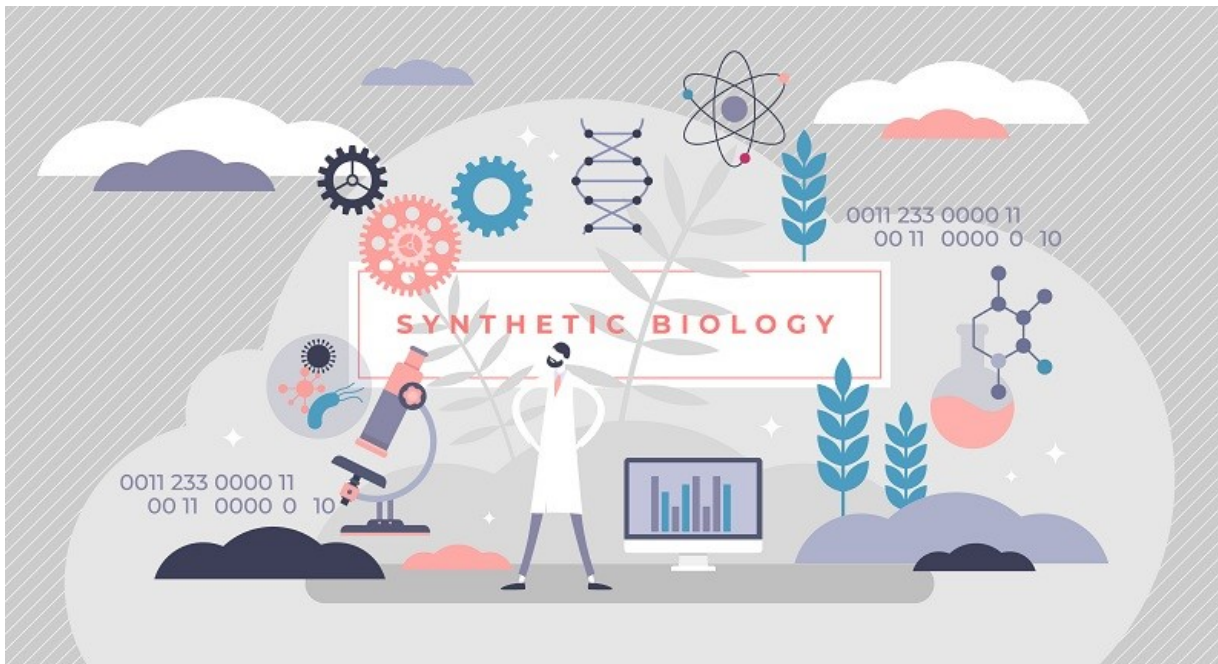


Synthetic Biology



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3rd Interim report of the Central Committee on Biological Safety Period: June 2018 - December 2021

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1 Introduction

The Central Committee for Biological Safety (ZKBS) has been monitoring synthetic biology for more than ten years in order to competently and critically accompany current scientific developments and to evaluate them with regard to biosafety. In 2012 and 2018, the first and second interim reports on this monitoring were published. While the first report focused on research activities in Germany, the second interim report looked at scientific developments worldwide.

The ZKBS has conducted a continuous monitoring of publications on synthetic biology since the second interim report. Selected publications which, in the opinion of the ZKBS, are particularly typical and relevant for the individual research subfields of synthetic biology are regularly presented on the ZKBS's homepage (https://www.zkbs-online.de/ZKBS/EN/SyntheticBiology/Current_developments/Current_developments_node.html)

This report briefly presents the developments in the various research subfields on the basis of the publications selected by the ZKBS for the homepage, evaluates them with regard to a potential threat to biological safety and assesses whether they are covered by the scope of the Genetic Engineering Act (GenTG) and the European Directives, respectively.

For an introduction to the field of synthetic biology, please refer to the interim reports already published.

2 Developments in the research subfields of synthetic biology and evaluation by the ZKBS

So far, there is no generally accepted definition of synthetic biology. In its monitoring, the ZKBS focuses on five areas that are generally seen by researchers and other stakeholders as the research fields of synthetic biology. These are:

- Design and synthesis of genes and genomes
- Design of genetic signalling circuits
- Design of customised metabolic pathways
- Minimal cells: Genome reduction and generation of protocells
- Xenobiology.

In addition, since 2018 the ZKBS has also listed publications on methods impacting on synthetic biology on its homepage. The results of the continuous monitoring are briefly summarised below. For a complete list of the short summaries, please refer to the ZKBS's homepage (https://www.zkbs-online.de/ZKBS/EN/SyntheticBiology/Current_developments/Current_developments_node.html)

2.1 Design and synthesis of genes and genomes

Some of the developments already described in the first two reports were continued and advanced. For example, a synthetic *Escherichia coli* genome with a size of 4 Mbp was synthesised, in which two codons were recoded [1]. DNA as a storage medium was used to dynamically store large amounts of data or to record cell lineage [2, 3]. Synthetic promoters, developed among other things for plants [4] and for *Saccharomyces cerevisiae* [5], contribute to standardisation in the synthesis of genes and genomes.

Assessment of the ZKBS: As already described in the last two interim reports, the possibilities for synthesising complete genomes are constantly progressing. However, a *de novo* design of genomes has still not been reported, meaning that *in vitro* synthesised genomes continue to be built on the basis of natural genomes. This means that a comparative risk assessment is still possible.

The *in vitro* synthesis of genes and genomes *per se* does not fall under the GenTG as long as the nucleic acid segments produced are not introduced into the genome of living organisms. If newly synthesised and modified genomes are introduced into living organisms, this constitutes a genetic engineering operation according to the GenTG, unless these modifications can occur naturally by mating or natural recombination.

2.2 Design of genetic signalling circuits

Foreseen applications of genetic circuits are in medicine and environmental diagnostics. On the one hand, genetic circuits seem to be becoming more complex; on the other hand, efforts towards a more stable expression of the circuits and their outputs as well as a regulation by novel inputs are described. In addition, more applied examples can be observed. Examples of complex circuits are the processing of multiple inputs by a metabolic perceptron [6] or flexible logic gates constructed from proteins and used for post-translational control [7]. Stable gene expression independent of copy number or the localisation of a circuit in the genome has been described, for example, by Segall-Shapiro *et al.* [8] or Frei *et al.* [9]. Novel inputs for circuits can be, for example, electrical signals [10], photothermal signals via LED light [11], or light [12, 13]. Genetic circuits are being explored for use in cancer therapy. They can be used to measure the concentration of surface proteins (e.g. antigen density) or microRNAs in a cell and thus make tumour cells specifically recognisable, which can mediate their targeted killing [14-18]. Another example of an application are genetic switches that are incorporated into tissue to act as wearable sensors for small molecules such as toxins [19].

Assessment of the ZKBS: In the design of genetic circuits, precisely defined DNA segments, usually well characterised in terms of function, are combined with each other. The circuits are often introduced into model organisms that have long been known in research, using a biological safety measure. As already stated in the last two interim reports, genetically modified organisms are produced in the process that fall within the scope of the GenTG.

2.3 Design of customised metabolic pathways

Tailored metabolic pathways are used, for example, to increase biomass production in plants or to establish new pathways for CO₂ fixation [20-22]. A new carboxylation pathway for improved CO₂ fixation [23] can be combined with the CETCH cycle described in the last interim report [24]. The production of economically interesting molecules, such as synthetic cannabinoids in *S. cerevisiae* and the aromatic substance vanillin from a PET degradation product, was also described [25, 26]. Metabolic pathways were placed into vesicles to prevent a toxic effect of the resulting products [27].

Assessment of the ZKBS: For the development of novel customised metabolic pathways, existing genes are modified or genes from another organism are introduced into an already existing organism. This could enable the specific formation of hazardous substances. However, the production and handling of these organisms are fully covered by the GenTG.

2.4 Minimal cells: Genome reduction and generation of protocells

Top down: Production of minimal cells by genome reduction

Reduced genomes have been developed for yeast *S. cerevisiae*, among others, by bundling essential genes into a superchromosome [28] or by reducing the size of one of the 16 chromosomes by 58 % [29]. In the field of bacteria, for example, the genome of *Caulobacter ethensis* was reduced and an additional 56 % of all codons were replaced by synonymous codons [30].

Bottom up: Production of protocells

The production of minimal cells from biological building blocks focuses on the development of various functions that "viable" synthetic cells require. This may include the formation of compartments such as an artificial cell nucleus [31], structures for cell division [32] or cell movement [33, 34] or for energy supply [35, 36]. Many working groups are also studying communication between protocells, e.g. with the help of DNA [37, 38] or small molecules [39].

Assessment of the ZKBS: Minimal organisms produced by the targeted reduction of the genome are generally less adaptable to their environment, which is accompanied by reduced fitness and possibly also reduced pathogenicity. Most of these organisms can only survive under defined conditions, which is why an increased threat to biological safety cannot be identified. As provided for in the GenTG, the hazard potential can be well assessed by comparing the organisms with the original organisms.

"Bottom up" organisms designed from scratch are not covered by the GenTG, which is applicable to organisms whose "genetic material has been modified in a way that does not occur under natural conditions by mating or natural recombination" (§ 3 GenTG). According to the GenTG, the risk assessment is based on the known hazard potential of the donor and recipient organism. Protocells that are not based on a natural organism are therefore not covered by the GenTG.

To date, however, no protocell has been described that can replicate autonomously and can be considered as an organism. No risk to biosafety is currently expected from this field.

2.5 Xenobiology

In the field of xenobiology, some approaches on genetic code modification have been described. These included, for example, the development of a fail-safe code, in which each amino acid is encoded by only one four-base codon, which cannot be converted into another codon by mutation and is thus intended to protect against spontaneous mutation [40]. Also, an eight-letter alphabet in which the naturally occurring bases A, T, C and G are supplemented by synthetic nucleotides [41], or new base pairs on the basis of metals [42] have been described. Work on tRNAs that recode sense codons, for example, [43] or decode four-base codons [44] complement this work. In addition, some research on the insertion of non-canonical amino acids into proteins has been described. These were aimed, for example, at auxotrophy [45]. Bacteria that can synthesise a 21st amino acid and insert it into a protein have also been described [46].

Assessment of the ZKBS: The approaches pursued in the field of xenobiology are used to produce organisms whose genetic material has been altered in a way that does not occur under natural conditions through mating or natural recombination. In this context, non-natural bases are also regarded as genetic material. They are therefore subject to the GenTG. Many applications from the field of xenobiology also aim for orthogonality and thus reduced interaction with natural organisms. This can lead to an increase in biosafety.

2.6 Methods impacting on synthetic biology

Since the second interim report in 2018, the ZKBS has also included publications on methods impacting on synthetic biology in its continuous monitoring. These are divided into applied methods and *in silico*, i.e. computer-assisted, methods.

Applied methods

In this area, research on the integration of nucleic acid segments using CRISPR/Cas9 [47], a sequencing method for a non-natural base [47] or approaches on data storage in DNA [48, 49] are listed. Techniques on the formation, division and colony formation of protocell precursors were also described [50–52].

In silico methods

Genetic data from synthetic biology applications can be converted into the *Synthetic Biology Open Language* (SBOL) for standardised data exchange. In order to make the often disordered datasets in repositories such as SynBioHub more accessible, Zhang *et al.* [53] developed a programme for sorting these data sets.

Assessment of the ZKBS: In the applied methods listed here, either the genetic material of an existing organism is modified in a way that does not occur under natural conditions through mating

or natural recombination, so that the GenTG applies, or methods are developed to generate protocells to which the GenTG does not apply (see section 2.4). The *in silico* method described does not involve handling an organism, so that the GenTG does not apply and no risk to biosafety occurs.

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