutions of complexity reduction at both the functional and the ecological levels can be contemplated. For example, the construction of refactored cells based on a non-universal codon table includes modularity and genetic isolation. Both controllability and genetic interactions of synthetic cells in the natural environment have to be considered as factors to assess the potential risk of synthetic organisms. Therefore, the approaches to reduce complexity in synthetic biology differ not only in their general strategy but also in their underlying concepts concerning safety and security aspects. I will briefly touch on this aspect at the end of this contribution.

4 Standardization and Modularization

Even purely technical systems sometimes display unwanted behaviour if the degree of complexity exceeds a certain level. This unpredictability usually results from the interplay of the large number of interacting parts and components. In biological systems, phenomena like stochastic noise and chance mutations further enhance this unpredictability (Maheshri and O'Shea 2007; Raj and van Oudenaarden 2008; Eldar and Elowitz 2010). Therefore, reduction of unnecessary complexity is a good means to gain better control in large-scale systems. The most prevalent approach in synthetic biology is the implementation of classical engineering standards. Living systems will be transformed into controllable technical devices by refactoring their genomes on the basis of a minimal chassis cell. These streamlined cells can then be used for useful purposes such as medical applications, generation of biofuel or to detoxify environmental pollution.

One of the first examples for this streamlining was the refactoring of bacteriophage T7 (Chan et al. 2005). In software technology, refactoring means the restructuring of existing computer code to improve readability and to reduce complexity. In the case of bacteriophage T7 all overlapping genes were disentangled and ordered in linear fashion. The viability and virulence of the refactored phage demonstrates that the redesigned phage has maintained the key features of the original and was still able to complete its life cycle. At the same time this indicates that the bacteriophage

genome contains no hidden features or genetic elements that have been overlooked. In addition, the refactored genome is much simpler to model and to manipulate. Therefore refactoring of existing biological systems also helps to understand the inherent design principles of natural living systems. This knowledge can then be used to design and construct novel cells that have never existed before and with properties not yet realized in nature. This situation resembles the enormous progress in organic chemistry in the mid-nineteenth century. This interplay between analysis and synthesis, the understanding of fundamental principles of chemical structure and reactivity allowed the synthesis of artificial organic molecules that did not exist in nature such as polymers and pharmaceuticals (Yeh and Lim 2007).

To refactor existing living systems or to design novel cells, synthetic biologists refer to the classic repertoire of engineering principles to reduce complexity. The most important aspect is the use of standardized modules whose functional properties are known and can be described quantitatively (Canton et al. 2008). Modularization has to be achieved at all levels, i.e. at the level of parts, devices and systems. Only then can these parts and devices be combined in all possible ways to construct higher-order systems with useful properties. To integrate such standardized parts and devices into more complex systems, engineers working at different levels then use an information hierarchy that facilitates communication (Endy 2005). This is possible since synthetic biologists can use these standardized parts and devices without full knowledge of their interior design. Since all modules are described in their functional properties in quantitative terms and use standardized input/output systems, they can effectively be regarded as "black boxes" (Endy et al. 2005). The information hierarchy may be best illustrated with engineers collaborating during construction of computers. Only standardization guarantees that engineers working at a high level of system integration, e.g. at architecture of central processing units, can communicate with engineers designing logical gates and vice versa.

Standardization in synthetic biology is best exemplified through the biobricks registry (http://partsregistry.org/) and the international student competition iGEM (Smolke 2009). This steadily expanding open-source depository of DNA sequences provides standardized biological parts and devices that can be used to assemble larger functional modules (Knight 2003; Canton et al. 2008). The long-term goal of this endeavour is to provide a toolbox for designing whole cells. While refactoring of small genomes such as those of bacteriophages might be reached in a single step, redesign of cells is normally accomplished in two steps. First, a cell with a minimal genome is constructed. Such a cell would contain only the essential biological pathways to avoid any adverse effects that might occur by interference with other pathways and metabolic processes. This makes the behaviour of this minimal cell much more predictable. The functionality of the simplified cell can then be expanded by implementation of additional genes designed for specific purposes. It thus serves as a reliable platform (chassis) for the build-up of tailor-made cells with useful properties. The idea of a chassis is an important aspect of these novel cells designed by rational principles and not by contingent evolution. The creation of a bacterial cell controlled by a chemically synthesised genome (Gibson et al. 2010)

demonstrates that it is even feasible to refactor a whole bacterial cell by designing its complete genome. This provides nearly unlimited possibilities to endow a minimal cell with all genes necessary and sufficient for stable and robust growth.

5 Orthogonalization and the "Genetic Firewall"

Another important aspect of refactoring is orthogonalization. This term is used in analogy to the design of electric circuits, where crosstalk between signalling channels has to be avoided for proper functioning. In synthetic biology, orthogonalization means the elimination of any unwanted interaction between components of biological processes that occur concomitantly in the same cell. If minimal chassis cells are endowed with new features by implanting synthetic parts and devices, it must be guaranteed that these novel functions neither affect each other nor the basal metabolism of the chassis cell. This requires careful design of all parts and components and can, for example, be achieved by using genes or proteins from unrelated species that are unlikely to interact with compounds of the host cell. Alternatively, synthetic signalling molecules can be rationally designed on the basis of existing sets of protein kinases and DNA binding proteins (Dueber et al. 2004; Pryciak 2009; Kiel et al. 2010; Lim 2010; Slusarczyk et al. 2012). Also synthetic expansion of the genetic code further enhances the level of orthogonality. Mutually orthogonal pairs of aminoacyl-tRNA-synthase/tRNA pairs have been generated in vivo to expand the genetic code. This allows selective incorporation of unnatural amino acids into proteins in vivo (Neumann et al. 2010).

Orthogonality can also be achieved at the level of whole cells. In this context, orthogonality indicates the biochemical and/or genetic isolation of synthetic cells from other natural organisms. This is reached by targeted alterations of basal metabolic and genetic processes. The most far-reaching alteration is the construction of cells that are based on chemistry distinct from that of natural organisms. The major challenge of such a 'xenobiological' approach is to construct "natural" cells from unnatural substances. In this respect, xenobiologists even claim to be the proper synthetic biologists, because the engineering branch of synthetic biology only seeks interchangeable parts from natural biology to assemble into systems that function unnaturally (Benner and Sismour 2005). As mentioned above, the major advantage of any xenobiological approach is the general isolation of these new forms of life from the natural world. Due to the changes in their information storage-molecules these cells are "invisible" to conventional biological systems and thus can be regarded as environmentally safe (Schmidt 2010). The xenobiological concept of biosafety by chemistry has also been propagated under the slogan "The farther, the safer." The idea behind this motto is that synthetic species with chemical constitutions as deviant as possible from that of natural species carries the least risk of dissemination and contamination of wild habitats, including the human body (Marlière et al. 2011; Herdewijn and Marlière 2009).

Even if realizations of fully xenobiological cells still seem to be far away, other options to reach orthogonality at the level of the organism have already been

achieved. To name but a few, incorporation of unusual or even toxic bases into the DNA has been shown to generate "chemically modified organisms" (Marlière et al. 2011). Elimination of one of the three translation termination codons has already been realized in *E. coli* (Isaacs et al. 2011; Lajoie et al. 2013b) and also the limits of genetic recoding in essential genes have been probed (Lajoie et al. 2013a). The feasibility of creating cells controlled by chemically synthesized genome (Gibson et al. 2010) makes it even possible to reassign more than a single codon. At least theoretically, an organism which uses a genetic code completely different from the universal would be totally isolated from any exchange with other living beings.

George Church, one of the leading figures in synthetic biology brought up in his book "Regenesis" the idea of creating mirror-like bacterial cells or even mirror-like humans (Church and Regis 2012). While the latter is clearly out of the question, the former might be an attractive option to create fully viable bacterial cells that are isolated from the natural environment. According to the physical and chemical laws such cells will behave exactly like the wild type form, with the only exception that all biochemical molecules of these cells would be stereoisomers of their natural counterparts. One immediate advantage of such cells would be that they are completely resistant to the attack of all existing bacteriophages. This demonstrates that orthogonalization can serve as a biosafety tool and allows the construction of synthetic cells which maintain their full inherent complexity without need to worry about their genetic interaction with the natural world. The concept of a genetic firewall does not reduce the inherent complexity of a biological cell but only minimizes its interaction with the environment.

6 Safety Aspects

As mentioned above, the different strategies to reduce complexity in synthetic biology come along with alternative concepts concerning safety and security of these synthetic constructs. For engineers, computability and predictability of refactored cells guarantees controllability. Chemists and biologists, however, are aware of the enormous complexity of living systems, and might trust more in genetic and biochemical firewalls. Both concepts have their pros and cons and might apply differently for specific applications. For example, the safety of cells that are cultivated in closed containments (fermenters) will be viewed other than that of cells that are planned to be used in the environment.

6.1 Safety by Computability and Predictability

A major claim of engineering synthetic biology is that its methods will guarantee high predictability and reproducibility. This claim is justified by the use of modularized and standardized parts that have been quantitatively characterized and thus

provides a high degree of computability. Therefore, the major tenet of this concept is that reduction of complexity by rational design provides us with control over these cells. They can thus be compared with a technical "system" whose behaviour is determined by its technical parameters. Quantitative description of modules and knowledge of their interactions within a network allows us to predict the future states of this system with high precision and reliability. This concept has its roots in the world view of engineers: complex systems like computers are built from simple components like graphics cards, processing units, integrated circuits, transistors etc. Each of these components is very well characterized and functions deterministically. This engineering principle allows the precise construction of highly integrated systems like airplanes which we often use, fully confident of their fail-safe design.

But within such a technical approach, a biological risk may come not only from the inherent unpredictability of any organisms that might be retained in spite of all engineering, but also from recent experiences with large-scale projects involving highly complex technologies, such as nuclear power plants or large electric power transmission grids. We experience in our daily life that even small-scale technology (like personal computers) often crashes. In this case, the computers just need to be rebooted. Failure of nuclear power plants, however, may result in nationwide blackouts or may even make large areas uninhabitable. In all these cases it is the large size of these highly integrated modular systems that obviously inherently bears a risk of unpredictable behaviour.

While genetically modified organisms, in which single or only few genes have been manipulated or been introduced, may be regarded as safe, the high complexity of organisms carrying diverse genes of different origin or even designed genes with no natural counterparts, may carry a risk similar to highly complex technical systems. This does not necessarily enhance the actual risk in terms of potential damage or danger but results in a remaining unpredictability, which maybe inevitably sticks to artificial cells. In contrast to many technical systems where risk assessments can be made more or less precisely (even for worst-case scenarios) this appears difficult for synthetic biology. The potential damage (if any) is hard to estimate and at the same time the probability of occurrence is nearly indeterminable. Therefore these systems are afflicted rather with uncertainty than with risk. Beside these deliberations on safety aspects, one might also have to consider security aspects. All technically "useful" devices can be misused by malevolent parties as weapons or for terroristic attacks. Therefore dual-use aspects of purported harmless material have to be considered under these assumptions. But this goes beyond the scope of this contribution.

6.2 Safety by Genetic Isolation '…the Farther, the Safer'

One of the strong arguments to follow the path of genetic isolation is the safety aspect under the motto: "The farther, the safer" (Marlière et al. 2011; Herdewijn and Marlière 2009). The idea behind this approach is to keep all the

unpredictability inherent to life, but to control it by efficiently preventing any interaction between artificial cells and natural cells. This can be reached by different means and to different degrees. Such artificial living beings are thus separated from nature by a genetic fence. Although this approach might be theoretically tight it leaves many observers with the same feeling one has watching wild animals in a zoo behind a glass window or a moat. One might think, "What happens, if…?" Already the announcement of the possible creation of mirror-like cells has sparked a similar reaction: "Mirror-image cells could transform science—or kill us all" (http://www.wired.com/magazine/2010/11/ff_mirrorlife/). Although we cannot predict by which means a genetic firewall might flop, the public is left with a strong feeling of uncertainty. In this case the fear is still enhanced by the unfamiliarity of such artificial creatures. Thus, the alien character of xenobiological organisms might be a severe disadvantage in any biosafety and biosecurity debate, although it is claimed that "the farther, the safer" could be regarded as a principle to make synthetic biology less dangerous. But the gain of having organisms that are unable to communicate or to admix with natural beings might be by far outweighed by the public's fear of the unknown.

7 Which Risk Remains?

As we have learned, it will be difficult to assess the risk of synthetic biology in general. Synthetic biology as engineering technology based on living systems, claims that reduction of complexity in one way or the other is the best way to create living cells that do not harm humankind or the environment. However, it is well known that even in classical engineering technologies, the construction of ever more complex systems is accompanied by an increase of inherent instability and uncertainty. Even for mathematics it was proven that every axiomatic system will contain statements that cannot be decided. Thus we are left with a level of uncertainty even in a world of complete predictability. Therefore it may be less important for synthetic biology to ensure the public of the general safety of their approaches, but to implement additional control mechanisms and information duties that may strengthen the public faith in this emerging technology.

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Characterizing Synthetic Biology Through Its Novel and Enhanced Functionalities

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Abstract What distinguishes synthetic biology from earlier approaches in biology and biotechnology? What are future applications that may possibly be realized through synthetic biology? What can be expected from synthetic biology with respect to the benefits it may provide as well as the risks it may pose? This chapter puts forward the idea that these questions, among others that regard the promises and threats of this new and emerging field of science and technology, can be explored by applying the concept of *functionality* to synthetic-biological structures and systems. Functionality, in this respect, is defined as a certain physicochemical or biological effect that can be brought about by a (synthetic-) biological object. This effect, in turn, has repercussions on the wider systems context the respective object appears in. Looking at the various hierarchical levels of biological life, functionalities that have already been realized through synthetic-biological approaches, as well as those that may be realized through future research and development, are systematically analyzed. Based on this analysis, applications that make use of these functionalities thus far, or may do so in the future, are presented. Furthermore, it is investigated how the functionalities may change the hazardous properties or exposure behavior of the respective structures or systems and thus potentially increase the risk associated with them.

1 Introduction

Given synthetic biology's very early stage of development, the characterization of this emerging field of science and technology is quite a challenging task. In this chapter, it is hypothesized that such a characterization may be successfully realized through an investigation into the *functionalities* of synthetic biological

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constructs (a more detailed definition of the term is given in Sect. 2.1). It is argued that a close examination of the functionalities of synthetic biological constructs may not only reveal the *kinds* of possible applications, but may also help assess their *qualities* and *capacities*. Furthermore, the identification and description of such functionalities would possibly allow for the determination of the potential uses and the potential hazards, respectively, of a biological construct. These potentials may be estimated even if the actual and concrete applications or contexts of application have not yet been established—which seems worthwhile, especially at the relatively early innovation stage that synthetic biology is in at the moment. Thus, functionalities can serve as the basis for both the characterization of the field and the assessment of its potential risks and benefits, possibly generating useful insights for the responsible shaping of this emerging technology.

Following an introduction of the main terms and concepts (Sect. 2), this chapter identifies, analyzes, and evaluates the functionalities that can be implemented through synthetic biology. The focus is on those functionalities that cannot—and probably will not—be realized or substituted by other technologies or disciplines. The starting point and, at the same time, backbone of analysis are the various synthetic biological approaches and their respective methods as well as their specific "loci of intervention" at the organizational levels of (synthetic-) biological objects (Sect. 3). Thus, the question being asked is: "Which *object* (here: the biological construct) is being used or modified in what *way*, and what is the object-related *outcome* to be achieved?" The next section (Sect. 4) examines the combinations of the specific synthetic-biological approaches with regard to the novel and enhanced functionalities resulting from these combined approaches. Additionally, possible applications and fields of application are derived and evaluated. The following Sect. 5 formulates and discusses some initial hypotheses with respect to potential risks possibly arising from the novel functionalities derived in the preceding sections. The chapter closes with a summary and conclusions (Sect. 6).

2 Definitions

2.1 Functionality

The central idea of this chapter is "functionality". Here, the term is defined as a certain effect that can be exerted by an object (in this case, by the synthetic-biological construct). This effect can, in turn, constitute or lead to a potential use or potential harm. These opportunities and hazards may be determined to a certain degree, but additionally they are context-dependent in their concrete specification and application. The object in question may be a structure, a process, or a system, whereas said effect may be of material (physical, chemical, or biological) or immaterial (energetic or informational) nature. The causes of the various effects, too, are rooted in the material and immaterial configurations (i.e. properties) of the objects. With respect to the topic discussed in this chapter, the functionalities relating to energy conversion, metabolism, structure forming, signal processing, adaptation, and reproduction are of greatest interest.

Functionalities can be viewed as one of the main characteristics of synthetic biological entities as implied, e.g., by the definition of NEST (2005, p. 5):

> 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*. [Emphasis added]

Similarly to the quoted definition, the focus of this chapter, too, lies on those functions/functionalities that have not (yet) evolved naturally. However, in this contribution the term "functionality" is preferred over the term "function" in order to direct attention to the *potential* of fulfilling a certain function rather than to the function itself.

All functionalities discussed in this Chapter have been summarized in Table 1.

2.2 Organizational Levels of Biological Objects

Characteristic of synthetic biology is the simultaneous targeting of each of the hierarchical and object-related organizational levels of (biological) life. Be it the molecular building blocks (DNA, RNA, amino acids, or proteins); the signal or metabolic pathways and the genome; or the material and structural organization of organisms as well as their sub-structures (organelles, cells, or organs) and super-structures (populations and ecosystems): synthetic-biological approaches use, modify, or fully artificially create biological structures, processes, and systems at each of these levels[1] —at least, this is what the scientific and engineering agenda of synthetic biology stands for (Benner and Sismour 2005; Purnick and Weiss 2009; Khalil and Collins 2010; Andrianantoandro et al. 2006; Heinemann and Panke 2006). The simultaneous targeting of various levels probably results from the insight that it is indeed the interdependent interaction of *all* these levels that enables natural biological structures, processes, and systems to fulfill the functions to the extent and in the way they do. Thus it is expected that all organizational levels are relevant, too, when it comes to modified or artificial biological structures, processes, or systems. Empirical findings from experimental biology do point in this direction by showing that modifications at only single levels create either very little positive effect (with respect to the intended function) or massive adverse effects, in the worst-case even leading to the break-down of the biological process or system (Kwok 2010).

2.3 Synthetic-Biological Approaches

There are two distinctive levels of "synthetic biological approaches." The first one is the "general" overall approach that comprises and describes—at a rather theoretical, epistemological meta-level—the overarching paradigm and basic modus

[1] A similar categorization into five "levels of complexity" is suggested by GR et al. (2008).

Table 1 Functionalities, their enabling structures and processes, their benefit potentials with regard to biological function as well as applications, and their exposure and hazard potentials (source: own)

Functionality, or combinations of functionalities	Functionality-enabling structure[1]	Functionality-enabling process	Benefit potential—with regard to biological function	Benefit potential—with regard to applications	Exposure potential	Hazard potential
• GROWTH	• cell	• anabolism	• autonomous self-construction of biological system	• tissue growth	• –	• (possibly) consumption of scarce substances for biomass build-up • exploitation competition (e.g. for food or construction materials) with natural organisms
• REPRODUCTION	• cell	• anabolism • → REPLICATION	• self-renewal • self-repair	• self-sustained manufacturing of biomaterials • self-sustained tissue growth • cultivation of strains (e.g. for chemical synthesis)	• increase due to increased persistence (formation of lines of generations) and due to increase in number (in case more than one offspring per parent survives)	• competition with or displacement of natural species/organisms
• EVOLUTION	• genes	• mutation (incl. novel combinations and loss of sequences)	• adaptability to changing environmental conditions (increase in fitness)	• specialization of functions and properties • performance increase or optimization (with regard to external requirements)	• increase due to increased persistence (survival) or increased fitness	• development of hazardous properties or functions (e.g. capacity to infect other organisms or to colonize habitats)

(continued)

Table 1 (continued)

Functionality, or combinations of functionalities	Functionality-enabling structure[1]	Functionality-enabling process	Benefit potential—with regard to biological function	Benefit potential—with regard to applications	Exposure potential	Hazard potential
• SELF-ORGANIZATION	• depending on level of self-organization: – molecules (e.g. DNA, RNA, proteins) – supra-molecular but sub-cellular structures (e.g. organelles, membranes) – regulatory networks – cells – organisms – populations	• molecular self-assembly • intra-cellular self-assembly and self-regulation • inter-cellular self-assembly and self-regulation	• construction of complex, three-dimensional structures • self-regulation of intra-cellular and inter-cellular processes (e.g. chemical synthesis)	• self-sustained construction or synthesis of various materials and chemicals • little or no external, top-down control necessary	• –	• uncontrolled self-assembly or self-regulation of biological processes with detrimental effects
• STRUCTURE FORMING	• proteins • nucleic acids • sugars (proteoglycan) • fatty acids (membranes) • cellular tissues	• chemical attractive or binding forces	• building various forms of containers, barriers, scaffolds, etc. necessary to hold, separate, or otherwise physically arrange chemical or biological components	• biomaterials (e.g. spider silk) • single-molecule reactions	• –	• toxicity or sensitization potential of resulting structures

(continued)

Table 1 (continued)

Functionality, or combinations of functionalities	Functionality-enabling structure[1]	Functionality-enabling process	Benefit potential—with regard to biological function	Benefit potential—with regard to applications	Exposure potential	Hazard potential
• METABOLISM (synthesis [anabolism] and degradation [catabolism] of chemical compounds)	• cell-free systems containing various ingredients • protocells • cells	• metabolic pathways	• effective use of chemical compounds as substrates • effective and efficient synthesis of very well defined, complex compounds • synthesis under physiological conditions	• effective and material-/energy-efficient production of chemicals that cannot be synthesized (in a sensible and feasible way) through chemical engineering • chemical synthesis under mild, environmentally friendly reaction conditions	• –	• detrimental degradation of valuable or essential materials of the natural (e.g. lignocellulosis) or built (e.g. metals, concrete) environment • interference with bio-geo-chemical processes (e.g. nitrogen-fixation, pH-regulation) • environmental pollution through hazardous chemicals produced by (synthetic-) biological structures or systems that are capable of replication/reproduction and have entered the environment • exploitation competition (e.g. for nutrients) with natural organisms

(continued)

Table 1 (continued)

Functionality, or combinations of functionalities	Functionality-enabling structure[1]	Functionality-enabling process	Benefit potential—with regard to biological function	Benefit potential—with regard to applications	Exposure potential	Hazard potential
• SENSING	• signal-sensitive proteins and nucleic acids (DNA and RNA)	• molecular recognition through: – binding – modification	• gaining and, in combination with → SIGNAL TRANSDUCTION, utilizing internal and external information	• recognition of pollutants (bioremediation) • recognition of pathogens (medicine, e.g. drug delivery)	• –	• –
• SIGNAL TRANSDUCTION	• integration of – signaling molecules (Proteins, RNA) – cellular sensors – cellular actuators – genetic circuitry	• signaling pathways	• utilization of internal and external information	• external controllability (through respective stimuli; in combination with → SENSING) • cellular computing (bio-computing)	• –	• –
•GENEREGULATION	• gene-regulative structures, e.g.: – promoters – enhancers – silencers – other molecules, e.g.: ◦ RNA ◦ DNA/RNA-binding proteins	• control mechanisms for transcriptional and post-transcriptional regulation	• generation of various well defined and specific outputs (gene products) depending on various well defined and specific inputs (→ SENSING; → SIGNALING)	• realization of components for activity control (e.g. switches, band-pass filters) • realization of bio-computing devices (e.g. logic gates, memory)	• –	• interference with genetic circuitry of natural organisms after gene transfer

(continued)

Table 1 (continued)

Functionality, or combinations of functionalities	Functionality-enabling structure[1]	Functionality-enabling process	Benefit potential—with regard to biological function	Benefit potential—with regard to applications	Exposure potential	Hazard potential
• MODULARITY	• a combination of – genes and gene-products resp. – (minimal) genome – minimal cell, protocell, micro-/nano-reactor	• standardization • →ORTHOGO-NALITY	• relatively easy construction, enhancement, and combination of biological systems • exchangeability of components between systems	• faster and cheaper development of biological structures and systems • increase in variety and diversity of biological structures and systems due to a practically infinite number of possible combinations (of the modules)	• –	• security: easier development and construction of pathogens or environmentally hazardous biological structures and systems intended for criminal or terroristic uses
• COMPARTMENTALIZATION	• structures build from – proteins – lipids	• self-assembly	• construction of cellular or sub-cellular organelles for: – separation and storage – chemical reaction under specific conditions in protected environments (→ ENCAPSULATION)	• realization of chemical reactions with unfavorable reaction equilibria through separation of reaction products • realization of complex chemical reaction pathways that require different reaction conditions at different reaction steps	• –	• –

(continued)

Table 1 (continued)

Functionality, or combinations of functionalities	Functionality-enabling structure[1]	Functionality-enabling process	Benefit potential—with regard to biological function	Benefit potential—with regard to applications	Exposure potential	Hazard potential
• ENCAPSULATION	• structures build from – proteins – lipids	• self-assembly	• transport (vehicle function) • synthesis (reactor function)	• medicine: drug delivery • enhanced synthesis through precisely controlled local reaction conditions	• increased due to increased persistence (higher resistance) and/or → MOBILITY (e.g. formation of spore-like structures)	• –
• ORTHOGONALITY	• genetic circuits • metabolic pathways • signaling pathways/ regulatory circuits	• separation on genetic, protein-, or metabolic level	• interference-free implementation of functional systems in cellular environments	• → MODULARITY • medicine: metabolic pathways for drug synthesis	• increase due to persistent chemical structures	• ambivalence: – significant *reduction* in hazard potential due to structural decoupling of natural-biological and synthetic-biological systems – significant *increase* in hazard potential due to principal inability of natural-biological systems to cope with structurally decoupled synthetic-biological systems (especially in case of completely decoupled systems)

(continued)

Table 1 (continued)

Functionality, or combinations of functionalities	Functionality-enabling structure[1]	Functionality-enabling process	Benefit potential—with regard to biological function	Benefit potential—with regard to applications	Exposure potential	Hazard potential
• SPECIFIC CHEMICAL BEHAVIOR (including non-natural chemical properties of biological structures and systems)	• various (bio)chemical entities, such as: – DNA – RNA – peptides – proteins – enzymes – lipids	• physical and chemical properties of the respective (bio)chemical entity	• very wide range of semi- or non-natural chemical properties for: – energy conversion through light-harvesting structures – catalysis of various chemical reactions (→ METABOLISM) – binding and recognition of substances (→ SENSING) – sensing of physical or chemical conditions (→ SENSING)	• very wide range: – conversion of solar radiation to electricity or to high-energy compounds – synthesis of bulk and fine chemicals – various forms of bio-sensing devices	• –	• detrimental chemical interference with natural-biological structures and systems as well as with the built environment
•SELF-REPLICATION	• DNA • RNA • peptides	• template-driven copying • auto-catalysis	• prerequisite for → REPRODUCTION • autonomous growth and/or increase in number of organisms	• easy mass-production of biological structures and systems for further utilization • efficient quality control through one-to-one copying of template	• increased (cf. → REPRODUCTION)	• –

(continued)

Table 1 (continued)

Functionality, or combinations of functionalities	Functionality-enabling structure[1]	Functionality-enabling process	Benefit potential—with regard to biological function	Benefit potential—with regard to applications	Exposure potential	Hazard potential
• MOLECULAR BINDING	• DNA • RNA • proteins	• physicochemical and structural properties of the respective binding molecules	• prerequisite for → SENSING and → MOLECULAR TRANSPORT	• detection and/or filtering of molecules with high precision (high resolution and specificity as well as low detection limit)	• –	• detrimental interference with natural-biological structures and systems caused by molecular binding (e.g. through blocking or activating) • damage of or functional interference with cells or tissue through infection by microbes, viruses, or phages through adhesion, invasion, or endocytosis mediating molecular structures
• MOLECULAR TRANSPORT (active and passive)	• DNA • RNA • peptides/proteins	• physicochemical and structural properties of the transporting molecules	• import (in combination with → MOLECULAR BINDING), internal circulation, and export of chemical substances relevant to → METABOLISM or → SIGNALING	• separation of chemical compounds with high precision • realization of complex chemical reactions that require the provision or evacuation of educts or products	• –	• –

(continued)

Table 1 (continued)

Functionality, or combinations of functionalities	Functionality-enabling structure[1]	Functionality-enabling process	Benefit potential—with regard to biological function	Benefit potential—with regard to applications	Exposure potential	Hazard potential
• MOBILITY (active or passive)	• active drive-systems (e.g. flagella) for self-propulsion (locomotion)	• generation of mechanical forces by using chemical or other forms of energy • passive transport through media (e.g. air, water) or other moving objects (though intrusion or adhesion)	• self-translocation to either escape less advantageous environments or to reach more advantageous ones	• through actively mobile biological entities: – active transport (e.g. drug delivery) – active structure forming • of actively mobile biological entities themselves: – active dislocation • active invasion of and/or spreading within industrial environments (e.g. reactors) or natural ones (e.g. bioremediation sites)	• increase due to dissemination	• –

[1] This column refers to the (modified) natural biological structures as well as to the synthetic-biological ones.

operandi underlying all specific and practical research and development projects within synthetic biology. The second one refers to the "specific" synthetic-biological approaches, that is, the concrete and practical projects themselves that are conducted by the scientists of synthetic biology and that deal with the material biological object. These approaches apply and extend established methods introduced by traditional disciplines, but they also develop new ones. This is why the difference between synthetic biology and traditional approaches is continuous rather than discrete, determined by the relative proportions of traditional and new methods applied (Giese et al. 2013). As shown in Sect. 4, it is in most cases only the combination of various *specific* synthetic-biological approaches that leads to the realization of the *general* approach of synthetic biology.

3 Specific Approaches

3.1 Nucleic Acids, Amino Acids, and Proteins (Level 1)

The design of nucleic acids, proteins, and other biomolecules is pursued to either establish a certain active function or to obtain a certain structural building material. These functions are controlled by the chemical and physical properties of the respective molecules, which in turn are determined by the molecule's elemental composition, spatial structure, charge distribution, etc. Biomolecules can be designed by altering known molecules through modification of the nano-molecular interactions, or by constructing molecules that do not occur naturally in living organisms (Behrens et al. 2011; Ball 2005). These altered or new molecules can exert their new properties or functions in principle in-vitro (i.e. cell-free systems) or in-vivo (i.e. in cells).

Based on recent progress made in biophysical description and computer aided modeling, a multitude of new methods and tools for designing functional molecules has been developed in various biotechnological and biochemical disciplines over the past years. These may now be applied in synthetic-biological research, bringing the rational, bottom-up construction of biological structures, processes, and systems with pre-defined functions closer to realization.

The functionalities primarily realized at this level are → SELF-ORGANIZATION,[2] → STRUCTURE FORMING, → ORTHOGONALITY, and the implementation of → SPECIFIC CHEMICAL BEHAVIOR of the biological systems. As mentioned above with regard to the definition of "functionality" (see Sect. 2.1), it is important to note that the improvements made as well as the transfer of existing or realization of novel functionalities are defined and evaluated against the naturally present biological structures or systems.

[2] For reasons of better readability and cross-reference, functionalities are set in small capitals preceded by an arrow ("→"). All functionalities discussed in this chapter are also summarized in Table 1.

3.1.1 DNA Nano-Structures and Nano-Components

While DNA molecules serve as the carrier of genetic information, their very specific physicochemical interactions (with each other) can also be used biotechnologically for the construction of well-defined biological structures. This kind of template-controlled self-organization opens up numerous possibilities for new applications such as the assembly of solid carriers for chemical reactions (Rothemund 2006) or the systematic synthesis of three-dimensional cell structures and cell organelles (Bath and Turberfield 2007). Although experiments utilizing DNA as structural material had already been conducted in the 1980s (Seeman 2010), it was not until a breakthrough in 2006 that the employment of a scaffold of long DNA molecules as a folding template enabled the reliable and robust assembly of complex structures (Nangreave et al. 2010) (see also Sect. 4.1.2).

3.1.2 Novel Nucleotides and Nucleic Acids

The utilization of artificial bases, which do not naturally occur in DNA, as informational entities allows for the extension of the genetic code (Benner 2004). The sugars and phosphate groups, which serve as the molecular backbone of DNA, can also be substituted by synthetic alternatives resulting in so called xeno-nucleic acids (XNA) (Herdewijn and Marliere 2009; Schmidt 2010). An extended genetic code allows for the incorporation of artificial chemical structures into anabolic processes, which enables fundamental manipulations of the chemical properties of biological systems. For instance, more than the twenty natural amino acids can be utilized; or other new ones may be developed and used. Also, novel forms of specific interactions of the single strands of DNA containing novel bases become possible. The novel chemical properties, in turn, allow for the realization of new applications as well as further new functionalities. In medical diagnostics, the specific pairing of novel nucleobases is already being used to significantly reduce noise in the detection of viral nucleic acids (Collins et al. 1997). Novel nucleobases may also represent a possibility to keep synthetic-biological structures and organisms isolated from natural ones, which constitutes a new functionality (→ ORTHOGONALITY) mainly discussed from a biological safety and security perspective (see also Giese and von Gleich 2015, this volume).

3.1.3 RNA Design

Chemically, RNA molecules are very similar to DNA. However, RNA may form complex three-dimensional structures. Moreover, RNA fulfills various catalytic and regulatory functions in the living cell. Compared to proteins, the rational design of functional RNA molecules (ribozyme, ribo-regulators, and ribo-sensors) is relatively well developed due to the relatively low complexity. For instance, the folding of RNA can be predicted quite reliably through in-silico simulations

(Isaacs et al. 2006). While traditional methods for the manipulation of RNA are based on the in-vitro evolution of molecules, recent methods apply three-dimensional modeling. With regard to modifications of cell networks, these novel methods represent an innovation that may open up virtually universal applications of RNA molecules (Khalil and Collins 2010; Saito and Inoue 2009).

3.1.4 Novel Amino Acids

Only recently, the canonical set of the twenty, naturally occurring amino acids could be extended. To an even greater degree than in the case of DNA and RNA, utilizing artificial amino acids as building blocks in proteins creates huge possibilities of modifying the properties of these proteins as well as tailoring enzymes to specific functions (Wang et al. 2006; Hoesl and Budisa 2011). At the level of proteins, the integration of novel amino acids can alter the properties of peptides or proteins, such as pH-sensitivity, temperature stability, enzymatic activity, structure, or fluorescence, which improves or enables their technical utilization (Lepthien et al. 2010). This may, furthermore, lead the way to realize novel functionalities at the level of enzymes or receptors, too.

3.1.5 Computational Protein Design

Proteins represent the primal units of the metabolic, energetic, and signaling functions of a living cell. Their three-dimensional structure is determined by their specific constitutional sequence of amino acids. The computer-aided rational design of proteins allows for the prediction of folding, electro-static bonds, and various other nano-molecular properties in order to engineer proteins for specific functions (Kortemme and Baker 2004). Over the past years, computational design developed complementary to the traditional methods of protein engineering. Very recently, it enabled the de-novo design of enzyme functionalities unknown to nature (Grunberg and Serrano 2010; Van der Sloot et al. 2009).

3.2 Genetic Modules and Circuits (Level 2)

The design of functional biological units (i.e. modules or devices) beyond the level of the molecular building blocks—e.g. metabolic pathways, genetic circuits, or signal transduction—is realized at the level of genetic information. Depending on the specific (gene) function that is to be achieved, this is pursued by combining genes not naturally occurring in one and the same organism. In most cases, an in-silico model serves as a starting point building upon a natural gene sequence, the functions and hierarchies of the genes involved, and the regulatory sequences of the respective genes. Then, the sequence necessary to implement the desired

function is chemically synthesized and transferred into a host system (chassis) via respective methods of genetic engineering. In the end, the targeted function is tested and further optimized through methods of directed evolution. Ideally, the abstract module is sufficient for the desired function and possible negative interactions with other metabolic, energetic, or signaling functions can be neglected.

The resulting functionalities are → METABOLISM and → SIGNAL TRANSDUCTION. Again, functionalities are realized if significant differences from natural biological structures are achieved. Regarding synthesis, these differences may refer to the substrate or to the product. Differences may also refer to quality or quantity, or to other performance criteria of synthesis. With regard to signal transduction, improved or novel functionalities may relate to the kinds of signals themselves or to the kinds of information processing operations being implemented (mathematical, logic, etc.).

3.2.1 Metabolic Engineering of Gene Clusters

"Metabolic engineering" constitutes a well-established biotechnological discipline. It refers to the analysis, quantification, and manipulation of cellular metabolic pathways in order to improve the synthesis (rate, yield, productivity, purity, etc.) of complex biochemical compounds. Through the modification and optimization of multiple genes at a time, organisms can be manipulated quite extensively, allowing for the synthesis of novel metabolic products and the utilization of novel substrates. For the past few years, this approach has increasingly been based on systems-biological modeling (Carothers et al. 2009).

3.2.2 Systems-Biological Construction

The description, bioinformatic modeling, and disintegration of (parts of) metabolic, regulatory, and signaling networks are pursued for the orthogonal (re)construction of technologically interesting biological functions (Marchisio and Stelling 2008; Greber and Fussenegger 2007). This domain may be viewed as the practical application of theoretical insights gained from systems biology. Although the genetic engineering methods applied have been established for quite some time, the descriptive approach is novel.

Being closely related to the approach of systems-biological (re)construction, the modules-based "bio-bricks" approach also aims at applying engineering principles to biological systems. However, in the case of the bio-bricks approach, demands on standardization, decoupling, and abstraction are higher (Endy 2005). The in-silico design of standardized information modules (DNA) with an orthogonal overall functionality contains all elements necessary for a certain function to be realized in a standardized host system (chassis), such as multiple genes and regulatory sequences. The physical modular representations of these functional units are provided through the design, too (Arkin 2008).

3.3 Genome (Level 3)

From a synthetic biology perspective, the genome represents the physical scaffold to incorporate the modules of genetic information that are necessary to realize the preferred (gene) functions. The provision of information-bearing molecules (based on nucleic acids) is fundamental to the construction of self-replicating and self-organizing or evolving systems. Respectively, → SELF-REPLICATION and → SELF-ORGANIZATION of (non-natural) biological structures and systems are the functionalities to be realized at this level. Additionally, fundamental modifications and the de-novo construction of genomes (through DNA synthesis; see Sect. 3.3.2) in combination with minimal cells, protocells, or micro-/nano-reactors (see Sects. 3.4.1–3.4.3) allows for the → MODULARITY of biological systems.

3.3.1 Minimal Genomes Through Reduction

The concept of "minimal genome" refers to the maximal reduction of a given natural genome to a minimal, essential set of genes. The objective is to reduce negative interactions and the evolutionary potential as well as to eliminate all functions that are not needed for the technical purpose the organism is to fulfill after integration of synthetic functional gene modules (Moya et al. 2009). Reducing a relatively simple microbial genome (top-down) to as few as possible fundamental genes and gene functions is realized through the application of common methods of mutagenesis and the use of restriction enzymes, sometimes facilitated by the bio-informatic prediction of gene functions (Feher et al. 2007).

3.3.2 DNA Synthesis

The chemical synthesis of multiple genes from their basic building blocks (i.e. nucleotides), as well as the subsequent assembly of these genes into a genome that functions in-vivo, serve as enabling technologies for the realization of synthetic biology. For several decades, the basic principles of chemical gene synthesis have been applied to smaller DNA segments. However, only in recent years, high-throughput technologies have allowed for a significant and still growing increase in the lengths of synthetic DNA molecules (Tian et al. 2009). To date, even the bottom-up synthesis of relatively small but complete genomes that are able to control living cells has been achieved (Forster and Church 2006; Gibson et al. 2010).

3.4 Cell (Level 4)

The cell membrane constitutes the layer that effectively separates the biological system physically, chemically, and biologically from its environment. At the same

time, it allows for the transfer of substrates and products of metabolism as well as the input and output of energy and information. Efforts are being made to orthogonally construct compartments that are enclosed by membranes resembling those of natural cells. The objective is to use such compartments as biological chassis that will house various kinds of genomes and enzymatic networks carrying out the functions required. Artificial chassis can be constructed by either using or mimicking membranes of natural cells, or by synthesizing them from non-natural organic or even inorganic compounds.

At the level of the cell, the functionalities realized at the lower levels can be integrated and utilized altogether. Moreover, based on → ENCAPSULATION and → COMPARTMENTALIZATION, → METABOLISM and → REPRODUCTION, as well as → EVOLUTION, there are functionalities that can additionally be achieved at this level (such as → ACTIVE OR PASSIVE MOBILITY).

3.4.1 Minimal Cells

Generally, the concept of minimal cells aims at creating (top-down) cells with structures and functions to as simple a level as possible. This holds for the membrane as much as for any other part of a functioning (i.e. living) cell. Since the lipid and protein metabolism of a cell also determines the composition of the cell membrane, structure and properties of the cell membrane can be altered through the manipulation of the respective genes, which can usually be achieved by using the traditional methods of genetic engineering (Moya et al. 2009).

3.4.2 Protocells

Recently, much progress has been achieved in the bottom-up de-novo synthesis of biological vesicles (i.e. synthesis starting from small molecular building blocks). Such artificial cell membranes can be produced through either reverse emulsion or micro-fluidic jetting (Schwille and Diez 2009). Afterwards they can be filled with nucleic acids and proteins. This allows for the construction of artificial cells whose content, lipid composition, and membrane-protein configuration can be precisely controlled (Richmond et al. 2011). In the long run, such constructs may be controlled by synthetic genomes and applied in, for instance, protein synthesis (Noireaux et al. 2011; Sole et al. 2007). Furthermore, additional compartments may be introduced that take over specific functions just as organelles do in natural cells (Roodbeen and van Hest 2009). Although biomimetic lipid-vesicles have been investigated within the origin-of-life research since the 1980s, the integration of enzymatic reactions into such structures combined with their self-replication have only now come into the realm of possibility. Thus, various new applications based on protocells, showing the features described, seem—at least theoretically—feasible (Szostak et al. 2001; Loakes and Holliger 2009; Porcar et al. 2011).

3.4.3 Micro-/Nano-Reactors

The function of spatial arrangement and control of a wide array (Urban et al. 2006) of (enzymatic) reactions can, in principle, also be performed by containers other than the described minimal cells and protocells (see Sects. 3.4.1 and 3.4.2). Examples include emulsions, micelles, organized thin films, and poly-electrolyte capsules (Shchukin and Sukhorukov 2004); viral protein cages (Uchida et al. 2007); or other kinds of containers constructed from organic (biological or non-biological) (Schwille and Diez 2009) or inorganic (Urban et al. 2006) micro- or nano-materials. Three-dimensional protein structures have recently gained much attention as they allow for a high specificity regarding structure and functionality (King et al. 2012). Even more importantly, protein envelops can be tailored through computational design and the proteins self-assemble to the final structures.

4 Combinations of Specific Approaches and Potential Applications

While each of the above-described specific synthetic-biological approaches on its own already enables the realization of certain functionalities, it is the combination and integration of several specific approaches that particularly allows for the implementation of enhanced and novel functionalities. Typically, evolutionary path-dependencies are to be overcome by combining many and diverse approaches. Apart from technological developments with regard to laboratory equipment in the life sciences, recent progress in fields such as bio-informatics and systems biology has made these combinatorial approaches possible and feasible. In the following sections, some of the combined approaches are exemplified and the functionalities that result from these are presented.

Regarding the possible combinations of specific synthetic-biological approaches and resulting functionalities, there are three main domains that can be distinguished, as shown in Fig. 1. Each domain is discussed in more detail in the following sections.

4.1 Combining Novel Molecular Building Blocks with Genetic Modifications and De-Novo Creation

One domain of combinations comprises the systematic (re)design of DNA sequences encoding either genes or regulatory functions. It is based on the new methodological possibilities of, on the one hand, modifying the fundamental molecular structures of life (such as RNA, DNA, amino acids, proteins—see Sect. 3.1) and, on the other hand, synthesizing genetic modules (such as "BioBricks"—see Sect. 3.2).

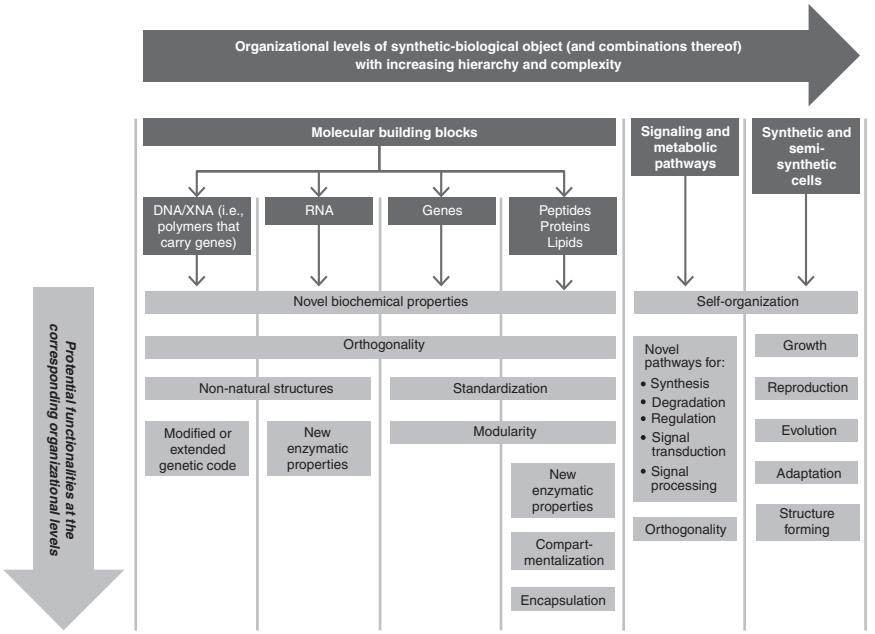

Fig. 1 Schematic representation of functionalities and the biological level they are occurring at (source: own figure)

4.1.1 DNA (Genes)

With regard to DNA, novel functionalities result from modifying natural or constructing artificial mechanisms of → GENE REGULATION, → SIGNAL TRANSDUCTION, or → METABOLISM. Most effort is put into altering gene regulation (Guido et al. 2006), constructing artificial gene networks (Ellis et al. 2009; Marchisio and Stelling 2008), and standardizing functional gene modules. In doing so, switches (Gardner et al. 2000) and circuits generating oscillating signals (Elowitz and Leibler 2000) could be realized already. The construction of cellular memory (Ajo-Franklin et al. 2007), pulse generators (Basu et al. 2004), logic gates (Anderson et al. 2007), and band-pass filters (Sohka et al. 2009) follow the idea of electronic components and circuitry to an even greater extent. Apart from *intra*cellular functions, such structures can also be applied to generate and process signals used in *inter*cellular communication, controlling the behavior of populations (Basu et al. 2005). Even more so, the integration of electronic and biochemical components have already been realized in living cells (Weber et al. 2009).

Based on the functionalities described, for instance, novel kinds of biosensors can be developed (Kobayashi et al. 2004). In the application field of bioremediation, gene expression in these biosensors may be initiated through substances present at the remediation site, alleviating the problem of artificial external stimuli necessary with current bioremediation techniques (de Lorenzo

2009). Other possible areas of application which are especially interesting are in the field of medicine: screening for new pharmaceuticals in drug discovery (Weber et al. 2008); specifically antibiotic bacteriophages (Lu and Collins 2009); applications in the production of biopharmaceuticals and in gene therapy (Weber et al. 2007); or bacteria able to recognize and invade tumor cells (Anderson et al. 2006).

4.1.2 DNA (Nano-Structures and Nano-Components)

Since the discovery of DNA as a building material for the construction of artificial objects in the early 1980s (Seeman 1982), a number of interesting functionalities have also been realized within this field of application (Sacca and Niemeyer 2012), such as → STRUCTURE FORMING and → MOLECULAR BINDING or → MOLECULAR TRANSPORT. Nanometer-sized structures composed of DNA may be systematically loaded with chemical functional groups, nanoparticles, or other biomolecules in order to fulfill specific functions. For instance, DNA nano-chips may be used to produce or detect reactions of single molecules (e.g. through binding of proteins), or to analyze sequences of RNA or DNA. By combination with other molecular structures, hybrid materials, such as nano-electronic components, might be realized. Apart from two-dimensional structures, "DNA nanotechnology" or "DNA origami" allows for the construction of various three-dimensional objects (e.g. cubes, tubes, or stacks of tubes) that may be further equipped with functional molecules (Sacca and Niemeyer 2012). For the future, even "DNA nanomachines" are envisioned that contain chemically fueled, mechanically active parts such as molecular motors (Bath and Turberfield 2007).

4.1.3 RNA

Initiated through the discovery of hitherto unknown natural RNA molecules (Dethoff et al. 2012), a number of synthetic biology researchers are working on the redesign and de-novo construction of RNA. Up till now, RNA molecules offer a multitude of functionalities: → SENSING structures responding to changes in, for instance, temperature; → MOLECULAR BINDING and → MOLECULAR TRANSPORT, in some cases thereby also regulating the activity of these molecules; → SPECIFIC CHEMICAL BEHAVIOR, e.g. for the catalysis of chemical reactions (as ribozymes), including cleavage and ligation of other RNA molecules; or → GENE REGULATION (Win et al. 2009). Modern design possibilities significantly contributed to improving and enhancing the (genetic) regulatory functions of RNA molecules (Suess and Weigand 2008). Ongoing research may yield new kinds of biosensors and regulatory switches, eventually—through the coupling of natural and artificial signaling networks—allowing for the realization of fully programmable cells (Isaacs et al. 2006).

4.1.4 Peptides and Proteins

One major field of rational design deals with peptides and proteins. As a sub-field of "protein engineering," borderlines to the field of synthetic biology are blurred and sometimes controversial. The objective is to either improve the natural functions or to establish completely new functions of peptides and proteins through complex, systematic modifications or de-novo design (Behrens et al. 2011). Novel functionalities implemented in proteins include catalysis for the synthesis of (bio-) chemical compounds (→ METABOLISM). This is primarily achieved through alterations in their specificities, as shown, for instance, for ligand-gated ion channels (Magnus et al. 2011). Also, employing biopolymeric building blocks ("tectons") to construct proteins, protein-like structures, or completely artificial objects of specific sizes and structures may enable novel functionalities such as → COMPARTMENTALIZATION and → ENCAPSULATION as well as various forms of active and passive → MOLECULAR TRANSPORT (Bromley et al. 2008). Such tectons that self-assemble from natural (such as amino acids), semi-synthetic, or synthetic molecules (monomers) are programmed to self-assemble into higher-order semi-synthetic or completely artificial structures, eventually delivering substantial contributions to resembling the functionality of natural cells.

4.1.5 Standardization and Modularization

Efforts to standardize "parts" and "devices" of biological "systems" in an engineering-like manner have been characteristic of synthetic biology since its early beginnings. As one distinct sub-field, it has been very influential with regard to the (public) perception of the whole field, especially through its fundamental claims (Endy 2005) and through hosting the "International Genetically Engineered Machine (iGEM) Competition." The basic assumption underlying this approach is that biological systems can, in principle, be designed and constructed, just as any other technical, especially electronic, system, through combination and integration of standardized, interchangeable modules. Thus, an initiative has been started by the "BioBricks" Foundation for creating, collecting, and making publicly available standardized *biological* modules. The rationale behind this strategy is that the design and construction of artificial biological systems of virtually any functionality would (only) become feasible if a (relatively small) number of standardized modules were used; in contrast, the conventional approach of individually designing and constructing each biological system of specific functionality from scratch is regarded as being too laborious, time-consuming, and expensive. → MODULARITY of standardized parts and devices itself may be viewed as a functionality in its own right.

To date, combining standardized biological modules at the level of DNA, RNA, or proteins also often produces a number of undesired side-effects. Averting these negative effects constitutes one of the main tasks of the research into the construction of artificial biological systems via the "BioBricks" approach. Central to this endeavor is, among other, the → ORTHOGONALITY of the modules (Bujara and Panke 2010).

4.2 Design of Signaling and Metabolic Pathways

Through *pathway* or *metabolic engineering*, that is, through the modification and integration of functional DNA-sequences, metabolic pathways can be optimized or created de-novo (Prather and Martin 2008). The DNA-sequences may be taken from organisms originating either from within one and the same, or from several different genera, families, groups, or even kingdoms. Equipped with such optimized and novel anabolic or catabolic pathways, the respective cell becomes a biological production system comparable to a miniaturized industrial factory for the synthesis of chemical compounds (→ METABOLISM) (Na et al. 2010). Possible products include, among others, bulk, fine, and specialty chemicals of various kinds, such as fuels or pharmaceuticals (Carothers et al. 2009; Clomburg and Gonzalez 2010; Chang and Keasling 2006).

Beyond classic genetic engineering, many additional approaches and methods were needed for the realization of recent progress: Advances in protein engineering, for instance, greatly increased the catalytic performance of the enzymes involved by improving their substrate specificity and range, stability, and selectivity (Behrens et al. 2011) (→ SPECIFIC CHEMICAL BEHAVIOR). Synthetic promoters and optimizations of RNA (transcription), on the other hand, enhanced regulation of gene expression (Guido et al. 2006; Saito and Inoue 2009). Moreover, novel bioinformatic approaches together with ever more powerful computing technologies enabled better analysis and modeling of metabolic pathways and thus contributed to their optimization and de-novo construction (Brilli et al. 2008; Brunk et al. 2012).

As far as successful applications of synthetic-biology and/or metabolic engineering approaches are concerned, most progress has been made with the production of certain bulk and fine chemicals, especially in the food, energy, and medical sector (Erickson et al. 2011; Khalil and Collins 2010). This may be due to the fact that there has been a long biochemical and biotechnological tradition in research and development and in production, on the one hand, and that the demand and markets, especially for fuels and pharmaceuticals, are powerful pull-factors, on the other hand (Erickson et al. 2012).

In case of energy applications, microorganisms are used to convert plant- or algae-biomass into fuels that are compatible to existing infrastructures. Thus, fossil raw-materials are being substituted with agri- or aqua-cultural ones. Due to their higher energy content/density, efforts increasingly focus on the production of higher alcohols (Lamsen and Atsumi 2012) and fatty-acid derived fuels (Dellomonaco et al. 2010). Instead of producing biomass first and converting it to fuels in a second step, some more recent approaches aim at the direct production of fuels from water, carbon dioxide, and solar energy through the living organisms themselves (mainly algae and cyanobacteria), which circumvents the necessity of multiple energy conversions and thus may result in higher over-all efficiency (Anemaet et al. 2010). Examples include the direct production of hydrogen (Magnuson et al. 2009) and ethanol (Anemaet et al. 2010). However, it remains an open question if and to what extent modified natural or completely synthetic biological approaches to solar energy conversion into fuels can compete with non-biological solar energy processes and technologies (e.g. Fischer-Tropsch) (Blankenship et al. 2011).

Another promising field is that of biological materials, where the focus lies on those (combinations of) material-properties that cannot be realized (yet) through the application of conventional material science and engineering. In this regard, multi-functionality at the material level is to be realized. One prominent example in this respect is synthetic spider silk, a material that integrates a number of extraordinary properties usually not found in one single material, such as high strength and toughness combined with high extensibility and low density (Heim et al. 2009). However, the production of synthetic spider silk in heterologous microorganisms is confronted with a problem common to many other syntheses too that make use of living cells: The substances produced inside the cells have to leave the cells in one way or the other in order not to exert a toxic effect. In the case of synthetic spider silk, a secretion system optimized for the export of silk proteins could be coupled to the gene-expression mechanisms responsible for the synthesis of these proteins (Widmaier et al. 2009).

In general, the modification of the central carbon metabolism (of microorganisms) is the key to the implementation of novel metabolic pathways. This is because any biological (and thus also synthetic-biological) metabolite produced originates from one of only twelve precursors (i.e. intermediate metabolites) of the central carbon metabolism. Changes in this main metabolism thus have major repercussions. However, once the metabolism has been adapted to the changes, a variety of syntheses become possible. For instance, acetyl-CoA may be used as a precursor for various substances that can be applied as pharmaceuticals, nutrients, polymers, or fuels (Nielsen and Keasling 2011).

The (re-) design of *signal transduction pathways* may offer new possibilities for sensing and processing of internal and external signals (Fritz et al. 2007; Xie et al. 2011). Among others, intra- and intercellular signal transduction processes play an important role in the differentiation and spatial arrangement of cells, that is, in the process of tissue formation. Therefore, the implementation of modified or synthetic signal transduction pathways to form inter- or supra-cellular structures may promote progress in tissue engineering (Cachat and Davies 2011) as well as contribute to the realization of novel biomimetic materials (Basu et al. 2005). As far as the synthesis of complex and hierarchically structured biological materials such as spider silk, nacre, bones, or teeth is concerned, processes of → SELF-ORGANIZATION will probably play an important role (Cartwright and Checa 2007).

4.3 Synthetic and Semi-Synthetic Cells

On the level of the whole cell, various concepts and approaches aim at the development of a whole synthetic-biological cell, usually termed “protocell” or “minimal cell” (see also Sect. 3.4). Within this field of research, two main approaches can be distinguished: “bottom-up” and “top-down” (Jewett and Forster 2010). The first one refers to the *bottom-up* de-novo synthesis of cells from basic building blocks (Chiarabelli et al. 2012; Kuruma et al. 2009). To date, these building blocks are usually extracted from natural organisms of one or various different species,

then reassembled in a more or less (un)natural way, and finally put into a synthetic vesicle (e.g. liposomes) (Chiarabelli et al. 2012). However, some approaches aim at the construction of living systems, the molecular building blocks of which are chemically different from those of natural organisms (Fellermann et al. 2007). Compared to natural DNA, RNA, proteins, enzymes, etc., the artificial components used are much simpler with respect to structure and functionality, which is why the respective artificial system also shows a degree of complexity that is far below the complexity of even the simplest natural organism. Up till now, no artificial system exhibiting all features of living systems has been created (Sole et al. 2007).

In the case of the second general approach, on the other hand, synthetic cells result from the systematic, *top-down* reduction in structure and function of natural cells, usually via the controlled elimination of non-essential genes (i.e. a reduction of the genome, thus also named "minimal genome" approach) (Feher et al. 2007). Apart from scientific interest in the fundamental principles and mechanisms of life in general, and living organisms in specific, both approaches aim at providing a viable standard container ("chassis") and/or standard genome ("minimal genome") that can be equipped with almost any kind of biological (information on) structure and function to finally resemble a complete synthetic cell.

Pointing in a similar direction, micro- or nano-reactors (Amidi et al. 2010; Nourian et al. 2012) mimic cellular systems via the combination of vesicular or carrier structures with catalytic (enzymatic) functionality (Shchukin and Sukhorukov 2004; Urban et al. 2006). One idea is to develop self-organizing, encapsulated systems, based on standardized peptides and proteins, with the ability to self-assemble to functioning systems (Bromley et al. 2008). Such systems would possess properties and functionalities similar to those of natural living organisms, such as sensing and signal transduction as well as synthesizing capabilities. Their inability to self-replicate, though a disadvantage with respect to self-renewal and productivity, may be taken as a property that contributes to their safe use as well.

In essence, all three of the above described approaches—bottom-up or top-down development of protocells or minimal cells, and micro- or nano-reactors—aim at the development of platforms that can house all other components and devices discussed throughout this chapter. The overall goal then is the → INTEGRATION AND COMBINATION OF THE FUNCTIONALITIES that the various building blocks, components, and devices bear, to result in even more powerful sets of functionalities allowing for an almost infinite diversity of applications.

5 Potential Risks Derived from Synthetic-Biological Functionalities

The functionalities that can be realized through the application of synthetic-biological approaches may not only enable beneficiary uses in respective applications, as has been discussed in detail in the previous section, but they may also allow for

the prospective derivation of possible risks arising from them. In this section, the functionalities are examined with respect to such potential risks and some initial and preliminary hypotheses are formulated.

The interpretation of *risk* employed here is taken from the definition of risk in toxicology. There, the combination of a *hazard*—that is, the potential of the entity in question to cause harm to another object or subject—and *exposure*—that is, the potential of the respective objects or subjects to actually interact with that entity—determine the resulting risk. Thus, there is no risk in such cases where hazard is given but no exposure, and vice versa. In order to differentiate the two characteristic elements of risk, the functionalities have been investigated with respect to both hazard and exposure potentials (see also the last two columns of Table 1).

→ Reproduction constitutes one of the key functionalities that result in an increased exposure potential. Whereas non-reproductive systems will relatively quickly become smaller in number and eventually disappear (become extinct), reproductive systems may overcome (extant) due to the propagation of new generations. If the number of offspring per generation is higher than the number of parents, reproductive systems may even proliferate. In a similar fashion, → replication represents a functionality that will very probably lead to an increase in exposure potential of the replicating system.

The functionality of → evolution may increase both the exposure and the hazard potential of the synthetic-biological structure or system in question. This is because through evolution a system may acquire properties and abilities it has not had before. This includes any property or ability that constitutes novel, or increases existing, hazard or exposure potentials.

The → structure-forming ability of certain synthetic-biological entities may result in structures that exhibit toxic or other adverse effects on natural or built systems, and thus constitutes a functionality with a hazard potential.

Just as natural living systems, synthetic-biological systems may also be able to chemically convert one or several compounds into one or several different compounds. The process of synthesizing one larger compound from two or more smaller compounds is referred to as *anabolism*, whereas the opposite process is called *catabolism*; each process constitutes one of the two variants of → metabolism. The entirety of natural organisms is able to synthesize a large variety of (organic) compounds. Similarly, for almost any biologically synthesized organic compound, there is at least one kind of organism that can degrade it, usually by using it as a substrate. Altogether, natural organisms form a (dynamically) stable metabolic system where one group of organisms (called "producers") synthesizes organic "living" matter from inorganic "dead" matter. The organic matter is then used by a different group of organisms (called "consumers") to cover their energy and material needs. Finally, a third group of organisms (called "decomposers") degrades organic matter into inorganic matter, thus closing the cycle.

Synthetic-biological systems capable of metabolism potentially pose two kinds of hazard. First, a synthetic-biological system may be able to produce compounds that are alien (in high quantities) to the natural living world, mainly because there is no natural organism that synthesizes them (in such high quantities). In this

case, the "non-natural" compound may have adverse effects on natural organisms or ecosystems, since these have not been able to develop strategies to cope with such compounds. Second, synthetic-biological systems capable of metabolism will require the uptake and utilization of substrates from their environments. In application contexts, these substrates may be provided to the respective synthetic-biological organisms in a controlled manner. However, in those cases where synthetic-biological organisms are intentionally or accidentally released into the natural environment, they might uncontrolled or even uncontrollably utilize what they find there. Depending on the respective substance (i.e. the nutrient), synthetic-biological systems capable of metabolism may consume scarce resources and/or compete with natural systems for substrate. Moreover, such synthetic-biological systems may effectively utilize organic or inorganic compounds or materials as substrates that cannot (or not that efficiently) be utilized by natural organisms. In this case, those natural organisms or parts of the built environment that consist of such substrates are in danger of being used as substrate by these synthetic-biological systems and become (partially) degraded. Again, the hazard potential mainly results from the fact that the natural organisms possibly affected would not have developed any kind of protection or defense against this sudden and novel type of threat. If both hazard phenomena occur together and on a large scale, metabolizing synthetic-biological organisms may even interfere with bio-geo-chemical processes (e.g. nitrogen-fixation or pH-regulation), though this scenario appears to be highly speculative considering the current state of synthetic biology.

Examples of research activities that might result in the generation of both kinds of hazards can be found in the field of synthetic-biological research into biofuels: Many efforts are made to modify existing or develop novel organisms that can produce substances such as, among others, various alkanes, alkenes, alcohols, fatty acids, and isoprenoids as biofuels (Lamsen and Atsumi 2012; Peralta-Yahya et al. 2012; Wijffels et al. 2013; Nielsen et al. 2013; Jang et al. 2012). Many of these substances are highly toxic to most natural organisms. As long as these substances are produced in small quantities or low concentrations only—as is the case for those natural organisms the respective metabolic pathways are taken from—the potential hazards are relatively low, too. However, the aim of current research is to significantly increase production efficiency in various host organisms. This may eventually result in considerably higher quantities or concentrations and thus correspond to an equally higher hazard potential. Similarly, organisms are being developed that can efficiently degrade lignocellulose in order to synthesize biofuels or provide precursors for biofuels (You et al. 2012; Olson et al. 2012; Elkins et al. 2010; Jung et al. 2012; Garvey et al. 2013). Although there are organisms naturally capable of lignocellulose degradation, they are relatively inefficient in doing so. However, synthetic-biological research and development may succeed in developing organisms that are able to degrade lignocellulose significantly more efficiently, which might put the entire living plant biomass at risk.

Closely related to the hazard potentials described for metabolism are those that seem plausible for synthetic-biological systems with → SPECIAL CHEMICAL BEHAVIOR. This means that synthetic-biological systems that consist of, or make

use of, chemical structures that cannot be found in or utilized by natural biological systems may, in detrimental ways, chemically affect the natural living or the built environment.

Synthetic-biological approaches that aim at altering or constructing novel mechanisms of → GENE REGULATION constitute a certain hazard potential. This is, because these modified or de-novo regulation mechanisms may get transferred to natural biological systems via gene transfer and cause adverse interferences in these natural systems. Similar effects can be expected from synthetic-biological structures and systems that employ artificial mechanisms or strategies of → MOLECULAR BINDING.

→ ENCAPSULATION of synthetic-biological structures and systems may function as a means of protection against, or provide a vehicle for better transport in, the environment. Both effects could increase exposure towards such encapsulated systems. Whereas encapsulation refers to passive transport, active structures and systems that allow for → MOBILITY clearly increase potential exposure, since actively mobile synthetic-biological systems may intrude and spread in environments that were out of reach for immobile systems.

→ ORTHOGONALITY represents a rather ambiguous and ambivalent functionality. On the one hand, it may decrease the hazard potential, as orthogonal synthetic-biological systems may show less or no direct interference with natural biological systems on a material or functional base. On the other hand, one may also expect orthogonal synthetic-biological systems to potentially dominate, supersede, or edge out natural biological systems, because both systems would be "living" in the same environment and thus indirectly compete for at least some of the limited resources (such as space), which would correspond to a quite large hazard potential. Also, orthogonality would probably result in an increased exposure potential, because there were no natural biological degradation systems in place that would decompose the synthetic-biological ones.

Finally, some of the functionalities enabled through synthetic biology may rather indirectly generate or increase potential hazards or exposure. → MODULARITY, for instance, may ease the intended as well as the accidental integration of building blocks into existing biological systems, or the construction of novel biological systems. As beneficial this functionality may be for the rapid development of various useful structures and systems, it may result in a faster emergence of a more diverse set of harmful structures and systems, as well. Similarly, → COMPARTMENTALIZATION allows for higher complexity and diversity that may result in either more useful or more harmful synthetic-biological entities.

6 Conclusions

As an emerging field of science and technology, synthetic biology raises questions regarding the kinds and qualities of potential applications and benefits, as well as risks that are to be expected in the future. As has been shown in this

chapter, the conceptual approach via *functionalities* offers a practical and useful way of examining the capabilities and properties of (future) synthetic biological constructs. Also, potential hazards of and exposure towards synthetic-biological structures and systems can be assessed through an investigation of their respective functionalities. As the analysis in the previous sections revealed, functionalities can be realized either at individual levels of (synthetic-) biological objects (such as, among others, DNA, metabolic pathways, or the whole cell), or at several levels simultaneously. Many applications that build on such functionalities are already within reach, with many more to be accomplished in the medium or long term.

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Synthetic Biology: The Next Step Forward for Industrial Biotechnology

Gerd Klöck

Abstract Basic research in synthetic biology is rapidly advancing. A Google search for the term "synthetic biology" revealed more than 3 million hits (accessed 15 January 2014). 793 scientific articles indexed with this term are found in the Web of Science database for the year 2013. It can be expected, that applications developed by synthetic biology will be commercialized in the not-so-distant future. However, significant commercial use of "biological principles" in industrial biotechnology already exists. The adaptation of results of synthetic biology by industry will not only depend on their scientific "beauty," but also on existing economic and environmental constraints and the socio-economic context. In the following chapter, we will give an overview of the economic context of industrial biotechnology, and identify major opportunities for future applications of synthetic biology in industrial processes.

1 Introduction

Industrial biotechnology is the application of biotechnology for industrial production. It uses microorganisms or components of microorganisms—mainly enzymes—to generate industrially useful products. Industrial biotechnology today is a well-established technology in sectors as diverse as fine and speciality chemicals, food and feed, pharmaceuticals and healthcare, pulp and paper, leather and textiles and also bioenergy. By transforming biomass to products previously made from fossil carbon sources such as oil or natural gas, industrial biotechnology could improve the sustainability of production processes and could especially lead to reductions in greenhouse gas emissions (OECD 2011). However, biotechnological processes still have several major disadvantages compared to traditional chemical

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processing, e.g. low volumetric efficacy, unwanted by products from cellular metabolism, etc. (Chen 2012).

The fundamental concepts of synthetic biology—common cellular "chassis" and standardized molecular building blocks—offer, theoretically, the chance to overcome these obstacles; thus providing a new wave of industrial processes based on biotechnology. However, a synthetic biology redesign of industrial biotechnology will also be confronted with the inherent complexity of industrial systems, because any new solution must be implemented in the context of industrial engineering, in addition to considering economic, political and environmental constrains. In this chapter, we will focus on market data and production volumes of current biotechnological products to indicate the levels of complexity that can be expected during the development of new technologies.

2 Major Products and Emerging Markets for Industrial Biotechnology

Over the last 20 years, industry has developed a range of biotechnological processes on a multi-ton scale. To make clear what industrial biotechnology means in practice, the following paragraph will present recent data on production scale and—when available—market data for selected product groups (data in this paragraph from Demain 2007, unless otherwise stated). Pharmaceuticals are not included, the focus in this chapter lies on the potential (and limitations) of industrial biotechnology to compete with "traditional" chemical engineering based on fossil carbon resources.

Amino acids: The worldwide production of amino acids is about 3 million tons, 50 % of the production is monosodium glutamate, followed by lysine. The market for amino acids is currently about US$6 billion. Amino acids are widely used as food supplements, food ingredients (e.g. Vitamin C and E as antioxidants) or feed supplements. The worldwide production technology for amino acids is dominated by fermentation and enzymatic processes.

Nucleosides and Nucleotides: The group of substances is used as flavour enhancers in food and feed. In 2005 worldwide production was 15,000 tons. In Japan alone 2,500 tons of GMP and IMP are produced by fermentation. The market is about US$350 million. Desoxynucleoside analogues are of great importance in cancer and anti-viral therapy. The market volume for this class of pharmaceuticals was US$50 billion in 2005. Some production processes for these drugs make use of natural precursors.

Vitamins: The total vitamin market is currently about US$2.5 billion, the production of Vitamin B2 (riboflavin), Vitamin B12 and biotin are completely based on fermentation. Other industrial processes are used for other amino acids (e.g. Vitamin C, market about 10,000 tons, Vitamin E, market 30,000 tons). Other vitamins are produced by chemical synthesis, in some cases involving an enzymatic step to separate L-amino acids from a racemic mixture. Currently, 4,400 tons of

riboflavin is produced by fermentation, using *Ashhyra gossypii*. During fermentation, the industrial strain produces vitamin concentrations which are 40,000 times higher than is necessary for their growth. The riboflavin market is currently US$170 million.

Organic acids: Besides the well know examples of citric acid and acetic acids, many other organic acids are produced using biotechnological methods on a significant industrial scale. Examples are lactic acid (>400,000 tons at US$2–3 per kg), gluconic acid (50,000 tons, US$125 million), succinic acid (50,000 tons) and itaconic acid (18,000 tons at US$4 per kg).

Polymers: Speciality polymers from fermentation processes such as xanthan gum (30,000 tons, market US$450 million) and dextran for biomedical applications at US$100 per kg are well established industrial products. The Brazilian company Braskem can make 200,000 tons bio-based polyethylene from bioethanol per year.

Enzymes: Enzymes are important tools for biotechnological industrial processes such as the production of fructose syrup from corn starch (US production in 2012 was 9.2 million tons[1] at about 25 US cent/pound[2]), antibiotics and food production. Per year, 100,000 tons of glucose isomerase, 30,000 tons of nitrilase, and 40,000 tons of penicillin amidase are produced. The total market for industrial and therapeutic enzymes is currently US$4.6 billion. Amylases having approximately 25 % of the enzyme market share (Neddy et al. 2003). The protease subtilisin sells for US$200 million (mainly for detergents), the feed enzyme phytase for US$135 million, chymosin (cheese production) for US$140 million, and restriction enzymes and DNA polymerases for more than US$100 million each.

Enzymes converting cellulosic biomass to fermentable sugars represent a rapidly growing sector for the market. In the US, the government's goal is to produce 16 billion gallons of ethanol from cellulosic biomass per year until 2022 (Schnepf and Yacobucci 2013). According to recent company information, costs for enzymes degrading cellulose to fermentable sugars would be approximately 30–50 US cent/gal of ethanol produced,[3] resulting in potential sales for cellulases in the US of about US$4 billion. Since currently about 50 kg cellulase (e.g. Novozyme Cellic CTec3) are used to produce one ton of ethanol (or 160 g/gal) (Lane 2012),

[1] United States Department of Agriculture—Economic Research Service USDA ERS (2014). *U.S. high fructose corn syrup* (*HFCS*) *production, quarterly, by fiscal and calendar year.* (Online), USDA ERS. Available: http://www.ers.usda.gov/datafiles/Sugar_and_Sweeteners_Yearbook_Tables/Corn_Sweetener_Supply_Use_and_Trade/Table29.XLS. Accessed 5 February 2014.

[2] United States Department of Agriculture—Economic Research Service USDA ERS (2014). *U.S. prices for high fructose corn syrup* (*HFCS*)*, Midwest markets, monthly, quarterly, and by calendar and fiscal year* [Online], USDA ERS. Available: http://www.ers.usda.gov/datafiles/Sugar_and_Sweeteners_Yearbook_Tables/World_and_US_Sugar_and_Corn_Sweetener_Prices/Table09.XLS. Accessed 5 February 2014.

[3] Peder Holk Nielsen (2012). *The path to commercialization of cellulosic ethanol—a brighter future* (Online), Novozymes A/S. Available: http://www.bioenergy.novozymes.com/en/learn-more/presentations/Documents/A%20brigher%20future_PHN.pdf. Accessed 20 December 2013.

the hypothetical production of these cellulases would be in the magnitude of 2–3 million tons.

Alcohols: Beer and wine are among the oldest biotechnological products. Today, based on the volume of production, ethanol is the most important biotechnological product. The world production of bioethanol (defined as ethanol made by fermentation) is currently about 70 million tons (Licht 2006; Pimentel et al. 2007).

Law requires the industry to include bio-based fuels (currently mainly bioethanol) at 6.25 % of the total transportation fuel market in Germany until 2014 (Gesetz zur Änderung der Förderung von Biokraftstoffen July 15 2009). The renewable fuel standard (RFS2) lays down a legal framework for biofuels in the US until 2022. The goal is to produce 36 billion gallons biofuel (15 billion gallons ethanol from corn starch, 16 billion gallons from cellulosic sources).

Today, ethanol is mainly made from corn or sugar cane. Especially the enzymatic conversion of cellulose to fermentable sugars requires further optimized enzymes.

The production details for the enzyme are kept secret by companies, but one should keep in mind, that the production of the enzyme also requires substrates and energy to produce the biomass. Usually, the conversion rate from sugar substrates to biomass is below 50 %, the enzyme protein represents, according to the companies information (Simms-Borre 2010) 50–70 mg/g cellular biomass. The current situation may be that the enzyme production only is more energy consuming than can be delivered by the cellulose based ethanol (see below).

3 Feedstock for a Growing Industrial Biotechnology

The recent political discussion about biofuels and the competition with food production shows the main risk for the bio-based economy: availability of renewable carbon sources (OECD 2011; Somerville et al. 2010; Klepper 2011). Currently, the main feedstock for biotechnological processes is fermentable sugars and other simple carbon compounds mainly made by plants. The current worldwide production of sugar (sucrose) is about 190 million tons, at €0.3–0.4 per kg. Currently 25 % of the sugar produced is converted to ethanol, which requires 8 % of the worldwide crop production. At the same time, 10 % of the world's oil production is converted to biodiesel (Klepper 2011).

With increasing production volume for bio-based products, there will be a stronger competition with food and feed production in terms of price, availability of arable land, water, fertilizer, among others. One solution to this problem is to make use of cellulosic biomass from agricultural production such as a corn stover (see below). However, additional renewable feedstock for industrial biotechnology will have to be produced, most probably by photosynthetic carbon dioxide fixation. This process relies on the availability of light, especially solar energy. Depending upon the location, this can deliver 1,000–2,500 Watts/m^2. In the

process of oxygenic photosynthesis, this energy is converted to organic biomass at a theoretically maximal rate of 12 % (the energy content of organic biomass can be expressed by its net calorific value). In real world conditions, conventional crops, e.g. corn, have an overall efficiency of about 1–2 % which corresponds to a dry biomass yield of 30 tons/ha. Worldwide, sugar cane's mean yield is 35 tons/ha, with a maximum value of 68.7 tons/ha in Brazil. Typically about 1,000 Gal of bioethanol can be obtained per hectare and year from corn (Klepper 2011; Somerville et al. 2010).

Other photoautotrophic organisms such as microalgae show, at least in the laboratory, photosynthetic efficiencies of up to 9 % (Wijffels and Barbosa 2010). This corresponds to a theoretical biomass yield of 240 tons/ha. Microalgae are microscopic photosynthetic organism, which grow typically in freshwater and marine environments. Some microalgae are known for having very fast growth rates and minimal nutritional requirements compared to higher plants. Because microalgae can, in principle, be easily and quickly propagated, these organisms have been the focus of applied research investigating the generation of valuable biomass. Decisive factors in the current interest of algae include its ability to accommodate large quantities of lipids under certain circumstances, and the possibility to propagate microalgae in water resources which are not usually exploitable (salt, waste and brackish water), in areas that have remained unsuitable for agriculture. Microalgae would thus be able (at least in theory) to provide a sustainable source of biofuel such as biodiesel, without competing with agricultural production.

In theory, producing biodiesel from algae biomass should be a simple technical process. The algae must be harvested from the culture medium and dried before the lipids contained within it can be extracted and ultimately, converted into biodiesel. Various scientific working groups and companies have sufficiently demonstrated the essential feasibility of this procedure on a laboratory scale. In early January 2009, a Continental Airways passenger aircraft flew successfully for over an hour using a fuel mixture of algae and Jatropha oil. However, industrial scale microalgae cultivation for biomass production is still in its infancy. A more detailed consideration of energy requirements indicates that the current practice is not sustainable because of the negative energy balance associated with the production process. Culture stability, media recycling and harvesting are still a significant challenge and require further research. More basic research and field demonstration projects are necessary to advance understanding the possible environmental risk of large scale microalgae cultures as an alternative source of biomass (Klöck 2010).

4 Opportunities for Synthetic Biology

A more sustainable, bio-based economy also means producing a significant amount of energy and raw materials for other industries. Today, this is dominated by fossil carbon sources such as oil, coal and natural gas. Currently, the world's

yearly energy supply is 14 terawatts, more than 80 % from fossil carbon sources (33 % oil, 27 % coal, 22 % natural gas). In terms of production, this means 31 billion tons of oil and 7.7 billion tons of coal (Murphy and Hall 2010). This is about 600 times more than the current ethanol production. The challenge for a bio-based economy will be to replace at least a significant amount of these non-sustainable fossil resources by renewable feedstock. Per year, 6 billion tons of cellulosic residues from cereal crops, oil seed crops and other agricultural are produced (Somerville et al. 2010). This biomass might at least partly replace fossil carbon resources. However, this still requires considerable improvements in process optimization. Currently, the net energy balance (potential energy output vs. energy input) of bioethanol production from corn starch is 1.3 (the same as for biodiesel). For bioethanol from sugar cane it is 5, for coal the energy gain is 30, for produced oil 20, and for power from wind turbines 18 (Murphy and Hall 2010). This means, that for the production of bioethanol or biodiesel, currently we have to process much more biomass compared to oil and coal. There is obviously an urgent need for significant improvements of the biotechnological processes in terms of efficiency.

Compared to "traditional" chemical engineering, biotechnological processes have some advantages (specificity of reactions, low temperatures, low pressure etc.), but many more disadvantages (Chen 2012): Microorganisms have a complex biochemistry optimized for survival and reproduction under different conditions, not for production of a single compound in a controlled bioreactor. Microbes generally use one substrate at a time; conversion rates are often limited due to a variety of by-products (cellular biomass etc.). Aerobic processes require energy for aeration; the scaling up of aerobic processes is costly and difficult. Heterotrophic microorganisms need organic carbon compounds as substrates, and—most important, the wide variety of industrial processes shown above has been established using a single specific species or strain per process. This is like engineering without ISO or DIN norms, and makes process optimization in bioprocess engineering sometimes like inventing the wheel again and again.

There is, in our point of view, an industrial potential of synthetic biology. On a short term basis, synthetic biology could easily improve enzymes for existing industrial processes. Within the next decade synthetic biology could tackle some of the main problems which biotechnological processes have, compared to chemical processing: A technological breakthrough will certainly be a minimized microbial cell system, a "chassis" that is optimised for the conditions of industrial production (and not survival and reproduction) and can be used for different processes (Chen 2012).

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Beyond Genetic Engineering: Technical Capabilities in the Application Fields of Biocatalysis and Biosensors

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Abstract Synthetic biology allows the generation of complex recombinant systems using libraries of modular components. Two major near-market applications are whole-cell biosensors and biocatalysts for conversion of lignocellulosic biomass to biofuels and chemical feedstocks. Whole cell biosensors consist of cells genetically modified so that binding of a specific analyte to a receptor in the cell triggers generation of a specific output which can be detected and quantified. Since these systems are intrinsically modular in nature, with separate systems for signal detection, signal processing, and generation of the output, they are well suited to a synthetic biology approach. Likewise, effective degradation of cellulosic biomass requires a battery of different enzymes working together to degrade the matrix, expose the polysaccharide fibres, hydrolyse these to release sugars, and convert the sugars to useful products. Synthetic biology provides a useful set of tools to generate such systems. In this chapter we consider how synthetic biology has been applied to these applications, and look at possible future developments in these areas.

1 Introduction

As discussed in Schmidt (2015, this volume), 'synthetic biology' is at present not particularly well defined. The UK Synthetic Biology Roadmap (UK Synthetic Biology Roadmap Coordination Group 2012) offers this definition:

> Synthetic biology is the design and engineering of biologically based parts, novel devices and systems as well as the redesign of existing, natural biological systems.

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For many (but not all) practitioners, synthetic biology represents the application of key concepts which have been instrumental in the development of other engineering disciplines, but which have as yet been little applied to genetic engineering. One of the most important is modularity and standardization. Libraries of biological 'parts' are created in standard formats so that they can easily be combined in many different ways for different projects, rather than being specifically created and modified for specific projects. BioBricks (Knight 2003) represent one well known implementation of this concept. Another important concept is that constructed systems are incorporated into a 'chassis' which provides essential support functions. This may be a living cell, or a cell-free system. This contrasts with the usual conception of genetic modification as starting with a 'host' organism and modifying it to serve a particular function.

Perhaps the major current area of focus in synthetic biology is in the engineering of metabolic pathways for the production of fine chemicals, pharmaceuticals and other high value products (see Klöck 2015, this volume). In this area, synthetic biology can draw on a long and successful history of metabolic engineering, so that it may be difficult to draw a clear distinction. Two other application areas may offer clearer demonstrations of the benefits of a modular, engineering-based approach, albeit in different ways. These are whole-cell biosensors (French et al. 2011) and biomass conversion processes (French 2009; French et al. 2012). Here we will take a closer look at these two applications and consider how new synthetic biology approaches are moving them forward.

2 Whole Cell Biosensors

2.1 Biosensor Concepts

Whole-cell biosensors are analytical devices which take advantage of the capacity of living systems to detect and respond to chemical cues with high sensitivity and specificity, via the binding of biological molecules to specific ligands (Daunert et al. 2000; van der Meer and Belkin 2010). In general, a biosensor is a hybrid device consisting of a biological component, which provides the desired sensitivity and specificity, and an electrical transduction system, which converts the response to an output which can be monitored, analysed and recorded. The biological component may be an enzyme, receptor, transcription factor, antibody, or nucleic acid. In the case of a whole-cell biosensor, this is part of a living cell. On recognition of its ligand, it triggers a biological signalling pathway which results in a detectable response, or output, which can be detected (transduced) optically or electrically. Thus we can consider a whole cell biosensor as consisting of multiple modules (Fig. 1):

1. Detection: the recognition molecule binds its ligand and initiates the signaling cascade.

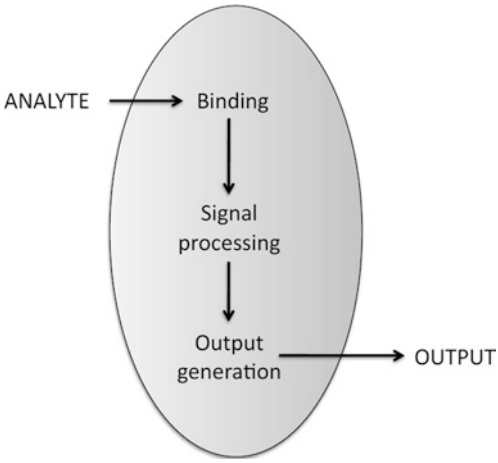

Fig. 1 Generic outline of a whole cell biosensor system. Note that in the case shown, the analyte must enter the cell and bind an intracellular receptor. This is typical of current systems, but future systems may allow binding of an extracellular analyte by a receptor embedded in the cell membrane

2. In vivo signal processing: this leads to a change in the states of various internal components of the cell.
3. Output: the changes cause the cell to generate an output signal which is related in some way to the initial binding event.
4. Transduction and post vivo signal processing: the output from the cell is detected leading to an electrical signal related to the concentration of the analyte.

Whole-cell biosensors are thus intrinsically modular, and are well suited to the engineering approach described above.

2.2 'Traditional' Whole Cell Biosensors

The concept of whole cell biosensors is not new. An early and highly successful example is the use of naturally luminescent marine bacteria (*Vibrio* and *Photobacterium spp.*) to detect toxic substances. Bacterial bioluminescence requires a continuous supply of ATP and NADPH. In the presence of toxic substances, this is disrupted and luminescence decreases. This is the basis for widely used toxicity sensors such as 'MicroTox' (Modern Water plc). The next development in the field was based on the ability of bacteria to detect specific substances and initiate a transcriptional response. Early examples involved mercury and arsenic, important environmental contaminants with major implications for human health. Mercury detoxification systems generally consist of a regulatory protein, MerR, which in the presence of mercuric ions, activates a promoter leading to transcription of *merA*, encoding mercuric reductase. This reduces toxic

mercuric ions to volatile elemental mercury, which can diffuse out of the cell. Arsenic is usually present in the biosphere as the oxyanions arsenate (AsO_4^{3-}) and arsenite (AsO_3^{3-}), toxic analogues of phosphate. Many bacteria possess a chromosomal or plasmid-encoded operon consisting of a promoter driving expression of an autoregulated repressor, ArsR, plus arsenate reductase, ArsC, which reduces arsenate to arsenite, and an arsenite efflux pump, ArsB or ArsAB. In the absence of arsenate or arsenite, ArsR binds the promoter and represses it; in the presence of arsenate or arsenite, ArsR releases the promoter allowing expression. Early whole cell biosensors were made simply by fusing the appropriate promoter to a reporter gene to generate a detectable output. Common reporters include β-galactosidase (LacZ, for which chromogenic and chemiluminescent substrates are available), bacterial and firefly luciferases, and fluorescent proteins such as GFP and its derivatives. Similar systems have been reported for detection of aromatic compounds and other organic molecules, simply by using promoters with appropriate response characteristics (van der Meer and Belkin 2010). Such systems can be highly sensitive and specific, but are rather complicated to use in the field, and pose particular regulatory issues since, in contrast to MicroTox and similar systems, they are based on genetically modified microorganisms. In addition, they require laboratory equipment (spectrophotometer, fluorimeter, or luminometer) to transduce the output. Thus they have not been widely adopted for commercial use, though several systems have been advertised (e.g., Aboatox mercury and arsenic sensors) (Aboatox 2010). One class of system has become commercially successful. These consist of cells in which an SOS promoter, responding to DNA damage, is fused to a reporter gene. Such cells can be used to assay mutagenicity of chemicals, as an alternative to the Ames test. One commercial example is SOS-Chromotest (Environmental Bio-Detection Products).

The modular nature and untapped market potential of whole cell biosensors makes them attractive targets for engineering-oriented synthetic biologists, including the mixed teams of engineering and biology undergraduates who compete each year in the International Genetically Engineered Machine competition (iGEM), a major showcase for innovation in synthetic biology.

2.3 Chassis Considerations

Whereas a ‘host’ can be any organism in which new genes can be inserted, the concept of a ‘chassis’ implies a system well enough understood that the performance of a new genetic module can be predicted. At present, only three living systems are commonly used as chassis: the Gram negative bacterium *Escherichia coli*, the Gram positive bacterium *Bacillus subtilis*, and the unicellular fungus *Saccharomyces cerevisiae*. In addition, cell-free systems based on *E. coli* cell extracts have been investigated (Chappell et al. 2013).

The great majority of whole cell biosensors, both pre- and post-'synthetic biology', are based on *E. coli*, for which the greatest variety of vectors and other genetic tools are available. *E. coli* grows rapidly on simple media and has a very simple life cycle. However, it lacks some features which would be useful for biosensor systems, especially the ability to produce a long lived dormant state. As a Gram negative bacterium, it possesses an outer membrane surrounding the cell membrane, which limits its ability to detect extracellular analytes, unless they are small and hydrophilic enough to pass through porins in the outer membrane and enter the periplasmic space (see Sect. 2.4 below).

B. subtilis is easy to manipulate, grows rapidly, and produces a long lived resting state known as endospores. Endospores are highly resistant to heat and desiccation, and can survive for many years in dried form (Nicholson et al. 2000). As a Gram positive bacterium, *B. subtilis* lacks an outer membrane, making it potentially better suited to detecting extracellular analytes. A number of reports have described the use of *B. subtilis* in biosensors. Luciferase-based biosensors have been reported for arsenic, antimony, cadmium and lead (Tauriainen et al. 1997, 1998), and β-galactosidase-based chemiluminescent and chromogenic endospore biosensors for arsenic, bacitracin and zinc (Date et al. 2007, 2010). We have previously reported an endospore-based arsenic biosensor with a chromogenic visual response for easy detection in the field (French et al. 2011) and are currently involved in a collaboration to develop an improved version of this (Arsenic Biosensor Collaboration 2013). *B. subtilis* is also a popular chassis in iGEM; for example, the winning entry in 2012, from the University of Groningen, involved engineering of *B. subtilis* to produce a pigment response in the presence of volatile compounds released by decaying meat (iGEM 2012).

S. cerevisiae is currently the most widely used eukaryotic chassis. It is well studied as a model organism, and many vectors and other genetic tools are available. Simple metal-ion biosensors can be implemented in *S. cerevisiae* (Baronian 2003; Leskinen et al. 2003; Peltola et al. 2005); however, its eukaryotic nature adds a layer of complexity to manipulation, and its advantage over bacteria is in cases where this eukaryotic nature adds some desirable functionality. For example, plant and mammalian signal transduction cascades, which are not functional in bacteria, can be transferred to *S. cerevisiae* allowing detection of biologically significant molecules such as plant and animal hormones (Mak et al. 1989; Bovee et al. 2008; Chen and Weiss 2005).

2.4 Detection Systems

Most reported whole cell biosensors are based on intracellular transcription factors which bind directly to their ligands and to DNA. This limits such systems to the detection of ligands capable of entering the cell. For many applications, it would be advantageous to detect ligands such as proteins and peptides, outside the cell.

This requires a signal transduction system which converts an extracellular binding event to an intracellular change in protein-DNA binding affinity. Several such systems are available. In bacteria, the most obvious choices are two-component sensor systems and chemotaxis receptors. These consist of a membrane-embedded sensor kinase, with an extracellular ligand-binding domain and intracellular kinase domain, and an intracellular response regulator. On binding of the ligand, kinase activity is altered, leading to increased or decreased rate of phosphorylation of the response regulator. In typical two component systems, this either increases or decreases its binding affinity for DNA, leading to activation and/or repression of relevant promoters. In the case of chemotaxis receptors, the response regulator, CheW, interacts with flagellar motor proteins to control motility. Many bacteria secrete pheromones to coordinate activities such as pathogenesis, competence and sporulation; in many cases, especially in Gram positive bacteria, these are peptides, and are detected by two component systems. Such systems represent an attractive target for modification to detect, for example, mammalian peptide hormones and similar biomarkers. However, progress towards this has been limited. A simpler goal is to apply these systems to the detection of their native targets, as a way to detect specific pathogens such as *Pseudomonas aeruginosa* (Massai et al. 2011) and *S. aureus*, major causes of hospital-acquired infections.

Two component sensor systems are interesting in being themselves modular; it has been shown that fusing the external receptor domain of one sensor kinase to the intracellular kinase domain of another can result in a hybrid protein which responds to the ligand of the first system by activating the response regulator of the second (Baumgartner et al. 1994; Levskaya et al. 2005). Recent structural studies (Casino et al. 2010) have begun to cast light on the signal transduction process. Furthermore, it has been reported that extracellular receptor domains can be modified by rational protein engineering to bind unnatural ligands (Looger et al. 2003), though some early results have proven difficult to replicate. This raises the intriguing possibility of generating a universal biosensor platform, with a standard transduction system and a library of sensor kinase receptor domains responding to different ligands.

In *S. cerevisiae*, detection of extracellular ligands may be achieved by modification of the mating peptide receptors Ste2 and Ste3. For example, this system has been modified for the detection of the plant hormone cytokinin (Chen and Weiss 2005). Since G-protein coupled receptors are abundant in mammalian cells, this would seem to be a promising avenue to explore for the detection of mammalian biomarkers.

2.5 Sensitivity Modulation and In Vivo Signal Processing

Two critical parameters in sensor design are the sensitivity (lowest analyte concentration for which a response can be observed) and dynamic range (range of possible outputs, corresponding to the range of analyte concentrations over which a

change in response is observed). Typical systems show a sigmoidal response. The exact nature of the response curve depends on the affinity of the receptor for the analyte, but due to the multiple stages between analyte binding and output generation, the nature of the relationship is complex (van der Meer et al. 2004) and in practice it is possible to 'tune' the response of the sensor through quite simple manipulations. For example, decreased background expression has been achieved in an arsenic biosensor through introduction of a second repressor binding site in the promoter region (Stocker et al. 2003). We have observed considerable changes in the response curve of an arsenic biosensor simply by altering the ribosome binding site of the repressor gene (French et al. 2011), and the 2010 Peking University iGEM team generated a family of mercury biosensors with different response curves by mutating the MerR-binding site in the promoter or altering the level of MerR expression (iGEM 2010). The modular, composable nature of BioBricks and similar 'parts' makes it easy to generate and screen many combinations of such elements. More rational design principles can also be applied to tune biosensors. For example, the University of Cambridge iGEM 2009 team provided a set of genetic cassettes consisting of a bacteriophage activator protein, transcription termination sequence, and bacteriophage promoter (iGEM 2009). These cassettes could be inserted into a biosensor construct between the analyte-responsive promoter and the reporter gene, so that the analyte-responsive promoter induced expression of the activator, which then led to expression of the reporter gene from the phage promoter. Different cassettes would provide different relationships between analyte concentration and reporter gene expression. The availability of as set of similar sensors with different response curves allows the preparation of sensors which give a 'bar-graph' like response, in which the number of wells showing a positive response gives a simple visual indication of the analyte concentration (Wackwitz et al. 2008).

Many early synthetic biology reports described the creation of biological analogues of electrical components such as toggle switches (Gardner et al. 2000) and oscillators (Elowitz and Leibler 2000). Perhaps the most obvious near-term application for such devices is to provide in vivo signal processing in whole cell biosensors. We refer to such genetic circuitry as 'object-oriented genetics', since it involves the development of genetic modules to perform specific 'calculations' and pass the result to another module. The most useful components in this context are logic gates (Wang and Buck 2012), which can be used to generate an output based on a combination of multiple input signals. Another application is to provide a multi-stage rather than continuous output; that is, one of several different and distinct outputs is generated according to the concentration of analyte. Such 'band-detecting' systems can be implemented in various ways. One early example was the arsenic biosensor designed by the 2006 University of Edinburgh iGEM team (Aleksic et al. 2007). This used two different arsenic-responsive repressor proteins to generate a multi-stage pH output. In the absence of arsenic, urease was expressed, leading to alkaline pH through cleavage of urea to ammonia. In the presence of low concentrations of arsenic, a sensitive arsenic responsive promoter would cause expression of a repressor protein to switch off expression of urease,

leading a neutral output pH. In higher arsenic concentrations, a less sensitive promoter was activated, leading to expression of β-galactosidase, allowing fermentation of lactose to give an acid pH response.

2.6 Novel Outputs

'Traditional' whole cell biosensors rely on a handful of widely used reporters. Quantitation of these signals requires a spectrophotometer, luminometer or fluorimeter. Considerable ingenuity has been devoted to the development of novel outputs for specific applications. For field use, it might be useful to have a visual output which does not require costly or labile chromogenic substrates. Several biosensors have been developed in which endogenous pigments are produced or modified in response to analyte concentration (Yoshida et al. 2008). The 2009 University of Cambridge iGEM team developed a set of genetic cassettes which could be used to generate red, orange, yellow (carotenoids), green, blue or purple (violacein and its precursors) pigments (iGEM 2009). Another approach to visual detection is the pH-based output (Aleksic et al. 2007). In this case the alkaline, neutral or acid response can be easily detected visually with a drop of pH indicator solution (de Mora et al. 2011).

For cheap quantitation, it would be desirable to have biosensors generate electrical outputs directly rather than via optical instruments. The pH based output (Aleksic et al. 2007) is one simple option, since pH can be quantified potentiometrically using a standard glass electrode or ion-selective field effect transistor (ISFET). Another option is to detect electrical signals generated by respiration. This can be accomplished amperometrically, as electrons are transferred from the bacterial respiratory chain to an electrode at a suitable potential. This detection system can be coupled to analyte detection if the analyte-responsive promoter induces biosynthesis of a soluble redox mediator such as pyocyanin (iGEM 2007) or expression of an outer membrane cytochrome which can transfer electrons directly (Goldbeck et al. 2013).

Re-engineering of cells can in principle allow closer integration of the biological and non-biological components of a biosensor. A number of steps in this direction have been reported, such as the Bioluminescent Bioreporter Integrated Circuit (BBIC), which incorporates a microluminometer and processing circuitry on a chip with a space for addition of bioluminescent biosensor cells (Nivens et al. 2004). Microfluidic devices incorporating luminescent or fluorescent biosensor organisms have also been reported, though external instrumentation was used (Diesel et al. 2009; Date et al. 2010). A more substantial re-engineering of biosensor cells to incorporate in vivo logic and signal processing, together with a direct electrical output for rapid and simple communication with electronic components, should enable a new generation of biosensor devices in the near future.

3 Biocatalysis and Biomass Conversion

3.1 Biocatalysis

The second application we discuss is in biocatalysis. Like a biosensor, a bioprocessing plant using a living biocatalyst is a hybrid entity consisting of a biological component (the biocatalyst organism) and a non-biological component (the remainder of the plant). Historically, the non-biological component has been designed to support the requirements of the biocatalyst; now, synthetic biology offers the potential to redesign the biocatalyst to suit the needs of the system. One area in which this is particularly significant is the conversion of lignocellulosic biomass to useful products. It is widely accepted that use of fossil carbon such as oil and gas should be decreased, due to limitations of supply, environmental damage due to extraction, climate effects due to net carbon dioxide production, and geopolitical issues related to uneven distribution. Lignocellulosic biomass, the inedible parts of plants, is the only conceivable resource which is available on a sufficiently large scale to replace even a significant fraction of our use of fossil carbon for fuels and chemical synthesis, without competing with human food production. Here we will consider how synthetic biology can aid in the implementation of such processes.

3.2 Natural Biomass Degradation Systems

Lignocellulosic biomass consists of long parallel fibres of cellulose chains, embedded in an amorphous matrix of hemicellulose, pectin, and lignin. Cellulose is a long polymer of D-glucopyranose residues linked by β-1,4 glycosidic bonds with each glucose residue rotated 180° with respect to its neighbours, so that the repeating unit is cellobiose rather than glucose. This leads to a linear structure held straight by intrachain hydrogen bonds, so that neighbouring chains can fit tightly together, held by interchain hydrogen bonds. This crystalline structure makes cellulose highly insoluble and very difficult to hydrolyse by chemical or enzymic means (Lynd et al. 2002). The hemicellulose component consists of shorter branched polysaccharide chains composed principally of D-xylose, L-arabinose, D-mannose and D-galactose as well as D-glucose (Scheller and Ulvskov 2010). Pectins are amorphous polymers of galacturonic acid with various sidechains. The most troublesome component is lignin, a three dimensional polymer formed by random polymerization of aromatic alcohols, linked by ether bonds. Lignin can not be hydrolysed, and in nature is degraded by oxidative processes; in commercial biomass fermentation processes it generally must be chemically and/or mechanically disrupted to allow access to the polysaccharide chains.

Despite these challenges, cellulosic biomass is generally biodegradable in warm, damp environments. Disruption of lignin is most associated with fungi,

especially ‘white rot’ fungi (Lundell et al. 2010). These attack lignin using oxidative enzymes such as lignin peroxidase and manganese peroxidase. The ability of filamentous fungi to exert physical forces may also be important. Degradation of hemicellulose and pectin is much easier and can be accomplished by a wide variety of fungi and bacteria. Covalent linkages between hemicellulose and lignin are hydrolysed by esterases, and hemicellulose is degraded by an array of enzymes including β-glucanases, xylanases, mannanases and arabinanases. Pectins are degraded by pectate lyases. This exposes the cellulose fibres to attack. In the paradigmatic process, based on studies of fungi such as *Trichoderma reesei* (the major source of commercial cellulase blends) (Peterson and Nevalainen 2012), amorphous (non-crystalline) regions of the chains are attacked by endoglucanases (EC 3.2.1.4), exposing a free reducing (C1) end and non-reducing (C4) end. These are then attacked by processive exoglucanases (cellobiohydrolases; EC 3.2.1.91, 3.2.1.176), which move along the chain releasing cellobiose units. These are finally hydrolysed to glucose by β-glucosidases (EC 3.2.1.21); this is essential as cellobiohydrolases can be strongly inhibited by cellobiose. In the case of aerobic organisms such as *T. reesei* and the Gram positive bacterium *Cellulomonas fimi*, these enzymes are secreted into the medium, whereas in anaerobic organisms such as the bacterium *Clostridium thermocellum*, they remain attached to the cell surface in a structure known as a cellulosome (Lynd et al. 2002). Interestingly, recent studies have expanded this paradigm. Oxidative enzymes of the GH61 and CBM33 groups seem to be widely involved in the degradation of cellulose and hemicellulose (Langston et al. 2011; Forsberg et al. 2011). Other proteins, known as amorphogens, interact with cellulose chains so as to disrupt their crystalline structure, increasing their accessibility to hydrolytic enzymes (Arantes and Saddler 2010). Furthermore, some non-canonical organisms, such as the highly efficient cellulose degrader *Cytophaga hutchinsonii*, appear to degrade cellulose by a novel mechanism which may not involve typical exoglucanases at all (Xie et al. 2007; Wilson 2009) (Liu and French manuscript in preparation).

Thus, biological conversion of lignocellulosic biomass to bioethanol, biobutanol and other useful products presents a challenge. Current processes involve chemical/mechanical treatment to disrupt lignin, followed by enzymic hydrolysis to release sugars, and then fermentation. A complicating issue is that the main ethanol-producing organisms, such as *Saccharomyces cerevisiae* and *Zymomonas mobilis*, are not able to assimilate the major sugars present in hemicellulose and pectin, and are reliant on glucose present in cellulose and as a minor component in hemicellulose. Since cellulose hydrolysis is much more difficult than hemicellulose hydrolysis, this means that a more expensive hydrolysis procedure is required.

The challenge for synthetic biology is to generate a microorganism which can be used in ‘Consolidated Bioprocessing’ (CBP) (Lynd et al. 2002), in which a single organism produces cellulases, hemicellulases and associated enzymes, takes up the products, and converts these in high yield to useful products, while avoiding

Table 1 Selected reports of engineering of common chassis organisms for generation of potential biofuel molecules

Product	Host	Titre	References
n-Butanol	*E. coli*	30 g/l	Shen et al. (2011)
n-Butanol	*S. cerevisiae*	2.5 mg/l	Steen et al. (2008)
n-Hexanol	*E. coli*	47 mg/l	Dekishima et al. (2011)
Acetone	*E. coli*	8.9 g/l	Bermejo et al. (1998)
Isopropanol	*E. coli*	143 g/l	Inokuma et al. (2010)
Isobutanol	*E. coli*	50 g/l	Baez et al. (2011)
Isobutanol	*B. subtilis*	2.6 g/l	Li et al. (2011)
Fatty acid ethyl esters (biodiesel)	*E. coli*	0.92 g/l	Duan et al. (2011)
Fatty acid methyl esters (biodiesel)	*E. coli*	16 mg/l	Nawabi et al. (2011)
Methylketones, C11–C15	*E. coli*	0.38 g/l	Goh et al. (2012)
Alkenes, C27–C29	*E. coli*	0.04 mg/l	Beller et al. (2010)
n-Butanol and longer chain alcohols (reverse β-oxidation)	*E. coli*	14 g/l	Dellomonaco et al. (2011)
Farnesol	*E. coli*	0.14 g/l	Wang et al. (2010)
Farnesene	*E. coli*	0.38 g/l	Wang et al. (2011)
Farnesol and farnesene	*S. cerevisiae*	1.0 g/l, 0.2 g/l	Choi et al. (2010)
Bisabolene	*E. coli* and *S. cerevisiae*	0.9 g/l	Peralta-Yahya et al. (2011)

being poisoned by its own products (French 2009). According to the genetic engineering paradigm, there are two major approaches to this:

1. Engineer a natural cellulose degrading organism to generate useful products.
2. Engineer an organism which can produce useful products to degrade cellulose.

The synthetic biology paradigm offers a third approach:

3. Engineer both cellulose degradation and product formation modules into a suitable chassis.

Here we will discuss progress towards these goals, and the ways in which synthetic biology can offer new alternatives. We will mainly consider biomass degradation and production of bulk chemicals such as ethanol and butanol, produced directly by fermentation of sugars released. However, we should also note that biosynthetic pathways for many other potential fuel molecules, such as alkanes, alkenes, fatty acids and their esters, and isoprenoids, have also been reported (Table 1). Some of these have also been produced from biomass, though reported yields so far are very low (Bokinsky et al. 2011). When considering the reports described below, it is also important to bear in mind the distinction between native insoluble ‘crystalline’ cellulose, such as filter paper or Avicel (microcrystalline cellulose), and soluble amorphous forms of cellulose such as PASC (phosphoric

acid swollen cellulose) or its analogue CMC (carboxymethyl cellulose), which are much more susceptible to hydrolysis.

3.3 Chassis Considerations

As noted in Sect. 2.3, three main chassis are currently used: *E. coli, B. subtilis*, and *S. cerevisiae*. In this context, *S. cerevisiae* is a special case, since it naturally produces large amounts of one of the most desirable products, ethanol. Thus, use of *S. cerevisiae* for cellulosic ethanol production combines approaches 2 and 3. *S. cerevisiae* is a fungus, hence capable of producing well studied fungal cellulases with correct glycosylation. It does not naturally secrete many enzymes, but is capable of secreting proteins if they are fused to the signal sequences of the a and α mating peptides. A major disadvantage is that it does not naturally ferment major hemicellulose sugars such as xylose and arabinose; thus a substantial fraction of the sugars released from biomass, including those easiest (cheapest) to release, are not available to it. Hence, much effort has been devoted to engineering strains of yeast which can ferment D-xylose and L-arabinose. Xylose fermentation can be achieved by expression of fungal xylose reductase plus xylitol dehydrogenase (Ho et al. 1998) or bacterial xylose isomerase (Karhumaa et al. 2007). Arabinose fermentation has proven more problematic, with cofactor imbalance generally leading to the major product being arabitol rather than ethanol (Karhumaa et al. 2006), and attempts to engineer strains which could co-ferment mixtures of xylose and arabinose were still more difficult, requiring prolonged post-engineering selection (Wisselink et al. 2009), though such strains have now been generated (Bettiga et al. 2009; Bera et al. 2010). This unexpected difficulty highlights the limitations of our ability to rationally re-engineer major metabolic pathways. However, synthetic biology has now produced a potential solution to such problems; the entire genome of *S. cerevisiae* is being resynthesised piecemeal, with incorporation of recombination sites between genes (Dymond et al. 2011). This will allow random 'scrambling' of the genome, and selection of strains with desired phenotypes. This is an interesting example of the substitution of highly parallel combinatorial reassembly and screening for rational design, a point to which we will return in the next section.

E. coli is generally the easiest chassis to manipulate, but since it does not naturally produce useful products, approach 3 is required. *E. coli* and its relatives (such as *Klebsiella oxytoca*) normally ferment sugars via the mixed acid pathway, producing a mixture of ethanol and acetic acid, along with lactic and succinic acids. However, these organisms are easily engineered to produce ethanol at high levels through the expression of pyruvate decarboxylase (PDC) and alcohol dehydrogenase (ADH) from *Z. mobilis*, plus deletion of genes involved in the mixed acid fermentation (Ingram et al. 1999). Such strains can produce 50 g/l ethanol from hemicellulose hydrolysate. An alternative is mutation of the regulatory region of pyruvate dehydrogenase so that it is expressed under anaerobic conditions, causing

a redox imbalance which leads to production of ethanol as the major product (Kim et al. 2007). *E. coli* has also been modified to produce high levels of n-butanol (Shen et al. 2011) and isobutanol (Baez et al. 2011), with product toxicity being alleviated by continuous removal of the product by gas-stripping. *E. coli* and *K. oxytoca* are able to assimilate all major biomass sugars including xylose, arabinose, mannose, and rhamnose; *K. oxytoca* can also assimilate xylooligosaccharides (Ingram et al. 1999). *E. coli* cannot naturally assimilate cellobiose, but can easily be modified to do this by prolonged selection (Kachroo et al. 2007) or expression of a periplasmic β-glucosidase or a cellobiose uptake system and intracellular cellobiose phosphorylase (Vinuselvi and Lee 2012). The major disadvantage of *E. coli* is its relatively limited ability to secrete enzymes into the medium. *E. coli* does not normally express a type II secretion system (the main terminal branch of the general secretory pathway, the major means of enzyme secretion in bacteria), though pathogenic strains secrete a few proteins via type I and type III secretion systems. However, a number of systems for protein secretion (Ni and Chen 2009) and cell surface display (van Bloois et al. 2011) are now available for *E. coli*. In one recent example, an ethanologenic *E. coli* strain was engineered to display an alginate lyase fused to the cell-surface protein Ag33, as well as an uptake and assimilation pathway for alginate degradation products; algal biomass was effectively fermented to 37 g/l ethanol (Wargacki et al. 2012).

B. subtilis is a less well characterized host than *E. coli*, but has the key advantage that it naturally secretes proteins at high levels, and will readily secrete heterologous cellulases. It is also capable of assimilating all of the major biomass-derived sugars, including cellobiose, and naturally secretes a number of biomass-degrading enzymes (Zhang and Zhang 2010), though it cannot naturally degrade crystalline cellulose. One major disadvantage of *B. subtilis* is that it prefers to grow aerobically and is not naturally fermentative, though in certain conditions it can ferment sugars to lactate and 2,3-butanediol (Nakano and Zuber 1998); while it can be modified to produce ethanol (Romero et al. 2007), reported levels are rather low (up to 8.9 g/l), insufficient for economical distillation. However, it may be well suited to the production of other classes of product such as isobutanol and other branched chain alcohols, produced via the amino acid biosynthetic pathway rather than via fermentation (Li et al. 2011). Thus, *B. subtilis* may ultimately be the most suitable of the current chassis organisms for recombinant biomass conversion systems.

3.4 Engineering for Cellulose Degradation: Progress to Date

The paradigm for cellulose degradation described in Sect. 3.3 would suggest that a cellulose-degrading phenotype could be achieved simply by co-expression of an endoglucanase, an exoglucanase and a β-glucosidase, with even the β-glucosidase being dispensable if the chassis has the capability to assimilate cellobiose. Many experiments along these lines have been reported in the literature. *S. cerevisiae*

Table 2 Assimilation of cellulosic substrates by engineered *Saccharomyces cerevisiae* strains

Enzymes	Substrate	Result	References
T. reesei EG II and CBH II, *Aspergillus* β-glucosidase (secreted)	Amorphous cellulose	3 g/l ethanol	Fujita et al. (2004)
T. reesei EG I, *Saccharomycopsis* β-glucosidase (secreted)	PASC	1 g/l ethanol, growth achieved (with peptone, yeast extract)	Den Haan et al. (2007)
Clostridium endoglucanase, *Saccharomycopsis* β-glucosidase (secreted)	CMC	11 g/l ethanol	Jeon et al. (2009)
Thermobifida processive endoglucanase Cel9A with or without *T. reesei* EG I, EG II, CBH I, CBH II	Amorphous cellulose	growth achieved (with peptone, yeast extract)	van Wyk et al. (2010)
T. reesei EG II and CBH II, *Aspergillus* β-glucosidase (surface display)	Acid-swollen Avicel	1.04 g/l ethanol	Apiwatanapiwat et al. (2011)
T. reesei EG II and CBH II, β-glucosidase 'cocktail δ-integration' (see main text)	PASC, rice straw	7.6 g/l ethanol, 7.5 g/l ethanol	Yamada et al. (2011)
Mini-cellulosome (cellulases expressed in *E. coli*)	PASC	3.5 g/l ethanol	Tsai et al. (2009)
Mini-cellulosome (enzymes expressed endogenously)	PASC, Avicel	1.8 g/l ethanol, 0.4 g/l ethanol	Wen et al. (2010)

can be engineered to assimilate cellobiose by secretion or surface display of β-glucosidase (Guo et al. 2011) or by expression of a cellobiose uptake system and intracellular phosphorylase (Sadie et al. 2011). As noted above, *B. subtilis* and many enteric bacteria can naturally assimilate cellobiose, and *E. coli* is readily modified to do so; it would therefore seem that only an endoglucanase and exoglucanase should be required. However, reported results to date have been rather disappointing, especially regarding direct fermentation of crystalline cellulose. The literature is extensive, and here we will consider only selected examples; a more comprehensive discussion is given elsewhere (French et al. 2012).

The majority of reports concern the modification of *S. cerevisiae* for secretion or surface display of cellulases, to enable direct fermentation of cellulose to ethanol. A number of reports have described fermentation of amorphous cellulose such as PASC to give low concentrations of ethanol, far below the 40 g/l or so which is considered necessary for cost-effective distillation (Lau and Dale 2009). Generally, the yeast is pre-grown to high density on a rich medium, so that it does not actually need to grow at the expense of cellulose, which may be energetically challenging due to the low energy yield of the homoethanologenic fermentation and the energetic cost of cellulase production. Representative examples are shown in Table 2. Both secretion and surface display have been attempted. Several recent reports describe attempts to mimic the highly efficient cellulosomes

of anaerobic bacteria, in which a large surface displayed 'scaffoldin' protein, with multiple 'cohesin' domains, binds an array of different cellulases via 'dockerin' domains (Fontes and Gilbert 2010). Yeasts were engineered to express a mini-scaffoldin with three cohesin domains, and cellulases with dockerin domains were expressed in *E. coli* and then added to the reaction (Tsai et al. 2009) or secreted from the same yeast strain (Wen et al. 2010). Results were not noticeably superior to those reported from other systems (Table 1). One proposed reason for under-performance is low exoglucanase activity, and codon optimization experiments have been undertaken to improve this (Ilmen et al. 2011). Another possible issue is inappropriate glycosylation of bacterial proteins such as scaffoldins (Suzuki et al. 2012). Balancing the different enzyme activities is another consideration; a procedure dubbed 'cocktail δ-integration' has been used to integrate multiple cellulase cassettes at random locations to yield a library of strains which can be screened for effective ethanol production (Yamada et al. 2010). However, the reported results are so uniformly unsatisfactory that it is difficult to avoid the impression of a deeper problem. In the case of engineered ethanologenic enteric bacteria, *K. oxytoca* has been modified to express endoglucanases CelY and CelZ of *Erwinia chrysanthemi* as well as the out operon required for their secretion (Zhou et al. 2001). The resulting strain was able to ferment amorphous cellulose producing up to 11.3 g/l ethanol, and also required reduced quantities of supplementary cellulases for fermentation of crystalline cellulose (Zhou and Ingram 2001). A strain of *E. coli* surface-displaying clostridial endoglucanase, exoglucanase and β-glucosidase fermented 10 g/l PASC to 3.6 g/l ethanol (Ryu and Karim 2011). In contrast to *E. coli* and *S. cerevisiae*, *B. subtilis* has some native ability to degrade biomass polymers, and relatively little modification is needed to improve this; for example, overexpression of endogenous cellulase Cel5 was sufficient to allow growth on PASC with a small amount of yeast extract (Zhang et al. 2011). Improved degradation of PASC and Avicel has been seen in strains expressing clostridial cellulases (Liu et al. 2012). Generation of mini-cellulosomes has also been described (Anderson et al. 2011; You et al. 2012). However, effective growth on crystalline cellulose, as seen in native cellulolytic organisms, does not seem to have been achieved.

3.5 A Synthetic Biology Approach to Studying Synergy in Cellulose Degradation

The general failure to achieve effective degradation of crystalline cellulose in recombinant strains suggests that an important factor may be missing. Examination of the genomes of natural cellulose degrading microorganisms reveals the presence of a large battery of biomass-degrading enzymes with overlapping predicted activities. For example, the recently sequenced *Fibrobacter succinogenes* genome showed 134 genes encoding putative biomass degrading enzymes, with 31 predicted to be cellulases (Suen et al. 2011). To consider a

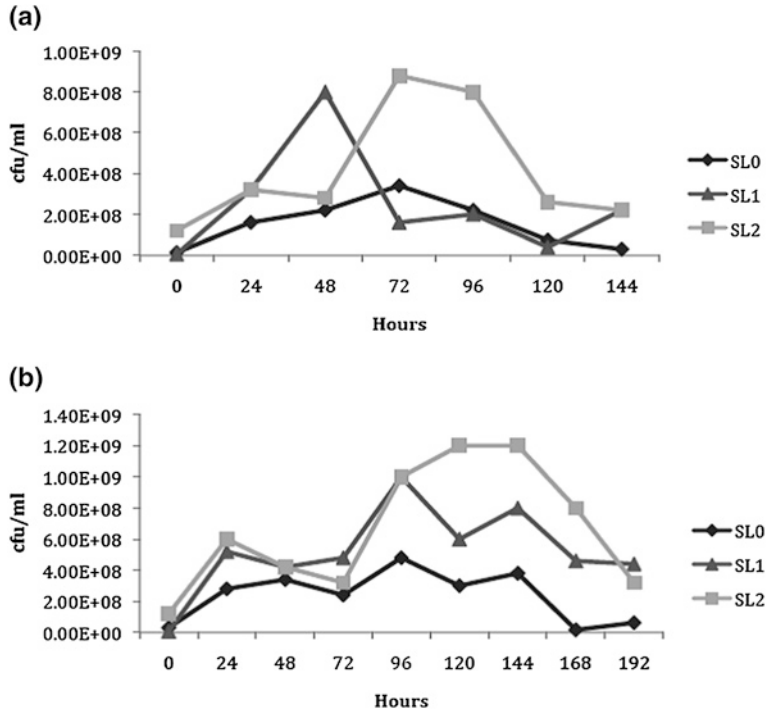

Fig. 2 Growth of *Citrobacter freundii* SBS197 expressing Cex and CenA at the expense of cellulosic substrates (Lakhundi 2012). *SL0 C. freundii* SBS 197 vector control; *SL1 C. freundii* SBS197 expressing *cenA* and *cex* from *lac* promoter; *SL2 C. freundii* SBS197 expressing *cenA* and *cex* from *spac* promoter. **a** Growth with 20 g/l filter paper +1 g/l yeast extract; **b** growth with 20 g/l Avicel +1 g/l yeast extract

well characterized example, *Cellulomonas fimi* secretes four well studied endoglucanases, three exoglucanases and two β-glucosidases (Stoll 2001), and our own annotation of the genome shows 97 putative genes involved in biomass degradation. The presence of multiple enzymes with overlapping activities suggests that synergy, both within and between enzyme classes (Lynd et al. 2002), plays an essential role. Studies of synergy are complicated due to the large number of potential interactions; both synergistic and anti-synergistic (competitive) interactions have been observed (French 2009). The tools of synthetic biology allow an empirical approach, which we refer to as 'combinatorial genetic engineering' (French 2009). A library of genes encoding putative biomass degradation enzymes, as well as accessory proteins such as amorphogens, is generated in a modular format which allows rapid parallel assembly of many combinations of genes and promoters. These are then used to generate a library of recombinant strains, which are screened for the ability to grow on cellulosic substrates. We have begun such a program, initially using the BioBrick RFC10 standard, with *Bacillus subtilis* and *Citrobacter freundii* as hosts (Lakhundi 2012)

(Lakhundi and French manuscript in preparation). *C. freundii* is a close relative of *E. coli*, so that standard *E. coli* cloning vectors and procedures work well, but possesses a native ability to assimilate cellobiose. Our preliminary experiments have shown that *C. freundii* expressing one endoglucanase (CenA) and one exoglucanase (Cex) of *C. fimi* shows some growth with filter paper or Avicel as main carbon source (Fig. 2), hence is a suitable test platform for in vivo synergy screening to achieve improved growth. Furthermore, different combinations of cellulases show varying effectiveness against different classes of substrate; for example, *C. freundii* expressing CenA and Cex grows best with filter paper, whereas strains expressing Cex with CenB, an alternative endoglucanase, have enhanced ability to degrade Avicel (Barnard 2012) (Barnard, Elfick and French manuscript in preparation). By extending this work, we hope to generate a large library of biomass degradation cassettes which can be screened against various biomass streams to determine the best enzyme combinations to allow growth and product formation in each case. Analysis of the results, together with detailed characterization of the enzymes involved, should also enhance our understanding of synergistic effects in biomass degradation, and enable us to develop a set of heuristics which can be used in designing biomass conversion systems.

3.6 Synthetic Biology Approaches to Inhibitor Tolerance

Growth of biocatalysts on biomass substrates is often limited by toxicity of the products produced (especially moderately hydrophobic compounds such as n-butanol) and also fermentation inhibitors such as furfural, furoic acid and hydroxymethyl-furfural produced during pretreatment. For cost-effective production of low value products, it is essential to produce a high titre of product so as to minimise downstream processing costs, hence limitations on growth may be a serious problem in developing commercial processes. Synthetic biology approaches have also been applied to increase tolerance to these compounds (summarized in more detail elsewhere) (French 2009; French et al. 2012). For example, 43 efflux pumps were screened in *E. coli* against 7 different biofuels, and several candidates were found to increase tolerance to terpenoids, though not to n-butanol (Dunlop et al. 2011). Screening of potential resistance genes directly from a soil-derived metagenomic library led to isolation of resistance determinants for important fermentation inhibitors (Sommer et al. 2010). Known tolerance determinants appear to operate via a variety of different mechanisms, including exporting toxic substances (Aono 1998), protecting proteins from misfolding (Okochi et al. 2008), and strengthening the cell membrane (Shimizu et al. 2005) as well as less obvious pathways (Woodruff et al. 2013a). This raises the interesting question of whether synergistic effects might lead to large tolerance increases (Woodruff et al. 2013b). Modular formats such as BioBricks are well suited to testing this hypothesis (Fletcher and French, manuscript in preparation).

4 Conclusions

In this chapter, we have seen how the modular, parts-oriented paradigm of synthetic biology is particularly well suited to the applications of biosensors and biomass conversion processes: one the one hand, because of the highly modular and engineered nature of whole cell biosensors, and on the other hand, because of the ability to perform 'combinatorial genetic engineering' to generate many combinations of parts which can be tested in parallel, where our knowledge is currently insufficient for rational design. Together with metabolic engineering or pathways for product formation (Klöck 2015, this volume), it is in these areas that we can expect commercial applications of the synthetic biology paradigm within the next few years.

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Protein Tectons in Synthetic Biology

The Expansion of Cellular Functionality Combining Chemical Biology of Small Organic Molecules with Protein Tectons—Unnatural Amino Acids, Protein Based Biohybrid Materials and De Novo Organelles

Stefan M. Schiller

Abstract The expansion of cellular functions via novel modular building blocks, namely unnatural amino acids, their site-selective genetically encoded cotranslational incorporation into proteins, requires the redesign and expansion of the translational network with additional components, the orthogonal tRNA and tRNA synthetase. At the next level protein tectons (tecton = architectural building block) constitute complex genetically encoded "material libraries" inside the cell. These protein tectons are architectural building blocks allowing for complex supramolecular self-assembly inside the cell, forming cellular compartments or constituting 3D matrix mimicry of the extracellular matrix outside the cell. In addition they form the basis for biohybrid materials in protein/enzyme engineering, nanotechnology and regenerative medicine. The defined modification of protein tectons utilizing chemical biology allows for the selective bioconjugation e.g. of unnatural amino acids, via bioorthogonal chemical reactions introducing novel chemical entities expanding the repertoire of "posttranslational" protein modifications in vitro and in vivo for various applications.

1 Introduction

Life—a complex phenomenon observed by man in nature and reflected within him, is the focus of many experimental and philosophical sciences. Since the discovery of the cell as the fundamental "unit" of life, mankind learned to

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analyze and modulate the molecular basis of life, applying the molecular sciences chemistry and molecular biology.

The ability to control cellular functions advanced from simple biotechnology used in preparing bread, brewing beer and fermenting vine, to a molecular understanding and redesign on the genetic level in recent decades. With the knowledge of many cellular signal pathways, metabolic energy and mass flux, transcriptional and translational control, as well as our ability to genetically encode many of these redesigned elements, mankind developed a set of tools allowing us to control cellular function e.g. for the production of biotechnological products, e.g. pharmaceuticals, enzymes and small molecules.

Nature uses compartmentalization, energy flux and de novo synthesis of multifunctional molecules to maintain a dynamic molecular system utilizing concepts such as self-organization and non-linearity, key elements constituting the complex phenomenon of life on its molecular/material level. Interconnected modularity is an essential phenomenon important to be implemented in approaching a system-wide control of cellular systems. The large number of known modular control elements, relations and interconnectivities of cellular processes, lead to the creation of "biological building blocks." This, in turn, allows implementation of modular elements expanding, substituting or redesigning cellular parts—the idea of synthetic biology. Ideally they should finally allow formation of a vast tool-box of compatible modules or systems to facilitate the assembly of functional systems of the living cell. This endeavor is still limited by our fragmentary insights into this complex system, and most complex systems in general. The known correlations of all linear or non-linear mass and energy fluxes, their separation and control in time and space, exceed our understanding at both the level of classical mechanics and quantum mechanics. Thus, our current approaches only allow us to control complex systems within a narrow window of often unreliably defined parameters. Nevertheless, our knowledge of the functional stability and reliability within predictable regimes of a large number of cellular elements allows for the efficient use of cellular systems for a large number of applications, including complex cellular manipulations.

With increasing complexity of the implemented synthetic elements provided by the "synthetic biology-toolbox" and the creation of robust synthetic subcellular systems, a different level of cellular functionality can be reached allowing novel cellular processes. The effectiveness and potential risks of reengineered cells will strongly depend on the ability of the cell to survive, to exchange genetic information and to grow/multiply. If complex genetic circuits, novel minimal organism and xenobiotic systems changing the cells capability e.g. to digest biomaterial and to interact with other living organism are of evolutionary advantage and maintain stability in the cells genetic repertoire, environmental risks can result.

DNA, as temporarily evolved system facilitating information storage, is the most important source of protein sequence information. Due to the complex differentiation and signal exchange leading to specialized tissue formation and complex organisms, multicellular systems are also the focus of many efforts in synthetic biology.

The combination of genetic tools from molecular biology with chemical biology expands the range of naturally available chemical functionalities. Chemical biology

applies synthetic methods, bioorthogonal concepts, novel reagents and analytical concepts from chemistry to understand, redesign and control biological systems in vitro and in vivo reaching into chemical synthetic biology (Sletten and Bertozzi 2009; Chiarabelli et al. 2009, 2013; Cravatt and Gottesfeld 2010; Doudna 2005). Therefore this chapter will highlight the functional scope of unnatural amino acids and the possibilities of bioorthogonal chemistry in vitro and in vivo.

The expansion of cellular functions and the introduction of new elements are predominantly encoded on the genetic level. This bioengineering route will be exemplified with several examples in this chapter. Amino acids, representing the letters of the translated alphabet of the genetic code, constitute the functional chemical entities of proteins. Hence, the expansion of the functional diversity of the amino acid repertoire would allow for an important expansion of the cells functional capabilities, highlighted in Sect. 2. The current applications and the vast number of possibilities of unnatural amino acids are largely under development and the focus of Sect. 3. The expansion of the structural and functional role of proteins is an important subject in synthetic biology. In Sect. 4 a new compartmentalization scheme utilizing amphiphilic de novo proteins forming "membrane-enclosed" organelles resembling the constitution of lipid based membranes is described. This scheme allows constitution of de novo organelles under direct genetic control. The last Sect. 5 shifts the focus from intracellular protein modules to the role of the extracellular matrix, and thus the material properties of proteins.

2 Redesign of the Translational Network Allowing for the Site-Selective Cotranslational Introduction of Unnatural Amino Acids: Xenobiotic Functions

The genetically encoded, site-selective cotranslational incorporation of non-canonical or unnatural amino acids (UAA) via the redesign of the translational network, allows the introduction of artificial xenobiotic functions into cells (Wang and Schultz 2004; Xie and Schultz 2006). Nature basically uses 20 different amino acids and, in some cases, selenocysteine (Berry et al. 1991; Bock et al. 1991) and pyrrolysine (Hertweck 2011; Fekner and Chan 2011; Ibba and Soll 2002) to access proteins via ribosomal synthesis. The important role of proteins in most structural, functional and regulatory processes of the cell raises the desire to introduce additional amino acids in order to expand protein function. The "interpretation" or the translation of the genetic code is facilitated at the level of the tRNA synthetase. The tRNA synthetase recognizes the amino acids and the corresponding tRNA and aminoacylates the tRNA utilizing ATP. At the ribosome the mRNA codon is paired with the corresponding anticodon of the tRNA determining the amino acid order in the growing peptide chain. Codon-amino acid correlations are complex and bear higher order correlations beyond the determination of the amino acid order: each of the three codon nucleotides has a general

correlation with a different, predictable amino acid property, depending on the position within the codon (Taylor and Coates 1989). Thus, alterations introduced at the genetic level have to be recognized as influential in a system wide context exceeding the direct codon to amino acid translation.

Strategies to expand the number of amino acids serving as building blocks for ribosome-mediated protein synthesis comprise processes such as re-coding, read-through or changes of the meaning of codon triplets of the universal genetic code. Read-through can be facilitated by suppression of stop-codons (UGA, UAG and UAA), non-triplet coding units, e.g. four-base codons (Magliery et al. 2001) or reassignment of evolutionary assigned codon triplets replacing a canonical amino acids proteome-wide with isosteric amino acids via selective pressure. Especially the latter one allows for a proteome-wide amino acid replacements—"unnatural organisms" (Budisa 2005). The dependence on the supply of such unnatural amino acids restricts such experiments to environments where the unnatural amino acid can be supplied.

The strategies used for the introduction of UAA (Budisa 2005) started early with the residue-specific, (Johnson et al. 2010; Cowie and Cohen 1957) method. The semi-synthetic in vitro amino acylation of orthogonal tRNA (Noren et al. 1989) was followed by site-specific incorporation techniques in vivo first developed in *E. coli* (Wang et al. 2001). The semi-synthetic in vitro amino acylation of orthogonal tRNA uses a chemically or enzymatically (Hartman et al. 2006) ligated aminoacylated tRNA for site-specific incorporation of the desired UAAs (Noren et al. 1989; Taki et al. 2001). Even though a large number of UAA have been introduced into various proteins (Ellman et al. 1992), the yields of mutant proteins are low and the in vivo applications are limited due to the small amount of aminoacylated tRNA microinjected into oocytes and the limited number of oocytes which can be injected. The inability to aminoacylate the orthogonal tRNA (Deiters et al. 2005) again inside the cell and the labor intense aminoacylation chemistry are further shortcomings. The residue-specific or global incorporation of UAAs is a powerful tool to access modified proteins with novel physical and chemical and properties allowing to add additional unnatural amino acids maintaining the use of all natural amino acids (Xie and Schultz 2005).

The reassignment of sense codons allows for the global—proteome wide replacement of one or several naturally occurring amino acids by their isosterical unnatural amino acid homologues, thus replacing the natural amino acid completely restricting applications where the natural amino acid can be omitted. In order to replace the natural amino acid, auxotrophic bacterial strains are used which are not able to synthesize the amino acid. These shall be replaced by the isosterical homologue. First the cells are grown on a medium supplemented with the natural amino acid which is exchanged for medium not containing the corresponding natural amino acid but the unnatural amino acid to incorporate structural homologs of the natural amino acid. All cellular proteins can be modified, even without sequence information using this approach. This method is useful for many applications providing good protein yields (Johnson et al. 2010). A limitation of this method is its dependence on close amino acids structural analogues which are tolerated by the tRNA synthetase.

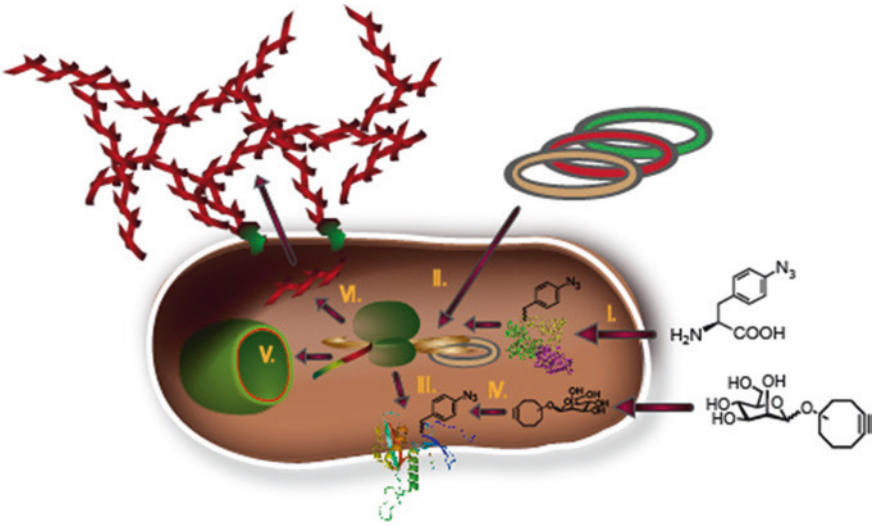

Fig. 1 All the approaches presented below are based on the concept of complementary modular elements encoded in various libraries ranging from genetic information to small synthetic molecules. Thus they reflect the modularity of functional cellular elements and expand them into individual and freely combinable molecular systems; expanding or rebuilding the cellular system on the one side and providing a library of highly defined molecules and materials for applications in vitro requiring molecules with exact properties on the other

Overcoming these constraints the genetically encoded, site-specific cotranslational incorporation of UAA in response to a stop codon utilizing an orthogonal tRNA (Wang and Schultz 2001) and tRNA synthase was developed (Fig. 1, I) (Wang et al. 2001). The introduction of the required stop codon e.g. the amber stop codon TAG is readily facilitated via site-directed mutagenesis (Shortle et al. 1981), e.g. Quik Change (Zheng et al. 2004). This approach allows the selective exchange of one amino acid for the UAA, or insertion of a UAA into an arbitrary sequence. Approximately 70 different unnatural amino acids have been added to the genetic code of *E. coli*, yeast (Chin et al. 2003) and mammalian cells (Liu et al. 2007b) expanding the genetic code via aminoacyl-tRNA synthetase/tRNA pairs (Liu and Schultz 2010). The spectrum of UAA with new functional site chain chemistries comprise azides (Fig. 1, I) (Chin et al. 2002b), keto groups (Wang et al. 2003), metal ion chelators, photocross-linking and photocaged moieties (Chin and Schultz 2002; Wu et al. 2004) e.g. benzophenone in *E. coli* (Chin et al. 2002a; Liu et al. 2007a), crystallographic probes (Xie et al. 2004), isotope labels for NMR (Deiters et al. 2005), posttranslational modification schemes (Deiters et al. 2006; Liu and Schultz 2006; Wang et al. 2007) and fluorophores (Summerer et al. 2006; Wang et al. 2006).

Utilizing this strategy site-specific UAA incorporation was achieved with high fidelity and yields of the target protein of up to 1 g/l in vivo (Young et al. 2010). The same method can be used in cell-free expression systems as well, which is

important for large screening formats such as high throughput screening (Albayrak and Swartz 2013).

The consequences of the expansion of the natural amino acid repertoire have to be reflected on the background of known amino acid and protein function, and their functional role in the cellular networks. Due to the tight regulation and stability of the regulatory systems, especially if they are important for sustaining life, the impact of additional functional elements such as unnatural amino acids can be regarded as minor. If unnatural amino acids have to be added to the cell culture if no metabolic pathways exist for their in vivo synthesis, potential risks of such newly added cellular components are small. Methods towards an easier supply of the unnatural amino include coupling bioorthogonal chemistries with artificial metabolism, e.g. the intracellular biosynthesis of azidohomoalanine and its incorporation into recombinant proteins (Ma et al. 2014). A first step towards an "autonomous system" has been developed as well. Biosynthesis of an unnatural amino acid generates a completely autonomous bacterium with a 21 amino acid genetic code.

> This bacterium can biosynthesize a nonstandard amino acid from basic carbon sources and incorporate this amino acid into proteins in response to the amber nonsense codon. The biosynthetic pathway for the amino acid p-aminophenylalanine (pAF), as well as a unique pAF synthetase and cognate tRNA, were added to *E. coli*. pAF is incorporated into myoglobin with fidelity and efficiency rivaling those of the common 20 amino acids. This and other such organisms may provide an opportunity to examine the evolutionary consequences of adding new amino acids to the genetic repertoire, as well as generate proteins with new or enhanced biological functions (Mehl et al. 2003).

The *E. coli* safety strain K12 used for this research is not able to replicated outside defined lab conditions, thus allowing for controlled risk management. The implementation into other organisms able to replicate in nature would need additional safety measures.

Without the presence of the unnatural amino acid the cell has to develop a way to encode an additional amino acid or find another utilization of the orthogonal tRNA and tRNA synthetase in order to take advantage of the additional elements of the translational machinery to develop risky phenotypes. Since the replication of these additional components is a metabolic burden for the cell, such elements are usually eliminated quickly. Thus, the potential risk of expanding the genetic code can be controlled.

3 Biohybrid Systems: Modifying Proteins Containing Unnatural Amino Acids—Membrane Proteins, Enzymes and Co

The use of an expanded genetic code allowing for the cotranslational introduction of unnatural amino acids allows for "precision protein engineering" with atomic precision. Due to the high level of translational control and reliability over the

accuracy of the protein sequence, many challenging questions can be addressed not possible with other methods. Below a number of examples are given where the site-selective incorporation of unnatural amino acids has been used. The first set of examples highlights the reassignment of sense codons using selective pressure in auxotroph strains, while in the second set of examples the site-selective suppression of the stop codon is highlighted.

Several classes of proteins are currently the focus of protein engineering utilizing unnatural amino acids. Important examples comprise enzymes and G-protein-coupled receptors (GPCRs). Lipases are important enzymes used in biotechnology. Fluorinated side chain amino acids have been used to alter their function/stability (Budisa et al. 2010). Additional new protein properties via fluorinated amino acids have been described (Merkel and Budisa 2012; Merkel et al. 2010) and applied to modify single-chain Fv (scFv) format protein, commonly used as analytical tool for diagnostic and therapeutic applications. "The usage of fluoroproline was exploited to enhance the thermal stability of scFv by replacing the natural proline on the framework regions of scFv that influence the folding or stability. To demonstrate the applicability of the approach, a bacterial cytoplasmic foldable and humanized anti-c-Met scFv (hu-MscFv) was used. The hu-MscFv proline sites were successfully incorporated with (2S,4R)-4-fluoroproline without affecting its structure and function by the in vivo residue-specific global replacement method which exploits bacterial auxotrophic system" (Edwardraja et al. 2011). In the field of X-ray structure analysis, the bioincorporation of telluromethionine into proteins was applied as a promising new approach for X-ray structure analysis of proteins (Budisa et al. 1997). Due to the ability to alter physicochemical properties of proteins by unnatural amino acids, a global replacement of tryptophan with aminotryptophans was used to generate non-invasive protein-based optical pH sensors (Budisa et al. 2002). Finally,

> ribosomally synthetized and post-translationally modified peptide natural products (RiPPs) are restricted to the 20 canonical amino acids. Microorganisms with an engineered genetic code are capable of delivering the biological, chemical, or physical properties of many unnatural or synthetic noncanonical amino acids, ncAAs (in different combinations of their numbers and chemistry) precisely defined by the chemist at the bench allowing e.g. for better membrane permeability and oral availability (Budisa 2013, 591).

The genetically encoded, site-specific cotranslational incorporation of amino acid derivatives containing an azide or benzophenon site group in response to a stop codon utilizing an orthogonal tRNA and tRNA synthase will be highlighted in several examples below.

G-proteins and G-protein-coupled receptors (GPCRs) are an important class of membrane proteins with important impact in many diseases, thus constituting the major target of most drugs. Elucidating the functions and interactions of membrane proteins and GPCRs is a difficult task, especially in vivo. Several approaches have been developed utilizing.

p-azidophenylalanine, e.g. for tracking GPCR activation using the azide as genetically encoded infrared probe (Ye et al. 2010). In order to investigate the

interactome of GPCRs, unnatural amino acids with bioorthogonal reactivity can be used. Site-specific incorporation of keto amino acids into functional G-protein-coupled receptors using unnatural amino acid mutagenesis is one example (Ye et al. 2008). Mapping the ligand-binding site on a GPCR using genetically encoded photo-cross-linkers (Grunbeck et al. 2011) allows us to control the start of the screening process. Genetically encoded photo-cross-linkers enable the mapping of the binding site of an allosteric drug on a GPCR (Grunbeck et al. 2012) helping to pin-point binding sites for drugs when no crystal structures of the target proteins are available. Such site-specific experiments can be conducted in vitro and in vivo also including the incorporation of molecular probes to study GPCRs (Daggett and Sakmar 2011). The incorporation of the unnatural amino acid p-benzoyl-L-phenylalanine (Bpa) into a G-protein-coupled receptor in its native context (Umanah et al. 2007) enables investigations under physiological conditions.

Other applications of Bpa are described for the study of autotransporters.

> Autotransporters are bacterial virulence factors that consist of an N-terminal extracellular ("passenger") domain and a C-terminal β barrel domain ("β domain") that resides in the outer membrane. The site-specific photo-cross-linking approach was used to gain insight into the mechanism by which the β domain is integrated into the outer membrane and the relationship between β domain assembly and passenger domain secretion indicating the applicability of the method for dynamic processes (Ieva et al. 2011).
>
> Investigating complex protein assemblies is often difficult and largely lacks 'generalizable' interaction screens. Studying ISWI, a part of the vast Snf2 family of helicase-related proteins, many of which constitute the catalytic cores of chromatin remodeling complexes, is such an example. Probing the conformation of the ISWI ATPase domain with genetically encoded photoreactive cross-linkers and mass spectrometry allowed us to take advantage of the UV-reactive p-benzoyl-p-phenylalanine. It could be shown that the ATPase lobes strongly rotate against each other, a movement postulated earlier to be necessary to achieve a catalytically competent state (Forne et al. 2012).

Even though Bpa is the preferred photoaffinity probe for in vivo applications, p-azido-l-phenylalanine (AzF) has been successfully applied as well. Used for novel insights into the pathogenicity of *Candida albicans*, interesting studies on molecular interactions of crucial central virulence factors have been conducted. Since methods for the analysis of direct molecular interactions of proteins in vivo are scarce, the genetic code of *C. albicans* was expanded with the synthetic photo-cross-linking amino acid AzF. Interacting molecules in close proximity of this unnatural amino acid could be covalently linked by UV-induced photo-cross-link, which makes unknown interacting molecules available for downstream identification (Palzer et al. 2013).

Another important application of genetic code engineering is altering posttranslational modification schemes, hardly to be realized with other methods. The spectrum of current methods and approaches comprises photocaged tyrosine, (Deiters et al. 2006) tyrosine sulfation as important post-translational modification widespread across multicellular eukaryotes but with very limited knowledge of its biological functions (Liu and Schultz 2006), and the introduction of lipid or carbohydrates in vitro (Wang et al. 2007). Currently, procedures are being developed which allow the use of glyco- and lipid building blocks with "clickable" functionalities to introduce lipid and glycoforms via an epitope building block tool-box. This allows the

transformation of the glycocode intracellularly to influence signal events (Fig. 1, III and IV–III showing the cotranslational insertion of p-azidophenylalanine followed by the modification with a clickable glyco-derivative synthesized in vitro and taken up by the cell).

3.1 Protein Material Libraries

In the last two subchapters several applications of protein tectons will be introduced. Tectons—architectural building blocks—are used to constitute cellular components (7.4) such as de novo organelles and designable extracellular matrix proteins/3D matrices, e.g. for personalized regenerative medicine (7.5). They require the ability to precisely assemble protein sequence blocks on the DNA level allowing access to libraries of various protein sequences. Currently several methods have been developed, e.g. the Protein Assembler-Technology (Schiller and Huber 2013) enabling access to large libraries with exactly defined sequences which can be freely combined to yield precisely controlled protein materials. Such modular molecular tecton-libraries introduce a combinatorial DNA-toolbox platform constituting defined protein based biohybrid materials (Huber et al. accepted).

Applications range from bio-inspired material science—nanobiotechnology—tissue engineering—to personalized medicine.

4 Protein Tectons I: Architectural Building Blocks Allowing for Complex Supramolecular Self-Assembly Inside the Cell Forming Cellular Compartments/Organelles

Key processes such as metabolism, energy conversion, replication… within and enlaced by compartments allowing to form chemical/molecular, electrical or energetic gradients, are fundamental requirements for all known forms of life. Thus, the ability to create compartments, as well as defined reaction spaces, is an essential feature in living systems. And as such, nanocompartments and reactors which can be added to the cellular tool-box of genetically encoded modules may allow us to expand the functional capabilities of the cell for many applications in biotechnology (Schreiber and Schiller 2013). Such elements are important modules in synthetic biology. The exact engineering and genetic programming of protein-tectons allows a direct translational and, therefore, genetic control over the formation of one of natures holy grails—the membrane enlaced organelle! Protein membrane based organelles (PMBOs) are a new class of protein-based compartments (Huber et al. in revision). They are comprised of amphiphilic block-domain proteins able to self-assemble spontaneously into vesicular organelles in vivo. The formation of vesicular structures based on amphiphilic proteins can be viewed in

close analogy to amphiphilic lipids. The amphiphilic proteins consist of a hydrophobic and a hydrophilic segment allowing the formation of dynamic vesicular compartments. In contrast to lipids, which are products of complex enzyme reactions, proteins have the advantage of ribosomal synthesis under direct genetic control. The organelle system allows for its covalent modification creating "de novo organelles" with small molecule modification in vivo (Huber et al. in revision). Figure 1, V. shows an amphiphilic protein-organelle within the cellular context. This approach can be envisioned to be the far-reaching beginning to utilize genetically encoded artificial organelles as nanoreactors, creating new combinations of natural and artificial catalysts in order to group chemical reactions via nanoscale assemblies of enzymes/catalysts. Implementing the molecular diversity of small functional molecules which are accessed by chemical biology approaches, in the context with the site-selective introduction of unnatural amino acids, opens up an additional level of control and functional expansion of PMBOs. These steps towards synthetic organelles are important to gain access to controllable "production-organism" in metabolic engineering, synthetic biology and biotechnology.

5 Protein Tectons II: Protein-Libraries and Mimicry of the Extracellular Matrix for Regenerative Medicine

Material libraries based on proteins may be viewed as physically detached from the usual tools and modular components in synthetic biology. Reflecting on the cellular control elements accessible via genetic engineering, one recognizes an important key player for complex multicellular systems—the extracellular matrix (ECM). The ability to program cells to express a redesigned extracellular matrix may have far-reaching consequences in tissue formation and gene-therapy-based treatment of many complex diseases involving cell-matrix interactions, e.g. in dermatology. The artificial ECM can be regarded as extracellular compartment and module controlling complex cellular behavior such as signaling, differentiation and proliferation—another core component of synthetic biology (Fig. 1, VI.).

Engineering this important element of complex tissues takes synthetic biology from the unicellular—intracellular level to the multicellular—extracellular—interaction and control level. Thus, biogenic materials systems accessed in a more modular and systematic level can be regarded as an outer border of synthetic biology not yet recognized, and implanted in the typical "tool-box" of modular components envisioned for synthetic biology requiring new technologies such as the Protein Assembler Technology (Schiller and Huber 2013).

Structural proteins (Pikkarainen and Kulonen 1972) such as silk (Porter et al. 2013; Holland et al. 2012; Liu et al. 2008; Shao and Vollrath 2002); resilin (Haas et al. 2000; Elvin et al. 2005; Andersen 2010); elastine (Wise et al. 2013; Heeger and Rosenbloom 1980; Vrhovski and Weiss 1998; Wise and Weiss 2009) constitute important mechanical, but also directed functional interactions. Recombinant silk (Xia et al. 2010), resilin (Su et al. 2013; Elvin et al. 2005) and elastine (MacEwan

and Chilkoti 2010; McPherson et al. 1996; Rodriguez-Cabello et al. 2011) allow for the engineering of materials with defined properties. These recombinant structural proteins are extraordinary because of the strength, resilience or elasticity of their native archetypes (Weisfogh and Andersen 1970; Bailey and Weisfogh 1961).

Contemplatable sequences comprise mainly repetitive and partly asymmetric motives for 3D-scaffolds with tunable elasticity and epitope presentation creating new opportunities for mimicking the extracellular matrix (ECM). Due to the difficulty of accessing such specialized sequences, the current recombinant DNA-technology needs to be revised. Current methods are mainly used to access individual proteins for intentional and rational design (Rodriguez-Cabello et al. 2011; van Hest and Tirrell 2001; Gomes et al. 2012). Strategies such as concatemerization and recursive ligation techniques (McDaniel et al. 2010; Meyer and Chilkoti 2002; Goeden-Wood et al. 2002) allow for the creation of defined sequences, but are limited if libraries of diversified long sequences are required. Polymerase chain reaction (PCR) based amplification strategies (e.g. overlap extension polymerase chain reaction—OEPCR) or rolling circle amplification (RCA) (e.g. overlap extension rolling circle amplification- OERCA) (Zhang et al. 2013; Amiram et al. 2011) have a focus on DNA-libraries with a statistical pool of structurally similar or quasi homogeneous, but different length sequences. They have the disadvantage that no defined individual sequences required for the intentional design of new material libraries can be yielded. Hence, a new method is currently being introduced, the "one-vector-toolbox-platform" (OVTP), designed to generate DNA-template entities (DNA-tectons) de novo (Huber et al. accepted; Schiller and Huber 2013).

Designable elastic resilin (Elvin et al. 2005) and tropoelastine (Wise et al. 2013; Mithieux et al. 2013) properties, both very versatile bioactive materials, are currently being implemented into modular molecular tecton-libraries; introducing a combinatorial DNA-toolbox platform constituting defined protein-based biohybrid materials (Huber et al. accepted).

These methods are applied to gain access to defined protein-based materials for applications in tissue engineering, regenerative medicine, nanoconfined protein tectons as building blocks for biohybrid structures for nano particle assemblies, biomineralization, protein formulation, and nanohybridmaterials for novel optical, plasmonic, electronic and magnetic properties.

Furthermore, one may always argue to understand the word "synthetic Biology" literally, thus focusing on the "synthesis of molecules" via biological systems.

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The Cellular Chassis as the Basis for New Functionalities: Shortcomings and Requirements

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Abstract By default synthetic biology refers to construction of synthetic genetic programs. Yet, programs must be expressed within a machine, and the elusive but multipurpose "chassis" is usually taken for granted. The program replicates while the chassis reproduces, showing that maturation, ageing and senescence are core processes which must be taken into account in order to explore realistic outcomes. Functional analysis reveals the essential functions that we need to consider. Some are listed in the present chapter, with emphasis on the role of information recruitment. This is a built-in process of living organisms whose outcome is the production of an ever young progeny as a way to cope with ageing and senescence. Life innovates using Maxwell's demons-like nanomachines. This is at odds with standard engineering practices, opening up new perspectives for synthetic biology.

1 Synthetic Biology, Beyond the Hype

Synthetic biology (SB) is the new fashionable trend in the exploration of life. Curiously, there are almost as many definitions of synthetic biology (SB) as investigators involved in its practice. The subject is perceived as new, not only because the progressive development of genetic engineering has recently been so renamed but, because there remains a flavour of vitalism associated with life: "synthetic" is understood as a convenient way of ridding all mysteries associated with the very idea of living organisms, perceived as differing from other physical systems. This is reminiscent of the state of affairs of chemistry in the 19th century, when there was a commonly accepted split between organic and inorganic chemicals (van den Belt 2009). This is also an underlying reason for all kinds of irrational behaviours when laypersons see the concept of life connected to that of synthesis.

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Briefly, in parts of the world where the concept of God or gods is pervasive, the idea of SB clashes with the creative behaviour of gods, and this upsets the corresponding creeds, with concomitant emotional reactions (Pearson et al. 2011).

To avoid these irrational trends, we will restrict our view of SB here to the following epistemological processes:

- Reconstructing and understanding (Porcar et al. 2011): ignoring the usual "black box" meant to account for living processes, SB reconstructs life to explore whether we understand what life is and uncover missing entities using engineering principles.
- Abstracting (Endy 2005): SB follows the laws defining life (see below how they are developed in the present reflection). Using these laws (i.e. abstraction) allows investigators to apply them, using objects of a different physico-chemical nature. This gives birth to what is now often known as *xenobiology* (Schmidt 2010).
- Engineering (Endy 2005): SB designs and standardises «biobricks» to construct programs using a «chassis» with man's interests as the goal.
- Evolving (Peisajovich 2012): SB combines design and evolution to use (still poorly understood) principles that drive adaptation. Remarkably, there is a built-in principle meant to trap information in living organisms; and this has consequences for engineering (Binder and Danchin 2011).

The idea that life could be amenable to synthesis is fairly ancient, and, as with any novelty, and in the absence of understanding of what life is, this idea triggered both wild hopes and irrational fears (Charpa 2012). In recent times, Stéphane Leduc (1853–1939) assumed in his book *La Biologie Synthétique* that life could be considered as a particular example of a manifestation of physics and chemistry (Leduc 1912, Chap. 2). He summarized his views as a fairly modern statement:

> Le programme de la biologie synthétique présente déjà de nombreux chapitres: la reproduction de la cellule ou cytogénie; la reproduction des tissus ou histogénie; la reproduction des formes générales ou morphogénie; puis la reproduction des différentes fonctions ou physiogénie, de la nutrition, de la circulation, de la multiplication, de la sensibilité; enfin la reproduction des molécules organiques ou chimie synthétique. […]. De tous ces chapitres de biologie synthétique, seule la chimie organique synthétique est constituée, reconnue, admise, les résultats, rapidement obtenus, établissent son importance. Les autres parties de la biologie synthétique, la reproduction des structures, des formes, des fonctions non seulement n'existent pas, mais leur étude n'est pas admise; il est difficile de voir pourquoi. En quoi est-il moins admissible de chercher à faire une cellule que de chercher à faire une molécule?
>
> The program of synthetic biology already displays many chapters: the reproduction of the cell or "cytogenesis"; reproductive tissues or "histogenesis" reproduction of general forms or "morphogenesis", then reproduction of the different functions or "physiogenesis" of nutrition, circulation, multiplication, sensitivity, and, finally, reproduction of organic molecules or synthetic chemistry. In all these chapters on synthetic biology, only synthetic organic chemistry is incorporated, recognized, accepted. The results, obtained quickly, establish its importance. The other parts of synthetic biology, reproductive structures, forms, functions not only do not exist, but their study is not allowed. It is difficult to see why: how is it less acceptable to try to make a cell than trying to make a molecule?

In the same way and more recently, James Danielli (1911–1984), well known for his insights into the structure of cell membranes (Danielli and Davson 1935), wrote presciently (Danielli 1972, 20):

> The age of synthesis is in its infancy, but is clearly discernable. In the last decade (1960–70), we have seen the first syntheses of a protein, a gene, a virus, a cell, and of allophenic mice. Nothing with such dramatic implications has ever been seen in biology before. Previously, plant and animal breeders have been able to create what are virtually new species, and have been able to do so at a rate which is of the order of 10,000 times that of average evolutionary processes. A further increase in rate is now on the horizon. We need a few additional "firsts" before this will occur: (1) to be able to synthesize a chromosome from genes and other appropriate macromolecules; (2) to be able to insert a chromosome into a cell; or, alternatively to (1) and (2), to be able (3) to insert genes into a cell in some other way; (4) we must also learn how to bring the set of genes, which is introduced into a cell, within the domain of cellular control mechanisms, so that they do not run wild in the cell. None of these problems appear to be of exceptional difficulty.

2 The Core Riddle of the Living Cell Factory: Babies Are Born Young

At first glance of this brief historical overview we may wonder, and ask whether there is a feature that makes life highly original, as compared to other material systems. The most obvious attribute of living organisms is that they build up a progeny. This is not quite as straightforward as we may think, when we recognise living organisms may be sterile. However, such organisms are simply borrowing time in that they need to be descended from fertile organisms, which we need to account for. Animal societies and plants may have classes of sterile individuals, but they are always directly connected to a fertile lineage. Indeed, were life limited to infertile individuals it would have disappeared, unless there existed a continuous process of spontaneous generation with a creation time shorter than the life span of individual organisms. This is quite unlikely with the chemistry of life as we know it. However this process probably existed during the period that preceded the origin of life.

Furthermore, this progeny displays a specific quality, not shared with most standard engineering contraptions, emphasising a time-dependent disposition: ageing organisms become senescent and die—which is the standard fate, while their descendants are born young. However, ageing is sometimes positive, as can be seen in the well-described process of "growth advantage in stationary phase" (the GASP phenotype (Navarro Llorens et al. 2010, 34)) a property generally foreign to standard engineering knowledge (remember the role of running in engines, however). Contrary to intuition, after mixing a population of young bacteria with an old culture, the old one outgrows the young one (Helmus et al. 2012).

These observations raise many questions: are they reconcilable with the idea of synthesising life or with scaling up? Which physical processes differentiate the creation of young entities from old ones? And, more technically, which genes allow information to accumulate in the young progeny?

3 Cells as Computers Making Computers

Before going further it might help to propose a short definition of life, so that we can use it when contemplating engineering life. We will not further justify this view here (for in-depth analysis and references see Danchin 2009a, c).

Life requires the simultaneous presence of three intertwined processes:

- Replication (making an exact copy) of a program (akin to a "book of recipes")
- Reproduction (making a similar copy) of a machine (named "chassis" by investigators involved in the SB domain (de Lorenzo 2011)) allowing the program to be expressed, while defining borders of the living entity, and displaying an inside and an outside
- Metabolism: a dynamic process allowing running the program via management of fluxes of matter and energy and allowing recursive information transfer and trapping (that is, coding information at two distinct levels, supported by different material supports, and related to each other by an essential asymmetry introduced from one level to a second level).

With this view, synthetic life asks that one places the program within a chassis and that the program is physically distinct from the chassis. The seminal experiment demonstrating the possibility of the transplantation of a whole genome that will further drive construction of a progeny, has experimentally established this separation (Lartigue et al. 2007). However there is a considerable constraint in the matching processes that allow the program to be expressed in a particular chassis (Itaya et al. 2005). This is not surprising as, even in the case of computers, portability of the Operating System (OS) is never guaranteed (Danchin 2009a).

SB usually aims at synthesising novel genetic programs, assuming that previously characterized chasses, preferably from generally recognized as safe (GRAS) organisms, will yield expected outcomes. Preferred candidate organisms are *Bacillus subtilis, Escherichia coli, Pseudomonas putida, Saccharomyces cerevisiae. Mycoplasma* species are essentially used as conceptual models meant for proof-of-principle experiments. Many others are possible, in particular belonging to the alpha-proteobacteria, that can give rise to autonomous organisms with a small genome (such as *Bartonella* species) and are supposed to be the ancestors of mitochondria (Andersson et al. 2003). Cyanobacteria are also interesting candidates because of their ability to fix carbon dioxide in the presence of light. However they usually display complicated chasses, with internal membranes, in particular.

4 The Minimal Genome, an Elusive Holy Grail

Understanding life requires a considerable level of abstraction. This can be seen in the evolving concept of the gene (Stadler et al. 2009), which has initially been identified as an abstract "character" that could be associated with a specific

feature of the organism (a phenotype) and manipulated by rules of logic (presence/absence); as well as apparent linkage groups (some characters tend to be dissociated from one another, whereas others go together at a high frequency). By contrast it was much easier to dissociate living organisms into well identified chemical components. This often leads to biochemistry being perceived as the ultimate proof of a biological concept, requiring identification of an individual object that can be defined by its molecular mass as well as catalytic or other structure-related properties. For this reason biology is still dominated by a structuralist view (as can be seen in the frequency of structure displays on the front cover of fashionable science magazines), where structures are usually thought to be enough to predict functions, in a more or less bidirectional equivalence. At the onset of genome programs this epistemological constraint brought about the quest for a minimum set of essential functions (Table 1, adapted from reference Danchin 1988) required to allow the development of life. Predominance of the structural view (wrongly) implied that the corresponding functions should be found by comparing genome sequences, looking for common genes (orthologous gene conservation).

This structuralist view of biology resulted in the widespread idea that the structure was enough to tell the function (which was indeed true in the case of the DNA double helix). As a consequence, because of the universality of the rule that defines the genetic code, the gene sequence was the ultimate label of a function, following the expected information flow: sequence → structure → function. With this view, comparative genomics, where sequences could be aligned with one another between different genomes, was the ultimate approach that would define a minimal set of sequences/structures. In turn, this would define the minimal set of functions required for making a living cell. The consequence of this common view was that it was expected that overlapping many genome sequences would result in the identification of a minimal genome that could be used to program the core chassis for SB constructs. This common view was illustrated by a first work benefiting from the sequencing of the first two complete (and very small) genomes, those of *Haemophilus influenzae* and *Mycoplasma genitalium* (Mushegian and Koonin 1996).

Table 1 Predicted genome sequence required for synthesis of the minimum set of genes necessary for life (Danchin 1988)

Process	Structure	Length (kb)
Replication	DNA wielding machinery	40
Transcription	Transcription + coupling with translation machinery	30
Translation	Ribosome: ribosomal RNA + 50–60 ribosomal proteins	60
	tRNAs + tRNA loading + polypeptide synthesis	80
Core metabolism	Building blocks and coenzymes	
Transport	Import and export	200
Energy management	ATP synthesis and electron transfers	
Specific casings	Creation of an envelope	100

This quest illustrates a general trend that unfortunately misses an essential point which is central to SB: life is built on in an abstract world, that of relationships between objects (Danchin 2003). This precludes confounding structure and function. Hence, the standard "Rosetta stone" stance, based on the bottom-up approach that uses sequence-based comparative genomics to identify conserved genes, is doomed to fail, at least if taken without a grain of salt (Acevedo-Rocha et al. 2013). There are no conserved orthologous genes in all extant genomes, despite conservation of proteins involved in the translation machinery in most of them (Lagesen et al. 2010). A way of circumventing this obstacle while keeping a bottom-up view is to use the fact that, during evolution, functional solutions that are successful tend to be preserved in the progeny. This observation gave rise to the concept of "persistent" genes, i.e. genes that tend to be present in a quorum of genomes (but not in all genomes, Fig. 1).

The persistent-genes set may be used as a minimal set to identify, bottom–up, a core functional set (Fang et al. 2005). These approaches, however, are not based on the need for understanding function first, and it is likely that they will miss some important functions, in particular ubiquitous functions that can be carried out by a variety of structures, as will be discussed below.

A diametrically opposite approach tries to see whether conceivable structures might fulfil the requested function by proposing a list of plausible functions. This method of exploring life is probably highly relevant, as it directly relates to the process of evolution. Living organisms evolve in such a way that if, during their life time

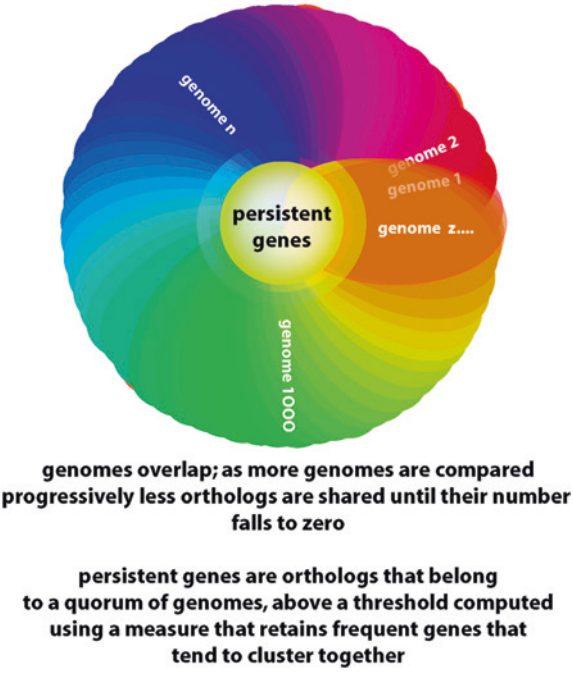

Fig. 1 The concept of gene persistence. Orthologs of persistent genes are present in a quorum of genomes

while producing a progeny they discover a structure that can fulfil a "useful" function (i.e. a function that increases the probability of having a numerous progeny), they will tend to keep the structure and transmit it to their descendants, either genetically (i.e. if the structure is coded in the genome) or epigenetically (i.e. if the structure is produced by any means that ensures heritability). Indeed, browsing known requirements for the development of life, a list of basic functions (ensuring reproduction of the chassis and replication of the genetic program) was proposed as an early incentive to support the first genome programmes by the European Commission (Table 1) (Danchin 1988).

At the time, the corresponding genes were supposed to be essential. It was however necessary to identify essential genes experimentally. Experiments were performed by many laboratories and it was observed that the number of essential genes varied according to the experimental method used, with a more or less incompressible minimum number of approximately 250 essential genes (Acevedo-Rocha et al. 2013). This was half of the number predicted as coding for essential functions (counting 1 kb for an average gene coding sequence, Table 1). Further work demonstrated that essential genes were located in the leading replication strand (Rocha and Danchin 2003) and that orthologs of the minimal set were conserved in a majority (but certainly not all) of genomes. By contrast, the genes that do not appear to be essential but are conserved and located in the leading strand of the chromosome's DNA, make a particular category, which doubles the number of essential genes. In fast growing organisms they further tend to be located near the origin of replication. Taken together these genes make a universal category which is most often present in genomes. They constitute the family of persistent genes (Acevedo-Rocha et al. 2013; Fang et al. 2005). Thus, 400–500 genes persist in a majority of bacterial genomes; they are not only involved in the three processes needed for life, but in maintenance and in adaptation to transient phenomena; a fraction manages the evolution of the organism and some cope with core intermediary metabolism.

When taking into account the schematic definition of life proposed above, it was possible to assign four major functional categories to these genes:

- information transfer
- compartmentalization
- intermediary metabolism
- stress, maintenance and repair

5 Structural Constraints in the Genome Layout

These categories are obviously highly non-random. The way the corresponding genes are distributed in genomes displays common features. In particular the cognate genes are expressed from the leading DNA strand, reducing transcription/replication conflicts (Rocha and Danchin 2003). The building up of a proper SB chassis should therefore maintain this particular organisation. As a matter of fact, the genetic program has a material support, DNA, which has hidden embedded

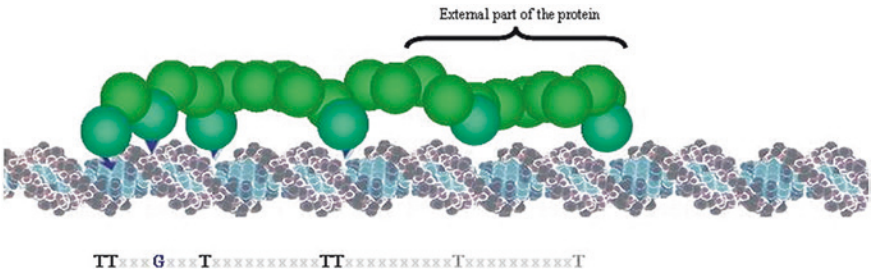

Fig. 2 DNA condensation. The genome DNA molecule is seeded with nucleotides that create binding sites for DNA binding proteins that result in DNA condensation (Larsabal and Danchin 2005), a process that allows the DNA molecule to be folded within a small space

constraints due to its chemical nature. In SB constructs it is not enough to have a DNA molecule with the right sequence, the molecule needs to be correctly folded to fit the size of the host cell. This is quite visible in the way genome transplantation has to be performed. Upon lysis of the donor cell, or total synthesis of donor DNA, the molecule is in an expanded filament form. As a consequence, it cannot enter the tiny host cell. This incompatibility has been experimentally circumvented by using polyethyleneglycol (PEG) which makes a large syncytium of host cells that can accomodate a decondensed DNA molecule (Lartigue et al. 2007). Once within the syncytium, the genetic program directs synthesis of proteins that condense DNA, making it fit to the size of a single hosting cell (Fig. 2, adapted from reference Larsabal and Danchin 2005).

Many further constraints stand out in extant genomes. For example, it has been repeatedly observed that a genome is interspersed with genomic islands preserving local biases in the codon usage (Yoon et al. 2005). In the same way common functional properties result in genes being clustered together (Fang et al. 2008). As a case in point genes involved in sulfur metabolism form islands (Rocha et al. 2000b), suggesting that there is a link between gene function and gene localisation in the chromosome (which is connected with localisation of gene products). These constraints are important for efficient engineering (Rocha et al. 2000a). Overall, putting together known constraints in genome sequences may result in the construction of a decision-tree for the design of proper engineering constructs. This should direct genome organisation in SB constructs aiming at successful gene expression.

6 Function First

Thinking in terms of function first is exactly the way designers work (Fig. 3). They define a master function for the device they plan to construct, and subsequently make a hierarchical list of parts, beginning with a variety of helper functions, each one the subject of specific downstream functional analysis. This procedure is well illustrated in the construction of a computer. Its master function is to run

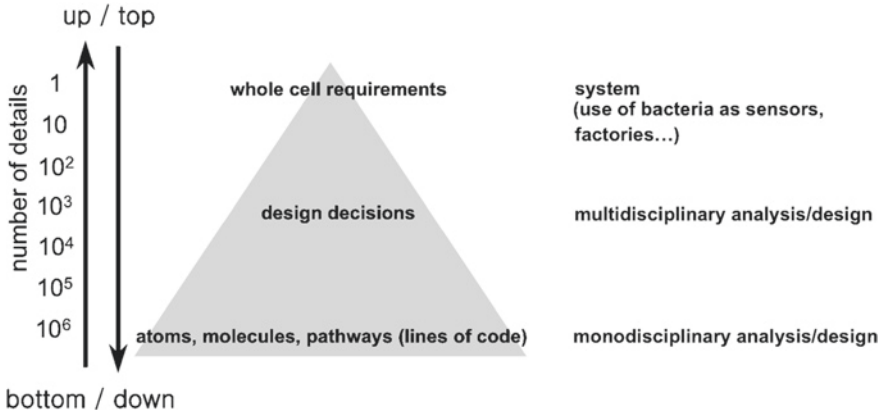

Fig. 3 Functional analysis. Designers usually proceed top–down, starting from a master function that is split into progressively finer details, with characterisation of helper functions

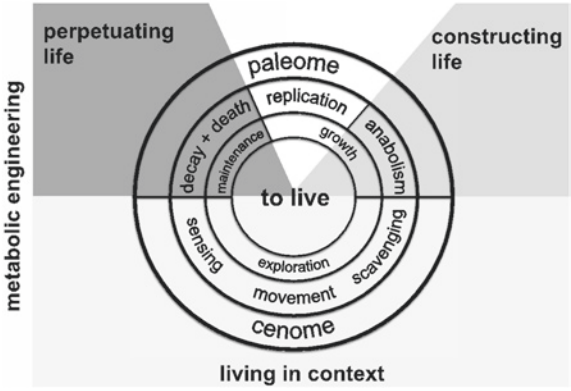

Fig. 4 The paleome and the cenome. Two genomes correspond to the two master functions driving life. The paleome runs the processes that allow cells to reproduce, while the cenome allows cells to explore their environment

algorithms, depending on highly specific functionalities that will define the type of computer: mainframe, for heavy computation, desktop for versatile use, PC for travel etc. Novel uses have been created with smart phones and tablets.

Depending on the point of view, the master function that drives living organisms is either their ability to create a progeny, or their ability to explore the environment in an organised way. This is reflected in the set-up of the genome, which displays two histories, embedded in two distinct functional sets. The former master function implies that the cells are reproduced, with concomitant replication of their genetic program. This master function is encoded in the *paleome*, the part of the genome that encodes functions (not structures, as seen above) conserved in all extant autonomously living cells. The latter master function, exploration, is encoded in the *cenome*, encoding an unlimited number of subfunctions involved in specific, means of exploration that differ between organisms and allow them to occupy their preferred niche (Fig. 4). The paleome mainly manages anabolism,

replication and maintenance (reproduces the chassis and replicates the program); the cenome manages life in context, i.e. explores the environment via sensing, managing movement, scavenging etc.

Up to this point, our view of functions has been fairly general. However, engineering requires specifying the minutest details. This implies making a list of further functions, some of which are rarely or never thought of to be relevant to life. Some are needed in all types of constructs. Others allow specifically designed outcomes. Here is a partial list of possible functions that must be considered, taking into account the five category of reality: matter, energy, space, time and information.

chassis structure:
- casings
- bumpers/buffers
- scaffolds
- microcompartments

sensory/motor:
- sensing
  - sensors for the outside
  - in the envelope (piezosome)
  - for the inside
- movement and localisation (of the whole cell)
  - flagella
  - fimbria
  - adhesins
- movements (inside)
  - chromosome partition (SMC proteins)
  - actin/tubulin

energy and matter flows:
- importers/exporters
  - small molecules
  - macromolecules (competence for DNA, protein export)
  - whole structures (bacteriophages)
- safety valves
- ATP synthase
- storage
- management of waste (trashing, shedding)

program and information transfers:
- replication
- transcription
- translation
- shaping: molecular chaperones

sensory/information:
regulation of gene expression (typically: two-component systems)
mechanosensing
Maxwell's Demons (use of NTP to reset functionality; e.g. ATP-dependent proteases)
Maxwell's Demons (measure of ageing and prevention or organisation of diffusion, e.g. septin-like GTPases)

These functions can be illustrated concretely. For example, to build up a bacteriophage such as phage T4, a scaffold is constructed and used as a vernier to make a tail of fixed length, with the proteins of the tail making a helix structure around the scaffold that is later disposed of. This structure is therefore not only a scaffolding device but also a measuring device (Arisaka 2005). In the same way, several scaffolds are used to produce the overall shape of the bacteria, which can be spherical, elongated, helicoïdal, branched, etc. (Celler et al. 2013). Remarkably, there may be a link between shape and distribution of specific genes in the genome (Tamames et al. 2001). In another example we can predict the existence of safety valves. Indeed, permeases are importers that often use energy (from electrochemical origin, or from ATP) to allow useful compounds to be concentrated within the cell. Because of sudden variations in the environment that would require exquisitely complex regulations to be dealt with, the cells have selected a solution that is similar to standard human design: they have created safety valves that export the overflow of the input compound, or a derivative when it reaches a dangerous concentration (Danchin 2009b).

Management of waste is also of major importance. During the process of construction of particular metabolites there is a significant amount of leftovers that need to be dealt with. In the same way, control of gene expression requires RNA turnover, and very short RNA segments (nanoRNAs) are produced continuously. They are degraded into the corresponding mononucleotides, which can be recycled into anabolic processes (RNA and DNA synthesis in particular) by nanoRNases. These enzymes in some cases also fill the function of degradation of 3′,5′-adenosine bisphosphate (pAp) which is a by-product both of the reduction of sulfate and lipid biosynthesis. In *E. coli* cysQ mutants require sulfite or cysteine for growth because they fail to degrade pAp, using CysQ; another enzyme, Orn, degrades nanoRNAs. Purification of pAp binding proteins from this organism identified CysQ and also protein Orn that hydrolyzes very small RNA molecules (nanoRNAs) (Mechold et al. 2006). Remarkably, complementation of an *E. coli orn* defect by libraries from a variety of bacterial genomes revealed proteins coming from unrelated structural descents, some of which were also able to hydrolyze pAp, i.e. could play the role that CysQ plays (Mechold et al. 2007). Furthermore, organisms such as *Mycobacterium tuberculosis* have counterparts of both *orn* and *nrnA*, while they also have a *cysQ* gene showing that degradation of nanoRNA is very important in this organism with a complex lifestyle (very long persistent life in particular) (Postic et al. 2012).

Another function that must be implemented in cells is that of clocks. If cells are computer making computers, they work in a highly parallel fashion. This requires clocks to synchronize activities. Clocks may be created by a large variety of biochemical processes, involving regulation of transcription and protein turnover, in particular. However it appears that some processes are not related in any straightforward manner to transcription regulation (Hosokawa et al. 2011). An engineer would ask the question: Is there a structural property in proteins that may be used for measuring time? This amounts to looking for time-dependent evolution of protein structures. Remarkably, two amino acids, aspartate and asparagine, do evolve in a time-dependent way, as they cyclise into L-succinimide within the protein backbone (Fig. 5, adapted from reference Danchin et al. (2011)).

This process is both fast and frequent, in particular at asparagine-glycine (AsnGly) motifs because of the intrinsic flexibility of glycine (Robinson and Robinson 2004), with an associated deamidation of asparagine. L-succinimide will either hydrolyse into L-isoaspartate spontaneously or after methylation (in 2/3 of the cases), or L-aspartate (in 1/3 of the cases). Subsequently, L-succinimide

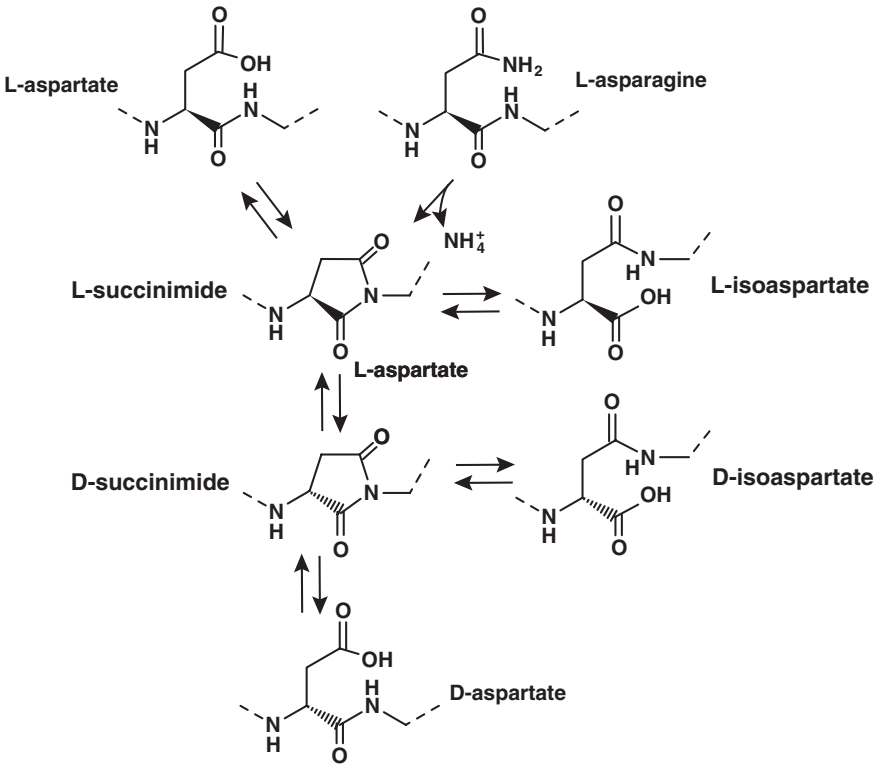

Fig. 5 Spontaneous evolution of asparagine and aspartate residues in proteins. Asparagine and aspartate residues cyclise into L-succinimide that will progressively lead to L-isoaspartate and via a series of isomerisations finally to D-aspartate, if context permits

will isomerise into D-succinimide at a slow rate, and then lead to formation of D-isoaspartate and D-aspartate residues. This process is general, and sequence context-dependent. As a consequence, proteins behave as clocks with a half-time for changing the structure of their backbone strictly dependent on their sequence (and the local environment in the physico-chemical parameters of the cell).

An important consequence of this type of behaviour is that information processing by cells goes far beyond information carried by the genetic program. This hints at the existence of deep processes managing information in all living organisms, in previously unrecognised ways.

7 A Challenge for Synthetic Biology: Information Trapping

A puzzling observation shows that we need to explore more in-depth the way synthetic biology takes into account the information-related functions of life:

- phage T7 has been redesigned according to engineering rules, tested using mathematical models and then transformed into *E. coli* cells; its design can be understood by human engineers (Chan et al. 2005)
- the synthetic phage forms lysis plaques; however, they are smaller than those of its natural counterpart
- the evolution of the synthetic phage to more virulent forms, making larger plaques, alters considerably the human construct (Springman et al. 2012)

How can this latter feature be understood? Back in 1974 John Hopfield stated that in order to identify important unexpected functions, we should explore metabolic reactions that use energy in an apparently expletive way: «*known reactions which otherwise appear to be useless or deleterious complications.*» This is the case observed, for example, with eFTu, eFTs, where GTP is hydrolyzed in a process controlling translation accurracy. Energy is used in the process to reset eFTu to its original state when a correct codon-anticodon pair has been formed, ensuring the right correspondence between a codon and its cognate aminoacylated tRNA. Another example is that of energy-dependent proteases. Degradation is exothermic: why should degradation processes use energy? A detour via the concept of Maxwell's Demon will tell.

Creating a link between information and entropy, James Clerk Maxwell invented an imaginary being, later named a 'demon', who could use a built-in information-processing function to reduce the entropy of a homogeneous gas (at a given temperature) (Maxwell 1871). Briefly, the demon is able to measure the speed of gas molecules and open or close a door between two compartments as a function of the molecules' speed, keeping them on one side if fast, and on the other side if slow. This will build up two compartments, one hot, and one cold, reversing time, and acting apparently against the second principle of thermophysics, since this set up forms the core of the work-producing steam engine.

The physics of information-processing derived a considerable variety of attempts to understand how Maxwell's demon could function. At a lecture in Göttingen attended by the most creative physicists and mathematicians of the time, Smoluchowski gave details of the way Maxwell's demon could be implemented as a trap door, permitting information to be coupled to availability of energy and material states of molecules in the environment (Smoluchowski 1914). Later on, Szilard proposed in a loose way to account for the relationship between infomation and entropy, and in the 1950s von Neumann followed suit, stating that each logical operation performed in a computer at temperature T must use an energy of kT ln2, thereby increasing entropy by k ln2 (von Neumann 1966). This remained the accepted consensus until the IBM company, which was concerned by the limits this would impose on computation, asked its engineers to explore the situation and propose possible remedies. Working at IBM, the limits of physical computation would have been reached rapidly, had the Szilard-von Neumann intuition standard been valid. Fortunately for computer sciences this view proved to be wrong. At IBM, Rolf Landauer demonstrated, 50 years ago, that computation could be made to be reversible, hence not consuming any energy (Landauer 1961).

In his conceptual work, Landauer showed that reversible, one-to-one, logical operations such as NOT can be performed without consuming energy. He also showed that irreversible, many-to-one operations such as ERASE require consuming at least kT ln2 of energy for each bit of information lost. The core of the argument behind Landauer's theorem can be readily understood. Briefly, when a bit is erased, the information it contains must go somewhere. This can happen in only two possible ways: either the bit moves to a place in the computer (or in the cell, if we consider cells as computers) corresponding to an observable degree of freedom, such as another place with a known bit in its memory. If so, it has obviously not been erased but merely moved. Or it goes into places with unobservable degrees of freedom, such as the microscopic motion of molecules, and this results in an increase of entropy of at least k ln2.

In 1973, Bennett extended Landauer's theorem, showing that all computations could be performed using only reversible logical operations, that is, without consuming energy (Bittencourt et al. 2012). But, where does the energy come from? To perform a logical operation, energy is commonly extracted from a store of free energy, then used in the processor that performs the operation, and finally returned to the initial store once the operation has been performed. We note here that in usual computers the store is a battery or an outside electric supply, whereas in cells energy is distributed throughout the matter of the cell. This may have considerable consequences for the computing power of cells (not discussed here). The property of reversibility has been implemented in real computers under the term "adiabatic logic", and real circuits have been described in detail to explain how this works. In the domain of SB, it is interesting to note that Tom Knight, one of the founders of iGEM at MIT, has been seminal in the actualisation of this work (Younis and Knight 1993). Hence, the connection between information theory,

computer sciences and biology is much deeper than most laypersons (and many biologists) might suspect.

Back to Maxwell's Demon: In a real computation, errors occur, and getting rid of them requires an irreversible operation, erasure of incorrect information and replacement with the correct information. Hence, this will result in consuming energy in order to restore the errorless situation. If energy were not consumed, then the system would be able to go backwards in time, and we would have created the perpetual movement. How does this work in reality? The situation is similar to the proposed action of Maxwell's Demon: measure, store information, use it via replication of the measurement to reestablish the initial state, and then erase the memory, to reset the initial state of the demon. Two logical processes are central to this action: REPLICATE and ERASE.

If the error rate is x bits per second, for example, then error-correcting processes can be used to detect those errors and reject them to the environment at an energy cost of x kT ln2 J s^{-1}, where T is the temperature of the environment. In fact, biological processes, even at the microscopic level, do not proceed bit by bit, but, rather are highly redundant and simultaneously change a fairly large number of bits. This is because at 300 K (the average temperature of life environment) the thermal noise is fairly high so that redundancy is necessary to increase the signal to noise ratio. And the usual "quantum" of energy used is that of hydrolysis of an "energy-rich" phosphate bond, typically hydrolysis of ATP to ADP or GTP to GDP.

Such error-correcting routines are the norm in biological processes, and function as working analogues of Maxwell's Demon, getting information and using it to reduce entropy at an exchange rate of kT ln2 joules per bit, and rejecting errors to the environment at a high rate to maintain reliable operations. This reflection is therefore at the core of what should be a renewed view of the process of ageing. This is obviously of particular importance for SB as the cell factories multiply and age (Binder and Danchin 2011; Danchin 2012).

8 What Does This Imply for the Future of Metabolic Engineering?

The common view of SB is that of a cell factory, which allows expression of complex programs that can answer many questions of metabolic engineering, as well as develop computation abilities (Daniel et al. 2013). However this view does not take into account the processes of ageing, which require specific maintenance steps that involve novel functions, usually not considered by the authors of SB constructs. Life has evolved many such processes, with the consequence that contextual information is constantly monitored, and sometimes trapped within structures and processes, making living cells information-trapping devices

(Binder and Danchin 2011). This is not a property that is current in standard engineering (although computers are now learning from the way they are used, beginning to make them information-trapping contraptions). This process needs to be taken into account in order to get output which match expectations:

- the engineering view of SB precludes that artificial cells be innovative
- it is possible to exclude the genes permitting accumulation of information
- the consequence is that, as with all factories, the cell factory will age and will need to be systematically rebuilt
- but this poses problems when applications require that industrial processes are scaled-up: this may not be possible, unless we can harness the function of the Maxwell's Demon's genes to the human goals.

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Hazards, Risks, and Low Hazard Development Paths of Synthetic Biology

Bernd Giese and Arnim von Gleich

Abstract In early stages of research and innovation a precise investigation of technological risks, as well as the analysis of particular beneficial features, is confronted with a lack of knowledge about exact process or product qualities, application contexts and intentions of users. Therefore, an appropriate identification of anticipated risks, accompanied by the achievements of synthetic biology, should rather focus on basic properties and functionalities of the objects of synthetic biology which will be exploited in future products and processes. Accordingly, the aim of this chapter is to determine major risk factors of synthetic biology creations with a focus on the technology itself. In consideration of the demand to cover these risks by appropriate counter measures, the question is raised, whether there are suitable strategies to achieve a high level of safety. In this regard, the discussion will be extended to feasible alternatives, e.g. by introducing trophic and semantic isolation strategies for synthetic organisms as an approach to overcome major drawbacks of classical biosafety mechanisms. Finally, functional reduction, a concept which is already aspiring to achieve efficient biosynthesis, is suggested as a measure for the reduction of risk-related functionalities. This strategy is worth further investigation if the full potential of synthetic biology is to be obtained in a safe and sustainable way.

1 Introduction

As technology assessment based on the precautionary principle is engaged in the early stages of innovation, the focus should be on technologies or interventions that have severe uncontrollable and irreversible effects. Especially where

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technologies or interventions provoke the dissolution of temporal and spatial boundaries, a precautionary approach is needed because in the case of failure the scope of corrective interventions is extremely limited. This makes a key difference to technologies, interventions and modifications whose reliability could be tested and improved by trial-and-error approaches. In practice, an application of synthetic organisms (including synthetic viruses) for medical purposes should in any case avoid non-intended proliferation or transfer of genetic information from the synthetic entity to the patient, not to mention an uncontrolled dissemination to any other person or organism.

Thus, technology assessment and design, in an early stage of innovation, analytically concentrates on constructs and interventions where failures with irreversible consequences have to be prevented, since later counter measures are not available. As path dependencies in an early stage of innovations are not yet fixed, alternative approaches may be developed more easily by applying a precautionary design to enable low hazard development paths.

In order to work with these approaches it is necessary to identify technologies or interventions of high concern. Characterizing criteria are needed to determine the special type of interventions or technologies that is connected with such unlimited consequences.[1] With the concept of 'depth of intervention' we try to focus at those technologies that intervene deeply into systems at different system levels. The hypothesis is that deep, remaining alterations of structures which are able to control certain phenomena at different systemic levels (e.g. interventions into atoms, molecular structures, genes) are often extremely powerful and able to cause unlimited chains of effects in space and time, as we have experienced so far with atomic energy (radioactive waste), synthetic chemistry (e.g. CFCs) and genetic engineering (deliberate release of GMO).

Focusing on the functionalities and the technological power of synthetic biology leading to technological opportunities on the one hand, and to hazard and exposure on the other hand, the two main elements of risk must be investigated in more detail. Without an exposure or a probability of occurrence for damage, there may be a potential hazard but no risk at all. Therefore, in addition to an assessment of hazards, the probability of exposure to a certain noxa and its determinants, the sensitivity of the exposed receptor as well as the probability of occurrence of adverse effects have to be determined as part of risk assessment.[2]

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[1] Unlimited consequences are defined as ramifications of causes and effects with a high range in space and time, ultimately global and irreversible.

[2] The concept of risk has different definitions. In the well-known current understanding risk originates from an adverse incident and its occurrence probability. This chapter refers to a definition of risk in an (eco)toxicological sense. Here, risk is defined as a function of hazard on the one side, and exposure on the other. Therefore, a hazard, caused by specific qualities and functionalities, is defined as the potential of an agent (entity or noxa) to cause an adverse effect on a receptor (e.g. organisms, systems, (sub)populations) (IPCS 2004). Exposure is defined as the concentration or amount of a particular agent that reaches a target organism, a system, or a (sub)population in a specific frequency for a defined duration (IPCS 2004). Special functionalities and qualities of

Thus, in an early stage of innovation it is possible to identify functionalities and qualities of concern, which may lead to severe hazard and/or high probability of exposure. But to identify and quantify risks much more knowledge is needed, especially concrete knowledge about the intended use and the final application context of products and processes based on synthetic biology. They are indispensable for the quantification of exposure to, or probabilities of the occurrence of, adverse effects. In the phase of basic research, where most of synthetic biology still resides, statements on risks combined with new biological constructs can be only vague or speculative, as long as the intentions and contexts of use are unknown. This lack of knowledge is definitely a severe problem and one could ask, whether it may be better to start technology assessment in later stages. But the advantage of early investigations and interventions is the high scope of action in a phase when path dependencies are still low. This predicament between knowledge and scope of action in different stages of innovation is mainly discussed as the Collingridge Dilemma (Collingridge 1980).

Thus, the focus in technology assessment of synthetic biology should rather be on what is already present and observable. That is, in cases of technology-push innovations the technology itself has to be investigated. For synthetic biology new or enhanced functionalities as sources of benefits (but also of hazards and exposure as well) represent the relevant characteristics for technology assessment. The investigation of specific and quantifiable risks must be the subject of forthcoming innovation stages of processes and products based not only on the functionalities of synthetic biology, but also on specific knowledge regarding intended use, probability of exposure and sensible application contexts like medicine or food production. Therefore assessment of hazards and high potential of exposure, based on basic functionalities and entities of synthetic biology and of their potential interactions with the living and non-living environment (including biogeochemical processes[3]) is currently our main subject in technology assessment of synthetic biology.

Furthermore, the ratio of options for opportunities on the one hand, and hazards on the other hand, may be investigated. High concern is not a sufficient reason to change the development path in all cases. There could be a number of good reasons for legitimizing measures and technologies with a severe depth of

Footnote 2 (Continued)
an agent, as the ability for self-replication, persistence in organisms and the environment (including bio-accumulation), mobility in environmental media and within organisms and—as an external driver—mass production, are therefore leading to a high probability of exposure. The notion of sensitivity (and bioavailability of an agent) of the exposed receptor is additionally important, because one and the same agent may lead to quite different effects in systems depending on the systems' states (developmental phase, trajectory, intensity, energy content etc.).

[3] Cf. Commission Decision, Annex Nr. 3.2.5 of Directive 90/219/EEC.

intervention and therefore high impacts in special application fields and under certain conditions.[4] However, the other way round a strong beneficial effect cannot always justify the use of a technology of high concern.

Finally, risks resulting from such hazardous interventions and technologies could be minimized by appropriate operating conditions (e.g. a complex containment). But a preferable strategy should concentrate on the decrease of hazards by designing low hazard biological processes and constructs to minimize or avoid elaborate measures in limiting the exposure of hazardous entities or processes to the environment when it comes to applications.

In consideration of the extended, improved or new functionalities, achievable by combining biological elements, it becomes clear that synthetic biology, with its attempts to overcome evolutionary path dependencies, opens up a large variety of new possibilities to create or just alter biological structures, processes and systems. Hence, in later developmental stages of synthetic biology, the multitude of beneficial effects will most probably correspond to increase in the number of adverse impacts as well. Highly powerful technologies are, as a general rule, combined with high impacts and unforeseen side effects (Anders 1958; Jonas 1985).

To prepare the ground for a discussion of high potentials of hazard and exposure and how to identify development paths with minimized risks, the following chapter focuses on the main technological hazard and exposure factors in the field of synthetic biology. The first part analyses and structures these factors in synthetic biology against the background of its expanded technological capabilities and functionalities; especially in comparison to genetic engineering. Based on this analysis a number of preventive measures is discussed in the second part of the article.

2 High Potentials of Hazard and Exposure Accompanying Some of the Expanded and Improved Functionalities of Synthetic Biology

Sources of potentially hazardous functionalities of modified or synthetic biological entities can be subsumed in categories that are related to characteristic qualities as well as the molecular basis of (biological) life. In the course of early assessments related to the impact of synthetic biology, it is essential to begin with the discussion

[4] The societal position regarding genetic engineering (GE) obviously reflects this policy, when more research into microorganisms and into medicines/vaccines is massively supported by the European population but applications concerning farm animals, food and plants have the weakest support (cf. Eurobarometer 1993).

Table 1 Sources of potential hazardous functionalities of biological entities, following Benner et al. (2011)

Combinatorial level of Hazards	Categories of potentially hazardous functionalities		
	Molecular Interactions		
	Persistency/Proliferative Potential	Ability to Evolve	Natural Molecular Genetic Basis
a)			
b1)			
b2)			
c1)			
c2)			
c3)			

of three categories in particular as sources for the emergence of hazards.[5] Table 1 represents these categories which are published by Benner et al. (2011):

1. the ability to replicate or proliferate or at least persist,[6]
2. the ability to evolve and adapt and
3. a natural or closely related molecular genetic basis, enabling an exchange of genetic information.

A fourth category should complement the list:

1. Molecular interactions of intermediates and products of altered or newly introduced (bio-) chemical reactions (including metabolic reactions) with organisms as well as organic and inorganic matter in the environment.

The latter applies to all biological entities, but especially to newly designed processes and organisms of synthetic biology with an extended spectrum of (bio-) chemical activities in comparison to natural organisms. However, the following discussion focuses on the emergence of hazards and increased exposure and on

[5] Cf. OpenWetWare, an information platform managed by the BioBricks Foundation: "All in all, biologically speaking, these sets of problems boil up to two things: horizontal gene transfer and excessive proliferation, although emergent properties of synthetic systems could make these problems worse." And: "Other bacteria that seem harmless could cause harm too if released in the environment because of a potential negative consequence of horizontal gene transfer or excessive proliferation which could disrupt the ecosystem." http://openwetware.org/wiki/How_safe_is_safe_enough:_towards_best_pratices_of_synthetic_biology#iv._Physical_harms, accessed: July, 03 2013.

[6] In this context an additional functionality which cannot be further discussed due to restrictions of space is mobility which increases inner and outer exposure, realized either passively by transport or actively by an entities own capacity to change its location.

potential risk reducing strategies in connection with proliferation, evolution and transfer of genetic information. Therefore, molecular interactions will not be investigated in more detail. But it should be noted that molecular interactions of new processes, products and organisms with the environment should in any case be the subject of thorough prior investigations before.

Single or multiple combinations of the above mentioned categories in modified or synthetic biological entities may result in different combinatorial levels of hazard and exposure factors (see Table 1).

Certain constructs of synthetic biology can be assigned to all of the presented categories (Table 1, combinatorial level a) since they have a natural biomolecular basis. As such, they are able to exchange genetic information with natural organisms and eventually also parasites in the natural environment. They may be persistent (not metabolizable by other organisms) and are able to proliferate because their metabolism is not dependent on a substance provided technically. Additionally, they are able to evolve and therefore able to change themselves and their qualities over time, and thus adapt to altering environmental conditions. Taken together—like all currently known genetically modified organisms—these constructs combine all qualities and abilities of natural life. Because every single category could contribute (especially to delayed) effects which are considered as relevant for environmental risk assessment[7] a combination of all categories results in the most hazardous constellation. Also synthetic phages and viruses or viral and phage-like systems may belong to this level if they are able to propagate with the help of a respective host organism.

If one of the categories is not present in a putative construct of synthetic biology (Table 1, combinatorial level b) three combinations are conceivable: (b1) the entity is not able to persist or proliferate like, for example, liposomes carrying functional biomolecules. Anyway, if the vesicular enclosure contains genetic information (Nourian et al. 2012), horizontal genetic transfer could cause detrimental effects in receiving organisms or ecosystems. (b2) Biological constructs could also consist of an unnatural biomolecular basis which may prevent genetic transfer (as will be discussed later) (Schmidt and de Lorenzo 2012). Nevertheless, they could represent a potential source of hazard if they are independent and able to proliferate. This interaction with natural organisms on an ecological level, with subsequent displacement effects from ecological niches as well as accumulation of non-degradable compounds (depending on their biochemical composition), is the most likely consequence of population growth.

Constructs belonging only to a single category (Table 1, combinatorial level c) include in vitro arrangements of biomolecules which are not able to evolve or at least to sustain its function without continuous energetic or metabolic support (c1) and in vitro systems based on unnatural nucleobases which may be exchanged during

[7] Cf.: Guidance notes on the objective, elements, general principles and methodology of the environmental risk assessment referred to in annex II to directive 2001/18/EC Commission Decision, 24 July 2002, (2002/623/EC).

replication and therefore show a rudimentary level of evolvability (c2) (Sismour and Benner 2005; Benner et al. 2011). If systems are simply self-sustaining and even able to proliferate, but realized without any components with genetic functions, then, at least theoretically, we have to include attempts to create a protocell or a self–replicating peptide[8] here too (c3) (Solé et al. 2007; Solé 2009; Lee et al. 1996). Although in this group the number of categories that could contribute to hazard and high exposure is limited to a single quality, they could as well have far-reaching consequences if they tend to concentrate in natural organisms and persist for long time periods in a free environment.[9]

Notwithstanding these complexity grades and combinations, a qualitative difference and thus a hierarchy results even from the different character of these qualities. Tucker and Zilinskas underline that due to their potential to proliferate and evolve, engineered microorganisms

> […] belong in a different risk category than toxic chemicals or radioactive materials. (Tucker and Zilinskas 2006, 31)

And a further differentiation between proliferation and evolution can be introduced: proliferation is able to constitute high, up to ubiquitous exposure and therefore, besides persistence, is a major factor for risks.

3 Critical Application Contexts

Risks posed by a technology, as well as benefits, not only depend on its functionalities, but also on specific application contexts and objectives. Even a low-hazard approach in virtue of low depth of intervention and minor effectiveness could lead to unlimited effects, if applied to an extremely sensitive system or system context (e.g. as a consequence of its high sensitivity or the criticality of its systems services for nature or society[10]). A prime example demonstrating the critical difference between initial objectives and unintended impacts of an application based on hazardous functionalities (in this case exposure relevant persistence of non-natural and highly mobile substances) is the historical case of chlorofluoro-carbons (CFCs): They were introduced as "safe" chemicals to replace toxic or inflammable coolants, such as ammonia and chloromethane. But due to their persistence and unnatural composition (the latter is also the reason for a lack of degrading mechanisms) CFCs have accumulated in the atmosphere and started to deplete the ozone layer, which in turn made it permeable for ultraviolet radiation. As a lesson for constructs

[8] Self-replication of living organisms depends on genetic information. But for less complex entities other forms of molecular self-organization are possible, as for example revealed by self-replicating peptides (Lee et al. 1996).

[9] E.g. the impact of prions (Norrby 2011), though they are also proliferative in a broader sense.

[10] E.g. tipping point characteristics of large-scale components of the Earth System, cf. Lenton et al. (2008).

of synthetic biology we still have to consider long-term consequences caused by their persistency or capability for proliferation in prospective unforeseeable application contexts. According to this challenge, in particular realizing synthetic-biological constructs which contain all of the above mentioned functionalities is of high concern if not alarming, because the resulting combination provides some evidence for problematic behavior. For instance, Dana et al. explain:

> […] unlike transgenic crops, synthetic microbes will be altered in more sophisticated and fundamental ways (such as elimination of metabolic pathways), making them potentially more difficult to regulate, manage and monitor. They might also have environmental impacts that are difficult to predict. (Dana et al. 2012, 29)

In particular (a) loss of control caused by a dissemination could be enhanced by evolutionary changes (Moe-Behrens et al. 2013), (b) traits can be transferred to other organisms by gene transfer (Moe-Behrens et al. 2013; Wright et al. 2013) including wild types and related species in the case of plants (Andow and Zwahlen 2006), (c) modified or synthetic organisms might change their qualities by the integration of advantageous naturally evolved genetic information (Schmidt 2010), (d) toxic interactions on the metabolic level (Holmes et al. 1999; Hilbeck et al. 2012) and (e) probable displacement effects could occur (Wright et al. 2013).

However, proliferation and persistence are major causes of concern, as are significant quantities of produced biological entities that cannot proliferate or persist. Thus, referring to Table 1, the ability to proliferate or to accumulate respectively, have to be regarded as the most important qualities for excessive exposure and—given unforeseen detrimental interactions with, or within not previously considered natural systems—for the emergence of risks.

Of course containment may reduce this hazard remarkably. But for the currently known and operated physical containment systems no guarantee can be provided for an overall and permanent isolation of the enclosed organisms. Organisms may escape simply due to mishandling, poor maintenance or accidents.[11] Accordingly, attempts to work with preferably known as harmless organisms or an implementation of biological safety mechanisms implemented into the organisms themselves should contribute to a lower risk in case of accidental release (Thomas and Nielsen 2005; Moe-Behrens et al. 2013; Wright et al. 2013).

Of much higher importance with regard to the release of organisms are all open systems. Again, differentiations are to be made. They begin with partially closed systems like open ponds for the cultivation of algae (Qin et al. 2012). Examples for completely open systems are the cultivation of genetically modified crops in agriculture, in situ bioremediation—in connection with synthetic biology, a frequently discussed topic in recent years (Schmidt and de Lorenzo 2012)—as well

[11] Cf. Wright et al. (2013, 1223): "Biology can achieve a lot in a contained environment; however, physical containment alone offers no guarantees. For example, no matter how ingenious a protective device or material may be for a GMM field application, an inventive way will eventually be found by an operator to compromise it. Failure in this case is a matter of when, not if. Although some form of physical containment is obviously prudent, inbuilt biological mechanisms remain crucial to biosafety."

as the application of genetically modified organisms to impact populations of natural organisms that are regarded as injurious, as in the fight against morbiferous insects (Lacroix et al. 2012). And finally, also the human body represents an open system from which genetically modified or synthetic organisms designed for medical purposes (Ruder et al. 2011) may disseminate. Pharmaceuticals are the best known example of this (Kümmerer 2010).

Most notably for higher organisms, practical experience revealed that in addition to modified organisms interfering with the environment, unforeseen interactions inside transgenic organisms may occur. Owing to several decades of investigating transgenic plants, a number of examples for unexpected new qualities are available (Breckling et al. 2003) like a change from self-pollination to cross-pollination (Bergelson et al. 1998, in Breckling et al. 2003) or increased sperm production (Pilson et al. 2002, in Breckling et al. 2003). These implications on the organismic level may have an impact on the ecosystems-level, a relation which is further explained in the chapter of Breckling and Schmidt on parallels in risk assessment of genetic engineering and synthetic biology (Breckling and Schmidt 2015, this volume).

We already had to learn from hazardous substances, that besides the possible loss of control due to functionalities of objects of synthetic biology like proliferation, persistence, evolution and genetic transfer one has to consider prospective deficits in surveillance due to quantitative or mass effects, when production quantities rapidly increase or when the widespread use of technologies for the synthesis and manipulation of genetic material becomes a reality. This tendency is supported by the progressing automation of laboratory procedures, the decline in prices for sequencing and synthesis of DNA and, not to forget, a growing interest of non-professionals (do-it-yourself and garage-biologists or biohackers) (Ledford 2010).

Therefore, unintended release of genetically modified and synthetic organisms from partially closed or open systems, their intended release (e.g. in agriculture or bioremediation approaches) as well as their spread as a result of cultivation outside state-controlled facilities of research institutions or companies requires the establishment of precautionary measures to avoid the unintended dissemination of the created organisms or their transgenic information.

For that reason the second part of this article introduces and discusses low-hazard- and low-exposure-strategies adapted to the increased technological potential of synthetic biology. To illustrate their advantages, not only over current 'end-of-pipe' strategies like physical containment but also over current biological containment strategies, the characteristics of practiced biological safety strategies and mechanisms will be discussed in a preceding paragraph:

For the case of intended or unintended release, currently practiced inherent safety mechanisms try to avoid survival of genetically modified organisms (GMO) as well as to prevent horizontal genetic transfer by the expression of toxins or the induction of auxotrophies[12] (Wright et al. 2013). These techniques are able to assist in the attempt to avoid the spread of recombinant DNA (rDNA) and GMOs.

[12] These "classic" auxotroph-strategies are based on a deletion or at least a deactivation of a gene whose gene product is essential for survival of the GMO (Wright et al. 2013).

But the accessible level of safety is still limited, even if safeguards are implemented in a redundant manner. Since all of these mechanisms rely on DNA-encoded sequences, malfunction due to mutation, recombination or loss of the respective sequences is quite likely. This deficit is reflected in the survival rates of GMOs with auxotroph- or toxin-based safety mechanisms and can only be reduced by their redundant implementation (Moe-Behrens et al. 2013).

Besides physically or biologically induced alterations of DNA a further difficulty for auxotroph-based systems emerges from the specific site in which the GMO resides. Depending on the specific habitat, cross-feeding of essential metabolites of auxotrophic organisms occurs, either due to active (secretion) or passive (death) release from other organisms as was shown by Wintermute and Silver (2010a, b).

Furthermore, also in case of a properly working safety mechanism which kills the unintentionally released GMO, its residual matter is another deficit of present strategies. Owing to the solid structure of DNA one cannot exclude that rDNA of dead organisms remains intact and will be integrated into the genome or mobile genetic elements of other organisms (Lorenz and Wackernagel 1994; de Vries and Wackernagel 2002).

Considering the growing number of possibilities for the dissemination of modified or synthetic organisms and their genetic information, it is a basic necessity to overcome the handicaps of present safety mechanisms. Hence, the following section describes two safety strategies that may overcome some drawbacks related to precautionary measures in terms of current inherent safety strategies as well as end-of-pipe technologies like physical containment.

4 Potential Risk-Reducing Strategies for Synthetic Biology

In order to identify ways to realize the technological potentials of synthetic biology with minimized risks, it makes sense to get an orientation from the above mentioned sources of hazard and exposure, namely the ability to proliferate, the ability to evolve and adapt and the natural biomolecular basis, enabling the exchange of genetic information.

Considering these categories, two strategies in particular are discussed in the literature:

1. a trophic containment,[13] where organisms are dependent on unnatural nutrients[14] and
2. a semantic containment,[15] where exchange of genetic information with natural organisms is prevented due to the unnatural character of their genome.

[13] Cf. Marlière (2009).

[14] Trophic containment basically resembles the auxotrophic approaches of current safety strategies.

[15] Cf. Marlière (2009).

Within the following passage advantages and disadvantages of the two options will be discussed. Subsequently, as an attempt to overcome their drawbacks, a further option is presented which may currently enable the safest way to benefit from the potentials of synthetic biology, not least in critical application contexts.

4.1 Trophic and Semantic Containment: Systems on an Unnatural Basis

With regard to approaches of synthetic biology a high potential for the implementation of biological safety is ascribed to a containment which is based on molecules that do not naturally occur in nature (Schmidt 2010). These unnatural biomolecules should replace—at least partially—native amino acids, the building blocks for proteins and peptides or common nucleotides for DNA and RNA. Current approaches could broaden the functional spectrum of the 20 natural amino acids (Hoesl and Budisa 2011), multiply combinatorial options for the four common nucleobases by the introduction of additional bases (Yang et al. 2006) and create new codon assignments and codon sizes enabling the expression of proteins and peptides with unnatural amino acids (Hoesl and Budisa 2011; Neumann et al. 2010). Attempts to change the components of DNA are not restricted to nucleobases. A synthetic genetic polymer whose backbone differs from DNA and therefore enables separation from the synthesis of natural genetic polymers, potentially represents a more thorough strategy which could also be combined with the above mentioned approaches to establish the basis of synthetic organisms (Schmidt 2010; Schmidt and de Lorenzo 2012; Herdewijn and Marlière 2009).

To illustrate the advantages of systems on an unnatural basis with regard to biological safety, their specific mechanisms have to be further explained. According to the altered molecular components the following semantic containment approaches can be separated:

1. By expanding the codon-reassignment approach (Hoesl and Budisa 2011) beyond the already taken 64 combinations of natural triplet codons to the use of quadruplets (Neumann et al. 2010), encoding of non-canonical amino acids as well as semantic isolation could be achieved. Due to the lack of respective tRNAs, tRNA synthetases and ribosomes, translation of quadruplet DNA outside a customized host would fail. Nevertheless, if a sequence is only locally modified, it may revert to a natural code by mutation or exchange of its sections.
2. The implementation of unnatural nucleobases into DNA (Henry and Romesberg 2003). Attempts include the implementation of new H-bond topologies (Yang et al. 2006), non-H-bonding base pairs using hydrophobic and van der Waals interactions to form the DNA duplex (Moran et al. 1997; McMinn et al. 1999) as well as size-expanded nucleobases (Lynch et al. 2006). Codons emanating from the new letters of the genetic alphabet could either exclusively contain solely unnatural new bases or a mixture of common and new elements.

Thus, the genetic code can be replaced or at least expanded. Exchange of genetic information with natural organisms would be prevented as long as naturally occurring polymerases and reverse transcriptases are not able to synthesize or transcribe DNA which contains unnatural nucleobases. In this regard it is noteworthy that only minor modifications or even unmodified enzymes are capable of synthesizing DNA from templates which contain unnatural nucleobases (Sismour et al. 2004; Yang et al. 2011). Additionally, an isolation strategy based on different letters of the genetic alphabet has to consider that some unnatural nucleobases might form mismatches with natural nucleobases during replication (Yang et al. 2011; Henry and Romesberg 2003). Depending on the selective pressure and the characteristics of the respective polymerases for DNA-replication, unnatural nucleobases can be exchanged by natural counterparts.

3. Applying a nucleic acid with a different backbone, a so called xeno-nucleic acid (XNA),[16] would be a promising way to separate genetically modified or completely synthetic from natural organisms (Herdewijn and Marlière 2009). The current variety of XNAs contains nucleic acids where the deoxyribose is substituted by different sugars and glycerol or a cyclohexenyl-unit (Herdewijn and Marlière 2009; Schmidt 2010). And even a peptide bond instead of the phosphate group of nucleic acid polymers through incorporation of a neutral pseudo-peptide backbone (Nielsen and Egholm 1999) seems to be a functional substitute for the natural backbone.
   However, it has been shown recently that by mutation, XNA-polymerases and even reverse transcriptases can be derived from natural DNA-polymerases (Pinheiro et al. 2012). Considering the potential to change and adapt the specificity of polymerases by point mutations, a safety strategy based on XNA alone seems to be not sufficient to ensure an enduring separation of synthetic and natural organisms. More reliable stages of separation from natural organisms might be achieved if XNA were applied in combination with the above mentioned approaches.
4. Far more comprehensive would be the approach of Church to create synthetic biological entities on a molecular basis having a reversed handedness compared to natural counterparts. A “mirrored” organism would then consist of DNA and RNA as L-isomers and proteins would be formed by enantiomeric D-amino acids instead of the usual L-isomers (Schmidt 2010; Church and Regis 2012).

A high level of reliability in genetic (semantic) isolation can be achieved by a combination of different approaches[17] and in particular with a combination of semantic and trophic isolation (Schmidt 2010). When semantic and trophic isolation are combined, survival of the respective organism would be dependent on

[16] The term xeno-nucleic acid (XNA) was first proposed by Herdewijn and Marlière for synthetic genetic polymers (Herdewijn and Marlière 2009).

[17] E.g. unnatural nucleobases in quadruplet codons.

"feeding" with in vitro synthesized molecular parts (e.g. unnatural nucleotides, amino acids and enzymes) which are necessary for the replication of its genetic information as well as transcription and translation into an amino acid chain. As long as synthetic organisms are not capable of producing all components required for their semantic containment, trophic isolation would complement semantic isolation.

But before both concepts, semantic as well as trophic isolation, can be considered as an option for the safe use of functionalities provided by synthetic biology, a number of critical qualities have to be addressed. A list of important requirements for XNA-approaches was created by Schmidt (2010, 328). Despite their frequent appearance in current debates on safety of synthetic biological systems, it is crucial to consider a number of important drawbacks that accompany these approaches.

First, as already mentioned in the list above semantic isolation could be compromised by an exchange or a mutation of the molecules involved in coding, replication, transcription and translation. Thereby the genetic firewall of semantic isolation would be lost, enabling a dissemination of hazardous functionalities (functionalities that may have caused the implementation of this approach) to natural species.

Second, due to evolutionary effects, organisms separated by a trophic isolation which is based on easily producible substances could become independent by emerging self supply with formerly in vitro synthesized molecules. For applications in open systems, or in the case of organisms that have been released into the environment, this adaption leads to a complete loss of control over the synthetic species. An absence of competitors or predators would be the basis for a spread of formerly isolated orthogonal entities.

And third, regardless of whether the mechanism of isolation works or fails, if unnatural molecules are part of the approach, one has to assure that structures and organelles made of these molecules, as well as the synthetic molecules themselves will be easily degradable in natural environments. Otherwise persistence of non-degradable compounds up to persistence of whole organisms will, most probably, cause serious problems[18] especially when these organisms are (a) able to proliferate and (b) become independent.

Finally, one has to consider that effective techniques to ensure biological safety in critical application contexts have to be rapidly available, because some applications are already well advanced (Folcher and Fussenegger 2012; Kitney and Freemont 2012). Hence, with regard to the drawbacks mentioned above, it is worth exploring more feasible approaches. The following section investigates the qualification of a strategy based on entities with a reduced spectrum of functions.

[18] As already known for persistent chemicals (e.g. CFC's) or more visually apparent as plastic waste in the oceans.

4.2 Safety by Functional Reduction

The plethora of functions in a natural organism, their complex interactions in gene regulation, metabolism as well as intra and intercellular communication hampers intentional construction and controlled function—which are central claims of synthetic biology (Cambray et al. 2011; Endy 2005). When pathways for signaling or synthesis should be implemented in so called "chassis"-organisms, plain cells with a small genome are the preferred choice, as Heinemann and Panke explained[19,20] (2006, 2793):

> As the complexity of existing biological systems is the major problem in implementing synthetic biology's engineering vision, it is desirable to reduce this complexity. One option is to reduce the genome of the host—the chassis—into which the new sequence is implemented, which would eliminate many possibilities for interference.

Ideally, in minimal-approaches an organism is stripped down to a basic metabolism necessary to keep the cell alive and able to proliferate. Genetically encoded devices and networks implemented afterwards in the chassis would then have less interference with cellular processes and thus enable easier control and regulation and should yield a higher production rate (Lu et al. 2009). Minimizing the complexity of an organism by cutting out all functions that are dispensable for the intended task may additionally offer the chance to get rid of risk relevant functions.

Avoiding proliferation and changes in genetic information of an organism would be most advantageous for improved safety by functional reduction. Where genetic stability is an important factor for functional stability as well.

A number of different options for a directed reduction of biological functions are currently available:

1. Minimal genomes/minimal cells
   If cells are functionally reduced, not only to ensure efficient protein expression without intracellular interference (Csorgo et al. 2012), but for their stronger dependence on artificial conditions, then, their potential to survive without support could be reduced as well. However, these cells would be still able to proliferate[21] and thus become independent when it comes to cross feeding with nutrients or metabolites provided by natural species.

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[19] See also Heinemann and Panke (2006, 2791): "Finally, there are strong ongoing efforts towards minimal (bacterial) systems and it can be expected that such systems—owing to their reduced complexity—have a much smaller number of cross-reactions, so that implementation of novel elements stands a much better chance of remaining functionally isolated."

[20] Cf. Jewett and Forster (2010).

[21] Ibid, (698): "Thus, if additional nutrients were supplied in the extracellular medium (and perhaps their uptake aided by encoding extra transmembrane transporters) it may be feasible to delete many more genes. This could take us down to a truly minimal, protein-coding cell: one sufficient for replication but not for metabolism of most small molecules."

2. Synthetic cells and protocells
   Developing a cell from the bottom up—from its basic molecular components—is presumably an ulterior option of realizing a functional reduction for special purposes. Nevertheless, using a minimal synthetic cell[22] and in particular specialized protocells as described by Solé et al. (2007) would bear the chance to realize important variants of functional reduction like non-proliferating organisms or entities without a genetic information component in the case of protocells.
3. In vitro-approaches
   Based on combinations of biological elements outside living organisms, an assembly of different molecules can—in addition to their presence in solutions—be achieved by close entrapment (e.g. in membrane vesicles) or immobilization on surfaces and in three-dimensional structures like gels (Park et al. 2009).

Among these options for functional reduction in vitro-approaches currently represent the most effective technologies for the realization of low-hazard- and low-exposure-development paths of synthetic biology. The last part of our article thus focuses on this promising opportunity of functional reduction not least for critical application contexts.

4.3 In Vitro Systems as a Sustainable Option for Applications of Synthetic Biology

In vitro-systems (also known as cell-free systems) have already been used for a long time in analysis as well as synthesis of biochemical processes and structures respectively. The investigation of protein synthesis, the discovery of the genetic codon structure and sequence (Nirenberg and Matthaei 1961; Leder and Nirenberg 1964) belong to these analytical milestones. For the purpose of product synthesis in vitro systems were used for biochemical conversions in standard analytical procedures like DNA-synthesis for polymerase chain reaction (PCR), restriction or ligation reactions as well as for the synthesis of compounds of economical value like proteins and peptides, metabolites and unnatural compounds. In vitro systems enable the synthesis of fine chemicals, new chemical compounds, fuels, biomaterials and therapeutics (Hodgman and Jewett 2012).

Two approaches can be distinguished to achieve an in vitro-assembly of molecular components: extracts from prior intact cells or a directed combination of purified or even in vitro synthesized molecules. The latter option, also known as synthetic enzymatic pathways, is cleared from potentially interfering reactions of a cellular background. But due to laborious purification procedures, establishing synthetic enzymatic pathways is more costly and time consuming than using

[22] Cf. Forster and Church (2006, 1): “Safety concerns for synthetic life will be alleviated by extreme dependence on elaborate laboratory reagents and conditions for viability.”

cellular extracts (Hodgman and Jewett 2012). Therefore, cell-free extracts are—at least up to now—the first choice for commercial applications (Swartz 2006; Hodgman and Jewett 2012).

Cells for extract preparations, or individually expressed proteins for synthetic enzymatic pathways, can be obtained in closed cultivations. Instead of cellular expression systems, proteins for synthetic enzymatic systems could be produced by in vitro systems as well.

In recent years, substantial progress in product recovery has been achieved, especially for protein expression using in vitro approaches.[23] However, in living cells (in vivo) much higher product recovery rates for expressed proteins are still the norm (Underwood et al. 2005; Wenzel et al. 2011). Nevertheless, despite a disadvantage in terms of productivity, in vitro systems could be the optimal choice for critical applications in which the use of open systems or an intended release of organisms increases the risk of uncontrolled spread and genetic transfer of transgenic or synthetic DNA (Forster and Church 2007). Here, the immobilization of molecules required for synthesis, metabolic conversion or regulation at surfaces or as tightly enclosed groups (Urban et al. 2006) would be an option for areas in which product recovery is not the decisive factor.

The intracorporeal administration of therapeutic devices is an application of the medical sector which could be improved by in vitro-approaches. A number of future medical approaches of synthetic biology rely on modified living microbial cells or viruses as delivery vehicles for therapeutic agents or as therapeutic device itself (Ruder et al. 2011). The identification and destruction of tumor cells by living bacteria is a frequently cited example in the context of medical applications of synthetic biology (Anderson et al. 2006). And a recently presented capsule for bovine insemination, which gets dissolved when the optimal conditions for fertilization are sensed, contains living cells as carriers of the sensor-effector mechanism as well (Kemmer et al. 2011). Here, vesicular enclosure of a therapeutic or diagnostic cell-free device and its production and release mechanism (Doktycz and Simpson 2007; Puri et al. 2009) would be a non-living alternative which might also avoid the risks of viral vectors[24] (Xu and Anchordoquy 2011). The application of hybrid approaches incorporating nanoparticles and their meanwhile great variety of functionalities for activation, release and signaling (Chen et al. 2013) would thereby enable synthetic biology to exploit certain potentials of nanotechnology as well.

Besides medicine, bioremediation is another critical application field, because a release of modified or synthetic living organisms is intended within the projected applications. An example for an application which abandons the full spectrum of functionalities of living organisms and concentrates only on relevant mechanisms is the application of chromate reductase coupled on polyhydroxyalkanoate

[23] Cf. e.g. Zawada et al. (2011).

[24] Cf. Xu and Anchordoquy (2011, 1): "While viruses offer greater efficiency of gene delivery, it is generally agreed that synthetic vectors would be preferable due to safety concerns, and viral vectors may be more suited for ex vivo applications."

granules, developed by Robins and colleagues. In combination with a cofactor regenerating enzyme[25] this quite "plain" system for ex situ remediation can transform the toxic and unfortunately water soluble industrial waste product hexavalent chromium into a nontoxic state (Robins et al. 2013). Systems based on this combination were suggested as an economical and safe solution for remediation of a number of pollutants (e.g. explosives).

In recent years in vitro systems have become an important branch of synthetic biology. As in cell-based systems, new regulatory mechanisms for transcription and translation are being developed. An increasing number of attempts even incorporate the elaborate process of protein synthesis after activation of a primary gene as an intermediate step leading to the transcription of a secondary gene. A recent example is a "toolbox" for the construction of regulatory circuits for transcription and translation including multiple stage cascades, AND-gates and negative feedback loops (Shin and Noireaux 2012). The "toolbox" consists of seven *E. coli* sigma factors and, according to its investigators, can be integrated in phospholipid vesicles. Another example at the threshold between in vitro systems and protocells is the work of Nourian and collegues who developed a microreactor for protein expression (Nourian et al. 2012). Here, DNA as well as the translation machinery are enclosed inside a lipid-vesicle and nutrients are taken up through its enveloping membrane (Nourian et al. 2012).

Regarding the development of regulatory circuits, it is worth mentioning that even the in vitro-version of a periodic transcription circuit, initially presented as an in vivo-approach by Elowitz and Leibler (2000), was successfully realized by Kim and Winfree (2011).

And RNA-based sensor and regulatory systems, also called "molecular automatons" have been realized in vitro as well (Isaacs et al. 2006).

These examples reveal that many constructions of synthetic biology can be realized as in vitro-systems. Moreover the preparation of in vitro-systems with minimized interferences due to the missing host cell background is potentially the most consistent way to follow the principles of synthetic biology which seek to establish predictable and rational approaches.[26,27]

Major benefits of in vitro-systems can be summarized in three points:

1. They provide unrivaled degrees of freedom in modification and control compared with in vivo approaches (Hockenberry and Jewett 2012; Forster and Church 2007).
2. By omitting requirements of a living cell for survival, growth and replication, processes could be reduced to the intended functions, hence interference with

[25] A dehydrogenase to regenerate the required cofactor NADH from glucose or formic acid (Robins et al. 2013).

[26] Cf. the NEST-Report of the European Commission (2005, 5): "In essence, synthetic biology will enable the design of 'biological systems' in a rational and systematic way."

[27] Cf. Forster and Church (2007, 5): "And engineering flexibility is much greater in vitro, unshackled from cellular viability, complexity, and walls."

different organismic pathways can be avoided (Jewett et al. 2008; Harris and Jewett 2012; Hockenberry and Jewett 2012).[28,29]

3. Organismic evolution and proliferation as hazard related aspects are excluded.

However, the lack of full cellular organization in in vitro systems reveals a disadvantage too: proteins are subject to aging processes and will not be renewed or protected as in an intact cellular context. But, at least partially, mechanisms of self-repair can already be compensated by optimized reaction conditions, optimized amino acid sequences to minimize covalent interactions or even the additional implementation of repair mechanisms (Hold and Panke 2009).

5 Conclusion

This chapter has shown that despite the (still) hypothetical variety of biological entities enabled by synthetic biology, a characterization of their potential hazardous functionalities can be deduced from basic capabilities like the potential to evolve, to proliferate and the application of a natural genetic code. In terms of risk assessment, the ability to proliferate is of particular importance. For example growing population numbers, enhanced exposure and—as an unintended consequence—genetic transfer and toxic interactions could gain quantitative relevance. A further reason for increased exposure would be a constellation in which persistency and accumulation of synthetic biological constructs complement each other. In analogy to products of synthetic chemistry this aspect should be especially considered when dealing with developing systems and organisms with an unnatural molecular basis.

Among the strategies, which try to exploit the technological potential of synthetic biology with minimized risks, a combination of trophic and semantic containment in particular, as well as a functional reduction of the required constructs, represents promising options. To assure a high level of safety by applying semantic containment, sophisticated combined measures, such as the combination of a different codon structure with an additional strategy of semantic containment are particularly effective. Nevertheless, even if such a combination is functional, when trophic containment gets lost due to an extended synthesis potential of synthetic entities, or if the required compound is provided by the specific habitat, a loss of control over the modified or synthetic organism may be the consequence. Furthermore, convenient strategies for trophic and semantic containment still

[28] Interfering background reactions as a cause for perturbed functions or diminished product recovery rates can occur in cellular extracts as well. However, extracts can be improved by mutation and selection of the required strains (Knapp et al. 2007).

[29] Hockenberry and Jewett (2012, 257) also mention the benefits for standardized elements in synthetic biology: “While the search for biological ‘parts’ has proven fruitful for in vivo synthetic biologists, many of these parts are still highly context dependent. In cell-free systems, these parts exist in a context outside of cellular adaptation and evolution and the results are therefore expected to be more tunable and reproducible.”

require an extensive amount of basic research. Functional reduction could thus serve as an alternative. And as one variant of functional reduction, especially in vitro systems could be a near-term path for the development of a low-hazard and low-exposure technology. They would allow tapping the potential of synthetic biology in critical application contexts where a release of living cells and the exchange of genetic information should definitely be prevented.

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Synthetic Biology and Genetic Engineering: Parallels in Risk Assessment

Broder Breckling and Gunther Schmidt

Abstract When introducing new technologies, or dealing with uncertain situations in general, risk assessment is an established methodology to systematically and reliably consider whether intended benefits are gained or whether unwanted adverse effects are likely to occur. The German Advisory Council on Global Change (WBGU) distinguishes different risk categories according to the probability and extent of any possible damage. Building on that, the Gene-Risk Research Consortium elaborated a hierarchical risk assessment approach to analyze possible impact of the cultivation of genetically modified organisms (GMO). This approach is also adaptable to risks involved in the development of synthetic life forms. Since the use of GMO affects different levels of organization addressed by different scientific disciplines and stakeholders, the potential risks of GMO cultivation have to be denoted as being systemic and require interdisciplinary as well as transdisciplinary co-operation. Synthetic biology can build on risk management solutions which have been established for the use of GMO—at least to the extent that comparable risk dimensions have to be covered. For both technologies, risk assessment has to consider a wide spectrum of cause-and-effect chains and the potential impact over long time spans and large areas of space. It must also consider potential self-dispersal and subsequent evolutionary processes in the ecosphere after intended or accidental release into open ecosystems. A holistic risk assessment approach to GMO was settled upon by applying the concept of emergent properties structuring possible effects for different levels of organisation considering that interactions on lower levels (molecules, cells, organisms) in composition are supposed to bring up new interaction types on higher levels (populations, ecosystems,

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landscapes, biomes). In comparison with this structured approach, the current legal regulations, as established in the EU, can be improved in coherence and systematization by the proposed approach, in particular with regard to different ecological and economic implications of GMO (and in parallel potential releases of synthetic organisms). This is especially relevant on the landscape level; for instance, as a comprehensive systematization of region-specific adverse effects on non-target organisms, complex coexistence issues related to different production systems or some social ecological topics. In conclusion, human intervention involving self-reproducing entities by means of genetic engineering, as well as development of new synthetic life forms, should always evaluate the complete set of causal interactions on all levels of physical, biotic and social organisation in order to minimise the probability of unintended, undesirable and even harmful effects as far as feasible through anticipative assessment.

1 Introduction

Risk assessment is widely used to evaluate the adequacy of strategies for managing uncertain situations (Breckling and Müller 1998). The assessment is applied where decisions have to be made based on significant uncertainties as to whether a particular action yields an intended benefit or could likely cause undesirable and unintended damage. The assessment considers the probability of desirable or undesirable and harmful effects as well as the extent of potential loss and damage and in the end whether this loss and damage can be mitigated. The acceptability of a decision is based on the results of these estimations and has to balance expected benefit and unintended losses not only for the applicant, but also for all directly or indirectly affected parties (Luhmann 1993; Williamson and Hulpke 2000a, b). It is necessary to consider not only individual scenarios, but also comparable situations where experience has been gained to evaluate a comprehensive picture of the situations that might occur and could require attention. *Completeness of the assessment across all relevant fields is crucial.* It is worth emphasising that an overall risk may be estimated by analytically composing estimations of partial risks, e.g., when the failure of a technical device is calculated from failure probabilities of its components.

Risk assessment applications relate to simple everyday decisions, as well as to situations where a complex scientific underpinning is required. Risk-related decisions in everyday life frequently rely on informal mental exercises and personal experience. However, when risks have to be evaluated according to legal requirements, the assessment needs to follow particular formal steps (von Kries and Winter 2012). Science-based risk analysis and risk management can involve, for example, normed toxicity tests, statistical applications, game theory, operations research, and methods from various other disciplines (WBGU 1999). They help to evaluate, e.g., economic strategies, technical solutions, chemical components, or environmental protection measures. Public controversies frequently

arise when a large number of persons might be potentially affected by emerging risks without being involved in risk decisions, and without receiving the intended benefits (Breckling and Müller 1998; Renn et al. 2007). Typical examples are the controversies about nuclear power plants, pesticide admissions, and genetically modified organisms. In these cases, applicants, regulators and the potentially negatively affected public represent stakeholder groups with very limited individual overlaps.

2 The GeneRisk Project: Outlining a Holistic Risk Assessment Approach for Genetically Modified Organisms—with Implications for Synthetic Biology

The GeneRisk Project was funded by the German Ministry for Education and Research from 2006 to 2010 as a part of social ecological research (Breckling et al. 2012b; Schmidt et al. 2009). The aim of the funding programme on social ecology was to investigate general principles that influence the impact of society on natural ecosystems and the feedback of how ecological conditions influence social development[1] (Becker and Jahn 2006; Keil and Jahn 2009). Risks play an important role in this context. Systemic risks are those which result from the interaction of a larger number of single events or cause-effect chains, which, in isolation, would not cause significant harm. Regarding systemic risks, the potential threat is caused by an unintended or unexpected overlay or by coincidences of particular single events which are not harmful when considered separately (Renn and Keil 2008).

The WBGU (1999) differentiates risk typologies according to the quantitative ranges of potential damage and the likelihood that it may occur. Risk types are named after figures from Greek mythology. Because of potentially high damage and difficulties in the determination of involved probabilities, the categories "Pythia" or "Pandora" can be assigned to genetic engineering. For many of the systemic risks, the assignment of a risk type is difficult because the extent of damage could be very high while the knowledge on probabilities is comparatively limited. Applications of genetic engineering are considered typical examples of such systemic risks.[2] Because of their self-replication, synthetic life forms are likely to share relevant risk characteristics with GMO if they are released into the environment. Therefore, the risk-discourse on synthetic biology can use and adapt valuable hints and conclusions from the extensive debate and experiences that exist on GMO safety (see e.g. the Biosafety Assessment Tool[3]). The development of risk

[1] http://intern.sozial-oekologische-forschung.org/de/724.php. Accessed 7 Feb 2014.

[2] http://intern.sozial-oekologische-forschung.org/de/692.php. Accessed 7 Feb 2014.

[3] http://www.inbi.canterbury.ac.nz/BAT.shtml. Accessed 7 Feb 2014.

strategies for synthetic biology can directly build on solutions which have been established for GMO—at least to the extent that comparable risk dimensions have to be covered.

The specific risk structure involved in genetic engineering as well as in synthetic biology originates from an intervention on the molecular level. Not all implications of a particular genetic alteration (or new assembly) become evident directly and immediately after the transformation event in the laboratory. Implications of the modification are forwarded through inheritance and can be amplified autocatalytically in the course of subsequent generations, where they cause effects in new and unforeseen contexts. In addition, the modified genotype can be potentially self-dispersing and can undergo undirected mutations and recombinations. As such, genetic modifications, and the organisms carrying them, can become subject to evolutionary processes as soon as they are released into the ecosphere (Breckling 2013). This is a problem in GM applications in agriculture. Accordingly, the risk assessment requires the consideration of a wide spectrum of cause-effect networks involving long time spans and large areas of space (Breckling et al. 2012c).

The legally prescribed risk assessment has to be applied before the admission of a GMO release. It is structured in a generalized way as to cover the majority of conceivable cases (Mertens 2008; Dolezel et al. 2009). It is not based on a coherent and deductively operating systematic approach, and it usually does not consider peculiarities of specific ecosystems or geographic regions where interactions of GMO and the environment take place. However, the particular steps of the regulatory routine require a case-by-case specification (Winter and Stoppe-Ramadan 2012; EFSA 2004).

In the GeneRisk Project, a systematic approach covering involved risks and cause-effect types was developed based on the hierarchical structure of the involved scientific disciplines (Breckling et al. 2012a). The concept of emergent properties is underlying this approach. It facilitates a coherent framework of topics to be investigated and a convenient possibility for considerations of completeness. The concept is not in contrast to the official legal approach, but is appropriate to check quality and adequacy of the respective assessments and the underlying data base. To familiarize our readers with this approach, we first present the emergence concept and then outline how to apply it to a derivation of GMO risks, followed by conclusions.

3 Emergent Properties as a Basis to Structure a Holistic Risk Assessment of GMO Across Different Levels of Organisation and Related Scientific Disciplines

As an ordering criterion for relevant cause-effect chains, the concept of emergent properties (Müller et al. 1997) was employed (Breckling et al. 2012a).

The various scientific disciplines have developed their own specific terminologies and methods to cover the particular properties of the domain in which they are applied. Though the scientific disciplines are distinct, there are also well-defined relations linking them. Disciplines can be ordered in the way the respective issues emerge from the interactions of the involved elements. Interactions on lower levels in composition give rise to new interaction types on higher levels (Müller et al. 1997). This systematisation starts with atomic physics from which molecular properties emerge, which give rise to chemical interactions—among which biochemistry forms a specific branch. Biochemical interactions give rise to cellular biology. Cellular biological interactions make up the substrate for organismic physiology. The activity of organisms facilitates the emergence of population ecology. Subsequently, the overall interaction of the particular populations in a given spatial setting brings up the properties of an ecosystem. The pattern of ecosystems constitutes landscape dynamics, embedded in different biomes, which all together let the biosphere dynamics emerge. On all these levels there are *specific characteristics* requiring a level-specific disciplinary expertise, level specific methods for investigation, which give rise to level-specific terminology. On any particular level of organisation, the interactions cannot be satisfactorily explained by applying the expertise, methods and terminology of another level.

A strategically targeted risk assessment is required to capture all these levels. Considering the context of systemic risks can explain why an interaction that is not actually remarkable on one level can become meaningful and relevant to trigger harmful effects on other levels. An expert on one level is not necessarily an expert on other levels without special qualification.

Examples of emergent properties in this context are

- Genes can change their activity and even the gene products of a given set of genes can differ depending on variations of the surrounding context (involved scientific terms: e.g. gene regulation, alternative splicing)
- The relevance of particular physiological constituents of a species can change according to characteristics of the ecological surrounding (involved scientific terms: e.g. competitive replacement of species, context-dependent transfer efficiency of food chains)
- Persistence or vanishing of a species in a regional spatial setting (involved scientific terms: e.g. metapopulation dynamics, landscape percolation)

A description of risk types involved in genetically modified insect resistant B.t. maize was presented by Breckling et al. (2012a) using the emergence-approach as outlined in Fig. 1. They started the assessment with the molecular alteration, addressing the possibilities of potential undesirable effects induced by it. Then they went through the different levels and asked which implications were brought up by changes observed on the particular lower levels. Here, we follow the main points of the argumentation. This structure provides a pattern which can be generalized and has a significant range of applications. In principle, it is also suitable to cases involving other relevant interactions on the molecular level. Examples include: pesticide applications, other fields of environmental chemistry

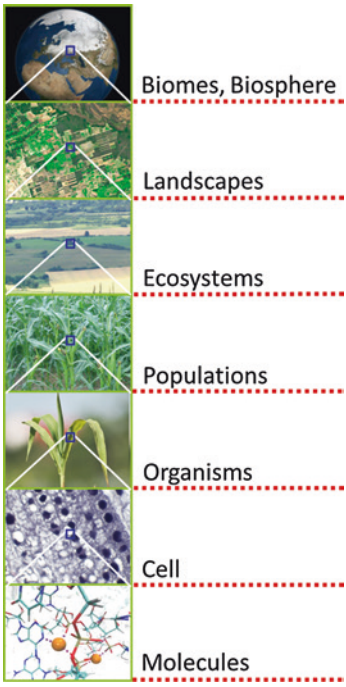

Fig. 1 Organisation levels considered in the risk assessment of genetically modified organisms. While the genetic modification refers to the molecular level, it is connected through cause-effect chains to higher levels, i.e. cells, tissues, organisms, populations, ecosystems, landscapes and biomes. The overall integrating level would be the biosphere

with ecological implications or targeted interference with the self-reproduction of organisms, either conventional or synthetic. Theoretically, artificial organisms, if released into the environment and self-reproducing, would require a complete analysis of the emerging "artificial ecology"—regardless of whether a release occurs intentionally or by accident.

4 Risk Considerations on the Molecular Level

The molecular level is the central starting point of risk analysis of GMO, as well as for developments in synthetic biology. The task on this level is primarily to understand the molecular interactions which are affected and potentially altered by the genetic modification; and to identify those which could become the potential starting point of a cause–effect chain which gives rise to undesirable or harmful results. This is more easily said than done since the metabolites involved in cellular physiology are not only numerous but can change according to external stimuli in a way that is quite difficult to predict. This is particularly difficult, because in living organisms part of the complexity is manifested (or expressed) only under specific external or internal conditions. External conditions encompass abiotic parameter like temperature or humidity; but also the biotic and molecular environment like viruses which may have a different effect when interfering with a

modified or synthetic organism, as compared to the conventional complement. So far, even capturing the balance of metabolites in a cell, which is technically possible through metabolomic techniques, has only been rarely applied in risk assessments of GMO in the course of admission for cultivation. Metabolomics deals with a quantitative representation of the entire set of metabolites in a cell. This should be considered as an essential requirement of risk assessment comparing the physiology of the altered genome with its conventional counterpart. The characterization of a completely synthetic genome might be a comparably complex task. The potential molecular interaction with conventional biological entities opens up a broad new research field of molecular ecology. Up to now, the risk assessment paradigms on the molecular level are dominated by additive assumptions and frequently downplay the interaction potential of the molecular entities. Both, the concept of substantial equivalence (Novak and Haslberger 2000) as well as the concept of familiarity (Andow et al. 2006; von Kries and Winter 2012), operate as if it was possible to add something to a cell—the transgene—without affecting its integrity in a significant way.

5 Risk Considerations on the Cellular Level

Cells represent a different organisation level since the molecular interactions are linked in a way which results in a functional entity. Describing properties of this entity is the scope of cellular biology (Pollard et al. 2007), which has developed its own methods and terminologies going beyond molecular approaches. It also operates on a different scale, though molecular techniques play a role on this level as well. Cellular biology in particular, addresses the interactions between cells giving rise to tissue developments. Light and electron microscopic techniques are important methods on this level, which links molecular processes with reactions to the physiological level of the entire organism. Risk issues on this level are the analysis of the formation of tissues affected by genomic alteration and whether the regulatory potential of cellular networks is affected as well.

6 Risk Considerations on the Level of the Organism

The level of the organism is special since it links the two domains of physiology and ecology. As a whole, the organism represents the framework of internal functional regulation and adaptation to external conditions as well as the reproductive unit. Environmental feedback acts upon the level of the organism and constitutes its fitness aspect. Fitness refers to the potential of a particular organism to contribute to the gene pool of the next generation (Gavrilets 2004). The organismic level plays a crucial role in the evaluation of the effect of a genetic transformation. The performance characteristics of a cultivated plant largely refer to the level of

the organism. Whether a genetically modified organism will be suitable for introduction into the market largely depends on to what extent the organisms' properties actually conform to market condition, the willingness of adoption of users and consumers, and the potential of the developer to place it in the appropriate and suitable market environment.

7 Risk Considerations on the Level of the Population

The population has characteristics which cannot be assigned to single organisms or individuals. In particular, this refers to the characterisation of collectives and their interaction pattern: size spectra, age distributions and spatial patterns can be relevant (Begon et al. 2009). The study of population processes is necessary in order to understand dispersal of species as well as genetic interaction probabilities within a species. The issue of gene flow, genetic marker frequencies and tolerance of changing environmental factors are investigated on the level of the population and are described with concepts and approaches of population ecology. Probability distributions play an important role on this level.

8 Risk Considerations on the Ecosystem Level

The level of the ecosystem addresses the interactions within the community of organisms (the biocoenosis) in a particular area and the interactions of biocoenoses and the environmental conditions in that area (biotope) (Loreau et al. 2001). A spatial delimitation is required for an ecosystem. Risk assessment on the ecosystem level encompasses the interactions of a GMO with other organisms and the way it interacts with the abiotic parameters. As an integrative system performance, the holon of the organisms active in a particular ecosystem brings up an energy flow through the system (formation of organic matter by the producers using an energy source) and a cycling of bio-elements as a result of trophic interactions within the community of organisms. Assessing energy flows and matter cycling within the system, and the way it depends on the biotic activities, is one of the dominating issues of ecosystem research (Odum 1971). A particular challenge is the estimation of the effects on biodiversity, because the diversity of organisms can be determined only approximately in relevant ecosystems.

Expertise and experience with the overall systemic interaction concept, as well as familiarity with the major groups of involved organisms, is an important precondition for establishing reasonable statements on this level. It is important to emphasise that these concepts are not relevant in research contexts dominated by laboratory applications. Though the ecosystem level is a key turntable for environmental effects of organisms through amplification, mediation or configuration in food chain relations, this field of scientific investigation is only marginally

represented in risk assessment (Breckling et al. 2012a). There are studies assessing the effects of some non-target organisms (Andow and Hilbeck 2004; Hilbeck 2001). These studies are, however, in most cases not embedded in an ecosystem approach according to the state-of-the-art in ecology.

9 Risk Considerations on the Level of Landscape and on the Regional Level

While an ecosystem is spatio-temporally delimited by definition according to a relative internal homogeneity criterion, the landscape level encompasses a network of different ecosystem types and investigates interactions between ecosystems and changes of the ecosystem patterns in larger regions (Wu and Hobbs 2002). Dispersal processes are relevant on this level since the success frequently depends on the type of ecosystem networks affecting the probability whether the distance of unsuitable habitats can be overcome by propagules and, thus, facilitating landscape percolation (Oborny et al. 2007). Furthermore, the landscape level is of high interest, since governance and regulatory decisions usually affect more than one ecosystem type and are of at least regional relevance. On this level, GMO risk considerations are currently most scarce. Gene flow estimations are among those tasks most relevantly needed in order to estimate potential transgene escape and its consequences for the larger biodiversity. On this level, the assessment is required to be region-specific.

10 Considerations of Effects and Risk Implications on Higher Levels

Are there relevant risk considerations on higher levels? Beyond regions come biomes (very large areas dominated by similar climatic influences) (Prentice et al. 1992) on the ecological side and larger regulatory units like sovereign states or supra-national associations. On that level, in particular, transboundary movement of GMO is an issue as regulated by the Cartagena Protocol on Biosafety (Meyer 2011).

11 Discussion

Risk assessment of GMO, as well as risk considerations in synthetic biology, reasonably start on the molecular level. It is apparent that molecular expertise, which is required to assemble the new organism, is not necessarily sufficient to assess consequences of its release on higher levels of organisation. In fact, in the course of risk assessment, molecular aspects contribute an essential, though only minor

part of the spectrum of interactions which need to be surveyed adequately. Starting from the molecular modification, subsequent scientific levels are affected. As we have emphasised, these levels can be delimited from each other according to emergent properties. When structuring the whole field of implications according to this systematisation, it becomes evident that the current regulatory practice (von Kries and Winter 2011) operates on an abstract basis. Crucial specific and structural questions are not explicitly addressed and tend to be missed in the actual risk assessment practice. This is obviously the case for metabolomic approaches, which are feasible to determine large numbers of metabolites simultaneously and quantitatively and also give information on metabolic responses within the cell to particular conditions under which the cell grows. There are deficits as well concerning effects of GMO on non-target organisms and the ecological context. Deficits have also been widely discussed concerning human and animal health aspects which can be assessed through long-term feeding studies (Seralini et al. 2012). Assessments of GMO concerning long-term ecological and economical implications of the particular production systems are scarce as well. Investigations on this risk dimension, especially concerning effects to the landscape, are almost completely missing, even though the considerable damages which have occurred in the context of GMO application largely refer to the higher levels, based upon the observance of unintended effects of GMO.

12 Experiences with Unintended Effects of GMO

Breckling (2010) lists prototypical cases of damage induced by GMO which can be assigned to different levels.

- The most prominent disaster was the Starlink-Maize scandal in the USA causing wide-scale economic damage of about one billion dollars (Bucchini and Goldman 2002; Thayer 2001). It can be assigned to the cross-regional level, since the maize variety with an admission only for animal feeding appeared in many food products during the year 2000 and after, and required large amounts of fiscal compensation.
- The problems with the Canadian Triffid flax (Schmidt and Breckling 2010) were even more surprising. A GMO, which was commercialised with negligible success, caused large-scale impurities which were discovered the first time 10 years later causing large-scale withdrawals of exports to various countries, not to mention the corresponding economic losses.
- More recently, the unintended dispersal of GM oilseed rape outside cultivation was discovered in various countries, where GM oilseed rape had no admission for cultivation (Kawata et al. 2009; Schönenberger and D'Andrea 2012). In Japan, Switzerland, and others, only import and processing is allowed. Removal from the natural ecosystems in which this occurred seems to be difficult.
- And lastly, recent findings connect the strong decrease in the populations of monarch butterflies in North America to the increased use of herbicide resistant

GMO. The herbicide application was the suspected cause for the pronounced decrease in the larvae's food plants (Dively et al. 2004).

The spectrum of risk-relevant relations is not complete when addressed on the scientific level alone. Social ecological topics have to be added. These issues are not dealing primarily with the topic of product safety, but can be quite important for the valuation of technology applications. With regard to GMO, this especially concerns food safety, diversity of production systems, and the sustainability of ecological goods and services (Farber et al. 2002). Accordingly, this requires a link to socio-economic and cultural approaches of risk investigation.

13 Risk Assessment and Conflicts of Interest

Anyone who has followed in detail the public controversy on GMO applications in agriculture has realised that specific interest-driven views, explicit or implicit business interest of particular actors, stakeholder networks (Waltz 2009), and Public Relation communication[4] all play important roles in the discourse. In opposition to business-related activities favouring GMO are conviction-based views brought forward by various civil society groups and Non-governmental Organisations.[5] For the entire spectrum of views there is scientific underpinning in terms of safety research (illustrating aspects of GMO) or risk research (investigating potentials risks and uncertainties).[6] In Europe, GMO are widely considered unacceptable as food items by a majority of consumers.[7]. The overall trend of economic concentration leading to decreasing alternatives in seed assortment on the market (Hilbeck et al. 2013), added to the fact that no increased harvest quantities through GMO cultivation can be realised, but rather the observance of increased use of agrochemicals (Benbrook 2012) reduces expectations. GMO—and this could be a parallel to synthetic biology—were advertised by its developers with the announcement of likely future benefits. A recent example is the BASF GM potato

[4] See e.g. the website http://www.gmo-compass.org. Accessed 7 Feb 2014, managed by the PR agency GENIUS (http://www.genius.de). Accessed 7 Feb 2014.

[5] See e.g. http://www.greenpeace.org/international/en/campaigns/agriculture/problem/genetic-engineering or https://www.campact.de/gentec/home. Accessed 7 Feb 2014.

[6] E.g. the bioscience resource project, see http://www.biosciencesresource.org. Accessed 7 Feb 2014, the research organisation *econexus*, see http://www.econexus.info. Accessed 7 Feb 2014, or the European Network of Scientists for Social and Environmental Responsibility, see http://www.ensser.org. Accessed 7 Feb 2014.

[7] Monsanto as the largest world-wide GMO producer announced a halt in approval activities for GMO cultivation in Europe (but continues to seek admission for import and for animal feed). Similarly, BASF stopped the commercialisation of its already admitted GM potato. See http://www.cbc.ca/news/business/monsanto-to-stop-seeking-gmo-approval-in-europe-1.1369375. Accessed 11 July 2014.

with the variety name "Amflora"[8] (Andersson 2004). It was approved for industrial use in the EU. Its intended advantage was to simplify processing. Despite its approval, the variety was not accepted on the market and finally withdrawn.[9] One of the reasons for market failure may have been that conventional varieties became available for the same industrial purposes without the GM-specific patent protection. If the diversity of current developments, the by far less diverse cultivation approvals and the even more limited number of successful GMO types on the market are compared, it becomes obvious that the technical feasibility of a particular genetic modification is not sufficient ground to assess the potential of a technology, its involved risks and benefits.

14 Conclusions

It seems possible to draw parallels and analogies in the developing field of synthetic biology. In this field of application, we also have targeted molecular interference with self-reproducing entities. In the case that synthetic organisms were considered for deliberate release, the complete set of causal interactions has to be checked during risk assessment, as is the case for GMO. If self-reproducing entities are released, it is required that the potential effects on all levels of biotic and social organisation are considered. This will assure that the probability of unintended, undesirable and harmful effects is minimised as far as feasible through anticipative assessment. If only contained use is intended, the reliability of the containment is crucial. Then the spectrum of interactions to be considered becomes narrower—as long as the reliability of containment can be secured against unintended as well as intended failure. Only then will utility and efficiency criteria, as established in technology assessment, have their fully intended effect[10] (EEA 2013).

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[8] http://www.gmo-compass.org/eng/gmo/db/16.docu.html. Accessed 7 Feb 2014.

[9] http://www.gmo-safety.eu/science/potato/263.amflora-potato-industrial-applications-starch-potatoes-renewable-raw-material.html. Accessed 7 Feb 2014.

[10] See e.g. the European parliamentary Technology Assessment network (http://eptanetwork.org), the EU project TAMO—Technology Assessment—Methods and Impacts (http://www.ta-swiss.ch/en/projects/biotechnology-medicine). Accessed 7 Feb 2014.

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The Regulation of Synthetic Biology by EU Law: Current State and Prospects

Gerd Winter

Abstract Assuming that synthetic biology (SynBio) will generate not only new benefits but also new risks to human health and the environment this article explores to what extent SynBio is already adequately supervised by the existing EU regulation of genetically modified organisms (GMOs). While the GMO regime is applicable to many kinds of SynBio activities, others are not covered, such as the complete replacement of the genetic material of a cell, the insertion of transgenes into an organism by other methods than those listed as qualifying as genetic engineering—or not—the construction of a protocell and minimal cell, the placing on the market of bioparts, and xenobiochemistry. The article then asks if the risk assessment methodology applicable to GMOs is suited for products from SynBio. This question is denied insofar as the familiarity principle which governs traditional GMO risk assessment is concerned. New and genuine methodology must be developed to identify hazards and evaluate risks. While the thrust of the article is on ex ante regulation, or administrative oversight, it also discusses ex post regulation, or civil liability for damage, concluding that liability schemes must also be adapted to the new characteristics of SynBio. In sum, it is time for regulators to take a closer look at SynBio.

This article is a part of a more comprehensive one that is being published in a book of an interdisciplinary working group of the Europäische Akademie zur Erforschung der Folgen wissenschaftlich-technischer Entwicklungen, Bad Neuenahr-Ahrweiler. I am thankful to the group members Michael Bölker, Nediljko Budisa, Margret Engelhard (its spiritus rector), Christian Illies, Rafael Pardo Avellaneda and Georg Töpfer for many seminal discussions. I also thank Bernd Giese and Broder Breckling for their helpful comments.

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1 Introduction

Synthetic Biology (SynBio) is being heralded for generating new benefits for society. These include such diverse areas as medicine, energy, fine chemicals, food, materials, environmental engineering, agriculture and even computer technology (Baldwin et al. 2012, Chap. 7; Church and Regis 2012). But it is also likely to cause drawbacks. Artificially designed and synthetically compiled organisms or genetic parts of them may escape containment, or may deliberately be released, and cause adverse effects to human health or the environment. Regulation is the major means of preventing this.

The prevention by regulation of such risks means that actors in research, development, production, trade and use of SynBio are subjected to a set of duties of caution concerning the effects on third parties or public goods. The fulfilment of theses duties is supervised by administrative bodies and liability for damages. Third parties may be given rights to claim protection against risks or compensation for damage.

The regulation can be ex ante or ex post, ex ante meaning that administrative oversight is involved before an activity may be undertaken, and ex post that the party is liable for any damage caused by his/her activities.

2 Regulation Ex Ante

Most closely related to SynBio is the legal regime on genetically modified organisms (GMOs). Other regimes (which are not considered here) are the regulation of chemicals and that of pathogens. The GMO regime consists of both EU and MS legislation. It is basically structured according to whether GMOs are handled in containment, or intentionally introduced into the environment, be it through release at a predetermined site or, after they have been placed on the market, through introduction anywhere.

In the EU any works or products based on genetic modification are subjected to a special legal regime for GMOs. In contrast, in the US processes and products are checked as part of the control regime for non-modifying processes and non-modified products. For instance, a genetically modified pesticide would in the EU need two market placement authorizations, one under the GMO and the other under the pesticides legislation, and in the US just one under the pesticides legislation (Lynch and Vogel 2001).

Before considering whether the EU's GMO regime is an appropriate regulatory tool for SynBio we need to examine if, and to what extent, the existing EU GMO regulatory regime is applicable to SynBio at all.

2.1 Applicability to SynBio of the GMO Regime

The GMO regime is, as already mentioned, applicable to the "contained use," the individual "release" and the wider "placing on the market" of "GMOs." The regulation of contained use is harmonized EU wide only in relation to genetically

modified microorganisms (GMMs).[1] Contained use of other GMOs is thus left to the regulatory competence of the Member States (MS).[2] In contrast, the regulation of the release and placing on the market of GMOs is standardized by EU legislation concerning all kinds of GMOs.[3] In any case, the core notion triggering the regulatory regime is a GMO. Its legal definition must therefore be explained and applied to SynBio techniques. The legal definition varies to some degree in relation to GMOs in general and GMMs, but the differences are not important in the present context.

An "organism" is legally defined as

> any biological entity capable of replication or of transferring genetic material.[4]

This already excludes any modified or artificial subcellular bioparts that are not capable of replication from the application of the GMO regime.

Further, a genetically modified organism is defined as

> …an organism, with the exception of human beings, in which the genetic material has been altered in a way that does not occur naturally by mating and/or natural recombination.[5]

Thus, for a GMO an organism must exist that is modified in certain artificial ways. For SynBio, this means that the GMO-regime only deals with activities which start with a real organism and modify it in specified ways. This excludes from the regime the complete synthesis of a known organism as well as the completely new design and synthesis of a new organism. In particular, bottom-up constructed protocells are not covered by the GMO regime.

The third element of the definition of a GMO is that the "genetic material" of the organism has been altered. The term "genetic material" undoubtedly includes the DNA and arguably also the RNA, considering the fact that the mRNA and tRNA, switched on by a gene, are part of the information process initiating the production of amino acids and through them of proteins. However, if by methods of the so-called xenobiochemistry (Budisa 2012) the amino acids are replaced by non-natural ones, and thus, new proteins emerge creating hitherto unknown properties of the organism, the operation is not one altering the "genetic material."

The fourth element is that the genetic material contained in the organism was "altered". This poses the question if "alteration" also includes the complete

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[1] Directive 2009/41/EC of the European Parliament and of the Council of 6 May 2009 on the contained use of genetically modified micro-organisms (Recast), OJ L 125, 21.5.2009, p. 75, Art. 1.

[2] The German Act on Gene Technology (Gentechnikgesetz—GenTG), for instance, extends its provisions on contained use to all GMOs. It however empowers the government to exempt those GMOs which are considered to be safe (Sect. 2a GentG).

[3] Directive 2001/18/EC of the European Parliament and of the Council of 12 March 2001 on the deliberate release into the environment of genetically modified organisms and repealing Council Directive 90/220/EEC, OJ L 106, 17.4.2001, p. 1, Art. 2 (1).

[4] Art. 2 (1) Directive 2001/18/EC.

[5] Art.2 (2) Directive 2001/18/EC.

replacement of the genome of a cell, such as in the experiment with mycoplasma bacteria of the Craig Venter Institute (Gibson et al. 2010). Based on a teleological reading this would, because of the unknown risks, need to be controlled even more than the mere modification. However, in a literal interpretation the full replacement is different from a mere alteration. Man may ask if this should be different in the case in which the inserted material consists of newly synthesized conventional components. But in this case the organism is not altered but remains the same both chemically and functionally.

The fifth element is that the nucleic acid molecules inserted into a host organism may have been "produced by whatever means outside an organism."[6] Thus, not only traits from existing organisms or a synthesized copy of them are covered, but also synthesized traits having a new design, such as those generated by the so-called xenobiology (Schmidt 2010; Budisa 2012).[7] This means that xenobiology insofar it induces artificial DNA or RNA is included in the GMO regime.

The sixth element is that that nucleic acid molecules must be *inserted* into a host organism. This excludes from the GMO-regime methods of reducing organisms to minimal cells because in this case genetic material is removed from, rather than added to, the organism.[8]

The seventh element, as mentioned, is that the alteration of the genetic material is done "in a way that does not occur naturally by mating and/or natural recombination".[9] The core techniques qualifying as not natural are listed in Annexes to the relevant directives. They include, inter alia, the insertion of nucleic acid molecules by means of a vector system into a host organism in which they do not naturally occur, or by direct introduction such as micro-injection, or by not naturally occurring cell fusion or hybridisation.[10] This implies, for instance, that the gene gun method used in the do-it-yourself networks (DIY-Bio) is un-supervised.[11]

In contrast to the positive list of techniques qualifying as genetic engineering, certain techniques are excluded from the GMO regime because although being more or less artificial they can (at least theoretically) also occur under natural conditions. These techniques are mutagenesis and certain kinds of cell fusion.[12] However, a whole bunch of "New Plant Breeding Techniques (NPBTs)"—arguably included in a broad understanding of SynBio—have been developed that although in

[6] Directive 2001/18/EC Art. 2 (2) together with Annex I A Part I (1).

[7] This technique was however, not unknown to earlier genetic engineering. For instance, the gene which encodes the PAT-protein and conveys tolerance of the herbicide glyphosinate was redesigned and thus differs from the natural PAT-gene. Example taken from (Bundestag 2011). The radical version would be the above cited mycoplasma experiment.

[8] For a description of this technique see (Budisa 2012, pp. 103–108).

[9] Art.2 (2) Directive 2001/18/EC.

[10] Directive 2001/18/EC Art. 2 (2) together with Annex I A Part I (1)–(3).

[11] How naïvely the networks operate can be studied from the video displayed at http://www.sueddeutsche.de/wissen/biohacking-bewegung-leuchtende-pflanzen-zum-selberbasteln-1.1875586-2 (visited 14.02.2014).

[12] Directive 2001/18/EC Annex I B.

Table 1 Defining GMOs in application to SynBio

Elements of definition	Elements not covered
The GMO must be an organism	Bioparts
The GMO must derive from an organism	Complete synthesis of an organism; bottom-up construction of a protocell
The genetic material must be altered	Complete replacement of the cell content, be it with conventional or new design
The inserted transgenes can be of any design and construction method	
Transgenes must be "inserted"	A minimal cell
Positive and negative lists of techniques of insertion	Not listed techniques (e.g. gene gun), new breeding techniques

principle "natural" are so deeply interfering that they can be as hazardous as GMOs in the legal sense. Such techniques include targeted site-specific mutagenesis, transgenesis as an intermediate step of breeding processes where the transgene is subsequently removed, or "cisgenesis" where genes from the same species or family are transferred (Parisi 2012; Raaijmakers 2009). Thus, a substantial part of new breeding techniques appear not to be captured by the EU GMO regime (Table 1).

In conclusion, SynBio, insofar as it works on existing living cells and alters their genetic material in a way that does not occur naturally, must be counted as a technique resulting in genetic modification and thus as subjected to the existing EU GMO regime. In particular, organisms in which the genetic content was modified by synthesized material of natural or artificial design are covered, even insofar as new genetic xeno-material is introduced. By contrast, the following SynBio products are not captured by the GMO regime:

- an organism which was synthesized, be it of natural or artificial design
- an organism in which the genetic material was completely replaced by known or artificial genetic material
- an organism into which genetic material was inserted by other techniques than vector systems, micro-injection, non-natural cell-fusion or hybridization
- an organism whose chemical derivatives (amino acids, proteins) were modified
- a protocell
- a minimal cell
- synthesized or extracted bioparts
- an organism whose chemical derivatives (amino acids, proteins) were modified by xenobiochemistry
- an organism resulting from new breeding techniques which although in principle naturally occurring are deeply interfering.

It appears that this result—important SynBio techniques not being covered by the GMO regime—is not adequately discerned by research institutions and governments.[13]

[13] See for Germany (Acatech et al. 2009, p. 34); (Bundestag 2011).

There are two ways of reacting to the fact that parts of SynBio escape the scope of the existing GMO regime: One is to widen the scope so that more areas of SynBio are covered, and the second is to introduce a new law. The first option is certainly easier to reach politically, but the second would be more appropriate because it could be based on a new approach which better matches instruments of administrative oversight with different categories of risk. This approach could even reflect the fact that some kinds of genetic modification qualify to be released from any prior regulation while others which are presently need to be subjected to it.

2.2 Adequacy of the GMO Regime

We now proceed to consider what principles of risk assessment are appropriate for SynBio. This shall be done by critically reviewing the risk assessment methodology that is presently applicable to GMOs. If they are found to be inappropriate, better methodologies for SynBio must be introduced either by the existing legal regime as enlarged in scope, or the new regime if that is established.

Products from synthetic biology, or SynBio products (SBPs)[14] will probably be fabricated and used in contained systems for a long time to come. Therefore, the relevant EU legal acts on contained genetic engineering operations must be consulted for their adequacy for SBPs. However, it is also possible that SBPs will be developed that shall intentionally be introduced into the environment, such as microorganisms for the treatment of contaminated water or soil; or for the production of energy from biomass (French 2014). It is less probable that SBPs will be placed on the market for random release, in the near future. But the possibility exists, for instance for microorganisms constructed for environmental management or energy fabrication. It must also be considered that a vibrant market has emerged for bioparts which provides services for contained R&D in SynBio. We will therefore first explore the regime for contained operations, and then the deliberate release of SBPs at certain locations as well as their market placement.

2.2.1 Contained Use

As already indicated, EU law on contained use of GMIOs only refers to genetically modified microorganisms (GMMs) leaving other GMOs to the legislative competence of the member states.

[14] I suggest the introduction of this term into the emerging debate on a regulatory scheme for SynBio. Alternatively one could consider “SynBio organism” (SO) as the core term, but this would not cover bioparts.

Risk Paths

Even if kept in containment, GMMs may cause risks for the researchers and workers. Moreover, they may unintentionally leak into the environment through persons carrying them out of the lab, or through solid waste, sewage or exhaust disposed from the lab. The same paths must be considered for SBPs.

Protected Goods

According to Art. 4 Directive 2009/41/EC Member States

> …shall ensure that all appropriate measures are taken to avoid adverse effects on human health and the environment which might arise from the contained use of GMMs.

The goods protected by the GMM regime are thus human health and the environment. Any "adverse effect" to them must be avoided.

Although this is not explicitely mentioned in the directive it has been discussed whether besides preventing risks GMMs must also provide a socio-economic benefit. When in the late eighties and early nineties the first facilities with contained systems were built for research on dangerous microorganisms, concerns were raised if the containment would be perfect enough to hinder any escape of GMMs. Considering that a residual risk of leakage cannot be avoided, it was debated if the unavoidable remaining risk should not be weighed against the benefits generated by the GMM. For instance, in a hearing on the construction of a BASF facility for the production of the pharmaprotein Tumor Necrosis Factor (TNF) a concerned citizen argued that TNF was ineffective if not detrimental as a medicinal drug so that the construction of a production unit for TNF constituted, as she called it, a senseless risk (cf. Winter et al. 1993, p. 34). Since then, the discussion about weighing risks against social benefits (or their absence) has faded away in relation to contained systems. It has however continued in relation to the deliberate release and market distribution of GMOs.[15]

Concerning highly problematic kinds of SBPs the same discussion may be reopened even in relation to contained systems.

Burden of Submission of Risk Related Data

Risk assessment is only possible if appropriate data are available. Generally, in administrative proceedings the authorities are responsible for collecting the relevant data (investigation principle).[16] Ultimately, this rule rests on the fundamental right to individual freedom, which implies that if a law imposes restrictions based on certain factual circumstances these facts must be identified and proven by the competent authority.

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[15] See further below.

[16] See Art. 337 TFEU and (v. Danwitz 2008, pp. 417–421).For Germany see Sect. 24 Administrative Procedure Act (Verwaltungsverfahrensgesetz—VerwVfG).

The burden of producing evidence can however be imposed on the individual by special legislation. This normally occurs, if an activity requires prior authorization or notification, because it is assumed that the activity is suspected to pose a risk and shall therefore only be allowed after detailed examination. The EU GMM regime is based on this assumption and therefore shifts the burden of data provision to the applicant.[17] It specifies which data have to be presented, limiting the scope to those data which are needed to assess whether the substantive protective standard (the protection of human health and the environment) is met.[18]

If the presented data are not sufficient to allow a prognostic assessment, the competent authority can request the submission of additional data.[19] If the available knowledge is not sufficient for this purpose, the applicant bears the burden of generating it, provided there are indications of risk.[20]

Knowledge relevant to an authorisation or notification proceeding may already be held by the administrative authority. If that is the case, the authority must make use of it in the authorisation procedure and cannot ask the applicant to reproduce it anew.[21]

It appears that these principles of data submission would also fit if an authorisation regime for using SBPs in contained systems was introduced.

List of Data to Be Submitted

In the case of contained use of highly hazardous GMMs the data to be submitted by the applicant comprise the following[22]:

(a) [...]

(b)

- the recipient or parental micro-organism(s) to be used,
- the host-vector system(s) to be used (where applicable),
- the source(s) and intended function(s) of the genetic material(s) involved in the modification(s),
- the identity and characteristics of the GMM,
- the culture volumes to be used;

[17] It is true, however, that Directive 2009/41/EC allows for exempting from its scope those GMMs which are considered to be safe (Art. 3 (1) (b) together with Annex II Part C of the same directive).

[18] Arts. 6–9 Directive 2009/41/EC.

[19] Art. 10 (3) (a) Directive 2009/41/EC.

[20] This requirement can be based on Art. 4 Directive 2009/41/EC as interpreted in view of the precautionary principle according to Art. 191 (2) (2) Treaty on the Functioning of the European Union (TFEU). On the necessity of indications and thus the exclusion of a zero risk approach see European Court, Case T-13/99, judgment of 11 September 2002 (Pfizer), paragraphs 144–148.

[21] See the clause “if necessary” in Art. 10 (3) Directive 2009/41/EC.

[22] Directive 2009/41/EC, Annex V Part C.

(c)
- a description of the containment and other protective measures to be applied, including information about waste management, including the type and form of wastes to be generated, their treatment, final form and destination,
- the purpose of the contained use, including the expected results,
- a description of the parts of the installation;

(d)
- information about accident prevention and emergency response plans, if any,
- any specific hazards arising from the location of the installation,
- the preventive measures applied, such as safety equipment, alarm systems and containment methods,
- the procedures and plans for verifying the continuing effectiveness of the containment measures,
- a description of information provided to workers,
- the information necessary for the competent authority to evaluate any emergency response plans, if required under Article 13(1);

While the data listed sub (c) and (d) might be transferable to the situation of hazardous SBPs those sub (b) reflect the fact that the object of assessment is genetic modification of existing organisms. This may be appropriate for SBPs that are based on existing organisms. However, for new SBPs lists of required data must be developed that are better targeted to the specific risks of such SBPs. Where interpolations from donor, vector and recipient organisms are not possible specific tests concerning the resulting organism must be required. Moreover, as the GMO regime only covers living organisms, risks from bioparts, individually and in combinations, are not addressed by the data list.

Assessing and Categorising Risk and Containment

Risk prevention measures should differ depending on the severity of the risks caused. The more hazardous the use of an organism is the tighter the containment must be. This is also the logic applied in the EU GMM regime. Four risk categories are distinguished corresponding to an increasing intensity of containment measures. These categories are described as Class 1: no or negligible risk, Class 2: low risk; Class 3: moderate risk; and Class 4: high risk. The four risk classes are correlated with four containment classes. These consist in clusters of measures concerning the construction of the lab (e.g. isolation), the equipment (e.g. negative pressure), the system of work (e.g. restricted access, clothing), and the treatment of waste (e.g. inactivation of GMMs). [23]

[23] Art. 4 (3) and Annex IV of Directive 2009/41/EC.

The risk assessment serves to classify any use of GMMs into one of the four risk and containment classes. A two-step procedure is recommended for this exercise[24]:

- Procedure 1
  Identify potentially harmful properties (hazard) of the GMM and allocate the GMM to an initial class (class 1 to class 4), taking into account the severity of the potentially harmful effects.
  and
  Assessment of possibility of harmful effects occurring by consideration of exposure (both human and environmental), taking into account the nature and scale of the work, with containment measures appropriate to the initial class allocated.
- Procedure 2
  Determination of final classification and containment measures required for the activity. Confirm final classification and containment measures are adequate by revisiting Procedure 1.

When assessing the risk of the resulting GMO, the hazards of the donor as well as the resulting organism must be considered, i.e.[25]:

1. the recipient micro-organism;
2. the genetic material inserted (originating from the donor organism);
3. the vector;
4. the donor micro-organism (as long as the donor micro-organism is used during the operation);
5. the resulting GMM.

The following endpoints must be examined[26]:

Human health considerations:

- expected toxic or allergenic effects of the GMM and/or its metabolic products,
- comparison of the modified micro-organism to the recipient or (where appropriate) parental organism regarding pathogenicity,
- expected capacity for colonisation,
- if the micro-organism is pathogenic to humans who are immunocompetent,
- diseases caused and mechanism of transmission including invasiveness and virulence,
- infective dose,

[24] Commission Decision of 27 September 2000 concerning the guidance notes for risk assessment outlined in AnnexIII of Directive 90/219/EEC on the contained use of genetically modified micro-organisms, Annex Nr. 2.

[25] Annex III A (2) Directive 2009/41/EC.

[26] Commission Decision of 27 September 2000 concerning the guidance notes for risk assessment outlined in AnnexIII of Directive 90/219/EEC on the contained use of genetically modified micro-organisms, Annex Nr. 3.2.5.

- possible alteration of route of infection or tissue specificity,
- possibility of survival outside of human host,
- biological stability,
- antibiotic-resistance patterns,
- allergenicity,
- toxigenicity,
- availability of appropriate therapies and prophylactic measures.

Environmental considerations:

- ecosystems to which the micro-organism could be unintentionally released from the contained use,
- expected survivability, multiplication and extent of dissemination of the modified micro-organism in the identified ecosystems,
- anticipated result of interaction between the modified micro-organism and the organisms or micro-organisms which might be exposed in case of unintentional release into the environment,
- known or predicted effects on plants and animals such as pathogenicity, toxicity, allergenicity, vector for a pathogen, altered antibiotic-resistance patterns, altered tropism or host specificity, colonisation,
- known or predicted involvement in biogeochemical processes.

These parameters will have to be revisited in relation to SBPs. Based on accumulated experience, lists of typical organisms and treatments have been compiled for GMMs. However, concerning SBPs, it is questionable if the research activities can already be categorized in a like manner. They are still very diverse, and risk related knowledge is scarce. Moreover, the risk classes and containment measures mainly refer to the hazards of the donor and receiver organisms. It appears that for the more radical interventions of SynBio into the genome, genuine methods of assessment must be developed. This is all the more the case in relation to bioparts, protocells and minimal cells. Obviously, more discussion with scientists is needed in this regard.

2.2.2 Introducing SBPs into the Environment and Placing SBPs on the Market[27]

As already indicated, EU legislation, and in particular Directive 2001/18 categorises the introduction of GMOs into the environment as the deliberate release at a particular site and the introduction into the environment at any site after GMOs have been placed on the market. Both the release and the placing on the market must be authorised. [28] An authorisation of market placement of a GMO implies the subsequent introduction into the environment at any location, unless the allowable

[27] The following analysis is based on (von Kries and Winter 2012).

[28] Articles 5 and 6; 13–15 Directive 2001/18/EC which provide differentiated procedures of notification, risk assessment, commenting and final decision.

locations are restricted by conditions of the authorisation. [29] Concerning genetically modified food and feed, including seeds, a special regime has been established which takes precedence over the general regime which will however not be treated in this article because SynBio is still far from involving food and feed.[30]

We can treat the deliberate release and the market placement together because the risk prevention criteria and risk assessment methodologies are largely the same for both activities, with certain variations due to the larger geographical scope of introductions into the environment of GMOs which are authorised for market release.

Risk Paths

According to Art 4 (3) Directive 2001/18/EC Member States shall ensure that potential adverse effects on human health and the environment, which may occur directly or indirectly through gene transfer from GMOs to other organisms, are accurately assessed on a case-by-case basis.

Correspondingly, an environmental risk assessment (ERA) must evaluate risks

> whether direct or indirect, immediate or delayed, which the deliberate release or the placing on the market of GMOs may pose … [31]

The distinction between direct and indirect effects means that not only those adverse effects caused by GMOs in direct contact with endpoints (e.g., a human being, animal or plant absorbing a GMO) have to be prevented but also those which are mediated by intervening factors. Annex II of Directive 2001/18/EC defines indirect effects as follows:

> "indirect effects" refers to effects on human health or the environment occurring through a causal chain of events, through mechanisms such as interactions with other organisms, transfer of genetic material, or changes in use or management.

On this basis one could differentiate indirect effects further into natural causal chains (horizontal and vertical gene transfer, food chain, etc.) and chains mediated by human practices (such as agricultural change in pesticide use and crop rotation, etc.).

Concerning the distinction between immediate and delayed effects, the Commission Guidance on the environmental risk assessment gives examples for delayed effects such as the GMO developing invasive behavior, several generations following its release.[32]

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[29] Parts B and C of Directive 2001/18/EC.

[30] Regulation (EC) No 1829/2003 of the European Parliament and of the Council of 22 September 2003 on genetically modified food and feed (Text with EEA relevance), OJ L 268, 18.10.2003, p. 1.

[31] Art 2 (No 8) Directive 2001/18/EC.

[32] Guidance Notes on the Objective, Elements, General Principles and Methodology of the Environmental Risk Assessment Referred to in Annex ii to DIRECTIVE 2001/18/EC, OJ L 18, 07.11.2003, p. 32.

In addition to alerting the risk assessment to direct/indirect and immediate/delayed effects the ERA must also consider different environments exposed to the GMO[33]:

> For each adverse effect identified, the consequences for other organisms, populations, species or ecosystems exposed to the GMO have to be evaluated.
>
> Moreover, there may be a broad range of environmental characteristics (site-specific or regional-specific) to be taken into account. To support a case-by-case assessment, it may be useful to classify regional data by habitat area, reflecting aspects of the receiving environment relevant to GMOs (for example, botanical data on the occurrence of wild relatives of GMO plants in different agricultural or natural habitats of Europe).

This rather ambitious programme, relating to genetically modified plants, was further elaborated by Guidance of 2010 of the European Food Safety Agency (EFSA).[34] It concentrates on interactions of the plant on the levels of organisms and ecosystems.[35]

While this analytical framework looks comprehensive, a note of caution is, however, appropriate: The fate of the GMO in the various environments may prove to be too complex to be examined. This is particularly true if the GMOs introduced into the environment are microorganisms. It is telling that in that regard the pertinent EFSA Guidance somewhat wearily states as follows:

> Predicting impacts of GMMs and derived food or feed on complex ecosystems can be difficult due to continuous flux and spatial heterogeneities in ecosystems creating a myriad of potential microbial habitats in which interactions between GMMs and their products with the indigenous organisms and/or abiotic components can take place. It is recognised that an ERA cannot provide data of a GMM or its products, which would cover all potential environmental habitats and conditions. Consideration of environmental impact (damage) should, therefore, focus on environments in which exposure is most likely or in which, when relevant, viable GMMs could potentially proliferate.

Protected Endpoints

Human health and the environment

EU law has established that, for the deliberate release of GMOs, as well as for contained use, the protected goods shall be human health and the environment. These shall be kept safe from 'adverse effects. 'All appropriate measures' must be taken to prevent these.[36]

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[33] Annex II Directive 2001/18s. 4.2.2 and Commission Guidance Sect. 3 3rd hyphen.

[34] (EFSA 2010).

[35] See further on a multilevel approach of risk assessment of GMOs and SBPs (Breckling and Schmidt 2015, this volume).

[36] Art. 4 Directive 2001/18/EC.

What are adverse effects? Is the mere presence of a GMO outside the field of release, per se, to be considered as adverse effect? Prevailing court practice and doctrine negate this. They posit that the adverse effect must be a result of such presence, like the damaging of non-target species from an insecticide plant. The justification given is that the law only addresses the specific risks of genetic engineering, which shall be only health and environmental risks.[37]

Concerning SBPs this might be seen differently. It could be argued that given the early stage of R&D in this area and the radically artificial nature of SynBio, SBPs should not be allowed to spread at all. Any release would then have to be contained. Alternatively, if SBPs were constructed to only survive under artificial conditions, one could consider their safe release into the environment, because they would immediately die off there. However, this would not apply to organisms which are intended to survive and perform in the open environment.

Socio-economic benefits

GMO releases may create benefits for the producer and consumer. Is this to be weighed against the risks to human health and the environment? Such an analysis is envisaged in the genetic engineering legislation of some countries.[38] It is, however, only scarcely present in European GMO legislation.[39]

When pursuing this request two brands of risk-benefit-consideration should be distinguished: a risk-tolerating variant which would allow any risk that is outweighed by benefits, and a risk-averse variant according to which only residual risks can be outweighed by benefits. An example, for instance, of the second variant would, in relation to seeds, be the agricultural benefits of certain genetic modifications, such as the subsequent non-use of pesticides, the use of less water and reduction of chemical fertilizers. Thus, a residual risk to certain parts of the environment could become acceptable, if the overall eco-balance of agriculture were to be improved.

Concerning the release of SBPs into the environment, socio-economic benefits should also be introduced as an additional requirement; but only after its risks

[37] See for Germany Administrative Court (VG) Berlin, decision of 12.09.1995—14 A 255.95, in: Eberbach/Lange/Ronellenfitsch, Recht der Gentechnik und Biomedizin, Entscheidung Chap. 4 on § 16 GenTG; VG Braunschweig, judgment of 12. 9.1995—14 A 255.95, No. 27.

[38] For Germany see § 16 paras. 1 und 2 GentG, according to which "harmful effects on the protected goods listed in § 1 No. 1 must not be incurred if unacceptable in view of the objective of the release." Unacceptability in view of the release objective can be understood as a kind of weighing risk versus benefit. German scholars tend to reject such interpretation, arguing that this would be incompatible with the relevant EU law. See also Art. 10 of the Norwegian Gene Technology Act: "In deciding whether or not to grant an application, considerable weight shall also be given to whether the deliberate release will be of benefit to society and is likely to promote sustainable development." This provision has, however, rarely been applied in practice (Spök 2010).

[39] See the rather enigmatic opening clause ("… other legitimate factors") in Art. 7 and 19 of Regulation (EC) 1829/2003.

where assessed and found minimal, not as a vehicle to outweigh significant risks by higher valued benefits.

Cultural factors

The rejection of GMOs by the majority of the population in a number of countries can be explained by cultural factors. This skepticism is based on a conglomerate of concerns including extreme precaution, criticism against neglecting the evolutionary wisdom, doubts about whether the promised benefits are not already available from existing organisms, political will as well as ethical concerns and religious beliefs. The cultural factor is not well represented in national and international law as a legitimate justification for trade restriction. For instance, it was not even considered in the resolution of the WTO panel on EC restrictions concerning the marketing of biotech products.[40]

The ECJ has shown understanding for the cultural factor in *Commission versus Poland* but finally rejected it by splitting the issue into three parts: Insofar as extreme precaution was alleged, the Court said that this does not dispense from the normal standard applied in the EU; concerning the opponent political will it held that the MS must neglect it once an EU legal act has been adopted; and concerning ethical and religious beliefs it held that the strength and spread thereof was not sufficiently proven. [41]

It is submitted that the cultural factor should be given a more legitimate place in regulatory designs.[42]

Data to be submitted

A long list of data has been compiled that must be submitted for an application for release of GMOs. It comprises[43]:

- Information relating to the GMO
- Characteristics of (a) the donor, (b) the recipient, or (c) (where appropriate) parental organism(s)
- Characteristics of the vector
- Characteristics of the modified organism
- Information relating to the conditions of release and the receiving environment
- Information relating to the interactions between the GMOs and the environment
- Information on monitoring, control, waste treatment and emergency response plans

[40] WT/DS 291, 292/293/ R 29 Sept. 2006.

[41] ECJ Case 165/08, judgment of 16 July 2009 (Commission v Poland) paragraphs 54, 55, 58, 59.

[42] See further (Pardo Avellaneda 2014 forthcoming).

[43] Annex III of Directive 2001/18/EC.

This list would have to be thoroughly checked for its suitability for SBPs releases. Once again, it must be considered that more and more research is aiming at replacing traits from parental organisms by synthesis and, even more importantly, by artificial design.

The ERA, as outlined by Annex II Directive 2001/18/EC, focuses on those paths of risk with human health and the environment as endpoints. Other endpoints, like the coexistence with non-GM agriculture, the economic benefit and political as well as cultural values, are hardly considered (Dolezel et al. 2009, p. 27). However, should these aspects become a legally required part of the risk management, then information has to be provided and assessed which is methodologically clear and rich in substance.

The stepwise generation of knowledge

Towards the end of the nineteen-eighties, when the deliberate release of GMOs was approached, knowledge about the involved risks was still highly undeveloped. Even today, there remain gaps in our knowledge. Nonetheless, to enable the release of GMOs and acquire knowledge, the step-by-step principle was introduced: incremental generation of knowledge in parallel with decreasing containment of tests.[44]

The step-by-step principle is characterised by recitals (24) and (25) Directive 2001/18/EC as follows:

> The introduction of GMOs into the environment should be carried out according to the "step-by-step" principle. This means that the containment of GMOs is reduced and the scale of release increased gradually, step by step, but only if evaluation of the earlier steps in terms of protection of human health and the environment indicates that the next step can be taken.
>
> No GMOs, as or in products, intended for deliberate release are to be considered for placing on the market without first having been subjected to satisfactory field testing at the research and development stage in ecosystems which could be affected by their use.

The following sequence of steps has emerged in practice:

- laboratory
- greenhouse
- small-scale release with strict containment (not specified in law)
- larger-scale release with more relaxed containment
- placing on the market
- subsequent measures covered by the authorization
- subsequent Member State measures based on safeguard clause

[44] The step-by-step procedure goes back to OECD reports, including OECD, Safety considerations for biotechnology, 1992 (available at www.oecd.org/dataoecd/8/3/2375496.pdf).

The substance of the step-by-step principle was somewhat specified by Commission Guidance which says that "data from each step should be collected as early as possible during the procedure." It points to the possibility that "simulated environmental conditions in a contained system could give results of relevance to deliberate release," such as the simulation of behaviour of microorganisms in the laboratory, and of plants in greenhouses.[45]

The step-by-step principle is an instrument of societal learning. In the initial phase of European genetic engineering legislation, it was at the fore of public debate and became a legal requirement as outlined. With the amendment through Directive 2001/18/EC, monitoring has become an additional instrument. In order to increase safety, and at the same time facilitate the release and market distribution of GMOs, it was emphasized that those issues which, for reasons of time or scale, cannot be solved at one level can be clarified through monitoring at the next level. Monitoring can therefore be seen as a phase of learning following the release or market distribution, respectively. This concerns especially the investigation of effects which cannot be researched on an experimental basis, such as complex interactions on population and ecosystem levels, or cumulative and long-term effects.

As to procedural aspects, the applicant must submit a monitoring plan that contains the following information:[46]

1. methods for tracing the GMOs, and for monitoring their effects;
2. specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques;
3. techniques for detecting transfer of the donated genetic material to other organisms;
4. duration and frequency of the monitoring.

The monitoring programme is then determined as a condition for the release authorisation. The operator is responsible for implementing the programme and reporting results to the authority.

It is submitted that the step-by-step-principle, including self-monitoring, should also be used in relation to SynBio. Of course, the methodology must still be adapted to the various strands of SynBio and its peculiarities.

Steps in the analysis and assessment of risks

It is characteristic for the risk assessment in form of the environmental risk assessment (ERA) that it processes the data successively in pre-defined steps. The staggered evaluation of risks is finally followed by the risk management, which translates the scientifically informed risk evaluation into measures, i.e. the

[45] Commission Guidance Chap. 3.

[46] Art. 6 (2) (V) and Annex III C of Directive 2001/18/EC.

authorisation, the conditions for the authorisation and, if applicable, the rejection of authorisation.

According to Annex II of Directive 2001/18/EC and the respective Commission Guidance the ERA consists of six steps. Using the language of the Annex the steps can be summarized as follows:

In step 1, the inherent characteristics of the GMO are to be identified. They present factors (or "hazards") that can lead to risks depending on environmental conditions and usage.

In step 2, the potential consequences of each established adverse effect have to be evaluated. The evaluation concerns organisms, populations, species and ecosystems interacting with the GMO. Particular emphasis is given to the expected magnitude of the consequences. The latter can depend on the genetic design, the established adverse effects, the number of released GMOs, the receiving environment, the manner of the release and the control measures taken as well as on a combination of all these factors.

In step 3, the likelihood of the occurrence of each identified potential adverse effect is to be evaluated; here, each effect is examined individually, taking into account the risk factors, the number of released GMOs, the likelihood and frequency of gene transfer, the receiving environment and the conditions of the release.

In step 4, the different magnitudes of consequences (high, moderate, low or negligible) of every risk factor are linked to the different degrees of their likelihood (high, moderate, low or negligible). In addition, the overall uncertainty for each identified risk has to be described, including assumptions and extrapolations made at previous levels in the ERA, different scientific assessments and viewpoints, and the uncertainties contained in each evaluation.

In step 5, management strategies for risks from the deliberate release (or marketing) of GMOs are to be developed. The risk management is to be designed in a way so that identified risks can be controlled and that uncertainties can be covered. Safeguarding measures (coated seeds, isolation distances, etc.) have to be proportionate to the levels of risk and uncertainty.

In step 6, the overall risk of the GMO is determined. This consists of a summary of all identified risks and uncertainties of the examined application, taking into account the magnitude and likelihood of the adverse effects as well as the previous release of other GMOs. The achieved risk reduction caused by the management measures must also be considered.

Core to this 6 step procedure is the distinction between inherent factors of a GMO, adverse effects of these factors through interactions on the levels of the organism, populations, species and ecosystems, the magnitude of each adverse effect, and its likelihood. In addition, the uncertainties of the assessment shall be described. Safeguarding measures shall also be taken into account. This sounds thorough and comprehensive but may not sufficiently reflect the fact that SynBio is too diverse and unstructured to allow for a standardisation of risk assessment. For instance, the fact that much of the produce of SynBio is claimed not to survive under real world conditions must be integrated into the methodology. Likewise, the focus on organisms does not reflect possible risks from bioparts and minimal cells.

Familiarity

The major innovation needed in risk assessment for SynBio will be that the familiarity principle must be modified and finally even abandoned, because the newly designed organisms are intentionally more and more alienated from the genome of existing organisms.

The status of the familiarity principle in the GMO risk assessment can be summarized as follows: Risks to human health and the environment can be caused by traits of the non-modified parental lines and of the genetic modification. The concept of familiarity (or—using about the same approach—comparison with similar organisms or substantial equivalence), which goes back to an OECD paper of 1993, suggests that only effects of the genetic modification should be assessed. This is reasonable; otherwise the applicant could be blamed for adverse effects that are already contained in the parental line. However, critiques have alleged that, by focusing on the modification, the concept of familiarity cuts the organism into pieces and disregards effects of the newly created organism as a whole. Rather than assuming firm knowledge of the unmodified organism, one should rather look for the unexpected, the unfamiliar in interactions between the existing cause-effect network and the newly introduced GM component (Breckling 2004, 52–59).

Asking what the law demands in this regard, it should first of all be noted that the concept of familiarity is not conveyed by the wording of the substantive standard expressed in Directive 2001/18/EC. Rather, Art. 4(1) states comprehensively that the release and the placing on the market of the GMO must not cause any adverse effects. The annexed rules on the ERA, however, state that a comparison with non-modified organisms

> will assist in identifying the particular potential adverse effects arising from the genetic modification. [47]

The new EFSA Guidance of 2010 unwisely reinforces this approach by making the "comparative safety assessment" the core yardstick of risk assessment.[48]

Whether called comparative or not, the examination is not allowed in any case to imply that the transgene has to be considered in isolation. Unintended position effects and mutual reactions at all organismic levels are rather the consequence of genetic modifications and have to be considered to their full extent. Upon closer look this is also envisaged by the EFSA Guidance of 2010. Therefore, the Annex on ERA is still right to regard the comparative approach as a heuristic, rather than constitutive, tool of the risk assessment.

Concerning SynBio, however, even this heuristic function will lose ground with the growing alienation from parental lines of the new synthetic organisms. New methods of risk assessment must be developed. It is suggested that such methodology should start with risk-related analysis of the main strands of developments of

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[47] Annex II Directive 2001/18/EC, C.

[48] EFSA Guidance Chap. 2.

this technology. Subcellular parts and protocells, for instance, do not pose a risk of replication and through that of risks attached to life forms, such as becoming dominant in ecosystems. Rather, they are to be evaluated in terms of criteria used for chemicals, such as toxic, carcinogen, mutagen and allergen properties, persistence and bioaccumulation, as well as exposure analysis. Xenobiology is claimed to be safe because resulting organisms can only survive under very artificial circumstances. However, this is not necessarily true, so that scenarios and tests must be developed to prove this assumption. In addition, criteria used for chemicals should be applied. The major challenge will be to develop methods for the vast and ever-expanding works of those kinds of genetic engineering which are increasing the degree of artificiality even more. Specific tests must be developed in order to identify risks. Specific risk abatement technology must also be developed.

As all this costs time and effort, it appears to be advisable to establish a moratorium for the release into the environment of SynBio organisms, as well as a moratorium for the placing on the market of such organisms insofar as this entails any release into the environment.

3 Regulation Ex Post

Regulation ex post makes an actor liable to remedy or compensate for damage he or she has caused. There are various legal bases for such liability, general ones and ones specifically created for GMO-related risks.

The general scheme is tort liability. It presupposes that damage was intentionally or negligently caused to human health or material assets by an operator. The burden of proof, in principle, lies with the victim. Tort liability seldom leads to convictions because the causation and negligence are difficult to prove.

More specific and promising from the victim's perspective is strict liability for GMOs which has been introduced by some countries including Germany. Art. 32 of the German Genetic Engineering Act (Gentechnikgesetz- GentG) provides:

> Where any properties of an organism that result from genetic engineering operations cause the death of a person or injury to his/her health, or damage of property, the operator shall be obliged to give compensation for the damage ensuing therefrom.

No intention or negligence is required. The proof of causation is facilitated in two ways:

Causation from genetic engineering operations is presumed if the damage was caused by genetically modified organisms. The burden of proof that this was not the case lies on the operator.[49]

If the victim brings a prima facie proof that the damage was caused from genetic engineering operations of an operator the operator must disclose

[49] Art. 34 GentG.

information "about the type of and steps involved in the genetic engineering operations performed" by her.[50]

In addition, the liability does not only extend to the victim's own damage but also covers expenditure incurred by her for the restoration of damage to the environment. If, for instance, a bacterium which has been gene-coded for an infectious animal disease, escapes from the laboratory and causes a disease to bees, the operator is liable to pay for the forgone fruit yield and for the restoration of the bee population.

Directive 2004/35[51] establishes a third basis for liability. The concept does not introduce an additional right of a victim against an operator, but empowers and obliges administrative authorities to intervene. This is possible, i.e., if any deliberate release into the environment, transport and market placement of genetically modified organisms causes environmental damage.[52] The administrative authority can order the operator to take remedial action. NGOs are given rights to sue the authority if it remains passive.

Overall, SynBio as far as it is subject to the GMO regime, faces rather strict liability rules. As the special rules all refer to GMOs, they do not apply to technologies or products outside this scope. For this reason it must be considered whether the liability should be extended to those parts of SynBio which do not consist of GMOs in the legal sense, i.e. completely new organisms, organisms whose genome was completely replaced, organisms into which transgenes were inserted by other techniques than those contained in the positive and negative lists, organisms modified by xenobiochemistry, protocells, minimal cells, and bioparts.

4 Conclusion

Other than official statements by governmental and scientific bodies assume[53] the existing regulatory framework cannot be relied on as an adequate means of controlling risks from synthetic biology. Various kinds of SynBio are either not captured by the present regulation, or not appropriately treated by the present risk assessment methodology. This study suggests that the risks from SynBio should carefully and systematically be examined. On such basis new regulation should be introduced. This could be done by extending the scope and improving the risk assessment of the existing regulation on genetically modified organisms, or by taking a new approach that addresses biotechnology in a broad sense, including GMOs, SynBio, new breeding techniques and possibly further variants.

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[50] Art. 35 GentG.

[51] Directive 2004/35/CE of the European Parliament and of the Council of 21 April 2004 on environmental liability with regard to the prevention and remedying of environmental damage, OJ L 143, 30.4.2004, p. 56.

[52] Art. 3 para 1 and Annex III of the directive.

[53] See Footnote 13 above.

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Biotechnology, Modes of Action, and the Value of Life

Joachim Boldt

Abstract Synthetic biology can give rise to euphoric utopian scenarios, as well as to frightening dystopian narratives. These scenarios and narratives are not based on a consequentialist analysis of synthetic biology as a value neutral means to given ends. In contrast, the hopes and fears of these future scenarios are based on an apprehension of different modes of action that are meant to be prevalent in synthetic biology. Utopian visions highlight synthetic biology's potential to contribute to a society that lives and acts in "cooperation" with nature, whereas dystopian scenarios interpret synthetic biology as "disrupting" our connection to nature. It is argued that these differences can be philosophically spelled out in terms of a distinction between "communicative" versus "instrumental" modes of action. On this basis, a proposal is made to address what it might mean for a biotechnology to adhere to the communicative mode of action. In general, a communicative technology prefers to induce change by exploring and making use of the inner tendencies and interests of an organism. In contrast, an instrumental technology favors redesign to efficiently achieve given ends. As it turns out, the current main characteristics of synthetic biology, by and large, render it an example of the instrumental mode of action. As a consequence, from an ethical point of view extra effort is needed to make ideas of careful step-by-step approaches to innovation plausible in synthetic biology settings.

1 Introduction

In April 2013, a group of young scientists presented a biotechnology project on kickstarter, a fund-raising platform on the internet. Their aim was to develop a set of genes that can be inserted into plant genomes in order to create glowing plants.

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In a not so distant future streets may be lined with trees that glow at night and replace traditional electric street lights, the group imagined. They raised almost $500,000 in 6 weeks. The project was announced as the first "synthetic biology" project looking for public funding. It comprises, in an intuitively accessible way, some of the main characteristics of this new, emerging biotechnology (Callaway 2013).

As a popular description of the field has it, synthetic biology is the design and fabrication of new biological parts, devices, and systems; or the re-design of existing, natural biological systems (Synthetic Biology Community 2013). Obviously, taking a look at the second half of this definition, synthetic biology is akin to genetic engineering. Similar to synthetic biology, genetic engineering comprises genetic modification ("re-design") of existing organisms ("natural biological systems"). At the same time, synthetic biology has the potential to intervene more radically in the genetic makeup of an organism than genetic engineering. This is what the first part of the definition indicates. Genetic design and construction of whole organisms is clearly beyond the scope of traditional genetic engineering.

Currently, synthetic biology research is, in many cases, still close to genetic engineering. Nonetheless, its aspirations to construct DNA-sequences as large as whole genomes with predefined functions are reflected in its current research methodology. In synthetic biology, information technology and engineering principles join biology in order to enable the effective and efficient design of DNA modules. In some strands of synthetic biology these modules are meant to adhere to certain standardization guidelines in order to ensure that they can be freely combined. Like Lego bricks these "BioBricks" may be used to create organisms with desired functionality. The BioBricks database is an open source registry, freely accessible to everyone.

Regarding the depth of genetic interference, the glowing plant project is also still closely related to genetic engineering. Nonetheless, it shares many characteristics with synthetic biology's far-reaching aspirations. The glowing plant project is based on the ideas of a student team that took part in the iGEM competition in 2010. The iGEM competition is a yearly synthetic biology student competition at MIT (iGEM stands for "international Genetically Engineered Machine"). Students are requested to hand in designs of DNA sequences that may alter the functionality of microbes in unique and useful ways. The DNA sequences are supposed to become part of the BioBricks registry. In 2010, a team from Cambridge (Cambridge 2013) put together a DNA design that enabled the bacterium *E. coli* to glow in different colors. Looking for useful applications for their technique, they came up with the idea of lighting streets with bioluminescent trees.

The iGEM pedigree of the glowing plant project provides contiguity to the modularization and standardization framework of synthetic biology. Furthermore, just as the iGEM competition fosters creativity, the glowing plant project is a product of creative imagination as much as it is the result of science and engineering. Lowering the need for carbon based energy production by replacing electric street lights with growing plants is an innovative solution to a well-known problem that

may even be seen to possess poetic dimensions. Moreover, the creative aspect of the glowing plant project also reflects the way in which natural plants are transformed. This transformation is not an improvement to the tree's natural functions. The tree's new property, i.e. the ability to glow in the dark, does not necessarily improve any of its other useful functions for humans. It does not, for example, optimize the tree's ability to cast a shadow or cool down the microclimate. Working along these lines would be genetic engineering's approach. By contrast, if one integrates the ability to emit light into a tree, one adds a feature which is unique to the individual tree in question. Engineering a glowing tree is thus also creative in the sense that it adds a novel function to the organism.

Finally, the unusual funding scheme utilized by the glowing plant project bears witness to the project's contiguity with the lifeworld of a young IT community. With regard to synthetic biology, "biohacking" comprises, for example, setting up public biotechnology labs and posting internet videos on how to assemble centrifuges (Biba 2011). Looking for funding on internet public-funding platforms can be seen as another variation of turning hacker ideals into reality. The glowing plant project is therefore in holding with IT hackers' ideals of transparency and their critical stance on industry-funded research.

Seen through the prism of the glowing plant project, synthetic biology is characterized, firstly, by an engineering framework of modularization and standardization, secondly, by a creative approach to building and re-building living organisms, and, thirdly, by displaying hacker ideals of transparency and the positive effects of doing research as part of, and with the help of, non-institutionalized "crowds."

2 Utopian and Dystopian Scenarios

On a larger scale, these features can become part of both utopian and dystopian visions. On the dystopian side, synthetic biology's aspiration to create novel life forms by synthesizing DNA sequences as large as whole genomes makes us think of synthetic organisms overtaking and destroying natural ecosystems and the planet. This vision bears close resemblance to the "Grey Goo" scenario that came up for the first time in the context of emerging nanotechnology research and applications. According to the "Grey Goo" scenario, self-replicating nanorobots may come to use up all the edible matter on earth [the term "Grey Goo" was originally introduced by US-American engineer and author K. Eric Drexler in his book "Engines of Creation", (Drexler 1990)]. In a similar way, Michael Crichton's 2002 novel "Prey" (Crichton 2002) depicts a team of scientists chasing highly dangerous and aggressive swarms of artificially evolving microorganisms gone astray.

On the utopian side, synthetic biology can relieve the economy of the need to use carbon-based energy and resources. In addition, synthetic biology can enable humans to conform the functions of plants (and non-human life in general) to

human needs. This could lead to a future in which the ability of life to adapt to new environments, and to transform itself in unprecedented ways, will be used to better humankind. Clothes that change their surface according to the weather; and houses and bridges that consist of living trees are cases in point. In this vein, the scholar and "sustainability innovator" Rachel Armstrong has published an e-booklet on synthetic biology and its future impact on society (Armstrong 2012).

Dystopian and utopian synthetic biology scenarios do not only differ with regard to the way they contemplate the consequences a new biotechnology may have. The utopian vision, in particular, brings to the fore that in these scenarios synthetic biology is embedded in broader ideals of humankind's relationship to nature and the environment. In the utopian vision, synthetic biology is represented as a technology that stands in for values such as harmonious cooperation and stewardship. Likewise, albeit less apparent, the dystopian scenario, in many of its instances, interprets synthetic biology as another—and this time perhaps decisive—step on humankind's path to alienate itself from nature. The aspiration to rebuild and recreate nature is read as an attempt to neglect one's dependency on nature. This attitude constitutes hubris and ultimately, so the dystopian story goes, must lead to catastrophic results.

3 A Common Story About Technology and Ethics

Interpreting synthetic biology as a way of understanding and relating to nature runs counter to the common idea that a technology is a value-neutral means to a number of diverse ends. To develop novel, more efficient technologies, then, is to increase human power and human abilities to change nature following human interests and needs. According to this idea the ends to which a technology is used, not the technology itself, are to be judged ethically. Basically, this means-end framework of interpreting technology is, ethically speaking, a consequentialist model of judging the ethical value of a given technology.

If one were to ask at this point why it is that we in western society promote power-increasing technologies, the answer can be twofold. On the one hand, striving to increase one's own abilities to change nature according to one's interests can be understood as a *fact* about human behavior. As a matter of fact, one may say, humans are driven by a desire to analyze, build, rebuild and make use of natural processes. On the other hand, one may argue that the desire to devise new technologies is an ethical *demand*. In order to argue in favor of this claim, one may point out that efficient means are a necessary prerequisite of the ability to do anything, including realizing good ends. On this account, if one has a moral obligation to realize good ends, one also has an obligation to devise the best and most efficient means to reach these ends.

If one argues along this second line, the notion of an ethical value of technology in itself is reintroduced at a second level. Primarily, one holds that the ethical evaluation of technology as a means to diverse ends depends on the ethical

value of the ends that one brings about by using the technology in concrete circumstances. Nonetheless, on a second level, one claims that looking for novel and more efficient technologies, and promoting them is a moral obligation since these activities enable a society to efficiently live up to ethical challenges.

4 Modes of Action in Utopian and Dystopian Scenarios

It is becoming apparent, even if one thinks about technology along the consequentialist means-end line, one can incorporate the idea that doing scientific research and developing technologies is, in itself, an ethically worthwhile activity. In this general respect, the means-end model need not differ from the way in which utopian and dystopian scenarios interpret synthetic biology. Nonetheless, neither utopian nor dystopian scenarios share the means-end model's assumption that the relevant ethical characteristic ascribed to developing novel technologies is the increase in power to change the natural state of affairs. Both the utopian and the dystopian scenarios distinguish ways of relating oneself to and understanding nature that have an effect on how one acts and how one develops technologies. That is to say, the scenarios determine modes of action.

These modes can be roughly described as follows: the dystopian vision emphasizes that if one progresses too boldly into uncharted scientific and technological terrain, one may be unpleasantly surprised by the effects of one's actions. Especially, if one proceeds to genetically create an organism that differs in substantial aspects from natural ones. Rather than altering the behavior of natural organisms by way of external stimuli, or genetically altering natural organisms in minor respects, the newly-designed organism may run out of control in unforeseen ways. Dystopian visions thus make use of the intuition that it is wise to follow a careful step by step strategy. The dynamics of living nature, it is assumed, are incalculable. We may infer from the dystopian narratives that there are technologies which are close to nature, and others that depart from nature in the sense that they rely less on natural, given entities, attempting to construct and engineer novel objects instead. According to the means-end model this step from altering to creating is a way of becoming able to design entities which conform to human interests potentially more perfectly than any natural "design" can do. According to dystopian scenarios, leaving nature's mold is dangerous, even if doing so, based upon our knowledge and the available technological abilities, promises to lead to more efficient means, and to more valuable ends. From this point of view there is a mode of action that relies on and remains close to nature, and another one that is characterized by distancing itself from nature. It is the second mode of action that dystopian visions warn against.

It is worth noting that part of the persuasive power of this distinction rests on the type of entities which biotechnologies are directed towards. Biotechnologies, such as synthetic biology, do not build machines, but living entities. A living entity, and this is an everyday assumption, acts upon and reacts to an environment according to that which one, when regarding higher organisms, calls a "will."

A will may be fairly stable, but a will may also change without being forced to do so by external, observable causes. Thus, the concept of will is closely related to notions of degrees of freedom. Now, if one introduces freedom as one of the features which constitutes a living being, it is plausible to anticipate unexpected outcomes of engineering, and the releasing of novel organisms that have not yet adapted to and found their place in an environment.

Before returning to this point in more detail later, a closer look at how modes of action are distinguished in utopian visions will help to further clarify what is at stake. In a certain sense, utopian visions reverse the interpretive order of dystopian narratives. Like dystopian visions, utopian scenarios also make use of the idea that a technology can be close to or depart from nature. In contrast to dystopian visions, though, the utopian story equates synthetic biology with a technology close to nature. Moreover, synthetic biology is understood as a technology that enables harmonious cooperation between living nature and humans.

The term "cooperation" further qualifies what "closeness to nature" might mean. Utopian visions emphasize that by the means of synthetic biology, the will of non-human living beings may come to conform to the needs and interests of humans, to the effect that organisms "voluntarily," so to speak, do what is good for humans. At the same time, humans using synthetic biology no longer need to resort to less sustainable technologies. In this sense they too become subjected to a transformation of the will since synthetic biology transforms their way of living.

Again, as an aside that will be picked up later in more detail, interpreting synthetic biology as a technology of cooperation is particularly appealing if one describes synthetic organisms as willfully acting and reacting living beings that possess a degree of freedom. Only living beings thus understood can form an independent counterpart and become the subject of "operations" themselves when confronted with attempts to establish co-"operation."

To summarize, firstly, utopian and dystopian scenarios distinguish a mode of action that is cooperative and close to nature, in contrast to a second one that diverges from natural models and may hence be called "disruptive." In this respect both scenarios resemble each other and both stand in contrast to a means-end model of understanding technology in which the only ethically relevant criteria to systematize technologies are efficacy and efficiency.

Secondly, the two scenarios disagree fundamentally about where to localize synthetic biology. On the one hand, dystopian scenarios place synthetic biology on the disruptive side of modes of action. The cooperative side is taken up by technologies that are less oriented towards designing and creating. Utopian scenarios, on the other hand, interpret synthetic biology as a cooperative technology. Paradigmatic instances of the disruptive mode of technological action are, from this perspective, carbon-based, non-sustainable technologies.

Two questions will guide the line of thought that is to follow. Firstly, it will be asked from a philosophical and ethical point of view whether a distinction between cooperative and disruptive modes of action can be further elucidated and made plausible. Secondly, it will be asked where on this scale, from an ethical and philosophical point of view, synthetic biology is to be located.

5 Two Modes of Action: Communicative and Instrumental

Philosophically speaking, cooperation is a prime example of ethical behavior of one person towards another. This relation is, to begin with, characterized by recognizing the needs and interests of another person and by providing assistance. If this happens, the behavior of two (or more) persons is turned into cooperation, since the actions of those involved are all guided by the same set of needs and interests.

Moreover, if one ascribes to another person the ability to act responsibly and morally, this includes an ethical demand on oneself to always communicate with the other person before attempting to change behavior by interventions that enforce behavioral change. If one, for example, reacted to apparently confrontational, ethically questionable behavior by directly intervening into the brain of the other person, this reaction shortcuts the ethical demand to acknowledge that the other person has equal ability to act as reasonably as oneself.

If one witnesses behavior that appears not to conform to a conviction of the common good, a first reaction is to figure out the reasons behind this action. This is already a communicative act. One asks the other person why she does what she does. If one is unsatisfied with the answer and still does not understand how this action and this reason can be part of an attempt to attain a common good, one may try to convince the other person of a better course of action. The resulting debates between two persons, addressing the notion of a common good, can render certain reasons, and actions, as *truly* good ones.

This supposed ability to adjust individual reasons for action to the notion of a common good is exactly what is meant when we regard ourselves and others as capable of moral and immoral behavior. Both the asking and the attempt to convince presuppose the assumption that the other person has free will and reason, that she can change her will according to reasons, and that these reasons are oriented towards the notion of a common good.

Jürgen Habermas has introduced the term "communicative action" to denote this behavior and its corresponding ontological and epistemological assumptions (Habermas 1984). Ontologically, communicative action presupposes the idea of another person who can make up his own will according to reasons that are oriented towards a common good. Epistemologically, communicative action posits the assumption that in a debate about what is regarded as reasonable action, there is no way to omit the debate. It is not possible to learn the truth about the common good by conducting experiments, for example.

Habermas delineates the characteristics of communicative action in contrast to instrumental action. In instrumental action, one acts towards pre-defined ends. The task of reflection and debate in this case is to determine the best means to bring about the intended effect. It is a debate between those who have agreed on an end, and are not themselves objects of the intended alteration of the state of affairs. If, for example, one comes to the conclusion that it is necessary to alter the self-destructive behavior of a psychiatric patient by medical intervention, the

debate about how best to do so involves medical experts, not the patient. In the paradigmatic cases, though, the objects to be altered according to instrumental specifications are non-human and non-living entities.

Now, in the case of non-human living entities the two modes of action may come to overlap. Firstly, from the point of view of communicative action, although verbal communication with animals is not possible, paraverbal and non-verbal communication is, in some cases, feasible. Moreover, the assumption inherent in communicative action that one should not change questionable behavior by genetic or neurologic intervention without strong, necessitating reasons. In the encounter with non-human living beings, making first contact can make great sense. In many cases we assume that it is ethically more valuable to provide animals with what they apparently need, to gain trust, and in doing so change behavior, than to intervene medically and technically. Engaging in this kind of relationship, in turn, implies setting aside one's own interests and changing one's own behavior. Hence, one can and often does follow the behavioral ideals implicit in communicative action in relation to animals as well.

At the same time, especially with regard to lower organisms, we assume that it is appropriate, both as a matter of fact and a matter of ethics, to treat them as means to our ends. In these cases, we follow the instrumental mode of action. In the case of higher, non-human organisms, we apply as a matter of fact this mode of action as well. This is usually the latest point at which the controversial social debate reveals diverging opinions on whether this treatment can be ethically defended.

6 The Role of "Biocentrism"

So far, it has been argued that we can and often do follow the communicative mode of action in the case of animals and that we sometimes think of this way of acting as ethically valuable. Apart from this observation, is there anything from a theoretical point of view to be said in favor of these practices and assumptions?

The answer to this question hinges on whether the ontological and epistemological assumptions implicit in the communicative mode of action can be applied in the case of animals as well. Thus, the question becomes whether it is feasible to think of animals as possessing a will, to follow motivational entities akin to reasons and to possess degrees of free will?

On the one hand, if one adheres to the Kantian sources of the theory of communicative action, the answer must be a definite "no" in both cases. On the grounds of his theoretical philosophy that cherishes forces and universal, deterministic laws as concepts that appropriately describe the order of reality, Kant assumes—at least in his *Critique of Practical Reason*—that the behavior of animals has to be understood according to this model as well. The only exception to this rule is human behavior, where freedom and practical reason come to play decisive roles.

On the other hand, there are conceptions in the philosophy of biology that attempt to render plausible the assumption that explanations in terms of reason and

free will can be partially attributed to animals as well. Firstly, we must ascribe the ability to act responsibly, to be guided by reason and to possess free will to ourselves. Secondly, human and animal behavior can be explained with reference to the same basic explanatory principles. Therefore, it is argued that as a consequence, one must conclude that reason and freedom are—more or less dormant—aspects of animal behavior as well.

The 20th century bioethicist and philosopher Hans Jonas has developed a position of this kind in his philosophy of biology (Jonas 1966). Jonas shows, for example, how the processes of metabolism display the ability of even the most basic organisms to form an identity that remains the same, even if all its molecules have been replaced. The detachment of organismic identity from material identity is, according to Jonas, the first step of the development of life towards more elaborate forms of self-determination.

In the same vein, aforementioned Jürgen Habermas does not, as one might presume, pursue the Kantian source of the theory of communicative action, but emphasizes, at least in some remarks, the ethical relevance of the factual kinship of animals and humans. He does so with direct reference to Hans Jonas (Habermas 2003).

Details of the arguments of this position aside, it is important to note for the purposes of this paper that if one distinguishes between communicative and instrumental modes of action, one has to introduce and spell out a conception of general characteristics of life that contain degrees of reason and freedom. Communicative action cannot be described within the framework of a theory of action or a philosophy of technology alone, since it presupposes ontological and epistemological assumptions that differ from those of other modes of action. If one does not restrict the validity of these presuppositions to the realm of human life, they become the basis of a theory that ascribes direct ethical relevance to non-human living beings as well. In this sense, the theory of communicative action can lead to a "biocentric" ethical stance that is not utilitarian.

7 A Typology of Instrumental and Communicative Biotechnology

If one distinguishes communicative from instrumental modes of action and accepts the biocentric interpolation of communicative action, it becomes possible to outline a scheme of criteria according to which biotechnological approaches can be judged with regard to whether they constitute an instrumental, rather than a communicative type of technology.

A general and partly metaphorical way to describe what is at stake ethically in communicative action is to say that adhering to the ideal of communicative action is to be prepared to learn from living beings, and to attempt to act in accordance with them. To induce alterations in the behavior of other living beings can become necessary if, firstly, an organism cannot live up to its own interests due to impairment or disease, or, secondly, if the behavior of an organism interferes

substantially with its own or the interests of other living beings. In the first case, biotechnological interventions can be immediately justified as measures that enable an organism to act in its own interests. In the latter case, one can introduce a step-by-step scheme that translates the ideal of "living in accordance with" that one ought to adhere to as far as possible into terms of method, level, and extent of the biotechnological intervention.

7.1 Aim of Intervention

Biotechnological interventions can aim at restoring natural capacities, or at altering or enhancing capacities. Again, given that the ideal of communicative action is to accomplish behavioral change by conviction, biotechnological interventions can be counted as effective if they enable an organism to remain in a state in which it can enact its instincts and interests. This preference reflects the ethical relevance of the distinction between therapeutic and non-therapeutic medical interventions. While therapeutic interventions are understood from the point of view of communicative action if they provide conditions that enable a living being to interact with its environment and recognize, adapt and follow its instincts and interests; non-therapeutic interventions cannot be justified on these grounds.

7.2 Method of Intervention

Methods of biotechnological intervention can be reversible or irreversible, local or systemic, they can have long-term or short-term effects on behavioral characteristics. Given that the ideal of communicative action is to accomplish behavioral change by conviction, somatic alterations ought to be as limited as possible with regard to time and place. That is to say, generally speaking, reversible, local, short-term effect technologies fit better into the framework of communicative action than their irreversible, systemic, long-term alternatives.

7.3 Level of Intervention

Biotechnologies can address an organism or a part of an organism at different levels. They can be interventions at the level of, for instance, organs as well as intracellular, molecular processes. These interventions will have different effects on the behavioral identity of an organism, given that different somatic levels have different bearings on behavior. An intervention in the overall genetic make-up of an organism will potentially have a greater effect on the way an organism behaves in its environment than an intervention in a cellular compound of an

existing organism. Hence, the closer the level of intervention of a biotechnology is connected to an organism's behavioral identity, the more instrumental, prima facie, is the character of that technology.

7.4 Extent of Intervention

Firstly, biotechnologies can be distinguished according to the extent of intervention into an organism. A biotechnology may replace whole genomes of organisms, or it may be restricted to adding or replacing short strands of DNA. To take another example, a biotechnology may be directed at replacing whole organs of an organism or it may be confined to substituting parts of organs. The more a biotechnology aims at substituting wholes instead of parts, the more it becomes instrumental in character. Since the ideal of communicative action is to change behavior by convincing instead of enforcing change, the more parts of a whole which a technology can leave untouched, the more it tends to act in accordance with communicative ideals.

7.5 Degree of Novelty of Resulting Objects

If a technology includes the ability to replace whole genomes—or organs, etc.—and if the substituting genome is not a copy of the natural one, the resulting organism will, to a high degree, be novel as compared to its natural ancestor. Accordingly, the novelty of an object that a biotechnology is able to engineer can count as another indicator of the biotechnology's position on the scale of modes of action. Again, a high degree of novelty indicates that the biotechnology in question departs significantly from the communicative ideal of convincing.

7.6 Affected Organism

Lastly, from a commonsense point of view, there can be no doubt that biotechnological interventions are more controversial and stand in need of stronger ethical justifications than interventions into lower living beings, let alone single-cell organisms. Biocentric approaches often have difficulty reproducing this ladder of increasing value, if they attempt to reconstruct it at all. Biocentric approaches usually assume that the factual feature a being must possess in order to be an appropriate object of ethical respect is not to be able to reason, but to have interests, to experience pain, or to be alive. If a being possesses, for example, the ability to form and follow interests, one may distinguish the urgency of the ethical demand not to frustrate these interests according to the assumed amount of harm

that neglecting the interest implies for the organism in question. The amount of experienced harm need not, in turn, correlate to whether the affected organism is a "higher" or "lower" animal.

The biocentric approach presented here follows a different path, though. It does not substitute the concept of reason and its ethical significance with a different concept altogether. Instead it assumes that concepts such as being alive and having interests can be understood as partial realizations of what it means to possess reason. Since the ladder of what one may interpret as increasingly free and reason-guided behavior closely resembles a commonsense understanding of higher and lower organisms, the model of communicative action is capable of recognizing the ethical relevance of the kind of organism that is affected by a biotechnological intervention. From the perspective of communicative action, biotechnologically interfering with the interests of a higher animal carries more ethical weight than interfering with the interests of lower animals.

8 The Place of Synthetic Biology

Now, where is synthetic biology to be located on the scale of biotechnological interventions into higher living beings? First of all, as a caveat, one needs to bear in mind that just as there is no unambiguous boundary between instrumental and communicative action, synthetic biology is not a monolithic technology but comprises a number of different research approaches and strategies. Still, if one emphasizes synthetic biology features such as the engineering framework and the focus on a creative approach of building and re-building living organisms, synthetic biology presents itself as an instrumental rather than a communicative technology, utopian scenarios notwithstanding.

The engineering framework and the corresponding approach to designing DNA sequences as standardized modules suggests treating single cell organisms as machine-like entities. The title of the iGEM competition ("genetically engineered machine") bears witness to this tendency. Since a machine is not an appropriate object for communicative action, synthetic biology's engineering framework leads to thinking along the lines of instrumental action instead. Likewise, the creative approach of synthetic biology favors engineering novel organisms according to predefined interests, instead of making cooperative use of existing organisms. Thus, if standardization, modularization and engineering novel forms of life are at the core of synthetic biology, synthetic biology will have to be reckoned with as a highly instrumental technology.

To be sure, this does not mean that there are no ethical arguments which support developing and using synthetic biology. From the perspective of communicative action, classifying a biotechnology as instrumental does not necessarily imply that there are no reasons at all justifying its use. If the ends to which synthetic biology is meant to be deployed are sufficiently important, and if more communicative means cannot bring about these ends, there are good reasons to proceed with this technology. What it does mean, though, is that synthetic biology solutions to societal and ecological challenges are not considered to be first-choice even if they

apparently promise to be more efficient than alternative, less instrumental solutions. Apart from the question of how to normatively justify and understand this reluctance, one may interpret the emphasis of communicative action on acting in accordance with an organism's interest as assuming that, as a matter of fact, the behavior resulting from an organism's natural interests is particularly reliable in the long-run and when faced with unexpected stimuli.

In addition, localizing synthetic biology on the instrumental side of the biotechnology typology does not imply that synthetic biology cannot comprise reversible, local and short-term methods of intervention. Synthetic biology interventions can, of course, incorporate many of these and other restraints, often in the guise of safety precautions. Nonetheless, from the point of view of an instrumental technology such as synthetic biology, these measures are not an internal part of the instrumental logic of developing efficient means to specific ends. An extra effort and a second line of reasoning are needed to supplement considerations of efficiency with those regarding safety. By contrast, the internal logic of communicative action, in itself, contains restraints on the temporal and spatial scope of an intervention.

9 Concluding Remarks

Synthetic biology has been identified, through the lens of a biocentrically extended theory of communicative action, as an instrumental technology. As such, synthetic biology does not comprise in itself restrictions with regard to scope and extent of its interventions; and it does not from within its own logic recognize an inherent value of living beings, their interests and behavior. Now, neither of these two issues presupposes that using synthetic biology must inevitably lead to the kind of catastrophic results as envisaged by the dystopian scenarios. But, one may understand dystopian scenarios as pointing to the fact that one needs to supplement the logic of instrumental technology in order to render plausible careful step-by-step approaches to innovation. At the same time, utopian scenarios overrate the degree to which synthetic biology is understood to cooperate with living nature. The synthetic biology of today is characterized by engineering ideals of modularization, standardization and targeted design of novel forms of life that do not fall easily under the rubric of cooperative communicative action. It remains to be seen whether the synthetic biology of tomorrow can incorporate more of the internal logic of cooperative action.

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Synthetic Biology as Technoscience and the EEE Concept of Responsibility

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Abstract Two fields of reflection on synthetic biology are related to each other: the debate on the understanding of the specific scientific character of synthetic biology on the one hand with reference to the notion of technosciences, and the debate on Responsible Research and Innovation on the other. The target is asking for the consequences and implications of classifying synthetic biology as a technoscience which implies blurring the traditional distinction between basic and applied sciences—for attributing and distributing responsibility. To this end, the EEE model of responsibility will be introduced (empirical, ethical, epistemological). Building on this concept the specific responsibility constellation in the field of synthetic biology will be analysed. Concluding, the necessities of conceptualising ethics as an accompanying reflection on the scientific and technological advances including the consideration of their relationship to the governance of science within the democratic system are taken under consideration.

1 Introduction and Overview

In this chapter I would like to relate two fields of reflection on synthetic biology to each other: the debate on the *understanding* of the specific scientific character of synthetic biology on the one hand with reference to the notion of *technosciences* (Kollek and Döring 2012), and the debate on *Responsible Research and Innovation* (Grunwald 2011; von Schomberg 2012) on the other. The target is asking for the consequences and implications of classifying synthetic biology as

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technoscience—which implies blurring the traditional distinction between basic and applied sciences—for attributing and distributing *responsibility*.

Synthetic biology is confronted with high expectations for innovation, primarily in the fields of energy, health, and sustainable development. The knowledge gathered by molecular biology, nanotechnology, biotechnology and information technology shall be combined to implement new functions in living systems by modifying bio-molecules or the design of cells, or by designing artificial cells and, perhaps, complete organisms. Therefore, the *convergence* of different fields of science and technology is crucial to this approach (Roco and Bainbridge 2002).

The traditional self-understanding of biology as natural science aiming at *understanding* processes of life is challenged by synthetic biology (Ball 2005) which shifts its target to *redesigning* life or *newly creating* living entities far beyond the understanding of how life works. This shift transforms biology, on the one hand, into an engineering science (de Vriend 2006), while it simultaneously remains, on the other, a cognition-oriented science belonging to the field of "new and emerging sciences and technologies" (NEST). This two-fold structure of synthetic biology prevents classifying it either as applied or as basic science and allows for referring to the ongoing debate on *technosciences* (see Sect. 2).

The postulates of 'responsible development' in scientific-technological advancement, and of 'responsible innovation' in the field of new products, services and systems have been discussed for some years now with increasing intensity (von Schomberg 2012; Grunwald 2011). Responsible innovation adds explicit ethical reflection to shaping technology and innovation, and involves normative questions of responsibility and their backing in ethical theory (Grunwald 2012a). Beyond ethical reasoning, any reflection on NEST developments must necessarily involve epistemological consideration of the status of the prospective knowledge on developments under consideration and on the uncertainties involved. Furthermore, responsible research, development and innovation also have to deal with *empirical* issues of power distribution, of the involvement of stakeholders and users, of organising governance and communication processes, etc. Therefore, the concept of responsible development and innovation has to integrate ethical, epistemological, and empirical issues (EEE).

In this chapter, I will explore the consequences of the classification of synthetic biology as technoscience for the responsibility debate and the respective governance. To this end, I will first briefly explain the classification of synthetic biology as technoscience (Sect. 2) and introduce the EEE model of responsibility (Sect. 3). Building on these argumentations I will then look for the specific responsibility constellation in the field of synthetic biology referring to the notion of technoscience which results in a differentiated picture of the distribution of responsibility (Sect. 4). Concluding, I will point to the necessities of conceptualising ethics as an accompanying reflection on scientific and technological advances, including the consideration of their relationship to the governance of science within the democratic system (Sect. 5).

2 Synthetic Biology as Technoscience[1]

The biological self-concept aiming at an *understanding* of life processes based on traditional natural science is reframed in synthetic biology. Synthetic biology no longer is satisfied with investigating life which already exists but aims at a redesigning or even reinventing nature. A turn towards artificial forms of life is characteristic of all definitions whether created by designing anew, or redesigning existing life (Grunwald 2012a). The targeted design of cells requires ample understanding of all essential sub-cellular processes and interaction. However, the current body of knowledge is still far from sufficient. In the ongoing research activities of synthetic biology, the main aim therefore is to gain insight into structures and functions of natural systems which is closer to analytical science rather than engineering. By moving more and more towards engineering, design and creation, synthetic biology will develop more towards an engineering science (de Vriend 2006) with a duality of cognition and design (Banse et al. 2006).

Living systems are examined within the context of their technical function, and cells are interpreted as machines—consisting of components analogous to the components of a machine which have to co-operate in order to fulfil the overall function. For example, proteins and messenger molecules are understood as such components that can be duplicated, altered or newly compounded in synthetic biology. A "modularisation of life" is thereby made as well as an attempt to identify and standardise the individual components of life processes. In the tradition of technical standardisation, gene sequences are saved as models for various cellular components of machines. Following design principles of mechanical and electrical engineering, the components of living systems shall be put together according to a building plan in order to obtain a functioning whole. The recombination of different standardised bio-modules (sometimes called 'bio-bricks') allows for the design and creation of different living systems. With the growing collection of modules, out of which engineering can develop new ideas for products and systems, the number of possibilities grows exponentially. The engineering approach of synthetic biology can easily be seen by looking at the language used: this is classical language of engineering, especially of mechanical and electrical engineering as well as that of informatics. The area of life under consideration is thus modelled as an ensemble of machines:

> Although it can be argued that synthetic biology is nothing more than a logical extension of the reductionist approach that dominated biology during the second half of the twentieth century, the use of engineering language, and the practical approach of creating standardised cells and components like in an electrical circuitry suggests a paradigm shift. Biology is no longer considered 'nature at work', but becomes an engineering discipline (de Vriend 2006, p. 26).

[1] This section builds on earlier work of the author on the understanding of synthetic biology. In particular it extends to what has been published by Grunwald (2012a, b).

Examples of such changes in the language are: haemoglobin as vehicle, synthesis of adenosine-triphosphate as generator, nucleosome as digital database, polymerase as copy machine, or membranes as electrical fences (Grunwald 2012a). The language used by synthetic biology proves it to be epistemologically bound to a technical view of the world and technical intervention.

This is one side of the coin—however, there is also another. The scientific-technical development of the past decades has made the traditional border between technology and the sciences more permeable. One aspect of this is that technical interventions in the sphere of molecular biology have led to genetic engineering, which can be understood as a classical (natural) science but as technology as well. This observation led to the notion of *technoscience* (Latour 1995) describing recent developments in science and engineering as overcoming traditional borders. This diagnosis also applies to synthetic biology (Kollek and Döring 2012). In particular, it has consequences for the assignment of responsibility because the traditional border between technology-oriented applied science and cognition-oriented basic research is disappearing While traditionally basic research is confronted with expectations to take over responsibility only for the research process itself but not for possible later out-comes in terms of technology, the situation in applied science is different. Because its target is to develop knowledge to be used and applied, e.g. in technology, the reflection on responsibility issues related to those applications intimately belongs to applied research. Following the diagnosis of synthetic biology being a technoscience belonging to both areas or none of them—gives rise to the question of distribution of responsibilities specifically regarding this situation.

3 The EEE Concept of Responsibility[2]

The notion of responsibility assumes a—more or less—clear meaning and idea of this responsibility. However, this might be misleading, at least in the field of science and technology. Concerns have been expressed (Beck 1986) that responsibility would be an empty phrase without reliable meaning; that it would merely show the character of an appeal and of moralisation of conflicts, that it would not be able to contribute to problem-solving, that the uncertainty of knowledge about future consequences of today's decisions would render any considerations of that responsibility ridiculous. Thus the complex governance of modern science and technology involving many actors would lead to the effect of "thinning" responsibility.

[2] **This section develops further clarifications on the notion of responsibility given in Grunwald (2012a, c). The notion of an EEE concept builds on earlier work but is presented here for the first time in this form.**

These concerns require a more in-depth look at the concept of responsibility (Lenk 1992; Grunwald 1999). Responsibility is neither a quasi-ontological predicate nor a natural object, but the result of a social process, namely of an act of attribution, whether actors attribute responsibility to themselves, or if the attribution of responsibility is made by others. The process of attributing responsibility takes place relative to *rules of attribution* (Jonas 1979). Assignments and attributions of responsibility take place in concrete social and political spaces. These involve and affect concrete actors in concrete constellations—therefore putting emphasis on the socio-political dimension of responsibility (Grunwald 2012c) which can be investigated empirically by the social and political sciences. Thus, attributions and assignments of responsibility, *ex post* as well as *ex ante*, are part of life-world practices and of the governance of the respective area. Often those processes are implicit and rely on established and recognized practices; in cases of ambiguity, indifference or conflict, however, they must be made explicit.

The notion of responsibility is often characterised by changes and alterations to sentence structure and word placement which are used to validate intention in the con-text of responsibility (Lenk 1992). A four-place reconstruction generally seems to be suitable for discussing issues of responsibility in scientific and technical progress:

- *someone* (an actor, e.g. a synthetic biologist) assumes responsibility or is made responsible (responsibility is assigned to her/him) for
- *something* (such as the results of actions or decisions, e.g. for avoiding biosafety or bio-security problems) relative to
- *rules and criteria* (in general the normative framework valid in the respective situation, see Grunwald 2012a, Chap. 3, e.g. rules of responsible behaviour given in a Code of Conduct) and relative to the
- *knowledge available* (knowledge about the impacts and consequences of the action or decision under consideration, including also meta-knowledge about the epistemological status of that knowledge and uncertainties involved).

Though the first two places are, in a sense, trivial in order to make sense of the word "responsible," they indicate the fundamental social context of assigning responsibility which inevitably is a process among social actors. The third and fourth places open up essential dimensions of responsibility: the dimension of rules and criteria comprise principles: norms and values being decisive for the judgment of whether a specific action or decision is regarded responsible or not . This constitutes the *ethical dimension* of responsibility. The knowledge available and its quality, including all the uncertainties, form its *epistemic dimension*. My thesis is that relevant questions arise in all of these three dimensions and that all three dimensions must be considered in prospective debates over scientific responsibility of synthetic biology and beyond, in new and emerging science and technologies NEST (Grunwald 2012c):

- The *empirical dimension* of responsibility seriously considers that the attribution of responsibility is an act of specific actors which affects others. It refers to

the basic social constellation of assignment processes. Assignment of responsibility must, on the one hand, take into account the possibilities of actors to influence their actions and decisions in their respective fields. Issues of accountability and power must be taken into account. On the other, attributing responsibilities has an impact on the governance of that field. Shaping that *governance* is the ultimate goal of debating issues of assigning and distributing responsibility *ex ante*. Relevant questions are: How are capabilities, influence, and power to act, as well as decisions taken in the field, considered? Which social groups are affected, and should they help determine the distribution of responsibility? Do the questions under consideration concern issues to be debated at the "polls" or can they be delegated to groups or subsystems? What consequences would a particular distribution of responsibility have for the governance of the respective field, and would it be in favour of desired developments?

- The *ethical dimension* of responsibility is reached when the question is posed for criteria and rules for judging actions and decisions under consideration as responsible or irresponsible, or for helping to find out how actions and decisions could be designed to be (more) responsible. Insofar as normative uncertainties arise (Grunwald 2012a), e.g. because of ambiguity or moral conflicts, ethical reflection on these rules and their justifiability is needed. Relevant questions are: What criteria allow distinguishing between responsible and irresponsible actions and decisions? Is there consensus or controversy on these criteria among the relevant actors? Can the actions and decisions in question (e.g., about the scientific agenda or about containment measures to prevent bio-safety problems) be regarded as responsible with respect to the rules and criteria?
- The *epistemic* dimension asks for the knowledge about the subject of responsibility and its epistemological status and quality. This is a particularly relevant issue in debates on scientific responsibility because, frequently, statements about the impact and consequences of science and new technology show a high degree of uncertainty. The comment that nothing else comes from "mere possibility arguments" (Hansson 2006) is an indication that, in debates over responsibility, it is essential that the status of the available knowledge about the accountable future is determined and is critically reflected upon from an epistemological point of view (Grunwald 2012a, Chap. 10). Relevant questions are: What is really known about prospective subjects of responsibility? What could be learned through more research, and which uncertainties are pertinent? How can different uncertainties be qualified and compared to each other? And what is at stake if worse comes to worst?

Debates over responsibility in technology and science frequently focus exclusively on the *ethics* of responsibility (Durbin 1987). However, regarding the analysis given so far, this is only part of the field and neglects the empirical as well as the epistemological dimension of responsibility. It seems that the familiar criticisms towards responsibility reflections (see above) of being simply appellative, of epistemological blindness, and of being politically naïve, are related to narrowing responsibility to its ethical dimension. The brief theoretical analysis above showed

that the issue of responsibility is not only one of abstract ethical judgment but necessarily includes issues of concrete social contexts. Governance factors must be treated empirically as well as the issue of the epistemological quality of the knowledge available. Meeting those criticisms and making the notion of responsibility work is claimed to be possible by considering the EEE dimensions of responsibility together.

4 Synthetic Biology: The Responsibility Constellation

In this section I would like to briefly unfold the responsibility constellation specific to the field of synthetic biology by referring to ongoing debates on responsibility in this area (Grunwald 2012a, c). As a first step, an impression which might be the subject of responsibility in current synthetic biology should be given.

4.1 Synthetic Biology: Subjects of Responsibility

A first task to make the notion of responsibility more tangible is to clarify those issues of responsibility we are talking about, or *should* talk about in the field of synthetic biology. This seems to be a prerequisite to any substantial responsibility debate avoiding a mere rhetorical use of this term. Possible subjects of responsibility debates and assignments in synthetic biology could be, on the one hand, future developments resulting from current research. Most people would think about those issues first. On the other, however, there are also issues of current research itself. The following list of elements could be understood as possible subjects of responsibility in synthetic biology, and shall give an impression of what the ethics of responsibility could include in this field; though it cannot claim to be comprehensive:

- the *goals and objectives, even visions* of current research in synthetic biology: these could be confronted with questions of whether they are responsible or could be made responsible by modifications
- envisioned, projected or even merely imagined *products* of synthetic biology in terms of materials, technological systems and services based on knowledge provided by synthetic biology. These might include highly welcome outcomes, such as new and better drugs; but also problematic and unwanted developments such as biological weapons
- possible future *knowledge* of synthetic biology which could influence not only our engineering capabilities, but also our understanding of life and of ourselves
- consequences for *actor constellations and power distribution*: how could developments emerging out of synthetic biology influence power constellations and influence, e.g. in the related economies?

- the *science system*: we might ask for its ability and willingness to develop and establish reflective accompanying procedures to monitor and assess the ongoing research in synthetic biology, with respect to social, political, ethical, cultural and other dimensions: are the preconditions of taking over responsibility fulfilled by current structures and institutions in science?
- *research funding*: funding policies clearly influence the advance of synthetic biology. Therefore, the direction and the themes of research funding in synthetic biology are subject to possible responsibility debates. In particular, facing scarcity of resources, the current priority-setting in the allocation of financial and personal resources to synthetic biology research might be considered more or less responsible regarding other, perhaps more urgent, fields of research
- the *legal and political framework* which would influence the further advance and direction of research in synthetic biology (e.g. regulation or incentive programmes)
- *current research*: it might be assessed with respect to responsibility criteria, e.g. precautionary measures, safety of the researchers, observance of animal protection rules in case of animal experiments, etc.
- providing knowledge-based and normatively reflected *policy advice* could also be seen as a subject of responsibility in this field
- increasing society's *awareness* with regard to advances of synthetic biology, and supporting an open dialogue about the further direction of research might also be a subject of responsibility assignment.

This list shows a high variety of different types of subjects of responsibility. Partially, they are directly linked with specific actors; partially it is not clear to whom which aspect of responsibility should be assigned. The variety of subjects, in combination with the variety of actors possibly made responsible, opens up a broad field of debate about legitimate, adequate, effective and efficient distributions of responsibility in our field of consideration. Obviously, developing a comprehensive responsibility theory of synthetic biology would go far beyond the scope of this essay. Therefore, I will restrict myself to a few fields in the remainder of this chapter.

4.2 Dimensions of Responsibility

The list presented above clearly illustrates that the empirical constellation is heterogeneous and will involve different types of actors, reaching from the biologists themselves to regulators, funding agencies and policymakers, up to civic organisations and private citizens. Assignment of responsibility must, on the one hand, be based on normative pictures of how society should work and how science should serve society, e.g. on ideas of a deliberative democracy or on ambitious concepts of modern governance of science in society (Siune et al. 2009). On the other hand, empirical investigation of the mutual relations of actors, and their capabilities to

influence specific developments, must also be considered. Responsibility assignments and resulting distributions are a complex mixture in regard to combination of normative and empirical insight. For the field of synthetic biology it would be valuable to consider this constellation in more depth (see few remarks in Sect. 5).

Defining sensible subjects for responsibility debates strongly depends on the epistemological dimension. A fundamental challenge to responsibility debates about far-ranging future developments in science and technology is the inevitably high degree to which material other than knowledge is involved. Future scenarios of the development of synthetic biology, of its useful outcome to society, and of the consequences of the real-world use of those products, systems and services are highly uncertain. In the context of responsibility, the question arises whether future products, systems and services based on synthetic biology's knowledge could be sensible subjects to responsibility assignments today at all. The following quote taken from a visionary paper on synthetic biology supports serious doubt about this:

> Fifty years from now, synthetic biology will be as pervasive and transformative as is electronics today. And as with that technology, the applications and impacts are impossible to predict in the field's nascent stages. Nevertheless, the decisions we make now will have enormous impact on the shape of this future (Ilulissat-Statement 2008, p. 2).

These statements express (a) that the authors expect synthetic biology will lead to deep-ranging and revolutionary changes, (b) that our decisions today will have high impact on future development, but (c) we have no idea what that impact will be. If this were true, there would be no chance of assigning responsibility; even speaking about responsibility would no longer have a valid purpose. Any ethics of responsibility would be obsolete because of a missing subject (Bechmann 1993): our complete lack of knowledge about future developments, and their relation to today's decision-making. This would make reflections on the desirability or acceptability of those future developments impossible; or would make completely arbitrary any conclusions on today's attribution of responsibility. Analogously, the critics of speculative nano-ethics (Nordmann 2007; Roache 2008; Grunwald 2012a, Chap. 10) have pointed out that no legitimate conclusions could be drawn if the ethical reflection addresses merely speculative and arbitrary futures ("mere possibility arguments," cf. (Hansson 2006)). The epistemological task is to examine both the cognitive and evaluative content of the prospective knowledge used in responsibility debates to describe the subject of responsibility as clearly as possible. In this context the vision assessment approach has been proposed in order to uncover the epistemological and ethical grounding of NEST visions (Grunwald 2009). It aims at uncovering the epistemological and normative ingredients of future statements in order to permit more well- informed and more rational formation of opinion, assessment and decision making on the attribution of responsibilities.

These considerations show that debates on responsibility in synthetic biology should not rely on mere speculative futures as subjects of inquiry. The difference between technoscience and traditional engineering sciences, is rooted in its

character (see above), in that reliable images of future products and technological systems are difficult to achieve. As a consequence, responsibility considerations in synthetic biology today relate mainly to current research rather than to future products, their consequences and expected, but epistemologically unqualifiable, innovations and risks (IRGC 2009, p. 7). This diagnosis focuses on the responsibility of scientists as individual professionals and that of science as a system to current research as a main subject of responsibility.

It is thus not surprising that the well-known conference held in Asilomar in 1975 is repeatedly cited as a model for future steps in the field of synthetic biology (Boldt and Müller 2008). That conference took place under circumstances in which a global spirit of optimism regarding genetic engineering could be observed, while at the same time the first signs of public criticism and demands for state regulation could be heard. The outcome of the conference was that genetic engineers committed themselves to taking responsibility and exercising caution. Interpretations of the conference are controversial (Grunwald 2012c). On the one hand, it was praised as a positive example of science proactively assuming responsibility; on the other hand, it mainly served the purpose of pre-empting state regulation so that genetic engineers could carry on conducting their research with as little interference as possible (de Vriend 2006). The recent controversy on the role of self-regulation in synthetic biology (Maurer et al. 2006) versus the claim of civic organisations involved in the governance of that field (Grunwald 2012a, Chap. 7) may be interpreted as a follow-up to the earlier controversy on the interpretation of Asilomar. This points to the same critical issue of determining the adequate relation between science's autonomy and society's claim of involvement in the governance of science (Siune et al. 2009). This issue makes clear that classifying synthetic biology as technoscience makes it more difficult to deal with the socio-political context of responsibility compared to the debates on basic and applied research. While science's autonomy is usually regarded with high value in basic research; society's voice and involvement in applied research is frequently welcomed. Thus the situation in synthetic biology seems to be ambiguous because it does not belong to only one type of research. Instead, a kind of "NEST-ethics" (NEST: new and emerging sciences and technologies) seems to be required (Swierstra and Rip 2007) which might be regarded as one of the predecessors of the idea of Responsible Research and Innovation (Grunwald 2011; von Schomberg 2012).

In view of the existing experience with genetically modified organisms and their regulation, and of the often speculative nature of reflections on the consequences of synthetic biology, it is not immediately clear what the *specific* challenges are that synthetic biology poses to the *ethical* dimension of responsibility considerations. The moral issues posed by synthetic biology resulting in challenges to responsibility can be classified according to the different normative frameworks and sets of rules that are affected: the question regarding how to deal with risks, normative uncertainties about the moral status of artificial living things, and the issue of human hubris or "playing God."

In view of the fact that, compared to traditional gene technology, synthetic biology leads to a further increase in the depth of man's interventions in living systems, and that the pace of innovation continues to increase, the precautionary principle will tend to become even stronger, in as much as we operate in the same normative framework (Paslack 2012). The responsibility of scientists will form a major issue in the run-up to *adequate* regulation. In particular, issues of bio-safety and bio-security are frequently discussed (de Vriend 2006). The ethical dimension touches questions such as: how safe is safe enough, what risk is acceptable according to which criteria, and is it legitimate to weigh expected benefits against the risks, or are there knock-out arguments morally forbidding cost/benefit comparisons? All these questions are well-known to many fields of risk ethics (Rescher 1983; Shrader-Frechette 1991) but must be answered anew in the particular context of synthetic biology.

The production of new living things or technically strongly modified ones by synthetic biology will raise the question of their moral status. With respect to its moral status—and various bioethical positions differ on this considerably—a difference in principle is made between the living and nonliving objects of ethical reflection, the question will be whether synthetically produced living things are also accorded this moral status. Assigning different moral statuses to such forms of "life" could lead to different answers on the questions of responsibility.

In synthetic biology, man moves from being a modifier of what is present to a creator of something new, at least according to the visions of some biologists:

> In fact, if synthetic biology as an activity of creation differs from genetic engineering as a manipulative approach, the Baconian *homo faber* will turn into a creator (Boldt and Müller 2008, p. 387).

In 2005 a high-level expert group on behalf of the European Commission called it likely that work to create new life forms will give rise to fears, especially that of synthetic biologists "playing God." Concerning responsibility issues the question could be (and is!) raised whether humans would run out of being able to act responsibly at all if they started "Playing God". However, this type of argument seems to be more an indicator of uneasiness with fast scientific advance rather than an ethical argument per se.

In summarising these thoughts and regarding the focus of this chapter on the consequences of classifying synthetic biology as a technoscience, it becomes clear that it is primarily the epistemological dimension of responsibility which makes a difference to traditional sciences. The combination of the "engineering" approach of synthetic biology with its openness to applications and its enabling character leads to a situation where the subject of responsibility should be seen more in the process of current research rather than in speculative future products. Taking over responsibility therefore means being responsible for current processes of research, defining the research agenda, determining objectives and goals and supporting current societal debates on synthetic biology instead of talking about responsible or irresponsible future outcomes of synthetic biology.

5 Concluding Remarks

The specific responsibility constellation of synthetic biology which is related with its character as technoscience is complex in particular because it includes, on the one hand, issues of current research in accordance with good scientific practice and established moral standards and, on the other, far-ranging but highly speculative visions and expectations. Responsibility always has to be assigned in a respectively present situation, with respect to present expectations and rules of assignment. Keeping this in mind will allow us to derive some orientation for related responsibility debates in the years to come.

5.1 Responsibility Today Facing Future Prospects

The increasing possibilities for the recombination of life "modules" such as those that are studied, duplicated and modified in synthetic biology (Sect. 2), make the possibility of "shaping technology" in the "strong understanding" (Grunwald and Hocke-Bergler 2010, p. 160) seem unrealistic. Even the promise of prospective impact research and the assessment thereof seem unrealistic. However, there is a whole spectrum of other possibilities of influencing the governance of synthetic biology by assigning responsibilities ("weak understanding" according to (Grunwald and Hocke-Bergler 2010, p. 160)). This opens up two types of options for shaping and influencing: (1) the design of current research and (2) the design of current debates on synthetic biology. Both options offer the opportunity to talk constructively and substantially about responsibility subjects and constellations. It is noteworthy in this context that both draw from the current situation, not from issues of a speculative future society in which synthetic biology could or would have major impact:

1. Taking the widespread impossibility of prospective impact research seriously can focus promising design on the *current research* of synthetic biology. This occurs factually and demands no prospective analysis, but can be confronted, for example, with the well-known concerns of "bio-safety" and of "bio-security". Or the possibilities and limitations of a "do-it-yourself" technology could be considered. Along the way, the next research subjects can also be debated over along with decision processes and criteria. Perspectives and experiences of responsible research and innovation can add to the inter- and trans-disciplinary insight and design process.
2. Likewise, without a glance into the future, we can debate on visions for the future and possibly also on other "futures" of synthetic biology, since these are voiced *today* and determine a good portion of the social debate, which ranges from expectations of salvation from the looming global energy crisis to the fear of "playing God." Design extends here to contributions to the social debate—with possible, but not definite consequences for the further pathway

of synthetic biology. Design is meant here as the shaping of the social context of debates over synthetic biology so that "responsible innovation" and "responsible research and development" are possible. The significance of conceptual, heuristic and hermeneutic questions grows. In the, often times, speculative estimation of the development potential of synthetic biology, it is important to clarify what is at stake in all these considerations. A "hermeneutic technology assessment" (Grunwald 2012b) would on the one hand clarify current debates as well as prepare for up and coming debates in which it could then, for example, be about concrete technology design. Within this context, a "vision assessment" (Grunwald 2009) would study the cognitive as well as evaluative content of tech-based visions and their impact. They would be the fundamental building blocks of a cognitively informed and normatively oriented dialogue—a dialogue, for example, between experts and the public; or between synthetic biology, ethics, research funding, the public and regulation. In the assignment of responsibility in synthetic biology, the realisation and support of such a dialogue is, without a doubt, of major importance—and it would affect many actors such as biologists, journalists, policy-makers, civic organisations and even private citizens.

5.2 Responsibility Reflection as Concomitant Activity

Since the very beginning of ethical reflection on science and technology, a discussion has been ongoing about what the appropriate relation in time is between scientific-technological advance and reflection on responsibility. The rapid pace of innovation in technology has led to concerns that ethical deliberations often come too late (Moor and Weckert 2004). Reflection then could, at best, only act as a repair service for problems which are already out in the open. In contrast, the "ethics first" model postulates comprehensive ethical reflection on the possible impact *in advance* of the technological development. It is in principle possible for responsibility ethics to reflect and discuss the normative implications of items long before their entry into the market because scientific and technical knowledge will make early ideas available about the items, their capabilities, and their societal impacts (both risks as well as chances). However, responsibility reflection in the "ethics first" model has to deal with the situation that the relevant knowledge about technology and its consequences is uncertain and preliminary—the epistemological dimension (see Sect. 3 in this chapter) will restrict its feasibility.

Responsibility reflections and assignments made during the very early stages of a development in synthetic biology could provide orientation for shaping the relevant *process* of scientific advance and technological development (for example, with regard to the question of equity, or of the risk of misuse). As the knowledge of synthetic biology grows, and with it the development of products and services, it will then be possible to continuously concretize the—initially

abstract—estimates and orientations on the basis of newly acquired knowledge; and finally, to carry out an ethically reflected technology assessment with specific assignments of responsibility. In this sense, responsibility reflection in all three dimensions is an ongoing process accompanying scientific and technological advances. In the course of this reflection its subjects will change (see Sect. 4.1). As well, as new actors will appear in the range of possible persons and groups to whom responsibility may be assigned.

Currently, there is the chance and also the time for concomitant reflection, as well as the opportunity to integrate the results of our reflection into the scientific agenda and design of technology, thereby contributing to the further development of this promising field of advanced science and technology (similar to what (Moor and Weckert 2004) expected for accompanying reflection on nanotechnology). In view of the still visionary nature of the many prospects in synthetic biology, and of the very long time spans within which the realization of certain milestones can be expected, the chances are good that responsibility reflection and the social discussion will not come too late. On the contrary, they can accompany scientific-technical progress critically and, in particular, can help influence science's agenda by providing ethically reflected advice, without sharing naïve and unrealistic expectations of shaping technology in a "strong understanding" (see above).

5.3 Responsibility Reflection Must Be Embedded in Democracy

A frequently mentioned question is *which* responsibility *should* be attributed to scientists in the field of synthetic biology. The answers often demand that scientists are supposed to reflect on the consequences of their actions in a manner that constitutes a complete assessment of the technology. This is often done with the implicit hope that scientists—if they assessed the results of their own actions comprehensively—would make judgments in a responsible manner and act accordingly, and that negative and unintended consequences could be largely, or even completely, avoided (cf. in this direction also (Presidential-Commission 2010, p. 13).

However, there are obstacles and limitations to be observed. There is a need for *social* consultation, deliberation and evaluation (on state sponsorship of research, on government policy toward science and technology, and on regulating the context of technical development by means of legislation, judicial decisions, or economic measures) extending beyond the capability of individual scientists. Relevant actors, stakeholders and citizens must be involved due to a modern understanding of the governance of science (Siune et al. 2009), even if this makes the empirical, socio-political dimension of responsibility much more complicated. Neither individual scientists nor disciplines such as synthetic biology or even philosophy can address these questions alone with any expectation of success. Scientists in

synthetic biology are experts in their fields, but not in the possible social consequences of their actions; and not for the question of the acceptability of uncertain risks and dealing with them.

When it comes to assigning responsibility, a broader approach is therefore necessary; one that does justice to the realities of an extensive division of labour, citizens' claims for democratic participation, and the specific *regularities in the sciences*. Responsibility must be shared among science, politics, authorities, and the democratic public. In particular, the need for transparency in the sciences, politics and the public sector, demands strict and democratic deliberation on the agenda of synthetic biology (Habermas 1968).

To take demands seriously for participation by a democratic public as well as for decision-making processes that are politically legitimate, however, does not free synthetic biology of all responsibility. These fields are justifiably expected to provide transparent information to the public. This is particularly true for potentially worrying developments. Faced by such developments, society might initiate ethical reflection or technology assessment in order to systematically analyse and evaluate the challenges ahead. The specific responsibility of scientists to provide information at an early stage lies in the fact that they possess particular cognitive competence in their own area, and are the first to have certain information. This responsibility also extends to participation in interdisciplinary and social dialogues, as well as political counselling. Science, including synthetic biology, is part of society, not something external to it. The expectation of science is that it reflects on its role in society and actively accepts this role in its many aspects.

Summarizing these thoughts briefly shows that it is essential to consider the ethical, the epistemic, and the empirical dimension of responsibility altogether, rather than restricting the debate to one or two of them. Taking this result seriously implies that responsibility issues should not be dealt with by ethicists alone, but by interdisciplinary teams involving also philosophers of science, political and social scientists, governance researchers and the biologists themselves—in cooperation with independent actors outside the field of science.

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