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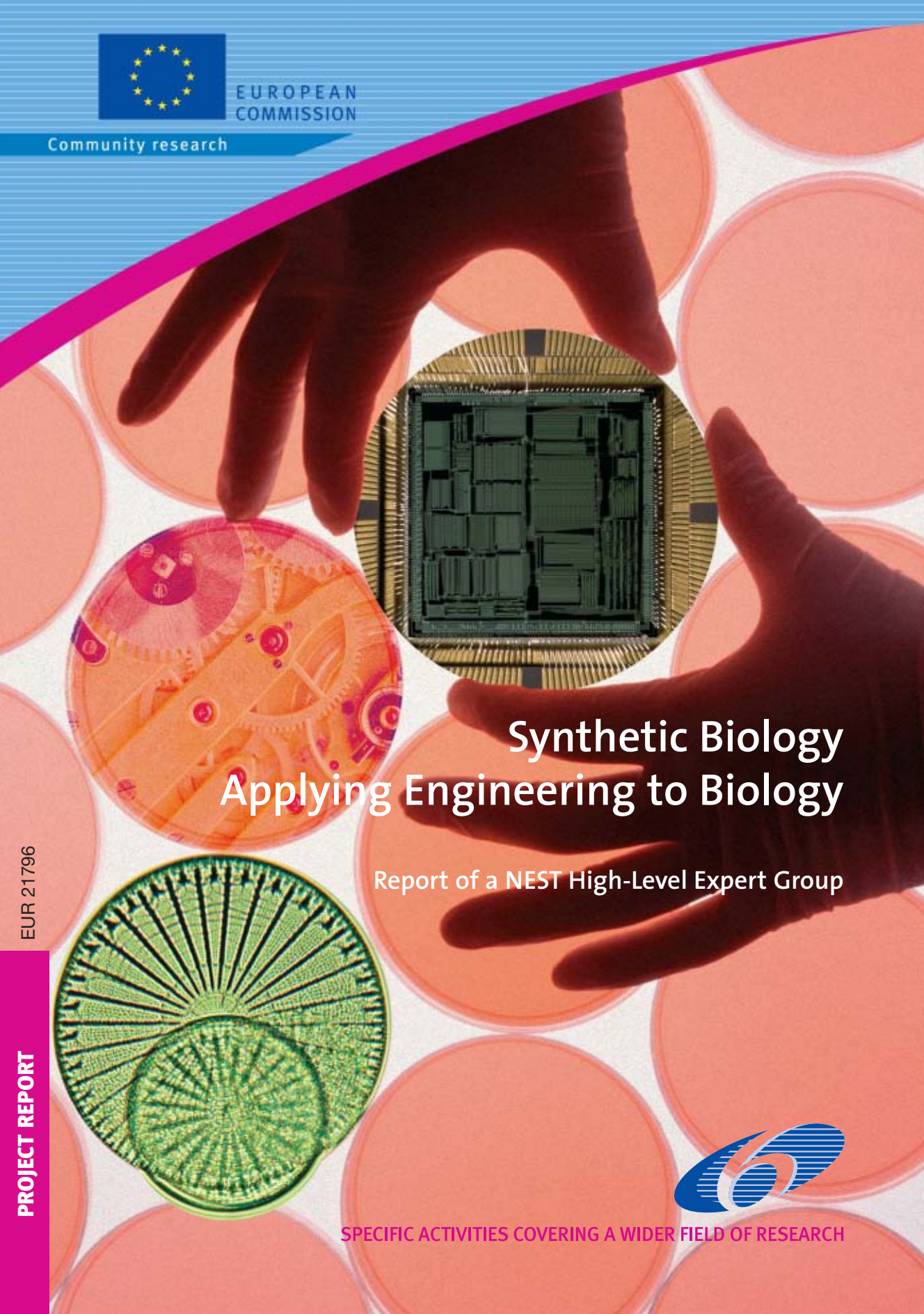

Synthetic Biology
Applying Engineering to Biology

Report of a NEST High-Level Expert Group

EUR 21796

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**NEST - New and Energing Science and Technology -
is a research activity under the European Community's
6th Framework Programme**

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EXECUTIVE SUMMARY

What is Synthetic Biology?

Synthetic biology is the engineering of biology: the synthesis of complex, biologically based (or inspired) systems which display functions that do not exist in nature. This engineering perspective may be applied at all levels of the hierarchy of biological structures – from individual molecules to whole cells, tissues and organisms. In essence, synthetic biology will enable the design of 'biological systems' in a rational and systematic way.

Opportunities for Europe

Synthetic biology is a field with enormous scope and potential. In many ways its current situation can be compared with the very early days in the development of the computer industry: it has the capacity to change quite fundamentally the way we approach certain key technologies, such as medicine and manufacturing, but at this very early stage it is hard even to guess where the most important applications will turn out to lie. However, it can be expected that synthetic biology will create highly generic capabilities for the use of bio-inspired tools and processes that will be applicable in industry and the economy. It is likely that open and public scientific knowledge will be embedded very quickly in an unrivalled set of technological "solutions", representing an arena, which will have vast implications for the ownership and control of intellectual property. It is obvious that Europe should invest in this area, in order to create the necessary intellectual and physical infrastructures, and capture a share of the valuable intellectual property that is at stake.

Synthetic biology is a nascent field, and there is currently no systematic, global effort to coordinate the developments in this field. Much of the research so far has been pioneered by individual groups in the US, and the European research community has been relatively slow to embrace the field. At the same time, there is a tremendous pool of expertise within the EU community that could be tapped to help a European programme in synthetic biology to develop. What is needed, and is not established in the US either, is a framework for coordinating the current research, fostering a community of researchers (particularly among younger scientists) and creating a forum for the establishment of clear goals, shared tools and agreed standards. It is also important to address ethical and safety concerns, and to address potential or perceived risks of synthetic biology from the very beginning, so that future development work can be done in conditions of public trust.

Why is synthetic biology emerging now as a field of research?

There are several reasons for this, both technical and fundamental. At the level of basic biological science, it has become clear that an understanding of the way cells work requires more than simply a list of the 'parts', as provided to some degree by genome sequencing for example. We need to know how the parts operate together – how genes and proteins modify each other's behaviour, for example, and how they interact to form modules and circuits analogous to those in electronic systems. This understanding, which is advancing within the field known as systems biology, is providing the conceptual tools needed for the rational construction and redesign of such 'biological circuitry'.

Systems biology relies heavily on the development of new tools such as computer models of complex systems, bio-informatics, and experimental techniques for exploring gene interactions. At the same time, the methods of standard biochemical and biotechnological research, such as the chemical modification of proteins and the splicing and rearrangement of genetic information in DNA, have

advanced to the stage where they can be used for the purpose of redesigning the fundamental molecular interactions and pathways of living cells. And the development of techniques for rapid synthesis of DNA with specified sequences has made it possible to build wholly synthetic, highly complex collections of genes and even to synthesize living organisms from the genome up.

What can synthetic biology achieve? What are the applications?

Potential applications of synthetic biology range very widely across scientific and engineering disciplines, from medicine to energy generation. For example, designed microorganisms might be capable of producing pharmaceutical compounds that are extremely challenging for existing methods of chemical or biological synthesis. While several pharmaceuticals are already produced biotechnologically using genetically engineered organisms, the capacity to design complex synthesis pathways into such organisms could greatly expand the repertoire of products that can be made this way. Engineered biological 'devices' based on modular assemblies of genes and proteins might also be able to act within the body to detect and respond to changes in the state of health – a kind of autonomous, molecular-scale 'physician' that can combat disease at a very early stage in its development. Such devices could also be used for tissue repair and cell regeneration. By such means, synthetic biology might provide the tools for medical intervention at the molecular level, obviating the rather crude surgical or pharmaceutical tools currently at our disposal.

Just as rationally engineered organisms might supply new drugs, so they could be designed to make useful materials (such as biodegradable plastics) from cheap and renewable raw materials, or to convert such feedstocks to fuels such as hydrogen and methanol. This could make the chemicals industry more environmentally friendly and sustainable. The ability of biological systems to control the structure of materials at the molecular level could also provide access to materials with new and improved properties, or devices such as machines and electronic circuitry structured at ultra-small scales.

Engineered organisms and biological structures might also serve as sensors and detection systems with improved sensitivity and autonomous operation, for example for the detection of pollutants in remote environments or the sensing of explosives and biological-warfare agents. Such detection capabilities might be coupled to the ability to degrade and destroy dangerous substances.

What is needed?

The NEST activity under FP6 has provided a starting point for synthetic biology activities in Europe. These activities should be continued and taken up by other organisations and future funding programmes. Building on this Expert Group report, what is needed now at European level is a detailed strategy, including a clear roadmap, which develops a set of necessary actions, such as infrastructure development, education and training activities, safety strategies, etc.

Funding is needed both to support basic research and to enable the establishment and maintenance of the infrastructure that synthetic biology requires: for example, an open-source repository for the molecular and genetic components and modules that should form the set of standardized parts on which 'biological engineers' can draw.

The profile of synthetic biology in Europe would be greatly boosted by the hosting of a major international conference on the topic. So far, there have been no more than a handful of such conferences worldwide.

The interdisciplinary nature of synthetic biology creates a need for educational initiatives at all levels, from undergraduate to experienced researcher, in order to foster the skills and shared language needed for the discipline to thrive. Specialists in different disciplines will need to develop a working knowledge of each

other's *modus operandi*, and in the long term it would be desirable to create a new breed of researchers who are familiar both with fundamental biology and with the methodology of engineering, as well as having requisite skills in areas such as computational sciences and chemistry. This will require integrating synthetic biological concepts into standard educational syllabuses.

PART I

WHAT IS SYNTHETIC BIOLOGY?

Definition and scope

Synthetic biology is concerned with applying the engineering paradigm of systems design to biological systems in order to produce predictable and robust systems with novel functionalities that do not exist in nature. Just as all engineering disciplines maintain a fruitful relationship with the fundamental sciences that underlie them, synthetic biology will seek to use and expand the mechanisms that control biological organisms using engineering approaches. These approaches will be applied on all scales of biological complexity: from the basic units (design and synthesis of novel genes and proteins, expansion and modification of the genetic code) to novel interactions between these units (regulation mechanisms, signal sensing, enzymatic reactions) to novel multi-component modules that generate complex logical behaviour, and even to completely or partially engineered cells.

Bringing the engineering paradigm to biology will allow us to apply existing biological knowledge to biotechnological problems in a much more rational and systematic way than has previously been possible, and at the same time to expand the scope of what can be achieved this way. The introduction of design principles such as modularity of parts, standardization of parts and devices according to internationally recognized criteria, and the (reciprocal) adaptation of available abstract design procedures to biological systems, coupled to novel technological breakthroughs (such as cheap mass synthesis of large DNA segments) that allow the decoupling of design and fabrication, will fundamentally change our current concepts of how to manipulate biological systems. In this sense, synthetic biology is not primarily a "discovery science" (that is, concerned with investigating how nature works), but is ultimately about a new way of making things. By adapting natural biological mechanisms to the requirements of an engineering approach, the possibilities for re-assembling biological systems in a designed way will increase tremendously.

While several of the fundamental scientific issues and current applied objectives of synthetic biology overlap with those in other, more mature fields, especially biotechnology and systems biology (see below), synthetic biology should be properly seen as a completely new discipline, which brings a systematic, application-driven engineering perspective to biology.

Just as in chemistry about a century ago, biology now seems poised to enter an era where significant advances in understanding will derive from a fruitful dialogue between theory and experiment, from analytical and synthetic efforts, and from interdisciplinary interaction with the chemical, physical, engineering and computational sciences. The potential for interaction with nanotechnology is especially apparent and appealing. It is often said that biology is the only existing nanotechnology that really works. But if we want to exploit this 'natural nanotechnology' for applied, engineering objectives, we will ultimately need to be able to intervene and to modify it at the level that synthetic biology is exploring.

It can be anticipated that the major change that the field of synthetic biology will bring is the synergistic **integration** of existing disciplines: not just biology and engineering, but also computer modelling, information technology, control theory, chemistry and nanotechnology. Ulitmately, it is likely that the analytical and synthetic approaches to biology (that is, systems and synthetic biology), as well as the *in vitro* and *in vivo* ('bottom-up' and 'top-down') approaches, will fully complement each other.

The scope and ambition of synthetic biology in many ways parallels the development of synthetic chemistry as part of organic chemistry.

Organic chemistry started out as a largely analytical science, concerned with the purification and characterization of products from natural sources. That is to say, it was a discovery-based science that aimed to elucidate the properties of natural entities. As the body of knowledge about structures and chemical reactivities of natural products grew, synthetic chemistry developed as a complement. Initially the main purpose of synthetic chemistry was to confirm the molecular structures deduced by analysis as well as the production of more such materials for study. However, inherent to the synthetic reproduction of natural molecules is the capacity to produce modified versions. Ultimately, this led to synthetic routes to molecular structures that are not found in nature, some of which mimic or improve on properties found in natural molecules.

Biology has now reached the stage where a sufficient amount of genetic and biochemical data on biological systems has been acquired to enter the synthetic stage. In analogy to synthetic chemistry, synthetic biology is not content with "explaining" or simply reproducing the behaviour of natural systems. Rather, synthetic biology aims to go one step further by building, i.e. synthesizing, novel biological systems from scratch using the design principles observed in nature but with expanded, enhanced and controllable properties. The complexity of such a 'design' goal makes an engineering approach imperative.'

Together and in dialogue with these synthetic efforts, a theoretical framework of the behaviour of biological systems and subsystems is being assembled as a part of the discipline called systems biology, which feeds into the synthetic biology knowledge-base. Thus, in many ways, synthetic biology requires a higher level of understanding than can be obtained from a purely empirical approach to biological systems. In return, by reconstituting systems along carefully crafted design –routes, synthetic biology will yield (in addition to its technological products) a much expanded understanding of the chemical, biophysical and dynamic parameters of those systems and their relation to function and behaviour.

Traditional biotechnology tries to tackle a technological challenge by manipulating existing biomolecules, cells or organisms. To that extent, the same is true of synthetic biology. But whereas biotechnology has tended to proceed in an ad hoc or empirical manner, which has typically restricted the degree of modification that can be reliably controlled or the goals that can be achieved, synthetic biology permits rational design and redesign of living systems at a deeper and more complex level. It will provide a 'biotechnology that really works'.

Systems biology is a science-based discipline that aspires to study a biological system at various levels in its entirety, ranging from cell networks to cells and complete organisms. It involves the mapping of pathways, gene and protein interactions and logical 'circuitry' of natural organisms at the cellular, tissue and whole-organism level and the integration of this information into a computer model. Its primary goal is to attain a quantitative and predictive understanding of a biological system.

Thus systems biology provides the analytical framework in which synthetic biology operates. The design approach of synthetic biology is heavily dependent on the ability to quantify the relevant information on the appropriate, frequently complex levels. Such high-throughput/high-resolution type of analysis is developed in systems biology. Moreover, simulation tools and models developed in systems biology could and will be used in synthetic biology to design and engineer novel circuits or components. In this respect, one could argue that synthetic biology is the design counterpart of systems biology. The corresponding design process requires advanced technological abilities (e.g. targeted manipulation of large numbers of components) as well as reliable model-based predictive capabilities, for instance, to explore entirely new design spaces. Therefore, it will be quite some time before synthetic biology can design the same level of system complexity that systems biology is currently already addressing.

THE VISION OF SYNTHETIC BIOLOGY

Where is the area going in the next 10 to 15 years?

The development of synthetic biology may best be compared metaphorically with the development of the computer industry and the impact it had on many industries and businesses. About 30 years ago, only a few companies were involved in the computer industry, and the functions and applications of computing were rather restricted and specialized. However, the application of system engineering design principles such as the development of standardized components for electronic engineering and circuit design – that is, the decoupling of fabrication as such from the exploitation of these parts in complex devices – along with the increasing scope and potential of computer software and a broader view of how the computational capacity of microprocessors might be exploited in technological applications, led to the vast expansion of computers from being centralized data banks and 'number-crunching' machines into devices that are central to everything from process engineering to communications technologies to fundamental scientific research, as well as finding their way into home applications.

In the same way, synthetic biology could also revolutionize the biological and biotechnology industries and maybe even biology as a science. To achieve this, it will be important:

- to invent, construct and test basic parts of complex synthetic systems with well controllable, programmable and robust behaviour. The parts may be of several types: (1) **input parts** that sense a designed circuit's environment, e.g. interfacing to biological signals and converting them to a different "biological format", amenable to processing by a synthetic system; (2) **internal parts**, relating biological information inside a synthetic system and processing this information; (3) **output parts**, interfacing the output of the synthetic system to the endogenous biological components. The internal parts should ideally be orthogonal to the natural biological environment, in that they should have minimal side-effects on the host biological system and on the other hand be affected only minimally by the host. However, a more advanced integration of parts in the future should imply that they take part in cellular generation/degradation processes together with all other endogenous components.
- to invent ways to efficiently integrate parts into complex synthetic systems that will to some extent alter cell biology and provide a cell with novel functions and/or capabilities.
- to develop a common framework for characterizing the parts so that some or all of them may be standardized. (This objective is being currently pursued through MIT's Registry of Standard Biological Parts[1]). When synthetic biology matures into an industrially relevant discipline, this standardization may lead to specialization and a division of labour between the designers of basic components – specializing for example in molecular architecture or interface design - and the designers of complex systems – specializing in the assembly of pre-designed basic components, thereby pretty much reflecting the hallmarks of a mature industrial sector.

[1] http://parts.mit.edu/

What can the field deliver?

Synthetic biology will drive industry, research, education and employment in the life sciences in a way that might rival the computer industry's development during the 1970s to the 1990s.

Due to the fundamental change in methodology that it entails for the modification of living organisms, synthetic biology may be able to fulfil many of the promises that traditional biotech is still struggling to fulfil, in such important areas as:

- Biomedicine
- Synthesis of biopharmaceuticals
- Sustainable chemical industry
- Environment and energy
- Production of smart materials and biomaterials
- Security: counter-bioterrorism

While traditional biotechnology has had some notable achievements in several of these areas, they have generally been slow and expensive to develop. A typical approach in bioengineering is to develop cells or molecular components with new functions using empirical, evolutionary processes that may involve screening of vast libraries of candidate systems and are hard to optimize. In essence, today's biotechnologist needs to master a very broad array of complex technologies in order to achieve a goal. By potentially re-organizing biotechnological development in line with the principles of synthetic biology, research & development are likely to proceed much faster and in a much more organized way. Introduction of design rules, separation of design and fabrication, adherence to standardized biological parts, and so on, are likely to aggressively tackle the problems encountered by the traditional empirical approach.

Because of its rational, knowledge-based approach to biological design, synthetic biology will allow such goals to be attained more quickly and cheaply. It will also enable developments that cannot obviously be brought about by evolutionary and screening procedures – for example, the coordination of complex sequences of enzymatic processes in the cell-based synthesis of useful organic compounds.

The analogy with computer technology is again illuminating here in terms of the change in outlook and capabilities that synthetic biology will occasion. Early computers were used to solve highly specialized and complicated problems. Today, computing has become so cheap that it is a pervasive aspect of technology, used for routine tasks in areas such as communication, retailing and leisure. Likewise, when the engineering of biology becomes easy, reliable and cheap, it will be used not only to solve currently intractable problems at the leading edge of applied science but for more routine applications that can at present be only speculated about. In other words, while synthetic biology will at first simply accelerate existing research and development for our currently most appealing applications of biotechnology, it might later expand its scope far beyond what it perceivable today. Examples of such current "high-impact" fields for biotechnological research are detailed below.

Biomedicine

Complex molecular devices for tissue repair/regeneration

One of the most fascinating possibilities for synthetic biology could be the development of small macromolecular assemblies composed of a sensor and a group of enzymes, which could be used to sense damage in for example blood vessels and proceed to repair them by dissolving plaques and stimulating endothelial regeneration. Similarly, other machines could be designed to help re-establish the integrity of the collagen network and so forth. This will require a conjunction of protein design with good physiological knowledge of the systems to be repaired.

While the assembly of such complex tasks is impossible with our current understanding, it is easily perceivable how such an assembly could be achieved once the synthetic biology design principles are consistently applied to adapt all the participating units.

Smart drugs
Synthetic biology might speed up our advances towards a synthetic molecular ensemble that encapsulates a drug in an inactive form. A smart drug includes a diagnostic module that is programmed with medical knowledge; it is capable of directly sensing of molecular disease indicators and making a diagnostic decision. This decision is then translated into drug activation. Ideally, a smart drug will be delivered to a patient like a regular drug; however it will only become active in cells affected by a disease.

Biological delivery systems
Synthetic biology could help in the design of organism-friendly devices that will sense (for example) changes in particular hormones and will proceed to secrete a chemical or biological compound in response. This requires the development of biosensors, an encapsulation material and an enzymatic reaction that releases the stored drug.

Vectors for therapy
One obvious application of synthetic biology (owing to advances in DNA synthesis) is the design and modification of viruses to deliver healthy genes to the target tissue in an efficient way, promoting specific recombination and integration of synthetic genes with the existing genome. Similarly, viruses that can recognize specific cells and target them for destruction will fall into this category.

Personalized medicine
In analogy to the advances in systems biology that will provide understanding of the complex traits of specific diseases, synthetic biology will equip us with the tools to adequately address theses complexities by allowing us to synthesize personalized drugs. Such biopharmaceutical drugs will be adapted in their mode of action (for example via specific glycosylation patterns), formulation, dosage, and release kinetics to the specific requirements of the patient. Only once medicines with such subtle differences can be manufactured reliably at a small scale – something that synthetic biology could enable – is this vision achievable.

Cells with new properties that improve human health
It is not far-fetched to imagine that we will be able to modify human cells, like stem cells, to achieve new functions not present in our body, and to introduce them back into the donor. One could think of cells involved in the immune response being programmed to recognize specific viruses or bacteria and target them in a more efficient way than our existing immune system does. This approach could be especially effective for combating new infectious diseases. Similarly, we could imagine making cell lines able to very quickly degrade toxins or chemicals used in biological warfare. It would be particularly appealing to reprogram cells in order to make them regenerate organs, the way lower vertebrates such as the axolotl do.

In vivo synthesis of small-molecule pharmaceuticals

Complex natural products
The ability to harness, combine, modify and adapt a multitude of biological pathways at will by mass DNA synthesis will open up new and efficient routes to natural and non-natural products, a pillar in the development of novel medicines. Engineering these pathways, for example into bacteria will provide (among other things) access to naturally active classes of compounds that have previously been too complex to

synthesize. By the same token, synthetic biology could allow efficient and facile reorganization of modular proteins, for example in the synthesis of antibiotics.

Engineered biosynthetic pathways will be used to manufacture increasingly complex natural products with desirable pharmaceutical properties (e.g. artemisinin). Organisms in which an expanded genetic code is incorporated for the synthesis of proteins offer the potential of expanded chemical capabilities of enzyme catalysis and therefore the potential to synthesize improved versions of natural products. Ultimately this could lead to the design of synthetic pathways for novel drugs based on peptides (which need not be synthesized on ribosomes), polyketides and polysaccharides.

Expanding the chemistry of life

Inherent in the design-driven focus of synthetic biology is the question of expanding the molecular basis of living systems – for example, incorporating altered and/or novel modes of (bio)chemical reactivity into living organisms. One such instance would be the creation of single or multi-celled organisms containing proteins that are comprised of non-natural amino acids (or which are modified by the addition of non-natural sugars), or that contain genetic material composed of non-natural nucleic acids or membranes comprised of non-natural lipids. Expanding the chemistry of life in this way can be expected to have wide-ranging consequences for medicine and biotechnology. Work in this direction has already begun and some examples are outlined below.

Expanding the genetic alphabet

One very promising way to expand the chemistry of life is by extending the genetic alphabet so that it contains more than four characters (A, T, G, C). This could allow new types of information to be genetically encoded, with wide-ranging applications in nucleic acid and protein chemistry.

Nucleic acids

Nucleic acid drugs (for example for anti-sense or RNAi based therapies) hold great therapeutic promise but currently suffer from a number of shortcomings inherent in natural DNA/RNA chemistry. Modified nucleic acids (with for example altered nucleobases and/or backbone structures) can be easier to transport across membranes. Several such molecules show much increased therapeutic potency, but are currently difficult to mass-produce at an economic level, calling for organisms with an expanded genetic alphabet. The same rationale applies to another promising class of nucleic acid therapeutics called aptamers.

Nucleic acids have also been recognized as versatile components for the synthesis of nanoscale structures and devices. An increasing array of nanosensors, switches and tweezers based on DNA and complex replicable DNA geometries can be imagined and realised. Again, *in vitro* or *in vivo* expanded genetic systems will allow the synthesis and replication of nanodevices with a wider spectrum of physico-chemical properties and functionalities.

Proteins

Organisms with an expanded genetic code (i.e. in which more than the canonical 20 amino acids can be incorporated into proteins) will allow the manufacture of protein drugs with novel or enhanced properties – for example, enhanced serum half-life – which might revolutionize monoclonal antibody applications in therapy. Examples of such non-natural protein drugs are already making their way into industrial application (for instance, a modified human growth hormone produced by the US company AbLynx).

Novel imaging & targeting methods
Introducing novel chemistry into 'bio-orthogonal' reporters (BORs), which are immune to the natural chemistry of the cell, enables the design of biomolecular sensors that operate independently from natural protein networks and pathways. BORs might then be used to sense a particular cellular state or pathological transformation (for example, aberrant glycosylation in cancer cells), allowing sensitive detection and therapeutic targeting.

A sustainable chemical industry

Environmentally friendly production of chemicals
As the world's fossil fuel reserves are coming to an end, chemistry needs a new raw-materials base. Synthetic biology might be the tool that enables this change, drawing from the same concepts as laid out earlier for *in vivo* synthesis of small molecule pharmaceuticals. One can imagine a set of organisms that reflect in their synthetic capacity the 'product tree' of today's organic chemical industry: first, microorganisms that produce efficiently the bulk chemicals that supply today's raw materials, and progressing to microorganisms that make ever more complex chemicals from these ingredients in ever more complicated combinations of synthetic pathways.

Environment and energy

Bioremediation
The rational modification of bacteria and other microorganisms such as fungi to eliminate toxic waste from soil has been a Holy Grail in remediation technologies for many years. Improved abilities to design complex behaviour and degradation capabilities as well as adaptation strategies within ecosystems might bring this dream into reach.

Production of energy
Just as our societies need to seek alternatives to fossil fuels as raw materials for bulk chemicals production, so they must replace such sources, ideally with renewables, for energy generation. Again, synthetic biology can help to make this transition possible and dependable. The challenge is to design a set of converging chemical pathways that allow an essentially quantitative conversion of readily available solar energy and natural or waste materials to (for example) biofuels.

GMO safety
Encoding transgene genetic information in non-natural nucleic acids might provide a safer path to genetic modification, as the presence of the transgene would at all times be dependent on the external supply of non-natural nucleic acid precursors. A genetic modification could therefore simply be removed from a transgenic plant, say, by withholding the precursor close to harvest.

Smart materials and biomaterials

There are several ways in which engineered proteins, viruses and organisms might assist in the development of new materials.

Synthesis
Biology achieves synthesis with atomic precision, for example in the construction of complex heteropolymers with exactly reproducible structures. The cellular machinery of transcription and translation has already been commandeered, via genetic engineering, to produce artificial peptide-based polymers with precise chain lengths and useful materials properties, such as silk-like or bioadhesive domains; synthetic biology will expand the range of potential target materials. Engineered cells will

make polypeptides with non-natural amino acids that have good materials properties such as cross-linking ability and useful electrical or optical behaviour. Proteins with artificially evolved recognition properties can provide the 'glue' for binding other materials together in highly selective ways.

More generally, biology provides both the inspiration and the machinery for conducting *programmed synthesis*, in which the molecular structure of the products is precisely specified by being encoded into the synthetic apparatus.

Organization

One of the major challenges for nanotechnology and nanomaterials engineering is to gain control of materials manipulation at these scales. Biology suggests several ways in which this control can be achieved. For example, motor proteins have been used to transport nanoparticles in a directed fashion, while DNA has been used as a patterning template for enabling the assembly of nanoscale objects at precisely defined locations on a surface. These approaches might allow for precise positioning in, say, the production of nanoscale electronic circuits or of molecule-based memories.

Integration

While it has proved possible to use individual biological components such as motor proteins, protein channels and light-harvesting molecules in proto-technological systems, the big challenge is to integrate and synchronize such components in functional systems, in a manner analogous to the way they operate in cells. For example, coupling photosynthetic machinery to motor proteins could enable light-driven molecular motion. It might turn out that the simplest way of achieving this degree of cooperation between components is to encode and express them in engineered or synthetic organisms in a manner analogous to the current engineering of metabolic pathways.

The areas of materials synthesis and processing that might benefit from synthetic biology include:

- biomedical materials
- microelectronics and information technology
- development of tough composites
- sensors and actuators
- materials for energy conversion

Risks and Rewards

It is clear from the above that synthetic biology is an emerging discipline with a huge potential and scope. It 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, cars or planes. Although we are far away from this point, this is clearly where the field ultimately will lead us. We can expect huge benefits as a result, but also – as with any other potent advance in science – there are risks. It is obvious that genetic manipulation of organisms can be used, or can result by chance, in potentially dangerous modifications for human health or the environment. The possibility of designing a new virus or bacterium "à la carte" could be used by bioterrorists to create new resistant pathogenic strains or organisms, perhaps even engineered to attack genetically specific sub-populations. Thus, the combination of engineering with the possibility of synthesizing whole genomes is clearly problematic.

We will not be able to eliminate the possibility of abuses of synthetic biology, any more than we can do so for other technologies. However, there are steps that can be taken to minimize risks. For example, controls and regulations can be imposed on 'parts suppliers'. In particular, companies that provide synthetic DNA sequences to order should check what they are making, and to whom they are supplying it. This will require a genomic databank of potential pathogenic microorganisms and viruses, toxic genes and gene circuits. Some DNA synthesis companies already apply such screening procedures – but it is not yet a legal requirement. It will probably be useful to set up an international committee that will explore the possible misuse of the technology and develop guidelines of how to prevent it. These guidelines should then be reflected in new laws that will regulate the exchange and access to materials and suppliers.

It has been suggested that, in addition to abuses of synthetic biology at the scales of organized terrorist groups or even biological warfare initiatives at a national level, there is a danger of the development of a 'bio-hacker' culture, in which lone individuals develop dangerous organisms much as they currently create computer viruses. Certainly, the basic technologies for systematic genetic modification of organisms are widely available and becoming cheaper; and while such modification currently requires considerable technical expertise and resources, it would be wise to anticipate this development as a possibility. Synthetic biology is already recognized and discussed as a topic within the hacker community. It is not easy to see how this sort of sociopathic activity can be prevented by the scientific community, any more than computer scientists can prevent computer viruses – it is an issue for law enforcement rather than science. But there is at least the possibility of aiming to 'deglamourize' such activities at an early stage. The chances of very serious harm being done within a 'hacker' culture are probably small: more serious abuses will surely be less common, but more threatening.

There is a pressing need to examine whether existing safety regulations for the management of engineered microorganisms provide adequate protection against inadvertent release of 'synthetic' pathogens. In particular, who is responsible for ascertaining and quantifying risks, and for implementing any clean-up measures that might need to be undertaken? There has been discussion about the insertion of 'autodestruct' modules into the genetic circuitry of engineered organisms so that they die after a certain number of rounds of cell division. Little is known so far about the feasibility of such measures, or about how robust they will be to random mutations.

In terms of risks, abuses and safety measures, it is not obvious that there is any aspect of synthetic biology that is qualitatively different from the way such issues apply to biotechnology and genetic modification, aside from the far greater capacity for manipulation and control that synthetic biology will afford (which has both

positive and negative implications). But it seems likely that the notion of creating entirely new life forms will also stimulate debates about the proper ethical boundaries of science: to some, this is sure to seem like 'playing God'. As was the case for reproductive technologies and stem-cell research, it seems likely that we do not as yet possess a conceptual ethical framework that can provide a common context for such debates: the science may have outstripped our ethical points of reference. We feel that such a debate should nonetheless be welcomed, but caution that it will be productive only if we can develop a more sophisticated appreciation of what is meant by 'life' than is current in popular discourse.

Any discussion of the potential risks of a technology as powerful as synthetic biology must inevitably sound rather alarming. But it is also important with a new technology of this sort to consider also the risks, and indeed the ethics, of not developing it. The basic techniques necessary for conducting some form of synthetic biology already exist and are publicly accessible; so indeed are the genome sequences of many pathogens. Thus, many of the risks of potential abuses exist already; and the tools of synthetic biology themselves offer the most powerful means of counteracting such threats. Insofar as they offer new and effective means of detecting and eliminating harmful biological agents, these tools will also be effective in developing defenses against existing, more conventional means of bioterrorism and biowarfare.

Quite aside from such issues, one can argue an ethical case for developing synthetic biology as a 'biotechnology that works' for the development of new drugs, particularly ones that might provide effective and affordable treatments for diseases such as malaria that present major health hazards and causes of fatalities in developing countries.

In this respect, it is encouraging to note that the ethical and safety aspects of synthetic biology have already become an integral part of the discussions in the scientific community. The synthetic biology "inaugural" conference 2004 in Boston dedicated an entire session to the topic, and the current proposals for European conferences include such activities in their proposals as well.

WHAT ACTIONS SHOULD BE PROMOTED?

Although there are different initiatives going on in USA, Japan and more recently in Europe through the NEST instrument, synthetic biology is a young discipline that needs some nurturing to flourish.

Research

Synthetic biology in Europe needs to agree as soon as possible on a set of common scientific goals in the broadest sense and on ways on how to implement the required degree of standardization in the science and engineering community. Any activity that contributes to this goal should receive a high priority. Specifically, activities such as the first European conference on synthetic biology or workshops on this or similar topics should receive support. Within the scientific community, funding organizations like ESF and EMBO should be approached to involve them in corresponding activities. Furthermore, contact with National Funding Agencies needs to be established and integrated in this effort in order to avoid duplications. Such efforts would benefit from Specific Support Actions or Coordination Actions, and on a larger scale from IP initiatives.

Infrastructure

The future development of synthetic biology will require a consistent application of the engineering design paradigm (see above). This implies the need to define suitable standardization procedures for biological parts, and facilities where such parts can be stored and controlled. Although the requirement for a physical repository will become smaller over time (due to decreasing costs in mass DNA synthesis), this curatorial function will be essential for the success of synthetic biology in its initial phase. In the initial phase, preparatory actions for allowing access to the crucial technology of DNA synthesis could be considered. Such initiatives would benefit from infrastructure funding in the 7th Framework Programme.

Education

Synthetic biology is a truly interdisciplinary endeavour that will draw on expertise from all the natural and engineering sciences. However, the availability of the correspondingly trained individuals is very scarce. Consequently, there is a need to train such scientists at various levels, from post-doctoral researcher (for more immediate needs) to undergraduate student (allowing a more in-depth interdisciplinary training). Appropriate tools that merit support are summer courses at undergraduate, graduate, and post-doc level as well as novel forms of teaching such as the MIT-inspired summer competition in synthetic biology for undergraduates and graduates. It should be ensured that such activities always include consideration of the ethical aspects. Irrespective of such "ad hoc"-type of measures, the long-term goal should be to build synthetic biology into standard educational structures.

PART II

SCIENTIFIC ACTIVITIES AND CHALLENGES

This section aims to provide an overview of current activities worldwide in synthetic biology, as well as some of the major questions and hurdles that these initiatives face. Thus, it can be regarded as a survey of the current state-of-the-art, and an indication that synthetic biology already exists as an active field with many facets.

Classification of activities

1. Device fabrication and characterisation

a. Input devices: cell surface proteins, sensors, input parts
b. Regulatory elements: inverters, logic gates, transcription, translation, phosphorylation, etc.
c. Output devices: pathways, etc.

2. System design and synthesis

a. Developing a hierarchy of parts, devices and systems (standardized)
b. Creation of synthetic organisms: top-down, bottom-up. Building gene networks and circuits, programmable systems
c. Building artificial cells or compartments, either replicating or not
d. Materials and nano-technology
e. Developing genomes from using non-natural nucleotides, proteins from non-natural amino acids
f. In-cell synthesis of chemicals, materials and biopharmaceuticals

3. Enabling Infrastructure

a. DNA synthesis and sequencing
b. Micro-fluidics
c. Protein engineering, directed evolution
d. Computer aided design tools: simulation of gene circuits
e. Measurement, measurement of noise and variation

Description of activities

1. Device fabrication and characterisation

a. *Input devices: cell surface proteins, sensors, input parts*

Although there is abundant literature on biosensors, in general most efforts in this area deal with biosensors in medicine but not with their direct link to synthetic biology. A seminal paper by Hellinga[2] and co-workers reports the use of a protein design algorithm to reengineer a sugar-binding protein from *E. coli* to bind various new substrates: molecules of the explosive TNT, the metabolite lactate and serotonin, a compound used by brain cells to communicate. The team members plugged the redesigned protein into an engineered gene circuit, which they stuck into a bacterium so that it glows green when it detects its target chemical. Similar

[2] http://www.biochem.duke.edu/Hellinga/hellinga.html

microbial biosensors could be used for subsea monitoring, detection of environmental pollutants, and in medical diagnostics.

There are other scant reports about groups trying to develop sensors and to couple them to biological systems. For example scientists at the University of Alberta are trying to develop a plant whose leaf shape or flower colour changes when a land mine is buried below it. The plant's roots would have to be genetically altered to detect explosives traces in the soil and to communicate that information to the leaves or flowers. In another example, Mrksich[3] developed a biological input/output capability consisting of experimental tools and predictive models that can be used to interact with biological systems, all based on the well understood and easily manipulated 'yeast a' factor signal transduction pathway. In single cell measurements by newly developed optical tools, the group of Walt[4] has developed a system in which genetically engineered cells express different reporter molecules and can be screened simultaneously for drug candidates or environmental toxins.

b. *Regulatory elements: inverters, logic gates, transcription, translation, phosphorylation, etc.*

Devices can be regarded as combinations of parts that perform useful elementary functions, out of which entire systems can be composed. In analogy to electrical engineering, they are characterized by relatively simple input-output relationships such as logical operations on the inputs. Engineered functions such as simple feedback loops, amplifiers and switches can be classified into the same category. As devices represent the 'building blocks' for engineered systems design, important aspects are standardization and reliability.

Most of the efforts in device development have focused on appropriate wiring of genetic constructs on prokaryotes, where it is possible to exploit some of the "naturally engineered" modularity of cellular regulation. Examples include early investigations in the Serrano group[5] on the role of feedback in gene regulation, using designed components. For simple logical gates, simple natural bacterial gene modules called operons served as example devices. In terms of engineering design, an inverter (yielding 'high' output at 'low' input and vice versa) was established by Weiss[6]. The group of Collins focused on different implementations of switches[7] (bistable devices) as minimal requirements for implementing memory as well as on interfaces between circuits[8]. More recent developments concern (i) the engineering of logic gates in mammalian cells by Fussenegger[9], which may significantly extend the realm of synthetic biology to medical applications[10] and (ii) protein (domain) engineering for single-molecule synthetic devices initiated by Lim[11], in which complex logical behaviour at the protein level in response to different intracellular signals is implemented by combining protein modules into a single "actuator".

In contrast to the fabrication of parts, device development is being done in an uncoordinated, ad hoc manner. Systematic approaches, e.g. for establishing a library of well-characterized (logical) devices appear to be the next logical step. For practical use in synthetic biology, however, improving the reliability of devices

[3] http://chemistry.uchicago.edu/fac/mrksich.shtml
[4] http://chem.tufts.edu/faculty/walt/
[5] NATURE 405: 590-93 (2000)
[6] Thesis, MIT (2001)
[7] NATURE 403: 339-42 (2000)
[8] PNAS 100: 7714-19 (2003)
[9] http://www.biotech.biol.ethz.ch/
[10] Biotech. Bioeng. 87: 478-84 (2004)
[11] SCIENCE 301: 1904-08 (2003)

(which are strongly affected by noise and leakage in gene expression), seems a major challenge.

2. System design and synthesis

a. *Developing a hierarchy of parts, devices and systems (standardized)*

This topic is pretty much at the heart of synthetic biology. In order to integrate a large variety of independently optimized parts into a larger system architecture, we need to define the interfaces in such a way that any novel part can be reliably and robustly inserted into the system. This is pretty much in contrast with the current reality in biology where essentially each part of the system is a unique – messy - result from millions of years of evolution and frequently is subject to a large amount of cross-regulation from functionally rather distant elements of cellular function. On the other hand, attempts – conceptually on the level of whole cell organization, experimentally on a simpler level - have been made to organize the processes in a cell into functional modules (protein synthesis, DNA replication, etc. on whole cell level) suggesting that this modular approach has a real equivalent in the organization of life. In the biological world, modularity is based on chemical isolation, achieved either by spatial separation (organelles) or chemical specificity (a compound belongs to glycolysis because it interacts only with enzymes of glycolysis). One (relatively) straightforward approach to reproduce such a level or organization would be to separate the components into many different compartments and to regulate their interaction at specific points, for example by exploiting microfluidic technology. Such organization would prevent the need to optimize the chemical interactions between modules, because they would be separated by a physical barrier.

On a more molecular level, attempts to define interfaces have already begun, for example the biobrick and the NOMAD concepts[12] of assembling DNA blocks into plasmids or the proposal of the MIT working group on synthetic biology to establish a standard for promoter "strength" (polymerases/second, PoPS) Furthermore, it is conceivable that model-based protein engineering technologies will allow one to use specific "key" proteins as scaffolds into which novel functions can be engineered while the output part of the scaffold remains the same. This way, it is also conceivable that interfaces can be designed between different regulatory circuits.

b. *Creation of synthetic organisms: top-down, bottom-up. Building gene networks and circuits, programmable systems*

In order to be able to carry out rational strain-engineering experi-ments, it would be desirable to have a strain of minimal complexity that is completely characterized. In addition, it is desirable to have a strain in hand that is still able to grow on cheap media, but whose metabolic capabilities have been reduced to a minimum so that any activity like engineering novel pathways can proceed with a minimum of intermediates diverting the flow of materials into wrong pathways.

There are a small number of projects that try to **reduce the genome size of bacteria to a bare minimum** (the top-down approach **to synthetic organisms**). In particular, two organisms have been addressed: *E. coli* and *B. subtilis*. And there is at least one minimal genome project that follows the reverse (bottom-up)approach, which is to start with a bacterium that already appears to be stripped down to a bare minimum.

[12] http://parts.mit.edu

Synthetic gene circuits are at the core of synthetic biology. Experimentally built circuits are simple input-output systems so far, with a small number of components. Nevertheless, they are good starting point for the next generation of such circuits. Synthetic gene circuits constructed so far have been used to modify cell behaviour and to perform measurements in single bacterial cells. However, new work on gene networks in cancer cells, carried out in the frame of the EU funded NEST project NETSENSOR[13], promises to take this concept one step further by applying the concept to actually carefully monitor the mechanistic cause of cancerogenic behaviour and illicit an appropriate response.

Weiss[14] and Knight[15] pioneered a number of approaches in synthetic circuits. They proposed to use gene regulation elements as logic gates, i.e. small computing units. Collins[16] built a bi-stable switch and later was able to use it as a sensor for intracellular signals (DNA damage). He also designed very useful novel regulatory motifs, such as ribo-regulators. Lim[17] works in the area of cell signaling; he demonstrated synthetic logic gates based on modified kinases. Endy and Knight work to enable the design and construction of large-scale integrated biological systems. They are currently exploring the application of three past engineering lessons: (1) standardization of components, (2) component abstraction and (3) decoupling of system design from system fabrication. Bar-Ziv[18] works on synthetic gene circuits, in particular focusing on their *in vitro* construction.

There is a parallel use of synthetic gene circuits as tools to study biological phenomena or to test theories regarding gene regulation mechanisms. This direction was developed by Becskei, Serrano, Elowitz, Leibler and others.

"Programmable synthetic systems" extend beyond the realm of synthetic biology: they include molecular ensembles with interesting emergent properties, usually related to information processing. They are driven by an attempt to realize complex logic using molecules. Various directions in this field include molecular computing, molecular automata, chemical logic gates, etc. Some of the results in these fields may be used in a biological context, making them potentially useful for synthetic biology.

Benenson and Shapiro focus on the development of autonomous bimolecular computing devices, in particular finite automata, and their integration with the biochemical environment[19]. In particular, they are driven by potential biomedical applications, i.e. molecular-level diagnostics and treatment ("smart drugs"). Stojanovic and Stephanovic study complex logic systems based on ribozymes[20]; their aim is to couple their systems to external molecular signals to activate drugs. So far they have demonstrated an RNA and DNA-based automaton that plays Tic-Tac-Toe. Winfree, Seeman and Reif strive to utilize various processes of DNA self-assembly to perform logic operations, computations and controllable behavior in general[21, 22]. The utility of these approaches in the context of biological systems is unclear, but these works may certainly provide inspiration for synthetic biology research. De Silva[23], Ghadiri[24] and a number of other researchers implement

[13] http://netsensor.embl.de/
[14] NATURE 428, 868-871 (2004)
[15] http://www.broad.mit.edu/annotation/microbes/mesoplasma_florum/background.html#ref
[16] PNAS 101, 8414-8419 (2004)
[17] SCIENCE 299,1061-1064 (2003)
[18] PNAS 100, 12672-12677 (2003)
[19] NATURE 429, 423-429 (2004)
[20] NATURE BIOTECHNOLOGY 21, 1069-1074 (2003)
[21] NATURE 394, 539-544 (1998)
[22] NATURE 407, 493-496 (2000)
[23] CHEMISTRY-A EUROPEAN JOURNAL 10, 574-586 (2004)
[24] JOURNAL OF THE AMERICAN CHEMICAL SOCIETY 126, 11140-11141 (2004)

Boolean logic using molecular systems. The systems work in vitro, but may inspire novel approaches in synthetic biology.

c. *Building artificial cells or compart-ments, either replicating or not*

The 'bottom-up' construction of cell-like entities has both fundamental and applied goals. It could tell us something about the minimal requirements of life-like systems that can grow, divide and evolve – and thus allow an exploration of the possible systems that could have appeared on the early Earth. And such 'cells' could act as 'factories' for the synthesis of biological molecules that are easier to design, control, adapt and sustain than natural cells are, such as in the NEST project NEONUCLEI[25], funded under the 6th Framework Programme: To realize the concept of personalized medicine, we need to reconsider modes of production because the concept of running millions of different production processes for complex biomolecules is unsustainable. An alternative might be to develop easy-to-multiplex self-assembling particles, being capable of sustaining gene transcription for a time period, which is long enough for the production of the required quantities of "personal biopharmaceuticals".

Also in this direction Libchaber has reported lipid vesicle-based systems containing pared-down genetic machinery (DNA and ribosomes) that can generate proteins when provided with the raw ingredients. He was able to perforate the membrane with pore-forming proteins, allow nutrients to get into these protocells[26].

The PACE consortium[27], funded under the IST-FET activity of the 6th EU Framework Programme, aims to "focus on the IT potential of truly artificial cells: addressing both the technical opportunities of programmable artificial cells and an evolutionary roadmap to producing them under the control of current computers." It does not yet seem clear to what extent these 'artificial cell' initiatives will be based in robotics as opposed to truly biologically derived chemistry.

Szostak aims to develop model 'protocells' that can replicate. These will most probably be based on ribozymes with replicase activity, encased in a membrane of fatty acids. Szostak is part of an international collaboration called ProtoCell[28], which aims "to understand and harness the basic principles of chemical living systems".

Luisi[29] has devised self-replicating micelles and vesicles made from hydrolysable surfactants. The hydrolysis process, which generates new vesicle-forming molecules, is catalysed by the vesicles themselves, enabling them to display replication-like behaviour. Luisi is aiming to incorporate these structures into a minimal cell, and has been able to put DNA and plasmids inside them. His group is also exploring a minimal RNA-based cell in collaboration with Szostak and Bartel.

d. *Materials and nanotechnology*

While there is now a fairly well established tradition in molecular nanotechnology of deriving inspiration from biological solutions to 'engineering' problems, increasingly researchers in this area are making use of genuinely biological materials and systems for technological ends. This typically involves the chemical modification of biomolecules, sometimes at the level of altering their genetic coding, and so to this extent such work can be considered a part of synthetic biology.

[25] http://www.neonuclei.soton.ac.uk/
[26] http://www.rockefeller.edu/labheads/libchaber/libchaber-lab.html
[27] http://134.147.93.66/bmcmyp/Data/PACE/Public
[28] http://www.protocell.org
[29] http://www.plluisi.org/grl_luisi.html

Belcher[30] is exploring a wide range of biological materials for nanotechnological uses. In particular, she has devised an *in vitro* selection procedure for isolating peptides that will recognize and bind to a range of inorganic materials (for example, semiconductors like ZnS, CdS, GaAs), providing a potential interface between the biological and inorganic worlds.

In closely related work, Sarikaya[31] is developing genetic and biochemistry (combinatorial) strategies to make biopolymers (proteins and other macromolecules) that can act as templates and 'adhesives' for assembling functional inorganic particles and thin films. Vogel[32] and Hess[33] have modified and developed the kinesin/microtuble system for nanoscale directed transport. They are interested in using these biomolecular machines as 'molecular shuttles' and as tools to create complex materials, repair tiny defects on surfaces or in living cells, and to store and retrieve information.
Montemagno[34] has used several natural proteins, modifications thereof, and protein assemblies in nanotechnological contexts. In 2000 his group modified the rotary molecular motor ATP synthase so that it could be attached to nanoscale metal pillars and drive a nanoscale nickel rotor bound to the protein spindle. They have also used the proton pump bacteriorhodopsin to drive protons against a chemical gradient across the proton-exchange membrane of fuel cells, reducing proton leakage and increasing the device efficiency. Montemagno's group is now seeking to incorporate these devices into larger-scale integrated systems such as 'biosolar cells'.

Francis[35] is chemically modifying viruses so that they can act as templates for inorganic crystallization (for example, to form metallic/magnetic nanowires), and as drug-delivery vehicles. Erlanger[36] has 'linked immunology with nanotechnology' by making antibodies that bind to C_{60} and single-walled carbon nanotubes. Zhang[37] is designing peptide materials based on natural self-assembly principles. He has created peptide amphiphiles that form membranes in which the photosystem of green plants can be kept 'active' *in vitro*, so that it might effect light-induced electron transport for solar-power generation.

Bayley[38] is exploring the expression of protein-based materials that form porous sheets, fibers, adhesives and elastomers. His group has engineered abductin, a protein found in the elastomeric inner hinge ligaments of bivalve mollusks, to create elastomers for use in thin film technology and in microfluidic and energy storage devices, and are developing technological applications of α-hemolysin, a bacterial toxin that forms a heptameric transmembrane pore, in areas including drug delivery and the construction of biosensors.

e. *Developing genomes from using non-natural nucleotides, proteins from non-natural amino acids.*

As indicated earlier, several groups are seeking ways to incorporate new building blocks into biologically generated polymers – for example, non-natural amino acids and nucleotides. Others are looking for *de novo* polymers that mimic some of the properties of biopolymers, such as non-natural oligopeptides with new folds and secondary structures. The motivations here are often for fundamental science, but

[30] http://belcher10.mit.edu/
[31] http://faculty.washington.edu/sarikaya/projects/projects.html#BiomimeticMaterials)
[32] http://www.nanomat.mat.ethz.ch
[33] http://faculty.washington.edu/hhess/
[34] http://www.cnsi.ucla.edu/faculty/montemagno_c.html
[35] http://www.cchem.berkeley.edu/francisgrp/
[36] http://www.research.hs.columbia.edu/Faculty_Profiles/profiles/erlanger_bf.htm
[37] http://web.mit.edu/lms/www/ and http://web.mit.edu/shuguang/www/resume.html
[38] http://bletchley.tamu.edu/homepage/)

such work might also yield drugs that interact with natural proteins and nucleic acids in new ways, or peptide-based materials with new structures and properties.

Schultz[39] has developed methods to genetically encode novel amino acids in bacteria. Schultz's group has synthesized a completely autonomous bacterium that not only genetically encodes the novel amino acid aminophenylalanine but also biosynthesizes it from basic carbon sources. They have extended this methodology to yeast cells, in which 5 non-natural amino acids have now been genetically encoded.

In 1984, Benner reported the chemical synthesis of an artificial gene encoding a designed enzyme. He and his coworkers introduced expanded DNA alphabets in 1989, and developed these into an 'artificially expanded genetic information system' which enables the synthesis of proteins with more than 20 encoded amino acids.

Kool[40] is exploring artificial genetic coding schemes that use non-natural nucleotides. His group isaiming to use such approaches to engineer a new telomere-maintenance pathway for human cells, and they have chemically modified DNA with the aim of detecting the small genetic mutations that cause cancer and drug resistance. These molecules are being tested for application in pathogenic bacteria and for detection of leukemias. Tirrell[41] is combining organic, biological and materials chemistry to make new kinds of polymers with controlled architectures. In particular, he pioneered the incorporation of artificial genes into microbes that encode novel peptide sequences with interesting materials properties (for example, forming liquid-crystal phases, hydrogels, and materials for tissue engineering), as well as developing methods for incorporating non-natural amino acids into these peptides (such as fluorinated variants).

f. *In-cell synthesis of chemicals, materials, and biopharmaceuticals*

The field of metabolic engineering is traditionally close to the definition of synthetic biology, as it has always been aiming at creating industrial methods for the production of chemicals and usually also exploits rational model-supported, engineering rooted approaches for the design of experiments. However, as metabolic engineering typically involves the exploitation of the whole cell, it also has to cope with a very high complexity that is typically not well amenable to rational analysis. In other words, it has often relied on 'tinkering' rather than rational "design-based" engineering, frequently leading to only minor re-engineering of cellular properties. Nevertheless, given sufficient manpower and time, the approach has resulted in some very successful industrial projects with some model bacterial systems, in particular *E. coli* and *B. subtilis*, leading to novel production systems for vitamins and fine chemicals.

However, the approach has been extended in two directions: [i] towards the production of novel products, most prominently the production of isoprenoid derivatives with microbes and the production of novel pharmaceutical intermediates. These projects require frequently the reassembly of alien and large pathways in bacteria that are suitable for cheap production processes. Here, the current abundance of genomic and metagenomic sequence data opens up novel opportunities in the area of *in vitro* assembly of artificial biocatalytic pathways that would allow novel routes to rather complex, typically pharmaceutically important, fine chemicals. [ii] Alternatively, the issue of complexity of the production system

[39] http://schultz.scripps.edu
[40] http://www.stanford.edu/group/kool
[41] http://www.cce.caltech.edu/faculty/tirrell/research.html

can be addressed, as in the NEST project EUROBIOSYN[42]. By using comprehensive genomic models of bacteria followed by targeted knock-outs, it is possible to create isolated, modular pathways which allow in principle realization of multi-enzyme pathways. Such pathway modules will in the future be combined to realize particularly difficult and complex synthesis problems.
Finally, another EU project under the NEST synthetic biology initiative is promising to help paving the road to truly personalized medicine by revolutionizing the access to hundreds of thousands of highly specific biopharmaceuticals for human therapy. Only once medicines with such subtle differences can be manufactured reliably at a small scale – something that synthetic biology could enable – is this vision achievable. The HYBLIB project[43] aims at realizing this promise by dramatically shortening development times for specialized antibodies.

[42] http://www.eurobiosyn.org
[43] http://www.dkfz.de/en/b120_cbpl/mitarbeiter/frank_en.html

3. Enabling Infrastructure

a. DNA synthesis and sequencing

As biologists learn to shape cellular circuits and their molecular components, developments in the automated chemical synthesis of DNA are allowing entire genomes to be designed and assembled. Venter's lightning-fast synthesis of a virus in November 2003[44] was a testament to the expanding capacity of DNA synthesis machines. By some estimates, **by 2010** machines will be able to generate sequences about a million base pairs long — roughly the size of the genome of *Chlamydia*, which causes a common sexually transmitted disease, and a quarter the size of *E. coli*'s genome.

"Bacterial genomes are within the range of current DNA-synthesis technology," according to John Mulligan, president of the DNA-synthesizing company Blue Heron Technology in Bothell, Washington[45]. But bacterial genomes must be embedded within a cell and its attendant biochemical machinery, making them much harder to synthesize than viruses. Nevertheless, attempts are under way. In November 2002, Venter made a high-profile announcement of his intention to build a simple bacterium starting with machine-made DNA.

A new methodology similar to that used to make computer chips can generate oligonucleotides in thousands of tiny reaction wells and release the sequences synthesized. These are then assembled by enzymes.

It seems that this is a mature field in which there are already many biotech companies[46]. Commercial suppliers routinely offer syntheses of 10,000-40,000 base pairs. There is no technical reason why larger constructs will not become available in the future. Commercial plasmid-synthesis companies currently construct large pieces of DNA for less than $1.5 per base. The price continues to drop, making the contract-synthesis of an entire bacterial genome feasible at costs comparable to pre-clinical drug development. Just as cheap transistors preceded the computer revolution, commoditisation of DNA synthesis will spur huge changes in biological construction. The capability to cheaply synthesize DNA is so powerful that no one quite knows what to do with it. Market pressures are already prompting biotech companies to speed up the DNA synthesis process.

The only barrier at the moment is due to the fact that chemical reactions are prone to errors, and a major barrier that slows down DNA synthesis is the need to correct errors and verify the correctness of a molecule that can have hundreds of millions of base pairs.

b. *Microfluidics*

The application of microfluidic devices – structures that direct complex flow patterns and chemical operations on the micrometre scale – is rapidly developing into a fundamental experimental technique, for example driven by the systems biology-induced requirement for highly automated, parallel experiments. In two areas, the main advantages become very clear: in the area of analysis, where several thousand analyses can be conducted at the same time, and in microTAS (micro-total analysis system, lab-on-a-chip) applications, which allow the miniaturization of entire sequences of processing steps onto chips (e.g. on-chip PCR, on-chip protein purification). Looking towards emerging application areas, synthetic biology will

[44] PNAS 100, **15440-15445, 2003**
[45] http://www.blueheronbio.com/
[46] An example of a German company doing synthesis: http://www.atg-biosynthetics.de/

benefit tremendously from this development. For example in the area of biosensors, miniaturization of sample preparation and actual measurement will allow highly precise, robust, and long-term analysis of even difficult samples. Furthermore, in view of the expected future increases in DNA synthesis capacity, microfluidics will be indispensable in the design of economic DNA synthesis protocols that are required to make the synthesis a genuinely routine tool in the lab.

Synthetic biology will also rely on the exploitation of microfluidic technology at a more fundamental level. It is clear that the intrinsically stochastic mechanism of living cells will prevent the required precision in prediction of cellular functions, which will drive the need to establish single-cell analysis systems at the micro-level. On the same note, one way to achieve truly novel functionalities in synthetic biology will be to trigger the behaviourof designed biological systems with easy-to-manipulate external stimuli such as electronics, light, magnetic fields or mechanic forces. This will inevitably require precise control of reagents, components and systems in time and space at the micro-level.

Finally, microfluidic systems are in a manner of speaking the functional, man-made equivalents of cells, which may transform the task of cellular organization by chemical specificity into the alternative of organization by spatial separation.

Pressing issues in micro-fluidics are already mainly driven by the life-sciences community. A large set of basic tools (pumps, valves, mixers) is available that is currently integrated into diverse applications. Another intensively researched topic is the application of microfluidics to parallelize growth experiments and to increase the complexity in operations that can be handled by these microfluidic systems.

c. *Protein engineering, directed evolution*

Although protein engineering has existed already for many years (since the early days of biotechnology), this discipline may in some cases also be considered part of synthetic biology. This is for example the case when the engineered proteins with unique properties are part of a more complex synthetic system such as, for example, gene networks, complex sensor systems, logic circuits, etc.

In these situations, an engineered protein will have to be much more than simply a fusion protein composed, for example, of two or three functional domains derived from other proteins. Typically, such an engineered protein will have to be able to read an input signal and to produce an output signal.

The seminal work of Lim's group demonstrated how complex logic decision procedures can be rationally manipulated not only on genetic but also on protein level, when the specific combination of protein modules is directing cellular responses to input signals in different directions[47].

d. *Computer aided design tools: simulation of gene circuits*

Simulation of gene circuits belongs to the set of basic technologies that are common to systems biology and synthetic biology. The simulation of biological/biochemical networks was first attempted in the 1960s. Today a large collection of *general* modelling and simulation tools for biological circuits, both commercial and open-source products, is available. In a community effort, development of a standardized model exchange language (Systems Biology Markup Language, SBML), a software

[47] SCIENCE 301 1904-1908 (2003)

broker for SBML-based communication between tools (Systems Biology Workbench, SBW), and software libraries and model repositories has ensured compatibility of currently ~80 modelling and simulation tools[48]. Most of these tools, however, are not supported by large-scale funding and corresponding consortia, hindering software integration and commercial-quality software production. A few initiatives constitute exceptions to this, namely the E-Cell project[49], the Virtual Cell[50] and BioSpice[51].

Because these tools have been developed for systems biology, however, in general they do not have the capabilities of their equivalents in electrical engineering, such as standardization of model components (supplying, for example, re-usable standardized parts in the engineering of genetic circuits) and the optimization of circuit design. Standardization of representations for biological circuits and their elements has only recently begun[52] and first attempts at using optimization methods for the design of artificial genetic circuits were recently published[53]. The BioSpice project in the U.S. (a DARPA-funded large-scale collaboration) appears to be closest to an integrated design tool for synthetic biology for several reasons: (i) its origins in the electrical circuit design tool Spice and corresponding operation, (ii) the ability to build tool chains that allow for a consistent handling of information involved in modelling, from storage of experimental data to simulation, and (iii) the availability of a variety of simulation and analysis methods. Certain aspects such as circuit optimization and the analysis under conditions of intracellular noise, however, will have to be added.

Hence, thanks to earlier efforts in systems biology, general modeling and simulation tools do not appear to create a bottleneck for synthetic biology, but nevertheless there is still a need to turn these models into design tools. In terms of software development, large-scale initiatives currently only exist in the U.S. and in Japan, with a fragmented - yet potentially compatible - tool landscape emerging in Europe too. Access to these tools is in most cases not a limiting factor, however, because the majority of them follows open source principles. It can be anticipated that open source and open standard developments - with commercial harvesting opportunities – will become more prevalent because this is commonly a requirement of (U.S.) funding agencies and scientific publishers[54].

e. *Measurement, measurement of noise and variation*

Most of the measurement techniques relevant to synthetic biology are single cell measurements. These techniques include fluorescent labeling of proteins (using GFP and other xFP's), indirect labelling of RNA using RNA-binding fluorescent proteins, and indirect binding of DNA using fluorescent proteins. Such measurements are performed using various microscopy techniques. In some cases, single molecules may be detected and counted. These techniques provide a much more detailed understanding of the cellular processes, in particular gene expression. For example, standard deviations and fluctuations may be measured. These data may be very useful for designing artificial networks at the cellular level.

[48] www.sbml.org
[49] www.e-cell.org; lead by M. Tomita, Keio Univ., JP
[50] www.nrcam.uchc.edu; lead by J. Schaff, Univ. Conneticut, U.S.
[51] www.biospice.org; lead by A. Arkin, UC Berkeley, U.S.
[52] Kitano (2003)
[53] Feng et al. (2004)
[54] DARPA Workshop on "Open on Tool and Software Infrastructure for Systems Biology", Washington / DC, Feb. 2005

Elowitz[55] measures statistical parameters of gene expression, such as fluctuation in input-output relationship between transcription factor and its regulated gene ("noise"). The data obtained in his experiments might be very useful for designing successful gene circuits. Singer[56] and colleagues develop methods to directly visualize various molecules on a single cell level, and measure the kinetics and diffusion properties of transcription-translation sequences. Xie and colleagues [57] have recently developed a unique method to measure single events of protein translation in living cells. Alon[58] is mainly interested in systems biology, but his lab also does some experimental work including single-cell measurements, combined with modeling.

[55] SCIENCE 307, 1962-1965 (2005)
[56] SCIENCE 297, 836-840 (2002)
[57] ABSTRACTS OF PAPERS OF THE AMERICAN CHEMICAL SOCIETY 228: U230-U230 199-PHYS Part 2, (2004)
[58] NATURE GENETICS 36, 147-150 (2004)

Additional References

Useful literature and website of groups that are active in the field of synthetic biology. (classified according to the type of activity).

I. DEVICE FABRICATION AND CHARACTERISATION

I.b. REGULATORY ELEMENTS: INVERTERS, LOGIC GATES, TRANSCRIPTION, TRANSLATION, PHOSPHORYLATION, ETC.

Representative papers:

- Becskei A., Serrano L. "Engineering stability in gene networks by autoregulation" Nature 405: 590-93 (2000).
- Gardner T.S., Cantor C.R., Collins J.J. "Construction of a genetic toggle switch in Escherichia coli" Nature 403: 339-42 (2000).
- Isaacs F.J., Hasty J., Cantor C.R., Collins J.J. "Prediction and measurement of an autoregulatory genetic module" PNAS 100: 7714-19 (2003).
- Weiss, R. "Cellular computation and communications using engineered genetic regulatory networks" Thesis, Massachusetts Institute of Technology (2001).
- Kramer B.P., Fischer C., Fussenegger M. "BioLogic gates enable logical transcription control in mammalian cells" Biotech. Bioeng. 87: 478-84 (2004).
- Dueber J.E., Yeh B.K., Chak K., Lim W.A. "Reprogramming control of an allosteric signaling switch through modular recombination", Science 301: 1904-08 (2003).

II. SYSTEM DESIGN AND SYNTHESIS

II.a. DEVELOPING A HIERARCHY OF PARTS, DEVICES AND SYSTEMS (STANDARDIZED)

Drew Endy (MIT, Boston, USA)
PoPS (polymerases per second, how to standardize biological parts)
Website: http://web.mit.edu/be/people/endy.htm
Representative papers:

- Endy & Brent, Nature 409: 391-395 (2001)

Tom Knight (MIT, Boston, USA)
Biobricks (plasmid assembly systems, how to standardize biological parts)
Website: http://web.mit.edu/synbio/www/

Registry of Standard Biological Parts (MIT, Boston, USA)
Database and DNA repository of simple and complex biological parts that may be combined to implement simple biological systems. These parts are used by students in the Intercollegiate Genetically Engineered Machine (iGEM) summer competitions.
Website: http://parts.mit.edu

Leland H. Hartwell (Seattle, USA); John J. Hopfield (Princeton, USA); Stanislas Leibler (Rockefeller, New York, USA) and Andrew J. Murray (Harvard University, Boston, USA)
Modular biology (modules as an organizational principles in cells)
Website:http://genomics.princeton.edu/hopfield/Biography.html;
http://www.rockefeller.edu/labheads/leibler/contact.php;
http://www.mcb.harvard.edu/Faculty/Murray.html
Representative papers:

- Hartwell *et al.*, Nature 402: C47-C52 (1999)

II.b. BUILDING GENE NETWORKS AND CIRCUITS, PROGRAMMABLE SYSTEMS

Ron Weiss (Princeton University, USA)
Weiss together with Tom Knight (MIT) pioneered a number of approaches in synthetic circuits. They proposed to use gene regulation elements as logic gates, i.e. small computing units. Some of the recent work was done in collaboration with F. Arnold from Caltech.
Website: http://www.princeton.edu/~rweiss/

Representative papers:

- Programmed population control by cell-cell communication and regulated killing
  You LC, Cox RS, Weiss R, Arnold FH
  Nature 428 (6985): 868-871 Apr 22 2004
- Directed evolution of a genetic circuit
  Yokobayashi Y, Weiss R, Arnold FH
  Proceedings of the National Academy of Sciences 99 (26): 16587-16591 DEC 24 2002
- Optimizing genetic circuits by global sensitivity analysis.

  Feng X., Hooshangi S., Chen D., Li G., Weiss R., Rabitz H.

  Biophys. J. 87:2915-202 (2004).

James Collins (Boston University, USA)
Collins is one of the most prolific scientists working in the field of synthetic biology and in particular synthetic gene circuits. He built a bi-stable switch and later was able to use it as a sensor for intracellular signals (DNA damage). He also designed very useful novel regulatory motifs, such as ribo-regulators.
Website: http://www.bu.edu/dbin/bme/faculty/?prof=jcollins
Representative papers:

- Programmable cells: Interfacing natural and engineered gene networks Kobayashi H, Kaern M, Araki M, Chung K, Gardner TS, Cantor CR, Collins JJ Proceedings of the National Academy of Sciences 101 (22): 8414-8419 JUN 1 2004
- Construction of a genetic toggle switch in Escherichia coli
  Gardner TS, Cantor CR, Collins JJ
  Nature 403 (6767): 339-342 Jan 20 2000

Wendell A. Lim (University of California, San Francisco, USA)
Lim works in the area of cell signalling; he demonstrated synthetic logic gates based on modified kinases.
Website: http://www.ucsf.edu/limlab/
Representative papers:

- Rewiring cell signaling: the logic and plasticity of eukaryotic protein circuitry
  Dueber JE, ***Yeh BJ***, ***Bhattacharyya RP***, ***Lim WA***
  Current Opinion in Structural Biology 14 (6): 690-699 Dec 2004
- Rewiring MAP kinase pathways using alternative scaffold assembly mechanisms
  Park SH, Zarrinpar A, Lim WA
  Science 299 (5609): 1061-1064 Feb14 2003

Drew Endy (MIT, USA)
Focuses on enabling the design and construction of large scale integrated biological systems. He is currently exploring the application of three past engineering lessons: (1) standardization of components, (2) component abstraction and (3) decoupling of system design from system fabrication.
Website: http://web.mit.edu/be/people/endy.htm

Roy Bar-Ziv (Weizmann Institute, Israel)
Focuses on synthetic gene circuits, in particular their *in vitro* construction.
Website: http://www.weizmann.ac.il/materials/barziv/
Representative papers:

- Principles of cell-free genetic circuit assembly
  Noireaux V, Bar-Ziv R, Libchaber A
  Proceedings of the National Academy of Sciences 100 (22): 12672-12677 Oct 28 2003

Kramer B. P. (ETH, Zurich, Switzerland)
Focuses on developing gene circuits in eukaryotes
Website: http://www.fussenegger.ethz.ch/people/bkramer/
Representative papers:

- An engineered epigenetic transgene switch in mammalian cells
  Kramer BP, Viretta AU, El Baba MD, Aubel D, Weber W, Fussenegger M
  Nature Biotechnology 22 (7): 867-870 Jul 2004

Yaakov Benenson and Ehud Shapiro (Weizmann Institute, Israel)
Focus on the development of autonomous biomolecular computing devices, in particular finite automata, and their integration with the biochemical environment. In particular, they are driven by potential biomedical applications, i.e. molecular-level diagnostics and treatment ("smart drugs").
Website: http://www.weizmann.ac.il/mathusers/lbn/new_pages/Research_Biological.html
Representative papers:

- An autonomous molecular computer for logical control of gene expression
  Benenson Y, Gil B, Ben-Dor U, Adar R, Shapiro E
  Nature 429 (6990): 423-429 May 27 2004
- Programmable and autonomous computing machine made of biomolecules
  Benenson Y, Paz-Elizur T, Adar R, Keinan E, Livneh Z, Shapiro E
  Nature 414 (6862): 430-434 Nov 22 2001

Milan Stojanovic (Columbia University, USA) and Darco Stefanovic (U of New Mexico, USA)
Study complex logic systems based on ribozymes; their aim is to couple their systems to external molecular signals to activate drugs. They demonstrated an RNA and DNA-based automaton that plays Tic-Tac-Toe.
Website: http://www.cs.unm.edu/~darko/biomolcomp.html
Representative papers:

- A deoxyribozyme-based molecular automaton
  Stojanovic MN, Stefanovic D
  Nature Biotechnology 21 (9): 1069-1074 Sep 2003
- Deoxyribozyme-based logic gates
  Stojanovic MN, Mitchell TE, Stefanovic D
  Journal of the American Chemical Society 124 (14): 3555-3561 Apr 10 2002

Erik Winfree (Caltech), Nadrian Seeman (NYU), John Reif (Duke University)
Strive to utilize various processes of DNA self-assembly to perform logic operations, computations and controllable behaviour in general. The utility of these approaches in the context of biological systems is unclear, but these works may certainly provide inspiration for synthetic biology research.
Website: http://www.dna.caltech.edu/~winfree/
Representative papers:

- Translation of DNA signals into polymer assembly instructions
  Liao SP, Seeman NC
  Science 306 (5704): 2072-2074 Dec 17 2004
- Logical computation using algorithmic self-assembly of DNA triple-crossover molecules
  Mao CD, LaBean TH, Reif JH, Seeman NC
  Nature 407 (6803): 493-496 Sep 28 2000

De Silva A. P. (Queens University, Belfast, UK), Rezha Ghadiri (Scripps Research Institute, USA)
They and a number of other researches implement Boolean logic using chemicals and biochemicals. The systems work in vitro, but may inspire novel approaches in synthetic biology
Website: http://www.ch.qub.ac.uk/staff/desilva/apds.html
http://www.scripps.edu/research/faculty.php?tsri_id=1364
Representative papers:

- Molecular-scale logic gates
  de Silva AP, McClenaghan ND
  Chemistry-A European Journal 10 (3): 574-586 Feb 6 2004
- Boolean logic functions of a synthetic peptide network
  Ashkenasy G, Ghadiri MR
  Journal of the American Chemical Society 126 (36): 11140-11141 Sep 15 2004

II.f. IN-CELL SYNTHESIS OF CHEMICALS AND MATERIAL

Keasling (UC Berkeley, USA)
Arteminisin production (next-generation anti malaria drug) by designing artificial terpenoid pathways in *E. coli*
Website: http://cheme.berkeley.edu/people/faculty/keasling/keasling.html
Representative papers:

- Martin *et al.*, Nature Biotechnol. 21:796 (2003)

DuPont Corporation (Delaware, USA)
1,3-propanediol in *E. coli* for the manufacturing of a novel polymer, Sorona
Website:

DSM (Delft, The Netherlands)
Production of 7-ADCA for semisynthetic cephalosporine-type antibiotics with *P. chrysogenum*
Website:
Representative papers:

- Schoevaart & Kieboom, Chemical Innovation 31(12):33 (2001)

Mads Nielsen (Copenhagen, Denmark)
Metabolic engineering – using model based approaches (like flux balance analysis, metabolic models) to manipulate microorganisms into the efficient production of chemicals
Website: http://www.itu.dk/people/malte/
Representative papers:

- Akesson *et al.*, Metabolic Eng 6:285 (2004)
- Bro & Nielsen, Metabolic Eng. 6:204 (2004)

Kyowa Hakko Kogyo Corporation (Tokyo, Japan)
Developed the metabolic coupling approach where several gene products are coupled for an artificial reaction network over several bacteria.
Website: www.kyowa.co.jp/eng/index.htm
Representative papers:
- Koizumi *et al.*, Nature Biotechnology 16: 847 (1998)

Wang (Columbus USA)
Developed an alternative concept to the Kyowa H. group, termed "superbeads" – many different enzymes are localized to one bead.
Website:
Representative papers:
- Zhang *et al.*, Methods in Enzymology 362:106 (2003)

Wandrey (Jülich, Germany)
Designer-bug concept: by using multiple gene knock-out strains bacteria are driven towards theoretical yield maxima
Website: http://www.fz-juelich.de/ibt/wandrey-e
Representative papers:
- Zelic *et al.*, Biotechnol. Bioeng. 85:638 (2004)

III. ENABLING INFRASTRUCTURE

III.b. MICRO-FLUIDICS

Stephen Quake (Department of Bioengineering, Stanford University, USA)
Developed the first truly integrated microTAS featuring 1000 chambers and 3547 valves but only 22 inlets
Website: http://bioengineering.stanford.edu
Representative papers:
- Thorsen *et al.*, *Science* 298: 580-584 (2002)

Richard Mathies (Physical Biosciences Division, UC Berkeley, USA)
pioneered many of the available microfluidic DNA analysis systems
Website: http://www.lbl.gov/pbd/about/people/mathies.htm
Representative papers:
- Paegel *et al.*, Current Opin. Biotechnol. 14, 42-50 (2003)

Marc Madou (Biomedical engineering, UC Irvine, USA)
microfluidics and its applications to transcriptomics
Website: http://www.eng.uci.edu/faculty_research/profile/mmadou
Representative papers:
- Madou & Florkey, Chemical Reviews 100: 2679-2691 (2000)

George M. Whitesides (Harvard University, Boston, USA)
elaborating on the chemical aspects of microfluidics nad the bioengineering/microfluidics interface
Website: http://www.chem.harvard.edu/faculty/whitesides.html
Representative papers:
- Sia & Whitesides, Electrophoresis 24: 3563-3576 (2003)

Klavs F. Jensen (Departments of Chemical Engineering and Materials Science & Engineering, MIT, Boston, USA)
microfabrication and microfluidics for (bio)chemical reaction systems
Website: http://jensengroup.mit.edu/
Representative papers:
- Jensen, *Chem. Eng. Sci.,* 56, 293-303 (2001)

MESA+ Institute for Nanotechnology (University of Twente, The Netherlands)
Major institute covering all aspects of nano- and microtechnology
Website: http://www.mesaplus.utwente.nl

Institut für Mikrotechnik (IMM, Mainz, Germany)
Major institute covering all aspects of nano- and microtechnology
Website: http://www.imm-mainz.de

Institute for Analytical Sciences (ISAS, Dortmund, Germany)
Major institute, focus on miniaturization, proteomics, and metabolomics
Website: http://www.ansci.de

Hiroyuki Noji (Institute of Industrial Science, University of Tokyo, Japan)
Combinations of single molecule analytic and micro-fabrication techniques
Website:
Representative papers:

- Microfabricated arrays of femtoliter chambers allow single molecule enzymology.
  Rondelez *et al.*, Nature 433:773-777 (2005)

III.e. MEASUREMENT, MEASUREMENT OF NOISE AND VARIATION

Michael Elowitz (Caltech)
Focuses on measuring the statistical parameters of gene expression, such as fluctuation in input-output relationship between transcription factor and its regulated gene ("noise"). The data obtained in these experiments might be very useful for designing successful gene circuits.
Website: http://biology.caltech.edu/Members/Elowitz
Representative papers:

- Gene regulation at the single-cell level
  Rosenfeld N, Young JW, Alon U, Swain PS and Elowitz MB
  Science 307 (5717): 1962-1965 MAR 25 2005
- Stochastic gene expression in a single cell
  Elowitz MB, Levine AJ, Siggia ED and Swain PS
  Science 297 (5584): 1183-1186 AUG 16 2002

Robert Singer (Albert Einstein college of Medicine, New York)
Singer and colleagues develop methods to directly visualize various molecules on a single cell level, and measure the kinetics and diffusion properties of transcription-translation sequences.
Website: http://singerlab.aecom.yu.edu/
Representative papers:

- Gene expression and the myth of the average cell
  Levsky JM and Singer RH
  Trends in Cell Biology 13 (1): 4-6 JAN 2003
- Single-cell gene expression profiling
  Levsky JM, Shenoy SM, Pezo RC and Singer RH
  Science 297 (5582): 836-840 Aug 2, 2002

Xiaoliang Sunney Xie (Chemistry Department, Harvard University, Boston, USA)
Xie and colleagues have recently developed a unique method to measure single events of protein translation in living cells.
Website: http://bernstein.harvard.edu/pages/AboutProfXie.html
Representative papers:

- Probing gene expression in living cells one molecule at a time.
  Xiao J, Cai L, Yu J, Yin JL, Friedman N, Markson J, Xie XS
  Abstracts of papers of the American Chemical Society 228: U230-U230 199-Phys Part 2, Aug 22, 2004

Uri Alon (Weizmann Institute of Science, Israel)
Alon is mainly interested in systems biology, but his lab also does some experimental work including single-cell measurements, combined with modeling.
Website: http://www.weizmann.ac.il/mcb/UriAlon/
Representative papers:

- Dynamics of the p53-Mdm2 feedback loop in individual cells
  Lahav G, Rosenfeld N, Sigal A, Geva-Zatorsky N, Levine AJ, Elowitz MB, Alon U
  Nature Genetics 36 (2): 147-150 Feb 2004

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The NEST PATHFINDER initiative on «Synthetic Biology» was launched in 2003. Following two calls for proposals, 8 projects have been selected for funding. These projects apply design and engineering principles to biology with the aim to construct new functionalities and novel artificial systems based on sub-cellular biological building blocks.

A high-level expert group was established in 2005 with the aim, to examine, forecast and describe this new and emerging scientific field, its potential impact and support needs. The present report summarizes their findings and recommendations.

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