

Marine creatures helping in medicine

The tell-tale glow

Colors from the deep: a great many marine animals have luminescent characteristics that provide a valuable service to active ingredients research.





Marine researcher: Russian biophysicist Professor Eugene S. Vysotski combs the sea for luminescent organisms on his expeditions.

The depths of the world's oceans are often brightly colored: fish, crabs and jellyfish glow in a multitude of hues. In most cases it is unclear what purpose these luminescent substances serve, but they are nonetheless an important help to science: in their search for new medicines, Bayer researchers are pinning their hopes on a luminescence gene of sea crabs.

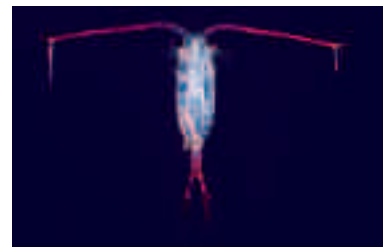
It happened in summer 1997 at a picnic in a forest near Moscow: it was the first time that Dr. Peter Spreyer, a biologist from Bayer HealthCare in Wuppertal, and Professor Eugene S. Vysotski, a biophysicist from Krasnoyarsk in Siberia, had spoken together. Their conversation was in English, and their subject was luminescent marine organisms, Vysotski's specialist area. Their dialogue is still going on today. "I was just traveling through, visiting colleagues at the Institute of Bioorganic Chemistry in the Russian Academy of Sciences," recalls Vysotski. "It was a lucky coincidence for me that I met the people from Bayer." The meeting was no coincidence at all, however, but deliberately set up, if you listen to Peter Spreyer: "In 1996 and 1997, the Bayer Board of Management started an initiative to pave the way for cooperation with Russia; the country was just beginning to open up to

the west. Dr. Hans-Joachim Zeiler from Innovation Management had been assigned the task of identifying potential cooperation partners and institutions in Russia for life sciences. We traveled to Moscow with a number of colleagues several times looking for partners for research projects. The aim was to find new tools for pharmaceutical research."

Spreyer had shortly beforehand started developing the first prototypes for screening processes using luminescence genes, known as luminescence assays, in Wuppertal. This was why he was visiting biophysicist Professor Yuli B. Alakov, a luminescence specialist, in the Shemyakin Institute in Pushchino, 110 kilometers to the south of the Russian capital. Alakov told him about his Siberian colleague Vysotski: "At the moment he is on board a boat on the White Sea, fishing for luminescent organisms, but I must introduce you to him if there is an opportunity." A couple of months later, at the picnic in the forest, the opportunity presented itself...

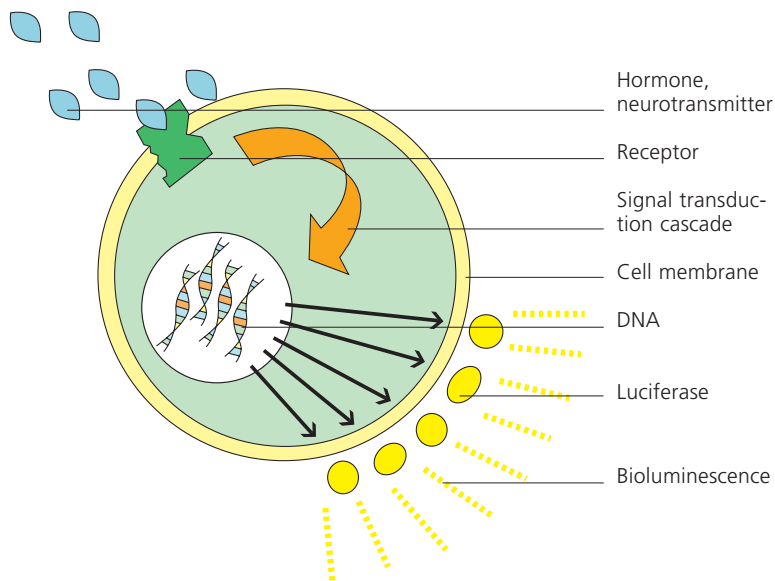
Cut to Wuppertal, Germany, May 2004. The robot in Dr. Bernd Kalthoff's laboratory makes a click-click sound. The 40-year old biologist manages the department for cellular test systems, and thus oversees three generations of laboratory robots, without which the rapid search for active ingredients would be unthinkable. The veteran amongst the three machines is hard at work.

Click-click: and once again the metal



Medical helpers from the sea

Metridia longa, from the glands of which the Russian biophysicist Eugene S. Vysotski isolated the enzyme luciferase Lu164, belongs to the Copepoda family. Although "longa" means long, the crab is only between 1 and 1.5 millimeters in size. It lives in cold waters, especially in Arctic and Antarctic zones. Why *Metridia longa*, in contrast to other copepods, not only makes light, but can also secrete its luminescent substance as a bluish shimmering cloud into the sea, is as yet unexplained. Vysotski and his colleagues suspect that it could be a defensive mechanism that fools its predators into attacking the cloud of light while the crab escapes, but at the moment this is only hypothesis. With other luminescent organisms, the function of the luminescence is clearer: the angler fish (*Melanocetus*) uses bioluminescence to lure its prey, while the glow worm (*Photinus pyralis*) uses its light when seeking a partner.



The way to the light

In the selective search for active ingredients, a cell is genetically manipulated in such a way that it produces the “reporter” luciferase. It then indicates by means of luminescence whether an active substance, such as a hormone, a neurotransmitter or a potential new medicinal product, has bound to the receptor on the cell membrane. Inside the cell, the luciferase gene is interpreted by a cascade (signal transduction cascade) of biochemical reactions. Depending on the information which it finds there, the cell starts to luminesce or goes dark.

arm of the robot has taken a plate of tiny cell cultures from the incubator and placed them under a camera system. Sensitive sensors record and later show on the computer screen the tell-tale glow which only occurs when a highly specific chemical reaction takes

place. Their aim is to find a substance which triggers this reaction. The glow comes from luciferase Lu164 – the enzyme from a tiny crab fished out of the White Sea by Professor Vysotski that spring. When coelenterazine, a small molecule which also

comes from sea crabs, is presented to this enzyme, it oxidizes it and a quantity of blue light is released. The enzyme Lu164 is already an established tool in the search for active ingredients in Bayer Health-Care’s high-throughput screening laboratories – a rapid rise to fame for a protein.

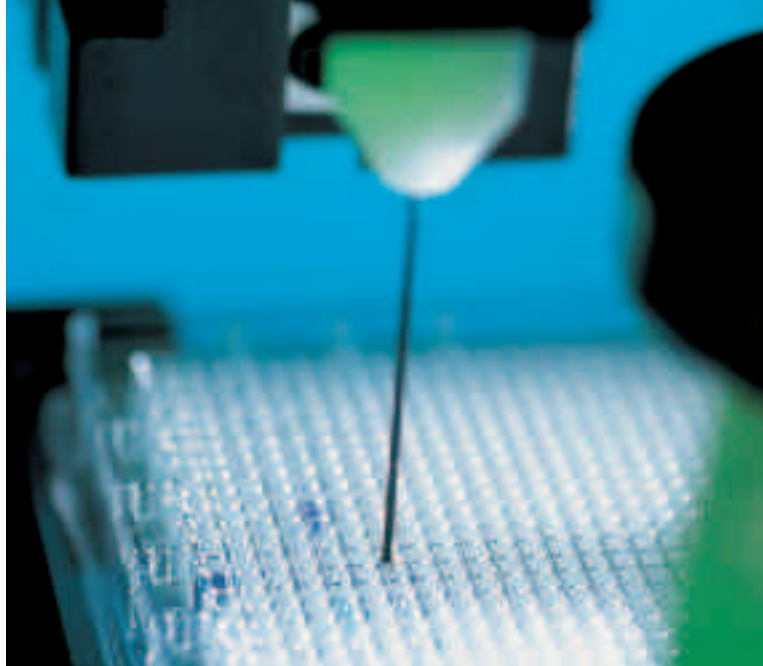
Luciferase reports what is really happening in the cell

“We use the luciferase gene as a reporter gene,” says Kalthof. “Like any other reporter, the enzyme tells us what is happening in the cell.” The complicated procedure is best explained by means of an example: Bayer is constantly searching for new active ingredients to combat coronary heart disease, which is the most common cause of death in the western world. Patients who have reached the first stage of this disease – stable angina pectoris – could for example be helped with a medicinal product that decreases their heart rate and so reduces their oxygen demand. Another enzyme plays a part in regulating the heart rate, however, namely adenylate cyclase. This enzyme produces a universal “messenger” known as cAMP. In a myocardial cell, cAMP determines how quickly and how strongly the cell contracts. They are not working with myocardial cells in Dr. Bernd Kalthof’s laboratory, however, but with ovarian cells from hamsters. These are easy to



Luminescent substance:
Dr. Stefan Golz demonstrates a luminescence reaction – the principle of luciferase.

Search for active ingredients:
In preparation for the test, a pipette needle distributes substances on to a microtiter plate. Any hits are then signaled by the luminescent substance luciferase (see picture below).



grow, reproduce and test in cell cultures. And however unlikely it may sound: in the search for active ingredients, it doesn't matter in the least what type of cell is used. This is because the "signal cascade", i.e. the sequence of reactions which ultimately lead to the up-regulation or down-regulation of the universal messenger cAMP, is the same in hamster ovarian cells as in a myocardial or nerve cell.

The only important thing is that the right receptor is located in the cell membrane, almost like a switch, which can be used to turn the signal cascade on or off from the outside. What is also important is that the aforementioned reporter is inside the cell reporting on whether cAMP has been formed or not.

Dr. Stefan Golz takes care of both these tasks. The 36-year-old Bayer HealthCare laboratory manager is responsible for the development of cell-based screening assays and the development of new reporter genes: at his suggestion, experienced genetic engineers reach for their biochemical construction kits and fit the appropriate genes into the hamster ovarian cells: the gene for the membrane switch (receptor) and the luciferase (reporter) gene. Not forgetting the "promoter", i.e. the starting signal for interpreting the reporter gene. The promoter only springs into action when cAMP is present in the cell: only then is luciferase formed, only then is the tell-tale glow created – de-

pending on whether the switch is "on" or "off".

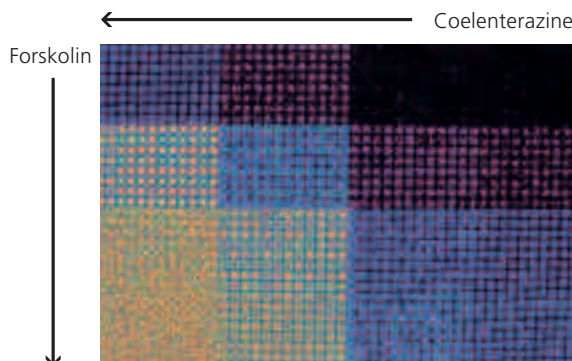
1.5 million substances tested in seven days

Complicated? "Yes, I found it very complicated at first," says Golz. "But once the preliminary genetic engineering work is done, it's really quite simple." So simple, in fact, that the robots can now take over some of the work: for example, they dispense the cell culture liquid into the wells on the microtiter plates routinely employed by Bayer, using the finest pipettes. Each such plate has precisely 1,536 wells, making it not much bigger than a bar of chocolate. An amount ten times smaller than a raindrop fits into each well. There are around 400 hamster ovarian cells in this liquid. After they have been "seeded" onto the plate, they are grown for two days in the incubator so that they form a cell layer at the bottom of each well. Only now does actual screening be-

gin: a small amount of liquid is drawn up from the wells, buffer liquid is added to it and then – this time using even finer pipettes – a microdroplet of "substance" is added to each well. A different substance in each well, of course.

The droplets contain chemicals of different size and characteristics, and they all come from Bayer's substance library. This collection contains about 1.5 million different substances, making it one of the largest in the world. Yet it only takes some seven or eight days to run them all through robot screening.

"If one of the 1,200 substances on the microtiter plate causes the cells to glow, then we've found an agonist," says Golz. "The screen shot from the camera system, virtually a photo of our microtiter plate, reveals it to us as a glowing dot." If, on the other hand, an inhibitor is needed, as for example with the angina pectoris remedy for the enzyme adenylate cyclase, then the scientists stimulate the glow with



Luminescent cells

Genetically modified cells grow in the wells of a microtiter plate filled with forskolin. They all contain luciferase Lu 164. The cells only glow when coelenterazine is added in sufficient quantities. There is a clear dose relationship: the more forskolin and coelenterazine, the brighter the light emitted from the cells.



Catch in the White Sea

Bayer researchers are cooperating with Russian marine scientists in the search for new avenues in medicine. In the White Sea, south of the Barents Sea, biophysicist Professor Eugene S. Vysotski caught the luminescent crab at a depth of 50 to 100 meters. *Metridia longa* forms part of the plankton there – the plethora of tiny marine organisms on which fish and other larger marine animals live.

a standard agonist, forskolin. Then they look for dark holes on the plate, which prove that the substance being tested has “switched the light out”. All this is a long, long way away from the home of *Metridia longa*, a copepod crab which measures just 1 to 1.5 millimeters and which the Bayer scientists have to thank for their luciferase Lu164. “This luciferase has a highly specialized characteristic,” emphasizes Kalthof. “It is secreted from the cell. We don’t have to destroy the cells to see the luminescence as we unfortunately used to have to do with other luciferases used in the past, such as glow-worms, for example.”

There is no question that the luciferase from Russia is a valuable aid to research. There are already efforts within Bayer not only to use the patents that acquired from Vysotski’s luminescence treasure chest for the scientists’ own work, but also to market it to other experts. “It’s fantastic what Vysotski brings up from the sea!” says Golz enthusiastically.

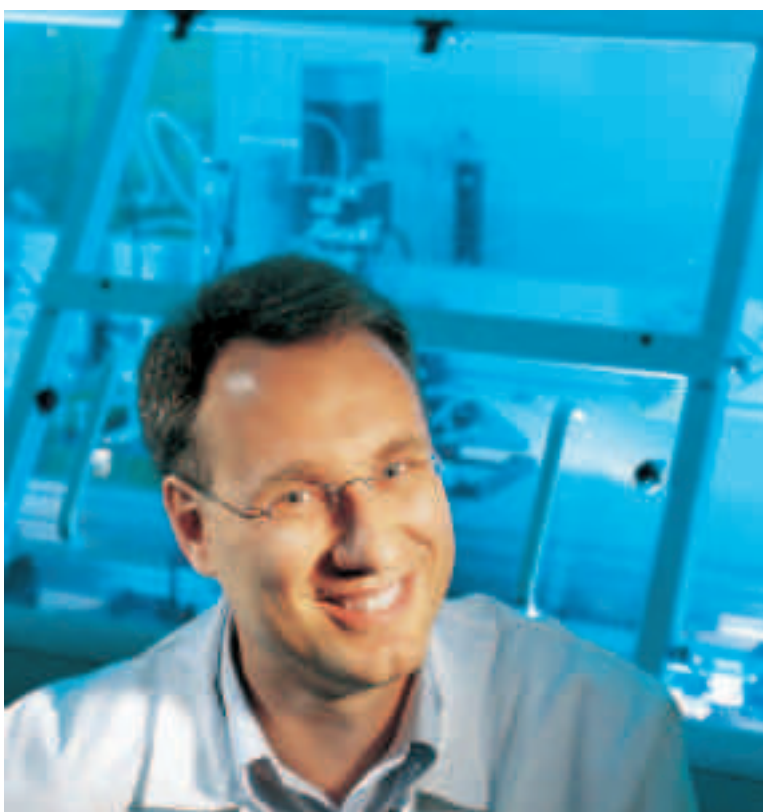
Glowing organisms, like stars in the night sky

What can a Siberian “landlubber” find so interesting in luminescent marine animals? Vysotski waxes lyrical: “If you’ve ever had the opportunity to see

the many luminescent organisms crowding together in the night sea, you will never forget it. It’s like the night sky, covered with millions of stars, only the tiny organisms are the small stars and the larger luminescent animals are the giants amongst them. Many comb jellies are like bright lights. They grow as large as a man’s fist and their light shines bright.”

For what purpose did nature create all this? And what biochemical tricks do the organisms use to achieve it? These are questions which have long interested the Siberian scientist. Since that picnic in the forest near Pushchino, Vysotski has been occupied with a new question: how can we use the light proteins as cellular “reporters”? “The co-operation with Bayer has expanded my research horizons,” he says. Distances are of no importance when interests are shared between Wuppertal, Krasnoyarsk and the White Sea.

Active ingredients researcher: Dr. Bernd Kalthof in front of the “hit picker” that he uses to prepare the promising substances found using luciferase for further experiments.



www.lifesci.ucsb.edu/~biolum/ “The Bioluminescence Web Page” is an award-winning site that provides information on bioluminescence. The site is hosted by scientists at the University of California in Santa Barbara.