The Formula for **Lobster Shell**

Lightweight and flexible, yet strong: The properties of the lobster shell derive from a sophisticated structure of chitin and calcium carbonate.

For gourmets, they are mainly a nuisance. For **Helge Fabritius**, however, they are a treasure trove of information. At the **Max-Planck-Institut für Eisenforschung** in Düsseldorf, the biologist investigates the construction principles of lobster and crab shells. In the process, he is uncovering how arthropods produce versatile material properties using a very limited choice of basic materials.

TEXT **ALEXANDER STIRN**

Photo: iStockphoto

Helge Fabritius enjoys a nice lobster, especially if it is freshly caught and served in a small restaurant somewhere on the coast. However, he wouldn't be disappointed if the lobsters were to arrive on ice. On the contrary, this makes them particularly valuable for him. This is because the 41-year-old is less interested in the white flesh or the sweet flavor, but is much more fascinated by the stuff the gourmets would discard: the lobster shell and its intricate structure.

Helge Fabritius is a biologist. He works in Düsseldorf, at the Max-Planck-Institut für Eisenforschung (Iron Research). This may sound like a paradox, but there is a straight-forward explanation: Scientists at the Düsseldorf-based institute, on the search for novel materials, have long since moved beyond the standard ores and metals. They now look at so-called composites, too – an area where Mother Nature, with her methods for evolving particularly sophisticated materials that have been tried and tested through millions of years, sets an excellent example.

"Virtually all natural composites follow more or less the same rules," says Helge Fabritius. "The exoskeleton of arthropods is a good model for learning how nature solves different problems." Still, the intention is not really to imitate nature. Rather, Fabritius wants to gain new insights, new ideas and, ultimately, inspiration for new materials.

The basement of the brick building in Düsseldorf is cool, and a gentle breeze can be heard. Almost like on the coast – but the sound and the breeze here emanate, not from the sea, but from the air conditioning system. The system is necessary to compensate for subtle variations in temperature in the room where Helge Fabritius' favorite instrument is set up: a scanning electron microscope. Stable ambient conditions are necessary for the device to deliver optimal results.

A BIOLOGIST IN AN IRON RESEARCH ENVIRONMENT?

Helge Fabritius deftly turns the palm-sized knobs on the control panel. He scrolls the image, changes the magnification and adjusts the focus. On screen, an image appears that seems to reveal a tangle of grey twine. In actual fact, however, it shows the inside of a lobster shell.

According to the stories circulating at the institute in Düsseldorf, Helge Fabritius owes his presence in the laboratory to a lobster dinner. Apparently, Dierk Raabe, Director of the institute and head of the department of Microstructure Physics and Alloy Design, was dining on one of these animals in a restaurant one evening, when suddenly the shell caught his interest. He soon assigned the task of studying the lobster carapace to a physicist, a chemist and an engineer. They were to establish how this surprisingly stiff and, at the same time, lightweight material is structured.

The scientists began to study the material, using all the tricks available to them in metals research. However, the success they had hoped for eluded them, since the material simply did not behave like a metal. It was then that Helge Fabritius received an e-mail. At the time, he was working on his doctoral thesis in Ulm. The subject of the thesis: the biomineralization of isopods (woodlice). "Iron research? At first, I thought someone was pulling my leg," Fabritius says, laughing. "Why did they want me to give a lecture on tiny calcium carbonate spherules in isopods?" Nevertheless, he gave the lecture. And not long after, Fabritius was offered a postdoctoral position in iron research.

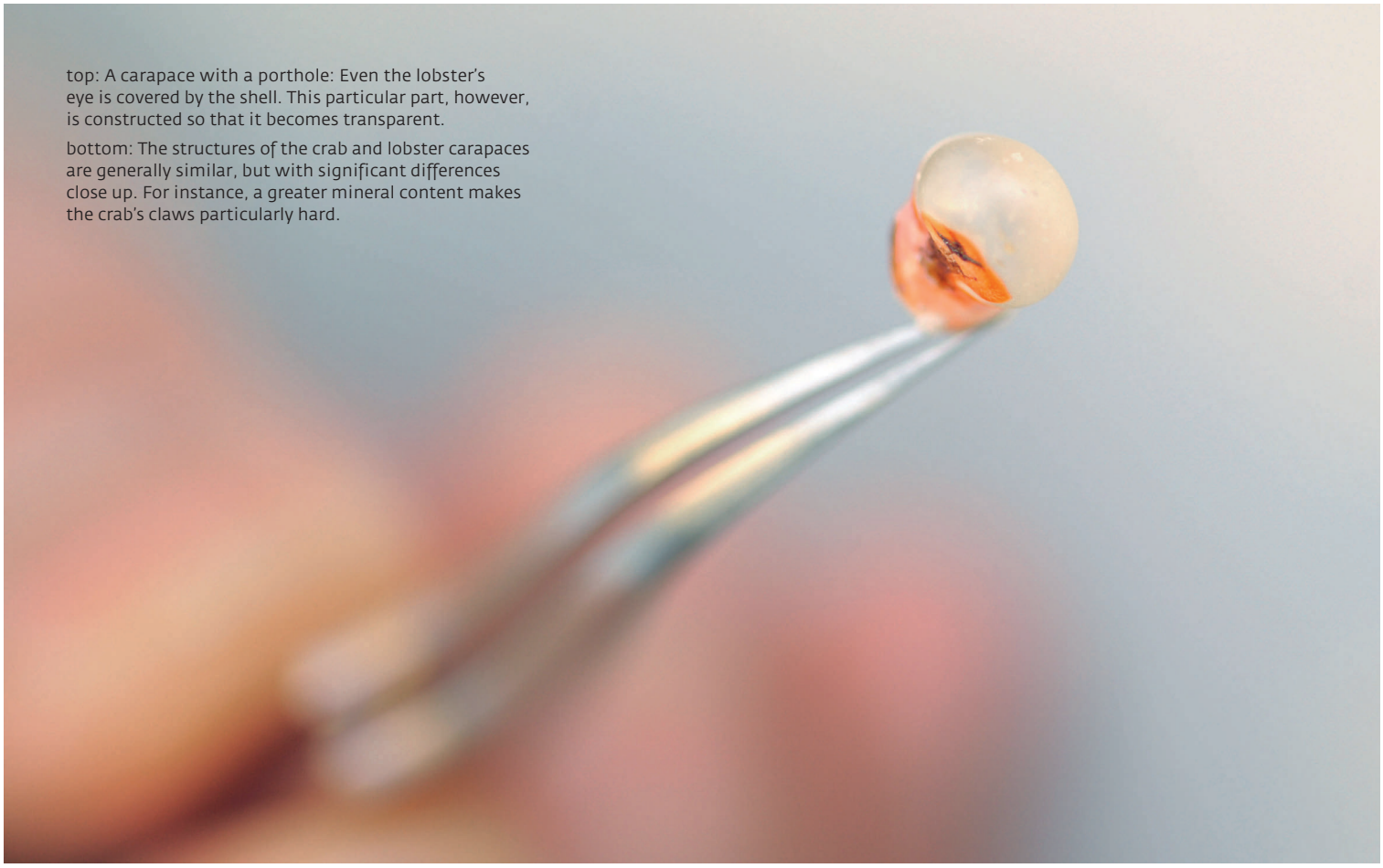
That was in 2005. Since then, lobsters have become a permanent feature on the scientific "menu" at the Max-Planck-Institut für Eisenforschung.

Helge Fabritius holds up a lobster shell. He taps it with his finger and it makes a hollow sound. It is lightweight, as if it were made of styrofoam. It is flexible, yet firm. And, above all, it is versatile. Like any arthropod, lobsters have a so-called exoskeleton. The shell covers the entire body – similar to the human skin.

In order for this to work, the material must provide a wide array of different properties. The lobster needs hard parts, such as the carapace and the extremities. It also needs movable parts

top: A carapace with a porthole: Even the lobster's eye is covered by the shell. This particular part, however, is constructed so that it becomes transparent.

bottom: The structures of the crab and lobster carapaces are generally similar, but with significant differences close up. For instance, a greater mineral content makes the crab's claws particularly hard.



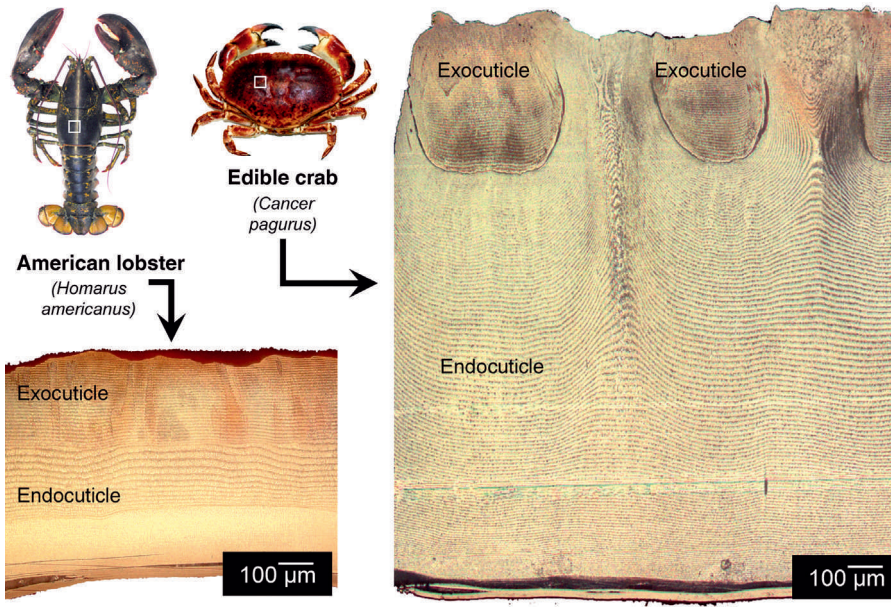
for the joints. Moreover, it needs flexible membranes that seal the gaps between segments and prevent the leakage of body fluids. It also needs transparent components to allow its photoreceptors to see through the exoskeleton. Finally, like all other arthropods, the lobster must renew its skeleton when the old shell becomes too small.

However varied the requirements placed on the lobster shell may be, its basic construction principle and the smallest components are always the same (as in all arthropods). It is by changing the arrangement and the composition of the smallest building blocks that nature has adapted the material to the different functions. "What we have here is a natural, multi-functional material, as it were. And since such materials play an increasingly important role in meeting new technological requirements, this is, of course, very interesting to science," Fabritius explains.

The first results are encouraging: they show that the exoskeleton in crustaceans is a classic composite that combines organic and inorganic constituents. Its structure follows a strict hierarchical organization, from the molecular level up to the final skeletal element.

The basic component is a sugar – more precisely: N-acetylglucosamine. The sugar molecules form chitin, a long-chain polymer that is abundant in nature. Twelve to eighteen of these chitin molecules assemble in a small filament that biologists call a fibril, and that is five to seven nanometers thick (one nanometer is one millionth of a millimeter). The fibrils are covered with a thin layer of proteins, and arrange themselves neatly in parallel rows on the epithelium – the outermost cellular layer of arthropods – forming a continuous, closed layer of fibrils.

The cells then start to produce the next layer under the first one, but the orientation of the fibrils changes by a few degrees, thus creating a stack of fibrous layers in which the fibrils are always twisted against each other by a certain angle. Fabritius calls this arrangement the "plywood principle." Just as in plywood, where two thin sheets of wood are laminated at right angles to each other to create a more stable material, the layers of twisted fibrils also form an extremely resistant material.



Lobster and crab shells under the optical microscope: Images of polished cross sections of the carapace show differences in thickness and organization of the different cuticle layers. The exo- and endocuticle of the two species display distinctive microstructures with differing mineralization.

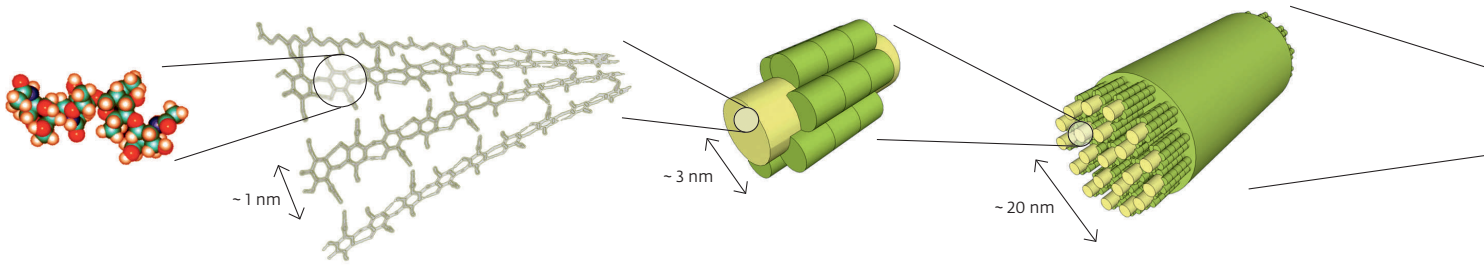
LOBSTER OR CRAB, THE MICROSTRUCTURE TELLS

"That is what we see in the electron microscope," Helge Fabritius says, and zooms deeper into the black-and-white image of "twine." Indeed, with a bit of imagination, superimposed fibers can actually be seen that, one by one, seem to rotate with respect to the image plane until they appear to be moving toward the observer. "When you see something like this for the first time, it's really confusing," Fabritius admits. "The only thing you can do is look at many different samples and gather more experience in order to reconstruct the formation process."

The biologist moves the sample in his microscope to a different part of the lobster carapace, closer to the surface. Here, it is impossible to discern individual fibers. "Sometimes you ask yourself, where is the map, or the user manual?" Fabritius says with a smile. He adds that it helps to stop focusing on particularities in the image and instead look for inconspicuous patterns that are repeated – the basic structure of the material.

To gain insight into the microstructure, Helge Fabritius has to look at the material from all angles. He cuts or breaks the shell into pieces, coats the samples with platinum and places them in the electron microscope. This means that he doesn't necessarily observe the lobster structures from the side. The twisted layers may also be cut obliquely. This creates confusing patterns, similar to the grain in wooden boards, where the original growth rings are very difficult to spot. "For practice, I sometimes even look at furniture and try to understand how the pattern could have formed," he says. >

Photos: Frank Vinken (2), MPI für Eisenforschung (right)



N-acetylglucosamine molecule

∂-chitin (alpha-chitin)

Chitin nanofibril coated with proteins

Chitin fibers

Even if the shell – the so-called cuticle – features the same basic structure in all arthropods, it shows great variation in the details. Fabritius presses a knob. As in a sushi bar, the next prepared sample slides into view in the electron microscope. It is the shell of a crab. After just a few seconds, the blurry image becomes clear. Compared with the lobster, it is much more structured. The individual fibrils are bundled in thick fibers, much like the strands of a rope. These bundles are placed adjacent to one another in layers, and the layers, in turn, are stacked horizontally twisted against each other.

On closer examination of the lobster shell and the crab carapace, pores can be seen that pervade the structure from top to bottom in the shape of long canals. They are formed while the epithelial cells create a new carapace before molting. Tiny bulges therefore protrude from the cell surfaces of the lobster or crab epithelium, and the layers of chitin fibrils must be woven around them. Gradually, elliptical, helically twisted pore canals are formed.

A keen eye for particularly fine details: Helge Fabritius in front of a scanning electron microscope, which he uses to investigate the microstructure of exoskeletons.

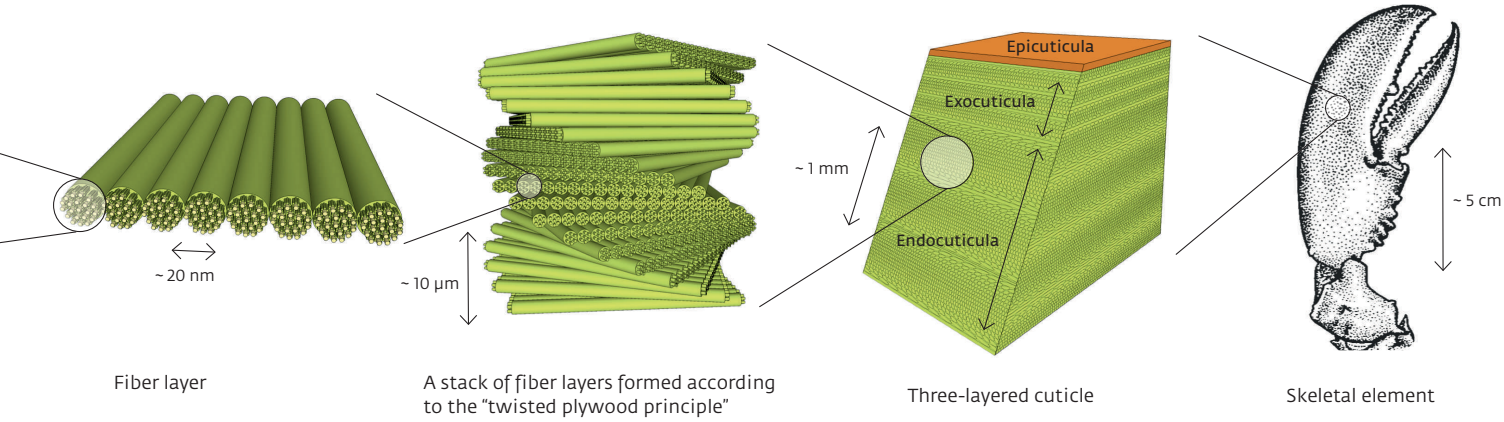
Initially, they serve as a transport system for the minerals that the animals use to quickly harden the soft skeletal structure of the new carapace once they have shed the old one.

When Fabritius zooms even further into the electron microscope samples, the particles that constitute the second main component of the natural composite become visible: essentially, they consist of different types of calcium carbonate, similar to the limescale deposits we know from kitchens and bathrooms. In the lobster shell, the particles are arranged between the chitin fibrils in the form of tiny spherules. In some parts of the crab shell, similar particles create solid tubes around the fibers. The type of crystal lattice and the shape and number of calcium carbonate particles determine the hardness of the exoskeleton, which may vary both according to species and within one shell.

Which other elements constitute the mineral particles besides calcium carbonate can't be established, even with a high-resolution electron microscope. However, Helge Fabritius' favorite instrument performs another trick: besides the electrons, which are ultimately responsible for the black-and-white images, the microscope also excites X-rays, the energy of which is characteristic for each element contained in the sample, making it possible to determine the composition.

This technique, which is called energy dispersive X-ray spectroscopy, can produce colorful maps of the inside of crustacean shells. Red represents calcium; yellow, magnesium; and blue, phosphorus. The distribution of the colors is every bit as varied as the properties of the exoskeleton: for instance,

Photo: Frank Vinken, graphic: MPI für Eisenforschung



The structural hierarchy of the lobster shell (from left): N-acetylglucosamine (sugar) molecules bond to the biopolymer chitin. The chitin molecules assemble into chitinous fibrils that are wrapped in a protein cover. They cluster to form chitin fibers, which in turn assemble into a horizontal layer of fibers. The fiber layers are stacked twisted, similar to the layers of a plywood board. Finally, these stacks form the lobster cuticle, which consists of three layers with varying structures. The endo- and exocuticles give the shell its mechanical stability, while the epicuticle, which mainly consists of proteins and waxes, protects the lobster from harmful environmental influences.

the images of the crab shell show regions with low calcium content containing a particularly large quantity of magnesium. But they also reveal areas with hardly any magnesium. Magnesium atoms in calcium carbonate crystals cause irregularities in the crystal structure, increasing the stiffness of the mineral.

CRUSTACEANS CONTROL THE MINERAL PHASE IN THEIR SHELLS

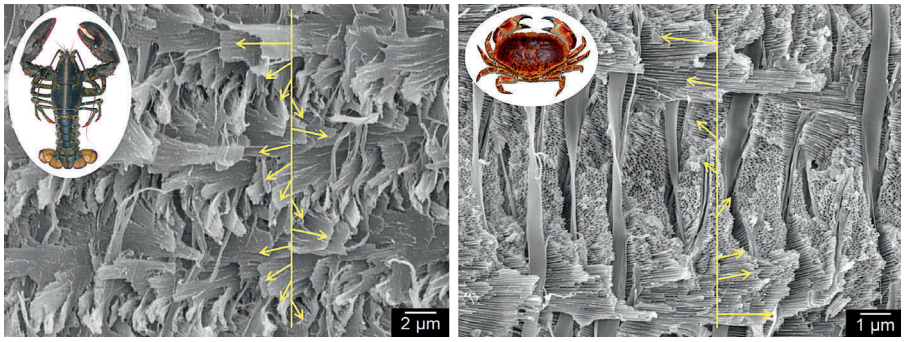
The elemental maps alone can't establish the chemical composition of the mineral phase. For this, they need to be combined with data obtained from another instrument: the confocal Raman microscope. In this microscope, the sample is illuminated with a laser beam. The material scatters the light, and the wavelength distribution is analyzed. Sharp peaks in this light spectrum stand for different molecules – carbonate, phosphate or calcite.

In conjunction with the elemental maps, the curves provide interesting insight into cuticle mineralization. For example, in the lobster, only a thin layer close to the surface of the shell consists of crystalline calcite – a particularly resilient compound. It is evidently designed to protect against attacks and wear. In the regions below, the carapace is hardened with amorphous calcium carbonate. This material has slightly poorer mechanical properties, but is more soluble, thus facilitating molting. On the whole, crustaceans are very particular about the type and quantity of minerals they use to make up different parts of their shell. Fabritius demonstrated that the claws, which need to withstand particularly strong forces, have a comparatively high mineral content. The carapace, in contrast, contains fewer minerals, making it less heavy. Crabs can't escape attacks by swimming away. They therefore rely on a thicker shell and use more resilient compounds in their carapace.

Helge Fabritius is not satisfied with just viewing, identifying and interpreting the material. He wants to understand the implications of the interaction between structure and composition. Mechanical tests offer the first clues: tiny indentations, star-shaped or circular, can be found on the polished cross section of the lobster carapace in the electron microscope. They were made by the tip of a so-called nanoindenter with which Fabritius probes fibril bundles sectioned in differently oriented planes. This means that he sometimes probes the fibers perpendicularly to their long axis, then obliquely from the side; finally, he presses the tip into the fibers in the longitudinal direction. The instrument then measures how far the tip will enter at a specified force. The measurements can be used to calculate the hardness and elasticity of the material. "As expected, the fibrils are stiffer in the longitudinal direction," Helge Fabritius explains.

He also uses conventional tensile, compression and shear tests from materials testing. They shed light on the mechanical properties of the overall structure. Helge Fabritius picks up a tiny reddish plate from a plastic box

Microstructure of lobster and crab shells: The electron micrographs show cross-fractured fiber bundles. The arrows illustrate the rotation of the stacked fiber layers.



Photos: MPI für Eisenforschung

A material that comes in many shapes: The exoskeleton of arthropods, such as the horseshoe crab, a tropical jewel beetle, or the edible crab (from left), consists mainly of chitin and proteins that have been structured differently according to the requirements of each species. Crustacean shells also contain minerals. The tropical jewel beetle forms photonic crystals within the cuticle, producing the iridescent color.

and places it on his fingertip. It is a neatly cut piece of a lobster's carapace, two millimeters thick and eighteen millimeters long. Its two ends are slightly wider, so that the test equipment can pull on them.

These proven methods from metal research are pushed to their limits when studying lobster shells. The scientists working with Helge Fabritius spray the surface of the carapace sample with an irregular graphite pattern. This is the same technique materials scientists use on steel samples before measuring their tensile strength. During the test, the graphite pattern is distorted together with the sample. Based on the distortion, a computer program calculates how the material is deformed in each location. However, this pattern is too coarse to offer an understanding of how fine variations in the lobster structure influence the mechanical behavior. "There is still major scope for refining the measurement techniques," Fabritius says.

Nor is this the only challenge. In order to show which properties the material provides the lobster with, the cuticle must be studied while moist and in a state that is as close to the natural one as possible. Lobsters and other crustaceans must thus be prepared on ice and studied as quickly as possible. Yet for some measuring techniques, such as nanoindentation, moist samples are not suitable. In any case, scientists trying to delve deeper into the structures are gradually seeing the limitations of existing measurement techniques, which is why computer modeling and simulations have become an essential complement.

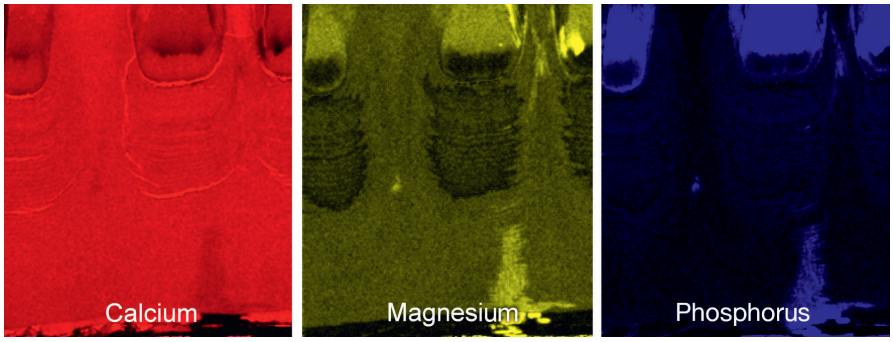
THE IDEAL MATERIAL, BUT NOT FOR MAN-MADE TECHNOLOGY

"As an experimental biologist, I had had little previous experience with simulations; now I know what they can do," relates Helge Fabritius. "I can forget about the material complexity that makes interpreting experiments so difficult, and instead concentrate on a single parameter." Together with scientists from the Computational Materials Design department at the Max-Planck-Institut für Eisenforschung and a colleague who now works in Bulgaria, Fabritius first had to develop suitable models.

This was done using, among other methods, ab-initio calculations. Based on the atomic distances and binding energies in the chitin molecules and calcium carbonate, the computer calculates the mechanical properties of the substances – on a scale that can't be accessed with experimental methods. The data is incorporated in mathematical models to describe the structures as realistically as possible.

Helge Fabritius was impressed by the results. If, for example, he feeds the model with the size of the mineralized particles, the volume fraction of the chitin fibers and the number and dimensions of the pore canals, it will calculate which mineral content will achieve the greatest stiffness. "We compared the simulation results with experimental data and found that almost all values were in the range of the best prediction," he says. From the lobster's perspective, and given the means available to it in nature, the shell is therefore ideally constructed – but only from the lobster's perspective. "The materials in the exoskeleton are always optimized for their function in the respective species – not for human applications," Helge Fabritius says. "You can't use them to produce something for human conditions that can't be manufactured to the same effect with less effort and at lower cost using conventional materials, such as plas-

Elements represented with colors: Energy dispersive X-ray spectroscopy reveals the elemental composition of the cuticle. The crab's carapace contains relatively little calcium in the outer layer, which has a greater magnesium and phosphorus content.



Photos: Frank Vinken (top, 3), MPI für Eisenforschung (bottom, 3)

Photo: Frank Vinken

tics or metals." Merely imitating nature wouldn't offer any particular benefits.

"What we're really looking for are the design principles of the material," Fabritius explains. That is why he is currently studying the cuticles of insects. They are also made up of chitinous fibrils, arranged according to the twisted plywood principle. Nevertheless, when looking at the details, their construction plan is different from that of the lobster or the crab. For one thing, insects don't use minerals. Special modifications to the shell structure provide insects with their colors.

On the exoskeleton of a tropical snout beetle with a greenish iridescent shell, Helge Fabritius and his colleagues found tiny scales. In the scales, the chitinous fibers form a diamond structure with air-filled cavities, thus creating a so-called three-dimensional photonic crystal. Such a material will reflect only light of a certain color, determined by the dimensions of the lattice and its orientation with respect to the incident light. Materials scientists in Düsseldorf are now able to produce structures of this type in the laboratory. Not only do they look pretty, but they can be used as gas sensors, since the color of the material changes when a gas with a refractive index different from air penetrates the pores.

According to Helge Fabritius, all this information can be used to draw lessons for future composites. The large pore canals in the lobster carapace that reduce the weight of the animal without compromising stability could be a great example of how to produce lightweight but strong materials.

The developed model should come in handy for this. The more data the scientists collect, the more accurate it becomes. The biologist hopes that they will be able to use it to make predictions "sometime soon." For instance, the program could suggest a fiber, a structural environment and a mix of minerals that could be used to produce a composite with the desired properties in the laboratory.

Helge Fabritius picks up the lobster shell again, turns it over in his hand and looks at it closely. "I think the future holds more examples of us using biological principles in technical applications," he says, "but with modification, so that they fit in the greater context." ◂

Subjecting biological materials to the tensile machine: Helge Fabritius tests the tensile strength of a sample that is clamped between two movable crossheads (top left). Two cameras, flanked by two light sources, document its deformation during the test.

GLOSSARY

Arthropod cuticle
The outer cover of arthropods. It is formed by epidermal cells, which correspond to the skin of vertebrates, and serves as an exoskeleton through adaptations in structure and composition.

Chitin
A polysaccharide that is the main constituent of arthropod shells.

Exoskeleton
The outer shell that arthropods, such as crustaceans and insects, use to stabilize their soft bodies.

Fibrils
Small fibers that may be composed of different substances, such as proteins, cellulose or chitin, depending on their provenance and function.

Nanoindentation
Method used to test the hardness of a material. A tip is pressed into a surface at a specified force. The hardness and elasticity are calculated based on the penetration depth and contact surface.