Synthetic Biology Foresighting

Technologies & Markets
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Executive summary

- Synthetic biology is an emerging and highly promising field, which is attracting considerable interest from academia, industry and the VC community.
- The technology can address market needs in a variety of sectors and so potential applications are diverse and wide-ranging.
- Application areas include bioenergy, chemical synthesis, drug development, biosensor development and tissue engineering. These are of high commercial and societal value.
- Opportunities lie in the utilisation of synthetic biology to meet existing technology and market needs in these application areas.
- The technology is continually advancing. Recent technology developments include:
  - whole genome transfer (Lartigue *et al*., Science 2007)
  - minimal cell projects, SC2.0 and Clean Genome® *E. coli*
  - significant cost reduction in DNA synthesis
- However despite recent advances, enabling technologies still require significant development in order to realise the synthetic biology vision. Opportunities exist in the development of these enabling technologies.

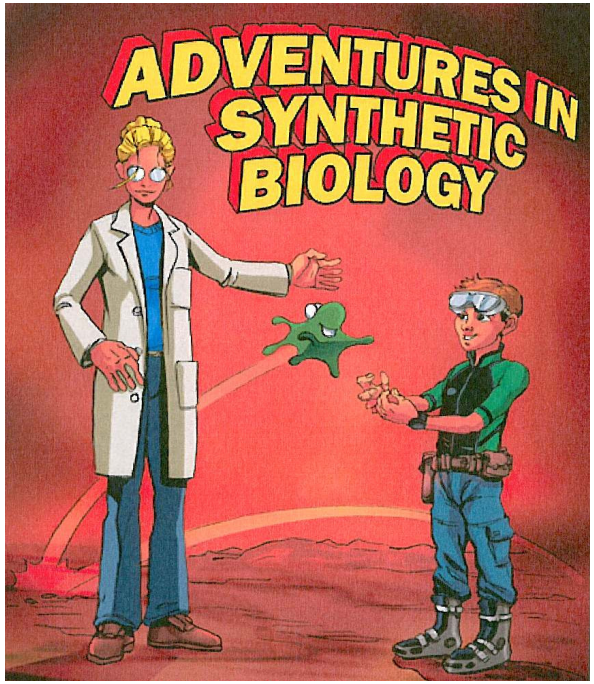
Executive summary 2

- The embryonic nature of the field offers significant opportunities to both new and established players.
- The immaturity of the area is limiting and progress is likely to be hindered by:
  - the lack of quantitative understanding of complex biological systems, which will impact on both the design and ultimate functioning of synthetic biology-based devices
  - development needs of foundational technologies
  - relative lack of established / experienced researchers restricting the rate of expansion of this field
  - unresolved IP issues
- Without sufficient funding to minimise the time taken to put the foundational technologies and characterisation into place, a significant cooling in interest in the subject may occur, perhaps analogous to the 'hype and cooling' cycles seen in Artificial Intelligence and DNA computing.
- Biosafety and ethical concerns have been raised by numerous parties. These must also be considered. Ensuring these issues are addressed at an early stage should minimise risk.

Introduction to Synthetic Biology

Introduction to synthetic biology

Synthetic biology is the application of engineering concepts to the construction of biological systems



Front cover, *Nature* Nov. 24th 2005

Synthetic biology can be defined as

- *the design and construction of new biological parts, devices, and systems and*
- *the re-design of existing, natural biological systems for useful purposes*

http://syntheticbiology.org

It has potential applications in numerous fields including bioenergy, drug development, chemical synthesis and biosensors.

Biology meets engineering

- Synthetic biology is based on the application of systems design to complex biological processes. It is an emerging area with high scientific and technological potential. The field opens up the possibility of manipulating living systems and their component parts in a rational way, akin to the way in which engineers design new machines. Critically, it involves the synthesis of novel biological systems, which are not generally found in nature.

- Synthetic biology aims to design and assemble biological 'systems' (e.g. metabolic pathways, genetic networks) from well defined biological components. This 'hierarchical module-based' approach views sub-cellular biology as a system of interacting modules, DNA elements as functional parts, signalling pathways as functional entities, and cell metabolism as a molecular conversion tool.

- Just as technicians can now assemble standardised, off-the-shelf electronic components to build computers, synthetic biologists foresee a day when engineers will assemble well-characterised biological components into robust biological systems.

- Such constructed systems will have numerous applications and will be required for the validation of systems biology models.

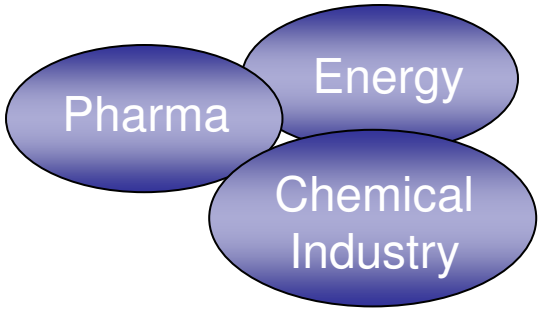
Market need

A wide variety of industrial sectors could benefit from synthetic biology. In order to begin to understand the impact that synthetic biology could have on these markets, it is helpful to examine the technology needs that synthetic biology could address within each sector and then search for commonalities.

There appear to be 2 clearly defined needs that are common to a number of markets and which synthetic biology could address:

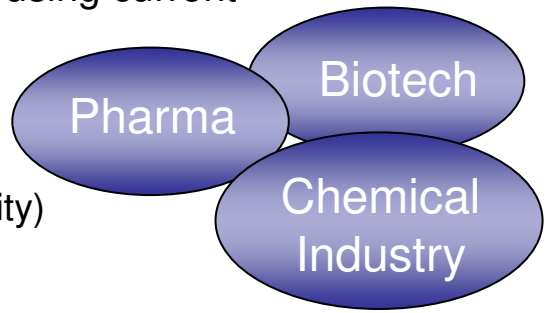
- **The requirement for alternative production routes**

  In order to: reduce costs
  enable scale-up
  utilise an alternative substrate
  increase yield
  remove waste
  produce compounds, which cannot be synthesised using current methodology



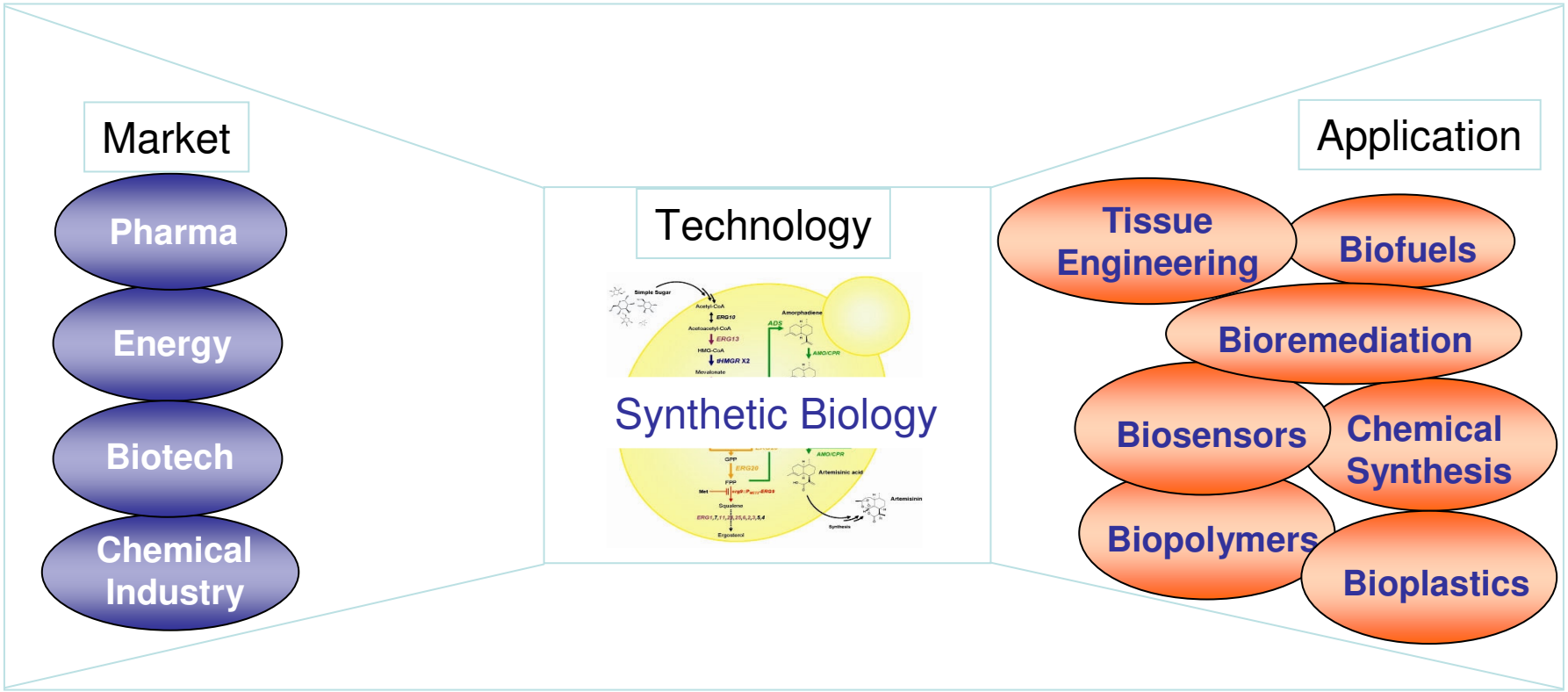
- **The need for <u>novel</u> properties**

  Such as: physical properties (e.g. biodegradation, biocompatibility)
  functionality (e.g. sensing capabilities, novel enzymes)



Synthetic biology meets market needs

The emerging field of synthetic biology could address these common needs and so positively impact several industrial sectors as evidenced by the numerous potential applications.



Given synthetic biology's early stage and the numerous sectors it could influence, it is impossible to assess quantitatively the impact that synthetic biology could have on these markets. Instead, this report provides an insight into synthetic biology's potential in each sector along with the technology advances required to progress this field.

Synthetic biology requirements

Synthetic biology is an emerging technology. For this field to advance significantly, we need to develop:

- Algorithms and software for design, analysis and assembly
- Tools to synthesise very large pieces of DNA
- A library of well characterised, standardised, modular components that enable plug and play and exhibit predictable behaviour
- A 'chassis' organism to act as host for the engineered system
- Vectors and methods to facilitate physical assembly of components

Synthetic biology does offer huge benefits but, as with any critical advancement in science, there are also risks. It is therefore important to address ethical and safety concerns and any potential or perceived risks at an early stage so that future developments can be fostered and established with the support of the general public. As a result, the following are also likely to be key requirements:

- Built-in security features to prevent accidents and abuse and to reassure the public
- Public acceptance of the technology and its application to areas of particular need

These requirements are discussed in detail in the following sections

Enabling Technologies

Technology requirements

Enabling technologies:

- DNA synthesis and genome assembly
- Chassis organism
- Well-characterised modular components
- Regulatory circuits
- Informatics and design concepts

Technologies:
DNA synthesis & genome assembly

From DNA synthesis to genome assembly

The ability to produce and rationally assemble DNA is a key technology requirement

Genome assembly is currently a 4 step process:

1. DNA synthesis: from chemicals (DNA nucleotides) to short DNA fragments (around 1 kb)
2. Combination of short fragments to larger fragments (typically between 20 and 40 kb)
3. Assembly of larger fragments into sub-genomic sets (e.g. a million base pairs)
4. Actual assembly of one genome (and only one) in a suitable cellular context

Technology currently exists that makes each step from DNA design to implementing a novel genome in a cell feasible. However, advances are still required to achieve this goal. An alternative approach to this 4-step process may be the massive editing of existing DNA backbones, 1000bp piece by 1000bp piece.

(kb or kbp: kilo base pairs = 1000 base pairs. A typical bacterial genome has 5 million bp)

DNA synthesis

DNA synthesis is a relatively well established technology. However, cheaper, quicker and possibly lab-based DNA synthesis could energise the field of synthetic biology and have widespread knock-on benefits in commercial and academic research.

Effort across a range of areas may be envisaged:

- Better algorithms for design of overlapping oligonucleotides, which will anneal correctly to generate the backbone of the desired construct.
- Parallel synthesis of oligonucleotides using inkjet microarray printing and microfluidic technologies will greatly reduce reagent volumes and hence cost.
- Improved methods for detection and removal of mismatches.
- Perhaps an entirely new chemistry for DNA synthesis.
- Cheap, quick, desktop synthesis.

Genome assembly

Although DNA synthesis appears to be a relatively well established technology, how to assemble *de novo* produced DNA sequences on a large scale into biological systems largely remains an unsolved problem. There is a clear need for technology advances in this area. Key technology barriers include the following:

- The road from oligonucleotide-based DNA assembly to genome engineering is presently very laborious even though the various elements required are emerging.
- Only the assembly of *de novo* synthesised DNA up to more than 30 kb is presently possible. This needs to be extended.
- Assembly of foreign DNA up to 900 kb/site is possible in *B. subtilis*. If gDNA was isolated from this strain for transplantation, issues remain. For example, how to remove the *Bacillus subtilis* DNA or how to incorporate incompatible genes? The molecular mechanisms of *Deinococcus radiodurans* may help address some of these issues.
- Although genome transplantation from *in vitro* to *Mollicutes* is possible, this method needs to be extended to other bacteria (e.g. with cell wall).

Requirement for automation

- Although DNA synthesis up to 1000bp appears to be a well serviced area, assembly of DNA to larger fragments is currently a key bottleneck. Many of the current approaches are highly repetitive and so amenable to automation.
- Microfluidics approaches provide an excellent opportunity for automation in a feasible scale.
- A number of lab protocols for molecular biology has been miniaturised to chip scale. Despite being vital for the advancement of synthetic biology, activities directed at miniaturisation for synthetic biology are virtually non-existent. The Bioprocess Laboratory at ETHZ plans to initiate projects to address this issue.
- A similar story holds for targeted genome reduction approaches (see 'chassis organisms'); these are highly repetitive manual activities conducted on a large scale.
- In general, once (sub)genome scale is addressed, protocols that were once effective will then have to be automated or even dramatically redesigned. Once we proceed to genomic perspective, the problem of automation will invariably appear.

Technologies:
Chassis Organisms

Chassis organisms

- Chassis organisms are a key technology requirement for synthetic biology. They are needed to act as hosts for the engineered system.

- Ideally, a chassis organism is an organism that has been so comprehensively adapted to a set of specific tasks that it serves as a starting point for numerous design projects. They are limited to the essential parts and so are necessarily reduced in size and adapted.

  This adaptation includes:

  - the elimination of all superfluous or redundant information, which may later complicate design and

  - the restructuring of remaining vital information according to the needs of orthogonality

Chassis organisms

Conventionally *E. coli* is used due to its relatively high degree of characterisation and the many specialised vectors and strains available. However, there are a number of disadvantages associated with employing *E. coli* as a chassis. For example:

- *E. coli* is Gram negative and has an outer membrane composed of potentially toxic lipopolysaccharide.
- *E. coli* lacks the main terminal branch of the General Secretory Pathway and so does not secrete most proteins effectively.
- *E. coli* does not form stable resting states so long-term storage requires freeze-drying or cryogenic freezing.
- Laboratory strains of *E. coli* are debilitated and non-pathogenic. However, other strains are pathogenic and so it may be easy to generate, accidentally or deliberately, pathogenic constructs in this host.

It may be advantageous to generate new chassis organisms for specific purposes and characterise them sufficiently so that they can compete with *E. coli* and become new standards. For example:

- Several attempts have been made to work with genomes that are by default small and could serve as the 'minimal chassis'. These include *Mycoplasma genitalium*, which is used by the Venter Research Institute, and *Mesoplasma florum*, which is used by the Knight group at MIT.
- *Bacillus subtilis* and *Lactobacillus* spp may also prove useful alternatives. These bacteria have the advantage of being non-pathogenic, Gram positive and efficient protein secretors.

Creating minimal genomic systems

There have been a number of approaches taken to reduce the genome of model bacteria:

- *E. coli* has been reduced by statistical processes by more than 30% and by directed, planned efforts by more than 15%; in the latter case eliminating recombinogenic sequences (transposons, IS elements) or potential virulence genes without experiencing negative effects.
- *B. subtilis* has been reduced systematically by 8%, again without major negative effects.

Adaptation of the remaining genome can then be achieved by editing or *de novo* synthesis.

The above approaches are most likely to succeed in the short run. However, it can be envisaged that in the future a wholly synthetic chassis may be constructed from the 'bottom up'.

Alternative minimal systems

- An alternative approach to minimal systems is the utilisation of *in vitro* systems that try to exploit only parts of the cell. For example, the protein translation apparatus for cell free protein synthesis or the enzyme complement to carry out multi-enzyme synthesis.

- These systems have the advantage of being able to be controlled to a much higher degree than *in vivo* systems. However, they are frequently less robust as they no longer use the cell's machinery to produce novel proteins.

- *In vitro* protein production has been a major topic in biotechnology over the last decade and could significantly benefit from synthetic biology approaches.

- Multi-enzyme catalysis could play a major role in sustainable chemistry (see application section). Enzymes tend to operate under similar conditions (in contrast to chemical catalysts), which makes assembly of multi-step reactions in one vessel relatively easy. This could significantly help to reduce the environmental footprint in fine chemistry. This approach could be particularly helpful for hydrophilic compounds that would otherwise have difficulty in crossing the cytoplasmic membrane e.g. biosynthesis of artificial sugars for pharmaceutical applications.

Chassis organisms: limitations

- The primary limitation of the chassis approach is that it rests on the assumption that the resulting redesigned minimal strain will still be active enough to be useful. This appears to be a reasonable assumption given the existence of relatively fast growing strains with very small genomes. However, these strains have evolved in parasitic environments and it is unclear whether bacteria such as *E. coli* will respond well to major rearrangements. After all, there are still a couple of hundred genes without assigned function even in the *E. coli* genome.
- An additional limitation is that chassis strain production is presently highly labour intensive and so will require either heroic efforts of single labs, the involvement of huge consortia or a significant push in laboratory automation of chassis strain production.
- It is likely that the road to chassis strains will not be straightforward. There will be many unexpected problems that will require re-evaluation of the underlying strategy. As a result, it will be difficult to manage such a project within a consortium. A successful chassis project is likely to require the intimate cooperation with a suitable functional genomics platform that can track the behaviour of the gradually emerging chassis strain on transcriptomic and proteomic levels and, if the final purpose is chemical production, on a metabolomic level.

Technologies:
Modular Components

Modular DNA components

- A library of well characterised, standardised, modular DNA components that enable plug and play and exhibit predictable behaviour will be a key enabling technology in this field.
- 'BioBricks' ™ have become a standard format and are supported by the Registry of Standard Biological Parts maintained by Massachusetts Institute of Technology.
- There are many advantages to be gained from working within or extending this standard rather than generating competing standards.
- BioBricks have standard ends, so that any two BioBricks can be combined in either order to generate a new BioBrick, which may be further combined with other BioBricks. Thus large and complex systems can be built up from a library of standard components.

http://parts.mit.edu/registry/

The assembly to the left shows 3 BioBricks assembled to create a simple device.

BioBrick tools

If BioBricks are to be widely adopted and, until DNA synthesis becomes considerably cheaper, it would be useful to develop a new generation of vectors for generating and assembling BioBricks.

For example:

- Vectors to allow direct cloning of blunt-ended PCR products automatically incorporating BioBrick prefixes and suffixes. This would remove the necessity for incorporating long non-complementary tails into primers, and remove a step in the construction process.
- Vectors with visually detectable (e.g. chromogenic) tags to facilitate assembly of BioBricks into larger systems.
- Broad host range vectors to allow use of BioBrick constructs in chassis (hosts) other than *E. coli*.

BioBrick tools

Informational tools will also be required. These include:

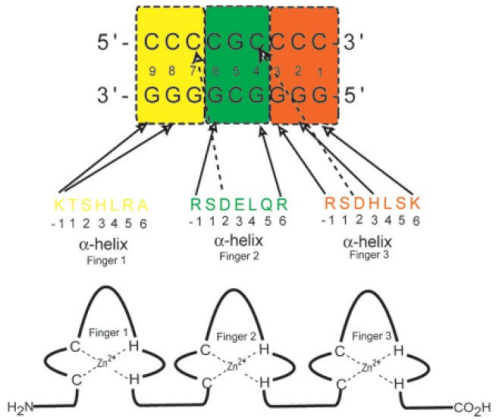
- Improved standard methods for characterising standard classes of BioBrick parts (promoters, ribosome binding sites, terminators) and incorporating quantitative data into databases.
  - Functional characterisation of parts giving dynamic performance measures with environmental context
  - Single cell kinetics, rather than population, stochasticity measures
  - Parameters of transcriptional, translational and post-translational control and the ability to measure them robustly
- Algorithms and software for utilising these databases to model BioBrick-based systems and simulate behaviour accurately prior to assembly.
- Methods to feed back results from laboratory tests of these systems into databases to improve future design projects.

Modular protein assembly

- The availability of modular protein domains would be a large step forward in the reliable design of complex biological systems. A protein with novel functionalities could be rapidly designed by combining the DNA elements coding for the domains and producing the novel protein.
- Essentially, the question is whether the success of the DNA level BioBricks strategy can be reproduced at the protein level. For which systems is it possible to mix protein domains in a way similar to how we combine different promoters with different ribosome binding sites and different genes? In other words, DNA fragments encoding protein domains would become novel BioBricks.
- Modular protein assembly would circumvent the need to evolve novel functionalities in demanding experimental settings. Instead, one would simply reassemble autonomous protein domains. Such an approach would prevent lengthy screening procedures at the beginning and would eliminate the need to design reliable and high-throughput compatible screening assays. Even if evolutionary approaches would be required later to improve certain aspects, the overall development time should be considerably reduced.
- Currently, the prime example for modular protein assembly is the zinc finger protein (ZFP) domain.
- In addition to ZFPs, modularity in protein domains is widespread in regulatory systems, in particular in eukaryotic systems. While this has been known for some time, it has never been as thoroughly investigated for its consequences for design as ZFPs. There are a number of autonomous domains around that can be easily and reliably coupled with other proteins.

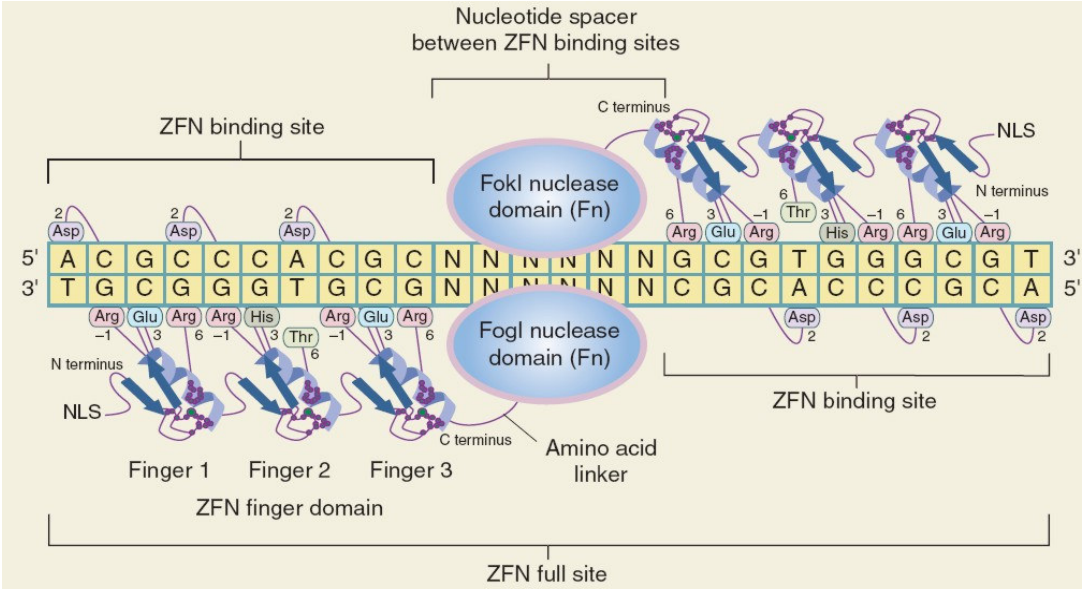
Zinc finger proteins

- Zinc finger proteins are used for selective DNA binding
- One ZFP recognises one specific DNA triplet. It is possible to generate ZFPs for essentially any DNA triplet. By combining ZFPs linearly, the DNA recognition sequence of the engineered proteins can be extended up to 24, which is sufficient to address unique DNA sequences in the human genome.



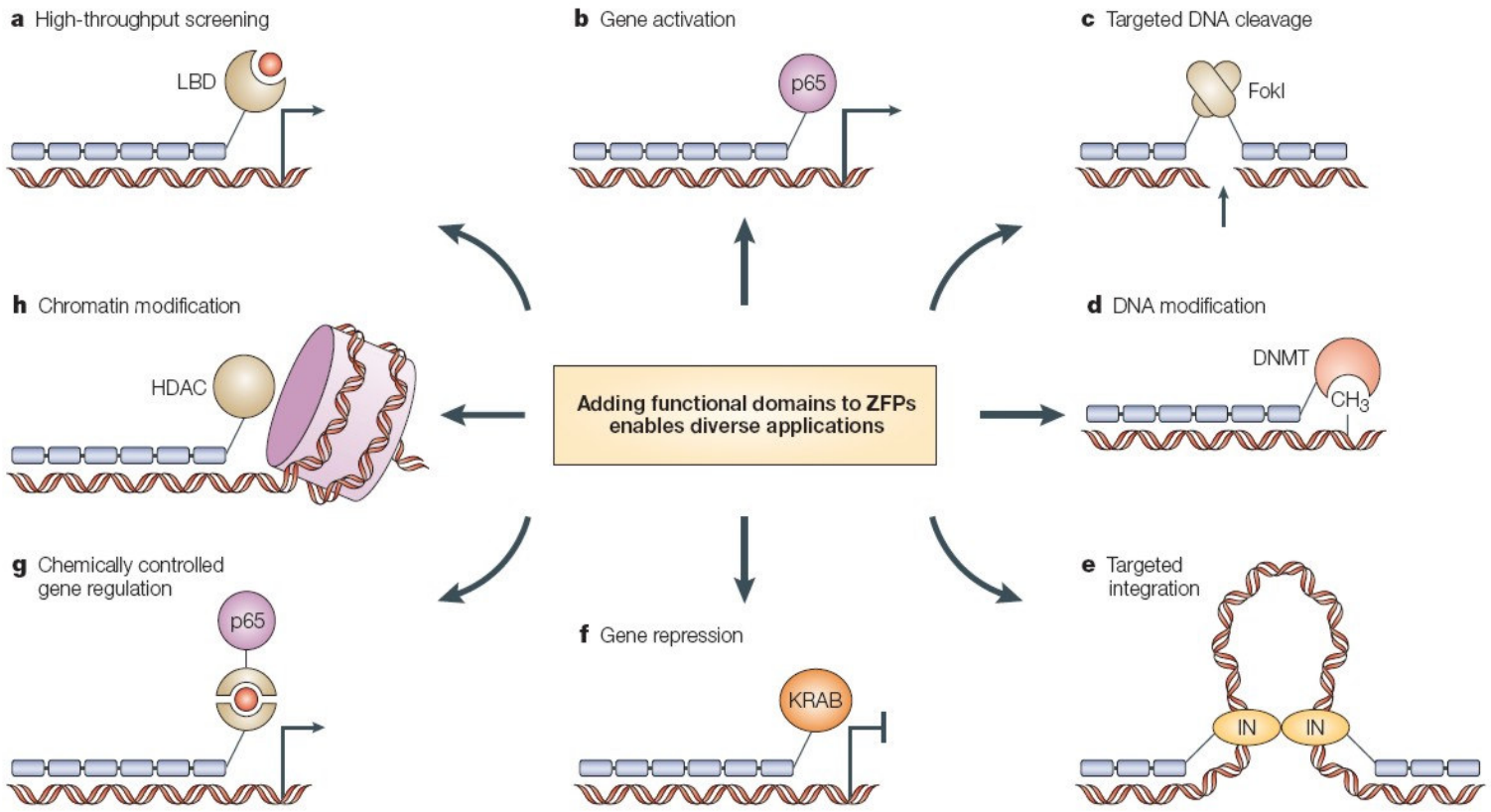
Example:

Two ZFPs, each containing three ZFPs and therefore specifically recognising a DNA sequence of 9 bp, localise a nuclease (FokI) to a target.



Zinc finger proteins

Zinc finger proteins can direct other functional protein domains to the DNA site of interest for a variety of applications.

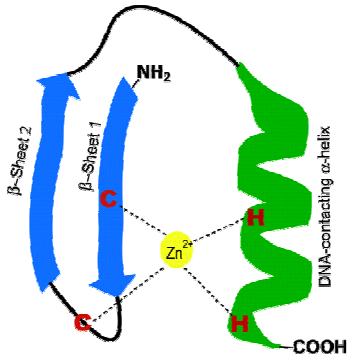


LBD: ligand-binding domain; p65: protein domain for activating transcription in eukaryotes; FokI: restriction endonuclease; DNMT: DNA methyltransferase; IN: retroviral integrase; KRAB: protein domain for repression of transcriptions in eukaryotes; p65 recruiting depends on presence of small molecule; HDAC: histone deacetylase

ZFP potential and limitations

- ZFPs have much potential. For example, their ability to address a specific DNA sequence selectively will be of prime importance in gene therapy. They have also other applications in drug development and production.

- However, there are a number of recognised limitations:
    1. The coverage of possible codons is not comprehensive
    2. Target side overlap leads to reduced specificities for selected sequences
    3. The assembly is in fact modular. Proteins with 3 ZFPs (recognizing 9 bp) can be assembled to rather specific binders. Proteins with 6 ZFPs (18bp, theoretically sufficient to address one single site in the human genome) are less specific than expected and are likely to still recognise several dozen sites in the genome
    4. Successful binding depends on the state of the DNA (only accessible chromatin is a useful target).



- In general, affinity seems to be high, but specificity can be a problem. This should be addressed by providing alternative ZFPs next to those available already (e.g. by phage display to obtain novel scaffolds and then rational design to improve the scaffold). It will be particularly important to identify the reasons for specificity loss in larger ZFPs and then translate this into design instructions to improve specificity.

Technologies:
Regulatory Circuits

Regulatory circuits

- Regulatory circuits refer to the design of artificial systems to control gene expression. Different regulatory behaviour can be 'programmed', depending on the system that is designed. For example, the bacterium can be designed to make its reaction to a particular stimulus conditional or to turn gene expression on and off regularly ('oscillator').

- As gene expression is the cornerstone of nearly any activity of a cell, rationally controlling the patterns of gene expression means controlling the behaviour of the cell.

- Many of the early experiments examining synthetic gene regulation were designed similarly to those experiments in electrical engineering. For example, the bacteria were designed to express genes if (and only if) two signals were present and thus were similar in action to an electronic AND-gate – hence the name regulatory 'circuits'.

- At the moment, this is mainly academic work. However, the implications are very clear. If complex artificial behaviour can be reliably and robustly programmed, then this will be extremely useful in a variety of settings, for example in gene therapy settings (in mammalian cells).

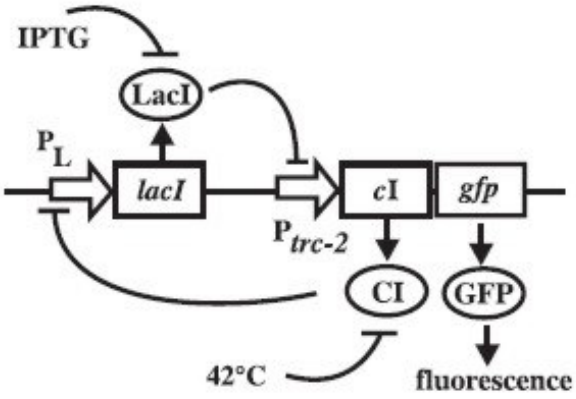
- This technology is absolutely crucial for designing complex systems. Any system that wants to do more than simply respond to one environmental stimulus needs to resort to complex regulatory strategies. Complex regulatory circuits will therefore be needed for any synthetic biology project where over 100kb of sequence is assembled.

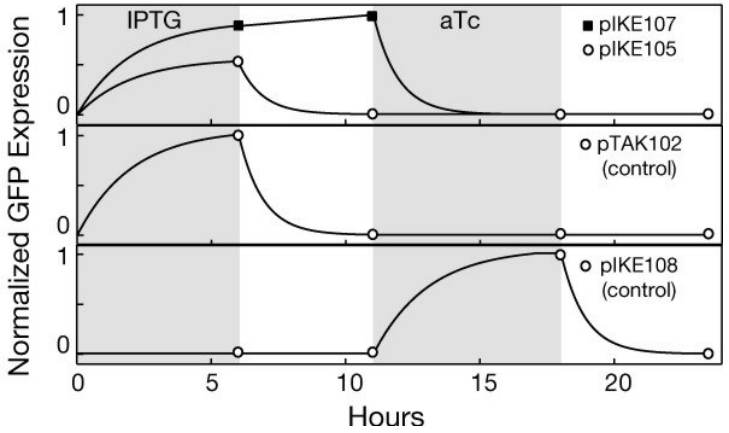
- This makes regulatory circuits a foundational technology for advanced synthetic biology.

Regulatory circuits

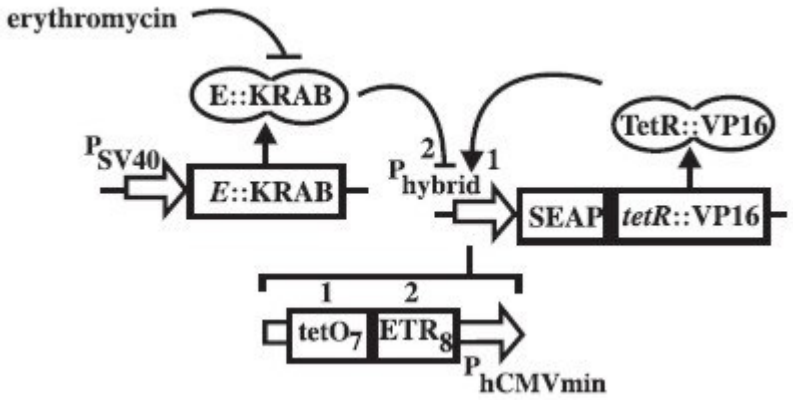
- The design of gene regulatory systems is the area in which the design component of synthetic biology is the most obvious and most advanced.
- The specificity of DNA/protein interactions and the limited burden that regulatory proteins impose on cellular systems ensure a relatively high level of orthogonality and allow a relatively high level of forward engineering.
- It is also the area where some of the limitations become most obvious, in particular the lack of experimental data required for design, the difficulty in accessing available data from the literature, and the lack of comparability between different experimental approaches.
- In the long term, the scarcity of design related data is a fundamental bottleneck that needs to be addressed. This should be addressed in several ways:
  - Data: this needs to be assembled in standardised experiments in standardised form by qualified personnel (basically a clearing station is required before the data can be used by all).
  - Technology: in many cases we do not have the means to assess these data with a meaningful measure; we need to develop the required technology (e.g.: how to determine 'promoter strength' by measuring the crossing of the RNA polymerase across the point of commitment).
- Nevertheless, some genetic circuits could already be implemented.

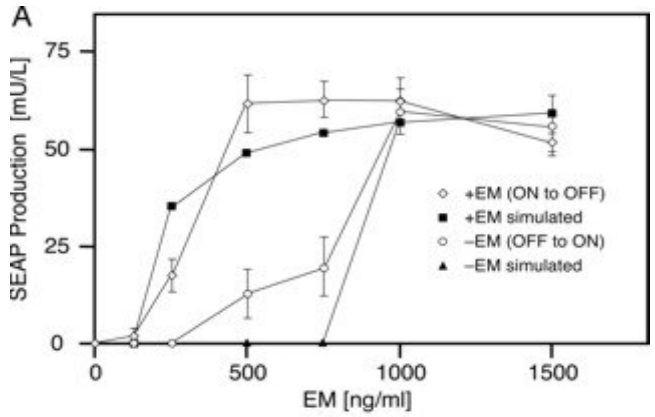
Regulatory circuits: examples





A toggle switch. Left: construction details. Right: properties. The circuit allows the cell to memorise signals it has received in the past, even if the signal is no longer present.





Hysteretic behaviour. Left: construction details. Note that the circuit has been realised in a mammalian system (CHO cells). Right: properties. When inducer (erythromycin, EM) is increased, a high concentration is required for turning the system on. When the concentration is reduced, a lower concentration is required to turn it off.

iti Life Sciences real possibilities

Technologies:
Informatics and Design Concepts

Informatics

The informatics goal: a ‘CAD/CAM’ (computer-aided design/computer aided manufacturing) or ‘IDE’ (integrated developer environment) system for synthetic biology.

- *In silico* simulation and automated construction of biological systems.
  - Database of information about available parts.
    - Issues surrounding ‘Digital curation’ for BioBricks
  - Simulation of developmental constraints
    - Predict & constrain evolution away from designed behaviour
  - Simulation of environmental constraints
    - Predict modification of designed behaviour under external influences
- A universal IDE to facilitate collaborative design across multiple centres.
- Feed information to an automated DNA synthesis/assembly system to generate the final construct.

Design concepts

- When introducing new metabolic networks, it may be advantageous to design them such that interactions with the existing components of the cell are removed i.e. the novel device and the chassis are decoupled.

- This may also aid in containment by preventing the genes from being passed to other organisms. For example, mRNAs that have been designed for dedicated ribosome pools cannot be read by other bacteria.

- There are two leading paradigms to achieve this aim: orthogonality and insulation

- Orthogonality

  The concept of orthogonality relies on the absence of interactions (other than those intended) between modules in the cellular machinery. For example, introduction of a non-canonical codon assignment to implement unnatural amino acids into proteins and adapting the entire genome of a chassis organism accordingly so that the old meaning of the codon is no longer present.

- Insulation

  Insulation coupled systems may be decoupled by physical partition. For example: use of co-cultures of cells with communication through quorum sensing.

Applications

Application areas

- One advantage of synthetic biology over 'traditional' genetic engineering is that systems can be constructed for the transfer of multi-gene traits to new hosts.
- For example, new metabolic pathways can be constructed or useful metabolic pathways from 'inconvenient' organisms can be transferred to hosts more amenable to large scale cultivation.
- As a result, synthetic biology has numerous applications. These include bioenergy, drug development and production, chemical synthesis, and bioremediation. Synthetic biology is also likely to have applications in tissue engineering and biosensor development.
- These applications are discussed in detail in the following section.

Applications: Bioenergy

Biofuel production 1: ethanol and butanol

- Many biologically produced substances can be used as fuels to replace non-renewable oil-based resources.*
- Most effort has been directed towards ethanol, which is produced in high yields by various yeasts and bacteria that can be easily grown on a large scale.
- The ability to produce ethanol can easily be transferred to new organisms, requiring only two genes (pyruvate decarboxylase and alcohol dehydrogenase).
- Ethanol has several disadvantages: it has a high affinity for water and cannot be effectively transported by pipelines thus necessitating the use of tankers, and it requires distillation for recovery, which requires a high energy input.
- Butanol is an alcohol with lower water affinity than ethanol. It can be produced by the bacterium *Clostridium acetobutylicum* (the ABE fermentation) together with acetone, another potential liquid fuel or chemical feedstock. The relevant genes could be transferred to a superior host to increase yields and reduce costs.

* ITI Life Sciences Liquid Biofuels Foresighting Report 2006

Biofuel production 2: hydrogen

- Much effort is currently directed towards the development of a 'hydrogen economy', since hydrogen burns cleanly, producing only water.
- Due to hydrogen's low molecular weight and extreme volatility, it is difficult to store and distribute but much research into this problem is underway.
- Hydrogen can be produced biologically in photosynthetic organisms by the action of hydrogenase enzymes. Hydrogen-producing algal bioreactors are under development.
- Engineering of photosynthetic organisms might greatly increase yields of hydrogen per unit area and time.
- Fermentative bacteria can produce hydrogen through the action of formic hydrogen lyase. Yields are not high but this is under investigation as a method of producing hydrogen from agricultural wastes.

Biofuel production 3: hydrocarbons

- Arguably, the ideal product would be liquid hydrocarbons, which could be processed by the existing oil-processing infrastructure to generate renewable fuels that could act as drop-in replacements for current fuels.

- Biodiesel is generally made by esterification of fatty acids from plant oils but can also be produced microbially via the fatty acid biosynthesis pathway.

- Plants produce long-chain hydrocarbons as cuticle waxes but the biochemistry of this process is poorly understood. If production could be increased and directed towards some internal compartment, plants might be used to generate hydrocarbons, which could be 'cracked' to generate liquid fractions.

- Some algae, such as *Botryococcus braunii*, produce large amounts of liquid hydrocarbons but are expensive to grow on a large scale. The biochemistry and genetics are poorly understood. Further research, such as a genome sequencing project on this organism, could provide information required to transfer the hydrocarbon production trait to more useful organisms.

Biomass conversion

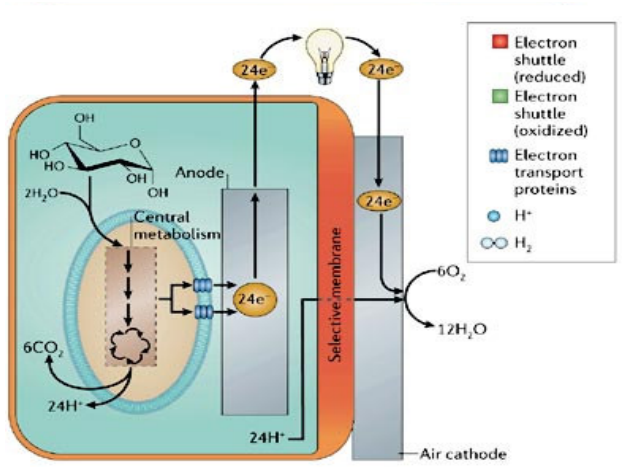
- Cellulose is a hugely abundant and renewable resource and ultimately should replace crude oil as a source for organic materials.
- Cellulose is difficult for microorganisms to degrade, due to its partially crystalline structure and the presence of a lignin matrix in woody materials.
- Some bacteria and fungi are capable of efficient cellulose degradation but do not produce any useful products and are generally not amenable to genetic manipulation.
- Degradation of cellulose and lignin requires the concerted action of multiple enzymes of different classes.
- Attempts to transfer cellulose-degrading ability into industrially useful organisms by transfer of one or a few genes have not produced useful results.
- If cellulose is to become a useful feedstock, a major effort will be required to develop a system encoding a suite of synergistically active enzymes, which can be transferred to a new host.

Biofuel production: the ideal system

- To provide a genuinely renewable large scale source of fuel, the system must assimilate atmospheric carbon dioxide either directly (by photosynthesis) or indirectly (by effective degradation of cellulose).
- The system must produce the desired product with high yield, channeling a large proportion of its carbon or energy flux towards the appropriate pathways.
- The system must not be poisoned by its own products; thus, if liquid fuels are to be produced, the organisms must be able to tolerate high levels of these. Solvent tolerance is a complex multi-gene trait.
- The organism used must be suitable for cheap large scale growth.
- Natural organisms do not fulfill these criteria. Synthetic biology can combine all of these attributes into one organism, or a few interdependent organisms, to generate the ideal biofuel production system.

Biological production of electricity

- In microbial fuel cells, electrons from microbial respiration are passed to electrodes generating an electric current.
- Fuel cells can be operated using agricultural or food processing waste.
- Certain bacteria known as ‘electricigens’ are particularly efficient at transferring electrons to electrodes, possibly via electrically conductive appendages.
- Synthetic biology could be used to combine this trait with other useful traits, such as the ability to derive electrons from cellulosic material.



Glucose serves as an example fuel. The oxidation of glucose to carbon dioxide with direct electron transfer to the electrode surface. Glucose is taken into the cell and oxidised to carbon dioxide by typical central metabolic pathways, such as the tricarboxylic acid (TCA) cycle. Electrons derived from glucose oxidation are transferred across the inner membrane, periplasm, and outer membrane through electron transport proteins, such as c-type cytochromes. In this example, the system is illustrated with an air cathode rather than a cathode submerged in water.

Lovley. Nature Reviews Microbiology 2006; 4: 497

Applications:
Drug Discovery & Development

Drugs: screening formats in drug development

Problem: to design specific and robust drug screening assays rapidly

Synthetic biology answer: utilise modular proteins, such as ZFP, to facilitate the rapid design of high throughput screening tools.

Zinc Finger Protein Examples:

1) Target validation for drug screening. For example, activating protein domains (p65) can be attached to appropriate ZFPs and localised to the gene in question, whose expression is turned up. Where required, attachment to KRAB can also turn competing activities off.

2) Fusion to regulatory domains of proteins especially where assaying is difficult (multiple receptors target the same DNA response element), coupling different regulatory domains to different ZFPs and activating different reporters facilitates screening and allows the screening of several effects at once.

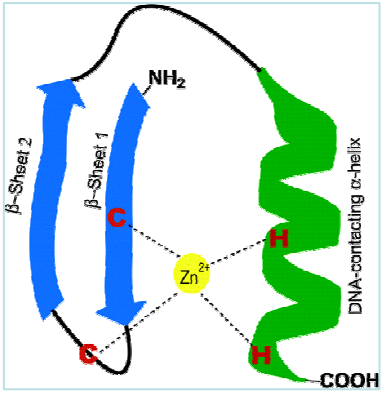
3) Fusion to functional domains of DNA-modifying proteins and localisation to suitable reporter via ZFP domains allows the monitoring of corresponding activities.

4) Realisation of two-hybrid-type screens, where the ZFP coupled to a binding domain represents one half, and another element coupled to activating p65 the other.

Drugs: drug target development

- Zinc Finger Proteins

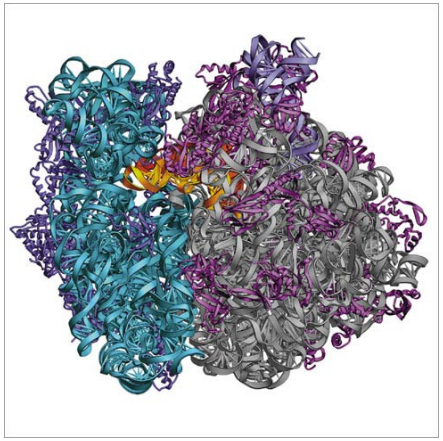
  Libraries of ZFPs can be used to perturb the cell and score phenotypes. The specificity of the DNA-binding domain allows target identification.



- Orthogonal systems

  The availability of orthogonal elements allows the detailed evaluation of cellular structures, the investigation of which would otherwise be difficult.

  For example: orthogonal ribosomes
  By providing a population of orthogonal ribosomes with an assay limited to this population, it is possible to study in detail the effect of mutations in the orthogonal population, which in turn should aid in the definition of novel drug targets.



Drugs: therapeutic strategies

Zinc Finger Proteins

- Genome editing i.e. exchanging a (disease-causing) gene by homologous recombination with a modified (curing) version initiated by localising ZFP-nuclease fusions to the gene in question.
- Gene regulation, by mediating changes in DNA-methylation or histone acetylation state, activating or repressing specific genes, suitable as an alternative to RNAi.
- ZFPs in general: though they have a high affinity for their actual targets, they also frequently have considerable affinities for other sequences as well, leading to off-target effects; in the worst case cytotoxic effects.

Modular proteins

- For example, polyketides (see next section).

Regulatory circuits

- Oscillators as triggers for cyclic treatments (insulin production in diabetes).
- Regulation of gene activity as a function of anecdotal exposure to small molecules (toggle switch triggered by antibiotics).

Drugs: drug production

- ZFPs:
  Triggering/turning off of specific functions (e.g. a monoclonal antibody of interest) by ZFP-coupled activated domains.

- Expanded genetic systems:
  1. Unnatural amino acids enable the external glycosylation of proteins chemically. This allows a) for simpler production systems (e.g. bacteria) and b) for more homogeneous glycosylation.
  2. Unnatural amino acids allow large scale manufacturing of proteins with unnatural amino acids, in bacteria as well as in mammalian cells.
  3. Additional chemical functionalities allow effective post-translational modifications, such as PEGylation to increase serum-half lifes of biopharmaceuticals

- Engineered bacteria for small molecule drug production:
  1. Reproduction of natural product pathways to produce low cost production systems
  2. Production of novel drug molecules

Lowered production costs: artemisinin

- Malaria disease control is hampered by the occurrence of multi-drug-resistant strains of the malaria parasite *Plasmodium falciparum.* Artemisinin, a sesquiterpene lactone endoperoxide extracted from sweet wormwood, is highly effective against multi-drug-resistant *Plasmodium* spp. but is in short supply and unaffordable to most malaria sufferers.

- Although total synthesis of artemisinin is difficult and costly, the semi-synthesis of artemisinin or any derivative from microbially sourced artemisinic acid, its immediate precursor, could be a cost-effective, environmentally friendly, high-quality and reliable source of artemisinin.

- Ro *et al*., described the engineering of *Saccharomyces cerevisiae* in order to produce high titres of artemisinic acid using an engineered mevalonate pathway, amorphadiene synthase, and a novel cytochrome P450 monooxygenase (*CYP71AV1*) from *A. annua* that performs a three-step oxidation of amorpha-4,11-diene to artemisinic acid.

- The synthesised artemisinic acid is transported out and retained on the outside of the engineered yeast, meaning that a simple and inexpensive purification process can be used to obtain the desired product.

- Yield optimisation and industrial scale-up will be required to raise artemisinic acid production to a level high enough to reduce artemisinin combination therapies to significantly below their current prices.

- This project is a collaboration between OneWorld Health, University of California, Berkeley, and Amyris Biotechnologies and is funded by Bill and Melinda Gates Foundation ($42.6M, 5 year grant).

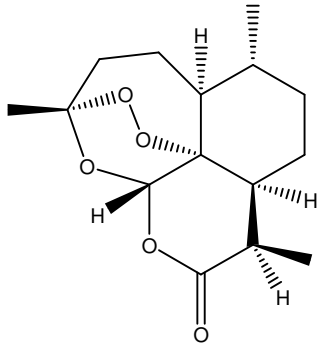
Excerpt from Ro *et al*., Nature 2006; 440: 940

Microbiological drug production

- The artemisinin project has an obvious synthetic biology component as the pathway has been assembled by *de novo* DNA synthesis. However, beyond this, it does not address many other aspects of synthetic biology such as orthogonality or re-utilisable parts.

- On this level, synthetic biology will most probably mushroom in the immediate future. However, apart from reducing the turnaround time between strain generations (which will be dramatically reduced), the long-term impact on synthetic biology will be limited as the efforts remain one-off activities adding something (even though considerably more than a few years ago) to a strain that remains as complex as before.

- In contrast, a valuable strategy for synthetic biology could be to rebuild the central pathways of secondary metabolism in order to deliver chassis strains to central intermediates of selected secondary metabolite product trees.
  For example: chassis strains to isopentylpyrophosphate, to geranylpyrophosphate, or to squalen for terpene/ioprenoid derivatives (such as artemisinin). Similar strategies could also be employed for the different alkaloid classes, eicosanoids, and to a certain extent for polyketides.



Artemisinin

Novel drug molecules: polyketides

In addition to its potential to lower manufacturing costs as evidenced by artemisinin, synthetic biology may also be employed to create novel drug molecules such as synthetic polyketides

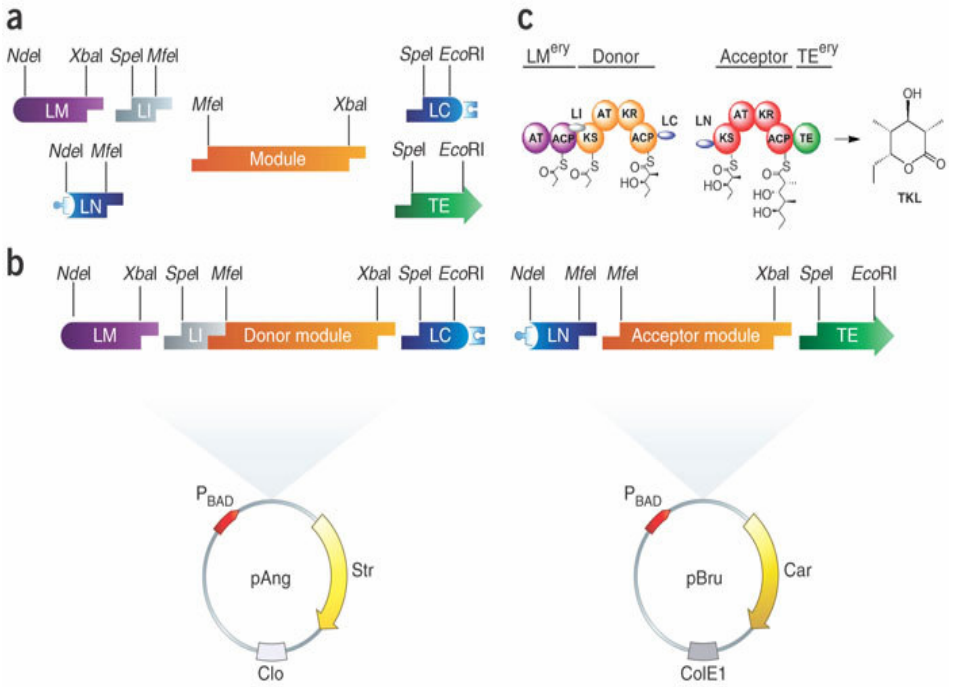
Synthetic Polyketides

- Polyketides are complex natural products produced by microorganisms in the soil. They constitute the biggest source of natural product based therapeutics and have proven therapeutic potential as antibiotics (erythromycin), immunosuppressants (rapamycin) and anticancer agents (doxorubicin).
- Pharma attach significant value to the polyketide compound libraries. Although microorganisms naturally generate polyketides with a variety of characteristics, this diversity could be extended further with the creation of synthetic polyketides resulting in considerable extension to existing polyketide compound libraries.
- Polyketides are produced in highly modular assembly lines of 'mega'-synthases. The DNA responsible for each block can be synthesised *de novo*, and these blocks of DNA can then be combined in many different ways to create different synthesis pathways and novel molecules.
- As novel molecules, polyketides are another excellent example of the potential of modular proteins in synthetic biology.

Novel drug molecules from modular proteins

Consequent re-design and *de novo* DNA synthesis of coding regions has allowed a very precise re-shuffling of protein modules in the mega-synthases and facile expression in *E. coli*.

In fact, the approach has lead to the identification of many novel polyketide molecules. However, the biological activity of these molecules remains unclear.



While the exploitation of the modularity has been a long-standing goal of recombinant biotechnology, it has only been met with limited success over the years. Those polyketides used as drugs are essentially natural polyketides. Kosan Biosciences, who have followed this approach over a long period of time, apparently no longer look for novel engineered polyketides but focus on the effective production (and marketing) of proven polyketides.

Production of biologics

- Therapeutic proteins are mainly produced using mammalian cell culture, the bacterium *E. coli*, or several different species of yeast. These all require aseptic growth in bioreactors.
- In recent years, considerable research has gone into production of therapeutic proteins and vaccines in transgenic animals and plants. Plant based systems have several advantages: they can be scaled up cheaply, can produce proteins to high levels, and are guaranteed to be free of animal viruses and prions.
- To enhance containment, post-harvest systems can be used in which non-transgenic plant material is infected by a recombinant virus encoding the desired protein. This could be improved by engineering of the plant host to reduce protease expression, prevent gene silencing, and ensure high expression from the viral promoter.
- Plant-virus systems could be co-engineered so that the pre-harvest plant does not contain any biologically active protein, and the virus cannot affect wild type plants. This 'insulation' would ensure containment and reduce public concern.
- Ultimately, synthetic biology could open a new avenue for biologics production through the design and 'bottom up' construction of a bio-processing system. Such a system would provide a well defined and controlled approach to therapeutic protein and vaccine production offering significant reductions in product development times and cost.

Drugs: summary

- Although ZFPs are well established, improvements are still required. In particular, specificity needs to be improved when ZFPs are directed to longer DNA sequences in order to reduce side effects.
- ZFPs can also be used to direct functional proteins to selected DNA sequences. Some of these biopharmaceuticals are in clinical trials (Sangamo).
- Application of ZFPs in drug screens and biopharmaceuticals production has yet to be explored fully .
- Manufacturing of complex drugs by redesigning whole pathways in, for example, bacteria is the most advanced of the various areas because the field benefits from vast prior experience. Here, developing new processes from scratch (like those that produced artemisinin) should be realisable in a relatively short time-frame. Expansion into other core secondary metabolite trees (chassis organisms) appears a promising track.
- Manufacture of novel drug molecules such as synthetic polyketides shows promise but has yet to deliver.

Applications:
Chemical Industry

Chemical industry

Synthetic biology has numerous potential applications within the chemical industry, offering significant cost reduction compared to traditional chemical synthesis as well as the promise of delivering new materials with novel functionalities.

This section will highlight the role of synthetic biology in two areas within the chemical industry:

1) Chemical synthesis

2) Polymers & Plastics

Chemical synthesis

- In conventional chemical synthesis, compounds are produced through a complex series of steps requiring significant input of energy and raw materials.
- Industrial biotechnology offers an approach that can simplify this process and cost-effectively replace the multi-step processes of traditional chemistry.

Fermentation

- Industrial biotechnology employs bacteria, yeasts and fungi for the fermentation of chemical products from raw materials such as sugars and oils.
- Crude antibiotics, for example, are produced using fermentation technology as they cannot be easily synthesised due to their structural complexity. Fermentation may also be a more cost-effective approach compared to conventional chemical synthesis. Conventional synthesis of vitamin B_2, for example, is currently a combined chemical-biotechnological route consisting of 8 steps. This complex route has recently been replaced by a simple fermentation step with production costs reduced by 40%.*
- Synthetic biology could significantly advance this area as evidenced by the semi-synthetic fermentation process employed in the production of artemisinin.

DuPont Tate & Lyle BioProducts

Tate & Lyle and DuPont's $100M joint venture has lead to the production of 1,3-propanediol derived from corn sugar fermentation process, the first to produce 1,3-propanediol from renewable resources.

Production consumes 40% less energy compared to petroleum-based propanediol and reduces greenhouse gas emissions by 20%. Performance benefits are also observed, which make it particularly well suited for cosmetics, liquid detergents and industrial applications such as anti-freeze.

*Bio-era. Genome Synthesis and Design Futures: *Implications for the US Economy.*
Royal Belgian Academy of Applied Science. *Industrial biotechnology & sustainable chemistry*

iti Life Sciences real possibilities

Chemical synthesis

Biocatalysis

- A large number of chemicals remain difficult to produce by fermentation as they cannot cross the membrane, are highly toxic, have a low yield and / or are co-synthesised with many by-products. These compounds may be produced by biocatalysis, where enzymes are used to catalyse the production of chemical entities from organic stocks.*
- Biotransformations have become an indispensable tool catalysing chemoselectively, regioselectively and enantioselectively challenging reactions but are typically used as one step in a series of chemical transformations.* There are a number of technology barriers that have limited the adoption of industrial multistep enzyme reactions:

  1) Assembly of a system of enzymes is economically challenging
  2) Due to the interactions of substrates, intermediates or products with the enzymes, the dynamic behaviour of the system is complex, not well understood and typically orientated towards cellular homeostasis rather than towards unnatural requirements of process productivity.*

- Several highly engineered strains have become available that over-express several recombinant genes during growth; these can be permeabilised or homogenised and used for multi-enzyme catalysis. However, the entire cell background is still present and so can potentially impact on reaction yield.*
- Synthetic biology has the potential to significantly advance this area through the assembly of multiple genes to create an artificial system of enzymes housed within a minimal genome host. This has the potential to remove key barriers to implementation of multi-step biotransformation. In addition, application of synthetic biology also raises the possibility of creating synthetic enzymes with novel functional properties.

* Meyer et al., Cur Opin Micro 2007; 10: 246

Protein-based polymers

- Protein-based biopolymers have become a promising class of materials for both the biomedical and chemical sectors largely due to their attractive properties (they have well-defined molecular weights, monomer compositions, as well as tuneable chemical, biological, and mechanical properties).
- These polymers must be produced in biological systems (initially micro-organisms or, in a later stage, plants)
- Opening up the number of amino acids that make a protein would substantially increase the scope of our molecular engineering capabilities. For example:

1. <u>Engineered silks from microorganisms such as bacteria or yeasts</u>
   Silks are promising biomaterials for:
   (i) medical purposes (tissue engineering scaffold, excellent biocompatibility and mechanical properties. Silk can also be fused to a variety of protein molecules to add functionality, such as angiogenesis-induction obtained by fusion to fibronectin-derived RGD-sites)
   (ii) performance material (superior mechanical properties)

   Problem: silk production is rather challenging both with respect to gene expression and also to downstream processing.

2. <u>Collagen manufacturing</u>
   - Huge potential in tissue engineering.
   - Suffers from a similar problem as silk with respect to gene expression and downstream processing. This is a problem common to repetitive amino acid polymers.

Protein-based polymers

- The functionality of protein-based materials can be substantially increased by insertion of unnatural amino acids. This would allow, for example, targeted fusion to other materials and novel chemical/structural/mechanical properties such as tailoring of degradation rate, increasing/decreasing stiffness of fibres etc.

- The availability of chassis organisms that can be used to insert unnatural amino acids into polymers on a large scale will be essential to realise the production systems.

- It should be noted that repetitive protein-based polymers (like silk and collagen) are typically difficult to produce in microorganisms. Production could benefit immensely from chassis strains that have been optimised for protein production, e.g. by benefiting from available chaperone cassettes.

Bioplastics

Metabolic engineering of bioplastics (PHA)

- Polyhydroxyalkanoates (PHAs) are polyesters that accumulate as inclusions in a wide variety of bacteria. They are considered to be an attractive substitute for petroleum-based plastics and to have particular applications in the biomedical sector due to their excellent biocompatibility.

- Well established experimental system: production by a large variety of bacteria among them many genetically well established.

- Opportunities for synthetic biology:

1. The properties of the material are highly dependent on the exact composition of the biopolymer. This is difficult to control by *in vivo* production. By providing highly tailored *in vitro* systems, synthetic biology can make a contribution to the production of highly regular polymers with presumably superior material properties.
2. It is unclear whether bulk bioplastics from bacteria are an opportunity. Generally, it is assumed that this would be too expensive. However, it is not clear whether the cost analysis would be more attractive if novel, highly engineered strains based on chassis organisms were used.
3. An additional issue in the manufacturing of bulk bioplastics is its purification with cell disintegration being the major problem. A cheap bacterial system that destroys the bacteria from the inside would be ideal. A rational design of this complex phenotype would be very useful, requiring a highly integrated regulatory decision process to trigger the self-destruction process at the optimal time-point.

Applications: Bioremediation

Bioremediation 1: microorganisms

Bioremediation is the use of biological activity to remove pollutants.

Natural organisms possess many pathways for the degradation of chemical pollutants, even those which do not occur naturally in the environment, but do not always degrade pollutants effectively *in situ.*

Genetic modification can create 'improved' organisms by:

- Combining multiple degradative pathways into a single organism.
- Ensuring that relevant pathways are always expressed at a high level, rather than requiring induction. For example, degradation of trichloroethene (TCE) in groundwater by natural organisms requires addition of toluene to stimulate production of the relevant enzymes.
- Transfer of pathways from slow-growing organisms to rapidly growing hosts. For example, transfer of the TCE-degrading enzyme methane monooxygenase from methanotrophs to pseudomonads.
- Addition of other useful traits such as production of biosurfactants, tagging for easy tracking in the environment, etc.

Bioremediation 2: plants

- Use of genetically modified microorganisms (GMM) for bioremediation has not been generally adopted because it is extremely difficult to obtain permission to release GMM into the environment and the relatively low value of bioremediation activities makes rigorous containment uneconomical.

- An alternative is to transfer microbial degradative pathways into plants so that plant roots express the relevant enzymes and destroy or sequester the pollutants of interest.

- Transgenic plants have been generated to degrade chlorinated solvents and explosives, to volatilise mercury, and to sequester other heavy metals.

- A major unexplored application is to engineer plants expressing microbial hydrocarbon degradation pathways, especially oxygenases, for the degradation of hydrocarbons in soil. Hydrocarbons are the major terrestrial contaminants and their bioremediation using natural organisms is already a valuable industry.

Applications:

Bionanotechnology, Biosensors & Tissue Engineering

Bionanotechnology

- Biological systems have the characteristic property of self assembly as well as the ability to specifically bind other molecules.
- By combining these traits, it is possible to use biological components to create nanostructured materials.
- For example, viruses can be engineered to express metal-binding proteins on their surfaces. The helical virus TMV (tobacco mosaic virus) has been used to generate nanowires by coating with platinum particles. These may have applications in the development of nanoelectronics. Engineered viruses could extend this to develop other nanostructured components incorporating a variety of different metals.
- Viruses can also be used as delivery systems for drugs. Tagging with metals allows the drug to be tracked by magnetic resonance imaging.

Biosensors

- Molecular recognition is one of the characteristic properties of biological molecules. Living organisms possess complex modular signaling pathways to initiate various responses on detection of a specific stimulus.
- These pathways can be ‘hijacked’ to produce biological sensors, which generate a quantitative response to a stimulus such as presence of a particular molecule.
- Bacterial biosensors have been developed for the detection of heavy metals such as mercury and arsenic, as well as for the detection of mutagenic chemicals. Synthetic biology could permit the use of more complex outputs and a degree of *in vivo* signal processing.
- More complex systems can be envisaged. For example, continuous *in vivo* measurement of glucose levels in diabetic patients linked to an appropriate response such as insulin release, if levels are outside a desired range.
- In agriculture, benign plant viruses could be engineered to generate detectable (e.g. luminescent or fluorescent) responses to report on the intracellular conditions within the plants, such as stress due to drought or nutrient insufficiency.

Tissue engineering

- Living organisms, from bacteria to animal cells, can organise themselves into complex three dimensional structures.
- The signaling pathways and adhesion molecules involved in these processes could be used to generate self assembling systems.
- Conventional developmental biology has identified 'modules' that, when activated, can alter cell shape or cause cells to come together to make specific elements of tissues – sheets, tubes, clumps of cells etc.
- These modules could be controlled by synthetic genetic circuits. This would allow the creation of self-constructing 'designer tissues'.
- Early applications are likely to allow living cells to add subtle biology to the rather crude engineering approaches taken now. For example, the addition of biological components to dialysis machines or biological linkers in bionics.
- Future applications are numerous and might include, for example, synthetic islet cells (networks developed to release insulin from engineered cells on demand) or alternatively cell systems that patrol the body to provide early warning of disease and perhaps even treat the emerging condition (e.g. atherosclerotic plaque detection and removal).

BioSafety and Ethics

BioSafety and BioSecurity

Synthetic Biology offers the possibility, or at least the public perception of the possibility, of generating, accidentally or intentionally, an organism that may pose a hazard to human health or to the environment.

Biosafety

- Synthetic microorganisms might escape from a research laboratory or containment facility, proliferate out of control, and cause environmental damage (by disrupting local biota or fauna, by becoming endemic, or degrading the local environment) or threaten public health*.
- There is a potential risk of gene-pool contamination.
- Some risks are indefinable at present. Synthetic genomes that have been assembled from entirely artificial BioBricks will lack a clear genetic pedigree and could have 'emergent properties' arising from the complex interactions of its constituent genes. Accordingly, the risks attending the accidental release of such an organism from the laboratory would be extremely difficult to assess in advance, including its possible spread into new ecological niches and the evolution of novel and potentially harmful characteristics.

Biosecurity

- Outlaw states, terrorist organisations, or individuals might exploit synthetic biology for hostile or malicious purposes.
- In the near future, synthetic genomics technology should make it possible to recreate any existing virus for which the complete DNA sequence is known.* This could mean the recreation of known pathogens in the lab (e.g. ebola).

* The Promise and Perils of Synthetic Biology, Tucker and Zilinskas

Recommendations

- Synthetic microorganisms appear to be less ‘fit’ than their natural counterparts and so are likely to die off rapidly in an uncontrolled environment. Nevertheless, it is important that measures are put in place to minimise the risks highlighted.
- Safety must always be a primary concern. Design software and DNA synthesis facilities should have built-in safety features i.e. ‘fail-fast’ mechanism.
- Public acceptance will be key. Synthetic biology must be presented in ways that explain the risk and benefits to the general public.
- National and international agreement is required to generate regulatory standards.
- Proactive input from the scientific community may help to prevent imposition of unnecessarily restrictive regulations.

Regulation

- The synthetic biology community is keen to regulate itself in order to ensure good practice and to address a range of concerns about their research and also to prevent overly restrictive regulations being imposed.
- Recombinant DNA offers a useful precedent. The safety and social issues surrounding its use were discussed by practising scientists at the Asilomar conference in 1975 leading to the development of a series of guidelines, which were adopted by the NIH.
- Effective regulation might reassure the public that new technology will be safe. It also facilitates investment; venture capital will not invest in risky industries that are not regulated.
- Companies such as Geneart and Blue Heron, who provide synthetic genes, are already taking steps to screen and validate their customers, as well as excluding customers from some countries.
- However, in 2006 a coalition of 38 civil society organisations called on synthetic biologists to withdraw proposals for self-governance of the technology and begin an international dialogue with society.
- Clearly there needs to be dialogue with policy makers, and indeed such work is being done by e.g. SYNBIOSAFE in Europe and SynBERC in the US.

Ethical considerations

- There are important ethical questions to be considered. For example, is it acceptable to experiment with organisms containing DNA from different types of animals, animals and plants or even DNA designed on a computer?

- Concerns have been expressed over the 'creation of life' by groups such as ETC in Canada. This international civil society organisation, which tracks developments in biotechnology, has called on the world's patent offices to reject the applications from Venter's group. ETC have said that these claims "signal the start of a high-stakes commercial race to synthesise and privatise synthetic life forms" and that Venter and his colleagues have breached a societal boundary without the public having had a chance to debate the far-reaching social, ethical and environmental implications of synthetic life.

- Venter himself has previously asked bioethics committees to examine the implications of creating synthetic life. The JCVI's policy team, along with the Center for Strategic & International Studies (CSIS) and MIT, have been conducting a 20 month study to explore the risks and benefits of this emerging technology as well as possible safeguards to prevent abuse, including bioterrorism. The group recently published its report after several workshops and public sessions. http://www.jcvi.org/research/synthetic-genomics-report/

Intellectual Property

Intellectual property debate

- Two key issues have been identified as critical*: the difficulty of assimilating new technology into the limits of existing IP protection frameworks and the tension between different methods of creating 'openness'.
- Synthetic biology poses a new challenge for existing intellectual property (IP) legislation. IP law covering biotechnology and software (both technologies integral to this emerging field) appear inadequate for synthetic biology and indeed both are areas that are already challenged.
- If the key to synthetic biology is modularity and the creation of libraries of standard parts that reliably perform simple functions then it is imperative that an intellectual property framework is found that can cope with the need to share parts but also continues to provide an incentive for innovation.
- Patenting has been critical to the life sciences sector, providing an incentive for innovation by allowing exclusivity. However, it is expensive and limits usage through licensing.
- In contrast measures that encourage openness, such as the open source model (source code freely available for improvement, modification and redistribution), allow broad use of technology at low cost and simplify subsequent innovation; however, they may not be able to provide a significant incentive for innovation.
- There is an on-going debate between advocates of open source and supporters of patent protection; it is likely that some combination will prove the most viable way forward.

* Synthetic Biology: The Intellectual Property Puzzle, Kumar and Rai

IP debate: protection 1

- US patents tend to have a low non-obviousness threshold, partly due to a reluctance to allow unwritten knowledge to be used in determining non-obviousness. This has led to many biotechnology patents being broader than necessary. Also, there is considerable evidence to suggest that broad patents on foundational research can slow growth in an industry and there are concerns that this may impede the potential of synthetic biology.(1)
- There is some evidence that in some areas of synthetic biology, foundational patents are being granted.(2) For example:
  - Applying electrical or chemical stimuli to genetically engineered cells for the purposes of producing at least one detectable output protein (Patent 7,020,560, Univ Tennessee)
  - The use of combinations of any nucleic-acid-binding protein and any nucleic acid to set up data storage (Patent 6,774,222, US Dept of Health and Human Services)
  - The use of a computer system to simulate operation of biochemical networks (Patent 5,914,891, Stanford Univ)
  - An iterative technique for optimizing the binding specificity of nucleic-acid-binding proteins (6,794,136, Sangamo Biosciences)
  - Methods for selecting DNA-binding proteins that bind with greater specificity in the presence of a DNA-binding ligand (6,706,470, Sangamo Biosciences)
  - Method for Producing a Synthetic Gene or Other DNA Sequence (Patent 7,262,031, Coda Genomics)

  It is unclear whether some of these foundational patents will hold up in court. Even if they do, where a single owner controls the foundational patent(s), the owner may recognise the profit potential of licensing the patent nonexclusively on standard terms, on the model of Stanford's licensing of its patented Cohen–Boyer recombinant DNA technology.(2)

1. Synthetic Biology: Caught between Property Rights, the Public Domain, and the Commons, Rai and Boyle
2. Synthetic Biology: The Intellectual Property Puzzle, Kumar and Rai

IP debate: protection 2

- In addition to the problem of broad foundational patents there is the possibility of a myriad of narrower patents (e.g. those covering parts). Since many products will be made up of numerous parts, patenting these could result in huge royalty stacking. In addition to such royalty stacking, the need to conduct rigorous and costly IP searches and to navigate your way around patents presents significant cost, time and risk that is likely to impede the field.

- However, patenting has been integral to the life sciences sector as it provides an incentive for innovation by allowing exclusivity. There is no direct equivalent in the world of free software.(2)

2. Synthetic Biology: The Intellectual Property Puzzle, Kumar and Rai

IP debate: protection 3

Various other forms of protection have been mooted. These include:

- Copyright or copyleft - licences that not only make source code freely available but also require those who distribute improvements to the source code to make the improvements available on the same terms. However, copyright law does not cover functional articles or methods of operation so is unlikely to apply to synthetic biology products.(1)
- Patent-based commons approach such as that created by the group Biological Innovation for an Open Society (BIOS). This group is using patent protection to force licensees to make patented improvements to these enabling technologies available to other commons members. However, this doesn't address the high cost of patenting; a single patent can cost tens of thousands of dollars to secure and the MIT Registry currently contains more than two thousand standardised parts.
- Statements of non-assertion by patentees. Many current synthetic biology patents are held by academic or government institutions, who might choose not to assert their patents against those working on an open source basis.(2)
- Contracts such as a 'clickwrap' license. This contractual alternative does not require an underlying property right. Instead, the contract simply imposes conditions as part of the price of access. One problem with such contracts is that they bind only those who receive the technology from the entity imposing the terms, so extremely broad restrictions on dissemination can be required.(1) Such licences could be used with BioBricks data.
- A tailored piece of legislation: *sui generis* property rights. However, these are difficult, uncertain and slow.

1. Synthetic Biology: Caught between Property Rights, the Public Domain, and the Commons, Rai and Boyle
2. The Economics of Synthetic Biology, Henkel and Maurer

IP debate: open source 1

- The idea of a synthetic biology 'commons' approach draws inspiration, in part, from the prominence of the open source software model as an alternative to proprietary software. Unlike proprietary software developers, open source software producers (such as those using embedded Linux) make their source code freely available for improvement, modification, and redistribution.

- Importantly, an open source model encourages companies such as software vendors to share parts but also allows them to protect the products made from the parts. The Linux General Public License (GPL) does not require makers to disclose their code to the general public until devices containing it have reached a mass market. This creates an 18-month window in which the code remains proprietary. The business models that drive this behavior are not specific to software and may well work in synthetic biology (see below).

- Companies in the embedded Linux industry use many business strategies to capture value. Synthetic biology companies could exploit most of them:

Open parts, patented products. Software vendors often share basic parts and modules, while protecting the products made from them. Synthetic biology companies could similarly share parts while patenting completed organisms. Companies that create organisms for individual clients may not need patent protection at all.
Shared development. Software vendors routinely share code, hoping that others will update it, identify and fix bugs, or write extensions. Synthetic biology companies could similarly learn from users and even competitors.
Establishing a user base. Software vendors rely on open source to attract users. Synthetic biology companies similarly want to see their parts used as early and as often as possible.
Other strategies. Software vendors participate in open source to demonstrate technical prowess to would-be clients, learn new product ideas, demonstrate social responsibility, and hire talented programmers. Similar motives should also operate in synthetic biology.

Taken from The Economics of Synthetic Biology, Henkel and Maurer

IP debate: open source 2

- It remains to be seen whether the benefits of the embedded Linux approach become too diluted by the likely longer timeframe required for incorporation of a synthetic biology part into a final product. However, there remains the possibility that a registry of parts could be open to any part maker that deposits its parts to the public domain after a set period of time. This could make it possible for companies to earn profits by patenting some parts while making others openly available.
- It has been suggested that the BioBricks Foundation, created by MIT scientists involved with the Registry of Standard Biological Parts, might serve to coordinate a synthetic biology 'commons'. The Registry has already placed its parts into the public domain, thus providing important protection against attempts to patent them, which might clog innovation.(1) Currently the data is available to anyone for any purpose.
- As currently envisioned by its proponents, a commons or public domain approach would be limited to parts and devices. Proponents of a commons approach do not envision extending the commons to include gene synthesis technologies.(2)
- However, one of the main problems with an 'open parts' type approach is that a reward limited to the commercial contributors' own use of the part will not provide the right incentive for more costly inventions (synthetic parts are likely to be more expensive to develop than software code). A solution to this may be metered protection. Software firms that write code for 'embedded Linux' usually sell their services to a handful of companies that make, say, DVD players or machine tools. However, the Linux GPL does not require them to disclose their code to the general public until devices containing it have reached a mass market. This creates an 18-month window in which the code remains proprietary, and rewards can be generated.
- Henkel and Mauer advocate such an embedded-Linux type solution, with open parts and metered protection.(3)

1. Synthetic Biology: Caught between Property Rights, the Public Domain, and the Commons, Rai and Boyle
2. Synthetic Biology: The Intellectual Property Puzzle, Kumar and Rai
3. The Economics of Synthetic Biology, Henkel and Maurer

Recent events 1

- In May 2007, the US Patent & Trademark Office published Patent application 20070122826 (International Publication No. WO2007047148). In this patent, entitled 'Minimal bacterial genome' scientists from the J Craig Venter Institute (JCVI) claim exclusive ownership of a set of essential genes and a synthetic "free-living organism that can grow and replicate" that is made using those genes.

- The new organism, named *Mycoplasma laboratorium*, would be the first ever entirely synthetic living organism whose genetic information is constructed from chemically synthesised DNA. However, the existence of the patent application isn't proof that the group achieved a fully-functioning organism at the time of the filing (October 2006).

- The inventors arrived at their minimal genome (consisting of 381 essential genes) by determining which genes within the genome of *Mycoplasma genitalium* (a naturally slimmed-down microbe with 485 genes) are essential and which are not. The application claims any synthetically-constructed organism that lacks at least 55 of 101 genes that they've determined are non-essential.

- It is unclear whether a group would infringe this patent if they created another organism that lacked some of the same genes that *Mycoplasma laboratorium* lacks. Even if granted it may be possible to engineer round it simply by packing a synthetic genome with extra genes to bring the total over 450. Others will want to use alternative genetic engineering staples such as *E. coli*.

- The patent application specifically claims any method of hydrogen or ethanol production that uses such an organism or any version of the organism. The research was partially funded by the US Department of Energy.

Recent events 2

- More recently (June 2007) researchers at JCVI published in *Science* results of work on genome transplantation methods allowing them to transform one type of bacterium into another by replacing one organism's genome with the other one's genome.
- The ability to transfer the naked DNA isolated from one species into a second microbial species paves the way for transplanting a chemically synthesised bacterial chromosome (containing the minimal genes outlined in the patent together with other application-specific genes) into a living organism and if successful, 'boot up' the new entity.
- Patenting of genes has been a controversial matter for many years but synthetic biology and this particular patent application takes the debate to a new level. The Canadian ETC Group has called on the world's patent offices to reject the applications
- Another patent (US patent 6,989,265) issued in January 2006 on a minimised *E. coli* genome may also be important. Based on the work of Fred Blattner at the University of Wisconsin, the patent contains claims that could cover any synthetic cell derived from an *E. coli* genome.
- The IP has already provided the foundation for startup Scarab Genomics, which offers a minimized version of *E. coli* K12 (15% of the genome deleted) with enhanced genetic stability and improved metabolic efficiency for gene cloning and heterologous protein expression applications.
- There are concerns that this patent could severely constrain onward research using this strain of *E. coli* and thus stifle innovation.

Financing

Commercial players

- $70 million for Series B funding to cover biofuels, led by Duff Ackerman & Goodrich Ventures, and previous investors Khosla Ventures, Kleiner Perkins Caufield & Byers (KPCB) , and Texas Pacific Group Ventures
  - Follows a large grant from the Bill & Melinda Gates Foundation awarded to Amyris and OneWorld Health (first nonprofit pharma in US) to develop artemisinin.

- $1.6m in Series B funding from the Life Science Angels.
  - Have patented a 'Method for Producing a Synthetic Gene or Other DNA Sequence'. Also working with UC Irvine to turn a common yeast strain (*Saccharomyces cerevesiae*) into an efficient producer of ethanol.

- $20m Series B funding from Highland Capital Partners, Flagship Ventures, KPCB, Alloy Ventures, and Khosla Ventures.
  - Founded by synthetic biology pioneers including George Church, Drew Endy and Jay Keasling.
  - Created a stretch of DNA more than 35,000 letters long (approx. ten genes present). Competing with Blue Heron Biotechnology, DNA 2.0 and Geneart.
  - Partnering with Agrivida (MIT spin-out) on 3rd generation biofuels.

Commercial players

LS9, INC.
the renewable petroleum company™

- $5 million raised in Series A funding from Flagship Ventures and Khosla Ventures. Applies synthetic biology technology to the production of proprietary biofuels. George Church is one of the founders.

SANGAMO
Sangamo BioSciences, Inc.

- Listed on Nasdaq since 2000, Sangamo Biosciences has broad patents on foundational technologies, in particular the engineering of zinc finger DNA-binding proteins (ZFPs).

  Licensing ZFPs to a range of large corporates, including:
  - Dow AgroSciences: the development of products in plants and plant cell cultures;
  - Lifescan (J&J): therapeutic cell lines as treatment for diabetes;
  - Novo Nordisk: evaluating for use in enhanced protein production;
  - Pfizer: research collaboration on enhanced protein production.

Commercial players

SCARABGENOMICS LLC
CLEAN GENOME® *E. coli*

- Technology developed at the University of Wisconsin. Has bioengineered the Clean Genome® *E. coli* by deleting over 15% of the *E. coli* K-12 genome, which could be used for a wide range of applications, including the production of biopharmaceuticals.

synthetic genomics

- $30m from VC Draper Fisher Jurvetson and two other investors. Set up by Craig Venter.
  - Has developed a minimal genome, *Mycoplasma laboratorium*, which is the first ever entirely synthetic living organism.
  - BP has taken an equity stake (June 07) in a deal to sequence the genes of microorganisms that live in fossil fuel deposits with a view to trying to determine ways to exploit properties of these microorganisms on an industrial scale.

Commercial players: DNA synthesis

BLUEHERON® BIOTECHNOLOGY

- Produces DNA sequences from 60 base pairs to well over 20,000 base pairs in length, including the first synthetic DNA fragment over 50,000 base pairs.

DNA 2.0

- Has developed two platform technologies, the DNA-2-Go™ gene synthesis process and DeNovo Genes™, a protein engineering and sequence optimisation technology.

febit

- About to enter the synthetic genes market; already active in synthesising oligos.

GENEART THE GENE OF YOUR CHOICE

- Leading DNA synthesis provider and first publicly listed gene synthesis company world-wide (listed in Frankfurt). Portfolio ranges from the production of optimised synthetic genes and the generation of gene variants and combinatorial biology (directed evolution) through to the production of DNA-based agents.

BP – Berkeley deal

- In February 2007, energy firm BP entered into a $500 million, 10 year research collaboration with the University of California, Berkeley, Lawrence Berkeley National Laboratory (LBNL) and the University of Illinois at Urbana-Champaign, to develop new sources of energy and reduce the impact of energy consumption on the environment.
- The funding will create the Energy Biosciences Institute (EBI), which will have approximately 25 faculty-level principal investigators housed at UC Berkeley and the University of Illinois. The institute will initially concentrate on three aspects of the biomass-to-biofuel equation: developing feedstocks; creating techniques for breaking down plant material to its sugar building blocks (particularly cellulosic feedstocks); and finding ways of fermenting the sugars into ethanol.
- Eventually, the institute expects to look at converting fossil fuels to energy with less environmental damage, maximizing oil extraction from existing wells in environmentally sensitive ways, and finding ways to store or sequester carbon so that it does not get into the atmosphere.
- BP had invited five universities to submit plans for an institute to explore the fuels and energy sources of the future.
- This deal has caused significant controversy with many claiming that BP has bought a chunk of America's premier public research institutions.
  - BP will co-own IP in some instances and receive exclusive (albeit time-limited) commercial licences as well.
  - The 50 BP scientists on campus are also not under any obligation to publish.
  - The director of the institute and other high-level positions will be proposed by BP.
- What remains to be seen is whether there are safeguards in place to protect academic integrity and independence.

Microsoft's interest

- In March 07 Microsoft Research announced the six recipients of the Computational Challenges in Synthetic Biology 2006 awards, totaling $570,000 (USD) in funding. The objective of this award was to stimulate foundational research in synthetic biology and DNA nanotechnology by identifying and addressing the computational challenges of two areas of synthetic biology:
  1) the re-engineering of natural biological pathways to produce interoperable, composable, standard biological parts;
  2) tools and information repositories relating to the use of DNA in the fabrication of nanostructures and nanodevices.

- The winners came from one Canadian and five US universities and covered topics such as:
  - Computational Interchange Standards for Synthetic Biology
  - Design and Synthesis of Minimal and Persistent Protein Complexes
  - BioStudio: A Collaborative Editing and Revision Control Environment for Synthetic Genomes
  - Identification of Standard Gene Regulatory Sequences for Synthetic Biology
  - Using Programmable Stacking Bonds to Combine DNA Origami into Larger, More Complex, Reconfigurable Structures
  - Noise Suppression and Next-Generation Cloning Vectors

- Microsoft stipulated that any results arising from the projects (including all intellectual property) should be broadly available by either 1) placing the results in the public domain; or 2) making the results available under a non-restrictive licence that allows modification and redistribution without any significant restrictions or conditions.

Government funding (Europe)

- An EC work programme on Synthetic Biology was set up through the NEST (New and Emerging Science and Technology) 'Pathfinder' initiative of FP6.
- 18 synthetic biology research projects are currently funded through NEST, to a total of over €22mln.
- The projects funded cover potential energy applications, nanodevices and biological computing, as well as some more structural projects, such as:
  - Emergence: this seeks to develop a foundation for Synthetic Biology in Europe, by defining standards, developing abstraction hierarchies, and looking for ways to protect IP rights without stifling openness.
  - SYNBIOSAFE is investigating the biosafety, biosecurity and ethical aspects of synthetic biology.
  - TESSY (Towards a European Strategy for Synthetic Biology) seeks to strengthen the European position by defining clear research goals as well as raising awareness.

Government funding (US)

- The Synthetic Biology Engineering Research Center (SynBERC) is a collaborative research effort between UC Berkeley, UCSF, Harvard and MIT to lay the foundation of synthetic biology. Funded by the National Science Foundation, it aims to develop:
  - a conceptual framework for designing small biological components (parts) that can be assembled into devices that will perform a well-characterised function under specified conditions;
  - a small number of chassis (stable, robust bacterial hosts with known responses) to host the engineered devices and to assemble several devices to accomplish a larger vision or goal;
  - a set of standards for the interactions of the parts and devices so that the devices can be built more readily and reproducibly.
- All parts will be made available as open source to other researchers and to the companies who are part of the SynBERC industrial consortium.
- Research is also carried out on human practices, including intellectual property and ethics.
- As part of its education function, SynBERC has developed iGEM: an international undergraduate student competition.

iGEM competition

- The International Genetically Engineered Machine (iGEM) competition is an international arena where student teams compete to design and assemble engineered machines using advanced genetic components and technologies.

- Student teams are given a kit of biological parts at the beginning of the summer. Working at their own universities over the summer, they use both these parts and new parts of their own design to build biological systems and operate them in living cells. These new parts are then entered into the Registry of Standard Biological Parts, for use on an open source basis.

- Over the last three years iGEM teams have managed to partially or completely build a variety of systems from biosensors to biological photographic film.

So far 57 teams have registered for iGEM 07 from all over Europe, US, China and Japan, including teams from Glasgow and Edinburgh universities. The iGEM competition finale is due to take place on 3-4th Nov at MIT, USA.

Scottish Activity

Scottish expertise

Scotland already has considerable academic strength in this area.

Expertise spans enabling technologies and application exploration. These include:

- megabase DNA synthesis
- BioBricks tools
- metabolic pathway assembly, insulation of genetic networks, genetic networks in yeast
- modeling of stochastic processes in single cells
- design of software and algorithms for systems and synthetic biology
- engineering of photosynthetic organisms
- new technologies for massively parallel synthesis of biological molecules
- biofuel (cellulose degradation, alkane synthesis)
- plant engineering (improved biomass yields and alkane production)
- tissue engineering
- plant viruses for assembling nano-structured materials
- plant viruses for production of therapeutic proteins
- glass microfabrication and microfluidics

UNIVERSITY of GLASGOW

THE UNIVERSITY OF EDINBURGH

HERIOT WATT UNIVERSITY

Conclusions

Conclusions

- Synthetic biology is an emerging and promising field, which has the potential to address market needs in a variety of sectors.

- The immaturity of the field offers significant opportunities to new entrants; with investment, Scotland could become a dominant player in this field. Indeed, Scottish universities are already active in this area.

- It is clear that opportunities do exist; these lie in two discrete areas:

    1. Development of enabling technologies

    2. Applications i.e. utilisation of synthetic biology to address existing market needs

Opportunities: enabling technologies

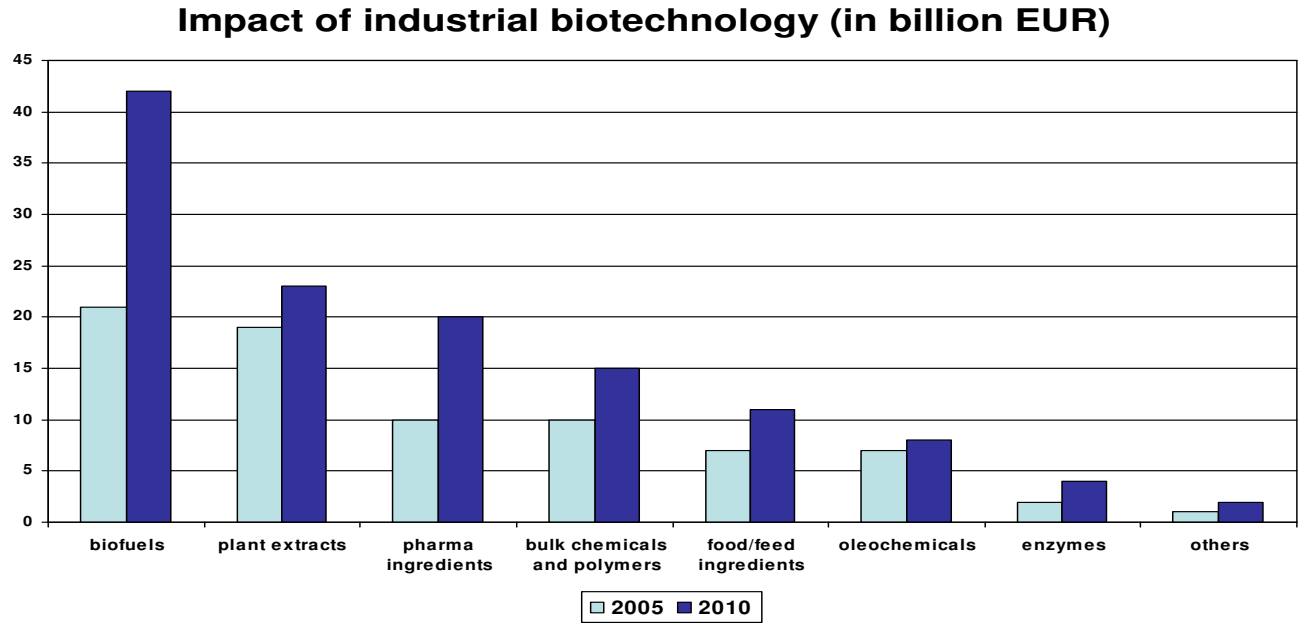
- There is a clear need to improve existing enabling technologies. Improvements here will accelerate the growth of the entire synthetic biology field and are key to the realisation of true synthetic biology, where a synthetic system with predictable behaviour is constructed from a biological blue-print.
- While a number of companies are operating in the DNA synthesis space, few are focussing on the development of other enabling technologies. This offers entrants the opportunity to become market leaders in select areas relatively quickly.
- However, while opportunities exist to build a business around enabling technologies, entering this market is not without risks.
  - Given the embryonic nature of the field, there is a danger that early enabling technologies may be superseded and patents bypassed. As a result, there may be a limited timeframe within which to obtain returns from an early investment in this area.
  - IP issues surrounding, in particular, part and chassis development should not be underestimated. An inability to protect developed technologies or conversely a lack of freedom to operate could significantly reduce commercial returns.
  - Biological systems are complex and our understanding of these systems lacking. This will hamper our ability to design parts and systems with predictable behaviour and is likely to restrict the growth of the synthetic biology field as a whole. This will slow the adoption rate of any enabling technologies developed and may limit their early use to research tools.
  - If foundational technologies are not developed within a reasonable timeframe, there is a clear risk that significant cooling in interest in the synthetic biology field may occur, analogous to the 'hype and cooling' cycles seen in Artificial Intelligence and DNA computing.

Opportunities: applications

- Synthetic biology has numerous potential applications and is particularly useful where there is a need to derive an alternative production pathway or develop a product with novel physical properties or functionalities.
- Many opportunities exist, particularly with respect to chemical synthesis, drug production and biofuel generation. Here a 'top-down', semi-synthetic approach may be employed to address the market need. Many do not consider such an approach true synthetic biology as it does not involve as much forward planning and rational design as would be ideal.
- Although the adoption of a semi-synthetic approach to address a specific market need would minimise the necessity to develop a suite of enabling technologies, some development of enabling technologies would still be required. However, this development activity would be focussed on delivery of the desired application rather than delivery of a standardised and well-characterised suite of technologies, which are required for the realisation of the true synthetic biology vision.
- This approach, where specific applications are addressed rather than enabling technologies, is unlikely to accelerate growth of the overall synthetic biology field. However, this could be at least partly countered by ensuring that any enabling tools developed are designed to be easily translated to other related applications, for example, development of a chassis optimised for protein production rather than production of one specific protein. Such an approach would also maximise returns on any initial R&D investment.

Applications: evaluation criteria

- Synthetic biology can help meet various market needs; a number of filters can be applied to facilitate the evaluation and ranking of these opportunities.
- First, market opportunity. As synthetic biology is in its infancy, it is difficult to assess quantitatively the impact that this technology could have on each market. However, insights can be gained when the potential economic impact of bio-based processes on key markets is considered.



2005: 77 billion EUR IB related sales in chemicals (7% sales of the chemical industry)
2010: 125 billion EUR IB related sales in chemicals (10% sales of the chemical industry)

Source: McKinsey & Co

Applications: evaluation criteria

- Second, public perception or more specifically potential public reaction to any resulting synthetic biology based product. Public backlash to synthetic biology, such as that witnessed against GMO, could impact product sales significantly . It may be prudent to address an application where consumers are likely to perceive the benefit of using synthetic biology to outweigh the risks associated with the creation of an artificial biological system. For example, production of biodegradable plastics.

- Third, regulatory hurdles. Several applications of this technology are likely to be governed by more stringent regulatory guidelines than others. Regulatory hurdles may be lower for extracorporeal applications, such as chemical synthesis and biofuels, for example or for applications where the synthetic systems are easily contained. It is also interesting to note the push from the synthetic biology community to regulate itself (as observed with nanomedicine*). This may serve to reassure the general public that biosafety and ethical issues are being considered and so lower the risk of potential public backlash to synthetic biology based technology.

- Lastly, technology challenges. Given the early nature of the field and associated immaturity of enabling technologies, it would be prudent to address a market need, where a 'top-down' semi-synthetic approach could be applied to meet this need. Applications where a truly synthetic, 'bottom-up' approach is required, will take longer and significantly more investment to bring to market.

* ITI Life Sciences Nanomedicine Foresighting Report 2005

ITI view

- Synthetic biology is a promising field with numerous potential applications.
- However, the 'bottom-up' construction of a wholly synthetic system from a biological blue-print in order to produce a system that delivers significant cost and performance benefits over conventional technology remains several years from realisation. Progress in the field over the next 2 years will dictate whether this will then be realisable within a 7 year time frame.
- Enabling technologies must be developed and biological understanding improved if this goal is to be achieved. Given the unresolved IP issues and degree of basic research required, development of the enabling tools may initially be best suited to an academic environment, although commercial opportunities do exist.
- A more 'top-down' approach to synthetic biology, where existing, natural biological systems are re-designed and engineered to perform specific tasks may also be taken to address some of the market needs highlighted. This approach will not be as reliant on enabling technology development and so may be realisable in a relatively shorter timeframe.
- However, the underlying complexity of biological systems and our lack of quantitative understanding of these systems remains a key barrier and is likely to limit the ability of synthetic biology to readily move beyond the current 'proof-of-concept' devices.
- Based on the above, ITI believes that while promising, the returns from synthetic biology are unlikely to be obtained within a near to-mid term timeframe. However, ITI does see returns opening up in a 7-10 year timeframe and so will maintain a watching brief on the synthetic biology space with a view to engaging with the field following further maturity.
- This foresighting report, however, has also highlighted the potential opportunities within industrial biotechnology. ITI believes that this area, in general, merits further analysis, particularly in the context of 'top-down' synthetic biology approaches.

Next Steps

What happens next?

We very much welcome dialogue with our Members in this area.

If you would like to discuss the report findings and associated opportunities with us further, please contact ITI Life Sciences at:

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For more information on ITI Life Sciences, please visit http://www.itilifesciences.com/

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