Researcher with ingenuity: Katharina Landfester has already registered more than 50 patents for nanocapsules. And because her team is continually pursuing new ideas, there will be more to come.

The all-purpose capsule

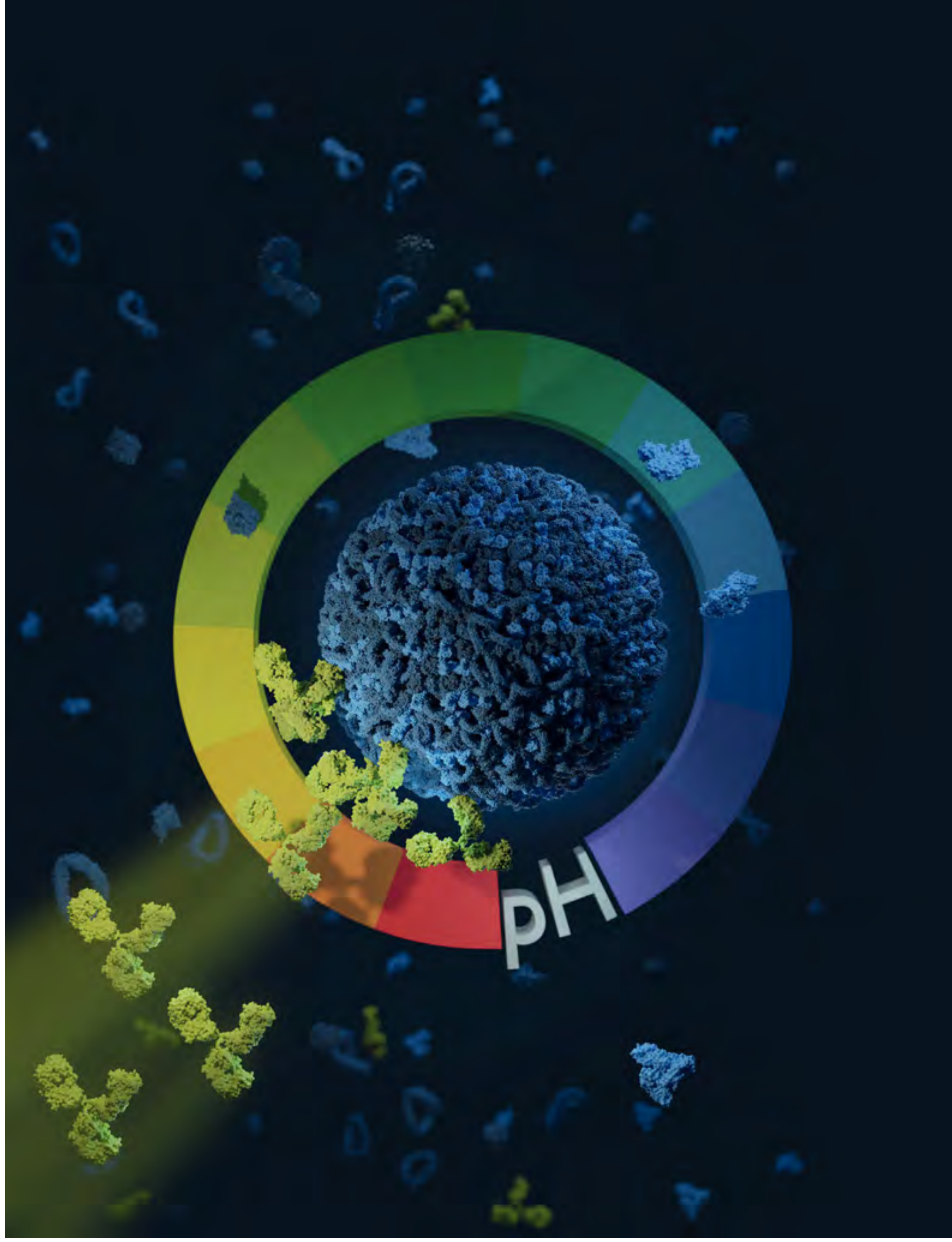
Katharina Landfester, Director at the **Max Planck Institute for Polymer Research** in Mainz, has opened the door to numerous applications. She has developed a technology whereby tiny containers can be specifically manufactured for almost any substance and equipped with various functions. Her team is now working on using nanocapsules as transporters for pharmaceuticals, as medical sensors, or as fungus treatments in wine production.

TEXT **TOBIAS HERRMANN**

When Katharina Landfester first held a glass of the milky liquid in her hand, she had no idea what was in it. The receptacle contained a mixture as unremarkable as its name: a mini-emulsion. Milk is a well-known example of a mini-emulsion. Tiny droplets of fat are distributed in a large quantity of water. These are held in suspension by proteins and lipids. But a mini-emulsion from Katharina Landfester's laboratory can do much more than milk. The chemist's team uses the droplets to produce nanospheres and nanocapsules that can be used as vehicles for numerous purposes. The particles can transport medications through the body or provide medically relevant data from the body. They are also useful for pest control in agriculture and for countless other technical applications.

The history of the multi-functional particles began in 1997. Back then, Landfester was working as a junior scientist in the Department of Markus Antonietti, Director at the Max Planck Institute of Colloids and Interfaces in Potsdam-Golm. There, she researched colloids and developed the first prototypes of the mini-emulsion droplets. However, she faced some obstacles along the way. "It was clear that we would have to take a close look at the physical-chemical processes in the mini-emulsions," explains Landfester. If these could be understood more precisely, it might be possible to produce a wide variety of nanoparticles in a targeted manner, controlled by the composition of the mini-emulsions. >

Photo: Frank Vinken



A question of environment: in an acidic solution (i.e. at a low pH), antibodies (yellow) attach themselves to nanotransporters (blue) without being damaged. In previous attempts to create a compound through chemical activation, the antibodies lost their function.

In 2003, Landfester – by now a professor at the University of Ulm – developed a method for producing emulsions in which all the droplets are nearly the same size. This worked both with emulsions of oily droplets in an aqueous environment and with mixtures of water droplets in oil. Landfester turned the emulsions into a versatile means of producing nanoparticles by allowing chemical reactions to take place on the surface of the droplets. For the first time, she succeeded in producing stable nanocapsules for various different purposes. Or, as Landfester puts it: "This allowed us to encapsulate virtually anything."

The chemist explains that the most important element of nanospheres is the shell. "The shell, which is only about 10 nanometers thick, must be absolutely leak-proof so that the encapsulated substance cannot diffuse out. But as soon as required, it must be possible to reliably open the shell." The researchers use enzymes, a change in temperature or pH value, or irradiation with UV light to do this.

THE CAPSULES NEED COVER AND A GUIDE SYSTEM

In her laboratory in Mainz, where she was appointed Director of the Max Planck Institute for Polymer Research, Landfester has been refining the chemical processes that turn nanocapsules into highly functional tools since 2008. Together with her colleagues, she has further developed the tiny capsules for various potential applications. Three ideas are particularly promising: a transporter for pharmaceuticals, a thermometer for cells, and a vaccine for grapevines.

The nanocapsules, which are intended to deliver an active ingredient to a specific focus of a disease, are perhaps the best example of how nanocontainers can be modified as required. This would allow the dose to be significantly increased where the active ingredient is most needed. The rest of the body would receive much less, and the side effects would be reduced to a minimum. This is a major advantage, particularly when determining the dose of cancer drugs for which there must be

Photo: Volker Mailänder / MPI for Polymer Research an appropriate balance between damage to the tumor and damage to the surrounding tissues.

Landfester's research team has already overcome several obstacles on the way to developing a cancer drug that is both more effective and better tolerated than other drugs thanks to the targeted delivery of active ingredients. Together with a team led by Volker Mailänder, who is both a doctor at the University Hospital in Mainz and a researcher at the Max Planck Institute, she has spent several years exploring the possibilities of the capsules. For example, they coated the surface of the capsules with proteins that immune cells or macrophages do not recognize as foreign. The camouflaged nanoparticles can then move through the human body unhindered by immune cells. And to ensure that the capsules penetrate only the target cells, they have built a kind of navigation system into them.

For this purpose, the researchers equipped the capsule shell with antibodies that are meant to guide the nanocapsules to the desired target. Nevertheless, it was precisely at this step that the scientists initially hit a brick wall. Mailänder remembers the difficulties well. "We wanted to chemically bind the antibody to the nanotransporter. In this process, also known as targeting, the surface of the nanotransporter is first chemically activated so that the antibody can dock to the carrier." However, this process always ended up altering or destroying the antibody, thus causing it to lose its effect. The researchers ultimately found a solution, albeit more or less by accident. "In order to be able to measure how effectively the antibody binds to the capsule, we performed a control experiment in which we did not activate the surface of the transporter but rather mixed nanocarriers and antibodies in a buffer solution." The scientists hypothesized that the antibody would be unable to bind to a non-activated surface and be easily removed by various washing processes.

To their great astonishment, the supposed negative control produced a better result than the actual experiment. However, this was not due to errors in the experiment: "Contrary to all previous findings, the non-activated nanocarrier obviously binds the antibodies to itself more strongly than the modified one," explains Mailänder. "We were faced with an enigma."

THE NANOTRANSPORTERS WORKED WELL IN MICE

The team found the answer in the slightly acidic buffer solution in which the antibodies unfolded easily and thus adhered firmly to the nanotransporter. This compound persisted even in media with a high content of other proteins – including blood. In contrast, the chemically activated carrier-antibody complex almost completely lost its effect.

After also overcoming this obstacle, the researchers tested their transporters in living organisms – and were successful. "We had already used nanotransporters in mice to transport some substances to the desired location," says Landfester, "and also released them there." The second step is performed primarily by enzymes. "We usually design the capsule shell so that it can be opened only by enzymes present in the target cells," says Landfester. The opening of some nanotransporters also differs depending on the pH value, which is different in cancer cells than in healthy tissue. So far, the scientists have encapsulated anti-inflammatory agents and drugs designed to specifically trigger immune cells. This vaccination should enable the immune system to more effectively fight cancer. However, before physicians can use this nanotherapy in practice, various tests, refinements, and clinical studies are needed. In the long term, however, the nano-submarines of Volker Mailänder and Katharina Landfester could make some treatments more effective and more tolerable.

The nanocapsules could not only improve therapy, but also biomedical research and diagnosis. To this end, Katharina Landfester and Stanislav Balouchev have developed a dual nanosensor that measures the temperature and oxygen content of a cell in real time. These two pieces of information are of particular interest for medicine. Whether protein synthesis, DNA repair, or signal molecules that dock to receptors – these constantly recurring biochemical processes that occur in every cell can be successful only if the temperature is right and the cell has the right amount of oxygen available. Deviations from the values may sometimes be the result of diseases. The appropriate measurements can therefore improve the understanding of what is wrong and also enable a diagnosis of the disease. >

For both measurements, dyes, which are transported in nanospheres consisting of a mixture of oil and wax to the diseased cells, play a decisive role. The dyes are stimulated to emit under red light, which also penetrates into deeper layers of the body. When measuring temperature, the color of the light that the dye molecules emit depends on how well they can move in the waxy nanospheres. These become increasingly soft, especially in the physiologically relevant range between 35°C and 42°C. The dye molecules come closer together more often in warmer environments. Because one molecule absorbs energy from the other during such an interaction and then emits more energetic light, the dye molecules emit yellow rather than red at a higher temperature of the tissue under investigation. In this way, they can be used as nanothermometers.

The nanospheres become an oxygen sensor because they contain dye molecules that bind a precisely known amount of activated oxygen contained in the sphere itself when excited with light. The resulting concentration gradient is balanced by oxygen diffusing into the cell. The more oxygen is present in the capsule environment, the faster the capsule refills with oxygen. As with a thermometer, the scientists calibrate their oxygen meter beforehand. They therefore know how fast

Photo: Christian Schneider / MPI for Polymer Research

An employee from Frederik Wurm's group drills a hole in a vine and attaches a plastic cup containing a few milliliters of a cocktail containing nanocapsules with a fungicide against the esca fungi, which is a dreaded vine pest.

this process takes place at a certain concentration. This enables them to deduce the oxygen concentration in the cell from the time when the capsule refills with oxygen.

The nanocapsules consist of biologically compatible components and are therefore harmless to the cell. However, this characteristic is accompanied by a significant disadvantage: within a few hours, they are degraded by enzymes. The dual nanosensor already works quite well in cell cultures, where it could one day help to investigate the effectiveness of active ingredients, for example. The researchers recently carried out the first experiments with mice. However, before they can test the method in human tissue, a lot of researching and experimentation is required. For this reason, Landfester describes the approach as "somewhat visionary."

Photo: Christian Schneider / MPI for Polymer Research

NANOBAIT WITH FUNGICIDE FOR LIGNIN-EATING FUNGI

An application beyond medicine that is already close to implementation is the protection of vines against fungal attack. This could help wine growers overcome their greatest adversary: esca. This is a group of fungi that eat their way through the trunk of the vine and decompose it – resulting in considerable financial losses every year.

The fungi ravenously attack the lignin, one of the main components of the vine. A team of researchers led by Frederik Wurm, group leader in Katharina Landfester's Department, take advantage of this. Using mini-emulsion technology, they produce tiny capsules of lignin and fill them with fungicides. For the treatment, they drill a hole in the trunk and attach a small plastic container containing a few milliliters of a suspension toxic to the fungi. The cocktail of nanobait is then drawn into the vine. There, the esca fungi are attracted by the lignin-containing shell of the nanocapsules and eat them. "They effectively dig their own grave," says Wurm. Because as soon as they open the capsule, the active ingredient escapes and does its job.

In the past, fungicides were simply sprayed on the vines. However, this treatment did not work for long and had to be repeated regularly. As a result, fungicide residues were detected in the grapes. And even with repeated applications, the plant protection agent did not effectively combat esca.

This novel method eliminates the fungus reliably and sustainably, even though significantly less of the fungicide is used here than in the spray treatment. "We conducted the first experiments five years ago, and the vines we treated then are still doing well," says the scientist. Because the fungicide was used sparingly, no traces of the fungicides were found in the grapes. A further advantage of the nano-fungus treatment is that it is a form of upcycling: the lignin for the nanocapsules is a by-product of paper production.

The method has been met with great interest from winegrowers and agrochemical companies alike. Talks are already underway with several companies about possible cooperation, says Wurm. A spin-off is also conceivable. The scientists are still carrying out various tests but hope to market the product soon. The esca fungi could then literally get their last meal.

"WE STILL HAVE PLENTY IDEAS"

The treatment of grapevines, the deployment of nanosensors and the transport of pharmaceuticals are just a few of the many possible applications of nanocapsule technology. Among other things, Katharina Landfester and her colleagues have already developed corrosion protection for airplanes, adhesives that gain and lose their adhesiveness as required, and a printer's ink with which electrically conductive polymers can be printed. Katharina Landfester has already filed around 50 patents in connection with the nanocapsules – the first during the late 1990s. While she was at Max Planck, she received ongoing support from the patent experts at Max Planck Innovation. Some patents for developments that Landfester made in Ulm were later transferred to the Max Planck Society. Her team will continue to employ patent experts at Max Planck Innovation: "We still have plenty of ideas about what we could do with the nanocapsules," says the researcher. ◂