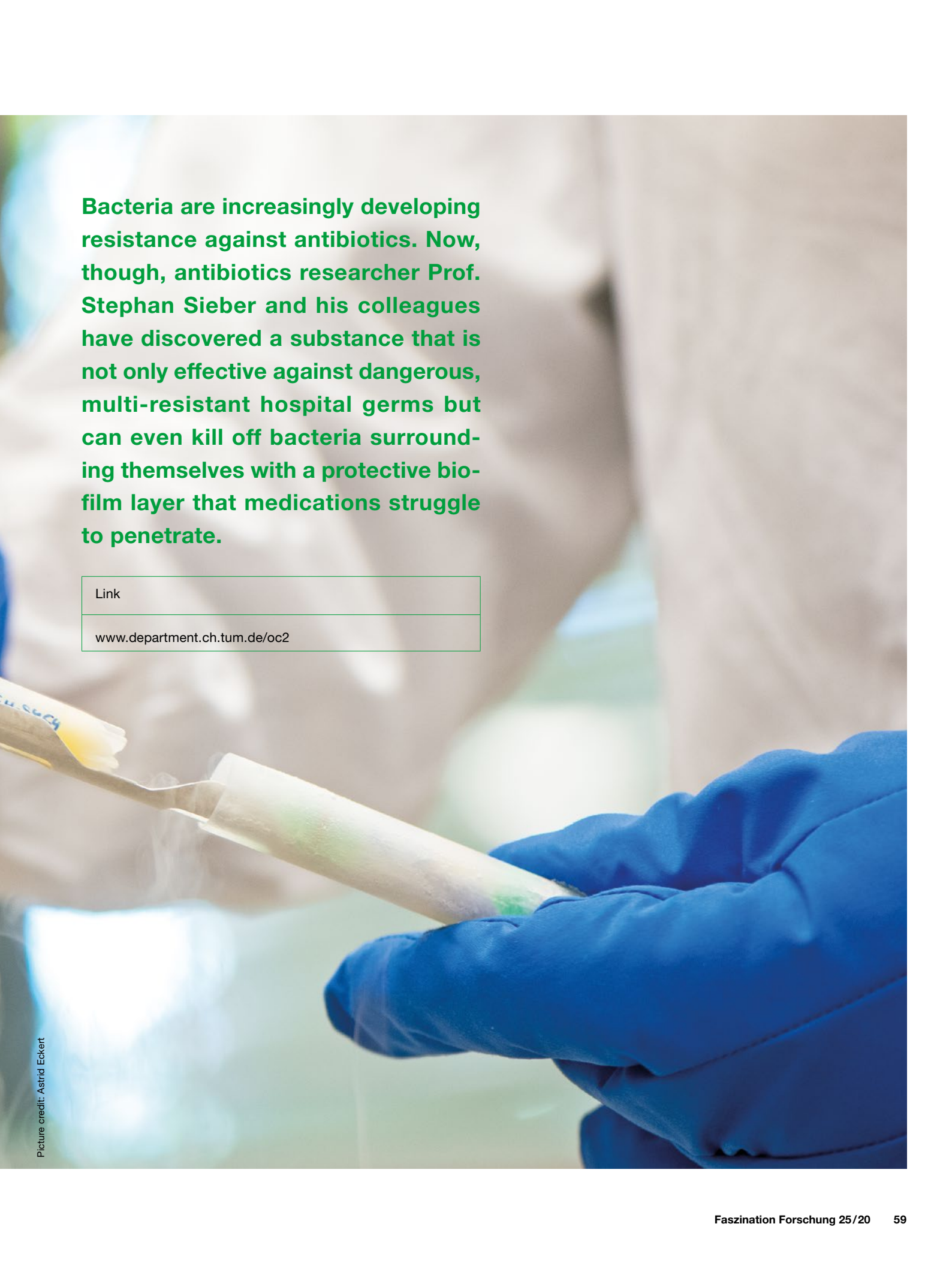


Fighting Superbugs with Superdrugs



Bacteria are increasingly developing resistance against antibiotics. Now, though, antibiotics researcher Prof. Stephan Sieber and his colleagues have discovered a substance that is not only effective against dangerous, multi-resistant hospital germs but can even kill off bacteria surrounding themselves with a protective bio-film layer that medications struggle to penetrate.

Link

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Krankenhauskeime im Klammergriff



Bakterielle Infektionskrankheiten können heute gut behandelt werden, denn seit rund 100 Jahren gibt es Antibiotika. Sie sind gut verträglich, günstig und effektiv. Viele Jahre lang wurden sie gerne verschrieben und großzügig in der landwirtschaftlichen Tierhaltung eingesetzt. Seit Ende der 1990er-Jahre gibt es jedoch ein zunehmendes Problem: Multiresistente Keime, heimisch in Krankenhäusern und lebensgefährlich für geschwächte Patienten. Effektive Antibiotika dagegen gibt es kaum. Stephan Sieber, Professor für Organische Chemie an der TUM, hat mit PK150 eine Substanz entdeckt, die gegen multiresistente Bakterien wirkt. PK150 greift die Bakterien an zwei Stellen an: Es führt zur unkontrollierten Freisetzung bestimmter Proteine, durch die sich das Bakterium selbst von außen auflöst. Und es stört die bakterielle Energieversorgung. So kann PK150 Bakterien sogar im „Persister“-Ruhestadium abtöten, selbst wenn sie sich zusätzlich mit einem Biofilm-Schutzmantel umgeben. PK150 soll jetzt zum Medikament weiterentwickelt werden. □

“The development pipeline for antibiotics around the world is readily estimable.”

Stephan A. Sieber

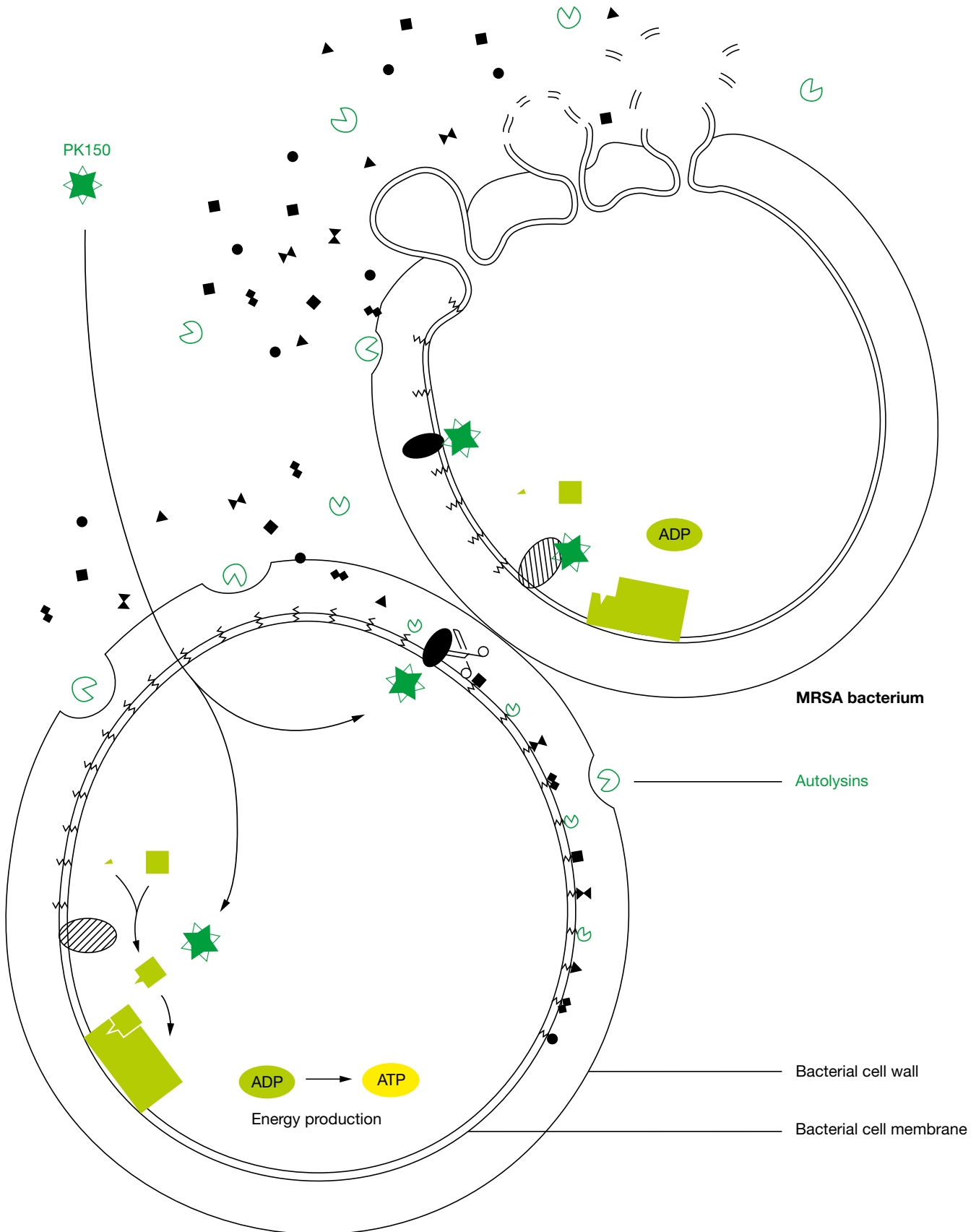
Klaus thought he had been exceptionally lucky when a routine health check uncovered a heart condition. As a slim, athletic man in his late 50s, nobody would have suspected he might have a heart condition. It was diagnosed at an early stage and he underwent surgery, which was successful. Six months later, Klaus was dead. He had become infected with hospital pathogens – multi-resistant bacteria that did not respond to any of the antibiotics that doctors administered. His body, which had been weakened by the operation, would not have been able to fight the infection at all without the support of these medications.

Nowadays, it is rare for anyone in the West to die from bacterial infections. Once-feared illnesses such as plague, cholera and typhus that depopulated entire regions in centuries gone by are no longer present in Germany. Around 1 million people die in Germany each year and around two-thirds of these mortalities are due to

cardiovascular diseases or cancer. In 2018, only around 0.1 percent of deaths were attributed to infectious bacterial diseases, a cause of death that doctors are required to report. We can attribute this to the strict hygiene standards and good quality of medical care in Germany today, as well as the fact that vaccinations are available against some pathogens. Above all, however, we have effective and highly tolerable antibiotics.

Antibiotic-resistant bacteria threaten global health

Since Alexander Fleming discovered penicillin in 1928, antibiotics have been overwhelmingly triumphant in the battle against bacterial diseases. Today, there are around 80 different classes of antibiotics, most of which were developed in the “golden years” of antibiotics research from 1940 to 1970. Antibiotics were seen as a panacea and doctors prescribed them with great regularity. They also came to be used heavily in animal husbandry and fattening. ▶



Graphics: edlundsepp (source: Markus Bernards, Stephan A. Steber)

PK150 blocks two key processes in the bacterium at the same time. It bonds with two enzymes in the bacterial cell. As a consequence, one enzyme (shown black), sets free proteins, including some called autolysins, which break down cell walls. The other enzyme (shown hatched), which is bound by PK150, is important for the bacterium's energy production. All in all, PK150 kills the bacterium by blocking its energy supply and by perforating its cell walls.

Since the late 1990s, however, the downsides of antibiotics have become clear. Bacteria are increasingly developing resistance against antibiotics, with some strains even becoming concurrently resistant to several substance classes. This gives rise to multi-resistant germs that become endemic in hospitals. Often referred to in the media as “super germs”, the World Health Organization (WHO) has identified antibiotic-resistant bacteria as one of the greatest threats to global health. In 2017, the WHO published a list of the 12 most dangerous resistant germs and called on governments to create incentives for the development of medications to fight these pathogens. Ultimately, too few new antibiotics have been developed to keep pace with rising resistance.

Big pharma has long since stepped back from the field of antibiotic development, leaving small and medium-sized pharmaceutical enterprises to fill the gap. Developing new medications costs billions and there is a significant risk that bacteria will become resistant once new drugs are launched on the market. What is more, antibiotics with new mechanisms of action are only used when standard methods fail – and their sparing usage generates little revenue. Prof. Stephan Sieber, antibiotics researcher and holder of the Chair of Organic Chemistry II at TUM, recently concluded a study with colleagues on the current state of antibiotics research. “The development pipeline for antibiotics around the world is readily estimable,” he says. “Of the 50 or so substances currently in the clinical phase, there are only a handful of genuinely new developments. The majority are variations of conventional antibiotics that have been once again enhanced to a certain degree.”

A cancer treating drug opens up a path

This makes it all the more gratifying for Sieber that he and his team have identified a candidate antibiotic in his aBACTER project that works differently to most antibiotics. Conventional medications inhibit either the formation of bacterial cell walls, bacterial protein generation or DNA replication. Sieber’s agent, on the other hand, attacks bacteria on two fronts – with both capable of killing the pathogens: His substance causes cells to break down their own cell walls and also blocks energy generation. The aBACTER research project has already been running for five years. In their search for antibiotics with new mechanisms of action, Sieber’s team of scientists came across a drug actually intended to treat cancer but which also has mild antibiotic properties. The TUM researchers created a series of chemical variants to improve the

medication wherever possible before testing it on MRSA – a dangerous multi-resistant bacterium and infamous super germ.

One variant, a molecule given the name PK150, hit the bullseye. Even in minute quantities, the molecule is capable of killing off MRSA bacteria. “It is important to use a low effective concentration so that the substance also has the lowest possible toxicity in the human body,” explains Sieber.

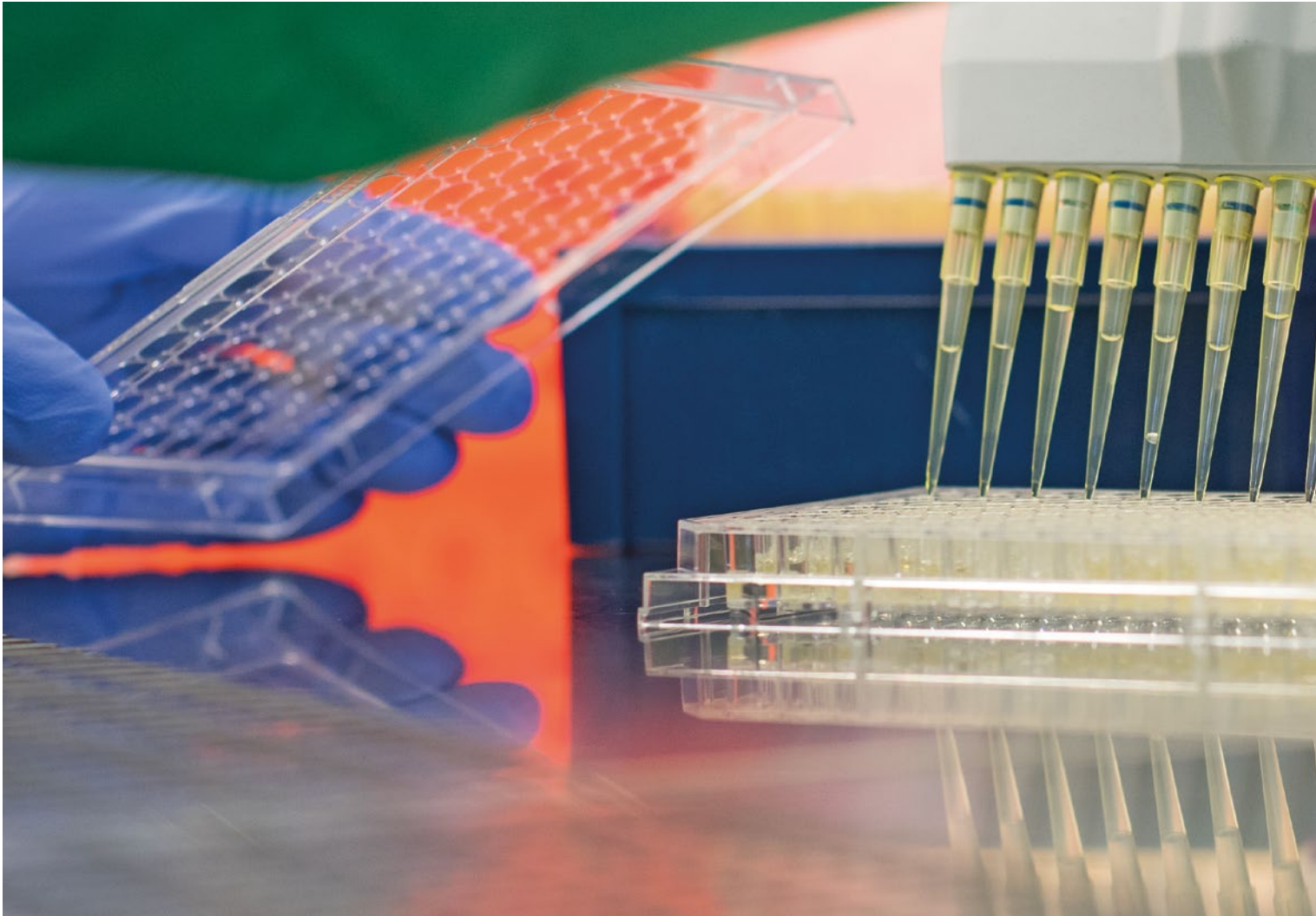
But is there a chance that bacteria might soon become impervious to PK150? Sieber’s team have made every effort to provoke resistance to PK150. The researchers have exposed MRSA bacteria to low concentrations of the substance for prolonged periods – so low that it is only able to kill off some of the bacteria. This gives the surviving bacteria the opportunity to alter their genome so as to render the antibiotic ineffective. In most cases, bacteria need four to ten days for this process. ▶

Prof. Stephan A. Sieber

Born in 1976, Prof. Stephan A. Sieber holds the Chair of Organic Chemistry II at TUM. After completing his doctoral studies in Marburg and Boston (Harvard), the chemist embarked on postdoctoral studies at the Scripps Research Institute in California, where he familiarized himself with target identification approaches. These techniques make it possible to search for binding partners for anti-cancer agents in human cells. As a recipient of an Emmy Noether scholarship at LMU Munich, he began to translate this technique to bacteria in order to shed light on antibiotics’ mechanisms of action. He accepted a position at TUM in 2009. In 2010, Sieber initiated AVIRU, which aims to use a new technique to get to grips with multi-resistant germs. His aBACTER project is funded by the VIP+ Program of the Federal Ministry of Education and Research (BMBF) and also won the Bavarian m4 Award in 2019.



Picture credit: Astrid Eckert



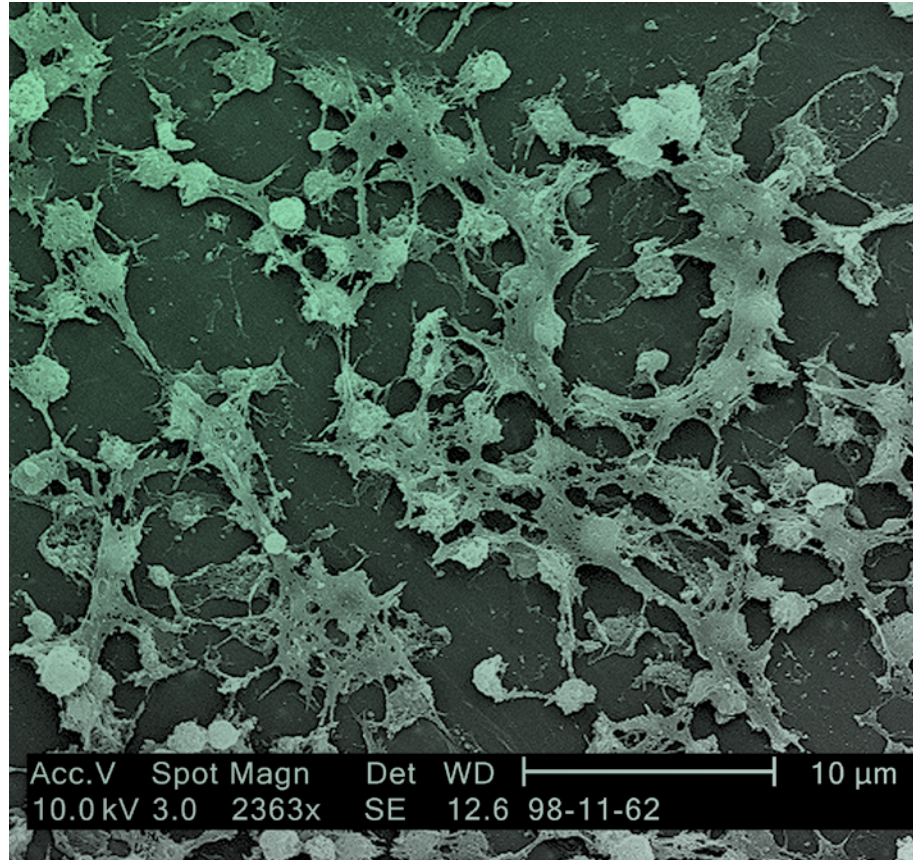
However, PK150 was still able to kill bacteria off after a month – to Sieber’s delight. “We were not able to make bacteria resistant to PK150 in the laboratory,” he said. “Not even when we used special substances to encourage genetic variation in the bacteria.”

Sieber and his team now want to find out precisely how PK150 works. They have identified that PK150 bonds with two enzymes in the bacterial cell. One enzyme regulates protein transport in bacterial cells. PK150 hyperactivates this enzyme, with fatal consequences for the bacterium – it results in the uncontrolled release of proteins, including some called autolysins, which break down cell walls. Normally, bacteria only need autolysins in very small doses to promote cell division. PK150, however, causes them to be released in such volumes that they essentially punch holes in bacterial cell walls.

The other enzyme that PK150 binds to is used by the bacterium for energy metabolism. PK150 blocks this process, which on its own would suffice to kill the bacterium. “The fact that PK150 blocks two key processes at the same time is likely the reason why the bacterium finds it so hard to become resistant to PK150,” surmises Sieber.

Tests with many resistant pathogens

The TUM researcher’s next step took him to the Harz mountains, to Wernigerode in Saxony-Anhalt, home to the Robert Koch Institute’s National Reference Center for Staphylococci and Enterococci. The facility collects resistant germs from across Germany and allowed PK150 to demonstrate that it is effective not only against MRSA but also against a whole host of other dangerous bacteria. Thanks to its novel mechanism of action, PK150 also



Scanning electron microscopic (SEM) image of *Staphylococcus aureus* bacteria, which were found on the luminal surface of an indwelling catheter. The sticky-looking substance woven between the round bacteria is known as biofilm. This biofilm protects the bacteria that secrete the substance from attacks by antimicrobial agents such as antibiotics.

Picture credits: Astrid Eckert, Janice Carr: phil.cdc.gov/details.aspx?pid=7484

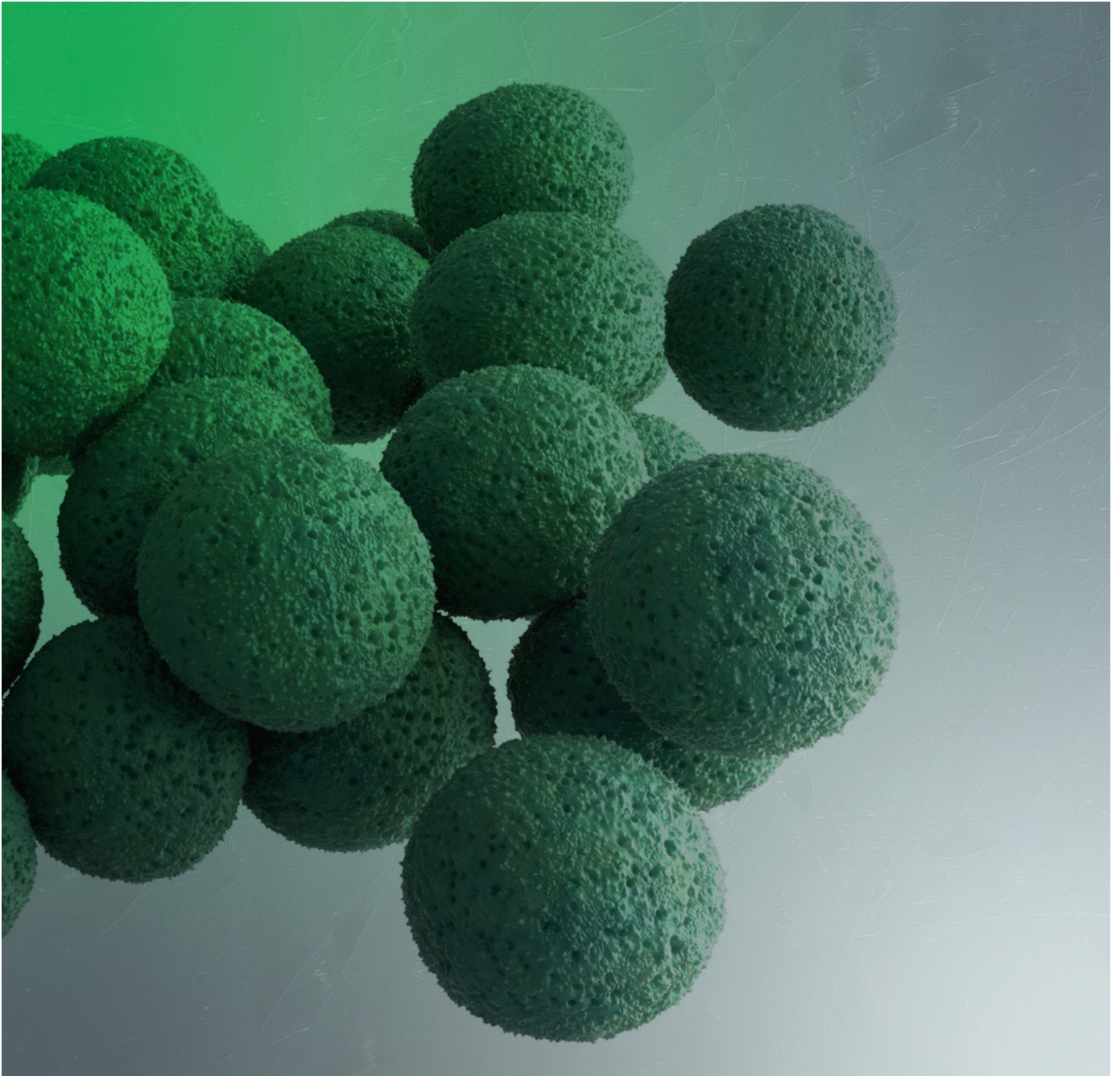
eliminated bacteria that other antibiotics cannot. This is because some bacteria tolerate antibiotics by, in effect, playing dead – they shift into an idle state in which they hardly divide and their metabolic processes are reduced to a minimum. A bacterium in this idle state is known as a “persister”. Given that many antibiotics target the division mechanism or an active metabolism, this state renders persistent bacteria safe from such agents. Groups of persistent bacteria sometimes surround themselves with a protective biofilm, a sort of thick mucus. This allows them to survive on an artificial hip or a catheter in the human body while protected against the body’s immune system and many antibiotics – and thus become the starting point for severe infections. Sieber’s team has observed that PK150 can both kill persisters and break down biofilms. ▶

Petri dish showing growing bacteria (left) as well as non-growing bacteria (right), which lack an essential gene. The protein encoded by this gene represents an attractive antibiotic target.



Bacterial colonies (small spots) are selected for subsequent studies.





3D illustration of *Staphylococcus bacteria*

“That is the most exciting of the substance’s qualities,” believes Sieber, “because there is no antibiotic on the market today capable of permanently breaking down biofilm.”

In the coming years, PK150 is to be developed into an active agent that can prove its credentials on patients in clinical studies. The TUM researchers have fixed their sights on infective or bacterial endocarditis – a condition

in which biofilms and hospital germs play a major role – and hope to have PK150 approved as a treatment. Before being approved as a drug, PK150 will need to prove that it is effective and tolerable in the human body. If this testing proves successful, there will be an entirely new class of antibiotics with which to fight many multi-resistant germs.

■ *Markus Bernards*