

PRESS FILE

SYNTHETIC BIOLOGY

ITS ORIGINS, APPLICATIONS AND POTENTIAL



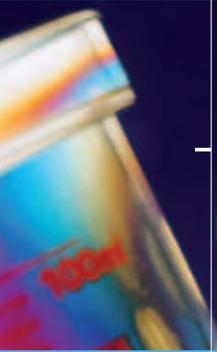
THE FOUNDATIONS OF SYNTHETIC BIOLOGY
THE FIRST APPLICATIONS

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December 7th, 2010

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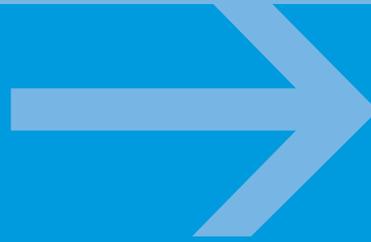
SUCCEED TOGETHER IN BIOTECHNOLOGY

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SYNTHETIC BIOLOGY: A SUMMARY



...Synthetic biology is an emerging science at the interface between biology, mathematics, computer science, physics and chemistry. It is founded on progress over the last few decades in biotechnology (particularly DNA sequencing and synthesis) and methods from the engineering sciences. It notably involves the deployment of a stepwise procedure with mathematical modeling, computer simulation, biological production and validation.

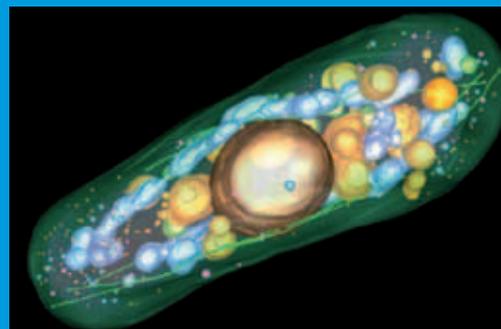
This methodical approach means that synthetic biology is used to design and then build biological systems with varying degrees of complexity. These systems reproduce the behavior of natural systems and may even have properties and perform functions which are not known or have yet to be discovered in nature. These new systems will help progress our knowledge of living systems and should result in a number of major applications.

Synthetic biology is a new science which differs from «conventional» genetic engineering in terms of its systematic methodology (rarely applied in biology), the complexity of the systems that it creates (when compared with one or a few genes transferred by genetic engineering) and the *de novo* construction of DNA sequences.

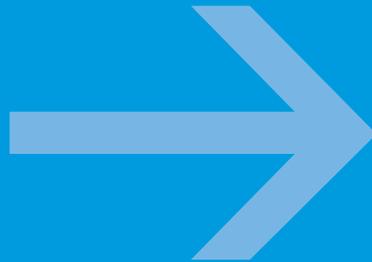
Synthetic biology draws together three different scientific approaches: (i) the generation of elementary DNA components for subsequent assembly, (ii) the synthesis of whole genomes and (iii) the construction of rudimentary cells.

A number of applications of synthetic biology are now emerging - particularly in the medical field. There are also applications in energy, materials, agrifood and the environment, corresponding to a large market.

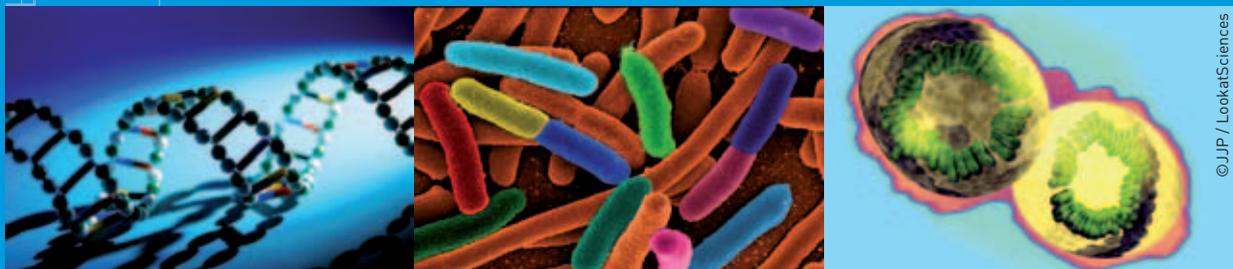
The United States is leading the way in synthetic biology but Europe (and France in particular) has clearly flagged up its intention to position itself scientifically in this field. In France, Genopole® constitutes one of the largest clusters of synthetic biology specialists from the public and private sectors.



KEY DATES



| | |
|-------------|--|
| 1912 | The term « synthetic biology » is coined by Stéphane Leduc, on the basis of his perception of biology, which was «successively descriptive, analytical and synthetic» (even though Leduc’s physical-chemical theory of life turned out to be erroneous). |
| 1953 | Watson, Crick and Wilkins determine the molecular structure of DNA , which carries the genetic information. |
| 1967 | The genetic code is fully deciphered. |
| 1972 | Initial gene transfer experiments in the bacterium <i>Escherichia coli</i> . |
| 1978 | Arber, Nathans and Smith discover restriction enzymes , which are able to cut DNA molecules at specific sites. This discovery prompts Szybalski to envisage the era of synthetic biology: «We will then devise new control elements and add these new modules to the existing genomes or build up wholly new genomes. This would be a field with the unlimited expansion potential and hardly any limitations to building ‘new better control circuits’ and synthetic organisms». |
| 1990 | Tom Knight (Massachusetts Institute of Technology, MIT) creates the BioBrick concept and builds a database of these functional DNA sequences. |
| 2000 | The journal <i>Nature</i> publishes reports on pioneering work by three groups, who demonstrate that it is possible to design complex regulatory systems and integrate them into bacteria (Elowitz & Leibler, 2000; T. S. Gardner et al., 2000; Becskei & Serrano, 2000). |
| 2002 | The first ever synthesis of a functional viral genome by Wimmer’s group (Stony Brook University, New York). |
| 2004 | Synthetic Biology 1.0 (the first ever international conference on synthetic biology) is held at MIT. |
| 2008 | The complete synthesis of a bacterial genome (<i>Mycoplasma genitalium</i>) by Gibson’s group at the J. Craig Venter Institute. |
| 2010 | Creation of «Synthia», the first bacterium with an entirely synthetic genome (<i>Mycoplasma mycoides</i> JCVI-syn1.0) at the J. Craig Venter Institute. The genome was incorporated into a bacterium related to <i>Mycoplasma capricolum</i> . |



DEFINITIONS AND TERMINOLOGY

... In «synthetic biology», the word «synthetic» should be understood in terms of its primary meaning – «formed by elements» - and not its biological meaning (the production of organic matter, i.e. biosynthesis). Synthetic biology consists in using human knowledge and our understanding of biological systems to design and then build complex new ones, which may have properties that are not found in nature.

This stepwise approach to design and construction is characteristic of the engineering sciences and explains why synthetic biology is sometimes also referred to as biological engineering.

It is worth bearing in mind that in an December 2009 article in *Nature* entitled «What's in a name?», twenty experts gave as many different definitions of synthetic biology - emphasizing that this is a very broad field whose boundaries have not yet been fully established!



WHAT ARE THE FOUNDATIONS OF SYNTHETIC BIOLOGY?

... **Synthetic biology was born in the 2000s as a result of conceptual and technological progress in the field of biology.**

THE CONCEPTUAL FOUNDATIONS

... It is probable that synthetic biology - a still-emerging science - will truly revolutionize biology and its applications. But was the emergence of this new branch of biology completely unexpected?

In fact, synthetic biology is the fruit of scientific logic; after having merely described the living world for centuries, biologists then sought to dissect biological phenomena and understand how they worked. Molecular biology and genetic engineering (born 40 years ago) turned biology into an explanatory science. Biological analysis has since been scaled up and large-scale «omic» biological methods have been developed.

Genomics, transcriptomics, proteomics and metabolomics are powerful tools for providing

an overview of systems by studying a species' entire genome, proteome (all the proteins synthesized by an organism) and metabolome (all the metabolites produced by it). These techniques generate a huge amount of information, which can then be fed into models. This is the goal of a new approach to biology: systems biology (also known as «integrated biology»), which seeks to accurately determine the interactions between genes, proteins and biomolecules in general, with a view to understanding the logic of living systems and achieving a dynamic vision of biological systems in time and space.

The ongoing development of systems biology is opening up the way for the construction of biological systems based on existing systems but whose properties can be controlled - for improving our understanding of life but also for designing systems of use in human fields of endeavor.

ITS ROOTS IN BIOTECH

... Even if the complete construction of cellular units falls within the scope of synthetic biology, most of the current work concerns the synthesis of functional modules of DNA or the reconstruction of genomes. Even though DNA constitutes only a small part of the cell's components, it carries almost all the information required for cells and organisms to operate and thus defines the latter's characteristics. Each individual being is unique because its detailed genomic composition is unique. DNA technologies constitute one of the cornerstones of synthetic biology.

Since 1944 (when Avery, MacLeod and McCarty discovered the genetic role of DNA), 1953 (when Watson, Crick and Wilkins established the double-helix structure of DNA) and then 1967 (when the genetic code was fully deciphered), progress in biotechnology has been astounding. Thanks to the use of molecular biology techniques, genetics has made giant leaps forward:

- the discovery of restriction enzymes in the 1970s enabled scientists to cut DNA into specific fragments and envisage cloning genes.

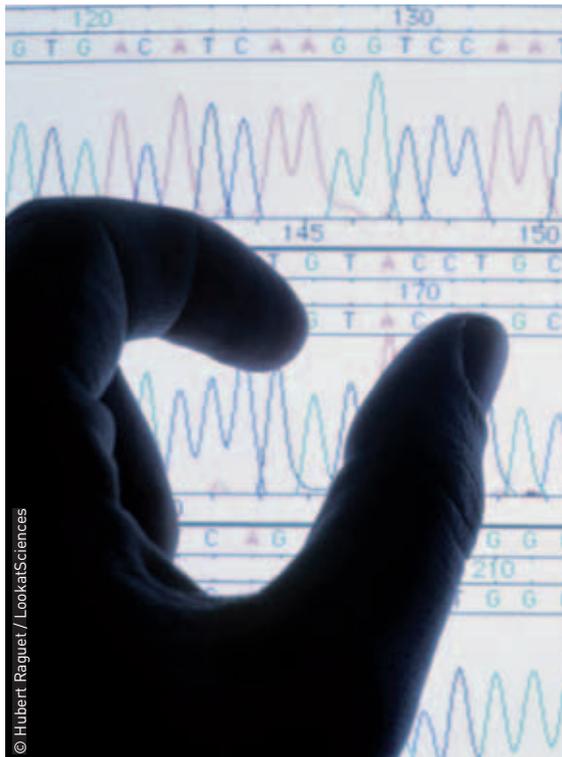
- the invention of DNA sequencing methods in the second half of the 1970s.

- gene transfer into bacteria was mastered in the early 1970s.

- in the 1980s, the invention of gene amplification in the polymerase chain reaction (PCR, a succession of cycles in which DNA fragments are copied many times) enabled the production of large quantities of DNA sequences of interest.

- the routine synthesis of specified DNA sequences became possible in the 1990s.

Recent progress in the reliability, rapidity and cost-effectiveness of DNA sequencing and synthesis constitutes the technological basis of synthetic biology. In 1977, it took several hours to read each «letter» (i.e. one of four nucleotides, denoted by A, T, G and C) of a DNA sequence, at a cost of \$0.1 per letter. In 2010, it takes just 24 hours to read the 3 billion letters in the human genome, at a total cost of \$1730 (i.e. \$0.00000058 per letter)! DNA synthesis currently costs less than €0.5 per base.



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A SHIFT IN BIOLOGISTS' EXPECTATIONS

... Since the Renaissance, when the leading humanists promoted the search for knowledge in its broadest sense and embraced all disciplines, the accumulated body of knowledge is so extensive that no one person can pretend to master all the sciences. Scientists have had to specialize and sharpen their focus.

Today, thanks to progress in telecommunications and growing awareness of the limitations of specialization, scientists are increasingly keen to interact with disciplines other than their own. Synthetic biology is the archetypal example of how important scientists believe it is to promote interactions between not only biology, physics, mathematics, computer science and engineering but also the human and social sciences.

→ TOOLS IN SYNTHETIC BIOLOGY

GENETIC TOOLS

••• DNA sequencing, DNA synthesis and genetic engineering are the key technologies used in synthetic biology. Large-scale genome sequencing is generating a pool of information in which biologists can look for the sequences that they will use to build synthetic systems. The synthesis and assembly of DNA sequences are essential for the construction of these synthetic systems.

However, synthetic biology is not simply a step forward in genetic engineering; in fact, it involves a novel methodology, adopted from the engineering sciences.

INTERACTION WITH A RANGE OF DISCIPLINES

••• By enabling scientists to read the genetic code and analyze its organizational structure, expertise in computer science and engineering has helped implement a true process for building synthetic biological systems in four successive phases: design, construction, implementation and validation.

The design phase consists in using the knowledge acquired in systems biology to develop a mathematical model of the desired biological system and then simulating the way it works in a computer model. By «running the model», it is already possible to explore the planned system's properties.

The construction phase involves the use of genetic engineering and other techniques (such as chemical synthesis, microtechnology and nanotechnology, for example) or indeed a combination of these technologies. However, the overall goal is to standardize processes and components.

The last two phases are the implementation and validation of the resulting systems via experimentation and the measurement of their biological properties. If the results do not correspond to those produced during the simulation, then the mathematical model must be recalibrated and triggers the start of another four-phase cycle.



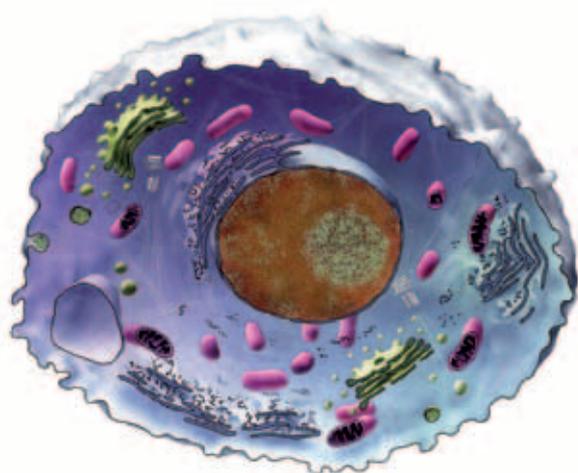
CURRENT APPROACHES TO SYNTHETIC BIOLOGY

... At present, synthetic biology labs tend to adopt one of three different scientific approaches.

The first «bottom-up» approach consists in building elementary DNA components which are stored as sequences in a circular plasmid and then assembled. The components are synthesized in a standardized process and have a precise function. Tom Knight at MIT was the first person to build this type of component, which he called a «BioBrick». He built up a collection of BioBricks, catalogued them in a database and founded the BioBricks Foundation to manage them. BioBricks can be used in synthetic systems with varying degrees of complexity. The prime objective is to use the brick to perform a useful function. More broadly, one can consider that this approach also includes certain metabolic pathway construction projects, which are designed to produce a desired chemical in a microorganism. This is clearly an application of science but the fundamental value is far from negligible because it sometimes prompts researchers to reconsider the fundamental paradigms such as «one gene, one protein» (an enzyme, for example) and «one enzyme, one function».

In contrast, the second «top-down» approach seeks to synthesize a whole genome after having reduced it to the smallest viable size. The most highly publicized example of this is the «Synthia» bacterium *Mycoplasma mycoides* JCVIsyn1.0, created by J. Craig Venter. The latter is not an entirely synthetic bacterium (as has often been wrongly stated) but comprises a synthetic, 1 million-base-pair genome inside an existing, natural bacterium. This constitutes major technological progress and may open up opportunities for studying how bacterial genomes operate (which we are a long way from completely understanding) and then modifying them. However, a few million base pairs would be enough for synthetic organisms suited to industrial applications.

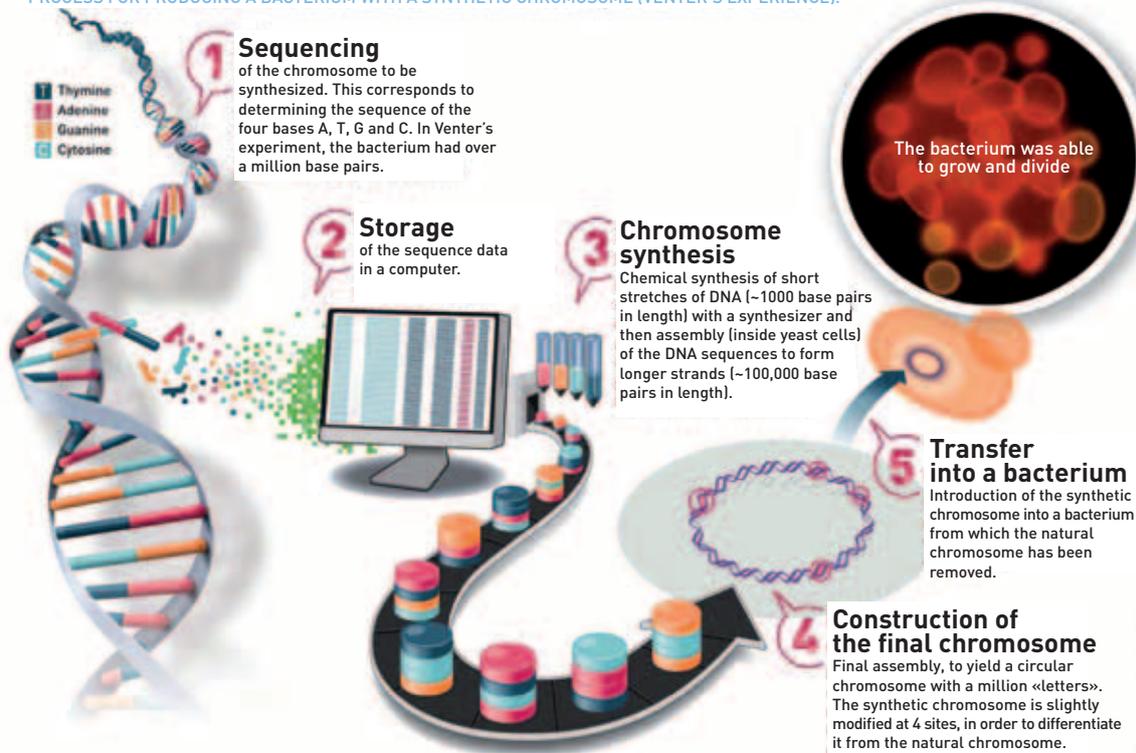
The third approach consists in assembling artificial components in order to reproduce an entire, functional, artificial cell. These components will include not only small DNA modules (as in the «bottom-up» approach) but also elementary cell components: enzymes, RNA and DNA-containing structures such as chromosomes. The elements will be contained within a lipid vesicle, mimicking the cell membrane. The absence of a clear definition of a «living system» is the first problem encountered by this approach. Scientists refer to a «proto-cell» because the current objective is to try to create a rudimentary cell which can function (i.e. perform biosynthesis), divide and react to changes in its environment. This approach seeks to understand how living cells work, identify the components which are essential for life and even understand the origin of life itself. For example, Luisi's group at the University of Rome's Biology Department is working on this approach.



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SYNTHETIC BIOLOGY

PROCESS FOR PRODUCING A BACTERIUM WITH A SYNTHETIC CHROMOSOME (VENTER'S EXPERIENCE).



APPLICATIONS

THE FIRST APPLICATIONS

••• **Synthetic biology has great potential for generating applications in fields as varied as healthcare, energy, materials, agrifood and the environment.** The construction of metabolic pathways for biomanufacturing compounds of interest appears to be one of the most promising uses.

Even though (i) science has not yet created any entirely synthetic organisms and (ii) the first completely synthetic genome is not large enough for use in biomanufacturing, applications have already emerged by using organisms into which tens of genes have been transferred for operation of a naturally absent metabolic pathway. The process for biomanufacturing artemisinin (developed by Jay Keasling's lab at the University of California in Berkeley) is being scaled up by Sanofi-Aventis.

Until now, artemisinin (a drug used in the treatment of late-stage malaria) was extracted from the plant *Artemisia annua* and was impossible to produce chemically. Biomanufacturing should decrease its production cost and improve batch supply and quality. Another example in the healthcare field is the collaboration between Sanofi-Aventis and the CNRS at Gif-sur-Yvette near Paris, which has led to development of a yeast that synthesizes hydrocortisone (a human hormone that is currently produced chemically).

Versant™ is a medical diagnostic assay which has been generated by synthetic biology; it is already being used to genotype hundreds of thousands of patients with viral diseases (particular AIDS and hepatitis). Bacterial biosensors have been developed to detect (for example) subtoxic doses of arsenic or the presence of explosives, with direct applications in monitoring drinking water and detecting antipersonnel mines, respectively.

FUTURE APPLICATIONS

••• Ongoing research in the United States and Europe will pave the way for new methods and novel compounds generated directly by synthetic biology. In the long term, a combination of synthetic biology and electronic/optronic nanotechnologies will probably enable us to build extremely small, sensitive hybrid components.

At present, there are few examples of industrial biomanufacturing. However, the synthetic biology market is forecast to be worth \$3 billion by 2016 and \$10 000 billion by 2026.

Biomanufacturing in the medical field requires the large-scale production of high-quality drug molecules - hence the importance of today's research work on the regulation of gene expression. At the opposite end of the scale, other research approaches are seeking to develop *in situ* biomanufacturing, i.e. the production of a drug or other compound specifically within diseased tissues and at a precise moment in time. In the longer term, synthetic biology could produce second-generation vaccines which neither rely on microbial surface antigens nor inactivated micro-organisms. This discipline could also elaborate tools for regenerative medicine; based on work in stem cells, synthetic biology could generate human cells with controlled properties, capable of colonizing defective organs.

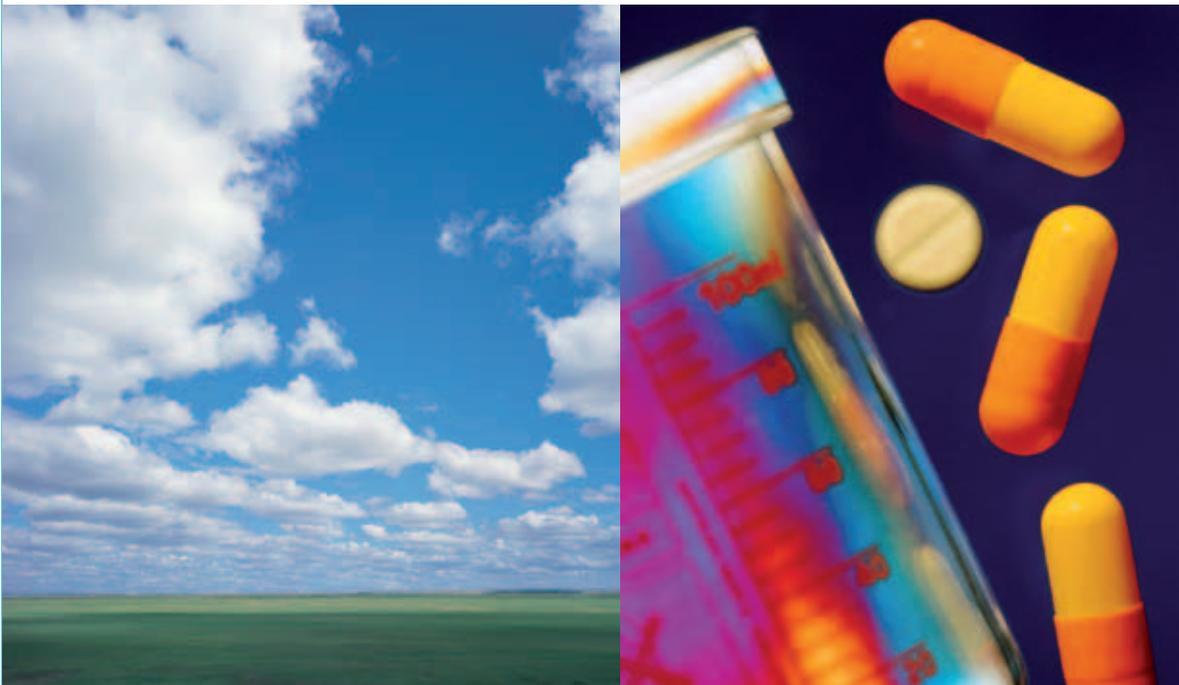
In fields other than healthcare, synthetic biology will generate new biomanufacturing techniques (which are less polluting than the current extraction or chemical synthesis methods) in the near future. These are likely to be used to produce bio-fuels, replacements for petrochemically derived materials, food ingredients and even synthetic meat!

Ongoing research clearly shows that it is possible to produce biofuels (such as biodiesel and ethanol) and hydrogen using a synthetic biology approach. The first biosynthetic fuels could be on the market within the next few years. The company Global Bioenergies (located on the Genopole® biopark in Evry) is carrying out research on how to produce isobutene from a sugar. The genes coding for the enzymes involved in this chain of reactions have been transferred into bacteria. For the moment, the sugars used in this type of production come from beet, sugar cane or cereal grains - all of which are food resources. However, by adding a few additional steps, it is possible to use the cellulose from waste (such as cereal straw). Ultimately, biofuel-producing organisms could be photosynthetic (microscopic algae) or given photosynthetic properties and would require only solar energy, CO₂ and water. This type of biomanufacturing would have clear environmental and cost advantages.



Synthetic biology is also showing promise in the development of highly sensitive sensors for the detection of changes in the environment or the presence of toxins or pathogenic bacteria. Biosensors could also be used non-invasively for medical diagnostics or implanted in the body permanently. When coupled with the *in situ* biomanufacturing of drugs, these sensors could lead to major progress in the prevention and treatment of disease.

Bioremediation (the biological processing of hazardous waste) is a field in which synthetic biology will certainly provide solutions. Jay Keasling's group at the University of California, Berkeley, has developed a strain of the bacterium *Pseudomonas* that is capable of destroying an organophosphate commonly used as a pesticide. The Genoscope/CEA Institute of Genomics at Genopole® is also developing strains of bacteria which degrade certain pollutants. Although the current work involves characterizing these strains and understanding their metabolism, it will serve a basis for developments in synthetic biology.



➔ KEY ISSUES FOR THE FUTURE

DEVELOPING AND STRUCTURING SKILLS IN FRANCE AND THE REST OF EUROPE

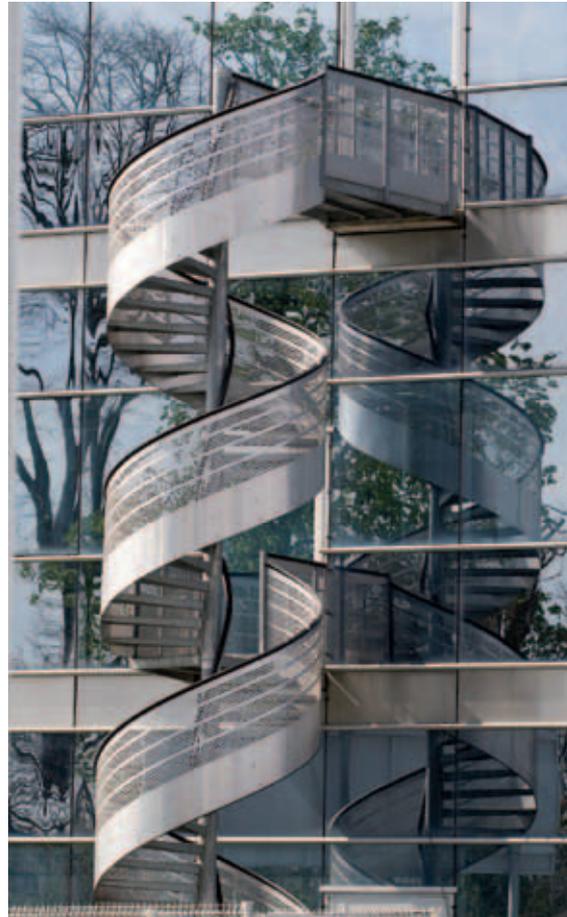
••• Even though the United States is still the main player in synthetic biology, Europe is starting to have a significant presence. An increasing number of groups are doing cutting-edge work on the subject. France has also committed itself to this approach - in particular at Genopole® Evry, which constitutes one of the country's most dense clusters of labs and companies specializing in synthetic biology.

RESEARCH

••• The growth of synthetic biology research in Europe requires access to funding and the implementation of dedicated infrastructure. However, the stakes justify these actions. For example, the largest ever grant in the history of life science research (a total of \$1 billion awarded to the University of California by British Petroleum and the United States Department of Energy) was for a research project in synthetic biology. It is clear that the above-mentioned applications in the energy field justify this concentration of resources.

In Europe, the New and Emerging Science and Technology (NEST) program identified synthetic biology as an innovative, promising field of research as early as 2003. Some projects have been funded, in particular those which seek to coordinate and stimulate research in Europe in this field. Even though the scientific community in this area was inexistent five years ago, there are now twenty or so European projects in operation.

France still has few synthetic biology groups but the latter are at the cutting edge of research in this field. Although there are no specific funding programs, synthetic biology does fall within a number of calls for proposals (particularly those issued by the French National Research Agency and the European Union's RTD Framework Programme). The French Parliamentary Office



for Scientific and Technological Evaluation could also help define a national strategy. France must invest in research and create the required infrastructure. This is happening at Genopole®, which has been thinking about and actively working on these themes since 2001, with a view to clearly defining its strategy and implementing the corresponding infrastructure, training courses and information dissemination activities. These joint efforts are needed to catalyze the emergence of a recent science with very attractive fundamental and applied outcomes but which is complex and subject to scientific and societal debate.

INDUSTRIAL ASPECTS

••• At present, several French companies are focusing purely on synthetic biology. These include AMAbiotics (Evry), Biomethodes (Evry), Collectis (Romainville), Global Bioenergies (Evry), Isthmus (Evry) and Metabolic Explorer (Clermont-Ferrand).

TRAINING

••• Synthetic biology will not emerge unless researchers and engineers are trained in this field. In parallel with the development of research and industrial activities in France and the rest of Europe, there is a need to set up specific training courses. The University of Evry-Val-d'Essonne opened a Masters in Systems and Synthetic Biology in 2010. The course is taught in English and is co-accredited by the Ecole Centrale de Paris, AgroParisTech, Sup Télécom Paris and SupBioTech. This Master will seek European accreditation in 2011, in collaboration with other major European institutions.



→ ANTICIPATING SOCIETAL ISSUES

SECURITY AND SAFETY

••• Today's research suggests that synthetic biology is capable of meeting society's key expectations, whether in terms of medical progress, protecting food resources or protecting the environment. But by building new genomes and cells, synthetic biology also presents new risks. The international scientific community must identify and mitigate these risks. The goal is to perform safe research (i.e. to promote safety) and avoid any misuse of synthetic biology applications (i.e. to promote security).

The issues relate to the endangerment of human health and the environment: the accidental release leakages of synthetic organisms, bioterrorism or military uses, for example. It may be possible to intentionally create new pathogens or organisms intended to spread a toxin. How can we prevent the abuse of synthetic biology? Do we have sufficient resources to prevent abuse? Are the regulations on genetically modified organisms and nanotechnologies sufficiently strict?

Even though synthetic biology is new, the associated risks do not greatly differ from those related to technologies used in this science. Some DNA synthesis companies («gene foundries») have already implemented security/safety checks on the sequences ordered by their customers.

To avoid any accidental proliferation of synthetic organisms in the environment, trophic containment solutions have been envisaged: the organisms would be modified so that their growth would depend on rare compounds (absent in the natural world). To prevent any risk of genetic exchange between synthetic organisms and natural organisms, one possible approach involves the use of novel nucleosides (other than those used in DNA) to constitute the synthetic organisms' genetic information. The Genopole® ISSB's Xenome project is working on this new form of nucleic acid: the «xenonucleic acid». Genetic isolation and propagation control will be particularly necessary for synthetic organ-

isms which, by virtue of their application, have to be disseminated throughout the environment (those used for bioremediation, for example).

It is important to engage in societal debate on these questions, so that we avoid creating thoughtless and unfounded fears of the applications of synthetic biology. This policy prompted Genopole® and the Ile-de-France Institute for Research, Innovation and Society (IFRIS) to organize a colloquium entitled «Life Science in

Society» in September 2010. A whole session was dedicated to synthetic biology. The objective was to think about how to organize debate on the subject and define the public concerned. This colloquium is the first of a cycle of five annual events.



TO PATENT OR NOT TO PATENT?

••• The question of patenting in synthetic biology is a delicate one; on one hand, it is reasonable to want to effectively protect processes or sequences developed by companies but, on the other hand, research must not be stifled by overly broad patents. Opinion on the subject is divided. Some people think that it is necessary to completely ban patents on gene sequences and living organisms. Others suggest «open source» systems, such as the BioBricks Foundation which manages the MIT's synthetic biology tools.

→ GENOPOLE® AND SYNTHETIC BIOLOGY

••• **Genopole®**, France's leading biopark, has designated synthetic biology as one of its priorities and currently constitutes the country's largest cluster of specialist labs and companies in this field.

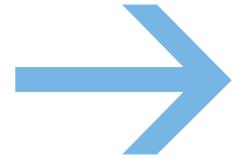
In 2001, Genopole® committed itself to systems biology by signing a collaboration agreement with the Institut des Hautes Etudes Scientifiques (IHES); this instigated a multidisciplinary work program on functional genomics. The Epigenomics Program (launched in 2002) federated several laboratories around this theme and prompted the creation (in 2008) of a CNRS unit (UPS 3201) and a Masters in Integrated Biology. Genopole® has progressively extended its activity to synthetic biology. A specialist Institute of Systems and Synthetic Biology (ISSB) was founded in 2010.

Genopole® also collaborates proactively with the University of Evry-Val-d'Essonne (UEVE) and helped found the latter's Masters in Systems and Synthetic Biology in 2008. The course is now run in collaboration with the Ecole Centrale Paris, AgroParisTech, Sup Télécom Paris and SupBioTech and is France's only dedicated training course on synthetic biology. The course will seek «Erasmus Mundus» European accreditation in 2011.

In order to promote French, European and international dialogue on this subject, Genopole® organized the international «ICSynthbio2010» seminar on synthetic biology on December 15-16, 2010. The meeting was chaired by Professor James Collins, a renowned expert on synthetic biology from the University of Boston. In June 2010, Genopole® also organized a working meeting for all the European research groups that had applied to the world-famous International Genetically Engineered Machines competition (iGEM) organized by MIT.



GENOPOLE®'S STAKEHOLDERS IN SYNTHETIC BIOLOGY



GENOPOLE® LABS WHICH SPECIALIZE IN SYNTHETIC BIOLOGY:

Laboratories:

- Genoscope – Institute of Genomics.
- The Institute of Systems and Synthetic Biology (ISSB), with five groups:
 - Bio-RetroSynt ;
 - MEGA ;
 - Metamorphosis ;
 - Synth-Bio ;
 - Xenome ;
- The Epigenomics Program.

A shared-access technology platform for synthetic biology.

The laboratories' outputs include:

19 research contracts (including 6 European Union contracts) in 2010
178 articles published since 2006

GENOPOLE® COMPANIES WHICH SPECIALIZE IN SYNTHETIC BIOLOGY:

- AMAbiotics
- Biométhodes
- Global Bioenergies

Detailed profiles of each of these laboratories and companies are attached as appendices.



TALKING ABOUT SYNTHETIC BIOLOGY

Antoine Danchin
Chairman of AMAbiotics and Honorary
Professor at the University of Hong Kong's
Li Ka Shing Faculty of Medicine

In your view, what are the major challenges in synthetic biology? Is synthetic biology an evolution or a revolution?

••• «Without any doubt, it's a revolution - but for a reason which is never mentioned. In fact, I believe that to understand, transform and rebuild life, it is necessary to introduce a new category of entity. Over the last few centuries, we've considered reality as being based on four entities, matter, energy, space and time. However, we've implicitly been dealing with a fifth one for some time now in a large number of fields: information. From that standpoint, one can, in summary, consider that living organisms are computers which make other computers. But a computer is made up of a machine and a program. In most of the work in synthetic biology, one really only considers the program. Furthermore, Craig Venter did not create synthetic life - just a (semi)-synthetic program. He needed a host cell and he didn't make it. This work revealed a hitherto masked property: the host cell no longer exists at the end of the experiment - another cell has replaced it, whereas the program that Craig Venter built is the same at the end. It is necessary to separate things on two levels: the cell reproduces (making a similar copy), whereas the program replicates (making an identical copy). We all know about this property because babies are born very young from an organism that is already old. The central issue in synthetic biology is, in my opinion, not at all metabolic engineering, the synthesis of novel compounds or even the replacement of one particular chemistry by another (for example, replacing tryptophan, one of the twenty amino acids used in most organisms, by the entirely synthetic analog 4-fluorotryptophan) - it's being able to subjugate the central process that captures information from the environment during reproduction in order to maintain the synthetic organisms in the state created by humankind. Living organisms manage to trap this information and so that's what we have to understand.

The central issue in genetics then becomes the need to understand genes and the functions they code for - functions which enable this information capture and sometimes even create information. In this context, what we call «natural selection» becomes a true physical principle, as follows: making space by using energy, to avoid destroying what is functional (or «information-rich» in this context, in other words). This vision of things is revolutionary. It means entirely reconsidering what we know about physics and applying it to biology as a particular physical system. The future of synthetic biology depends on close collaboration with «information physicists».

What types of initiative are needed to enable France's public and private-sector researchers to lead this field?

••• «French research is in a catastrophic state, for reasons which are too numerous to analyze here and which go back several decades. It is fashionable to criticize the academic rankings drawn up by Shanghai University and the *Times Higher Education* newspaper but what they show must be well founded to some extent. In brief, good governance practice would involve building a system that would make space (continuously and at a rate defined in a reasoned way) and use all the energy it can get (mainly funding - but not only) to avoid destroying what works well. In fact, it seems that a lot of energy is used to destroy things and that there's none left over to protect what is innovative. This type of process is particular in that none of the structures last forever, just because it's there. The structure can only persist if it generates innovation. This approach would empower all the inventive stakeholders to go on producing (if they wish so) until they die and would do everything possible to highlight and protect their innovations. Furthermore, this type of governance should apply whenever it is a matter of promoting creation, discovery or inventiveness».

TALKING ABOUT SYNTHETIC BIOLOGY

Marc Delcourt
Chairman and CEO
of Global Bioenergies

In your view, what are the major challenges in synthetic biology? Is synthetic biology an evolution or a revolution?

••• «Our field is metabolic engineering - one of the branches of synthetic biology. The discipline has emerged as a result of the very rapid growth of knowledge in genomics and sequencing techniques; it is a logical evolution. It's a «significant step forward», more than a revolution» helped by the fact that the genomes from many different organisms have been completely sequenced and standardized, low-cost DNA synthesis is available. For many years, chemicals were produced industrially by natural or modified micro-organisms, such as brewers' yeast

and fungi (for antibiotics). In an initial step, progress in molecular biology enabled us to modify microbial genomes in a targeted way. Today, the results obtained by Global Bioenergies prove that it is possible to create totally new metabolic pathways - pathways based on enzyme activities which do not exist in nature and involve totally new metabolites».

What types of initiative are needed to enable France's public and private-sector researchers to lead this field?

••• «Because Global Bioenergies' process is unique, the company is extremely well positioned in the French and European markets for applications in this field. We have attracted private investment and increasingly strong expressions of interest from major industrial players. However, new calls for proposals dedicated to synthetic biology are necessary if France's potential is to emerge. Few candidates responded to the last call; a certain number of groups are ready for the next one».

François Képès
Director of the Epigenomics Program

In your view, what are the major challenges in synthetic biology?

••• «In synthetic biology, some issues are fundamental: what, for example, are the minimum attributes of life? Which scenarios might plausibly underlie the origin of life? To what extent is one cell component independent of the others? Other issues are methodological in nature: for example, how can we engineer metabolic pathways and their regulatory circuits at the same time, that is to say the circuits which regulate the genes coding for the enzymes in these metabolic pathways? How can we modify the genetic material of living cells living without running the risk of horizontal transfer of these modifications to other organisms (i.e. the avoidance of genetic pollution)? An overarching issue in synthetic biology is the need to successfully perform technology transfer to industry. And another general issue relates to the need to promote true science-society dialogue on synthetic biology».

Is synthetic biology an evolution or a revolution?

••• «Synthetic biology marks a significant change in humankind's control over the biotope. In fact, it

constitutes a clear increase in our ability to rationalize the construction of objects based on or inspired by biology. It prompts us to proactively rethink how we engineer biology in a normalized, modular, hierarchical way. Even though it's still more about perspectives than reality, this goal will rapidly change the way we do biology».

What types of initiative are needed to enable France's public and private-sector researchers to lead this field?

••• «If French research in synthetic biology is to reach a high level, it must adopt a novel positioning and receive strong financial support. The need for a novel positioning is prompted by the clear American dominance in synthetic biology (68% of all publications in this field come from the USA, with just 17% from the European Union; France is in the top four countries in Europe). To create this positioning in a self-adjusting way, the solution is to trust the researchers. For strong financial support, a highly visible funding mechanism must be implemented at the national and European levels, in order to fund competing, fundamental projects. Subsequently, this funding could be channeled in part towards projects involving industry, in order to promote technology transfer. A decisive point is the need to deal equitably with all the various aspects of synthetic biology research».

Genoscope - CNS CEA/Genomics Institute



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MAIN TOPIC

\ Genomics \ Postgenomics

FIELD OF ACTIVITY

\ High-throughput production of DNA sequences \ Genome analysis \ Functional genomics \ Applications (research on biological solutions for the replacement of chemical synthesis).

KEYWORDS

\ Sequencing \ Genomics \ Biochemistry \ Metabolism \ Bioconversion \ Comparative genomics \ Metabolic Engineering.

RESEARCH THEMES

Since 1998, Genoscope has been responding to the high-throughput sequencing needs of the French scientific community (380 Mbases per day). Genoscope has also participated in international collaborative sequencing efforts such as the Human Genome Project (chromosome 14), plant genome projects (algae, the banana plant, *Arabidopsis*, *grapevine*, *rice*, etc.) and animal genome projects (*Tetraodon*, *Anopheles*, etc.), fungi (truffles) and has sequenced more than fifty prokaryotic genomes.

The Genoscope lab keeps up with the state of the art in sequencing and sequence analysis; 97.3% of our DNA sequence output is generated using the latest Flex and Illumina technologies. The sequencing facility is constituted by 3 Roche 454

GS FLEX Titaniums, 2 Illumina GAIIX and 1 Solid machines and 17 ABI 3730 capillary sequencers, which will be progressively replaced by new-generation sequencers. The current average daily throughput is around 10 billion bases.

Genoscope is currently focusing its research activity on the genomics of environmental micro-organisms in particular marine protists (the «TARA Oceans» project), the bacterial flora of the human digestive tract and those involved in effluent treatment.

The exploitation of sequence data (now extended to the identification of biological functions, notably in the biocatalysis field) is opening up new perspectives for developments in industrial biotechnology.

In the field of sustainable development, Genoscope is searching for biological solutions in synthetic chemistry, in order to reduce pollution and energy & fossil fuel consumption.

To this end, the centre has developed an enzyme activity screening center and a metabolic engineering laboratory. This research is performed in close collaboration with the UMR 8030 Metabolic Genomics research unit (cf page 31).

Industrial collaborations:

- Global Bioenergies
- Isthmus
- Suez Environnement



Supervisory bodies \ Genopole® \ Université d'Evry-Val-d'Essonne \ CNRS Director \ Jean-Loup FAULON

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MAIN TOPIC

\ Systems and synthetic biology

FIELD OF ACTIVITY

\ Modeling and engineering of biological processes with a (post-)genomics approach
\ Bioinformatics \ Metabolic engineering \ Epi-organization of the genome.

KEYWORDS

\ Engineering \ Modeling \ Simulation \ Macromolecular networks \ Epigenesis.

RESEARCH THEMES

The Institute for Systems and Synthetic Biology (ISSB) is structured into 4 research groups. Systems biology brings together experimental and theoretical studies with a view to modeling the functions of living systems (the MEGA and Metamorphosys research groups). Synthetic biology uses models taken from systems biology to design, build and validate new biological circuits inserted into micro-organisms (the Synth-Bio and Bio-Retro-Synth research groups).

- The «Modeling and Engineering Genome Architecture» (MEGA) research group analyses the topology of transcriptional networks in time (i.e. dynamic studies) and space. Its recent work has dealt with the functional organization of the nucleus, genome evolution & organization and the links between carbon metabolism and DNA replication. The group's theoretical work is prompting bench

experiments which examine the cell's regulatory networks on the genome-wide scale.

- The Metamorphosys group studies three aspects of the genome of the African clawed frog *Xenopus tropicalis*: **1)** genome structure (DNA transposons in *Xenopus* and their use in genome engineering experiments). **2)** expression of the genome during ontogenesis. **3)** genome evolution.

- The Synth-Bio research group is developing computational methods for designing biological and metabolic circuits within bacteria. These biological circuits are then characterized in vivo. Lastly, the bench data feed into established models and thus close the loop.

- The Bio-Retro-Synth group's research interests cover the use of retrosynthetic methods for designing and building new metabolic networks. Retrosynthesis consists in choosing a set of exogenous enzymes which, when introduced in a host organism, produce the desired target compound. The method is being applied to the production of drug compounds in bacteria.

ASSOCIATED «ATIGE» JUNIOR RESEARCH GROUPS:

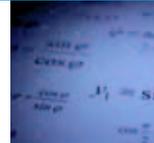
«Towards Reliable Synthetic Biology».
Leader: Alfonso Jaramillo
«Metabolic production of therapeutic compounds by bioretrosynthesis»
Leader: Jean-Loup Faulon

Industrial collaborations:

- Watchfrog.

Epigenomics Program

CNRS UPS 3201



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MAIN TOPIC

\ Synthetic systems biology and bioinformatics

FIELD OF ACTIVITY

\ Modeling and simulation of biological processes in a (post)genomics context
\ Epi-organization of genomes.

KEYWORDS

\ Modeling \ Simulation \ Engineering \ Macromolecular networks \ Epigenesis.

RESEARCH THEMES

The Genopole Epigenomics Program (founded in 2002 and whose slogan is «model to understand») aims first and foremost to be a forum for dialogue in order to catalyze research on complex biological problems via contributions from a range of disciplines: biology, computing, mathematics, theoretical physics, artificial chemistry and so on.

The Program simultaneously serves as :

- a vector for training researchers in disciplines other than their own.
- a visiting researcher program.
- a mainly French-speaking, multidisciplinary research network with regular meetings.
- a hotbed of pioneering science (stimulating the invention of new research subjects and supporting them through targeted, thematic activities).
- a joint service which centralizes Evry-based research efforts on modeling in biology.

All the activities funded by the Epigenomics Program are highly thematically targeted and are based around a small number of leading researchers.

AMAbiotics



Chairman \ Antoine DANCHIN **CEO** \ François GENDRE

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Web site \ www.amabiotics.com **Date of incorporation** \ Feb 1, 2010

FIELD OF ACTIVITY

\ A partnering research organization (PRO) which studies the relationships between microbial metabolism, nutrition and health.

KEYWORDS

\ Bioremediation \ Metabolism \ Genomics \ Bioinformatics \ Ageing \ Reactive oxygen species.

DESCRIPTION

Background

AMAbiotics' creation was based on Antoine Danchin's acknowledged expertise in metabolism and François Gendre's extensive experience in a major agrifood company.

Description of Products/Services/Technology

AMAbiotics' business is based on understanding the metabolic interactions within and between living communities (notably between complex organisms - such as humans - and micro-organisms. The balance between these interactions results from flows of chemical compounds, some of which are produced by the organisms themselves.

Understanding how these metabolic cascades arise in specific situations enables the company to identify metabolic states and then find ways to balance (or rebalance) them. AMAbiotics' research focuses on chronic pathologies requiring long-term drug use, metabolic imbalances and, more generally, ageing-related diseases. By using new genomic techniques and computer modeling, AMAbiotics is developing both proprietary and collaboratively owned portfolios of patents and patent applications.

Achievements/collaborations/highlights

- A member of the Microme European FP7 consortium.
- Creation (with the Fourmentin-Guilbert Foundation) of *Symplectic Biology*, a peer-reviewed journal for systems biology and synthetic biology.
- A patent application and high-level scientific publications.

Collaborations sought

Industrial collaborations for the exploitation of novel metabolic bioremediation approaches (metabolic impairments in humans, animals and plants) for combating the noxious effects of ageing and chronic exposure to chemicals.

- 5 staff
- **Strong points:** expertise demonstrated in the analysis of bacterial metabolism and the discovery of new metabolic pathways.
- **Strengths:** close relationships with world-famous partners (particularly in Europe and Asia) in the field of genomics and its applications.
- **Innovation assets:** a multidisciplinary approach to understanding the metabolism of communities of organisms, which combines *in silico* approaches (bioinformatics) and *in vivo* experiments.

Biomethodes



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Date of founding \ 6th Nov.1997

FIELD OF ACTIVITY

\ Genetic engineering applied to bioenergy, green chemistry and industrial biotech.

KEYWORDS

\ Biocatalysis \ Biofuels \ Biorefinery
\ Specialty enzymes.

DESCRIPTION

Background

1998-2000: development of the company's technology platform.

2000-2005: R&D collaboration with several major chemicals and pharmaceutical companies.

2005-2007: development of biocatalysis and bioenergy applications.

2008-2011: collaboration between Biomethodes and Virginia Technology/Oak Ridge National Laboratory (US Department of Energy) on the development of the OPTAFUEL/OPTACHEM biorefinery platform.

Description of the products/services/technology

Genetic, protein and enzyme optimization. Biomanufacturing system applied to industrial biotech.

The company has developed and exploited novel technologies (MMT® and THR®) for improving industrial enzymes. These technologies are protected by 3 patent families owned by the company and parts of the work have been published in top-rank scientific journals.

Customer references/collaborations/highlights

Development of the first process for transformation of lignocellulosic biomass into cellulose, hemicellulose, lignin, acetic acid. Implementation of the enzymatic hydrolysis of biomass.

Collaborations sought

Joint ventures in industrial chemistry, energy and the environment.

→ 12 patents → 12 salaried staff

→ **Strengths:** intellectual property, industrial feasibility, well positioned in the USA and Europe.

Global Bioenergies



Chairman \ Marc DELCOURT Member of the Executive Board \ Philippe MARLIÈRE

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FIELD OF ACTIVITY

\ Global Bioenergies is developing a bio-process for converting renewable resources into a hydrocarbon gas (isobutene).

KEYWORDS

\ Biofuel \ Bioenergy \ Renewable resources \ Isobutene \ Synthetic biology.

DESCRIPTION

Background

Global Bioenergies (established in 2008 by Marc Delcourt (CEO) and Philippe Marlière (project founder)) is one of the few companies in the world (and the only one in Europe) developing a bioprocess for hydrocarbon production.

Description of the products/services/technology

The process is based on establishing an artificial metabolic pathway in microorganisms. It uses plant-derived feedstocks, such as cane or beet sugar or carbohydrates obtained from agricultural or forestry waste. Since the resulting hydrocarbon is a gas, no purification work is required (in contrast to the distillation needed for ethanol, for example). This is expected to produce better environmental and economic performance levels than current approaches to biofuel production. By using cheap, tried and tested chemical processes, the isobutene gas can then be easily converted into liquid hydrocarbons (petrol, kerosene, diesel

and ETBE) and various polymers (tires, organic glass, plastics).

Customer references/collaborations/highlights

Global Bioenergies closed its first round of financing in early 2009 (several million euros from Masseran Gestion/BPCE). It has created a top-rank Scientific Advisory Board, built up its research team (around fifteen staff) and achieved proof of concept for the bioprocess. A second round of financing will be closed this year and will help fund the process industrialization stage. Global Bioenergies works in close collaboration with the Genoscope lab (at the CEA Genomics Institute), which has Europe-leading skills in sequencing, cloning, metagenomics and synthetic biology. The company also collaborates with the joint UEVE/CEA/CNRS LAMBE laboratory.

Collaborations sought

Global Bioenergies seeks to forge industrial collaborations on the implementation of a pilot.

- 18 salaried staff
- **Strengths:** a bioprocess based on the creation of a new, artificial metabolic pathway; major environmental and economic value.
- **innovation assets:** biomanufacturing of gaseous hydrocarbons.

→ GLOSSARY

DNA Deoxyribonucleic acid (so called because it contains the sugar deoxyribose).

A macromolecule composed of two chains intertwined to form a double helix. Each chain is formed by a linear sequence of nucleotides, each of which bears one of the four bases (A, T, G or C). In most living organisms, DNA carries the genetic information, i.e. the hereditary traits. In higher organisms, the DNA is highly compacted and forms (along with chromatin proteins) the chromosomes contained within the cell nucleus. In bacteria, the DNA is «naked» and is not compartmentalized within the cell.

RNA Ribonucleic acid (so called because it contains the sugar ribose).

A macromolecule composed of a single linear chain of nucleotides, each of which bears one of the four bases A, U, G or C. The cell produces RNA by copying DNA segments; this is what is known as «transcription». Most RNAs are used as templates for protein production.

BACTERIUM A unicellular microorganism lacking a nucleus and cell compartments but which contains a single, circular chromosome.

CLONING The isolation and amplification of a DNA fragment.

GENETIC CODE A code which enables the synthesis of proteins on the basis of the information carried by DNA. The linear sequence of the DNA bases is transcribed into RNA, which is then «read» by the cell machinery to produce a protein: each series of three bases «codes» for one of the 20 amino acids (the elementary constituents of proteins).

ENZYME A protein that is involved in the activation of biochemical reactions in organisms.

RESTRICTION ENZYME

An enzyme that cuts a DNA chain at a specific, short nucleotide sequence.

For example, the restriction enzyme EcoR1 specifically recognizes the sequence GAA TTC.

GENE A DNA segment corresponding to the synthesis of a protein, in most cases. A gene includes regions which the cell machinery transcribes into RNA and adjacent regions with regulatory functions. Some genes lead to the synthesis of RNAs with particular functions.

GENOME All the genetic information (DNA) in an organism, contained in each of its cells. In higher organisms, DNA is found not only in the nucleus but also in some other cell organelles (the mitochondria or the chloroplasts). The human genome is composed of 23 pairs of chromosomes, plus some mitochondrial DNA. Plant genomes additionally include chloroplast DNA.

NUCLEOTIDE The basic unit of the DNA or RNA chain. Each nucleotide is made up of:

- a base: adenine (A), thymine (T), guanine (G) or cytosine (C) in DNA and adenine (A), uracil (U), guanine (G) or cytosine (C) in RNA.
- a sugar (deoxyribose in DNA and ribose in RNA)
- a phosphate group.

PLASMID A small, circular DNA molecule which is often (but not always) found in one or several copies in bacteria, in addition to the bacterial chromosome. Plasmids are key tools in cloning.

PROTEINE A macromolecule formed by a variable chain of the 20 amino acids. Proteins are made up of between a dozen and a few thousand amino acids. A protein's three-dimensional shape (referred to as a tertiary structure or even a quaternary structure for protein assemblies) often has a significant impact on its activity. In cells, proteins act as enzymes or serve as structural components. Haemoglobin, collagen and polymerases are all proteins, for example.

SEQUENCING Determination of the linear sequence of the components in a macromolecule (nucleotides in DNA and RNA or amino acids in proteins). DNA sequencing is the most precise method for deciphering genetic information.

VIRUS A simple organism that does not have a cell membrane and is formed by one or more molecules of single-strand or double-strand DNA or RNA contained within a protective protein envelope (the capsid). Viruses are necessarily parasites of higher organisms, i.e. plants, animals or bacteria. Viruses that infect bacteria are called bacteriophages or phages. To replicate, a virus must penetrate into a host cell and «hijack» the latter's gene expression machinery.

For example, herpes is a DNA virus from the adenovirus family.

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