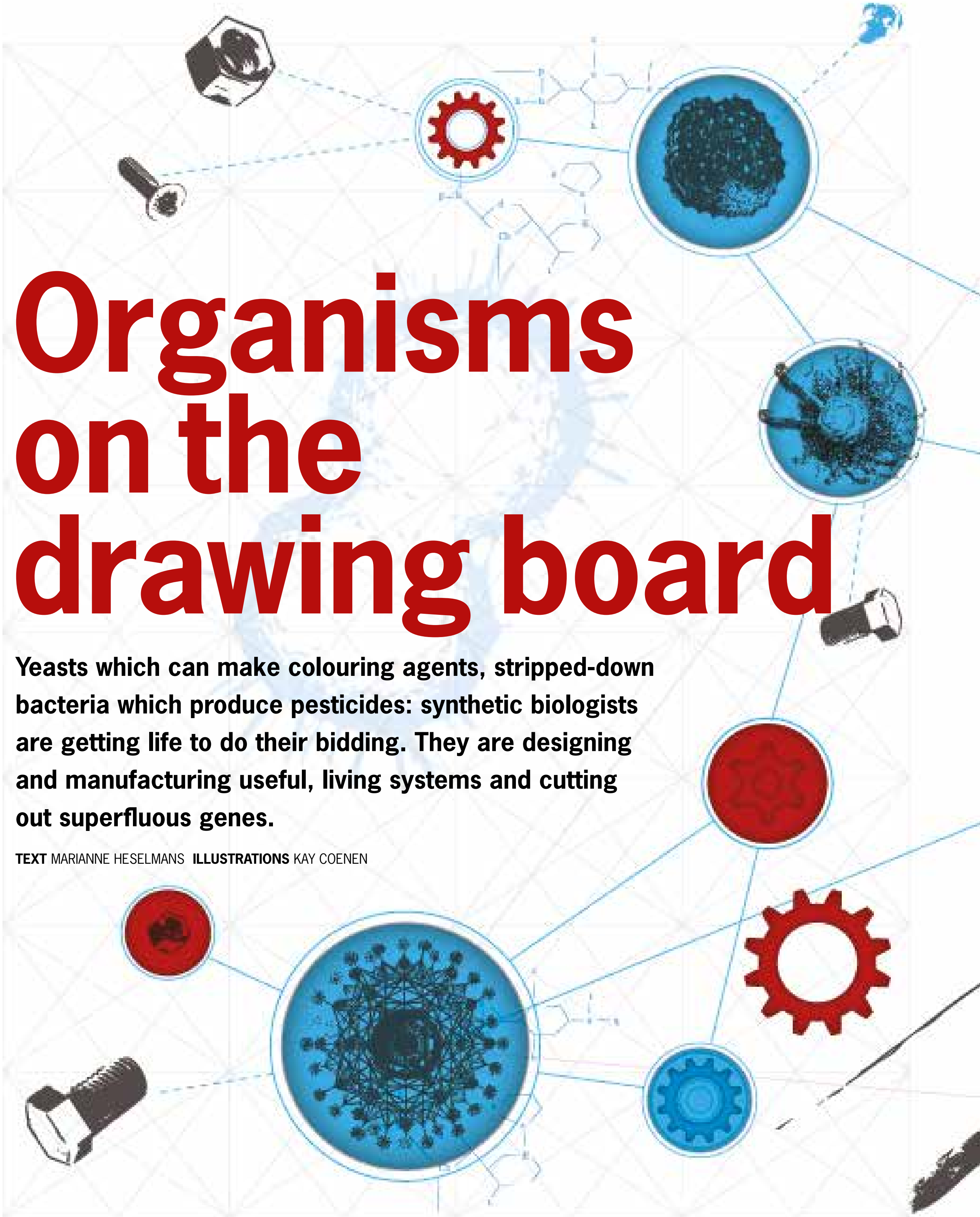
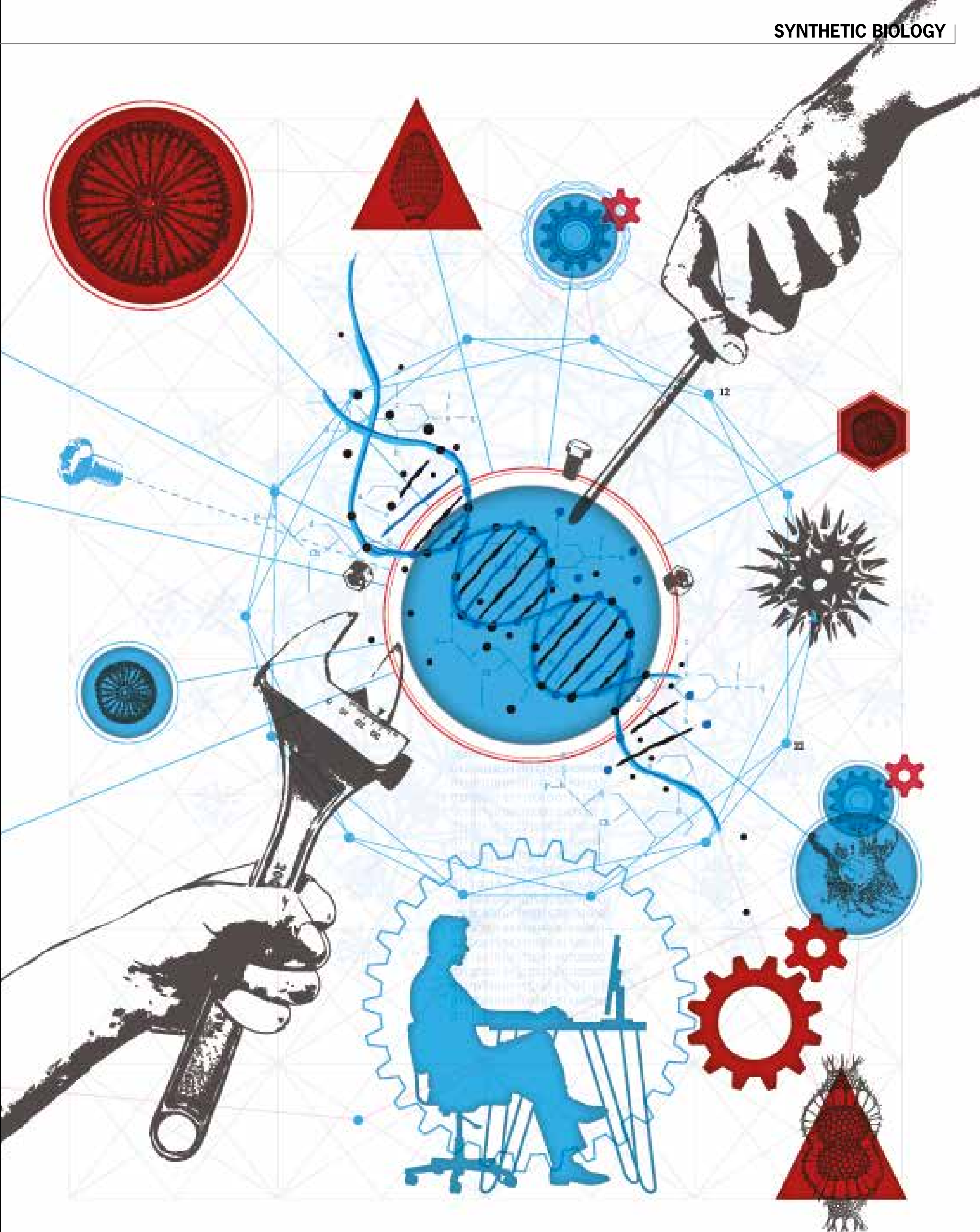


Organisms on the drawing board

Yeasts which can make colouring agents, stripped-down bacteria which produce pesticides: synthetic biologists are getting life to do their bidding. They are designing and manufacturing useful, living systems and cutting out superfluous genes.

TEXT MARIANNE HESELMANS **ILLUSTRATIONS** KAY COENEN



> **‘Twelve PhD students spend all their time modelling and are never in the lab’**

There is a yeast stored in a Wageningen UR freezer that has something in common with a grapefruit. It can use sugar to manufacture naringenin – one of the compounds that give grapefruits their bitter taste.

The same yeast is stored in a freezer at the technical university TU Delft as well. This is because it was made by Wageningen plant scientists and Delft microbiologists together. The Wageningen scientists developed the DNA for naringenin based on plant genes, while the microbiologists helped the yeast DNA to adapt so that the yeast could start producing this phytochemical efficiently itself. A yeast that can make phytochemicals could have its uses. It could make companies manufacturing flavourings and colourings less dependent on variable harvests.

Dirk Bosch, Synthetic Biology research team leader at Wageningen UR, refers to this piece of plant DNA for naringenin as a standardized ‘biomodule’. A company could combine this module with others, such as one which makes the yeast grow faster, or which converts sugar on receiving a particular signal. ‘Just as you design parts of a car and combine them to get the car you want,’ says Bosch. He even talks in terms of a ‘chassis’ which biomodules are attached to – in this case the yeast from Delft.

Terms such as ‘biomodules’ and ‘chassis’ are common parlance in the discipline known as synthetic biology. Synthetic biologists like to compare themselves to engineers who build cars, bridges or boats. They do not design and manufacture steel or plastic products, but useful living systems. These can vary from bacteria which manufacture plant substances to healthier bowels and little glass sheets with cells which light up when exposed to certain flavourings.

MILLION TIMES MORE

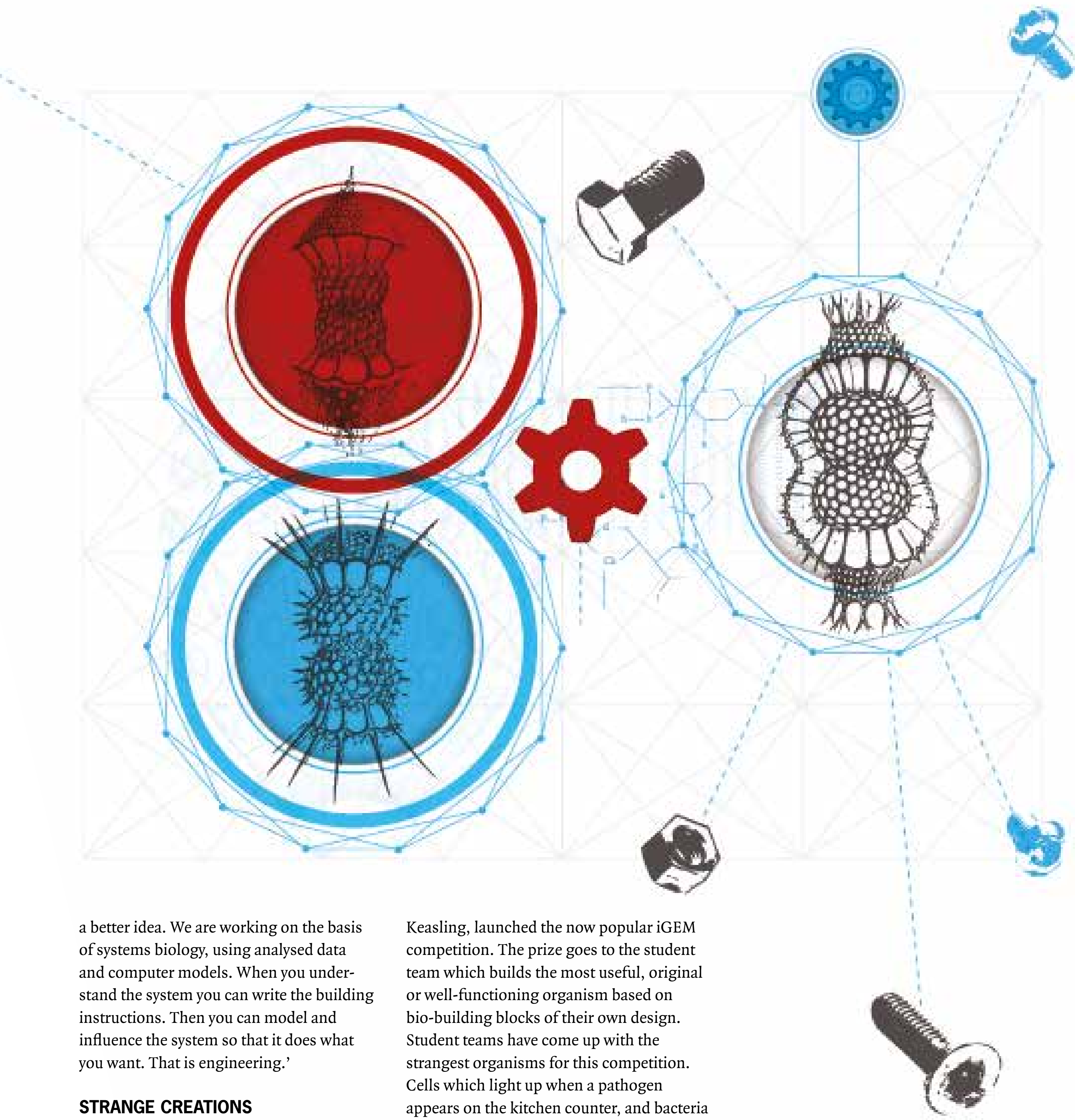
Typically, synthetic biology entails designing modules and organisms on the computer before they are actually created. The production process is therefore more systematic than the biotechnology of the past, which had a bigger element of trial and error. This means that expectations run high. Of the performance, for example, of bacteria and fungi which produce enzymes for making bioplastic out of maize waste and beet leaves. The new approach could enable them to produce a thousand or a million times as much enzyme per reactor tank, bringing down the price of the enzymes. This would make it more attractive for companies to stop using petroleum-based plastics.

In view of the potential of this engineering approach, Wageningen UR has included synthetic biology as one of the investment themes in its strategic plan. ‘Synthetic biology is not at all the same thing as genetic modification,’ emphasizes Vitor Martins dos Santos, professor of Systems and Synthetic Biology at Wageningen University, who leads this investment theme together with Dirk Bosch. ‘Genetic modification is a matter of introducing a couple of genes, seeing whether it works and then trying

LIGHT-EMITTING TONGUE DOES TASTE TEST

The cells or organisms which synthetic biologists tinker with are usually destined for a reactor. But there are many other possibilities. Dirk Bosch of Plant Research International shows a simple rectangular glass slide in his laboratory. ‘You can make an illuminable tongue with this,’ says the researcher. Working with Dutch firm Micronit Microfluidics, he developed a prototype for the fast detection of certain flavourings in, for example, a new variety of tomato or bell pepper. For laboratories this entails channelling juice through tiny little canals on the slide, upon which illuminable mammal cells betray the presence of flavourings. This is possible due to cells placed on the slide which have been given two genes. One is for a receptor from the human tongue or nose (there are 350 odour receptors in the nose). This detects flavouring X, Y or Z, depending on the type of receptor. On detection of the flavouring, the second gene emits coloured fluorescent light. This biological system with its different receptors was first designed on a computer.

‘An optical tongue like that also helps us understand which flavourings and aroma compounds people like best,’ says Bosch. ‘Because it is every bit as sensitive as a taste panel. It turned out that the human testers and the optical tongue stopped being able to taste Tabasco at the same dilution.’



a better idea. We are working on the basis of systems biology, using analysed data and computer models. When you understand the system you can write the building instructions. Then you can model and influence the system so that it does what you want. That is engineering.'

STRANGE CREATIONS

The term synthetic biology came over from the US about 12 years ago. There, a group of young professors, among them mechanical engineer Drew Endy and microbiologist Jay Keasling, launched the now popular iGEM competition. The prize goes to the student team which builds the most useful, original or well-functioning organism based on bio-building blocks of their own design. Student teams have come up with the strangest organisms for this competition. Cells which light up when a pathogen appears on the kitchen counter, and bacteria that can stuff themselves with iron. Last year a Wageningen team came second out of the 245 teams with its BananaGuard, a bacterium which targets and attacks >

the destructive banana fungus *Fusarium oxysporum* in the soil.
Synthetic biology means a lot of computer work. Martins dos Santos shows a diagram illustrating the carbon metabolism of a bacterium. This diagram is used by researchers for modelling. It includes about 1000 genes and enzymes, all linked by arrows. A diagram like this comes with a calculation model, created by modellers and bio-informatics scientists. 'Twelve of my PhD students and three post-docs spend all their time modelling and are never in the lab,' says the professor. They look at what happens 'in silico' in a computer simulation if gene A starts working five times as hard, or gene B is switched off. Do you get more product? Don't you get too many by-products? The results give people ideas for an improved design.

'Once you understand the system you can write the building instructions'

CHANGING BACTERIA

Martins dos Santos has just acquired two EU projects worth 16 million euros between them. Of that funding, 2.2 million will go to Wageningen. In one of the two projects, *Empower Putida*, his team is going to change the bacterium *Pseudomonas putida*. This adapted bacterium will then be put into enormous reactors where it will convert sugar into the raw materials for bioplastic and bioenergy. These include isobutanol and 1-butene, compounds which can currently be made more cheaply from petroleum. *P putida* bacteria with other built-in modules will use sugar to produce new pesticides such as the potentially powerful herbicide tabtoxin. Wageningen UR is working on this in collaboration with four other European scientific institutions and four companies, including the German corporation BASF and the Spanish multinational Abengoa.
The professor's story is full of engineering terms such as 'reprogramming', 'biofactories' and 'model-driven design '. 'Linking up biomodules is not the same as building something with Lego, of course,' says Martins dos Santos. 'It's about biology, so the modules often work differently in one system compared to another. But at the same time, bacteria or yeasts are somewhat comparable to factories.'
To be able to predict the effect of interventions in these living factories, the synthetic biologists aim for simple organisms. In nature, *Putida's* genome consists of 5500 genes. But for production purposes many pieces of DNA just get in the way: these include 'jumping' DNA fragments which move randomly through the genome, and genes for flagella, the hairs bacteria use to propel themselves along. So in a previous EU project the group already removed 15 percent of the genome.
The modules are then built into the 'stripped' bacterium. One of the EU team's aims is to design a module which will let the bacterium grow without oxygen. Oxygen disrupts the functioning of some enzymes that are essential to the production process. A second module that is under construction speeds up the growth of the bacteria and then makes it concentrate solely on making the desired molecule. The signal for this can be light. Researchers led by microbiologist Willem de Vos are creating gut bacteria with light receptors for various wavelengths such as red and blue. So a company will be able to link a module for 'react to blue light' with one for 'stop growing'. And one for 'react to green light' with one for 'now make isobutanol from sugar'. This makes it easy to steer microbial processes. 'You build modules in collaboration with many research groups,' says Martins dos Santos. 'That is another interesting aspect of synthetic biology.' The Wageningen groups working on this have joined forces in the Wageningen Centre for Systems and Synthetic Biology (WCSB).

ETHICAL ISSUES

The members of the centre will be doing more than just practising hard-core science. They will also be addressing such questions as: is a new bacterium safe? Is it permissible for the industry to describe flavourings made by yeasts as 'natural'? How do you make sure poor farmers don't suffer as a result of the new production method? Public acceptance of the new technology will also be studied.
Henk van Belt, assistant professor of Applied Philosophy, works on these kinds of social and ethical questions. One of the most important issues, in his view, is who gets access to synthetic biologists' research results. Patents could strengthen the concentration of power in industry, and concentration of power is one of the reasons genetic modification is unpopular with many NGOs. So Van den Belt is monitoring how synthetic biologists around the world patent their products.
'You see two tendencies,' says the philosopher. 'The group of professors behind the iGEM student competition has an explicit strategy of keeping basic modules accessible

in a 'Registry of Standard Biological Parts'. But we are also seeing that most groups are patenting biomodules.' This could lead to opaque 'patent clusters', he comments. There are hundreds or even thousands of patents, for example, on the fluorescent reporter proteins which are used a lot. This is not a problem for academic researchers and teams of students, because patent holders can't be bothered to pursue it if they use these proteins anyway. But for small businesses it can be problematic, says Van den Belt. They don't have the money, for instance, to find out who owns which patent. Or the patent-holder might refuse them a licence, which increases the concentration of power.

The Wageningen synthetic biologists do patent some discoveries, but not all. It is expensive to hold on to a patent and defend it if necessary, explains Dirk Bosch. So the decision depends on questions such as: do we need to recoup the investment? How many licence applications can we expect? And how easy is it to bypass the patent?

MAKING DRUGS

That synthetic biology can work is clear from the production process for artemisinine, an antimalarial drug. Artemisinine was harvested solely from the plant Artemisia annua, or sweet wormwood, until the aforementioned American Jay Keasling and his team managed around 2005 to get baker's yeast to make a miniscule amount of the drug. They did this by building in DNA for artemisinine production which they had designed on the computer. They hoped to use the yeast to make the production of artemisinine cheaper and more stable – less dependent on unpredictable harvests of Artemisia. The Bill and Melinda Gates Foundation put 40 million dollars into this pilot project and biotechnology company Amyris stepped up production. After many simulations and trials with new yeasts, Amyris succeeded in getting 25 grams of artemisinic acid per litre of end product, which is at least 1000 times more than the couple of micrograms the yeast was producing at first. Last year pharmaceutical firm Sanofi-Aventis produced 16 million courses of treatment with artemisinine from yeast.

There is a success story in the Netherlands too, partly thanks to Wageningen UR. Until recently, orange flavouring (valencene) could only be obtained using large quantities of orange peel. Since last year the Dutch company Isobionics has been marketing orange flavouring manufactured by *Rhodobacter* bacteria in reactors. The company aims to produce at least 1500 kilos of valencene this year, enough to give 90 billion litres of fluid a fresh orangey taste. For commercialization Isobionics has applied for a patent on the valencene research by Wageningen UR. The company hopes to become less dependent on orange peel, which is not always available in sufficient quantities.

'Using synthetic biology, reactors can be made which respond fast to new demand,' predicts Bosch. Demand from an ice cream producer, for example, who suddenly needs a lot more orange flavouring in the summer. A company such as Isobionics wants to be able to deliver the goods quickly. Another example would be a company that produces vaccines and wants to be able to respond immediately to an epidemic. Or a factory which converts crop waste into bioplastic and gets maize stalks one week and grass or beet leaves the next.

With all these different demands and seasonal changes, it is useful to have flexible biological systems which can produce whichever product is needed. Bosch: 'That is why we and the technical university of Delft have made that yeast which produces naringenin, which is used as a base in colourings, flavourings and anti-oxidants. Biomodules for various products can be combined with the module for naringenin.' Light receptors in the yeast could be of help here. Green light on the reactor: orange flavour. Blue light: orange colouring as well. Red light: grapefruit flavour. But that is a long way off still. ■

www.wageningenur.nl/en/synthetic-biology

STRATEGIC PLAN

INVESTMENT THEME SYNTHETIC BIOLOGY

Our knowledge about how cells, genes and enzymes work is growing fast. Microscopes are improving, chemical techniques are becoming more precise and methods of calculation are getting faster. Research consortiums, too, are getting bigger and more effective. Between them they have already mapped out the DNA of thousands of micro-organisms, hundreds of plants and dozens of animals.

All that information, stored in databases, offers more scope for synthetic biology: the systematic design and manufacture of living systems. Algae or yeasts, for example, which efficiently make products such as pesticides and vaccines, or fats and proteins for the food industry. Or biosensors which monitor water for toxic substances.

In view of synthetic biology's massive potential, Wageningen UR has made the discipline one of its five investment themes. Extra investment is aimed at increasing the chances of collaboration with research groups abroad. Natural and social scientists will also be joining forces to study the ethical and social implications of the new technology. What is life?

How can the scientific world and society together ensure innovation is ethical? Coordinator Vitor Martins dos Santos: 'We shall engage in dialogue with groups in society in every project.'