Biology at work

- Danish focus on synthetic biology

At the border between old and new disciplines, a new field of research – synthetic biology – is under development. Here, scientists are attempting to build biological nanomachines that can, for example, harvest the sun's rays, and sensors that can detect minute quantities of chemical substances within cells.

By Rikke Bøyesen og
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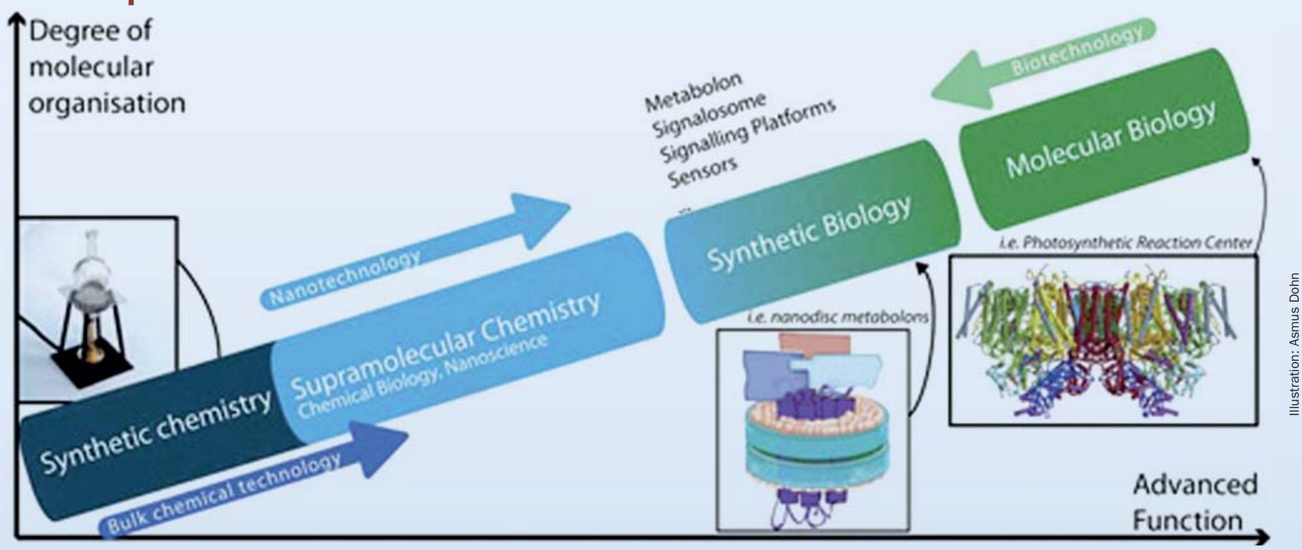
■ Over time, we humans have developed a multitude of machines which enable us to travel to the moon, communicate across continents, map the body's physical features and much more. However, despite our best efforts, we still find ourselves beaten by that elderly lady, Mother Nature. Over a period of millions of years, she has refined the biological machinery that constitutes our bodies, plants and all other living organisms. This biological machinery consists of molecules that organize themselves in relation to each other and that together act as efficient, miniature machines. Research in synthetic biology comprises the elucidation of nature's secrets: to understand how biological machines work and to recreate them with novel, custom properties enabling them to develop, for example, personalized medicine, sustainable energy and foods with health-promoting properties.


Foto: Colourbox

One of the primary visions in synthetic biology is to mimic plant photosynthesis, which can convert light energy into chemical energy in the form of e.g. carbohydrates and proteins.

Plant photosynthesis is a biological machine, which many scientists have tried for years to imitate. The photosynthetic processes enable plants to effectively and rapidly convert light energy from the sun into chemical energy in the form of e.g. carbohydrates, proteins and fats. With breathtaking precision, sunlight is captured by antenna molecules and converted into chemical energy in the reaction center itself. Only the green light waves are not harvested, which explains why plants are green! If we could replicate this process and use it in other biological systems - for example,

Synthetic biology in the classical disciplines hotspot



Synthetic chemistry has existed as a research field since the 1800s and aims to develop new chemical compounds i.e. new chemicals with specific, desired properties. This knowledge is the foundation of the research field of supramolecular chemistry, where chemical compounds are used as building blocks for the development of even more complex compounds, and where these compounds quite simply construct themselves. Supramolecular chemistry also covers the development of self-organizing systems whose performance is not borne by the individual molecule, but is created when molecules come together in larger units. Developments in nanotechnology have over the past 20 years given researchers the tools needed to synthesize molecules with desired chemical properties, and to develop new bespoke systems on this basis. On the other hand, we have the research fields of biotechnology and molecular biology. These research fields are based on a fundamental understanding of how biological processes take place in a coordinated manner in the cell because of interactions between a myriad of small efficient machines. Molecular biology and biotechnology involve the identification of the genes that code for individual processes, understanding their interactions and, on this basis, the design and construction of biological systems with new, desired properties. This might involve changing a cell's "programming" so it becomes programmed to accumulate or secrete valuable new compounds with desired functional properties. Using genetic engineering, it is for example possible to obtain plants that produce large amounts of polyunsaturated fatty acids as well as plants that produce biodegradable plastics.

to stimulate nerve cells in the human body by means of light - this would be revolutionary.

However, if we know that nature has mastered the processes we are interested in, why do we not merely mimic them? The answer is our lack of basic knowledge and technological development. For centuries, humans have been able to produce complex molecules in the laboratory by chemical synthesis. Yet, it is only in the last 20 years that we have been able to examine directly how molecules are organized and have, to a certain extent, learned to control them. Nanotechnology has given us the tools to see and work with the world's smallest building blocks - atoms. Work in the field of synthetic biology is carried out at the nano scale (i.e. where sizes are measured in billionths of a meter). Developments in plant biology, neurobiology, molecular biology, bioinformatics, biotechnology and nanotechnology together create the prerequisites for the development of the research field of synthetic biology.

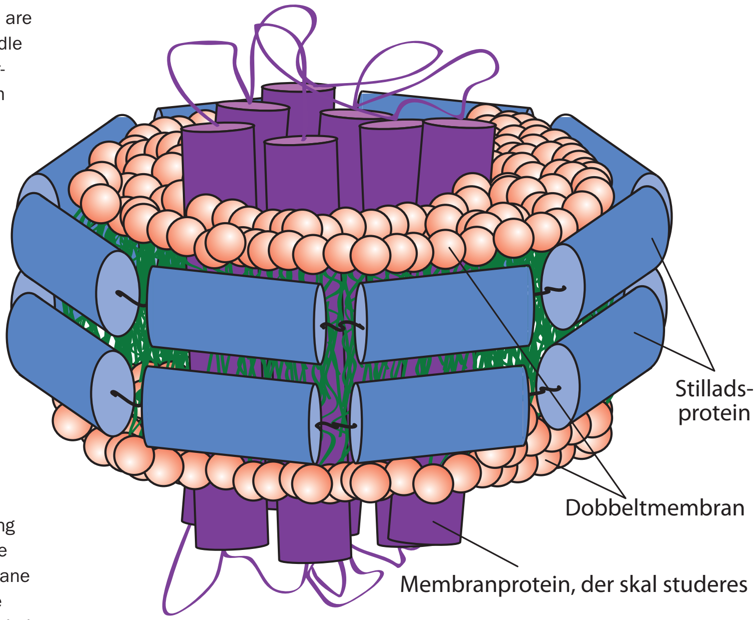
Membrane proteins – a research challenge

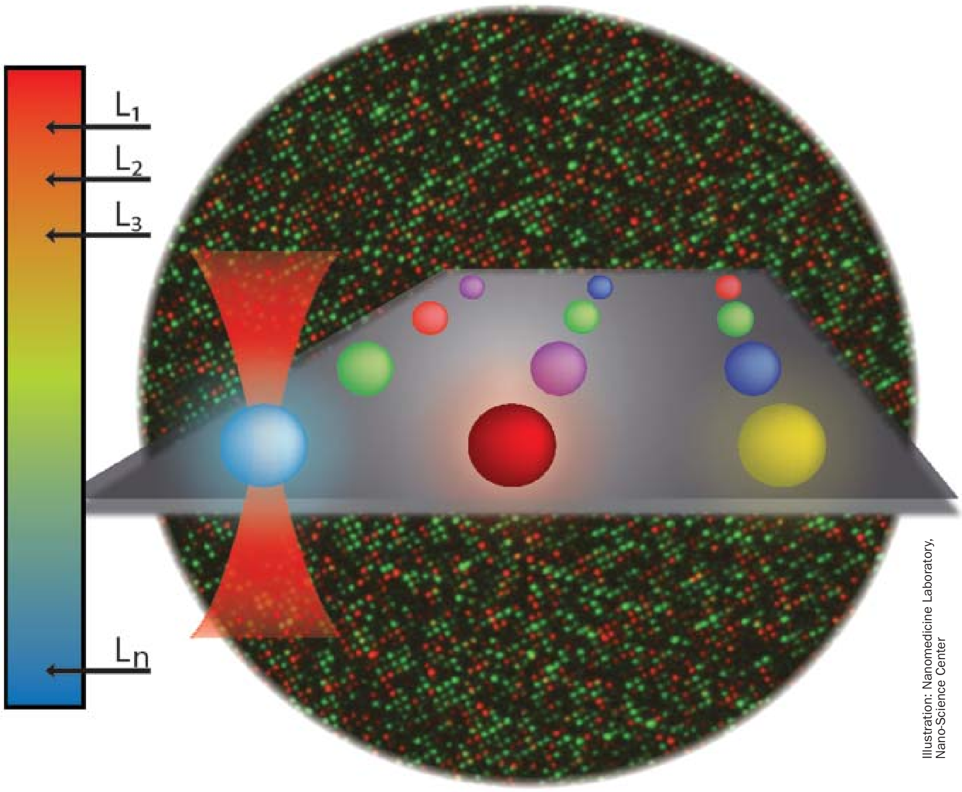
Research in synthetic biology at the Center for Synthetic Biology, University of Copenhagen has a special focus on the class of proteins that are not soluble, but are embedded in cell membranes. These membrane proteins play many key roles in cell function and intercellular communication. As receptors and transporters, they are the interface between the external environment in tissues and the cell interior. Membrane proteins also form the foundation of basic life processes such as photosynthesis, the formation of biologically active substances and biomass, energy storage through the creation of electrical potentials across membranes and the transmission of nerve impulses. By, for example, linking membrane proteins from the plant photosynthetic apparatus with enzymes that are normally located in other parts of living cells, we can take the first step toward utilizing sunlight to synthesize complex chemical substances independently of a living biological system. In this way, we may be able to move toward the development of some of the technologies that characterize a bio-based society.

Membrane-bound proteins are notoriously difficult to handle and thus, to characterize. A tool capable of addressing this challenge is the so-called nanodisc. A nanodisc can best be characterized as a small, nanometer-scale sample of a cell membrane surrounded by a stabilizing protein. Membrane-bound proteins can be placed in a nanodisc and studied while they are still active. This is instrumental for the characterization of these proteins, in understanding the structure of enzyme complexes and in mimicking them. Several research groups at the Centre have now attained experience working with nanodiscs - some prepare them, others insert proteins in them and still others analyze their structure and function. Their efforts are far from trivial, since they require isolated membrane proteins and technology for the preparation and purification of nanodiscs. The Center's researchers are carrying out pioneering work on the structural determination of nanodiscs and nanodiscs with

Membrane proteins and nanodiscs

Membrane-bound proteins are notoriously difficult to handle and thus also to characterize. To separate them from other membrane proteins, membranes must first be dissolved in a detergent (soap). As a result, many membrane proteins lose their activity. One way to avoid this is to instead incorporate membrane proteins in nanodiscs. These are small discus-shaped double membranes with a diameter of between 9 and 18 nm, surrounded by a "scaffolding protein" that holds the membrane in suspension without using detergents. This avoids the adverse effects on membrane protein activity seen in the presence of detergents. This is essential in order to characterize the proteins, as well as to understand the structure of enzyme complexes and mimic them. This work is far from trivial, since it requires isolated membrane proteins as well as technology for the collection and purification of nanodiscs.





Measurement of chemical substances: using fluorescent probes, it is possible to measure the transport of specific molecules into a small lipid bladder (liposome), and thereby determine the presence of chemicals in the surrounding fluid. A number of different attoliter liposomes (one attoliter = 10-18 liters), each containing a specific transporter, can be fitted onto a chip. These liposomes contain molecules that cause them to fluoresce in different colors, and in this way, it becomes possible to simultaneously measure the presence of a variety of substances with a sensitivity of only a few molecules.

incorporated membrane proteins. Researchers using several scattering methods and advanced data processing have developed a technology that makes it possible to directly see the structure of membrane proteins while they are active.

Synthetic biology - phase to phase

Work on synthetic biology can be divided into several phases. The first phase is to build a collection of functional biological, chemical or physical building blocks that may, for example, consist of membrane-bound proteins, nanodiscs, vesicles, nanowires or tailored molecules. The next phase is to assemble these building blocks in order to construct systems with new functional properties. The final phase consists of the linking of biology and electronics. An example of a phased project is the integration of semiconducting nanowires with pre-organized enzyme complexes and the development of new fuel and solar cells based on biological components.

The sun's rays are to be harvested

We all know that the sun's radiation heats the earth. However, few are aware that the solar energy reaching the Earth over a 1,3 hour period corresponds to the total energy humanity uses during a whole year. We humans are able to capture solar energy through artificial solar cells. With the help of these solar cells, we can now convert 25% of the sun's energy. Plants use the sun's energy more effectively and in a far more refined way. Using atmospheric carbon dioxide as sole carbon source as well as minerals absorbed from the soil, plants - with sunlight as an energy source - are in a position to synthesize all the organic compounds they require. However, plants can achieve more than this. Since they cannot "run away" when they are attacked by microorganisms and animals, they instead defend themselves by producing a number of often very complex bioactive defense compounds. Many of these compounds are used by humans as medical drugs to fight disease and alleviate pain. By consuming plants, humans obtain the essential amino acids and vitamins that we are not able to produce with the help of sunlight.

When plants form bioactive defense compounds, enzyme systems bound to the cell membrane control the key reactions. In the living cell some enzyme systems are connected to each other so that the product from one system can be utilized as the substrate for the next, while other systems perform their function alone. The function of these enzyme systems can now be studied by researchers, by incorporating them into nanodiscs or small lipid vesicles (liposomes). This permits the combination of enzyme systems in new ways, giving them novel properties in relation to how they function in the living cell. For example, research groups at the Department of Plant Biology and Biotechnology, lead by Poul Erik Jensen and Birger Lindberg Møller, are attempting to integrate one of the enzyme complexes from plant photosynthesis, which in the living plant cell is found in chloroplasts, with the enzymes that perform the complex syntheses of bioactive natural products. The latter enzymes are not found in chloroplasts, but are instead located in a special membrane system called the endoplasmic reticulum. By combining these two enzyme systems, they have managed to develop a system that produces useful, complex products using sunlight as an energy source (see box).

The long-term objective of this research is to produce a series of simple raw materials as well as complex bioactive natural products using light-driven processes. Production systems could include microbes, algae, mosses and plants, and eventually synthetic systems such as nanodiscs, with sunlight as energy source.

Light replaces expensive auxiliary molecules

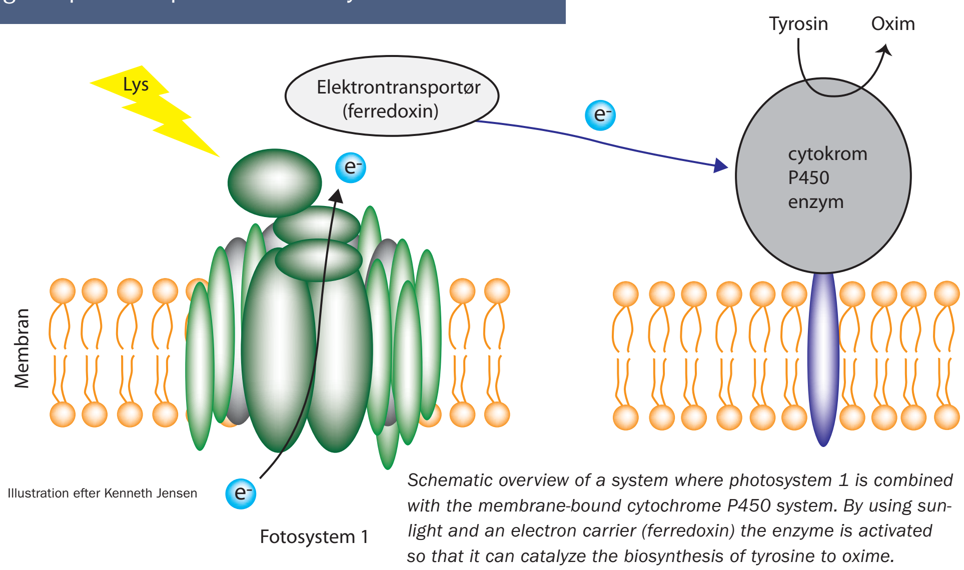


Illustration efter Kenneth Jensen

Schematic overview of a system where photosystem 1 is combined with the membrane-bound cytochrome P450 system. By using sunlight and an electron carrier (ferredoxin) the enzyme is activated so that it can catalyze the biosynthesis of tyrosine to oxime.

One of the goals of synthetic biology is the use of nanoelectrodes to transfer energy to biological processes. Hitherto, it has been necessary to utilize costly auxiliary molecules (eg. ATP and NADPH) to initiate these biological processes. If these auxiliary molecules can be replaced by the sun's energy or by an electric current, this will reduce production costs dramatically and be crucial for the commercial development of such systems.

Concrete examples are the so-called called cytochrome P450 enzymes. These are industrially interesting membrane-bound enzymes capable of catalyzing the complex hydroxylation processes that typically constitute key steps in the biosynthesis of a wide variety of compounds such as alkaloids, terpenes and flavonoids. These compounds often comprise very complex chemical structures and are therefore difficult, costly and time consuming to synthesize chemically.

In order for P450 enzymes to function, the presence of the coenzyme NADPH is required, which has made the industrial use of P450 enzymes economically unviable. Now, researchers at the Center for Synthetic Biology have successfully established a system in which the use of NADPH has been replaced by sunlight. This is done by connecting P450 enzymes to photosystem I, using the protein ferredoxin as electron carrier. This process is driven by light, without any requirement for the presence of expensive cofactors. The light driven system comprises purified components, which makes it possible to characterize the system. This light driven system has a catalytic activity double that of the NADPH-driven system, which underlines its biotechnological potential.

New possibilities for diagnosis and treatment

Medical drugs do not always give the desired effect, either because they are not targeted to individual needs or because they give rise to too many side effects. Focus on and work with membrane proteins in synthetic biology opens up endless new opportunities for the development of personalized medicine, as well as the improved diagnosis and treatment of diseases.

Researchers at the Department of Neuroscience and Pharmacology, led by Claus Juul Løland, Karen Martinez, Dimitrios Stamou and Ulrik Gether, work on constructing supersensitive nanosensors for amino acids, neurotransmitters, carbohydrates, toxins, metabolites and other molecules. This requires the accurate measurement of the content of various substances in very small quantities of body fluids such as blood, synovial and spinal fluid. Here, membrane proteins acting as sophisticated receptors and transporters play a central role.

Receptors can recognize chemical substances such as hormones, aromas, nutrients and ions within the cell's surroundings with impressive precision, binding to them and sending signals into the cell. They can translate this specific chemical recognition into information, which can for example induce the cell to change shape, grow or divide. The activation of a receptor can also induce the cell to release signal molecules and thus transfer information to other cells. Likewise, there are a myriad of transporters, which not only recognize chemical substances in the cell's surroundings, but which can also transport compounds into the cell and ensure that the cell has all the requisite substances available to ensure its survival.

Researchers are now able to incorporate receptors and transporters in either nanodiscs or in very small liposomes with a volume of only one attoliter (10^{-18} liters). This allows them to determine the presence of even extremely small quantities of chemical compounds in the environment. This technique will not only provide researchers with new opportunities for the diagnosis of disease, but could

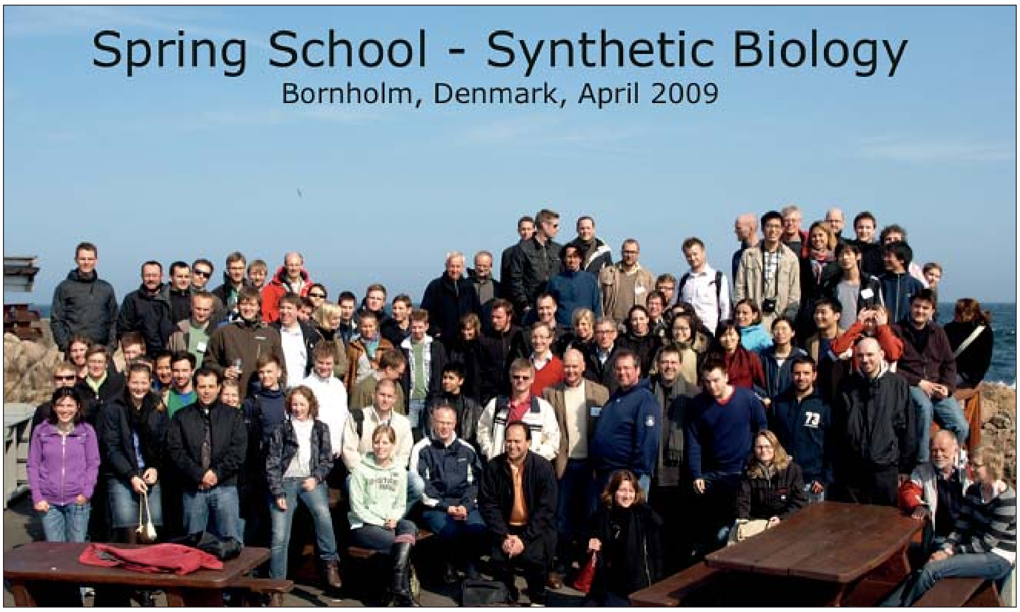


Photo from the Nano-Science Spring School på Bornholm, april 2009.

Center for Synthetic Biology

The establishment of the *Center for Synthetic Biology* at the University of Copenhagen was made possible through a grant of 120 million kroner from the Danish Ministry of Science, Technology and Development in 2009. The grant runs for 5 years and joins research groups in 7 institutes at the University of Copenhagen, each of which represents a key area within synthetic biology: chemistry and nanotechnology, molecular plant biology, molecular neurobiology and biophysics. In addition, philosophers from the Faculty of Humanities are associated with the project. The steering group behind the Center for Synthetic Biology consists of plant biochemist Birger Lindberg Møller (chairman), Department of Plant Biology and Biotechnology, and biophysicist Kell Mortensen.

also be used to screen for new medicines. In particular, it will be attractive to use this technique to simultaneously screen numerous target molecules for pharmaceutical effect, extremely rapidly and with minimal material expenditure.

Liposomes could also be used to remove toxins from the body, if they contain a transporter that can "vacuum" the surroundings of a given toxin. This could be relevant in connection with an overdose of medicine or other poisoning. The next step would be to integrate receptors with their effectors, for example enzymes, within the cell. This form of biological nanomachine can be used to activate or detonate the liposome in diseased tissue and deliver a drug contained in the liposome. One could also imagine that it could be possible to "deliver" specific proteins or protein complexes to diseased nerve cells, or to other damaged organs.

Much progress is yet to be made before nano-machines and -sensors can become a reality.

Great challenge and huge potential

Prospects for the utilization of commercially interesting systems, for example for the manufacture of personalized medicine and the light-mediated synthesis of complex bioactive natural products are years away. However, Danish scientists have started this process and are well prepared. Synthetic biology extends to several economically important sectors in Denmark: health and pharmaceuticals, energy and environment, agriculture and food, enzymes and proteins, chemistry, biochemistry as well as control and security. These are interdisciplinary research areas, where there are good opportunities to establish close contacts between basic research and industry for the benefit of Danish society. For example, Denmark already has a unique position within the biomedical industry, with companies such as Novo Nordisk, Lundbeck, Danisco, Novozymes and Chr. Hansen at the forefront of the global market based on a strong tradition within research and development. Synthetic biology will therefore in all likelihood play an important role in technology development in the majority of these sectors. Accordingly, research in synthetic biology can help to further Denmark's position on the research world map.

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