Transporting Toxins to Tumors

Developing drugs that eliminate cancer cells effectively and have few or no side effects – this is one important aim of the Research Group led by **Tanja Weil**, Director at the **Max Planck Institute for Polymer Research** in Mainz. Weil and her team of chemists convert proteins into traceable drug transporters for nanomedicine with the help of miniscule diamonds.

TEXT **PETER HERGERSBERG**

You could be forgiven for thinking you had misheard her at first: "I always found polymers to be a bit suspicious," says Tanja Weil, who completed her doctorate at the Max Planck Institute for Polymer Research and is now Director there. Polymers are long, often net-like molecules composed of numerous small chemical subunits that are repeated many times. The highly versatile and durable synthetic materials that have become indispensable to our everyday lives consist of these chains of molecules.

The fact that Tanja Weil was nevertheless initially wary of polymers owes not least to aesthetic factors: "I felt that the beauty and precision of organic chemistry had been left by the wayside here." The scientist, who switched to the MPI in Mainz from the University of Ulm in 2016, explains it in very simple terms: "In classical polymer chemistry, when you mix the monomers and press the start button, the reaction comes to an end at some point and that's it." In most cases, the product of such reactions is a more or less diverse mixture of chain molecules of varying lengths. Unsurprisingly, for her doctoral research, Weil also worked on one of the few polymer classes with a precisely defined structure: dendrimers – symmetrical tree-like molecules whose branches are assembled in a controlled fashion from the same basic chemical components.

POLYMERS AS VEHICLES FOR SUBSTANCES

The often uncontrolled growth that dominated in the test tubes of polymer chemists seemed less than optimal for the applications that Tanja Weil had set her sights on for polymers. She wanted to use the chain molecules as transporters for medical substances, for example to precisely maneuver cytotoxins to a tumor. The aim of this form of nanomedicine would be to wreak as much havoc as possible within cancer cells while causing no damage to the rest of the body. However, when chain molecules differ in length, they could also be loaded with different amounts of active substances. "But promising new approaches are now available that implement structural precision in polymer synthesis and make it possible to change this," says Tanja Weil. As she explains, it is essential that the polymers used in medicine be able to be loaded with an active substance in a controlled and reproducible fashion: "Patients would like their daily pills to contain the same dosage every day."

Leukemia patients, for example, could benefit from a treatment that specifically targets cancer cells: "With the standard treatment that has been used for acute forms of leukemia since the 1960s and 1970s, we ride roughshod over all blood-forming cells," explains Michaela Feuring-Buske, who is a professor at the Department of Internal Medicine III and at the Institute for Experimental Cancer Research of Ulm University Hospital and works on the development of new therapeutic ap-

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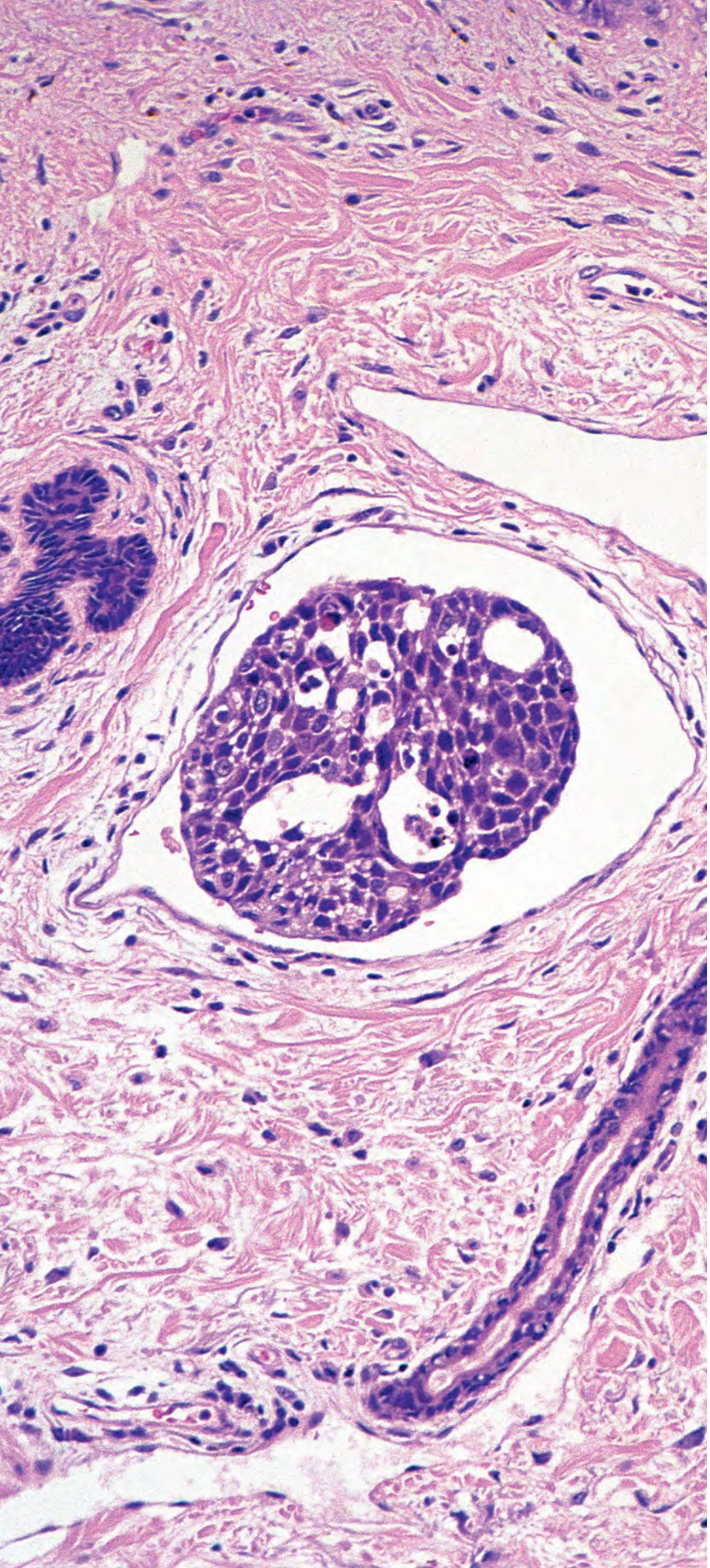


proaches for the treatment of leukemia. She has been working with Tanja Weil since her time as a professor in Ulm. The scattergun approach aimed at all rapidly dividing cells also affects healthy cells – with corresponding side effects: hair loss, nausea, a reduced white cell count and even damage to the cardiac muscle and the central nervous system, to name just a few. "So the development of a more targeted approach to cancer treatment is timely and a very attractive prospect," says the Ulm-based physician.

Tanja Weil's motivation for developing transporters for pharmaceutical substances probably originates from the time she spent working for pharmaceutical company Merz. She managed various positions there while also carrying out independent research at the Max Planck Institute for Polymer Research. Although nanocarriers that transport drugs to the exact location in the body where they are needed don't currently feature in the standard repertoire of pharmaceutical concerns, Tanja Weil has been working on their development with her colleagues since she returned to full-time academic research.

The list of requirements for a targeted drug transporter is long: it not only needs a protected loading space for the active ingredient, but it must also have a kind of address label so it can identify its destination. In addition, the drug carrier must be able to disguise itself to

Target of the nanoattack: Max Planck researchers in Mainz aim to use protein carriers to infiltrate cytotoxins into cancer cells, including breast cancer tumor cells (purple).

evade the vigilance of the immune system. It would also be nice if it had markers on it that would make it possible to track the active ingredient's progress through the body. And of course all of this must be tolerated by the body. There are very few materials that can fulfill these requirements.

PROTEINS AS PRECISION POLYMERS

For this reason, Tanja Weil and her colleagues came up with a new idea. They asked themselves whether the precision biopolymers, such as proteins, can be used as classical polymers. Proteins occur in different spatial forms in the body, but most of them tend to be stubby with bulges and dents. This specific spatial structure and the surface chemical properties mean that every protein molecule is a specialist. Some of them adhere to other proteins so they can carry out tasks together; others convert smaller molecules into substances the organism needs at a given time, or transport these molecules to a specific site.

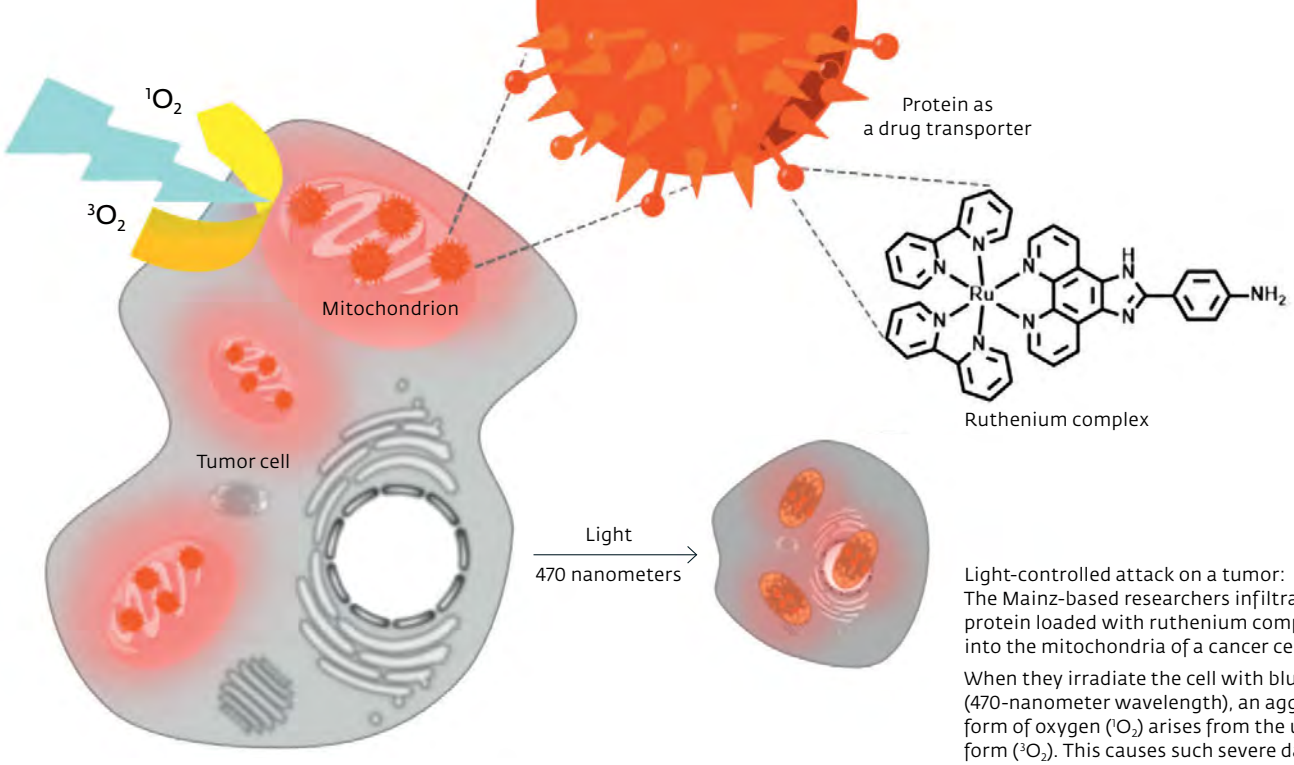
When untangled, however, a protein is always a chain molecule with a precise length and composed of a defined sequence of 21 different amino acid components. Absolute precision is required here, as a specific chain of amino acids is essential for the protein to be able to fold itself into the form that does the job assigned to it within the organism.

The properties of the amino acids are determined by characteristic molecular groups – functions, as chemists call them. Some of them work like chemical hooks to which other molecules, such as active drug molecules, can attach themselves. This is precisely what Tanja Weil and her colleagues do. They have developed some promising candidates for animal studies, in which potential drugs are now being tested. And it is very much a team effort, as Tanja Weil emphasizes. Indeed, the most ingenious solutions for a particular problem often arise only when a group gets together for a chat over coffee in her office.

This was also the case when Weil's group faced its first hurdle in the process of developing protein carriers for medical substances. To enable proteins to be used as polymeric materials, the entangled amino acid chains first had to be unraveled. This involved dissolving the bonds between links in the chain molecule located far away from each other and folding the protein into its biologically active form. To prevent the polymer from being entirely dismantled, however, the stronger chemical bonds between the individual links in the amino acid chain had to remain intact. This process, which biochemists refer to as denaturation, is simply what happens when you fry an egg: the heat breaks down the bonds that give the protein molecule its three-dimensional structure. The unfolded proteins coagulate, and the egg white solidifies. This renders the proteins useless for any other application except being served on a plate: "You can never get them back into solution," says Yuzhou Wu, a Research Group Leader in Weil's Department and now also a professor at Huazhong University of Science and Technology in Wuhan, China.

Orchestrating the team effort: When Tanja Weil and her colleagues work on converting proteins into nanotransporters for drugs, she often formulates the problems to which solutions are found during the team's discussions.

Photo: Thomas Hartmann



Light-controlled attack on a tumor: The Mainz-based researchers infiltrate a protein loaded with ruthenium complexes into the mitochondria of a cancer cell.

When they irradiate the cell with blue light (470-nanometer wavelength), an aggressive form of oxygen ($^1O_2$) arises from the usual form ($^3O_2$). This causes such severe damage to the cell that it dies.

Primarily through the tests that Yuzhou Wu carried out when she worked with Tanja Weil at the University of Singapore, the group found a way to denature proteins without causing them to coagulate. "Before or shortly after we unfold them, we graft water-soluble components, such as polyethylene glycol, to certain amino acids," explains Yuzhou Wu. "In this way, we keep the denatured proteins in solution."

Polyethylene glycol, known as PEG, not only makes the protein water-soluble, it also enrobes it in a cloak of invisibility. The appeal of the protein transporter lies in the fact that it is biodegradable, but this process shouldn't begin until the drug carrier has reached its destination – so the fact that the PEG attachments can hoodwink the immune cells is very helpful. The body's law enforcement units don't initially identify the unfolded protein, which means that it can circulate in the bloodstream for a longer period and its inevitable destruction as a foreign body is delayed.

Yuzhou and other members of Tanja Weil's group have already packed drugs in such cunningly disguised nanotransporters, such as Doxorubicin – a drug used in the treatment of acute myeloid leukemia, which affects mainly older people. Because this cytotoxin has strong side effects, the Max Planck chemists wanted to use a protein to transport it directly to the tumor cells. In this case, they used human serum albumin (HSA) from human blood. "We load HSA with Doxorubicin at the stage we can control most precisely, which is directly after denaturation," says Yuzhou Wu.

DRUG TRANSPORT INTO THE CELL ORGANELLES

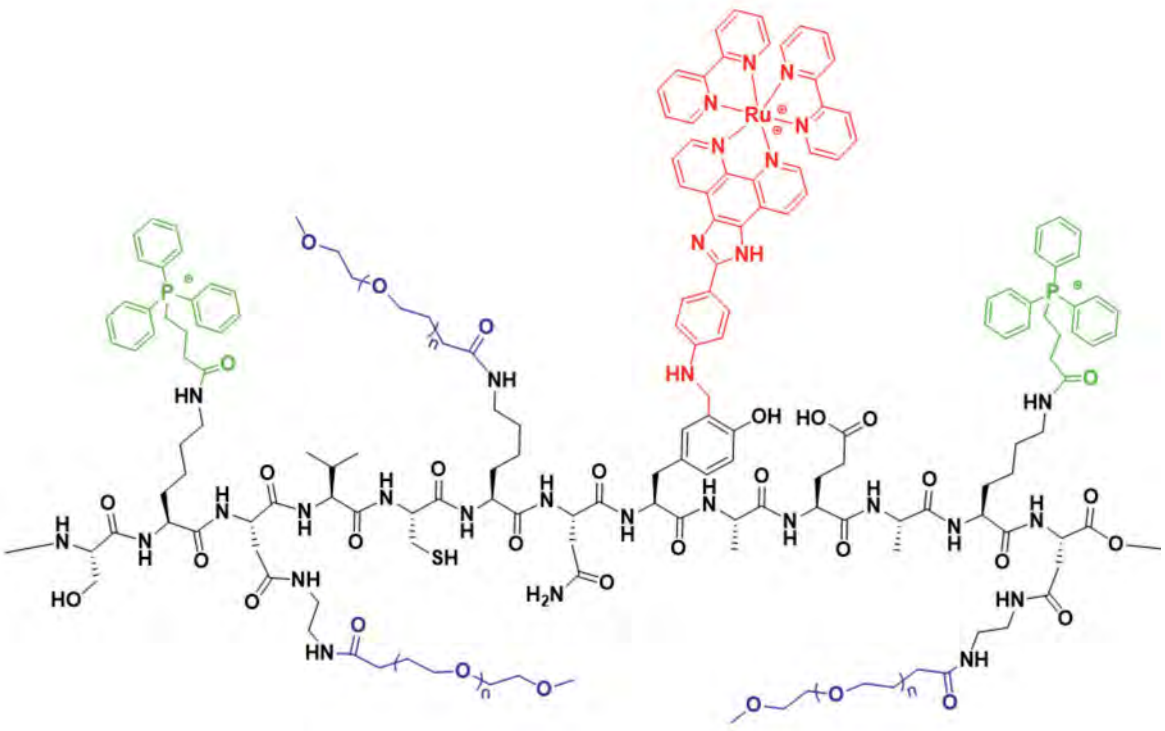
The researchers attach the drug molecules to thiol groups of the amino acid cysteine. "And because all HSA molecules have the same number of accessible thiol groups, each protein molecule carries around 27 molecules of the active ingredient," says Yuzhou Wu. Deviations occur only when no drug molecule finds its way to a thiol group in the reaction mixture.

And, as initial tests have shown, the concentrated loading of the drug on the protein is effective: a comparatively small volume of Doxorubicin-loaded biopoloymers succeeded in killing half of the cancerous cells in cultures containing different leukemia cells. The effect was also confirmed in a study carried out over a period of 12 weeks in which mice were initially injected with differently treated cancer cells. While mice with leukemia cells that had been treated only with Doxorubicin survived on average for 69 days, all of the animals whose cancer cells had been targeted by protein carriers loaded with the cytotoxin were still alive at the end of the entire study period.

Tanja Weil's group is now investigating an approach for optimizing the effectiveness of the transport of the toxin to tumor cells. The idea for this arose when a project wasn't as successful as the scientists had initially hoped. Sabyasachi Chakrabortty, a postdoc working in Weil's group, had packed an HSA carrier with around ten ruthenium complexes. The application of light energy to this precious metal produces an aggressive form of oxygen. In addition, the researchers equipped the ruthenium complex with a human hormone that bonds specifically with receptors on tumor cells.

"We then observed in cell cultures that the nanotransporters actually go into tumor cells and their toxicity increases when we irradiate them with light," explains the chemist. "The combination with the hormone also made them specific to cancer cells." However, compared with treatment with a pure ruthenium complex, the nanotransporter that targeted the tumor cells worked only slightly better. "We were very disappointed with this result," admits Sabyasachi Chakrabortty. However, the Max Planck chemists refused to simply accept it and asked themselves why it was that their targeted freight wasn't more effective and how they might be able to improve it.

Graphic: Journal of The American Chemical Society, ACS, Jan 2017



Graphic: Tanja Weil

Again, a crucial suggestion arose during a team meeting: they should try to transport the drug specifically to certain organelles in the cell instead of simply infiltrating it into a tumor cell and leaving it to its own devices. The chemists quickly identified the mitochondrion, the power plant of the cell, as a promising target for such an attack. They suspected that this organelle would respond particularly sensitively to the aggressive form of the oxygen. So Sabyasachi Chakrabortty programmed the transporters to head for mitochondria – he found a suitable molecular address label in a sort of catalogue that biochemists had already compiled for targeted navigation to the different cell organelles.

When the researchers applied the transporter to the mitochondria of the cancer cells, the effect was resounding: the cytotoxin was 200 times more effective than when the pure active ingredient was infiltrated using a nanotransporter. For each of the ten drug complexes on the carrier, this meant a 20-fold increase in toxicity. "That was really great," says Tanja Weil. The fact that even very small volumes of drugs can fight tumor cells effectively if they can reach the right site in the cancer cell could represent a major advance in oncology. University of Ulm professor of medicine Michaela Feuring-Buske, too, hopes to significantly reduce the side effects of cancer drugs in this way. It is also possible that this principle will apply not only to the substance that gets to work on the curative destruction of malignant cells when exposed to light, but also to other drugs.

The fact that a drug doesn't switch on until a button is pressed – that is, when it is illuminated at its target – is practical in the research context, but less so in medical use. The light that releases the aggressive oxygen doesn't penetrate deep into the body, and the cancer cells in the bone marrow that flood the body with useless blood cells can't be fought in this way.

However, working in collaboration with doctors at the Ulm University Medical Center, the team has found a potential application for this approach: following initial chemotherapy, patients with myeloid leukemia may be given a stem cell transplant to consolidate the treatment. If a suitable donor can't be found, the stem cells are taken from the patients themselves and the light-controlled therapy would offer a suitable method for liberating the stem cells from the cancer cells. For this method to succeed, the drug must cause greater damage to the malignant cells than to the healthy ones.

When the scientists from Mainz and Ulm were investigating this, they discovered something surprising: "We observed a trend indicating that the protein-linked ruthenium drug showed a preference for damaging cancer cells," says Tanja Weil – and it did this without the researchers having to target it directly at the cancer cells using complicated address labels. "We were unable to explain this at first and the doctors had no answer either," she admits. The idea that put the researchers on the right track arose, again, at a team meeting. They are currently pursuing this and may discover a new approach for targeted cancer therapy in the process.

DIAMONDS AS BIOLOGICAL NANOSENSORS

This prospect could also arise from another project on which Weil's group is currently working intensively: together with physicists at the University of Ulm, the scientists aim to use diamonds as biological nanosensors. "I always found diamonds rather boring as a functional material," says Tanja Weil. "They are very hard and beautiful as decorative stones, but not very interesting from the perspective of materials chemistry, as it is difficult to control their functionalization and morphology." That was until

Left Tanja Weil's team developed a serum albumin protein (black) into a versatile nanotransporter. The protein, of which only a part can be seen here, occurs in human blood. The researchers loaded it with different attachments to it: Polyethylene glycol (blue) makes the protein water soluble and protects it from rapid degradation in the body. The ruthenium complex (red) generates an aggressive form of oxygen that acts as a cytotoxin when exposed to light. The triphenylphosphine groups (green) navigate the drug carrier into the mitochondria, where the cytotoxin is particularly effective.

Right Christiane Seidler, Wenhui Dong and Tanja Weil (from left) examine solutions of protein-coated nanodiamonds. Due to targeted impurities with foreign atoms, the diamonds shine in different colors and enable the scientists to track the progress of the drug transport in cell cultures.

two physicists from Ulm, Fedor Jelezko and Martin Plenio, visited Weil's office one day and the three scientists had a conversation about what diamonds are capable of. Due to defects – minute flaws at which other atoms such as nitrogen sit in the carbon lattice – they can be used, among other things, as nanosensors for structural elucidation, for example in particularly sensitive magnetic fields. "I found the wide-ranging possibilities offered by this use of diamonds very exciting after all," says Weil.

A SYNERGY GRANT WORTH NEARLY TEN MILLION EUROS

The three scientists thus joined forces to carry out research on the diamond nanosensors. "Firstly, we liked each other, and secondly, we were convinced that we could make a scientific difference with our interdisciplinary knowledge and skills," says Tanja Weil. For her, this difference consisted above all in the fact that it would enable diamond sensors to track the progress of drug-loaded biopolymers in the body and possibly even in a cell. "It would be interesting to be able to observe in an MRI scanner whether the biopolymers we equipped with nanodiamonds actually go where we want them to," explains the researcher.

The enthusiasm with which Weil reports on this project wasn't initially shared by the research sponsors. The answer she received to the funding applications she submitted with her partners was always the same: it was far too uncertain whether it would actually be possible to implement the project. The scientists were deeply frustrated with this response. They eventually decided to make one last attempt to obtain funding and applied for a Synergy Grant from the European Research Council ERC – and were one of the 11 projects out of more than 700 submitted to be awarded a grant worth nearly ten million euros for their project. The researchers from Mainz and Ulm are therefore now working on transporters for nanomedicine that not only maneuver drugs directly to a tumor, but also can be observed in detail every step of the way. ◂

TO THE POINT

- **As biopolymers, proteins can be loaded with medical active ingredients that provide a precisely defined length and composition and are directed specifically at tumor cells.**
- **The effectiveness of cancer treatment could be increased significantly if such nanotransporters were able to infiltrate cytotoxins into cell organelles such as the mitochondria.**
- **The researchers expect that nanodiamonds will be suitable for use as detectable drug transporters whose path through the body can be traced right up to the target cells.**

GLOSSARY

Biopolymer: A chain molecule that is synthesized by an organism. In addition to proteins, biopolymers include polysaccharides and DNA.

Chemical function: The part of a molecule that determines its properties and behavior in chemical reactions.

Nanomedicine: The application of nanotechnology in medicine.

Photo: Thomas Hartmann