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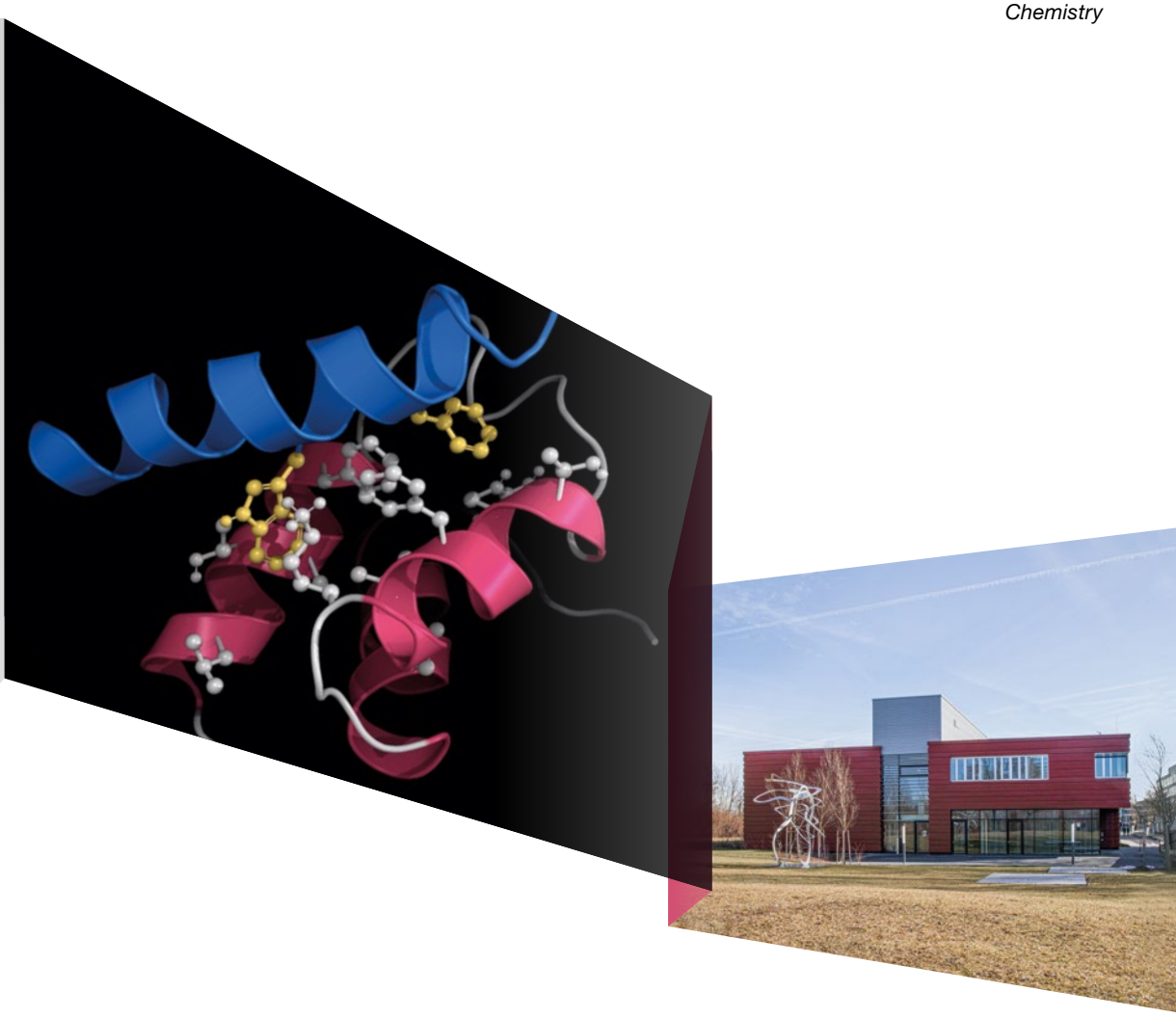
The professors (clockwise) Aphrodite Kapurniotu, Bernd Reif and Michael Sattler are working at the new NMR center to elucidate the structures and functions of complicated protein molecules.

Picture credit: Juli Eberle, Bayerisches NMR Zentrum



Rings, chains, loops, spirals: Structures like the ones shown here are investigated in the newly erected building of the Bavarian NMR Center at TUM on the Garching research campus.

How Proteins Work

Protein molecules are made up of chains of amino acids, folded in a complex way. Alongside the sequence of amino acids, this folding is crucial to their function. At TUM's new NMR center, researchers can now gain unique insights into these complex structures.

There are already seven NMR spectrometers in the hall of the NMR Center. Three more will move from other departments, and one of the world's strongest instruments with a frequency of 1.2 gigahertz and a magnetic field strength of almost 30 tesla will be added later.

Kurzfassung

Falsch gefaltet

D

Obwohl die Struktur vieler Proteine weitgehend durch die Abfolge ihrer Aminosäuren bestimmt wird, lässt sie sich bis heute nicht vorhersagen und muss experimentell bestimmt werden. Die Kernspinresonanz-Spektroskopie (englisch: Nuclear Magnetic Resonance, NMR) ist hierfür das umfassendste und leistungsstärkste Verfahren. Mit ihr können die komplexe dreidimensionale Raumstruktur und die interne Beweglichkeit von Proteinen exakt vermessen werden.

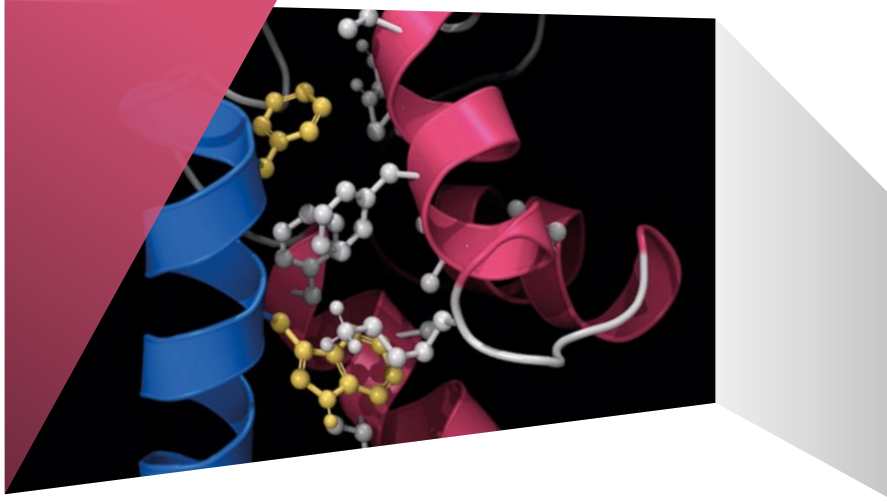
Das neue Bayerische NMR-Zentrum der TUM wurde im Oktober 2018 offiziell eröffnet. Sieben Spektrometer wurden bereits zusammengezogen, drei weitere warten auf den Umzug. Herzstück der Anlage wird in drei Jahren eines der weltweit stärksten NMR-Spektrometer mit einer Frequenz von 1,2 GHz sein. Mittelfristig wird es auf der ganzen Welt nur eine Handvoll solcher Geräte geben. Im Kernresonanz-Zentrum arbeiten Chemiker, Physiker und Biochemiker interdisziplinär zusammen.

So konnte Prof. Michael Sattler zeigen, dass sich auf der Basis der NMR-Spektroskopie Zielstrukturen für neue Medikamente gegen die Chagas-Krankheit ermitteln lassen. Die Professoren Bernd Reif und Aphrodite Kapurniotu sind in einer Reihe von Projekten, zum Teil auch gemeinsamen, auf der Suche nach Wirkstoffen, die sich zur Behandlung von Alzheimer eignen. Die NMR-Spektroskopie bietet hier völlig neue Ansätze, weil sie erstmals erlaubt, die Bindungsstellen, die bei den Fehlfaltungen eine Rolle spielen, mit hoher Auflösung zu betrachten. Wirkstoffkandidaten können so gezielt optimiert werden. □

Picture credit: Juli Eberle, Bayerisches NMR Zentrum

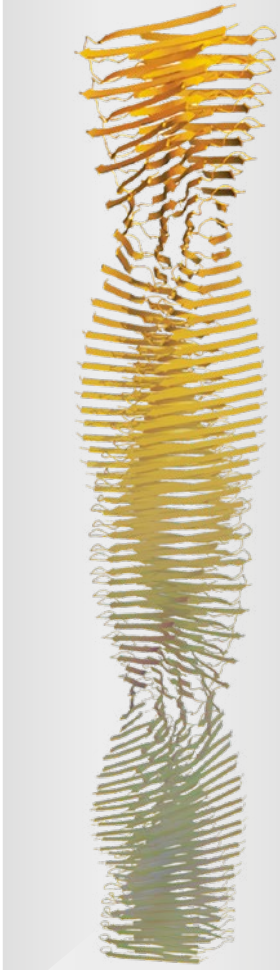
The BNMRZ cooperates closely with the researchers of the Center for Functional Protein Assemblies (CPA), whose new building is currently under construction in the immediate vicinity on the Garching research campus.

The systems are operated from the well-shielded control room attached to the spectrometer hall and all data are collected here.

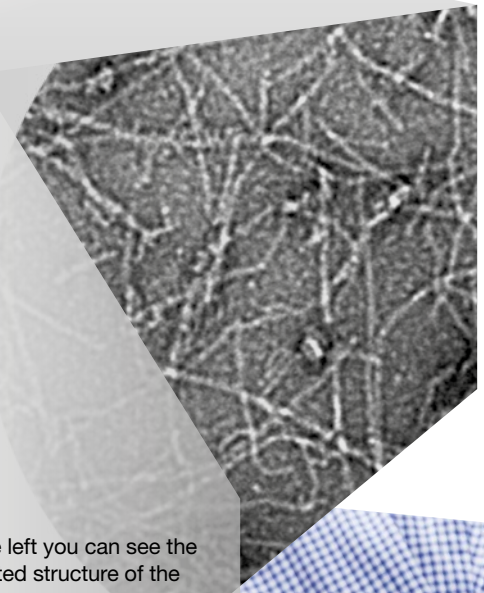


Prof. Bernd Reif holds up a small glass tube around five centimeters in height, but only around five millimeters thick. He shakes it carefully, observing the solution inside, which contains a dissolved protein. The structure of this protein was determined by one of the seven nuclear magnetic resonance (NMR) spectrometers arranged side by side in a 700 square meter measurement hall.

This hall is part of TUM's new Bavarian NMR Center (BNMRZ), officially opened in October 2018. Everything in the brand new, dark red building on the Garching campus revolves around nuclear magnetic resonance spectroscopy – a method that allows the precise measurement of the spatial structure of protein complexes and especially can assess their internal dynamics. One of the most comprehensive and powerful structural analysis methods in chemistry and biochemistry, it enables researchers to take images of the electronic environment of individual atoms and their interactions with neighboring atoms – and thus not only capture the structure but even the dynamics of complex molecules. It is one of the methods that gives atom-level snapshots also of proteins consisting of thousands of atoms. ▷



◁ On the left you can see the complicated structure of the fibrils when they are analyzed in the NMR spectrometer. Scientists are discussing the role of beta-amyloid and its misfolding in the development of Alzheimer's disease.



◁ In Alzheimer's disease, the spatial structure of the beta-amyloid protein changes, causing it to stick together in such long, insoluble strands. At the same time, more and more of the surrounding nerve cells die off.

The protein to be investigated using the NMR spectrometer is first dissolved in a liquid, which is then transferred into a 5 cm long and 5 mm thick glass tube. ▷

Over the last few months, the seven NMR spectrometers previously scattered across various different locations have been brought together at the Bavarian NMR Center. Now widely distributed around the airy hall with its eight-meter-high ceiling, they run around the clock, seven days a week. The fact that this room is so spacious and north-facing makes it easier for the air-conditioning system to keep ambient temperatures stable. The devices each contain a superconducting magnetic core, which must be cooled down to four and sometimes two degrees Kelvin and kept at that temperature with the help of liquid helium. "This is the only way to generate highly homogeneous magnetic fields with the required field strength," explains physicist Bernd Reif. The spectrometer hall is also well shielded from external potential sources of interference. Its location at the northwestern tip of the campus was deliberately selected so that neither the subway nor passing trucks would influence the sensitive data collection process.

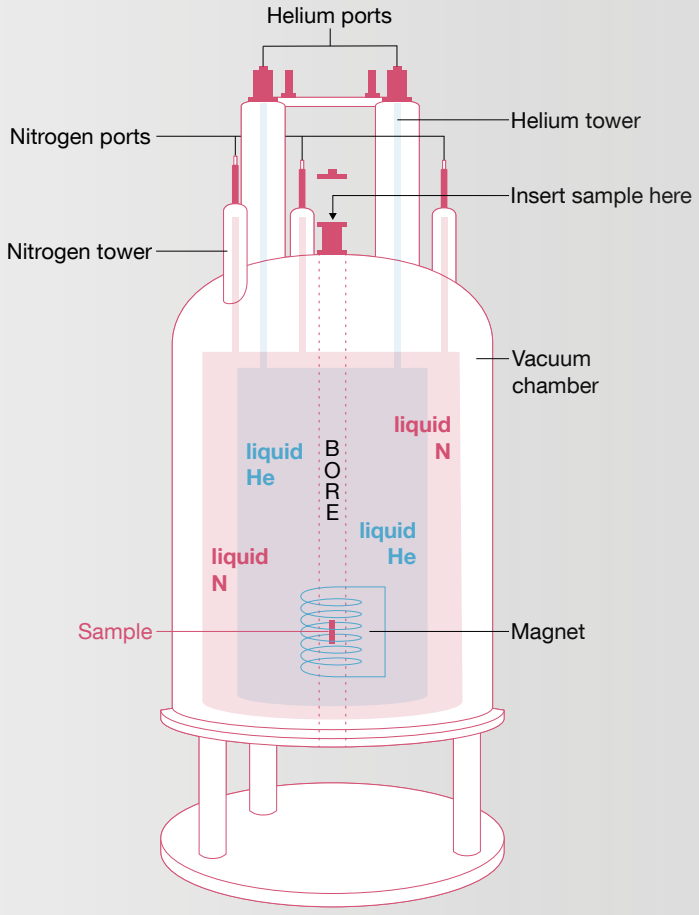
"We wanted a building where labs, offices and equipment are all close by," remarks Prof. Michael Sattler, chemist and Director of the Bavarian NMR Center. Ease of access holds many advantages, enabling closer and more efficient collaboration – not only in terms of research and joint publications, but also when it comes to training students.

Picture credit: Juli Eberle, Bayerisches NMR Zentrum
Graphics: Bayerisches NMR Zentrum, ediundsepp (source: Bayerisches NMR Zentrum)

The strongest magnetic field on Earth

Three more spectrometers from neighboring buildings are now waiting to be moved over. The only item missing then will be the core of the center: one of the world's most powerful NMR spectrometers with a frequency of 1.2 gigahertz and a magnetic field strength of almost 30 tesla – the highest permanent magnetic field on Earth to date. "TUM will be the first university in Germany equipped with a high-performance spectrometer of this kind," Sattler observes. The device is currently being developed by the company Bruker, with delivery planned in three years' time. It will then enable analysis of even larger and more complex proteins than before: "Higher magnetic fields mean a stronger signal and, at the same time, less background noise that would otherwise interfere with our measurements," explains Reif. ▷

NMR magnet



Compared to the sample, the NMR system is huge: The superconducting coil for generating the magnetic field works close to absolute zero. For cooling, it is surrounded by liquid nitrogen on the outside and liquid helium on the inside. However, the NMR sample is measured at room temperature.

The Garching location is an enormous well of expertise, promoting the development of new methods and acting as a central hub specialized in the chemistry of biological macromolecules. In his office, flooded with midday sunlight, Bernd Reif is discussing the broad spectrum of potential applications with Michael Sattler and Aphrodite Kapurniotu, a chemist at the TUM School of Life Sciences Weihenstephan. In highly simplified terms, NMR spectroscopy exposes molecules to a strong homogeneous magnetic field that aligns the nuclei of atoms like compass needles. Short pulses of radio waves are then applied, disrupting this order. During their reorientation, the nuclei release part of the previously absorbed energy as a radio frequency that decays over time, which is measured and then analyzed further by computer. “Each atomic nucleus behaves differently here due to its environment, so in the end we can assess the exact molecular structure of the protein,” reveals Reif. The data provide important information about the form and thus also the function of the molecule, such as how it interacts with other molecules. Not only is this an important step along the path to understanding how diseases develop, it can also be used to pinpoint targets for the development of new drugs.

By way of example, Sattler highlights a new drug candidate for Chagas’ disease. Findings from a joint study on this with scientists at Helmholtz Zentrum München and Ruhr University Bochum were recently published in “Science” magazine. Chagas’ disease is caused by trypanosomes, single-celled parasites, transmitted by blood-sucking insects known as “kissing bugs”. It damages many of the body’s organs, and around ten percent of cases still prove fatal. The drugs currently available have major side effects and are not able to destroy ▷

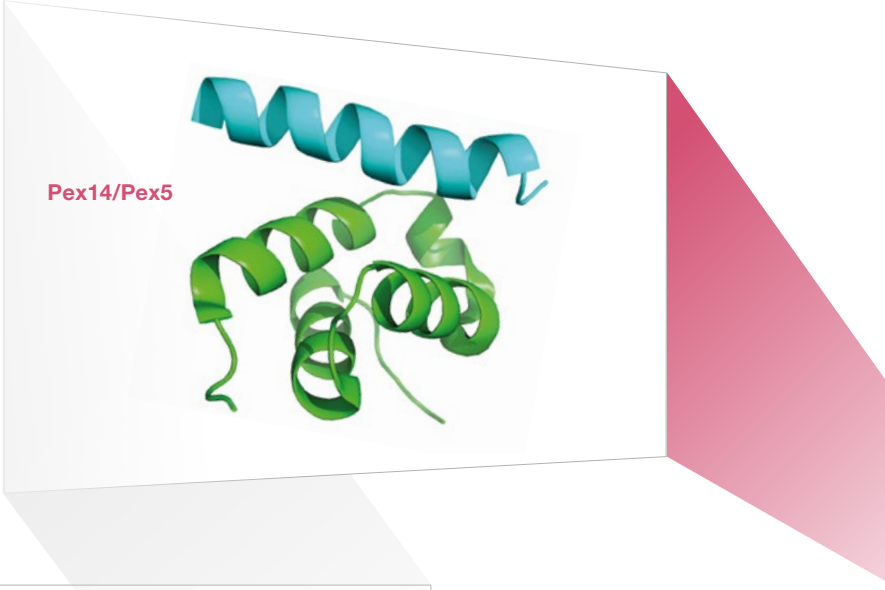
Due to the complexity of the protein structures, the researchers are using state-of-the-art technology, such as VR glasses, with which the spatial arrangement of the individual components can be visualized and intuitively better recognized.

Picture credit: Bayerisches NMR Zentrum

“Each atomic nucleus behaves differently here due to its environment, so in the end we can calculate the exact molecular structure of the protein.”

Bernd Reif, professor and expert in solid-state NMR

Trypanosomes, unicellular parasites that transmit Chagas’ disease, require enzymes for their metabolism, which they take up with the help of a special protein complex. The two proteins PEX14 and PEX5 have to bind to each other. A cartoon representation of the structure of this complex is shown here.

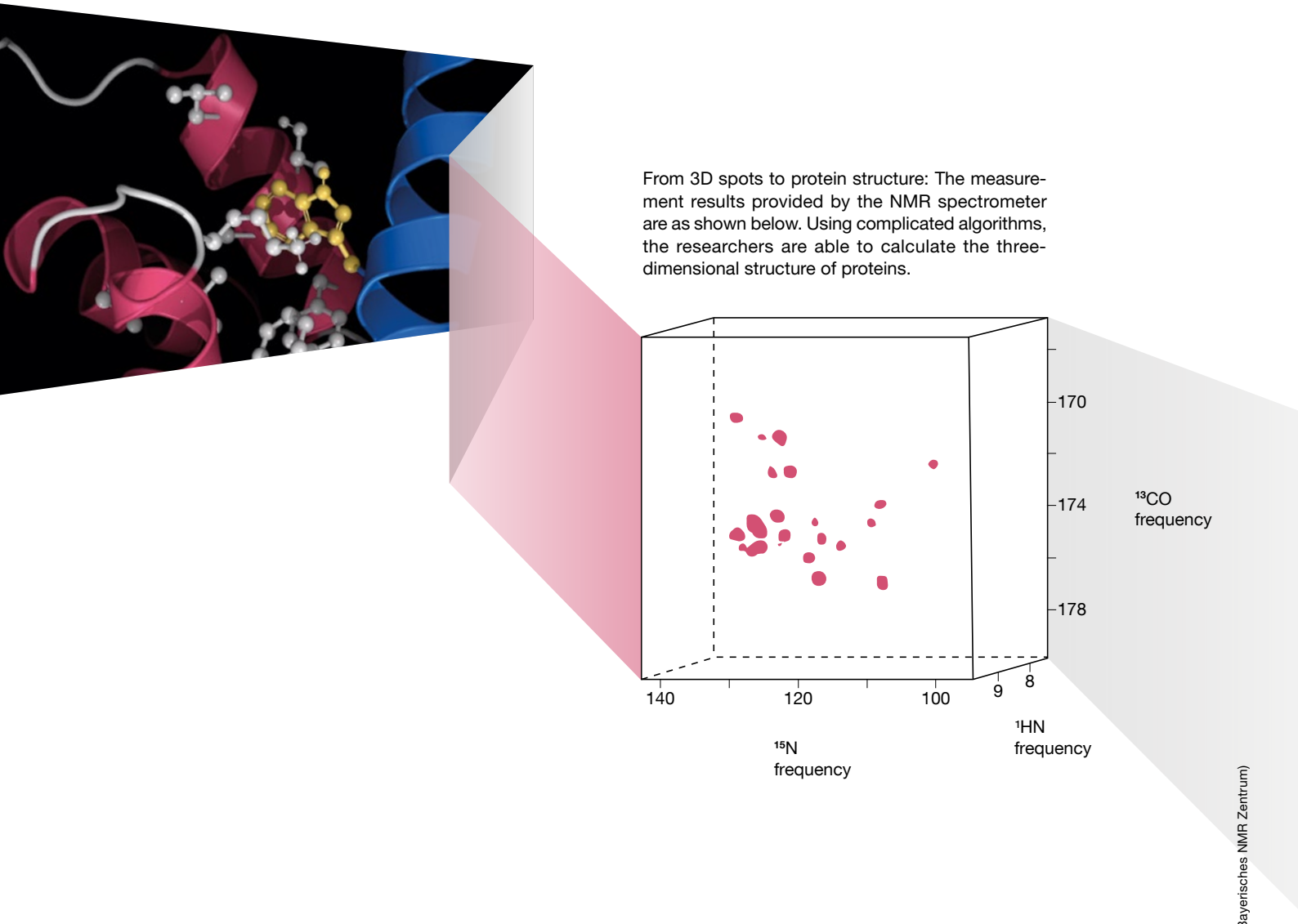


Folding is important

Proteins are made up of amino acids. When two amino acids join together, this is called a dipeptide, and a chain consisting of fewer than ten amino acids is an oligopeptide. Chains of between fifty and two thousand amino acids are referred to as polypeptides or proteins. In simple terms as a basic building principle, a protein can be viewed as a long chain on which various amino acids are strung together.

The exact sequence of amino acids determines the identity and function of the protein. However, proteins can only fulfill their biological function if they have been folded into a specific, complex spatial structure. This folding process takes place after their synthesis – or sometimes during it – due to interactions between amino acids within the molecule. In some cases, enzymes or certain auxiliary proteins, known as chaperones, are required for proper folding. Depending on which amino acids interact with each other, structures can form within the protein that look like spirals or leaflets.

A protein’s three-dimensional structure in space is crucial for it to function correctly. If this is disrupted, diseases can occur.



From 3D spots to protein structure: The measurement results provided by the NMR spectrometer are as shown below. Using complicated algorithms, the researchers are able to calculate the three-dimensional structure of proteins.

the pathogens completely. However, it is known that the single-celled organisms contain specific organelles called glycosomes that metabolize sugar, which is essential for the organism's survival. In order to achieve this, the glycosomes must first import certain enzymes with the aid of a special protein transport complex. And it is this mechanism that Sattler and his colleagues chose to examine more closely. Since they knew that the PEX14 and PEX5 proteins have to bind together to enable this transport, they used NMR spectroscopy to identify their precise structure.

With this as their basis, they identified and gradually optimized a small molecule that specifically binds to PEX14, thus preventing it from interacting with PEX5. As a result, Sattler explains: "This blocks the metabolism of the pathogens, so the trypanosomes starve to death." The next step is to continue developing the drug candidate and ultimately bring it to clinical trials. "Market-ready medication is still a long way off," acknowledges Sattler. "But we have been able to show that blocking the PEX14 protein is a promising novel concept for new agents to combat trypanosomes."

Picture credit: Bayerisches NMR Zentrum, Juli Eberle; Graphics: ediundsepp (source: Bayerisches NMR Zentrum)

When folding goes wrong

The function of a protein depends not only on the precise sequence of its amino acids, but also on its folding – its exact spatial structure (see box). Some diseases are associated with incorrectly folded proteins that form clumps inside or between cells. Over thirty of these protein misfolding disorders have been identified to date, with Alzheimer's probably the most prominent. While clusters of this kind are difficult to access with established methods of structural biology, they lend themselves well to investigation using NMR spectroscopy. In Alzheimer's disease, the spatial structure of the beta-amyloid protein changes, causing it to stick together in long, insoluble strands. At the same time, more and more of the surrounding nerve cells die off. Although this has long been a topic of discussion, it remains unclear what role beta-amyloid and its folding play here and what causes the nerve cells to die. And there are still no drugs to prevent the process. ▷

Based on the protein structure of PEX14 and PEX5, the researchers finally developed a chemical agent that binds optimally to PEX14 and prevents interaction with PEX5. This makes PEX14 inactive.

"We have been able to show that blocking the PEX14 protein is a promising novel concept for new agents to combat trypanosomes."

Michael Sattler, Director of the Bavarian NMR Center

Prof. Aphrodite Kapurniotu

Researcher with international outlook

Prof. Aphrodite Kapurniotu studied chemistry in Athens (Greece). After obtaining her degree in 1984 and her doctorate in 1990 in Tübingen (Germany), she moved to the US, spending 2 years as a postdoc at Rutgers University and another 1 year as a senior scientist at the Picower Institute for Medical Research (US). Returning to Germany, she was a group leader at the University of Tübingen between 1994 and 2002, while completing her postdoctoral thesis in biochemistry. From 2002 through 2007, she led a biomedical research group at RWTH Aachen University, before becoming Professor for Peptide Biochemistry at TUM in 2007.

Prof. Bernd Reif

From Berlin to Munich

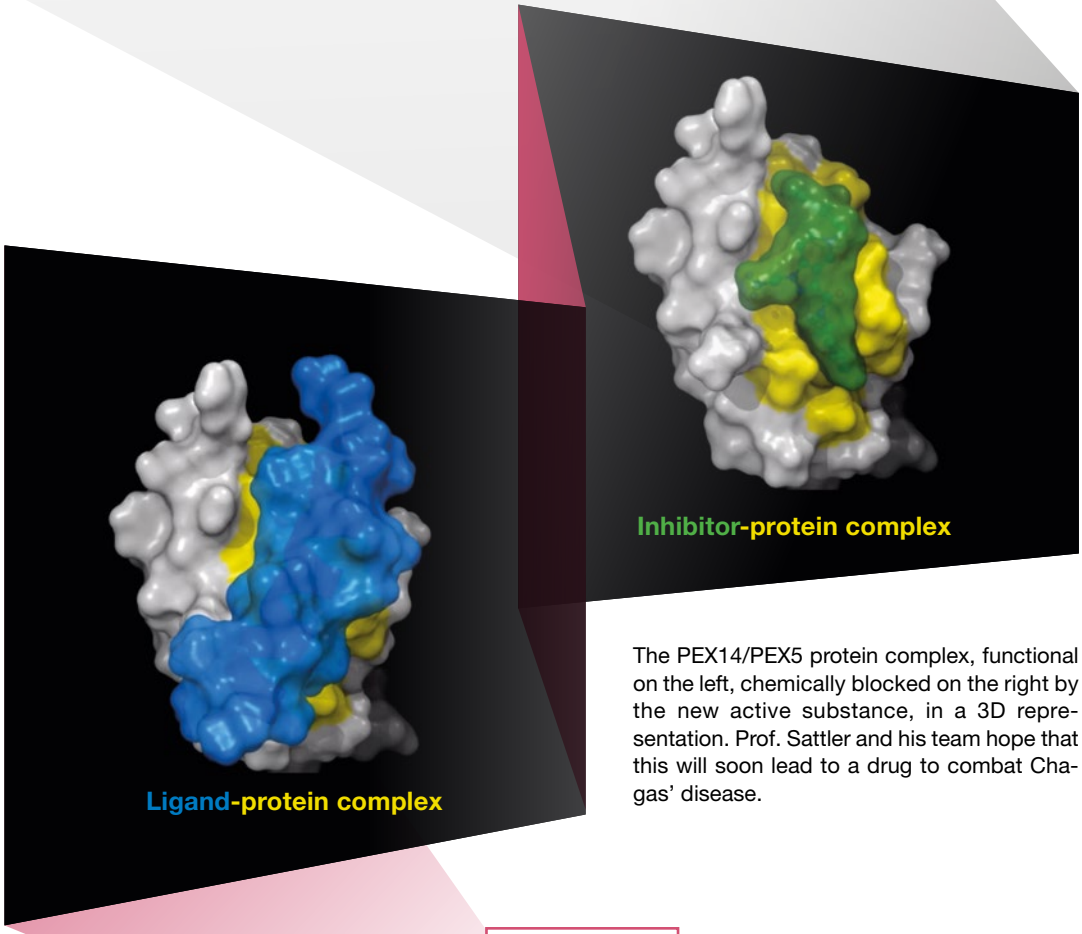
Prof. Bernd Reif studied physics at the University of Bayreuth (Germany), obtaining his degree in 1993. After completing his doctorate in 1998 at Goethe University Frankfurt, he pursued his studies as a postdoc at the Massachusetts Institute of Technology (MIT) in Cambridge (US) until 1999. He then returned to Germany to lead an Emmy Noether Junior Research Group at TUM until 2002. From 2003 through 2010, he worked at the Leibniz Institute for Molecular Pharmacology in Berlin-Buch, while also holding a professorship at the Charité university hospital in Berlin. In 2007, he became Coordinator at the Leibniz Graduate School of Molecular Biophysics in Berlin. He took up his position at TUM in 2010, and has also led a working group at Helmholtz Zentrum München since that time.

Prof. Michael Sattler

An NMR researcher from the very start

Prof. Michael Sattler studied chemistry at Goethe University Frankfurt (Germany), where he received his degree in 1991 and his doctorate in 1995, focusing on the development of NMR methods. He then spent two years in the US, working as a postdoc at Abbott Laboratories – a global pharmaceutical company. Back in Germany, he led a research group at the European Molecular Biology Laboratory (EMBL) in Heidelberg from 1997 through 2006. He has been Director of the Institute of Structural Biology at Helmholtz Zentrum München since 2007, while at the same time holding TUM's Chair of Biomolecular NMR Spectroscopy. He is also Director of the Bavarian NMR Center.

Picture credit: Juli Eberle; Bayerisches NMR Zentrum



The PEX14/PEX5 protein complex, functional on the left, chemically blocked on the right by the new active substance, in a 3D representation. Prof. Sattler and his team hope that this will soon lead to a drug to combat Chagas' disease.

What we do know is that healthy cells usually have enough clean-up workers on hand in the form of specialized proteins, which ensure that folding errors are corrected immediately or that the affected molecules are taken out of circulation. Bernd Reif is investigating their interaction with beta-amyloid protein. Meanwhile, his colleague, Aphrodite Kapurniotu, is focusing on chemical synthesis and developing molecules intended to block the misfolding and clump formation of beta-amyloid.

"NMR spectroscopy helps us to understand beta-amyloid interactions and thus continue improving the properties of potential aggregation inhibitors," Kapurniotu explains. In the future, the two professors intend to take results from the test tube further along the path towards medical applications. They are also keen to find out whether their results could be applied to other diseases associated with misfolded proteins. ■ *Karoline Stürmer*