Introduction

Synthetic biology includes the design and construction of new biological systems and devices, as well as and re-designing existing, natural biological systems for industrial utilisation. Synthetic biology is used for rational and systematic design and synthesis of complex systems, which may display functions that do not exist in nature. The conference will focus on the use of synthetic biology for optimization and design of cell factories in relation to chemical industry, energy and pharmaceuticals. But first of all, the conference will give a basic introduction to synthetic biology including concepts, methods, opportunities and limitations.

The conference includes a poster session covering a broad range of topics on biotechnology as well as a commercial exhibition of equipment, consumables and services to research and development in biotechnology.

The conference is organised in collaboration with Danish Society of Biochemistry and Molecular Biology as well as BioPeople.

Danish Biotechnological Society (DBS) is a scientific society established in 2006 in collaboration between Danish Biotechnology Forum and The Danish Society for Biochemistry and Molecular Biology. DBS is organised as part of The Danish Society of Engineers, IDA. The purpose of DBS is to facilitate networking within the field of biotechnology in Denmark, creating links between universities, research institutions, hospitals and companies. DBS represents professionals working with biotechnology and seeks to promote and communicate important issues on biotechnology, biochemistry and molecular biology to the public. DBS organises scientific conferences and meetings on biotechnology.
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# Programme

**May 26**

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Perspectives in Synthetic Biology

Peter Olesen

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Major research breakthroughs often take place in the interphases between classical research disciplines. The development of the SynBio area (synthetic or synthesis biology) is based upon converging and systems-oriented research and omics approaches in and between the biochemistry, molecular biology, biotechnology, bioinformatics, and nanoscience disciplines over the last decade. SynBio has in the last few years gained extreme speed and interest as a promising new research and innovation platform offering substantial technological developments needed for the realisation of a future biobased economy.

Nanoscience (nanobiology, nanowiring, nanoelectronics, nanomachines etc.) has great promises to deliver new hardware and software contributions to further development of the bioinformatics and biotechnological areas. Likewise, new developments in ‘in silico biology’ bring extreme speed and new technologies to the bioinformatics area: new generations of sequencing and annotation tools, synthetic genes and regulators and other molecular bricks and components – thus supporting major new steps in bioengineering. In this way, bioinformatics and bioengineering may also provide new molecular entities and processes that may lead to the development of new synthetic or semisynthetic expression and production systems at nanoscale.

Thus, to a large degree, the essentials of SynBio is the potential to understand, mimic and recombine nature’s molecular building blocks in new ways, using bioengineering and minimal cell design and molecular breeding technologies. This will allow the creation of new expression and production systems, ranging from cell-free systems over microbial/plant/animal/human cell factories to novel organisms. The resulting SynBio platform for research and innovation will have many diverse application areas such as new individualised drugs and health ingredients, healthier (functional) foods, more nutrition efficient animal feeds, biofuels, biomaterials, biocomputers etc.

In order to realise these potentials of SynBio it is of paramount importance to focus future research initiatives to foster and harvest innovative breakthroughs between the different research disciplines already mentioned – and maybe others not mentioned. Therefore the Danish Council for Strategic Research recommends a strong prioritization of advanced new research activities across the SynBio area, focusing on maximizing the synergies between the disciplines in order to release the breakthrough potentials.
Building synthetic cells, rewiring existing genomes and other advancements of synthetic genomics

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In May 2010 the synthetic biology team at JCVI announced the first self-replicating synthetic bacterial cell. The technologies developed during this process are now being applied towards bio-fuels research, drug discovery and vaccine production. Also, the capacity to rewrite the genetic code of entire organisms, remove or replace codons, coupled with the use of orthogonal tRNA-AARS systems, has opened up the possibility of in vivo sense codon suppression for the insertion of non-standard amino acids into proteins. It may now be possible to force dependence of synthetic cells on a 21st amino acid, one that is not available in nature.
Engineering Synthetic Mammalian Gene Networks – From Tools to Therapies

Martin Fussenegger

Department of Biosystems Science and Engineering (D-BSSE)
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Mattenstrasse 26, CH-4058 Basel, Switzerland

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Capitalizing on our latest advances in the design of heterologous mammalian transgene control systems we have designed the first prosthetic networks that sense, monitor and score (disease-) relevant metabolites, process off-level concentrations and coordinate adjusted diagnostic, preventive or therapeutic responses in a seamless, automatic and self-sufficient manner. We believe that the design of synthetic gene networks, which process molecular signals with near digital precision, may provide novel therapeutic opportunities. Therefore we invite you to join us on a journey exploring the latest generation of “traceless” transcription control systems, designing gene networks with highly complex expression dynamics and appreciate our proof-of-concept studies on prosthetic networks improving artificial insemination and enabling the treatment of gout.
Mammalian/Cancer cell manipulation via synthetic biology.

S. Schmidt¹, J. Trojnar¹, A. Riedel¹, H. Christiansen¹, P. Lund Hansen¹, C. Müller¹, A. Jaskot¹, I. Block¹, J. Mollenhauer*¹

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Synthetic biology comprises two major complementary approaches. Bottom-top strategies aim at recreating hallmarks of living systems from artificial building blocks to explore basic principles of life. Top-bottom approaches utilize and combine well-characterized building blocks or biological elements to introduce them into living systems with the goal to create synthetic systems with new properties.

Despite the availability of viral and non-viral systems, the possibilities for the manipulation of mammalian cells are still very limited. This is especially true, when more complex traits are to be introduced into the cells, which is the prospective next demand for high-throughput cell biology (functional genomics) as well as for highly targeted drug screens for personalized medicine and for the sophisticated manipulation of human stem cells for the purpose of regenerative medicine. Research and development in these areas requires the creation of cell models, into which multiple traits can be engineered in a standardized fashion, so that the models come closer to the complex scenarios in the living organism or in the individual patient.

To meet these future demands, we have been starting with a top-bottom synthetic biology strategy. We used and newly combined well-characterized genetic building blocks in a kit-like fashion, yielding a system that allows for mammalian cell manipulation in any desired direction, via an ordered array of events. In the first step, we convert a given cell line to a so-called acceptor cell line, which is now able to easily accept any of the downstream elements selected for manipulation via site-specific recombination. In a second step a first genetic/molecular trait is introduced into the acceptor cell line into a pre-defined site in the cellular genome. Performed in parallel, this yields genetically identical (isogenic) daughter cell lines, with either constitutive or inducible overexpression or knockdown of a cellular or reporter gene. Consecutively, these can be used to create isogenic second generation daughter cell lines via an analogous procedure, allowing for the creation of cells with any desired permutation of the afore-mentioned traits, e.g. double reporter cell lines or cells with inducible upregulation of one gene and constitutive knockdown of another gene.

We will provide examples for the manipulation of cancer cells. For example, the technique is used to construct large panels of hundred up to several hundred of stable cancer cell lines for functional genomics approaches and drug target discovery. Other applications, such as the construction of reporter cancer stem cells, synthetic cancer stem cells and in vivo reporter systems are also outlined. While this already provides powerful tools, the potential of the system is not yet fully exploited.
Synthetic biology, the technology counterpart of systems biology, aims at establishing novel, useful biological functions by suitably combining well-characterized parts. Especially when complex circuits - in terms of the number of components and interactions involved, or with respect to the dynamic behavior - are to be designed, computational engineering methods have to be an integral part of the approach. Here, we will focus on engineering concepts to achieve scalability and robustness (relative insensitivity to external or internal perturbations) of designed circuits. Both aspects are important for the field because the biology-based parts employed are not (yet) well-characterized, the circuits have to operate in a noisy cellular environment, and they cannot be completely isolated from the cellular context. Specific examples that illustrate the challenges of and possible strategies for rational circuit design include devices for digital signal processing, tunable synthetic oscillators, and physiological set-point controllers with clinical application.

These cases demonstrate that
(i) novel mathematical modeling and systems analysis methods are needed to enable efficient computational design of synthetic circuits, and
(ii) the design of synthetic systems is also valuable in testing and refining our current understanding of natural biological systems.
From *in silico* Analysis to Potential Antifungal Targets in *Aspergillus fumigatus*

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Aspergillosis is an infection caused by the opportunistic fungus *Aspergillus fumigatus*. Invasive aspergillosis is a major problem for immunodeficient patients including those suffering from AIDS and cancer or recovering from organ transplant. While several antifungal agents are available, mortality caused by the fungal infections is still high. Although new generations of antifungal drugs are appearing, development of new more effective drugs for treatment of aspergillosis and other fungal infections is required.

Coupling of antifungal design and gene essentiality has previously been introduced in *A. fumigatus* (Firon et al. 2003), but at that point the main limitation was the generation of a gene deletion strain collection. Despite the development of molecular biology tools, generation of mutant strain collections remains laborious and time consuming. In the present study, an *in silico* approach that relies on a genome scale model is proposed. The objective of the approach was to develop an alternative to the tedious systematic generation of deletion mutants and to bring forward new antifungal targets based on essential pathway analysis.

References:

Microbial fermentations are the core of biorefineries as this process ensures value addition when the raw material is converted to desired fuels and chemicals. The development of efficient cell factories that can be used as biocatalysts in microbial fermentations is often the most time consuming and R&D intensive part in the development of a biorefinery. Most current biorefineries involves ethanol production, but there is much interest to upgrade the production of ethanol to other more valuable fuels like butanol and diesel. This can be done through simple replacement of the biocatalyst, which in this case is the yeast *Saccharomyces cerevisiae*, and replacement of the biocatalyst can often be done in a plug-and-play fashion with little requirement for retrofitting of the production facility.

Besides its classical application in the production of bread, beer, wine and bioethanol, the yeast *Saccharomyces cerevisiae* is a widely used cell factory for production of fine chemicals such as resveratrol, and for production of pharmaceutical proteins such as human insulin and vaccines. In connection with the development of biorefineries for sustainable production of fuels and chemicals, there is much interest to use yeast as a cell factory due to its general acceptance in the industry, its robustness towards contaminations, its high alcohol tolerance, and its low pH tolerance. Further advantages of using yeast are that a large number of molecular biology techniques are available for this organism, there is a large experimental database available and there is an excellent research infrastructure.

In this lecture tools for advancing the design and engineering of novel, efficient yeast biocatalysts will be presented. This will involve both a number of novel synthetic biology tools that allows for rapid reconstruction of heterologous pathways, for tuning expression using promoter libraries and different expression vectors. Also different tools from metabolic engineering will be presented, e.g. methods for flux quantification and mapping of flux control. Finally methods from systems biology will be presented, as these may offer completely computerized design of cell factories in the future using so-called genome-scale metabolic models. Examples for how the metabolism of yeast can be engineered for production of novel fuels and chemicals will be presented, and it will be demonstrated how yeast can be engineered to produce a range of different products and hence can serve as a platform cell factory in modern biorefineries.
Bioengineering of glucosinolates production in yeast

B.A. Halkier¹, U.H. Mortensen², L. Albertsen², B. Salomonsen¹, M. Mikkelsen¹, J.K. Vester, F. Geu-Flores¹

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Epidemiological studies have demonstrated reduced risk of developing cancer upon consumption of diets rich in cruciferous vegetables. Key players in this chemoprevention are the natural products glucosinolates, in particular the methionine-derived glucoraphanin which is highly abundant in broccoli. Improved nutrition by functional foods or health-promoting dietary supplements is an attractive means for prevention of lifestyle-based diseases. Towards this goal, we have engineered the production of both simple and complex glucosinolates into tobacco (1,2) transferred the entire glucoraphanin biosynthetic pathway consisting of thirteen genes from Arabidopsis into the non-cruciferous tobacco by transient expression. The engineering involves the chloroplast-localized chain elongation machinery (5 genes) that converts methionine to dihomomethionine, and the cytosolic, ER-anchored core structure pathway (8 genes) that converts dihomomethionine to the glucoraphanin. More recently, we have embarked on development of a technology platform for engineering plant pathways exemplified by glucosinolates into yeast. Our progress in this respect will be discussed.

References:

Bioengineering of glucosinolates production in yeast

B.A. Halkier¹*, U.H. Mortensen², L. Albertsen², B. Salomonsen¹, M. Mikkelsen¹, J.K. Vester, F. Geu-Flores¹

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References:

Engineering the spatial organization of metabolic pathways

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In nature, enzyme proximity is used to efficiently regulate cellular metabolism. Several systems for ensuring close proximity of sequentially acting enzymes exist. Enzymes may be assembled in complexes, up-concentrated in an organelle or attached to cellular structures such as the ER membrane. By positioning enzymes in close proximity, loss of metabolites through diffusion and competing pathways can be minimized and accumulation of intermediates can be prevented. Although the proximity concept is used extensively to regulate natural pathways, the application of the concept in metabolic engineering has been limited. But mimicking nature’s way of organizing enzymes has great potential to increase the efficiency of novel pathways.

When cell factories are engineered to produce valuable metabolites, the pathways most often rely on heterologous enzymes as well as on the enzymatic machinery of the host. For such novel pathways, no optimal spatial organization can be expected to be in place. Presumably, this contributes to novel pathways suffering low productivity and yields as well as the formation of undesired side-products. In this study we have tested two different strategies for positioning sequentially acting enzymes in close proximity. One strategy was to fuse the genes encoding the enzymes and the other strategy was to attach enzymes to a protein-based scaffold. The effect of the two strategies was tested on two industrially relevant model pathways. One that leads to sesquiterpene production and one that leads to vanillin glucoside production in the yeast Saccharomyces cerevisiae. Enzyme proximity resulted in an up to two-fold increase in production. Moreover the strategy could be used in combination with traditional metabolic engineering approaches to further increase the production. This simple test case of synthetic biology demonstrates that
engineering the spatial organization of sequentially acting metabolic enzymes has great potential for diverting flux towards a desired product.
Bottom up assembly of minimal life

Steen Rasmussen\textsuperscript{1,2*}, Pierre-Alain Monnard\textsuperscript{1}, Martin Hanczyc\textsuperscript{1}, Anders Albertsen\textsuperscript{1}, James Boncella\textsuperscript{3}, Eva Bonzil\textsuperscript{1}, Filippo Caschera\textsuperscript{1}, Mark Dorr\textsuperscript{1}, Harold Fellermann\textsuperscript{1}, Maik Hadorn\textsuperscript{1}, Wendie Jørgensen\textsuperscript{1}, Philipp Loffler\textsuperscript{1}, Sarah Maurer\textsuperscript{1}, Kent Nielsen\textsuperscript{1}, Pernille Pedersen\textsuperscript{1}, Carsten Svaneborg\textsuperscript{1}, Michael Wamberg\textsuperscript{1}, Rafal Wieczorek\textsuperscript{1} & Hans Ziock\textsuperscript{3}

\textsuperscript{1}Center for Fundamental Living Technology, University of Southern Denmark  
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When seeking to assemble minimal life from the bottom up, the critical properties of life apparently emerge from the interconnected functions of three subsystems: information, metabolism and container. Such interconnected supramolecular systems, so-called protocells, will under the right circumstances be able to mimic the main functions of a living cell although in a very simplified manner\textsuperscript{1}.

Seeking to create minimal life from the top down leads us to a somewhat different picture, where construction of synthetic / streamlined genomes become the critical scientific issue\textsuperscript{2,3}. How to integrate the knowledge we obtain from the top down- and the bottom up approaches is a great challenge for our community\textsuperscript{4,5} and a good problem to discuss at this workshop.

In technical terms, our bottom up team explores ruthenium-based photocatalysis as metabolism, fatty acids vesicles, oil droplets and reverse micelles as containers and lipophilic XNA as minimal informational systems\textsuperscript{6,7}. Based on our experimental, computational and theoretical work we review protocell feeding, growth, division, motility, and information controlled metabolic production of containers\textsuperscript{8,9,10,11}.

Finally, we demonstrate preliminary integration of biochemical- and microelectromechanical (MEMS) systems where life-like information processing and material production occur and interact in different media\textsuperscript{12} and as such form a new frontier for synthetic biology.

\textsuperscript{1}Rasmussen S, et al., Protocells: Bridging nonliving & living matter, MIT Press, 2009  
\textsuperscript{3}Rasmussen S, Life after the synthetic cell – Bottom up will be telling more (2010), Nature, 465422a, May 20  
\textsuperscript{6}Rasmussen S, et al., (2003) Bridging nonliving and living matter, Artificial Life 9; 269  
\textsuperscript{8}Fellermann H, et al., (2007) Life-cycle of a minimal protocell – A dissipative particle dynamics study, Artificial Life 13; 319  
\textsuperscript{9}DeClue M, et al., (2009) Nucleobase mediated, photocatalytic vesicle formation from ester precursor molecules, JACS 131 931  
\textsuperscript{10}Toyota T, et al., (2009) Self-propelled oil droplets consuming “fuel” surfactant. JACS  
\textsuperscript{12}http://www.fp7-matchit.eu
Insulating, analyzing and designing biochemical networks

Sonja Billerbeck, Matthias Bujara, Christoph Hold, Rene Pellaux, Sven Panke

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Synthetic Biology plays an increasingly central part in the manufacturing of compounds in the pharmaceutical, chemical, and fuel industry. The underlying biological research has moved beyond the molecular reductionist dogma to a systems view. This has reinforced the notion that the reliable and predictable construction of novel biocatalytic systems is at odds with the complexity of biological systems and highlights the need for concepts that allow reducing this complexity in an attempt to recruit engineering approaches for systems design.

We are exploiting in vitro enzyme systems to implement biochemical pathways for the manufacturing of fine and other chemicals. While in vitro systems are less complex than in vivo system, they are laborious to assemble and typically still beyond our ability for comprehensive analysis, let alone for rational design. At the Bioprocess Laboratory, we are developing methods to facilitate the assembly of complex in vitro systems from cell free extracts based on strategies to insulate the network of interest from a complex background. In addition, we work towards methods for the highly time-resolved analysis of the system dynamics, which in turn allows to parameterization of adequate system-level models with unprecedented exactness. Together, these tools are expected to take us a long step towards the rational design of in vitro biochemical systems.
Lipid-Membrane Biotechnology and Synthetic Biology on the Nanoscale

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The functional nanoscale architectures of cells are made of lipid and proteins interacting in a highly coordinated fashion. We are interested in elucidating mechanisms driving nanoscale membrane rearrangements in-vivo, and also in using this knowledge to create functional artificial biomimetic architectures for synthetic biology applications.

Selected References


Photosystems and electron supply to redox reactions

P. E. Jensen*, K. Jensen, L. M. M. Lassen, A. Zygadlo Nielsen and B. L. Møller

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Photosystem I (PSI) from plants, algae and cyanobacteria utilizes energy harvested from sunlight to mediate light-driven electron transport across the thylakoid membrane and produce reducing equivalents for the metabolic reactions of the photosynthetic organism. Cytochrome P450s constitute a very large and highly versatile superfamily of membrane-bound enzymes that catalyse a wide variety of different reactions, the most frequent being highly stereo- and regiospecific monoxygenations that are often difficult to achieve using the approaches of chemical synthesis. In this project we are aiming at coupling PSI directly to a cytochrome P450 (P450) to develop a system in which the enzymatic reaction of some P450s is driven directly by the energy of solar light. As photosynthetic host organism we use the cyanobacterium *Synechococcus* sp. PCC 7002, which easily can be transformed, is fast growing and is tolerant to high light intensities.

In nature, PSI transports electrons from plastocyanin or cytochrome c6 on the luminal side to ferredoxin (Fd) or flavodoxin (Fld) on the stromal side of the thylakoid membrane. The electrons supplied by PSI may via the soluble electron carriers Fd or Fld be donated to ferredoxin NADP+ oxidoreductase (FNR), which reduces NADP+ to NADPH. *In vivo*, the electrons required for the reactions of the P450s are taken from NADPH through the NADPH-cytochrome P450 oxidoreductase (CPR) enzyme. The consumption of the costly cofactor NADPH constitutes an economical obstacle for biotechnological in vitro applications of P450s. Our recent results from *in vitro* experiments demonstrate that the delivery of electrons to the P450 may not have to involve the production and oxidation of NADPH, but that direct electron transfer from PSI via Fd to a P450 adjacent to the PSI is possible.

A supra-metabolon containing PSI and a cytochrome P450 provides the opportunity for direct utilization of solar energy for production of complex chemical products. In this synthetic enzymatic supercomplex, the energy of the photons harvested by PSI will be utilized to mediate electron transport to the cytochrome P450 enzyme. To facilitate highly efficient electron transfer, the fusion protein complex has been designed to keep the interacting proteins in close proximity to each other. Thus we are aiming at linking the P450 enzyme directly to one of the small subunits of PSI containing one transmembrane domain. Ultimately, the aim is to generate a system in which the fusion protein is stably expressed in the thylakoid membrane of *Synechococcus* and utilized to carry out the desired enzymatic reactions *in vivo*.
Production platforms for high-value bioactive diterpenoids

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Diterpenoids represent with 11% a substantial fraction of the currently ~43,000 terpenoid structures identified in the plant kingdom. While vascular plants universally produce diterpenoid phytohormones as part of the general metabolism, a few plants also accumulate diterpenoids of the specialised metabolism with an amazing structural diversity. Those plant species have been explored in modern and traditional medicine because of pharmacological activities that include anti-cancer, anti-malaria, analgesic, antimicrobial and antifungal, contraceptive as well as psychoactive properties. With very few exceptions, the bioactive diterpenoids are only produced in minute amounts, limiting, together with challenges in cultivation, extraction and purification the production using native plant resources. Furthermore, the high structural complexity makes chemical synthesis difficult. We are using genomics and systems biology approaches to accelerate the discovery and characterization of the genes, enzymes, and biosynthetic pathways. Based on their metabolite profiles and accumulation of specialised diterpenoids, we have selected ten plant species for high-throughput transcriptome sequencing. The deep sequence inventory was mined for terpene synthases and cytochromes P450. Heterologous expression of these new candidate genes and pathway engineering is underway in several different host systems with the goal to build sustainable production systems for high-value diterpenoids in microorganisms and Physcomitrella patens.
DNA nanotechnology for rational design of novel molecular devices

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DNA nanotechnology exploits the unique structural motifs and self-recognition properties of DNA to self-assemble pre-designed nanostructures in a bottom up approach. We have designed, assembled, and characterized a fully addressable 3D DNA box with dimensions of that can be opened in response to external “key” signals including specific RNA sequences or light [1]. Opening of the lid can be monitored optically by fluorescence resonance energy transfer and we show that the lid can act as a sensor for multiple sequence signals, opening up for several interesting applications. E.g. it allows us to restrict the transport of material in or out of the box in a controlled fashion. Importantly, the opening mechanism of the DNA box operates under native conditions and is readily taken up by cells. The DNA box can both sense and act, for example by combining a diagnostic sensor of complex signals with the controlled release of, or access to, a payload. It can also be programmed to act as an AND, OR or NOT gate in logic circuits.

The YoctoReactor® drug discovery technology platform enables the creation and interrogation of DNA-encoded, drug-like, small molecule libraries of millions-billions members in a single tube format. Hits are identified by molecular evolution in rounds of affinity selection, amplification and translation. Diverging from the concept of DNA as a linear template the YoctoReactor® uses the centre of DNA junctions, which constitute a yoctoliter (10e-24 L) volume, as a 3D chemical reactor to create DNA-encoded libraries of small molecules by positioning building blocks for reactions at the centre of the junction. This design is shown to be favorable for chemistry, fidelity and ultimately for library size.
Industrial biotechnology – A ”top down” approach.

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Industrial biotechnology was has been known for more about 100 years as an industry in its own right. Still most of the attributes of modern industrial biotechnology relies on the use of recombinant DNA technology. The first recombinant enzyme product was marketed in 1988. Following that the development of cell factories and of industrial biotechnology products has been developed in a radical way.

The cell factories for production of proteins and chemicals have been developed to be more productive, to produce cleaner and more stable products and to become more versatile. The products have developed from a product range of a few enzyme products, antibiotics and chemicals like citric acid to a very broad range of enzymes modified by protein engineering and chemicals that have started to replace petrochemical based products to a large extend. Fuel ethanol is e.g. already a mature industry in the US and Brazil and China has taken steps to catch up fast. In Europe M&G just initiated construction of the first commercial plant for cellulosic fuel ethanol in Italy. This development will likely change the world economy from oil based to bio based over the next decade. This development can only take place due to the dramatic progress in the field of industrial biotechnology during the past 20 years.

The development of the cell factories has been based on a rational design approach. The starting point has been highly productive natural micro organisms. These organisms have been improved by deleting unwanted genes and up-regulation or addition of desired genes as well as fine tuning of the primary and secondary metabolism. For 20 years this has just been referred to as host organism optimization. With the advent of synthetic biology this has also been termed “top down synthetic biology”. The implications of this to the industry will be discussed.