Experiments in miniature: the screening robot "octopus" performs thousands of biochemical reactions simultaneously, delivering an extremely high throughput. The reactions take place in plates 15 centimeters long, each of which contains 1,536 wells.

FULLY AUTOMATED HIGH-SPEED ACTIVE SUBSTANCE DISCOVERY

The million-molecule octopus

In their search for novel active ingredients for drug products, Bayer researchers can rely on highly efficient robots. They use fully automated ultra-high-throughput systems to investigate the pharmacological action of millions of substances and find candidates that could potentially mean the breakthrough to new drug products.

The main research assistant doesn't say a word – it just continues its analysis with extreme precision. The robot tests substances from Bayer's library at top speed, processing several plates at a time. "The octopus" is what it's called by its customers – the team of Bayer researchers in Wuppertal headed up by Dr. Bernd Kalthof, head of High-Throughput Screening Technology in Bayer's Pharmaceuticals Division.

"With our new ultra-high-throughput robot we can test the pharmacological action of up to one million substances daily," says Kalthof. 20 years ago, it would have taken a fully staffed laboratory about 100 years to handle the same workload.

Modular construction makes the robot flexible

Unlike its aquatic namesake's eight arms, the mechanical octopus in Wuppertal has only four appendages which it uses to process samples at extremely high speed. "The new automated system opens up completely new options in experiment design," explains Kalthof. It comprises different modules which the experts can combine in new ways to quickly integrate new methods into the high-throughput system as required. A vital factor for the system's high throughput – the number of samples tested per day – and flexibility is the infrastructure in which the fully automated octopus is embedded. A second robot prepares the reaction vessels with the test substances, allowing the system to process some 60,000 plates per year. This all takes high tech on every level. "Our computer systems also have to harmonize with the robot systems. So we have developed a special database infrastructure and proprietary analysis software," explains Kalthof.

Screening is the first step on the long road to a new drug product. As Kalthof describes the process, "We test each of the 4.1 million molecules in our substance library to see if they exhibit the desired effect." On average, the scientists will identify an effect in only one in 400 candidates. The identified active molecules are then characterized and enhanced by Bayer scientists working in interdisciplinary project teams. "Many of these molecules will not make it through, but if everything proceeds ideally, the drug development process will ultimately lead to a new drug product receiving regulatory approval after 10 to 12 years," says Kalthof. Many of the success stories of Bayer's drug products and development candidates began in active ingredient screening. For example, the predecessor to the octopus was where the starting compound for the active ingredient rivaroxaban was discovered.

The octopus needs to be fed with a special diet to perform its duties. One precondition for ultra-high-throughput screening – the technical term that experts use to describe a system capable of processing more than 100,000 samples per day – is miniaturization of the sample vessels. "We work with what are called microtiter plates, which contain 1,536 separate wells for biochemical reactions. Each tiny hollow can hold a volume significantly smaller than the average raindrop," says Kalthof. The plates in the octopus are already loaded with the active ingredient candidates. The researchers test their effects firstly on isolated proteins and secondly on living cells. For both test formats, they use luminescence- or fluorescence-based measurement meth-

The master of the octopuses: Dr. Bernd Kalthof uses the high-performance robot to look for the active ingredients of the future.

After screening about

10 years

of drug development follow.

Source: Bayer

Photos: Bernhard Moll/Bayer AG (2), Peter Ginter/Bayer AG (1), Marcus Höhn/Bayer AG (1), Michael Rennertz/Bayer AG (1), Maria Nemchuk/Broad Institute (1)

A robot in a protective atmosphere: Dr. Donald Bierer (photo left) loads the equipment used for catalyst screening. He and his team are looking for chemicals that will accelerate reactions and make the synthesis of some active ingredients possible. Dr. Anke Müller-Fahrnow (photo right) is head of Lead Discovery, which includes the screening laboratory in Berlin. Like the octopus in Wuppertal, this system is also fully automated.

ods. A change in the measured light signal reveals that a substance intervenes in the process that the researchers want to influence pharmacologically.

"We in pharmaceuticals research at Bayer have long been committed to automated robots," says Dr. Anke Müller-Fahrnow, head of Lead Discovery in Berlin. She and her team use a similar robot system in a second screening laboratory in Berlin. In Cologne, meanwhile, colleagues employ a comparable approach to test and optimize therapeutic antibodies: they analyze the binding characteristics of more than 10,000 antibodies. Robots also operate what are known as the preparation laboratories – substance libraries in Berlin and Wuppertal which supply the active ingredient candidates for all of Bayer's research. "Of course, our colleagues also regularly check the purity of all the substances in these libraries. That is likewise done fully automatically," says Kalthof.

Robots create completely new molecules

Automation is a leitmotif that runs through many areas of research at Bayer. The synthesis of new molecules for the substance library can also potentially be fully automated. If Bayer's chemists experience problems in certain reactions, they can get help in the catalysis screening laboratory. "We usually come up with solutions by adding or changing the catalyst – a chemical reaction accelerant," explains Dr. Donald Bierer, head of the Catalysis Screening Laboratory at Bayer's Wuppertal Research Center. At the heart of the platform is another robot, which is capable of conducting up to 192 reactions simultaneously. This miniaturized approach helps the researchers find solutions for the production of new active ingredients. "We pass on our results to the chemists, and in 90 percent of cases the reaction works on a larger scale as well. In the other 10 percent, we work together with our clients on site to find a solution," explains Bierer. His team cooperates not only with the researchers in Medicinal Chemistry but with many other departments at Bayer too. "That really has been our concept for success since we began planning the platform in 2012: with teamwork, we can achieve new solutions," sums up Bierer.

At that time, the fully automated catalysis screening laboratory – operating on a small scale and using a protective gas atmosphere – was the second of its kind worldwide and more or less a prototype. Today, the system is in routine operation and this approach is becoming more and more popular around the world. Bierer and the catalysis screening team were thus able to make possible the synthesis of active ingredient candidates that their colleagues were initially able to produce only in very small quantities, if at all. This enabled the substances to be characterized earlier in more detail in subsequent investigations such as preclinical studies or field trials for pharmaceutical or agrochemical compounds respectively. These active ingredient candidates form the basis for a comprehensive synthesis program, for example in Medicinal Chemistry. Thousands of slightly modified variants of the starting molecule are generated with the objective of finding the one with the best efficacy.

The demands on pharmacological screening are increasing. "So we are boosting our throughput in all areas so that we have a greater probability of suc-

Screening for rivaroxaban

The story of the active ingredient rivaroxaban began in the screening laboratories in Wuppertal in 1998. Back then, Bayer's compound library comprised 200,000 substances that were screened in the search for suitable lead structures. Rivaroxaban is used to reduce coagulation and thus to prevent thrombus formation. It inhibits Factor Xa, which plays an important role in the blood coagulation process. Today, rivaroxaban is approved for the prevention and treatment of thromboembolism, i.e. vascular occlusion caused by blood clots in the bloodstream, in more than 130 countries. The active ingredient is used in indications such as stroke prevention in patients with non-valvular atrial fibrillation.

A four-armed octopus: the unique system can screen Bayer's entire substance library, containing 4.1 million molecules, within a few days. The octopus was constructed by Bayer employee Georg Schmidt, shown standing with colleague Maike Günther.

cess," explains Kalthof. And the key to this strategy is his fully automated octopus in Wuppertal, which reliably carries out its daily duties like clockwork, thanks partly to Kalthof's colleague Georg Schmidt who, together with technicians and designers from supplier companies, developed the unique construction. "There weren't any off-the-shelf solutions for this kind of system," explains Schmidt. Bayer's scientists are already planning the next step. A second octopus is set to start operations in Kalthof's screening laboratories in mid-2017. "And then we'll finally have our eight arms," says the master of the octopuses. ■

"Robots in everyday laboratory work"

research *talked to Dr. Virendar Kaushik, Director of Biochemistry and Biophysics at the Broad Institute of the Massachusetts Institute of Technology and Harvard, about robots in chemical and biological laboratories. Bayer is collaborating closely with the institution on the East Coast of the United States, in particular in the fields of oncology and cardiovascular diseases.*

Virendar Kaushik

When did scientists start using robots in the laboratory?

Robots were first used in the 1980s in biomedical research – or more precisely, in active ingredient development. Back then, they were mainly used for sample processing, for example to prepare dilution series and divide liquids among reaction vessels or microtiter plates. Automation was designed to increase precision and at the same time reduce the number of monotonous, repetitive actions done by humans.

What applications are they used for today?

Nowadays, robots are used extensively in biological and chemical research. In active ingredient development, robots enable chemists to set up large substance libraries straight away and allow biologists to test thousands of these molecules in an in vitro experiment. All the researchers have to do is provide a large quantity of reagents, select the program and press start.

What role will robots play in science in the future?

I think that robot applications will continue to increase. In particular, the miniaturization of reaction mixtures means that many tasks now have to be performed by robots. Humans don't have the visual acuity and dexterity required for these experimental formats.