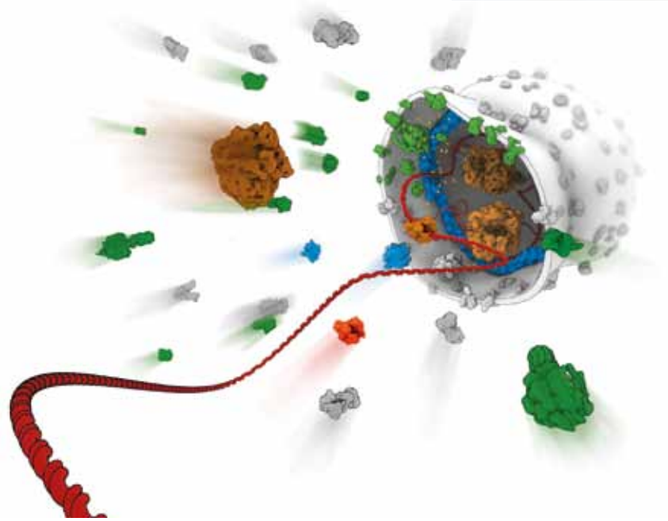
in 2013 it was shown that these effects can be repaired in a humanized yeast model of Parkinson's disease by a compound that is able to protect yeast cells from the toxicity of the disease proteins.
Altogether, research using budding yeast as model organism offers concrete opportunities to unravel functional mechanisms underlying human diseases. Discoveries may lead to the discovery of new ways by which diseases can be identified and offer new possibilities for therapies.

Synthetic biology: From engineering living systems to the bottom-up construction of synthetic cells

Bert Poolman and Vincent Noireaux



Artist's representation of a synthetic cell, showing metabolic processing (green), information processing (transcription and translation in orange), DNA in red, and reproduction/vesicle division (blue).
who is the author of the figure?

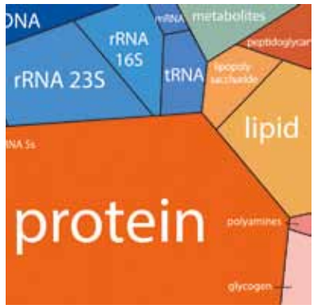
'What is life?' One of the most intriguing and difficult questions to answer. Richard Feynman argued that 'Everything that living systems do is done by atoms that act according to the laws of physics', and this is the basis we (all) build on. Today, it is still difficult to define life, even at the cellular scale. At the molecular level, however, it is well established that life is a system of self-sustained chemical processes. Complex networks of proteins, nucleic acids and small molecules sustain the essential processes of energy provision, gene expression and cell reproduction that characterize living matter. Biochemical networks direct cell growth and division, and through the uptake of nutrients, conservation of metabolic energy and the excretion of waste, they maintain a dynamic state far from thermodynamic equilibrium. In fact, a living system that reaches equilibrium is dead.

Creating synthetic life

One of the grand challenges in synthetic biology is to construct a living system such as a bacterial cell from molecular building blocks, that is, to assemble and engineer the components that allow a synthetic cell to grow and divide.
The prospect of creating synthetic life has inspired people for many years. The Venter Institute, for instance, has recently demonstrated that a *de novo* synthesized genome containing less than 500 genes can lead to viable cells. While creating a reduced cell by selectively removing components from a wild-type genome is an impressive achievement, this top-down approach neither reveals how the remaining gene products act together to create life nor captures the links between metabolism, compartmentalization and the information contained in DNA. As a result, it has not yet been possible to rationally design and construct, using a bottom-up constructive approach, a simple form of life based on a limited number of molecular building blocks. While our fundamental understanding of the individual building blocks of life is rapidly growing, putting a minimal set of components together so that life-like properties emerge remains a formidable, yet exciting challenge (see box).

Bert Poolman
Department of Biochemistry, University of Groningen, The Netherlands

Vincent Noireaux
Department of Physics and Nanotechnology, University of Minnesota, USA



Voronoi tree diagram of the composition of an *Escherichia coli*. Proteins (55% of the cell mass) and ribosomal RNA, contribute most to biomacromolecular crowding. Lipids, lipopolysaccharides and peptidoglycan are components of the cell envelope. who is the author of the figure? Figure very small, high resolution available?

Registry of Standard Biological Parts (screenshot from the website). Figure very small, high resolution available? cannot remake, the website has changed!

Crossing the border between death and life

Non-equilibrium systems are driven by the continuous flow of energy and matter, and can develop into a multitude of states, for example when the flow of matter is perturbed. Nature is an assemblage of many of such open systems, each of which can take its own path. The challenge is to construct and control such systems. This is an exciting area of science and technology where researchers try to cross the border from the "dead" molecules of chemistry to the living systems of biology.

How complex is a living cell?

A typical bacterial cell such as *Escherichia coli* has a volume of 1 femtolitre (= 10^{-15} L), a genome of about 4300 genes and a total number of macromolecules (proteins and nucleic acids) of 3 to 4 million, which is comparable to the number of components needed to build a major aeroplane. The proteins and nucleic acids (DNA and RNA) occupy about 20% of the volume of the cell. Such close (macromolecular) crowding is responsible for many essential biochemical and biophysical mechanisms ranging from protein folding to self-assembly of active structures. If we assume that proteins have a diameter of 5 to 10 nanometres, then each protein is at a distance of, at most, 2 nanometres from the surrounding macromolecules. In cytoplasm, the macromolecules continuously collide with each other, interacting as they move. These interactions influence the activity or function of the molecules. The molecular crowding creates excluded volume effects and complex diffusion modes, used by cells to form spatial patterns. The short intermolecular distances allow macromolecular surfaces (proteins, nucleic acids and membranes) to co-evolve and cells to maintain the dynamic structure of the cytoplasm. It is the system's dynamic structure and evolution potential that gives rise to the emergent properties of the cell, but this is something that we poorly understand.

A cell is not a random bag of macromolecules, of course, and we need to better understand the organization of the cytoplasm in time and space on the small physical scale. Outstanding questions such as 'How does a cell know when and where to divide?', 'How does a cell control the synthesis and assembly of complex molecular machines such as the ribosome?', 'How does a cell maintain a state far from equilibrium?' and 'How does a cell coordinate DNA replication with growth and division?' must be tackled in the near future.

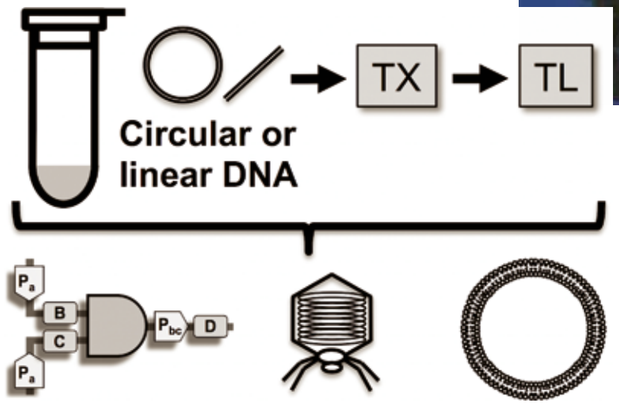
New approaches

To address these questions, it is important to develop new approaches to cell biology. Researchers have launched the Registry of Standard Biological Parts, a collection of genetic parts that can be mixed and matched to build synthetic biology devices and systems. The Registry was set up to make biology easier to engineer, but much more is needed in terms of the standardization of biological parts, not only at the level of DNA but also for proteins and other (macro)molecules. At this moment, the Registry is used by students in the annual iGEM (international Genetically Engineered Machine) competition to solve real-world problems by building biological systems with standard, interchangeable DNA parts. As the engineering is done in existing forms of life (e.g. bacteria such as *Escherichia coli*) the approach is top-down.

True understanding of "molecular life" will come from the design and synthesis, from scratch, of systems with increasing complexity (bottom-up assembly using molecular components). Concurrent with such an enterprise is the responsibility of the multidisciplinary research community to transform biology into a real engineering discipline by developing the "nuts and bolts" of biology. Here, the famous quote of Richard Feynman 'What I cannot create, I do not understand' is very relevant.

Importantly, in recent years, tremendous advances have been made in the bottom-up reconstitution of basic cellular machinery. There has been rapid progress in the reconstitution and quantitative understanding of complex biological systems and processes such as complex membranes and transport systems, sophisticated DNA processing machinery, complex cytoskeletal systems, self-organized spatial protein patterns, and cell-free gene expression. In addition, the possibilities for genome engineering have exploded with the development of powerful DNA assembly methods and the CRISPR technology.

In conclusion: A cell is an extremely complex system made of many different molecules. We do not know how these molecules interact to form a living cell that sustains itself, grows and divides. However, most of the individual macromolecules that form microbial life are known and novel technologies are available to assemble the basic molecular components and let them interact in a functioning synthetic cell. The design, concepts and challenges to construct synthetic cells will be very attractive to (prospective) students, and their imagination will be crucial for tackling the challenges ahead of us.



Cell-free transcription-translation (TX-TK) systems have risen to be powerful and versatile experimental platforms for synthesizing complex biochemical systems in test tubes through the execution of gene circuits. Several platforms have been optimized specifically for synthetic biology, with a constantly expanding scope of applications such as rapid prototyping of regulatory elements and circuits, complete synthesis of bacteriophages from their genomes and bottom-up construction of synthetic cells. who is the author of the figure?