CONTROLLING CELLS USING OPTOGENETIC METHODS

Precision lead finding

It is many scientists' new favorite tool: optogenetics, a relatively recent discipline that makes it possible to control cells with light signals. Bayer researchers want to use it to improve substance screening and to discover active substances that might otherwise never have been found.

Photos: Gaby Gerster/Bayer AG (1), Sabine Bungert/Bayer AG (3), mauritius images/Science Source/ Biophoto Associates (1), private (1)

Chemical mass production: any one of the many black plates shown here could contain the lead structure for a new active ingredient. Biochemist Dr. Linn Schneider uses a high-throughput robot system to search for promising molecules for drug development.

Controlling molecules at the touch of a button – that sounds like science fiction. In the still young research field of optogenetics, however, precisely this is becoming reality. Optogenetics is a union of two disciplines that, at first glance, have little to do with one another: optics and genetics. Modern methods in genetics enable scientists to make cells light-sensitive and then control them with optical impulses.

A mere ten years have passed since neurobiologist Karl Deisseroth from Stanford University in the United States astonished experts with one of the first optogenetic experiments. He managed to control brain cells in a mouse using light rays and to make the animal go round in circles. The potential uses nowadays extend far beyond neurobiology. Scientists in many different disciplines are working on this ▶

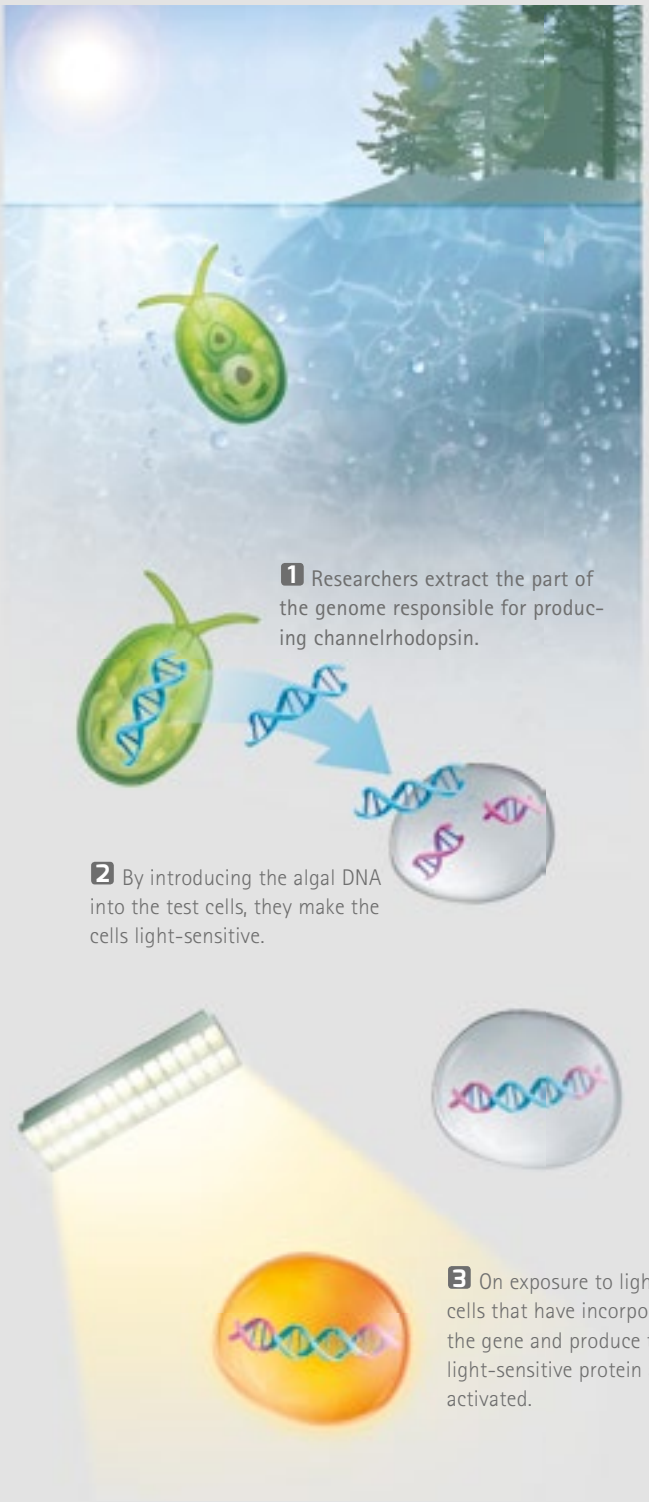
4.1

million active substances are stored in Bayer's substance libraries.

Source: Bayer

Optogenetics: light switch for cells

The freshwater alga Chlamydomonas seeks out places with favorable light conditions. It does this using the light-sensitive protein channelrhodopsin, which it can produce itself.



promising method worldwide, including Dr. Linn Schneider from Bayer's Pharmaceuticals Division and Dr. Arunas Damijonaitis from the Crop Science Division.

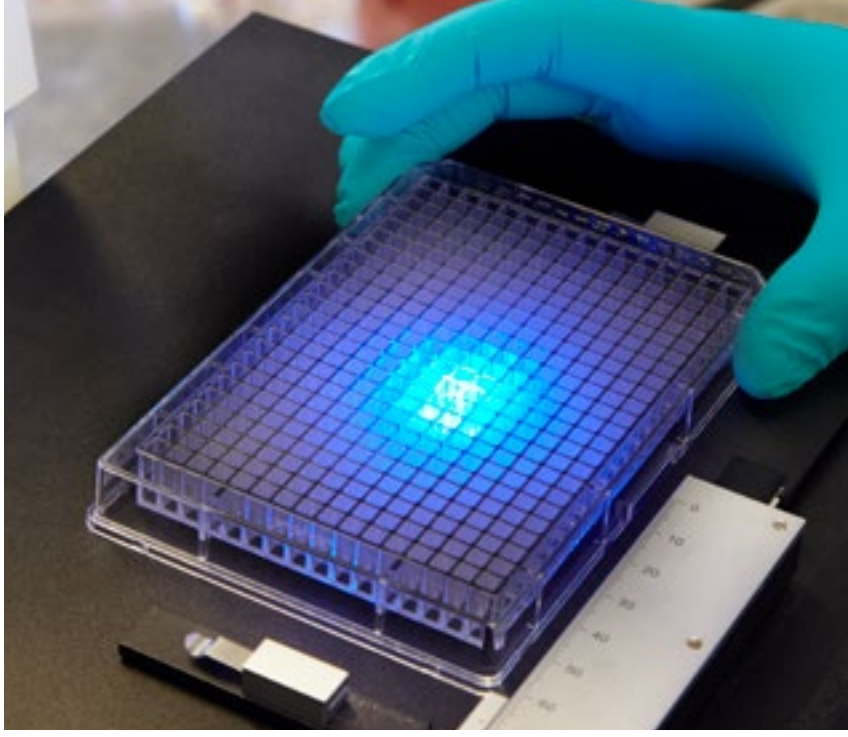
Anyone entering Schneider's laboratory in Wuppertal cannot fail to notice a complex robot system taking up a good third of the large room. "That's our octopus," says Schneider. Unlike its deep-sea namesake, the machine has only four arms instead of eight. The octopus is a fully automated high-throughput screening (HTS) system. Bayer researchers use it to test the pharmacological action of millions of substances. After identifying a target protein that might be associated with a particular disease, the scientists place it in a test cell together with a fluorescent indicator. In the octopus, these cells then encounter potential active substances, in other words molecules from Bayer's own substance libraries. If a substance binds to the target protein as desired, the indicator lights up indicating pharmacological activity. A camera records the corresponding spot on the microtiter plate in which the substance is held in one of 1,536 wells, and one important first step in the development of a new medicine is done.

Finding a suitable drug candidate for a medicine is a time-consuming task, in view of the sheer number of potential active substances; to start with, there are 4.1 million substances stored in Bayer's libraries alone. With the aid of optogenetics, however, Schneider and Damijonaitis want to improve the HTS process as part of the cross-divisional "Life Science Collaboration" initiative, as the conventional approach using chemical indicators has a number of disadvantages.

> *In the best-case scenario, we want to activate cells with one wavelength and measure them with another.*
>
> Dr. Linn Schneider

The biggest drawback is the unreliable hit rate. The cells that are the focus of biochemists' attention exist naturally in different states. "Some are active, others are passive," explains Damijonaitis. "But binding often takes place only in the active state."

Optogenetic methods could be useful in research into various biochemical processes. Scientists are focusing in particular on ion channels, i.e. proteins that, depending on their state, allow the passage of electrically charged particles. This is where scientists see the most common ground across divisions. A multitude of biological processes take place via ion channels. Their state depends on the membrane potential of the cell, in other words the electrical voltage between the inside of the cell membrane and its surroundings. In cell cultures, the distribution of ion channel



Optogenetics for crop protection: Dr. Arunas Damijonaitis (photo left) uses microtiter plates (photo right) to test large numbers of active substances simultaneously. With optogenetics and light impulses, he is making this method more reliable.

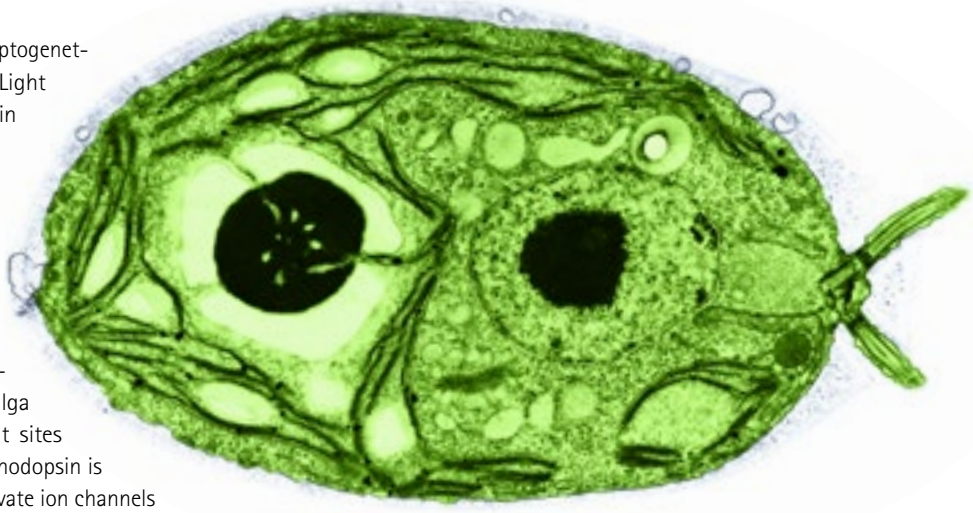
state is random. Since active compounds are often better able to bind to active ion channels, passive ion channels are not deemed important in providing any usable information in screening.

Scientists would therefore like to have a switch for cells. On a small scale, this already exists in electrophysiology, where researchers influence the membrane potential of cells with electrodes, resulting in their activation. However, this is a complex, rather slow process and one that is often not feasible for high-throughput screening.

Cell switches made possible through optogenetics, on the other hand, are much better. Light impulses activate ion channels in cells in thousandths of a second, switching them on more or less instantaneously. To do this, researchers use a light-sensitive molecule, known as the "optogenetic tool". In recent years, the optogenetic toolbox has been expanded to include a number of light-sensitive molecules. The best-researched of these is channelrhodopsin, used by the fresh-water alga Chlamydomonas reinhardtii to seek out sites with favorable light conditions. Channelrhodopsin is a light switch that researchers use to activate ion channels to increase their screening hit rate.

Introducing the switch into the cell is the genetic aspect of optogenetics. Researchers achieve this by inserting the part of the algal genome responsible for production of channelrhodopsin into the cell. The DNA acts as a kind of guide by which the cell pieces together proteins – in this case specifically the molecular light switch (see also *research* 30, "Light switches for molecules").

For Schneider's optogenetic research, the octopus screening robot was equipped with LED lights. This allows it to irradiate the substances with light of any color. The light spectrum also ▶



Light switch donor: movement of the freshwater alga Chlamydomonas reinhardtii is light-dependent. It is the source of the gene for the light-sensitive protein used in optogenetic experiments.

How optogenetics helps in the testing of thousands of substances

Researchers usually seek active substances by means of high-throughput screening (HTS). 1,536 miniature experiments with different active substances run simultaneously on what are known as microtiter plates. Screening is successful if a test substance binds to a target molecule. A light signal shows the researchers which of the experiments has been a success.

Conventional high-throughput screening

Since not all cells are activated and take part in the experiment, the light signals received by the researchers on the microtiter plates are weak.

Cells exist naturally in different states, either passive or active. An **active substance** can bind only to active cells and make an **indicator substance** light up.

Optogenetic high-throughput screening

Since all cells are optogenetically activated, the researchers receive clear light signals if experiments are successful.

The **active substance** binds to all available cells. Optogenetics consequently increases the reliability of HTS and speeds up the search for suitable active substances.

includes ultraviolet, in other words light that is invisible to the human eye. "In the best-case scenario, we would want to activate with one wavelength and measure with another," says Schneider.

This works if optogenetics is used to introduce not just switches but also sensor molecules into the cell. They perform the role of indicators and show whether binding between the active substance and the target protein occurs. Unlike chemical indicators in conventional HTS, they are not applied to the substance/cell mixture as a chemical but are introduced into the cell itself as DNA. "That, too, is progress, as it avoids misleading incidental effects," says Damijonaitis. "In addition, the new sensors measure ion flows faster and more selectively," adds Schneider. Until now, scientists had only been able to demonstrate electrical charges, irrespective of their element. With suitable sensors, they can now distinguish ions from one another.

In their work, Schneider and Damijonaitis are guided by recent scientific developments. Initial results with the new screening method are so promising that Bayer is providing additional

Preparations at the clean bench: Dr. Ursel Collienne prepares cells for optogenetic experiments. The researchers want to make them light up.

Key progress: with optogenetic methods, we avoid misleading incidental effects.

Dr. Arunas Damijonaitis

funding for their work for two years. With two postdocs appointed specifically for the "Life Science Collaboration", up to eight scientists are now working across divisions on optogenetic tools.

The potential applications are diverse. For Damijonaitis and his research for Crop Science, the top priority is ion channels. "Many of the insecticidal crop protection agents on the market address ion channels. Since ion channels in insects are completely different in some respects from those of vertebrates, we can target these ion channels specifically, without posing a risk to humans."

Even though Schneider and Damijonaitis work in different Bayer research fields, they share a common goal: "We want to discover something that we couldn't find with conventional systems," says Damijonaitis. In other words, both want to shed light in the darkness.

Interview

Alexander Gottschalk

"Potential for the medical field"

Biochemist Professor Alexander Gottschalk from the University of Frankfurt is one of the leading scientists in the field of optogenetics worldwide. research *talked to him about problems and opportunities in this young research discipline.*

For what purposes are optogenetic methods being used?

Optogenetics was originally used in neuroscience. Light-sensitive proteins are now also being used in cell biology. Any number of things can be controlled with them from the outside, whether gene expression, modification of the structure of cells or cell movement.

How does the protein know which cell to modify?

Genes, the "blueprints" for proteins, bear a kind of access code for this. A gene consists essentially of two segments: the promoter, a type of address, followed by the DNA sequence, which codes for the protein.

How do you introduce the light-sensitive proteins into the nerve cell?

To do this, we have to genetically modify the cell. In more complex organisms, such as a mouse, the genome that is to be incorporated and that is to make the cell light-sensitive is packed into a virus. The harmless gene vector introduces its genome, often in the form of RNA, into the cell, where it is translated into DNA and becomes permanently integrated into the host-cell genome.

From then on, the modified genetic information serves as the source for the optogenetic tool, i.e. the protein.

Could this be used in the future in medicine?

Potentially, yes. The proviso, however, is that the light for activation is able to reach even the affected cells. The process might work, for example, in degenerative diseases of the eye's retina. If photoreceptor cells die off with advancing years and no longer sense light, the cells that have previously only transmitted the light signal could themselves be made light-sensitive. Similar approaches are available for hearing.