

# NERVE SCAFFOLDING FROM A TEST TUBE

TEXT: ANDREAS KNEBL

66 Numbness, immobility and, in the worst case, paraplegia – the severing of a nerve pathway – often has permanent consequences. This is because the extracellular matrix, which provides support for the neurons, is also damaged during the injury. Tanja Weil and Christopher Synatschke, who work at the Max Planck Institute for Polymer Research in Mainz, are looking for a replacement for this support material. And they have already made an important find.

Weil quickly got to the bottom of things. Her colleagues had observed something puzzling about a peptide from the envelope of the HI virus. Peptides consist of amino acids, the basic building blocks of all proteins, and perform countless functions in living organisms. In the case of the peptides from the HI viral envelope, the question arose as to what role they play in the infection of host cells. Initial tests

showed that the effect of the peptides changed when they were left in a solution for a long time. When the researchers led by Weil examined the solution in question, they found that after some time, the peptides in it had arranged themselves into fibrils. Their fibrous structure caused the peptides to become sticky and provide support for both the HI virus and the host cell. “In the scanning electron microscope images, we were able to see the cells clinging to the fibrils,” says Weil. This accidental discovery launched a whole new research project.

At the time, Weil was working as a polymer chemist at the University of Ulm. But word of her observation quickly spread among her colleagues. One of them was Bernd Knöll. The cell bio-

logist works on therapeutic approaches to repairing injured nerves. At the time, his office was on the same floor as Weil’s. The short distances enabled a lively exchange. And so the two developed the idea of using the peptide fibrils as a kind of trellis or scaffold for nerve cells.

## Replacement for the supporting matrix

The research approach was as follows: if the host cells of the HI virus can cling to the peptide fibrils, nerves should be able to do the same. If this assumption is confirmed, the peptide fibrils could help injured nerves to heal. In most cases, severed nerve fibers do not grow back together on their own.



Chemical customization:  
a Max Planck team in Mainz  
is creating specific peptides  
that form networks and support  
the healing of nerves.



PHOTO: KATRIN BINNER FOR THE MPG

This can result in numbness and paralysis. A deep cut in the hand, for example, can leave a person unable to feel or move a finger. After a severe injury, a nerve lacks the necessary support to heal independently. This is because not only the nerve but also its extracellular matrix is damaged. This complex protein scaffold provides support for the nerves. When that support is missing, there is a gap that the two ends of the injured nerve cannot bridge. At that point, only surgery can help the severed nerve to heal. The surgeon attempts to suture the

two nerve endings together or connect them with a piece of nerve taken from another part of the body. But Knöll and Weil are taking a different approach. They rely on peptides delivered to the wound site to replace the extracellular matrix, thereby allowing the nerves to regenerate.

The collaboration continued even after Weil moved to Mainz to become the Director of the Max Planck Institute for Polymer Research. There, Christopher Synatschke joined their “Synthesis of Macromolecules” working

group. The chemist had already gained experience with peptides in various international working groups. As a postdoctoral researcher and later group leader, he now took over responsibility for the cooperative project with Knöll in Weil’s working group. Two years later, the team reported its first success. By using peptide fibrils, the researchers succeeded in improving the regeneration of injured nerves.

Weil and Synatschke agree that self-assembling peptides, such as the

On the way to a new therapy: Tanja Weil and Christopher Synatschke are looking for peptide structures that can be used to treat nerve damage – maybe someday even spinal cord injuries.

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PHOTO: KATRIN BINNER FOR THE MPG





sequences from the HI viral envelope, are the ideal substitute for the extracellular matrix. “They allow us to imitate nature,” says Synatschke. “We are creating a structure that is close to the natural environment of the nerves – yet much simpler.” Self-assembling means that the peptides assemble themselves into larger structures without external influence. Some of these self-assembling peptides (SAP) form fibrils. These fibrils are only about 10 nanometers thick and 0.1–1 micrometer in length. The fibrils of some SAP consolidate to

form larger networks. These can serve as scaffolds for nerve fibers that are up to 20 micrometers thick. For comparison: a human hair has a diameter of about 50 micrometers.

The special features of SAP arise from the interaction of the individual amino acids. The peptide sequences that Synatschke works with consist of only a few amino acids. Each of these amino acids has certain properties. For example, one building block may be positively charged on the surface, while another is hydrophobic (i.e., water-repellant). The characteristics of the individual amino acids give rise to completely new and not easily predictable properties in the peptide compound – such as the tendency to form fibrils.

## Multi-variant peptide structures

In order to better understand how peptide sequences and self-assembly are related, Synatschke and his colleagues studied numerous sequences. Synatschke’s team specializes in the targeted production of peptides with a desired sequence of amino acids. Once the peptides have been synthesized, the scientists end up with SAP in a powdered form; this is added to a solvent and mixed with water. In addition to the sequence, the conditions within the solution also play a role in how the SAP behave. If the researchers change how acidic or basic the solution is or how long and at what temperature it is stored, the properties of the structure that emerges from the SAP also change. Synatschke started his experiments with 27 sequences based on the peptide from the HI viral envelope discovered in 2011. His working group first investigated whether and to what extent the different peptide sequences lead to the formation of fibrils. In collaboration with Knöll’s working group, the researchers next applied a thin layer of each of the materials to individual Petri dishes. They then

cultured nerve cells in them, before going on to examine the cultures under the microscope to see whether the cells found support on the substrate and, if so, how well the nerve fibers had developed.

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### SUMMARY

Severed nerve tracts usually do not heal on their own because the extracellular matrix surrounding them is also damaged.

At the Max Planck Institute for Polymer Research, researchers are replacing the scaffolding structure of injured nerves with fibril-forming peptides so that the nerve endings can grow back together.

In mice, severed facial nerves healed better when injected with a solution containing self-assembling peptides (SAP).

In the long term, the research team also hopes to find a way to use SAP to heal nerve injuries in humans.

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Weil emphasizes the importance of these basic investigations. In contrast to the highly complex extracellular matrix of nerves, the peptide scaffolds are comparatively simple in structure. This allows them to conduct scientific studies that enable rapid progress. “Because we are familiar with the building blocks of the SAP, we can replace them individually and thus change the properties. We can then examine the structure in detail, study the effect of the SAP within the biological system, and learn from it.”

The researchers quickly found several properties that are important for the interaction between SAP and nerves. For example, SAP that have positively



charged surfaces interact strongly with nerve cells. The number of the solubilized SAP that attach to each other to form fibrils is also important. If this proportion is large, these peptides are particularly well-suited to forming a support structure. The research team also discovered that SAP that form thicker fibers provide a better scaffolding for nerves. But a survey of all 27 sequences presented Synatschke with a puzzle: there were peptide sequences that were positively charged and formed high numbers of fibrils. However, they did not act as scaffolds for the nerve cells in the Petri dishes. Collaboration with Tuomas Knowles from the University of Cambridge helped the team in Mainz to figure out what the peptides

were missing. Knowles found what he was looking for in the infrared spectra of the various SAP. His detailed evaluation showed that those SAP that are particularly suitable as nerve scaffolds form fibrils with a high  $\beta$ -sheet content. At the molecular level, this structure of the amino acid chains looks like an accordion or a sheet of paper folded in a zigzag pattern and describes how the individual peptide chains are arranged within the fibrils. Synatschke and Weil thus found another crucial characteristic that SAP must have in order to provide a scaffold for nerves.

The team led by Knöll then took the three best SAP from Synatschke's 27 candidates and tested them on living

mice that had lost control of their whiskers because of an injury to the facial nerve. The researchers injected a solution of SAP at the sites where the nerves had been severed. Then, over the next three weeks, they studied how the nerve fibers regenerated. They initially saw no clear difference between mice injected with peptides and the control group. However, as the study progressed, it became apparent that the nerves healed better when a peptide scaffold replaced the injured extracellular matrix. A prerequisite for this was that the peptide structures remained as a stable framework at the wound site throughout the healing process – even though they are biodegradable.

## Improved nerve function

The positive influence of the peptide scaffold on the regeneration of the facial nerve was verified by the scientists through various observations. They demonstrated that severed nerves in the mice tissue that were supported by a peptide scaffold re-connected better than the nerves of the control mice. This result was also confirmed during the functional check. The mice that the researchers had injected with a solution of scaffold-forming peptides recovered better from the injury and were able to move their whiskers in a more controlled manner than the control animals after three weeks.

The experiments in the Petri dishes and on the mice made it clear that the scientists had found an ideal material when they discovered SAP. "By accurately differentiating the distinctive features of SAP, we have developed an understanding of their fundamental relationships," says Synatschke. "We want to build on this." Together with the group led by their colleague Tristan Berau, the researchers are now using computational methods to search for peptide sequences that are even better suited as a neural scaffold

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Healing bond: the fluorescence microscope image shows how a nerve cell (green) uses special proteins (red) to attach itself to conjoining nanofibers (purple) that are made of peptides.



IMAGE: BERND KNÖLL/MPI FOR POLYMER RESEARCH

than the sequences from the HI viral envelope. Using new approaches from computer science and the processing power of modern computers, they can screen millions of possibilities in search of the three desired properties without having to conduct elaborate experiments. They can then test the most promising candidates in the laboratory. “This speeds up the research process immensely,” says Weil. “By doing so, we hope to find novel sequences that have no prototype in nature but which have exciting properties.”

The next research collaboration with Knöll is also already in the pipeline. After successful experiments in the peripheral nervous system, the researchers are now venturing into the central nervous system. The challenge of healing nerves in the brain and spinal cord is much greater because the nerves here generally do not regenerate at all. Accidents in which the neck or spine is severely injured often lead to paraplegia. In the coming years, Bernd Knöll, Tanja Weil, and Christopher Synatschke want to modify the peptide scaffolds in such a way that they also enable the healing of nerve damage in the body’s central control centers.



## GLOSSARY

### EXTRACELLULAR MATRIX

is the name of the tissue component consisting of proteins and carbohydrates in which cells are embedded.

Nerve cells require the extracellular matrix in order to grow.

### SELF-ASSEMBLING PEPTIDES (SAP)

These are short amino acid chains that autonomously form larger structures such as fibrils.

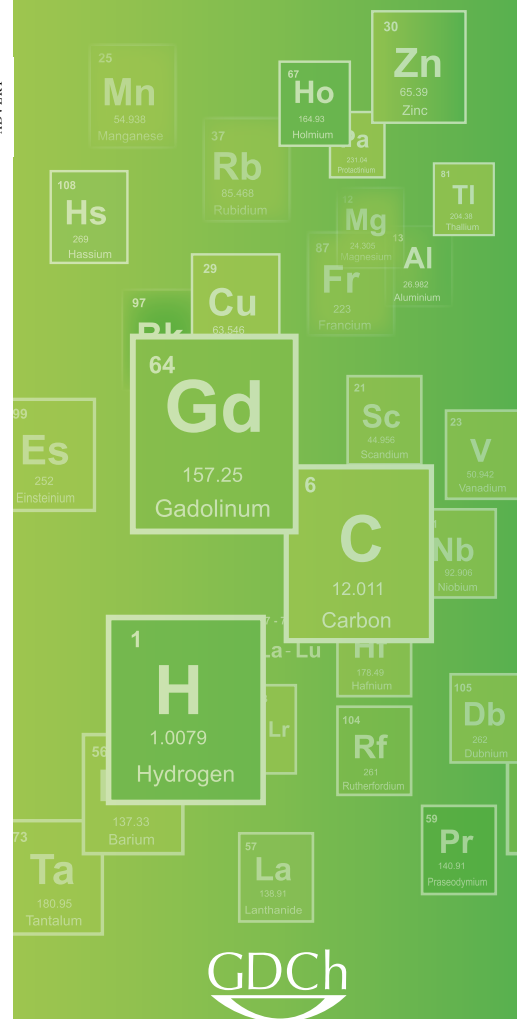
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