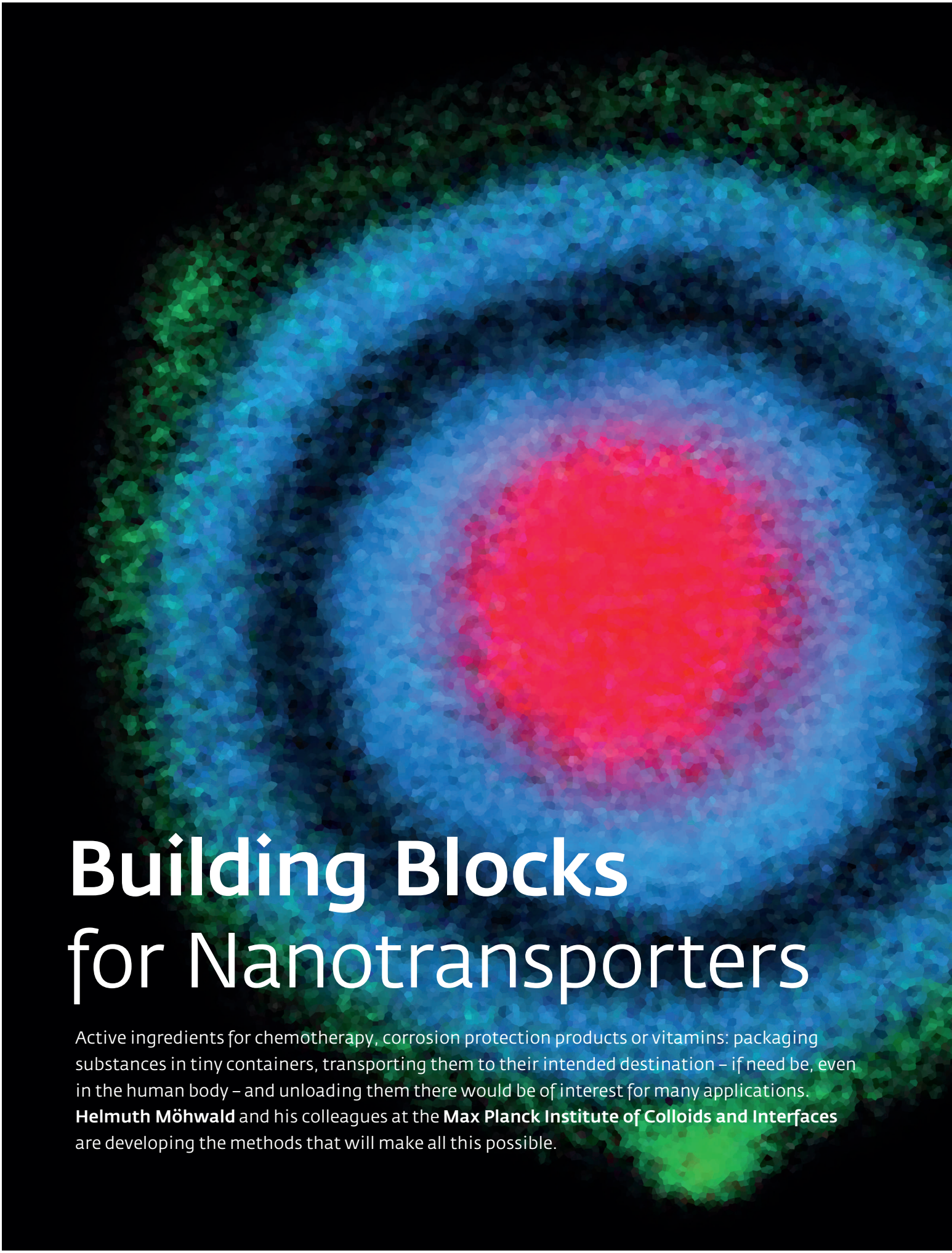


Building Blocks for Nanotransporters

Active ingredients for chemotherapy, corrosion protection products or vitamins: packaging substances in tiny containers, transporting them to their intended destination – if need be, even in the human body – and unloading them there would be of interest for many applications. **Helmuth Möhwald** and his colleagues at the **Max Planck Institute of Colloids and Interfaces** are developing the methods that will make all this possible.

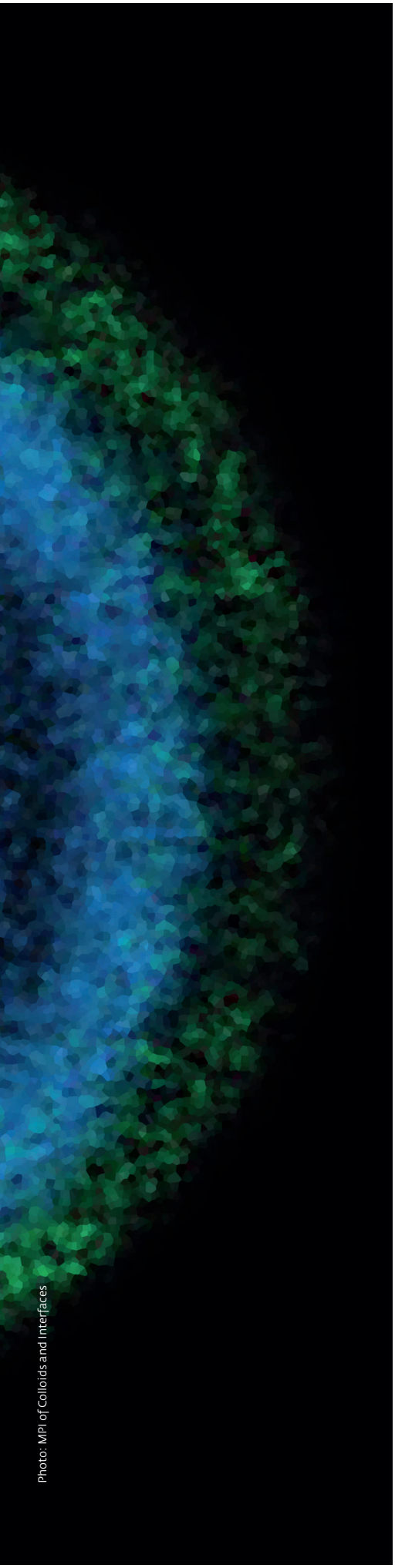


Photo: MPI of Colloids and Interfaces

TEXT **REBECCA WINKELS**

Even a simple train ride from Berlin to Potsdam goes smoothly only because everything's well organized: the train rolls into Berlin's main station, the passengers open the doors and crowd in to the sound of "all aboard!" Then the train unerringly finds its way, through a rather complex rail network, to Potsdam station. There, the doors open again and an automated message politely invites the passengers to leave the train. Passengers don't notice just how complex it is to plan and operate such everyday transport systems until things don't work: providing suitable places and mechanisms for getting on and off is just as important as the route and a control system that guides the train to the right place.

A transport system that is intended to head for somewhere much smaller than a train station to deliver a nanoscale cargo is no less complicated. Potsdam, or more specifically Potsdam-Golm, is exactly the right place to go to find out how all this works. There, at the Max Planck Institute of Colloids and Interfaces, is where, for more than 20 years, Helmuth Möhwald and his colleagues have been developing micro- and nanocapsules that can do just that. The scientists are investigating how to create micro- and nanotransport systems, for example to deliver pharmaceutical substances to their intended target in the body and release them there.

Russian doll capsule: A number of different-colored hollow spheres nested inside one another can accommodate various substances.

This could provide a solution to, among other things, the dilemma doctors face when they have to treat cancer with toxic chemotherapy agents that not only inhibit the growth of tumor cells, but also damage healthy tissue. Releasing active ingredients right at the focus of the disease is one solution to this problem. In addition, in many diseases, medicines are intended to act over an extended period. Transport capsules of the kind being developed by the Max Planck researchers are capable of releasing active ingredients slowly, thus providing long-term treatment for a disease.

A SIMPLE PRINCIPLE WITH A WIDE RANGE OF BENEFITS

Micro- and nanocontainers for transporting materials would also be useful in fields other than pharmaceuticals. They could be used in corrosion protection, biochemistry, surface coating and micromechanics. "The wide range of potential applications make development work particularly attractive," says Helmuth Möhwald, who has been a Director at the Max Planck institute since 1993, and who has also headed the "Interfaces" department there since that time. "A simple principle can provide the basis for a wide range of benefits and solutions to the most varied problems."

"In principle we now have the ability to produce polymer containers that measure just a few hundred nanometers in size and that can ferry any active ingredient to almost anywhere in the body and release it there. It is difficult

to achieve all the required properties at the same time," says Möhwald. "The fact that we can now do this successfully is a massive step forward compared with our capabilities when research began here in Golm."

Initially, he and his colleagues investigated chemical, biological and physical processes that take place at interfaces between biological membranes and their gaseous or liquid surroundings. Biological membranes consist of a double layer made up of fat molecules, proteins and polysaccharides. To facilitate their investigations into membrane properties, the researchers used one half of the membrane as a model, and initially coated it with individual molecules. The resultant monolayers on a surface allowed them to gain a better understanding of the properties of biological membranes, and to investigate the interactions between interfaces and their surroundings. The emphasis at this point was on the phospholipid monolayer, which is distinguished by a particular domain structure and many different phases. The monolayer varies between a solid and a liquid state when it interacts with binding proteins, DNA strands or other biomolecules.

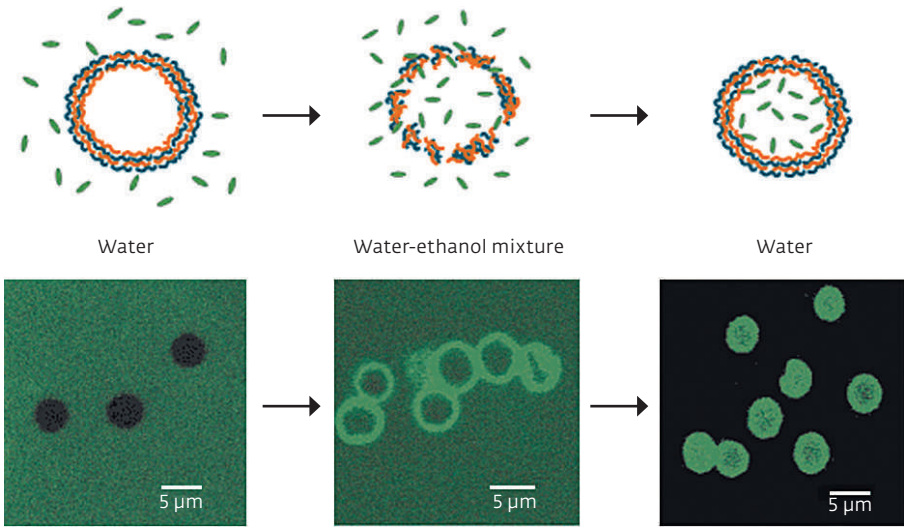
The Potsdam-based scientists are building on the findings from these investigations by developing organic multilayers of negatively and positively charged polymers, which are simpler to produce and handle than, for instance, lipid membranes. They are exploiting the electrostatic interactions between the differently charged molecules: they dip a material with a negatively charged surface into a solution with positively charged molecules, causing the latter to be deposited on the surface. This results in a positively charged layer exactly one molecule deep and approximately one nanometer thick. "This is an example of self-organization, where we merely exploit the interactions of the molecules with one another, and the individual layers delimit themselves from one another, as it were," says Helmuth Möhwald.

SOME CARGOES NEED SPECIAL LOGISTICS

In the next step, the surface then binds negatively charged molecules. In this way, the researchers can build up any number of alternately positively and negatively charged layers, both on a flat surface and around a particle that is to be packaged for transport. The size and shape of the vehicles then depend on the material enclosed, and may be individually specified for each substance. However, not every cargo can be packaged quite so simply. In such cases, the Potsdam-based scientists rely on another logistical solution. By carefully modifying the conditions in the polymer solution, such as temperature, concentration and pH, they can dissolve the core, giving rise to hollow spheres that have the dimensions of the templates. Producing a transporter for drugs in this way often proves to be a particular challenge, since nanocapsules are often the only suitable option for transporting active ingredients, as only very small containers can pass through the cell membrane. However, producing polymer vehicles smaller than 100 nanometers in size is tricky: such tiny hollow spheres can easily clump together into an unusable conglomerate. The researchers prevent this from happening by working with highly diluted solutions in which the finished hollow spheres virtually never come into contact with each other. "Ultimately, it's more com-

Photo: David Ausserhofer

Tatiana Kolesnikova prepares the process for introducing polysaccharides labeled with fluorescent molecules into the polymer capsules (left). She investigates the properties of the capsule walls, for example their thickness and roughness, with an atomic force microscope (right). Before turning the instrument on, she closes the lid of the foam-lined box to protect the sensitive measurement from vibrations.



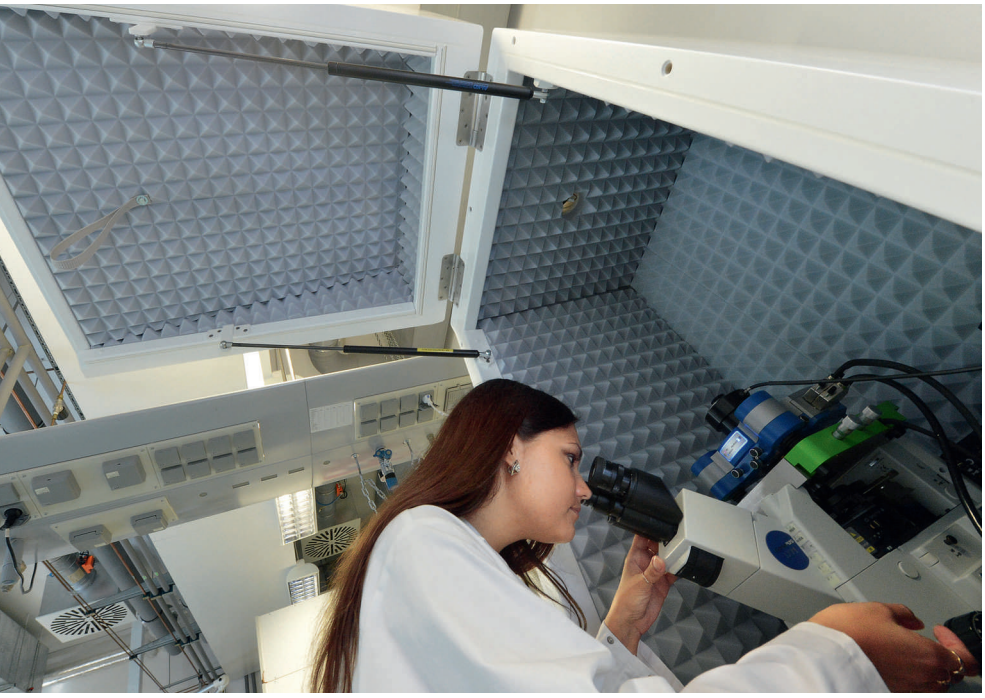
The art of packaging in a test tube: One mechanism for introducing substances into the micro- and nanospheres makes use of an effect of the solvent. In water, the capsule wall is watertight (left); in a water-ethanol mixture, it becomes permeable, and the luminous green test load penetrates into the hollow spheres. The polymer wall closes again in water.

Photo: David Ausserhofer; graphic: 2001 American Chemical Society

plex than producing larger vehicles. But even so, we've discovered various usable methods," explains Helmuth Möhwald.

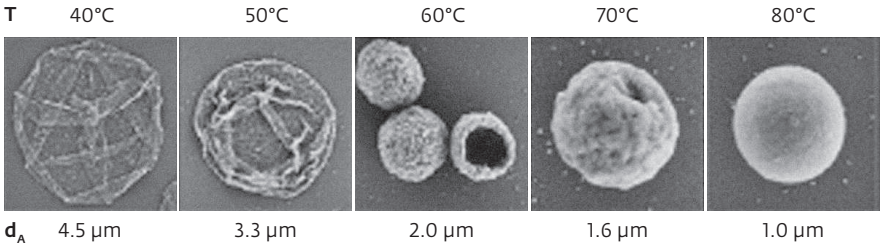
Then drugs or other substances have to be brought into the resultant containers. In the same way as a train door that opens to allow boarding and stays closed during the ride, a capsule should accommodate the active ingredient and not let it back out until it reaches its site of action. The researchers aim to achieve this by designing the capsule wall in such a way that it becomes permeable when required. As the scientists in Potsdam discovered, the thickness and density of the capsule wall, and thus its permeability, can be controlled just by altering the temperature and salt content of the solution.

For instance, increasing the temperature causes the molecular building blocks of the capsule wall to move more vigorously, and the shell softens. This can have two diametrically opposed effects: either the container shrinks or it swells. If the hollow spheres contract, the bonds between the differently charged polymer layers break and their constituents mix and coalesce. As a result, the wall becomes thicker and denser and encloses the active ingredient in the interior. However, if the layers start to move, this can have precisely the opposite effect. If the differently charged layers move apart, they no longer have a neutralizing effect on each other. The identically charged particles in an individual layer then become more sensitive to each other – they repel one another and move apart. This causes the polymer wall to expand, making it thinner and more permeable.



In order to close the wall again, the researchers neutralize the electrical charges with a salt, making the wall thicker. "Wall permeability can thus be systematically and reversibly controlled," says Tatiana Kolesnikova, a postdoc at the Max Planck institute. "We can thus decide when active ingredients pass through the capsule wall." Using this principle, she and her colleagues not only fill the spheres with active ingredients, but also release the substances where they're needed.

However, temperature isn't the only way to control permeability. It can also

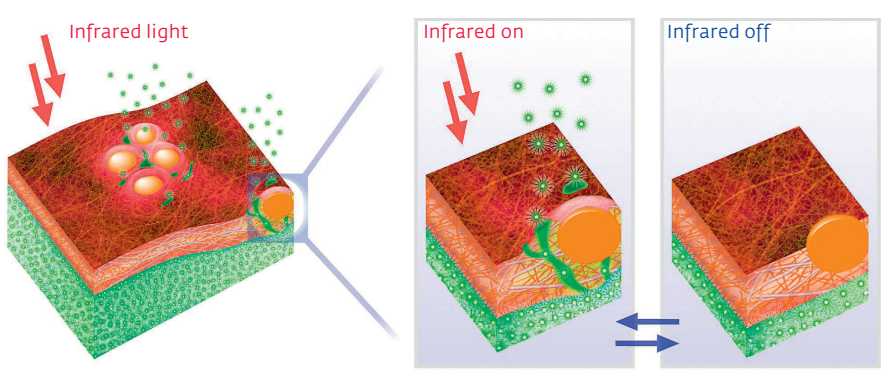


Shrinkage in heat: The polymer capsules contract as the temperature rises. As they shrink, the walls become more rigid and the spheres retain their shape, even under a scanning electron microscope.

be controlled by varying the pH-value. In cancer cells, for example, the pH-value is lower than that of healthy tissue. If a capsule carrying a cytotoxin payload infiltrates a cancer cell, the capsule wall becomes more permeable and the active ingredient escapes. "We can also use a similar mechanism in corrosion protection applications, because a different pH-value prevails in the vicinity of a corrosion spot than in the undamaged material," explains Möhwald.

However, if active ingredients are to be unloaded as accurately as possible and in the greatest possible concentration at a specific location in the body, the container needs proper doors that can be opened from the outside. The Max Planck researchers' chemistry set has something suitable for this, too: molecules or nanoparticles that can be built into the shell and act as door openers. For instance, if the scientists pack the capsule wall with gold particles, they can open the wall with pinpoint accuracy using an infrared light stimulus: the gold particles heat up in the infrared light and warm their surroundings, and the contents pass through the shell at precisely this point. "We can also achieve targeted substance release with ultrasound, microwaves or chemical reactions in which the polymer is, for example, broken down by enzymes," says Kolesnikova. The Potsdam-based researchers have also developed corresponding building blocks that can be integrated into the vehicle shell and open the containers by means of these mechanisms.

Hatchways in the capsule: The gold particles, depicted as spheres, in the polymer shell act as antennas for infrared light, which heats the precious metal while being particularly compatible with tissue. The capsule wall melts in the immediate vicinity of the particles, releasing the fluorescent green particles. As soon as the light beam is switched off, the wall closes again.



LIME TEMPLATES FOR MULTIFUNCTIONAL CAPSULES

Which particles the researchers use to produce the shell and what size they make the nanocapsules depend on the active ingredient they want to transport. However, gold particles, which absorb infrared light, are of particular interest especially for medical applications. Radiation of this wavelength can penetrate tissue to a depth of one centimeter, and the particles absorb light around one million times better than dye molecules. "This means that, in a cell, we can target a capsule that releases an active ingredient at a precisely specified time. We can even release different active ingredients simultaneously or in succession," says Möhwald.

The researchers in Potsdam recently presented an elegant method for incorporating various building blocks with different functions into hollow spheres, thus producing containers of an exact size. They use calcium carbonate – lime – microspheres as templates. First, they fill the pores of the calcium carbonate particles with the nanoparticles that ultimately form the shell and perform various functions, and also with the cargo for the transport con-

Photos: The Royal Society of Chemistry (top); graphic: MPI of Colloids and Interfaces

Nanotransporter designer: Helmuth Möhwald and his colleagues have developed a wide range of methods for providing polymer capsules with various functions. The poster and screens in the background show atomic force microscope images of surfaces, for instance of the capsule walls.

Photo: MPI of Colloids and Interfaces – Göran Gnaudschun

tainer. They then mix the filled calcium carbonate spheres into a solution of short amino acid chains or other polymers. The molecules are deposited like threads in the pores and around the filled calcium carbonate spheres, and are crosslinked with a further protein or polymer building block to form a dense cocoon. When the researchers then remove the calcium carbonate with an acid, the nanoparticles arrange themselves into hollow spheres in the protein shell or polymer threads and take on exactly the same size as the calcium carbonate spheres.

"This method is easier to control, faster to implement and less costly than previous methods for producing such containers," says Möhwald. And in the case of vehicles for a medicinal cargo, the protein shell itself is extremely useful. It not only defines the size of the hollow spheres, but it also ensures that the containers don't clump together, and makes them biocompatible. The protein web, for instance, prevents the colloidal spheres from becoming unstable in the highly saline environment of the body.

SIGNAL PEPTIDES ACT AS INTERACTIVE ADDRESS LABELS

Irrespective of the construction plans the researchers follow to produce the micro- and nanocontainers for medical use, they must always select materials for the shell that don't induce an immune response, so the body's defense cells don't intercept the active ingredient vehicles before they reach their target. Alternatively, the scientists design the containers in such a way that they can escape from the immune system's phagocytes. For instance, macrophages, which eliminate any intruders or foreign bodies from the body, find it more difficult to grasp long, thin containers.

However, being able to smuggle an active ingredient container past the immune system and unload it in a targeted manner is helpful only if the means of transport can also find its way to the target. What a navigation device does for a car, signal peptides, acting as interactive address labels, do for the active ingredient containers. These signal peptides are appendages coupled to the polymer shell that fit only receptors on the target cell. They direct the capsules to their destination because they continue to travel around the body until their address labels dock onto the correct receptors on the target cell.

During years of research work, Helmuth Möhwald and his colleagues developed a range of methods for customizing micro- and nanocontainers with various properties. "All we need is an idea for a specific container property and we can do just about anything," says Tatiana Kolesnikova.

However, in order to be able to draw up detailed instructions for practical pharmaceutical use, the methods have to be optimized for each specific case, as the list of requirements for the capsules is determined by the substance to

be transported and its target and purpose in the body. They can't be too large, must adapt to the biochemical circumstances at the site of action, and should produce an ideal concentration of the active ingredient at the target. The technique used to produce the capsules, in turn, is determined by the precise design of the containers. "This is one of the reasons why industrial applications are still faltering. The methods must be highly specialized for specific active ingredients," explains Helmuth Möhwald. "That's complicated and costly, but from a scientific standpoint, possible."

COLLOIDAL SPHERES AS VALVES IN MICROPUMPS

Capsules of the kind developed by the researchers in Golm could, however, be used for applications other than combating disease. Indeed, Möhwald can see a whole spectrum of very specific and very different potential applications, for example in nutritional supplements. For example, vitamin drinks could contain high concentrations of vitamins while remaining as clear as possible. "They should look delicious and be effective," says Möhwald. "And that's just what we could achieve if we packaged the vitamins at high concentration in colorless nanocapsules."

The hollow spheres could also be of use as a component in automotive coating systems, because many of the substances currently used for corrosion protection, such as chromium(VI) compounds, are harmful to health and need to be replaced. However, the non-harmful active ingredients disturb the coating process and are readily dissolved out of the coating. This is why the scientists are currently developing capsules that enclose alternative active ingredients that aren't harmful to health, and that release them only when required.

However, acting as a container for substances to be encapsulated isn't the only potential application for these hollow spheres. The colloidal spheres are also suitable as micromechanical components, for instance as valves controlled by salt concentration in micropumps. A hollow sphere expands when exposed to an elevated salt content in its surroundings and can block a channel, while it shrinks when the salt concentration drops, opening the channel back up. "Our modular concept could therefore be used in many sectors," comments Möhwald.

However, especially in medicine, it's not enough for the micro- or nanotransporters to perform all the necessary functions reliably. They won't have a chance on the pharmaceuticals market unless they are user-friendly and are also accepted by patients. For example, plans to distribute an insulin preparation that diabetics could inhale came to nothing some years ago because patients preferred to continue injecting the medicine. It's unclear whether this was because of distrust of a new technique or for other reasons. In any case, the example shows how difficult it can be for a method that is successful from a scientific standpoint to actually make it into practical use. The basic researchers therefore still have some work to do, as Helmuth Möhwald explains: "We consider it our job to point out potential applications and hope that, at least for simple applications, we can demonstrate the practical benefits and convince industrial partners of them." ◂

TO THE POINT

- **Nano- and microcapsules that enclose active ingredients may be produced from many different materials and be given different functions.**
- **The colloidal spheres are suitable as containers not only for drugs, for instance in chemotherapy, but also for corrosion protection or as micromechanical components.**
- **The capsules and methods for producing them must be optimized for specific applications, and the scientists at the Max Planck Institute of Colloids and Interfaces have developed a range of options for achieving this.**

GLOSSARY

Colloids: Particles up to a size of approximately one micrometer, for instance of a polymer or droplets that are present in finely dispersed form in another medium, usually a liquid.

Permeability: The ability of a material to allow a gas or liquid to pass through it.

Phase: Area having a homogeneous chemical composition and identical physical properties. Steam above water and a layer of oil on water each constitute two-phase systems.