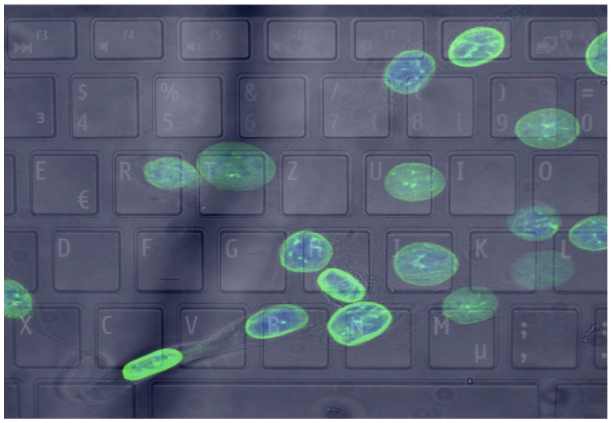
Karlsruhe Institute of Technology

Bundesministerium für Bildung und Forschung

Evidence Maps for Synthetic Biology Applications

+ A Discussion Map on Synthetic Biology and "Knowledge about Life and Its Origin"



These evidence maps represent a work-in-progress as part of the "Engineering Life" project (www.engineeringlife.de) supported by the Federal Ministry of Education and Research, Germany (Bundesministerium für Bildung und Forschung, BMBF). These versions of the maps were used as a discussion basis during the two-day expert workshop on "The Present and Future of Synthetic Biology" held in Karlsruhe on 21 – 22 June 2011.

When using these maps, or parts of them, please refer to them as:

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The Engineering Life project

http://www.engineeringlife.de
http://www.itas.fzk.de/eng/projects/2010/coen10_e.htm

The aim of the project is to reflect the theological and ethico-philosophical relevance of synthetic biology, to analyze its opportunities and risk potential, and to evaluate whether the current legal situation in Germany seems appropriate to account for possible risks. Special attention is paid to specific differences between synthetic biology and genetic engineering, to the concept of life and its implications, and to questions concerning governance options. In this way, the project will help to contextualize synthetic biology research and to enable a well-informed public and political debate.

Within the Engineering Life project, KIT's Institute for Technology Assessment and Systems Analysis (ITAS) is responsible for one of five subprojects. In its subproject, ITAS will analyse and assess the potential social benefits and risks of synthetic biology (including environmental, health, safety, security and socioeconomic aspects) as well as the various expectations and visions shaping the field ('vision assessment') and its knowledge politics. The results of the subproject will be useful when it comes to governance of synthetic biology and will also help to deal with the field's ethical, legal and broader societal implications.

Evidence Map
Synthetic Biology Applications (I)

Environment

State of the art

GE/SynBio

- GE E.coli and yeast for 1,3-propane-diol [1, 2, 3] and 1,4-butanediol [4, 5] (→ eg, plastics, solvents, adhesives)
- GE E.coli for lactic acid [6, 7]; GE algae and plants for direct 'photosynthetic' polyhydroxy-alkanoates PHA [8, 9] (→ biodegradable plastics)
- GE E.coli or algae for isobutene [10] and isoprenoids/farnesene (also 'photosynthetic') [11, 12, 13] (→ polymers, rubber, fuels, pharma-ceuticals)

- Reduces depletion of/dependence on fossil resources [14, 15]
- Biodegradable materials [15, 16]
- Potential to reduce green house gas (GHG) emissions [14, 17-19]

Production of chemicals from renewable sources

- Potential land-use/food com-petition issue [17, 20]
- Environmental impact (eg, GHGs, carcinogens, eutrophication) of bioplastics production may exceed that of fossil-based plastics [21]

Suitable feedstock may reduce/ avoid land-use/food compet-ition issue and other environ-mental effects [17, 20, 21]

Potential societal benefits

Environment

- Environmentally-friendly synthesis of chemicals/materials
- Biodegradation/removal of environmental toxins and contaminants
- Bioensors for monitoring of environmental pollution

Potential risks/ conceptional problems

Bulk production of bio-based polymers (+ fuels) could lead to competition with land needed for food production

Production by algae or biomass from perennial grasses grown on degraded land may prove beneficial [17, 20, 21]

Biosafety: Unintended or deliberate release (for *in situ* remediation) of GE/synthetic organisms may have negative impacts on indigenous flora/ fauna

SynBio approaches for potential 'biological containment' (eg, suicide [70-72] / xenobiotic mechanisms [54, 73-75]) and its verification (via genome "watermarks" [76-78])

State of the art

Addressing technical pro-blems seems possible; eg, genetic instability [27], noise [30-32], specificity [28, 29, 33]

- Maintenance of viability/ activity [34]
- Genetic instability, reporter noise, delayed response [34, 35]
- Often lack of specificity [34-36]

In-situ application requires release of genetically engin-eered organisms [36]

GE/SynBio

- Coupling (natural) pollutant-responsive gene-regulation units to reporter genes [22-25]
- 'Evolved' variants of natural sensor proteins [26, 27], ration-ally redesigned ones [28] or synthetic riboswitches [29] for new pollutants; may also control cell behaviour (migration) [29]

Environmental (whole-cell) biosensors

- Can measure bioavailability of pollutants [34, 36]
- (First-line) screening for general toxicity or specific pollutants [34-36]
- Cheap and simple large-scale field measurements (*in situ/ex situ*) [34-36]
- Integration onto microfluidic, electronic detection platforms [34, 35]

Data from a field study suggest that these issues may be not general ones [45]

Effects may be alleviated by systems biology/SynBio approaches [52-54]; see also above

- Survival of GE microorganisms (GEM), competition with indigenous populations may be an issue [52, 55-57]
- Potential problems of genetic/ phenotypic stability of GEM [52]

For *in situ* applications: possible impacts of GEM on natural flora by hori-zontal gene transfer of recomb. DNA [58, 59] or by indirect effects [60-63]

GE/SynBio

- GE bacteria for heavy metal [37, 38, 39] and radionuclide removal [40-42]
- GE bacteria [29, 43, 44, 45] and plants [46, 47] for organic chemical removal (eg, pesticides, explosives)
- Approaches to constructed multi-organismal consortia [48, 49, 50, 51]

Removal of environmental pollution by GE microorganisms (or plants): bioremediation

- Degradation of organic pollutants without destruction of site material, flora or fauna [64-66]
- May be used *in situ*, for pollutants present in low but relevant concentrations [52, 57, 64, 67, 68]
- May be less expensive [64]

Very few bioremediation data from field studies of GE bacteria exist [45, 69]

Pro arguments

Contra arguments/risks

GE/SynBio Genetic engineering/SynBio approaches (references for highly and moderately artificial work are shown in colour*)

Attenuating arguments

Experimental evidence

Hypothetical

*For artificiality criteria, see Annex, Table I

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Evidence Map
Synthetic Biology Applications (II)

Health

State of the art

GE/SynBio

- Genetic screening circuits for drug discovery [1, 2, 3, 4]
- RNA devices translating disease-associated molecular changes to cell death [5, 6]
- Synthetic circuit-based "prosthetic devices" to control pathogenic metabolites [7]

- Design of disease-specific pathways to screen for therapeutic compounds [8-10]
- Generation of molecular devices combining sensor and effector functions [8-10]

Synthetic circuits/devices for drug discovery and therapeutic applications

Systemic/targeted *in vivo* delivery for therapeutic devices often not solved (eg, when RNA- or gene-based) or can pose safety problems (eg, delivery by viruses) [11, 12]

Ex-vivo applications (cell-based therapies) may avoid targeting issue and facilitate delivery [13, 14]

Supply/lower prices may be met faster, and economically more beneficial to developmental countries, by other methods (eg, artemisinin) [25-27]

- Meet demand of natural products that are in short supply [26, 28, 29]
- Can substitute expensive chemical synthesis or extraction from natural sources [29-31]

Stable source for natural products (not vulnerable to climate conditions) [25, 26]

GE/SynBio

- Biosynthesis [15] and potential diversification [16, 17] of compound libraries
- GE E.coli for anti-cancer drugs: polyketides [18, 19, 20], taxol [21, 22]
- GE yeast and E. coli for malaria drug artemisinin [23, 24]

Biosynthesis of pharmaceutically active natural compounds

May threaten existence of drug-plant farmers in developing countries (eg, artemisinin) [25]

Example of artemisinin suggests that farmers may suffer from subsidy-driven (agricultural) overproduction, rather than from SynBio product to come [26]

Potential societal benefits

Health

- Novel drug discovery and diagnosis tools
- Novel therapies
- Biosynthesis of pharmaceuticals
- Better and more rapidly available vaccines

Potential risks/ conceptional problems

Dual use/biosecurity issue of synthetic pathogens

Potential biosafety issues:
- harmful effects of GE viruses and GE bacteria on patients and medical personnel

- Ecological damage by release of GE mosquitoes,

Governance options/standards for DNA synthesis [61-65], researchers/ projects [40, 66-69] or for controlling equipment and reagents [40, 61, 66] may impede misuse

Improved detection, prevention or treatment of diseases caused by bioterror agents

Improving specficity of viral and bacterial agents

State of the art

GE/SynBio

- Synthetic, chimeric antigens as diagnostic tools [32]
- Synthesized viral genomes to understand etiology/pathogenicity [33-36]
- Vaccines by synthetically attenuated virus engineering (SAVE) [37, 38]

- Synthetic vaccines/diagnostic tools may help to counteract bioterrorism attacks [9, 39]
- Governance options, see above

Synthesis (and sequence data) of viral genomes may allow/facilitate generation of pathogens by bioterrorists [40]

Synthesis of pathogens or components thereof for diagnosis and vaccine development

- Design of complex artificial antigens for better diagnosis and vaccines [9]
- Knowledge on origin and virulence of pathogens, rapid deployment of vaccines [39]

Progress in engineering target specificity or target-dependent expression of therapeutic products [51, 52]

- Potential side effects (eg, by bacterial toxicity, inefficient targeting, or gene transfer) [51, 52]
- Unexpected effects on viral pathogenicity [53]

For schemes requiring release of GE mosquitoes: potential ecological, ethical, social and legal issues [54-57]

GE/SynBio

- GE bacteria against cancer [41, 42]
- GE viruses to fight cancer [43, 44]
- GE bacteriophages against bacterial infections [45, 46]
- GE mosquitoes for disease control (eg, malaria, dengue fever) [47-50]

Genetically engineered organisms/viruses to fight disease

- Generation of targeted therapies (eg, specific to cancer cells) [51, 52]
- May overcome cancer-cell and bacterial multidrug resistance [51, 52, 58, 59]

Control of mosquito and parasite populations without toxic insecticides/drug resistance [57]

Potential development of resistance to introduced gene products [60]

Pro arguments

Contra arguments/risks

GE/SynBio Genetic engineering/SynBio approaches (references for highly and moderately artificial work are shown in colour*)

Attenuating arguments

Experimental evidence

Hypothetical

*For artificiality criteria, see Annex, Table I

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Generation of virus via synthesis of whole genome

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Evidence Map
Synthetic Biology Applications (III)

Energy/Biofuels

Energy

Biofuels (new generations)
- Less GHGs/CO_2-neutral
- Less land use/food competition
- Substantial replacement of fossil fuels by renewable ones

→ Climate change mitigation + energy security

Biosynthesis from molecules produced by plants

Potential societal benefits

State of the art

GE/SynBio

- GE E.coli for butanol [1-3] and branched-chain higher alkolhols [1]
- GE E.coli, yeast and other fungi for biodiesel or alkanes [4, 5, 6, 7, 8]
- GE microalgae for alkanes [6, 7]

Sugar → drop-in fuels

- Sugar directly available, no need for direct light conversion technology (eg, photobioreactors) [1, 9, 10]
- Drop-ins show higher energy density (compared to ethanol), fungible with fossil fuels and their infra-structure, can also replace marine and aviation fuels [1, 9, 10]

Often long-term net green house gas (GHG) emissions by land-use change [11-14]

Food competition (negative effects on food security/food prices) [15-17]

Food competition may depend on region/ feedstock [18, 19]

GE/SynBio

- GE yeast for ethanol [20]
- GE bacteria for isobutanol [21]
- E.coli for biodiesel [8]
- GE microalgae, yeast and other fungi for oil production [22]
- GE lignin-reduced plants [23]

- GE drought-tolerant plants [24, 25]

Lignocellulose → drop-ins, ethanol

- Higher fuel yield per land area, making nonedible plant parts available (lignocellulose) [10, 26]
- Usage of non-food feedstocks (eg, switchgrass), less competition for food [10, 26]

Net GHG emissions by land-use change (direct/indirect) [11-14, 27]

- Soil organic carbon loss by removal of feedstock residues [13, 28]
- Invasive feedstock plants [29-31]

Not necessarily net GHG emissions by land-use change [13, 32]

Potential risks/ conceptional problems

Biosafety: release of GE/synthetic organisms may cause ecological damage (higher risk of open systems)

- Closed production systems (eg, photobioreactors) [46, 49, 50]
- SynBio approaches for potential 'biological containment' (eg, suicide [65-67] / xenobiotic mechanisms [68-71]) and its verification (via "watermarks" [72-74])

Human rights: eg, displacement of indigenous tribes (by "land grab"), food/water security

Loss of biodiversity

Carbon neutrality

Can depend on feedstock, product, processing and land use [75]: perennial grasses on degraded lands or algae may prove beneficial [13, 32, 46, 76]

Proportion of fossil fuels that can be replaced sustainably?

- Calculations vary, influenced by feedstock, products and land use [49, 77-79]:
- Lignocellulosic and algal bio-fuels may replace substantial proportion [49, 80]

Proposed ethical framework for biofuel production [81] (and references therein)

Direct biosynthesis from light and CO_2

State of the art

GE/SynBio

- GE microalgae for ethanol [33, 34], iso-butyraldehyde/-butanol [35], butanol [36], methyl-butanol [37] or iso-prenoides [38, 39]
- GE microalgae for secretion of fatty acids (-> biodiesel) [40] or alkanes [41]

Light + CO_2 → drop-ins, ethanol by microalgae

- Enhanced light conversion [42]
- Direct fuel synth. strategies, secreted products [41, 43]

- Wastewater, flue gas CO_2 or energy from spent algal biomass [44-48]
- Feasability studies [49, 50]

- Large environ. footprint (H_2O, fertilizer, energy/CO_2 emissions) using current techniques [45]
- No industrial scale yet / economic viability? [51, 52]

High photosynthetic yields, (high energy yield per area); less need for (arable) land; use of brack/sea water; production of lipids and hydrocarbons [44, 51, 53]

GE/SynBio

- GE green algae with increased H_2 production [54-58]
- GE cyanobacteria with enhanced H_2 production [59, 60]

Light + H_2O + CO_2 → hydrogen by microalgae

- Improved light conversion [58]
- Improved efficiency of hydrogen production [54, 56, 57, 59, 60]

Process effectivity (eg, O_2-sensitivity) [61, 62]

- Hydrogen infrastructure [63]
- Potential enhancement of GHG effects [64]

- Hydrogen is a non-carbon based fuel (may reduce CO_2 emissions) [63]
- Microalgal based production may sequester CO_2 [51, 61]

Pro arguments

Contra arguments/risks

GE/SynBio Genetic engineering/SynBio approaches (references for highly and moderately artificial work are shown in colour*)

Attenuating arguments

Experimental evidence

Hypothetical

*For artificiality criteria, see Annex, Table I

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A Discussion Map (Draft):

Synthetic Biology and Knowledge about Life and its Origin

- Knowledge on minimal set-up for cellular life
- Could lead to basic cells ("chassis") to generate specialized microbial producer strains for biotechnology uses [1, 2, 4]

SynBio

- Minimizing genomes by planned, multiple deletions of dispensible genes [1, 2]
- Multiple designed alterations in genomes and accelerated cell evolution [3]

Minimal genomes/cells from existing cellular life

Finding the set of essential genes will require generation/testing of huge numbers of altered genomes [5]

Might be aided by automated, multiplex genome engineering and directed evolution approaches [3]

State of the art

'Top-down' approach
(starting from existing cells)

Potential societal benefits

Some potential ethical issues / conceptional problems

Knowledge about origin and nature of life

- "Minimal life"

Generation of self-sustaining biological systems may provide knowledge on conditions/components that can permit life to emerge, rather than on what (existing) life is

Synthesizing/creating life "ad libitum" may undermine a potential intrinsic value ascribed to life

Value/moral status of life may be more associated with features we perceive of/ascribe to certain life forms (eg, sentience, consciousness) than with life itself

"Creating artificial life is morally wrong" may be a major concern for public SynBio acceptance [33]

Accurate information/language could alleviate moral concerns about (current) work

Potential biosafety issues from "synthetic" organisms (gene transfer, health, ecology)

Orthogonal/xenobiotic SynBio approaches might prevent harm from gene transfer [34]

Potential dual use/biosecurity issues (genome synthesis/ assembly, genome evolution techniques)

Governance options/standards for DNA synthesis [35-39], researchers/ projects [40-44], or for controlling equipment and reagents [35, 42, 44] may impede misuse

'Top-down' and 'bottom-up' approaches may finally merge

'Bottom-up' approach
("Synthesizing life*")

State of the art

***Definition of ("minimal") life** [13]:
- Autonomous replication, reliant on input of small molecules and energy only
- Darwinian evolution: genetic variation and its phenotypic expression in survival and reproduction

Relation of protocell models to current life forms may remain elusive

SynBio

- Growth and division ('replication') of fatty-acid vesicles [6-11]
- 'Nutrient' (nucleotide) aquisition and nucleic-acid replication in membrane vesicles [12]

Models of early cells (protocells) distinct from modern cells

May help to evaluate potential routes for the emergence of life on early Earth [13-16]

May be aided by multiplex genome engineering and directed evolution [3]

Total '*de-novo* construction' would require design of DNA programme for synthetic network of 150-400 genes [5, 30, 31] - in contrast to current ability to (predictably) programme networks of a few genes only [5]

SynBio

- Reconstituted gene expression (ie, by purified components) in phospholipid vesicles [17-21]
- Assembly and transplantation of synthetic bacterial genomes [22-24]
- Multiple designed alterations in genomes and accelerated cell evolution [3]
- Genetic-code expansion by non-natural nucleotides (*in vitro*) [25-27] and orthogonal ribosomes (in cells) [28, 29]

(Semi-) Synthetic cells based on natural and/or non-natural components

Can lead to knowledge about current cellular life [5, 16, 31, 32]

Pro arguments

Contra arguments/risks

SynBio SynBio approaches

Attenuating arguments

Experimental evidence

Hypothetical

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Annex

Artificiality	Functions/entities	... combined with processes to generate them
High	**Generation/alteration of organisms or viruses via synthesis of whole genomes** or substantial genome portions	
	Generation of 'minimal' life *in vitro* using natural or non-natural molecules/molecular complexes, **or *in vivo*** by eliminating multiple dispensable genes	
	Functions occuring in nature	Transfer/generation of **complex pathways or genetic circuits**
		Generation involves **non-natural components ('parts')** (eg, non-natural nucleotides or amino acids, synthetic enzymes)
	Functions not present in nature	Generation of **comlex or less complex pathways or genetic circuits** (based on combination of natural components); **transfer/downregulation of several genes**
		Generation involves **non-natural components ('parts')** (eg, designed/'evolved' proteins or riboswitches with novel specificities)
Moderate	**Functions occuring in nature**	Transfer/generation of **less complex pathways or genetic circuits** (based on combination of natural components); **transfer/downregulation of several genes**
	Functions not present in nature	Generation via **simple genetic circuits** (based on combination of natural components)
Low	**Functions occuring in nature**	**Transfer of 1-2 genes only** [including simple reporter genes or gene/protein fusions]
	Functions not present in nature	

Table I An attempt to characterize artificiality of genetic engineering/ synthetic biology work.
Assigned degrees of artificiality (high, moderate or low) are based on characteristics of both the generated functions/entities and the processes used to obtain them. The basic three-level scheme of artificiality and properties to characterize artificiality of processes were adapted and refined from ideas of our "Engineering life" BMBF-project partners Hanna Wischhusen, Birgit Wiltschi and Wilfried Weber (BIOSS, University of Freiburg).

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