German Association for Synthetic Biology (GASB)

Joint Working Group on Synthetic Biology (GFGSB)

Submission of information on the trends in new technological developments in synthetic biology

Synthetic biology is a relatively new discipline bridging engineering with life sciences and computational modeling. It applies basic engineering principles for the modular, combinatorial assembly of biological parts into higher order complex signaling and metabolic structures. Key to the strategy is the implementation of mathematical modeling for the design and quantitative functional characterization of the molecular parts and for guiding the assembly, implementation, and optimization of the individual modules and networks.The standardization and modularity contribute to speeding up the engineering process as well as progressing designs through iterative engineering cycles of design-build-test-learn.
Synthetic biology tools allow for the rational, targeted design of production organisms, including microbes and plants, far exceeding the precision of traditional breeding and genetic engineering methods. It allows for precisely knowing which genetic modifications are introduced, thereby contributing to a tighter control over the product organism. This facilitates the establishment of reliable containment strategies and is the basis of evaluations by some regulatory bodies [1].

Classical genetic engineering and biotechnology rather focuses on modulating individual components, but designing, implementing and improving complex multigenic traits requires rational and systematic engineering strategies. Synthetic biology goes a step beyond genetic engineering and the development and application of new technologies improving the predictability of biotechnology. It represents a conceptual and theoretical-experimental breakthrough, expanding the range of applications and products to be potentially developed.

In contrast, the modifications of engineered organisms are perfectly tractable, thoroughly described, and allow for efficient control. Therefore, despite sometimes using some of the classical GMO technologies, broader incorporation of synthetic biology approaches into microbial- and plant-based applications will lead to the development of unforeseen applications in the next years as it is currently occurring in the biomedical/biopharmaceutical fields with major positive implications for the environment:

Synthetic biology makes use of a whole variety of molecular biology and genetic technologies, including CRISPR/Cas and other genome editing approaches. However not all applications of these technologies fall under the definition of synthetic biology, but only those in which the synthetic biology strategies and principles are applied (as described above).

If the product of a synthetic biology engineered organism, e.g. an edited organism (plant) is not different (environmental risk, nutritional aspects) from a corresponding wild type, a naturally occurring or randomly induced mutant, or a conventionally bred organism, then there are no scientific grounds for considering or regulating it differently. Given the various options for controlling the emergence of unwanted traits, regulatory oversight of synthetic biology in agriculture shall at least not be more restrictive than conventional breeding.

With regard to the current research, one can expect the development of unforeseen and beneficial applications to solve the major challenges of humankind, capitalizing on the numerous advantages of synthetic biology:

High potential to improve global food security
Enabling agriculture on previously non-arable land and more efficient usage of natural resources
Improving resource efficiency, translating to higher yields and a lower burden on ecosystems protecting biodiversity/sustainability
Facilitating a transition to a bioeconomy-based society
Establishment of biosafety measures, containment strategies and incorporating safety valves beyond the current technological level
Fostering capacity building and technology transfer/sharing
Democratization of technology development and global implementation

A more detailed view of the current development can be found below.

[1] EFSA Panel on Genetically Modified Organisms (GMO), Naegeli H et al “Evaluation of the existing guidelines for their adequacy for the molecular characterization and environmental risk assessment of genetically modified plants obtained through synthetic biology”
EFSA Journal (2021) e06301 DOI 10.2903/j.efsa.2021.6301

(a) Increased field testing of organisms, components and products derived from new developments in synthetic biology;

In order to verify the properties of a developed system outside of the controlled test environment, field tests are common and necessary. As the research field of synthetic biology is comparatively young, applications are rather in early development stages. It is, therefore, plausible to assume an increase of field trials in the following years.

In general, most synthetic biology applications may be considered genetically modified organisms (GMO), field testing is therefore performed under the control of national GMO release regulation. As a consequence, a multitude of Biosafety regulations should be in place to contain the organism to the field trail site, as well as monitoring mechanisms to assess the environmental impacts.

Examples of synthetic biology accompanied field trials encompass the testing as part of the "Target Malaria" consortium. In July 2019 researchers from Burkina Faso released marked, male sterile mosquitoes in order to see the impact of the genetic modification in a natural-like environment [2]. Afterwards the edited insects were recaptured and the population was monitored for several months to verify the absence of the transgene.

Field trails on the industrial site are exemplified by the currently running field test of the company Living carbon. Modified poplar trees with higher photosynthetic capabilities were successfully tested under greenhouse conditions [3] and are now tested in a forest in the United states [4]. The impact on the environment as well as the growth rates of these trees under real-life conditions are monitored. The idea behind planting these enhanced trees is to improve CO_2 capturing capabilities and to use them as a biological Carbon-capture and storage solution.

However similar yield improvements have already been shown for model plant species, where it has been demonstrated in field trial conditions, that yield can be increased by 40%, by engineering synthetic photorespiration bypasses [5]

Another example, which is already at the stage of commercialization is using synthetic biology products, namely engineered microbes for the bioremediation of environmental pollutants. The company Allonia is aiming to degrade or metabolize contaminants into upcycled, valuable materials. [6]

In the future more examples of synthetic biology might be implemented in the real world by field testing. This could include plants, which are able to produce Vitamin B12. Vitamin B12 is widely known to be the only vitamin that cannot be acquired directly from plant-derived foods since it is synthesized solely by a subset of prokaryotes. Therefore the production of Vitamin B12 within plants could contribute to counteract malnutrition. [7]

Another example of yield improvement, which potentially could be tested in the field in the future are rice plants with C4 photosynthesis implemented. Such plants potentially could increase the yield by 50% and therefore prevent mass malnutrition of the future growing population [8].

An example for an application which lies rather in the more distant future but might still be tested in the field, is engineering plants that are able to produce their own fertilizer by fixing nitrogen from air and could save more than 1% of the global energy consumption, normally needed for nitrogen fertilizer production.

[2]https://www.nature.com/articles/s41467-022-28419-0
[3] https://www.biorxiv.org/content/10.1101/2022.02.16.480797v2
[4]:https://www.nytimes.com/2023/02/16/science/genetically-modified-trees-living-carbon.html?smid=tw-nytimesscience&smtyp=cur
[5]South, Paul F.; Cavanagh, Amanda P.; Liu, Helen W.; Ort, Donald R. (2019): Synthetic glycolate metabolism pathways stimulate crop growth and productivity in the field. In Science (New York, N.Y.) 363 (6422), eaat9077. DOI: 10.1126/science.aat9077.
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[8]https://www.science.org/doi/10.1126/science.1220177
[9] https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.13347

(b) Increased development of technologies that genetically modify organisms directly in the field;

The use of small interfering RNA, thereafter called siRNA, for silencing gene expression represents a powerful and increasingly precise tool for diverse applications such as personalized medicine or reverse breeding [1; 2; 3].

However, siRNA does not fall under genetic engineering as it does not alter the genome of organisms, but only acts at the RNA-level of gene expression.

It is important for us to underline that we do not support uncontrolled release of living modified organisms or previously unexplored genetic modifications in the field.
We advocate in favor of preceding testing in a laboratory environment and testing in a confined environment and encourage assessing the risk of the resulting product of modification. We highlight that there are also advanced methods of biological containment for living modified organisms released into or directly modified in the field through kill switches, the incorporation of non-canonical amino acids or auxotrophies, which are subjects of research of increasing interest and can enable reliable control of the viability and spread of living modified organisms [4; 5].

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(c) A shift to the development of synthetic biology for environmental, conservation, agricultural and health uses (some examples are provided in paragraph 12 below);

(a) Applications intended for use in the environment in managed and wild populations:

(i) Genetically engineered nitrogen-fixing bacteria [1] have huge potential for improving crop yields while saving fossil resources and energy and other genetically engineered bacteria/viruses for applications in agriculture are constructed with synthetic biology methods. [2] Some projects are at the field trial stage.

(ii) Genetically engineered bacteria for environmental applications such as bioremediation, biodegradation [3] and biomining are currently at various stages of R&D, in some cases tested in field trials. Strains resulting from pathway and metabolic engineering are considered synthetic biology products.

(iii) Cyanobacterial production platforms, engineered for the photosynthetic production of fuels and fine chemicals in contained environments, have been reported recently. [11,12] The majority can be classified as synthetic biology due to profound metabolic engineering. Most examples are at laboratory and a few at pilot plant stage.

(iv) Engineered gene drives adapted to certain mosquito species have been designed for the control of vector-borne diseases through either population collapse or corrupting the ability to transmit disease. [5] Projects are moving from discovery-stage demonstrations of proofs-of-principle to the next phases of development; field trials have been reported in May 2021.

(v) An engineered Medea gene drive designed for control of crop pest *Drosophila suzukii* (spotted wing Drosophila) is at the laboratory stage. [6]

(vi) A gene drive system engineered for mice has been recently reported, for conservation purposes, control of vector-borne diseases and agricultural pests. [4] The work is at an early laboratory R&D stage.

(vii) Genetically engineered sorghum to produce a new synthetic kafirin protein, harboring ten cleaving sites, rationally designed to improve digestibility for food and feed. [7] R&D is at early field trial stage.

(viii) Transient modifications of agricultural plants through, for example RNAi spray (non-living biopesticide), are not considered synthetic biology as no inheritable traits result from the agents. Research is at laboratory and greenhouse stage. [10]

(ix) Insect delivery of modified viruses for the modification of crops (horizontal environmental genetic alteration agents HEGAAs). HEGAAs enable remote modification of the germ line of target plant species in the field. Depending on the complexity of the genetic construct, transformed organisms can be considered synthetic biology products. The technology has dual use potential, published research is at early laboratory R&D stage [8].

(x) Re-engineering corals with techniques as old as the domestication of plants and as new as the latest gene-editing tools may offer an option to rescue valuable marine ecosystems. Corals more resistant to abiotic stress, i.e higher temperatures, lower pH and bleaching could result from knowledge on genes that might serve as "master switches" controlling how coral copes with stress. Current research on the coral's microbiome serves to explore the potential for genetically engineering the microbes to help coral become more resilient. Research is at very early laboratory stage. [9]

(b) Applications intended for use in the laboratory:

(i) Development of protocells and minimal cells for basic, early-stage research. Protocells and minimal cells are, with regard to their dependence on other cells and cell components, non-living, non-replicable systems as stated in the text. Consequently, they do not require regulation.

(ii) Applications to produce non-native nucleotides and amino acids inside the cell via novel engineered synthetic pathways for basic research and production of pharmaceuticals. [13,14] An autonomous viable strain that both biosynthesizes a modified base and efficiently incorporates it into the genome without addition of exogenous nucleoside analogs has been reported. [15] The research is at early stage.

(iii) Chemically synthesized biological agents able to be replicated, e.g. synthetic horsepox [17] and polio viruses [18], the reconstituted 1918 flu virus [19] and ectromelia virus [20] are prime examples of synthetic biology. Due to the high pathogenic and dual-use potential, strict regulations of technology transfer and trafficking have to be implemented in addition to the well-established biosafety regulations for laboratory research with pathogens, including gain of function studies.

(c) Applications with intended use in both the environment and the laboratory:

(i) Genetically engineered bio-containment systems within the cell, primarily for use in the environment but also some laboratory applications are currently at various stages of R&D. [21] The constructs may fall under the definition of synthetic biology (e.g. auxothrophic strains, or strains containing pathways requiring synthetic substrates). These developments represent options for increased biosafety.

(ii) Biofoundries (i.e., highly automated service laboratories) grant cost-effective access to high-cost equipment and small-scale prototype evaluation and significantly accelerate the engineering of biological systems by providing higher reproducibility, throughput and ease of sharing of standardized protocols. [22,23] Products developed via biofoundries are in various stages of R&D, some already available on the market. Biofoundries have large potential for extending research collaborations to non-industrialized countries.

(iii) A genetically engineered plant producing recombinant polyclonal antibodies against snake venom toxins relies on virus-based expression systems to induce somatic expression mosaics in plant leaves, where each ‘tile’ in the mosaic functions as an independent monoclonal micro-production line yielding recombinant polyclonal cocktails. [24] This approach can potentially overcome the shortage of supply of anti-venoms from hyperimmunized animals. Research is at the laboratory stage.

(iv)
To increase the yield of photosynthetic CO_2-fixation, four general strategies have been pursued, at least partially: (a) improving the catalytic properties of RubisCO, (b) improving the working conditions for RubisCO through CO_2-concentrating mechanisms (CCM), (c) engineering synthetic photorespiration bypasses, and (d) engineering synthetic CO2-fixation pathways. Completely rewiring CO_2-fixation in plants, algae and cyanobacteria (d) is the most ambitious approach. [25-27] Some natural microbial CO_2-fixation pathways not based on Rubisco show advantages with regard to efficiency. However, their reconstitution in model organisms has not been successful so far, probably due to the complex interplay and interference with the host's native metabolism.
Current synthetic biology approaches based on metabolic retrosynthesis aim at completely novel, highly efficient CO_2-fixation pathways designed by a free combination of enzyme reactions. [25] These efforts are fueled by the discovery and rational engineering of highly efficient carboxylases and the general progress in computational enzyme design. The high degree of freedom theoretically allows for tailoring the conversion of CO_2 into virtually any desired product, while the synthetic nature of the pathways proves advantageous due to minimal interference with natural metabolism.

(v) Health: Plant natural products (PNPs), ubiquitous components of medicines, flavors, and fragrances, are challenging to produce. Engineering PNP pathways into new hosts requires metabolic engineering at the level of host, pathway, and enzymes, i.e. finding or modifying a suitable host to accommodate the pathway, planning and implementing a biosynthetic route to the compound, and discovering or engineering enzymes for missing steps. [28] Synthetic biology offers the option to improve the supply of pharmaceuticals while decreasing the dependency on rare plant species and thus relieving the burden on ecosystems and biodiversity.

(vi) Health: Recent advances in reprogramming microbial biosynthetic pathways through polyketide synthase and non-ribosomal peptide synthetase engineering are revitalizing the discovery and development of new natural products with therapeutic potential and medicinal applications. [29] This strategy is promising for finding novel, desperately needed antibiotic drugs.

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(d) Increasing sophistication of methods, including, for example, new genome editing techniques, more complex metabolic engineering, the recoding of genomes, and the use of artificial intelligence/machine learning for the redesign of biological systems;

It is important to note, that synthetic biology makes use of some of these technologies and approaches in the engineering strategies, however the sole implementation of these methods/technologies does not imply the generation of a synthetic biology product.

However here are some examples of the newly developed methods, which are also applied in the synthetic biology field:

Automated continuous evolution as developed by the Liu lab (Orthorep system) allows to select in vivo a single gene for adaptive traits (extensively reviewed ref [6])

Nevertheless, today the application of modern machine learning algorithms on biological data is solving problems that were once believed to be unsolvable [1], [2], [3|, such as the protein folding problem, which enables the design of entirely novel proteins with new functions. [2]
For the engineering of biological systems, this means that highly accurate predictions on the outcome of genetic interventions are possible [4]. In particular, artificial intelligence applications have already made a great impact [9] on several research frontiers.

The ability to recode entire genomes will open up completely new possibilities and the potential has already been shown the scientific literature, e.g. for the production of valuable compounds

(ref Liu).

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(e) The use of transient modification of organisms, including, for example, through the use of synthetic double-stranded RNA molecules, nano-particles, and genetically modified viruses;

(f) Ability to produce new synthetic biomolecules using non-canonical nucleotides and amino acids;

The use of non-canonical building blocks in biomolecules and microbial organisms offers great potential for biocatalysis and biosafety [1], [2]. With regard to biocatalysis, the incorporation of non-canonical amino acids into enzymes opens up the possibility of new reaction mechanisms [1], [3]. This allows combining the progress of synthetic organic chemistry with the opportunities of more environment-friendly biocatalysis [1].
In terms of biosafety, the incorporation of non-canonical biomolecules into microorganisms allows the creation of synthetic auxotrophies [2], [4]. These synthetic auxotrophies will be a powerful tool for locally- and timely-restricted field applications of genetically modified microorganisms [2], [5].
Even though the incorporation of non-canonical building blocks into biomolecules and organisms is currently associated with extensive engineering of the host genome [2], [5], advances in the assembly of whole synthetic genomes and DNA synthesis [6] will make non-

canonical biomolecules a common tool for synthetic biology in the future. These synthetic genomes can furthermore again be utilized to increase the biocontainment of the engineered organism by reassignment of the genetic code to be incompatible with any natural organism, so that the local restriction of the application of genetically modified microorganisms can be guaranteed [7]

[1] https://onlinelibrary.wiley.com/doi/full/10.1002/anie.201610129
[2] Mandell, D., Lajoie, M., Mee, M. et al. Biocontainment of genetically modified organisms by synthetic protein design. Nature 518, 55–60 (2015). https://doi.org/10.1038/nature14121
[3] https://chemistry-europe.onlinelibrary.wiley.com/doi/10.1002/cbic.201400060
[4] https://onlinelibrary.wiley.com/doi/10.1002/anie.201103010
[5] https://www.biorxiv.org/content/10.1101/2022.07.08.499367v1.full
[6] https://doi.org/10.1038/d41586-020-00511-9
[7] Young, Rosanna E. B.; Purton, Saul (2016): Codon reassignment to facilitate genetic engineering and biocontainment in the chloroplast of Chlamydomonas reinhardtii. In Plant biotechnology journal 14 (5), pp. 1251–1260. DOI: 10.1111/pbi.12490.

(g) The use of synthetic biology for non-biological purposes, for example, in data storage.

Deoxyribonucleic acid molecules theoretically have an exceptionally large (petabytes per gram) capacity for storing digital data and are considered superior to conventional data storage media with regard to their high durability if kept under appropriate ambient conditions. Combined with cellular replication, providing an efficient molecular read-and-write machinery, the technological concept yields promising options for the energy-efficient long-term storage of large amounts of data. Currently, the speed of the “writing” process, i.e. DNA synthesis, is a serious bottleneck.
[1] The diverse technical concepts and recent developments have been documented in Technical series 2022 as well as
[2] Bencurova E, Akash A, Dobson RCJ, Dandekar T. DNA storage-from natural biology to synthetic biology. Comput Struct Biotechnol J. 2023 Feb 2;21:1227-1235. doi: 10.1016/j.csbj.2023.01.045. PMID: 36817961; PMCID: PMC9932295.
[3] Hoose A, Vellacott R, Storch M, Freemont PS, Ryadnov MG. DNA synthesis technologies to close the gene writing gap. Nat Rev Chem. 2023 Jan 23:1-18. doi: 10.1038/s41570-022-00456-9. Epub ahead of print. PMID: 36714378; PMCID: PMC9869848.]