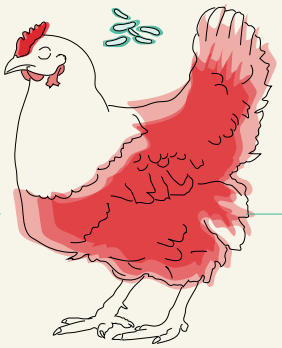
Bacteria



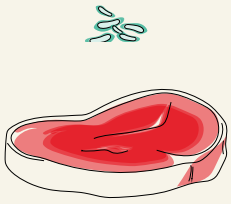
Antibiotics

The use of antibiotics can result in the development of resistant bacteria

Resistant bacteria can occur in livestock breeding

2/3

animal



Due to incorrect processing, resistant bacteria can turn up in meat

Manure containing resistant bacteria can affect fruits and vegetables

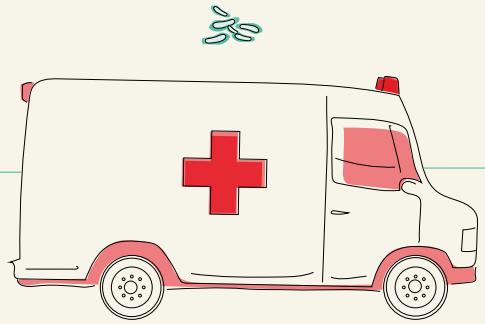
Persons working with animals can catch resistant bacteria

In this way, resistant bacteria spread from agriculture to humans

Graphics: ediundsepp; source: GERMAP2012

Overcoming **Antibiotic Resistance**

Pathogenic bacteria are becoming increasingly resistant to standard antibiotics – and overcoming this public health risk calls for fresh approaches. That is why Prof. Stephan Sieber and his team are on a quest for totally new targets and their corresponding inhibitors. Rather than killing bacteria, his anti-virulence strategy is looking to neutralize or "tame" them – removing their claws, so to speak.



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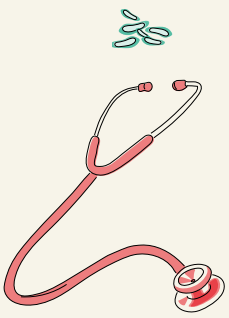
human

Hospitals account for
10–20% of antibiotic use

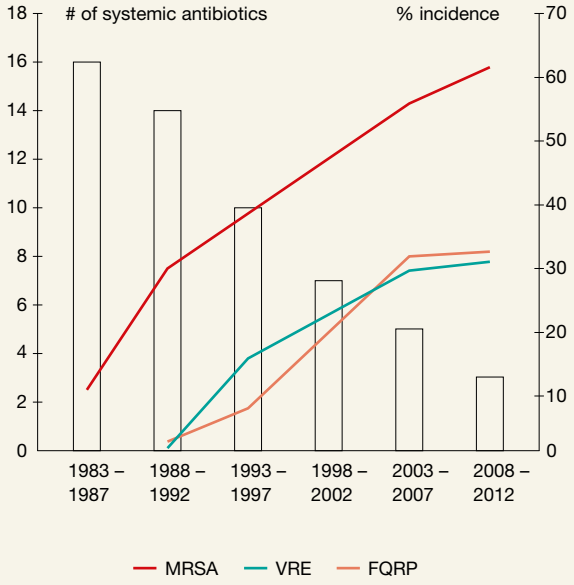
The use of antibiotics can result in the development of resistant bacteria

Antibiotics

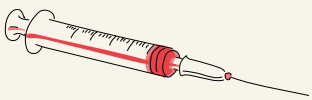


Resistant bacteria can develop inside the hospital or they can be brought in by patients. If they colonize doctors and nurses or contaminate surfaces, they can transfer to other patients

The use of antibiotics can result in the development of resistant bacteria



Between 1983 and 2012, the number of new systemic antibiotics approved by the US federal Food and Drug Administration continously declined. During the same time, the incidence rates with multiresistant bacteria rose. Shown here: methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococci (VRE), and fluoroquinolone-resistant Pseudomonas aeruginosa (FQRP).



7,500–15,000
deaths due to infections per year (estimate)

400,000-600,000
hospital infections per year

18,000,000
hospital patients per year (Germany)

Graphics: ediundsepp (source: TUM, GERMAP2012, Stellungnahme zur Antibiotika-Forschung, Leopoldina und Akademie der Wissenschaften in Hamburg, 2013)

Antibiotika-Resistenzen besiegen

Es ist eine gefährliche Kombination: Herkömmliche Antibiotika werden wirkungslos, weil immer mehr pathogene Bakterien resistent gegen Antibiotika werden – nicht nur gegen eines, sondern gleich gegen mehrere. Und es kommen kaum neue Antibiotika auf den Markt. Die Angriffsziele von Antibiotika in den Bakterien – die Bildung von Zellwand und von Proteinen sowie die Vervielfältigung der Erbinformation – sind abgenutzt. Deshalb ist es äußerst wichtig, neue Angriffsziele für Antibiotika oder Antibiotika-ähnliche Stoffe zu finden. Diesem Ziel hat sich der Chemiker Prof. Stephan Sieber mit seinen Mitarbeitern an der TUM in Garching verschrieben. Er verfolgt bei seiner Arbeit ein noch junges Konzept: die Anti-Virulenz. Hierbei geht es darum, die Bakterien durch Substanzen nicht abzutöten, sondern zu „zahnlosen Tigern" zu machen. Er spricht von Inhibitoren. Dies soll erreicht werden, indem sich geeignete Verbindungen an neue Angriffsziele in den pathogenen Bakterien binden und so verhindern, dass bestimmte Proteine, die zum Beispiel dem Ausbreiten der Bakterien im Körper und dem Kampf gegen das Immunsystem dienen, freigesetzt werden. Sind die Bakterien „zahnlose Tiger", kann das Immunsystem abschließend das Aufräumkommando spielen. Resistenzen können sich dabei nicht bilden. Sieber begann seine Forschungsarbeit mit ß-Lactonen, also Naturstoffen, die Bakterien nicht abtöten, aber bekämpfen. Mittlerweile konnte das Sieber`sche Team zwei Zielproteine in Bakterien identifizieren: Das erste ist ein proteinspaltendes Enzym (Protease) namens ClpP (caseinolytische Protease). Es schreddert nicht nur fehlgefaltete Proteine, sondern hat auch wichtige regulatorische Funktionen im Zusammenhang mit der Fähigkeit der Bakterien, den Menschen krank zu machen. Diese Fähigkeit bezeichnet man als Virulenz. ß-Lactone binden sich auf raffinierte Weise an das ClpP, sodass dieses durch strukturelle Veränderungen inaktiv wird. Damit wird das Bakterium an die Leine gelegt. Das zweite Angriffsziel ist ein sogenannter Transkriptionsaktivator, der aktiv wird, sobald die Bakterienzahl groß genug ist, um das Immunsystem erfolgreich anzugreifen. Er leitet dann in der Bakterienzelle das Ablesen wichtiger genetischer Informationen ein, sodass das Bakterium krankmachende Stoffe herstellen kann. Sieber und seine Mitarbeiter haben bereits die Substanz gefunden, die das verhindern kann. *Gerlinde Felix*

Link
www.oc2.ch.tum.de

We all have our dreams – whether it be a sports car, the lottery jackpot or Olympic gold. A passionate researcher through and through, Stephan Sieber dreams of finding active agents that will prevent the growing resistance to antibiotics becoming a full-blown crisis. His priority is to develop agents with concrete medical applications. Since taking over TUM's Chair of Organic Chemistry II in Garching in October 2009, he has worked doggedly toward this ambition, building up a team that now numbers 24 researchers. Emphasizing the pressing nature of the problem, he points to a chart that shows a dramatic drop in the number of systemic antibiotics approved by the FDA in the US since 1983 – and an ominous rise in multidrug-resistant bacteria over the same time frame. This is partly due to a general lack of new antibiotics being developed. And this trend looks set to continue. Hospital "superbug" MRSA is a case in point. Though short for methicillin-resistant Staphylococcus aureus (S. aureus), the bacteria in question has built up a resistance not just to methicillin, but to a range of other antibiotics as well. In a future without either new antibiotics or fresh approaches to battle the onslaught of bacteria, mild infections could become severe or even fatal. The challenges are huge. But Sieber sees the answers to these challenges in his team members, giving them as much freedom as possible to work independently. "I hope that I can continue to work with such excellent and inventive self-starters – the quality of our research is largely in their hands." The team is focusing on the search for new targets within the bacteria, and effective agents with the ability to inhibit these target proteins.

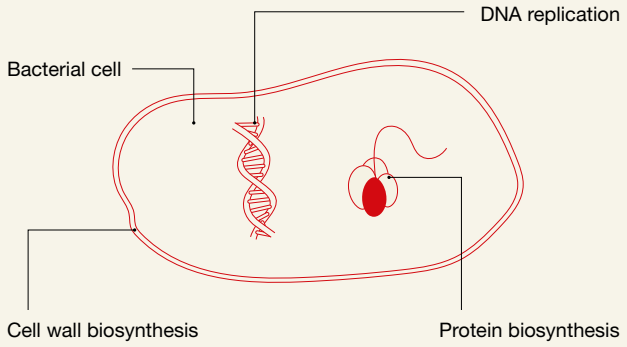
From hostile pathogen to toothless tiger

Each of us carries bacteria like S. aureus. As long as they are only present in small quantities, these bacteria are able to hide from the immune system. They produce autoinducing peptides (AIPs), which they release into the surrounding environment to communicate with other bacteria. Only when the AIPs – and thus bacteria – reach a critical level do the bacteria switch from "stealth" to "attack" mode. "At that point, they know they are powerful enough to wipe out the immune system," explains Sieber. When they go into attack mode, the bacterial cells produce a selection of proteins or virulence factors. These include proteins that enable the bacteria to spread through the body, destroy cells, feed on those cells and weaken the immune system. Other proteins can cause tissue death, blood ▷

poisoning (sepsis), severe organ failure and circulatory collapse (toxic shock). The bacterial processes targeted by conventional antibiotics – cell wall synthesis, protein synthesis and DNA synthesis – have been exhausted. Over the years, bacteria have developed ways to sidestep the attacks launched by antibiotics, for instance through genetic mutation, and become resistant to them. Hence Sieber's quest for completely new targets, building on the relatively recent concept of anti-virulence. Here, Sieber and his team are focusing less on the bacteria-killing potential of new substances, and more on their ability to prevent the bacteria from producing virulence factors. The immune system can then take care of the rest. The major advantage of this approach is the fact that there is no selection pressure so that, according to Stephan Sieber, the risk of building up bacterial resistance ought to be relatively modest. An added benefit is that the normal flora in the intestines are not destroyed.

"The combination of biology, chemistry and structural chemistry all in one building is hugely beneficial to our work. It saves us a lot of time and makes it easy to try out new ideas spontaneously."

Stephan Sieber



Conventional antibiotics attack bacteria by blocking the building of cell walls or the replication of DNA, or by hindering the synthesis of proteins. Bacteria have developed ways to sidestep these attacks and thus become resistant to them.

Beta-lactones make a comeback: disarming rather than killing

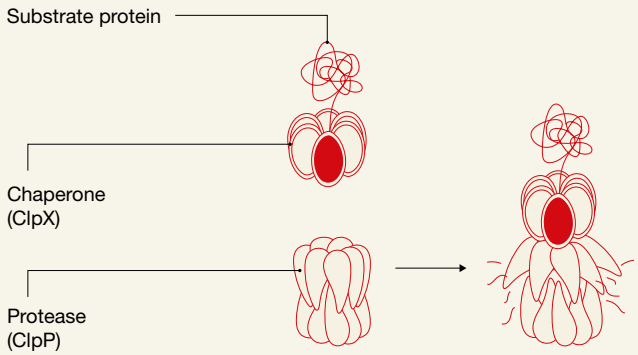
Now just turned 39, Sieber has been driven by a keen interest in bacteria and natural substances produced by organisms ever since working on his doctoral thesis in Germany and the US. Unsurprising, then, that on coming to TUM, the sporty chemistry professor chose to continue where he left off at Munich's Ludwig Maximilian University (LMU), researching the function of naturally occurring beta-lactones within the bacterial cell. A long-neglected substance class, ß-lactones are similar in structure to ß-lactams – a broad class of antibiotics that includes penicillin. In contrast ▷

Graphics: ediundsepp (source: TUM); picture credit: Eckert

Substances that do not kill the bacteria but prevent them from producing virulence factors help avoid resistance developing

Left: Chemist Prof. Stephan Sieber has a keen interest in bacteria and natural substances. His research focuses on finding natural substances that help disarm bacteria and thus offer an alternative to traditional antibiotics.

The protease ClpP acts as a sort of molecular scissors with 14 blades, snipping misfolded proteins into short segments. While ClpPs play a positive role in humans, they are also responsible for the pathogenic effect of bacteria.



to ß-lactams, however, ß-lactones are ill-suited to killing bacteria, leaving them sidelined by research. “But I was convinced that nature wouldn’t simply produce a second-rate solution,” recalls Sieber – and he proved to be right. As it turns out, ß-lactones can indeed prevent microbes such as S. aureus posing a threat to human health. This is because ß-lactones are anti-virulent, meaning that they strongly impair the pathogenic effect of S. aureus, in essence rendering the bacteria a “toothless tiger.” But which proteins do the ß-lactones bind to in S. aureus? To find out, Sieber turned to activity-based protein profiling (ABPP) to monitor ß-lactone behavior with proteins – a complex method he learned during his postdoc period with US researcher Benjamin F. Cravatt at the Scripps Institute in La Jolla. ABPP enables examination of bacterial cells in aqueous solution with tagged chemical probes. The probe used by Sieber is a ß-lactone and the attached tag is an alkyne, or unsaturated hydrocarbon, which would not normally be present in S. aureus. An additional reporter tag that reacts with the alkyne enables visualization of the ß-lactone target protein in S. aureus using fluorescent in-gel analysis (SDS-PAGE analysis) and identification by mass spectrometry.

Targeting a specific protease

Sieber and his team established that their ß-lactone probe attaches to a proteolytic (or protein-splitting) enzyme or protease – in this case, caseinolytic protease, or ClpP. “ClpP is a giant protease consisting of 14 subunits – so a tetradecamer – and looks like a double donut,” according to Sieber’s brief description. Proteases are vital proteins that ensure order within a cell – hence they are often referred to as “housekeeping” proteins. If the bacteria cause an infection, oxidative stress ensues, triggering misfolded bacterial proteins. Here, ClpP acts as a sort of molecular scissors with 14 blades; each of its subunits houses an active site that can snip misfolded proteins into short segments. Since a serine atom is located in each active site, researchers also refer to ClpP as a serine protease. However, ClpP is more than just a housekeeping protein: it is also responsible for the pathogenic effect of S. aureus and other types of bacteria. So if it were possible to deactivate this serine protease, that would deal S. aureus a serious blow – which is where the ß-lactones come in. The Munich research team has determined that ß-lactones with a particular structure make good inhibitors and ▷

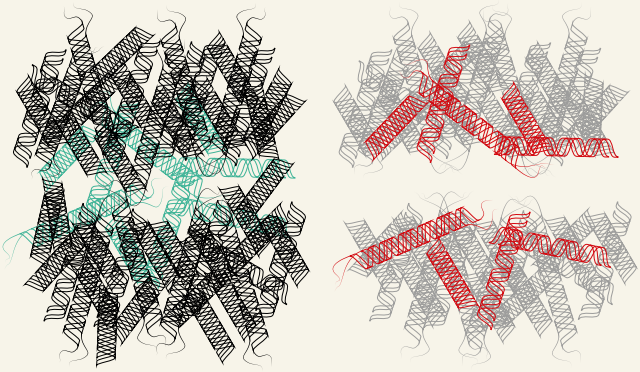
Picture credit: Ecker; graphics: edundsepp (source: TUM)

are able to bond very well with the reactive serine in a pocket in each of the ClpP's active sites – as long as the ß-lactone side chain is only eight carbon atoms in length. This bond renders the protease harmless. The structure of ß-lactones incorporates a characteristic electrophile four-ring form. "Without this quadruple ring and specific lactone geometry, the bond wouldn't be possible. But this is ideal," enthuses Sieber. So far, the Garching chemists have synthesized around 60 lactone derivatives in an effort to understand the exact relationship between ß-lactone structure and ClpP activity, and to identify the optimum candidates for a new antibiotic.

Double donut with a weak point

So how do the appropriately structured ß-lactones set about deactivating the ClpP? In one of two ways: If the ß-lactone has a side chain with residual hydrocarbons, they fill the pocket in each of the 14 active subunits, essentially disabling the blades of the molecular scissors. But the ClpP itself remains intact. Or – and this way only half of the active sites need blocking – they split the dual structure into two "donuts," so ending up with two heptamers. This is the case when the ß-lactone has an aromatic ring in its side chain. Each heptamer on its own is then completely inactive. "Incredibly, nature has fitted the ClpP with a predefined breaking point, which certain ß-lactones don't hesitate to use." Crystal structure analyses in collaboration with biochemist Prof. Michael Groll, based two floors above in the same building, have played an important role in revealing various secrets of the ClpP. Sieber views this extremely fruitful partnership with his colleague in crystallography as a major advantage over other research groups in the same field. Team members can just drop by at a moment's notice: "This combination of biology, chemistry and structural chemistry all in one building is hugely beneficial to our work. The physical proximity saves us a lot of time and makes it easy to try out new ideas spontaneously," declares Sieber. ▷

ClpP consists of 14 subunits – so a tetradecamer – and looks like a double donut



Right: Disarming bacteria: S. aureus with disabled ClpP cannot act on the blood sample (red), while untreated S. aureus does kill blood cells (dark area).

Left: Splitting the ClpP into a double "donut" disarms their virulent function: certain ß-lactones, which have an aromatic ring in their side chain, can do that.

Graphics: ediundsepp (source: TUM); picture credit: Eckert

"I hope that I can continue to work with such excellent and inventive self-starters – the quality of our research is largely in their hands." *Stephan Sieber about his team*

Promising inhibitors in the pipeline

Despite the general euphoria, it has to be said that ß-lactones come with a catch: they are unstable and need to be injected. So oral administration would not be an option. But: "We can do better than that," resolved Sieber, thinking that there must be more stable compounds that would also be suitable. Sure enough: "Working with the Max Planck Institute of Molecular Physiology in Dortmund, we used high-throughput screening of 138,000 chemical compounds to identify a few that can block or completely deactivate the ClpP even without the ß-lactone four-ring structure." The outcome was seven or eight compounds that bind to the ClpP's active sites. Each compound contains a phenyl ester and splits the ClpP into two "donuts." These phenyl ester compounds have actually proved more stable and effective than ß-lactones in tests to date. "But we don't yet know how they behave in animals." ß-lactones, on the other hand, have already proved effective in mice and could progress to clinical trials in three to four years' time – provided a major investor is found. Meanwhile, another group of new compounds with properties similar to antibiotics has also been identified. For the moment, though, Sieber is keeping them under wraps, divulging only that they do hold great potential.

Second target: stopping armament

The second target Sieber and his team have isolated within S. aureus is associated with production of the virulence factors previously described. When the bacteria determine that it is worth launching an assault on the human organism, a cascade of reactions is triggered in the bacterial cell. These culminate with a molecule capable of binding to genetic information – known as a transcriptional activator – which is slightly but effectively modified so that it can form a strong bond to genetic material from S. aureus. In this way, it initiates transcription of the genes that carry the blueprints for the bacteria's virulence factors or "weapons." So the transcriptional activator and necessary bond to the genetic material present another target for anti-virulence efforts. But what compound could be used to sabotage this bond to the genetic information? Here, Sieber turned his attention to alpha-methylene-gamma-butyrolactones – a large class of natural compounds – to select an inhibitor, synthesizing numerous derivatives with his team. And not without success: When these substances are added to S. aureus, hemolysin – one of the most important virulence factors – is no longer released, for example. A drawback of this particular source of hope, however, is that it is toxic to human cells. But Sieber is not giving up just yet: "We're trying to alter the compound accordingly. Though it's unfortunate that the toxicity stems from the exact part we need for the bond to the transcriptional activator."

Alongside his research efforts, Stephan Sieber also gives lectures – by preference on natural substances. His trademark black suit makes him TUM's best-dressed chemistry professor, as far as students are concerned. And every December, it accompanies him on his trips to the German Institute of Science and Technology (GIST) at TUM Asia, founded in 2002 in Singapore, where he holds intensive lecture series for chemistry students. At around two meters tall, his size alone sets him apart there – and he frequently gets caught on Christmas decorations on the escalators. Fortunately, in life, as in research, obstacles leave Sieber undeterred.

Gerlinde Felix

Picture credits: Eckert