



Research Report 2005 2006

Leibniz-Institut für Molekulare Pharmakologie
im Forschungsverbund Berlin e.V.

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Research Report 2005 2006

Scientific Board of the FMP

Prof. Rudolf Balling

Helmholtz-Zentrum für Infektionsforschung GmbH
Inhoffenstr. 7
38124 Braunschweig

Prof. Annette G. Beck-Sickinger

Universität Leipzig
Institut für Biochemie
Brüderstr. 34
04103 Leipzig

Prof. Matthias Bräutigam

Bayer Schering Pharma
Müllerstr. 170-178
13342 Berlin

Prof. Christian Griesinger

(Chairman since 22.01.2007)

Max-Planck-Institut für Biophysikalische Chemie
Am Fassberg 11
37070 Göttingen

Prof. Reinhard Jahn

(Chairman until 22.01.2007)

Max-Planck-Institut für Biophysikalische Chemie
Am Fassberg 11
37070 Göttingen

Prof. Hans-Georg Joost

Deutsches Institut für Ernährungsforschung
Arthur-Scheunert-Allee 114
14558 Nuthetal

Prof. Frauke Melchior

Universitätsklinikum der Georg-August-Universität
Zentrum für Biochemie und Molekulare Zellbiologie
Humboldt-Allee 23
37075 Göttingen

Prof. Herbert Waldmann

Max-Planck-Institut für Molekulare Physiologie
Otto-Hahn-Str. 11
44227 Dortmund

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The FMP's niche in the modern biomedical revolution

Throughout history, the greatest threats to human health have come from parasites – usually bacteria or viruses that have bred in domestic animals until a chance mutation allowed them to gain a foothold in the human body. These diseases remain the most serious health problem in many parts of the world, and new ones like AIDS or Ebola will continue to emerge, sometimes causing an immense amount of suffering before cures are found. But in developed countries, most infectious diseases have steadily lost ground thanks to vaccines, antibiotics, and other drugs. Those cures are products of the last great revolution in medicine, ushered in by Pasteur, Koch, and other scientists of the 19th century, which made it possible to combat these diseases because it discovered their causes – microorganisms. Today another revolution is happening in which scientists hope to learn to combat diseases which have a different origin, and the FMP is striving to play an important role in bringing it about.



Cardiac and genetic diseases, cancer, and degenerative conditions like Alzheimer's arise from inherent flaws within our own cells. Cures – if they can be found – will have to come from a deep understanding of how molecules function and change their behavior over a person's lifetime. Microscopes and dyes gave researchers of the 19th century a look at infectious agents, and the tools of genetics, chemistry, and physics are giving scientists a similar understanding of modern threats. It might be possible to repair molecules or design new ones to take over their functions. The same method can be used to fight infectious diseases which have not yielded to vaccines or have become resistant to antibiotics.

This approach is only likely to be successful through a combination of expertise from chemistry, physics, molec-

ular biology, computer science, and many other fields. It will require a detailed look at how molecules are put together and how they work with each other, and how those interactions build living systems. This is the FMP's interdisciplinary niche. The institute pursues questions that will be essential to create new drugs, yet are usually too basic to be taken up by the pharmaceutical industry. Its aim is to understand disease processes, identify potential targets for drugs, and take the next step – to screen the targets against an extensive in-house library of compounds to find substances that might be useful in therapies. Thanks to the FMP's extensive expertise in chemistry, the compounds can then be refined in hopes of having very specific effects on cellular processes.

The purpose of this report is to provide a snapshot of ongoing work at the FMP in the larger context of where biomedical research is heading. It contains a brief overview of the work of most of the groups and a few in-depth stories, chosen by the scientists, which are representative both of the type of projects carried out at the FMP and the interdisciplinary way in which they are carried out.

Most of the institute's work is focused on complex details that are difficult to understand without specialist knowledge. But the revolution that is happening in the life sciences will have a huge impact on all of our lives in the near future. It is the responsibility of institutes to help the public understand and adapt to those changes. We hope this report makes a small but meaningful contribution to that process. ■



Interview with Director Walter Rosenthal



What makes the FMP unique?

The FMP is unique partly because of its scientific vision and the people and tools we have assembled to accomplish that vision; it is also special because of the way in which we operate as a member of the Leibniz Association, which gives us a great deal of scientific and organizational freedom.

I came to the FMP in 1996. Like many other scientific institutes that had formerly operated under the GDR, the FMP was still in a process of evolution. Before the "Wende" it had been called the "Institut für Wirkstoffforschung." At that time there had been a good mixture of life scientists and chemists among the staff, because it had worked in conjunction with pharmaceutical companies. When a scientific council was appointed to guide the formation of the FMP, they originally wanted to make it into an institute with a focus on cell signaling. But the major development that we started in 1996 was to significantly expand structural biology within the FMP. My predecessor had already begun to push this area. Our vision was to establish a strong program in structural methods centered around NMR. That has worked out very well: scientists of the FMP have made very important contributions, not only in NMR applications, but also in the development of methods like solid-state NMR.

In a subsequent phase, we wanted to strengthen the chemistry activities again. Michael Bienert was here, doing peptide chemistry. To that we have added a Medicinal Chemistry group focusing on combinatorial chemistry and the Screening Unit. Soon we'll have a new group here that makes artificial proteins. What we have achieved is to establish a good mixture of cell biologists, signal transduction people, chemists and structural biologists. This mix represents a potential we should use to work on rather difficult projects. For example, several of our groups are working on membrane proteins such as receptors and channels; these are among the most important areas where structure elucidation and drug design are concerned, yet they are also among the most difficult. Finally, we now have groups working on protein-protein interactions, hoping to develop approaches to interfere with them pharmacologically. A research group funded by the German Research Council (DFG) has now been established with that focus.

We work without restricting ourselves to particular organs or diseases. The ideal projects require experience from groups from all the subdisciplines represented here at the FMP. Teams from these areas already work well together under our roof, but many scientists are still unaccustomed to working this way. Our aim is that everybody in the house – from PhD students to established scientists – should take advantage of the resources of the entire institute. When we hire we look for people who will be able to do that. And interdisciplinarity is also a hallmark of our new Leibniz graduate school, which has just been established thanks to an application submitted by Bernd Reif. It has been set up so that the students get real exposure to a spectrum of platforms and scientific themes. It's a good mirror of our overall concept.

You have said that institutes like the FMP are evolving a new role in the process of drug design...

Today we have this vision of a "rational" drug design which will operate not on a basis of blind trial-and-error, but on our knowledge of how molecules are structured and how they behave. The plan is to find better and easier ways to identify good targets for drugs, to quickly get a look at the details of their surfaces, and to engineer artificial molecules that will change their behavior. Achieving this will require a great deal of work collecting basic knowledge about the structures and functions of



Walter Rosenthal

molecules and processes in the cell. Large pharmaceutical companies will not invest in such high-risk basic issues, although it's clear that this type of research is essential to drug design. It is better carried out in an academic setting. So we're moving towards new types of partnerships between these communities.

Science has been changing rather dramatically over the past decade. Most research groups have access to a wide range of technologies that have enabled them to bring their work much farther along the road to applications. But there are still some important gaps. For example, as far as I know, there are no open platforms in Germany where academic researchers can carry out screens for small molecule inhibitors of the molecules they are interested in. They might be looking for something that can be developed into a drug, or they may simply need some sort of a probe that can be used for basic investigations of the cell. They will only get access to a screening platform by paying for it – which is usually too expensive – or by getting a company interested in the project. In most cases that's unlikely to happen.

We're helping to fill that gap by setting up a major new screening unit that will cater particularly to academic users. This will give a boost to their basic work; in the case of those interested in drug design, they will be in a better position to hook a pharmaceutical company. They may leave the facility with several hits – compounds that bind to potential drug targets. There are several more steps that have to be taken after that, including optimizing the compounds and testing them in cell culture systems or laboratory animals. The chemistry groups we have put into place can help there. Again, the farther along a group brings a project, the more likely they are to catch the interest of a company.

We're working in a context where, over the last 20 years, Germany has steadily lost its position as the world's major producer of pharmaceuticals. We hope that the platforms and expertise we have assembled will make the region attractive to those who want to invest in Germany and Berlin.

The Timoféeff-Ressovsky building in the evening



How does the FMP fit into the research landscape in Buch and Berlin? In Germany? In Europe?

Going back to the 1970s, there was the Institut für Wirkstoffforschung, the IWF, a part of the GDR Academy of the Sciences, which was housed in Berlin Friedrichsfelde. After the "Wende", scientific reviewers came in to evaluate its activities and to think about its future. They were impressed by some of its hallmarks, especially the interdisciplinarity. There was already a tradition of working with animal models, doing chemistry, research into signal transduction, and clinical pharmacology work. There was even a small company alongside the institute, a unique "academic industrial complex." That type of combination was a new approach for the West. The recommendation of the review was to transform the institute into the FMP, with a focus of molecular pharmacology. It was opened in January, 1992.

It was clear that Friedrichsfelde wouldn't do over the long term; the old buildings weren't adequate for our needs. It was very difficult to set up cell biology labs there, for example. The only place big enough to house our NMR machines was a big open building with a huge iron beam running across the ceiling. When asked why it was there, they said it had been used to hoist dead elephants or something, as once the buildings were used by a veterinary institute that worked for the animal park nearby.

The move to Buch was a very good decision. We have very strong partners on the campus that we can support and get support from, and are working closely with them on several very interesting projects. Our most important partner in Buch is the Max-Delbrück-Center for Molecular Medicine (MDC). The research concepts of the MDC and the FMP complement each other: while the molecular medical research at the MDC is particularly dedicated to diseases or clinical symptoms and their molecular explanations, the FMP investigates the functional and structural characterization of proteins as well as the development of strategies for influencing them pharmacologically.

The close connection to the MDC extends to the organizational level. Thus, large equipment is shared and jointly operated. Guest scientist contracts make it possible for scientists of one institute to use equipment belonging to the other. Both establishments send representatives to important committees of the other. The planning of cost-ly and long-term research projects as well as the

appointment of leading scientists takes place in joint agreement. The MDC and the FMP arrange and finance joint events for those studying for their doctorates.

In terms of Germany, the FMP is a member of the Leibniz Association, a national research organization similar to the Max Planck Society, the Helmholtz, and the Fraunhofer Associations. Leibniz institutes retain a great deal of independence but also profit from the association. They perform problem-oriented research of national interest and strive for scientific solutions for major social challenges.

Another hallmark of Leibniz is the fact that we promote very strong links to the university system. At the moment the FMP has six full staff with joint appointments (three full, two associate and one honorary professorships).

One particular feature of Leibniz is that its institutes are not guaranteed a permanent existence. Every seven years, the FMP – like every other Leibniz institute – undergoes a thorough scientific evaluation. The question is whether the institute is needed – whether it is fulfilling its mission, and whether the same scientific work could be accomplished without it. This challenges us to perform at a very high level and to be responsive to the shifting landscape of science. In some cases, of course, institutes are closed – or they leave the organization and find another mode of funding. But new institutes are joining. Very soon we will add a research laboratory in Berlin whose focus is rheumatology, and another new member will be the Berlin Natural History Museum.

This system also allows for expansion of institutions that are doing an excellent job. Despite a period of financial problems in Berlin, we haven't suffered – just the opposite. We have received budget increases and hope for more, particularly in investments into NMR and other major equipment. Recommendations for growth and other types of changes arise from the evaluations. We also have had quite strong support from the scientific council in achieving our plans.

As far as our relationships to Europe are concerned, the FMP has participated in several European research networks over the last years. Since October 2006 Enno Klußmann has been the first FMP scientist to coordinate a project funded by the European community (STREP "Identification of the therapeutic molecules to target compartmentalized cAMP signaling networks in human disease (thera-cAMP)," under the 6th framework programme).



Long Night of the Sciences (Chemistry Fair at the FMP).



Visit of the Berlin parliamentary fraction of the Free Democratic Party (FDP).

Facing the European deficit in Chemical Biology, the FMP coordinates activities in the field of small molecule screening. One of these activities, the infrastructure initiative "EU-OPENSREEN", will provide access to screening platforms for European academic groups. Thus, local expertises of research institutions like the Screening Unit of the FMP can be used throughout the entire European research area.

What's life like for scientists here?

We have junior and senior groups. As of November 2006, we standardized the system so that junior group leaders are not initially hired into tenure-track positions. They are very well funded for an initial period of five years; that can be extended for another four years. All of our junior group leaders have gone on to find positions, becoming chairs of departments or receiving C3 professorships. About two-thirds of our alumni move into academia; the remaining third go to work for drug and biotech companies.

The fact that we use tools similar to the pharmaceutical industry means that people who receive training here should be interesting candidates for biotech companies. To promote this link, the campus has set up a summer school – "From target to market" – to help prepare postdocs and predocs for careers in industry. The next course will be offered in September.

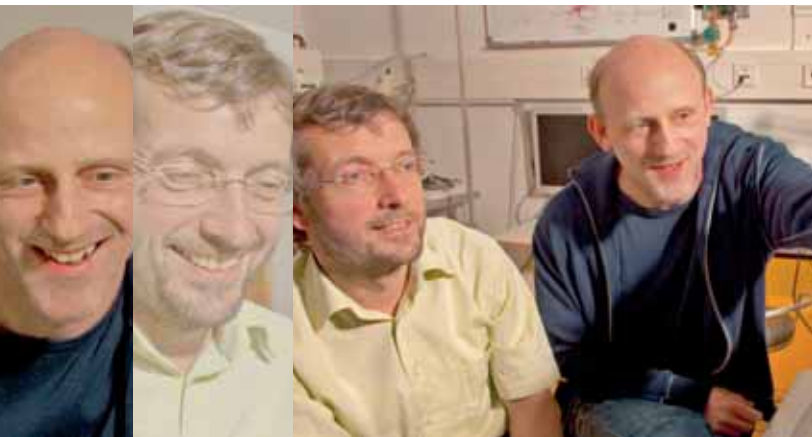
What do you consider to be the most significant bottlenecks for the institute and the field in general?

Right now I would like to add a team of synthetic chemists who can work on hits which we have identified in our Screening Unit. There is also a general bottleneck in academia when it comes to support for cell biologists and biochemists to synthesize new tools. We have one excellent group here, but that's not enough. As an important step toward a solution, we're setting up a national network of chemists and biologists (ChemBioNet). Our ambition is to further expand those activities and to become a national platform to promote this symbiosis of chemistry and biology.

In classical academia, you characterize a target and usually stop there. However, while maintaining the focus on basic research, we strive to extend our pipeline. We take projects to the point that we have small molecule inhibitors for a protein, including small molecules to disrupt protein-protein interactions. This is a significant contribution to narrow the gap between pharmaceutical companies and academia. ■

Trouble in the Waterworks

How cells in the kidney manage the body's supply of water



If it weren't for proteins called *aquaporins*, you wouldn't have to be stranded in the desert to die of dehydration. While a human body is 70 percent water, only a fraction of that comes from our daily intake of liquids and food. The rest – 99 percent – is won through recycling. Recovering that water is the job of cells in the body's waterworks – the kidney – and they only manage because they are leakier than other cells.

In the 1920s researchers figured out that the membranes of cells were made of fat molecules called *lipids*, put together in such a way that small quantities of water could simply slip through. But that amount was clearly not enough to supply the body's big drinkers, like kidney and red blood cells, so there had to be another way for water to enter. Fifty years later, this "water channel" still hadn't been found. Then along came Peter Agre, a young hematologist who was working at the John Hopkins School of Medicine in Maryland.

"The field was essentially stuck," Agre says, "but following the well known scientific approach known as 'sheer blind luck,' we stumbled upon the protein that is the answer to the question: do water channels exist?" Agre and his colleagues had found the first aquaporin, a discovery so important that he was awarded the Nobel Prize in 2003.

In the meantime, scientists at the FMP and elsewhere have learned a lot about how aquaporins work. These proteins float in the *plasma membrane* – the barrier between the cell and the world – where they create passageways. Proteins are folded in specific ways as they are made, and the folds of aquaporins leave a gap through the middle that permits the passage of water molecules. When the body needs water, aquaporins are added to the plasma membrane of cells; when enough has been absorbed, they are removed again. Cells know how much water to let in because of hormone signals that get released when the supply gets low. The hormone doesn't directly communicate with aquaporins; first it has to trigger another signal, called *cAMP*, which does that job.

Enno Klußmann began looking at aquaporins as a post-doctoral fellow in the research group of FMP Director Walter Rosenthal. He joined the lab in 1997 – "on loan" from the Günther Schultz's group at the Freie Universität Berlin, where he had attempted to identify a membrane-associated steroid hormone receptor. University regulations permitted postdoctoral fellows to remain no longer



Klußmann lab member Michael Gomoll

than five years at a time, and Schultz recommended that he join the group of another former student, Walter Rosenthal, at the FMP. "The idea was that I would leave for a few months and then go back," Enno says. "But Walter's lab was working on some fascinating things, and ten years later, I'm still here."

One of the group's projects was the study of aquaporins. "The way that aquaporins behave in the kidney is important and interesting," Enno says, "but it opens the door on some even more interesting questions. cAMP is a strong signal, found almost everywhere in the cell. It triggers a wide range of other processes often at the same time, in the same cell. That situation raised the question of how the cell controls the signal – how it manages to respond in specific ways, in specific places. Without controls, everything would be happening everywhere, all the time."

Many processes triggered by cAMP are linked to diseases – including hypertension, gastric ulcers, and heart disease. It turns out that some common drugs work by preventing proteins from "hearing" the signals. That's desirable if the drug blocks only one kind of activity. But there may also be unintended side effects. In the 1990s doctors discovered that lithium, commonly used to treat people with a psychiatric problem called bipolar disorder, was interfering with aquaporin. Patients on the drug developed a form of diabetes in which their bodies lost their ability to recycle most of the water. Even though they drank all the time, most liquids just passed right through without being absorbed. This was bad news because lithium was helping many bipolar patients, and it was also being considered as a supplement in the treatment of other diseases. A better understanding of aquaporins might be to help researchers find a way to prevent the side effects.

A drug that blocked all of cAMP's activity would be fatal, but maybe there were ways to stop a single process. Learning to do that might show how to block other



Walter Rosenthal, head of the FMP, and Enno Klußmann looking at cells with labeled AKAPs

processes. Enno and Walter hoped that an aquaporin called *AQP2* would make a good place to start.



Things went so well that Enno could set up his own lab in 2001. He brought along the aquaporin project, and has continued to work on it in collaboration with Walter and a network of researchers across Europe. He heads a project funded by the European Community to identify or create small peptides that can control cAMP signaling in processes related to human diseases; he and Walter have also been awarded a number of grants from the German Research Council (Deutsche Forschungsgemeinschaft) to study particular aspects of cAMP signaling and aquaporins.

If a protein needs to be active in a certain place, a good way to station it there is to tether it to a particular membrane. That's what happens with the system that activates aquaporins.

Enno says that understanding how cells control water channels – and thus the body's water supply – boils down to several questions. Those have to be tackled one by one, and over the past few years the FMP has made progress on them all. The first question is how the molecules are moved to the locations where they are needed.

"The molecules triggered by cAMP have to get to the right place to respond properly to a signal," Enno says. "The cell has a lot of internal membranes in addition to the plasma membrane, and they often serve as duty stations. If a protein needs to be active in a certain place, a good way to station it there is to tether it to a particular membrane. That's what happens with the system that activates aquaporins. *AQP2* is first stored in internal membranes before it is moved to the cell surface. Inside the cell it is installed alongside the machinery that controls it. The switch consists of the actual receiver of the cAMP signal, called a *PKA* protein, plus the anchor that attaches the *PKA* to the membrane, and other molecules. There are lots of types of anchors – so far, about 50 have been found. The anchors for *PKA* are known as *AKAPs* (A kinase anchoring proteins). The cell has so

many because each one is used to anchor a particular type of molecule to its proper place."

In 2001 it wasn't certain that aquaporin functions depended on the interaction of *PKA* with an anchor, so Enno and Walter decided to find out. For their experiments they used cells taken from rat kidneys, raised in laboratory cultures, which produced aquaporins. Microscope studies had shown that water channel proteins were held in the interior of the cells, in small membrane-wrapped compartments called vesicles, until they were needed. When the cAMP signal was received, the aquaporins moved to the plasma membrane. The scientists began shutting down proteins in the cells, hoping to block this movement. A success would mean that they had found a protein that had something to do with positioning the aquaporin. Since it was likely that an anchor was involved, one thing they tried was to use a small molecule, a synthetic peptide, known to prevent *AKAPs* from binding to *PKA*.

Cutting an anchor sets a boat adrift, and if an *AKAP* is disturbed, signaling molecules drift away from their duty stations – or never become attached there in the first place. This renders the anchor an interesting target for drugs. "You wouldn't want to block cAMP itself to stop a process – that would disrupt a lot of other important things going on in the cell that rely on the signal," Enno says. "The anchor is a much better point of attack."

Enno and Walter introduced the synthetic peptide that disrupts anchors into the kidney cells; they discovered that aquaporins were no longer arriving at the membrane. An anchor was obviously involved, but which one? In 2006 Postdoc Volker Henn, PhD student Bayram Edemir found a candidate: a protein called *AKAP18δ*. Microscope studies and other methods showed that *AKAP18δ* was installed alongside *AQP2* in the cell interior and moved along with water channels when they moved to the plasma membrane – "guilt by association."

Another version of the *AKAP18* protein influences the contraction of the heart; there, cAMP signals control a different type of channel which allows the passage of calcium ions into the cell. These channels are stimulated through another hormone. "The situation is similar to that of aquaporins," Enno says. "The hormone signal triggers a wave of cAMP signaling that opens channels in the plasma membrane. But only if the channel proteins are connected to anchors."

Cutting an anchor sets a boat adrift, and if an AKAP is disturbed, signaling molecules drift away from their duty stations - or never become attached there in the first place. This renders the anchor an interesting target for drugs.



Beta-blockers, which are commonly used in the treatment of cardiovascular disease, work because they tune down the heart's response to the hormone. The same thing might be achieved by blocking the connection between anchors and their partners, such as calcium channel proteins. "This makes the anchor a very good target for drugs," says Christian Hundsrucker, another PhD student in Enno's group. "The peptides we had been using to block AKAP-PKA interactions don't do so very effectively. So we began trying to make new ones."

The scientists began by taking a closer look at the docking site. Normally two PKAs form a pair by binding in a region called the DD domain. Two Rs joined in this way create a surface (RII) that plugs into the anchor. Structural pictures of anchors had already been obtained using NMR and X-rays, providing a model of one of the surfaces. This could be used in "docking" simulations - twisting and turning the RIIs until the molecules snapped

together on the computer screen.

Christian says that a coiled structure of the anchor fits into a pocket formed by RIIs. Whether the coil fits at all and how strongly it binds depend on the shape and chemistry of the two structures, which are determined by the amino acid subunits that make up the proteins. Christian hoped to make a molecule that would fit better and bind even more strongly to RII, which would then lock onto the domains of PKAs and prevent them from acquiring an anchor. Christian began with a fragment of AKAP18 δ and started to change its recipe.

Understanding how the surfaces of two proteins bind is an enormous chemical puzzle that would probably be impossible to solve without the help of sophisticated computer techniques. Christian and his colleagues enlisted the help of Gerd Krause's lab, who were using software to study protein binding. By scanning features of several different RII domains and their binding sites on

PDE molecules are found throughout the cell, and they help tune down the signal. That they can bind to an AKAP means that the anchor collects an entire toolbox of molecules that give the cell a fine level of control over the water channels.



AKAP proteins, the scientists could detect specific amino acids that seemed to be crucial to linking the molecules. Michael Beyermann's group, experts in building small proteins, helped design new versions of the binding domain by replacing those subunits with others that might make a better fit.

The resulting molecules were screened in heart muscle cells, and revealed that they broke the connections between anchors and their targets. "What this showed us is that we have identified the key points that permit binding between the anchor and its PKA partner," Enno says. "This gives us a much clearer strategy as we develop new synthetic molecules."

The dozens of known AKAPs may have evolved from a single ancestral molecule, inheriting a common "docking station" that allows them to bind to their main partner, PKA. The situation is a bit like the way a boarding ramp at an airport fits the hatches of different airplanes. You could design something that would prevent a plane made by one manufacturer from docking onto the ramp, but it would probably also interfere with other types of planes. In the same way, a drug that stopped one anchor from binding to its PKA would probably stop others. So the binding site between anchors and PKA proteins probably wouldn't be the best choice to interfere with. "The approach is valuable as a proof of principle to verify that pharmacological interference with AKAP function

is a feasible concept," Enno says, "because we know a lot about the function of AKAP-PKA interactions. Less is known about functions of AKAP interactions with other proteins."

Instead of disrupting the contact between the anchor and PKA, the scientists figured it would be better to aim a drug at another part of AKAP188 – its targeting region. "This is the 'address label' that sends the anchor to the same location as aquaporins," Enno says. "Interfere with that and the anchor gets lost, so it can't help aquaporins move."

One goal is to produce potential drugs; another is to give scientists tools to dissect other aspects of the molecules' activity. Aquaporins don't work forever, even when the cell is flooded with cAMP. A study begun by PhD student Eduard Stefan revealed one of the reasons. As the anchor links to an aquaporin, it also brings other molecules on board. One of them, called a PDE, is a sort of volume control for cAMP. It works by cutting a chemical link inside the cAMP molecule, leaving pieces that can no longer pass the signal.

PDE molecules are found throughout the cell, and they help tune down the signal. This fact, plus the cell's ability to install different sensors for cAMP in different places, is what keeps the cell from becoming overstimulated by the signal all the time. That they can bind to an AKAP means that the anchor collects an entire toolbox of molecules that give the cell a fine level of control over the water channels.

■ ■ ■

One of the lab's ongoing projects is to clarify yet another question about how aquaporins take up their proper positions in the cell. cAMP delivers the signal to move the water channel protein to the cell membrane, but what actually carries the aquaporin there? This is really a two-part question: transport through the cell requires a track to move things along and a motor to do the pulling. Enno says that scientists had been focusing on *microtubules*, a network of fibers that act as a scaffold to give the cell its shape and also function as tracks along which molecules are delivered throughout the cell. Motor proteins act as the locomotives, traveling down the microtubules with other proteins in tow.

"There was experimental evidence that artificially breaking down microtubules stopped the transport of aquaporins," Enno says. "And motors that use microtubules had been found in the same vesicles that contain aqua-



porins. This looked like another case of preassembly, in which aquaporins and the molecules that were needed to transport them were being wrapped up in the same package.”

However, another lab had recently done a study of all the proteins found in aquaporin vesicles, and they failed to turn up the motor proteins. Enno began to wonder whether microtubules were really the delivery route – after all, the cell had other transport systems. In 2005 a Japanese group did an experiment in which a drug was used to break down the microtubules in cells taken from kidneys. They discovered that even without a microtubule railway system, aquaporins could be delivered to the membrane. Pavel Nedvetsky, a postdoctoral fellow in Enno’s group, hoped to find an explanation.

“Some papers from 20 or 30 years ago had already hinted that another type of filament called *actin fibers* might be involved,” Pavel says. “So we began looking for another transport system, one that could move along these filaments.”

Working with other members of the group and international partners, Pavel made an important discovery: a motor protein called *myosin Vb*, known to shuttle other types of vesicles back and forth along actin fibers, was consistently found in the neighborhood of aquaporins. Motors often require adaptor proteins and other helpers to find and attach a cargo, and one of myosin Vb’s usual assistants is a protein called *Rab11*. This gave the scientists two ways to investigate whether myosin Vb was acting as a transporter for aquaporins – by interfering with the motor and with the adaptor.

Pavel first used a non-functioning version of the motor by removing the regions that attach it to filaments. He introduced it into the kidney cells and watched what happened when they were stimulated with the “thirst” signal. They no longer began taking in extra water. A look under the microscope showed why: aquaporins were trapped in internal vesicles, and were no longer being transported to the cell membrane. In another set of experiments, he interfered with Rab11’s ability to bind to the motor. The same thing happened; aquaporins became stranded.

“These experiments gave us an idea of some of the culprits involved in moving aquaporins around,” Enno says, “but they still didn’t tell us exactly what was happening. To understand what we found next, you have to realize that most of us believe that aquaporins get recycled.

That means that an aquaporin isn’t finished once it has moved to the plasma membrane. It helps absorb water as long as the signal to do so continues, but when the signal stops it has to be removed from the membrane. We think that it gets transported to an internal compartment, where it gets put on hold, and then it can be called up for duty again.”

Returning to the microscope, the scientists found that some aquaporins managed to make it to the cell membrane without myosin Vb, but once there, they couldn’t be shuttled back inside or out again. The cell handles new aquaporins a bit differently than those it recycles – other motors and helpers probably lend a hand. But myosin Vb and Rab11 appear to be essential in handling the recycling of the motor channels. Myosin Vb is thought to have a similar role in other recycling events.

“The aquaporin story is interesting when you think about diseases in which the body retains too much water, like hypertension or congestive heart failure,” Enno says. “In those cases the body still needs to be able to absorb water – just not so much. Maybe you could temporarily shut down the recycling center alone, without also turning off all the intake valves. To do that you’d have to get a good look at the recycling machinery, and find something you could perturb without disturbing the rest. That’s where these experiments are taking us.” ■



Bribing the ferryman

Smuggling drugs into cells and other adventures
with membranes



In Greek mythology, the dark river Styx separates the world of the living and the dead. It is traversed by a ferryman named Charon, a gloomy, glowering giant who floats up and down the river, guarding the entrance to the underworld. To appease him the Greeks buried their dead with a coin under the tongue – a bribe, or a fare – to secure a passage to the afterlife.

Smugglers throughout history have used bribes to move their wares cross borders, so it shouldn't be surprising that scientists are trying something like a bribe to slip drugs and other substances into cells. In this case the barrier that has to be crossed is also a sort of river: the cell membrane, two liquid layers of fats and proteins. One of its key functions is to keep out toxins, parasites, and a wide range of other foreign substances.

"It wouldn't do much good to design the perfect drug," says Sandro Keller of the FMP, "if you couldn't get it to a cell that was diseased, and then get it inside the cell to do its job. Managing that means designing a delivery vehicle that can get substances through the cell membrane."

Once inside the cell, drugs have to survive other cellular defenses that target and break down foreign substances. And ideally the substance should last a long time; otherwise, the drug would have to be administered continually, in such high doses that it would probably be toxic. The task is tricky enough when the cargo to be delivered is a small protein or chemical compound, like most of today's drugs. Now scientists are now hoping to insert other things into cells: DNA, RNA, large proteins, or complex molecules that can serve as probes of the inner workings of the cell. Getting these past the membrane will require understanding how it is built and how it functions, and that's the task Sandro and his laboratory have devoted themselves to.



It's not often that a young scientist goes directly from receiving his PhD to his first independent research position, without moving abroad and passing through a stint or two as a postdoctoral fellow, but that's the case with Sandro. It has been just a little over one year now since the transition, and he seems entirely at home in his office at the FMP; he has brought in some plants, and the bookshelves are full. But those who don't know him – including the organizers of some of the conferences he gets invited to speak at – still mistake him for a student. He's young, and looks young for his age, which might

make it difficult to pick out the group leader in the picture on the left. Sandro's fast track to leading his own group is the result of a success story: while working on his PhD, Sandro began taking an interesting approach to the study of the cell membrane and how to get cargos inside.

The membrane has two liquid layers, like a soap bubble. Both layers are built of fat molecules called *lipids*, interspersed with proteins. Many membrane proteins have a head region that hangs outside the cell, a tail that dangles in the interior, and a complex folded region packed between the two layers of the membrane. What happens in this zone has been difficult – often impossible – to observe, but that will probably be necessary to solve the problem of drug delivery.



Jana Bröcker preparing a sample for isothermal titration calorimetry.

Sandro would like to get a look at the regions of proteins embedded in the membrane, to understand how they are linked to each other and to lipids, and to watch what happens as molecules cross the barrier. The problem is that the inner space of the membrane is a bit like the zone of radio silence that astronauts pass through as

“We started with a simple bacterial protein called *Mistic*, which has four domains that pass through the membrane. We have created four separate, artificial molecules – one corresponding to each domain – that can be used as a model to study the behavior of the protein.”

they reenter the earth's atmosphere. It is hard to get any direct information from the zone. “The classical methods used to study the structures of proteins don't work with proteins that span the membrane. We have to be very creative and use less direct, biophysical techniques.”

One method that the group is using involved creating an artificial molecule that can function as a probe. The goal is to understand how the chemical subunits of proteins interact and cause the protein to fold in the presence of lipids. The folds determine the molecule's shape and functions. “We started with a simple bacterial protein called *Mistic*, which has four *domains* that pass through the membrane,” Sandro says. “We have created four separate, artificial molecules – one corresponding to each domain – that can be used as a model to study the behavior of the protein. These regions are called alpha-helical domains; they are found in many different types of proteins, and they play a crucial role in the structure and organization of the membrane. But we know very little about how internal forces in the molecules cause them to fold and interact in certain ways. We can manipulate these artificial molecules to test hypotheses about these processes and to expand what we have learned from *Mistic* to many other molecules.”

The project is the subject of a grant proposal that Sandro submitted to the German Research Council (DFG) and which was approved in spring, 2007. The grant will give Sandro two extra positions to pursue the project in his lab. Getting a glimpse of protein structures in the membrane would help solve questions about how other molecules enter the cell. Most methods used to observe this process have drawbacks, Sandro says. Some leave “artifacts” that make it seem as if a protein has crossed into the cell when it hasn't. Other techniques are restricted to one aspect of the problem; for example, they either show how a protein binds to the cell surface, or give hints about how it passes through – but not both. Different kinds of experiments give contradictory results.

“All of this confusing data has led to debates about how molecules enter,” Sandro says. “There has been quite a bit of discussion about the peptide *penetratin*, which manages to get through the barrier. Penetratin is a small module of another protein that has to move into the cell during the development of the fruit fly. Penetratin is like a key, and if you attach it to another molecule, it sometimes will escort that into the cell as well. What was unclear was whether penetratin somehow entered the cell on its own, or whether it came wrapped in a vesicle

– sort of a small membrane bubble. Cells often swallow up vesicles in a process called *endocytosis*. The process by which they absorb single molecules is different, and it's important to know which one is happening.”

In 2006 Sandro and collaborators at the FMP (Michael Bienert and Margitta Dathe) and at the Martin Luther University Halle-Wittenberg helped to demonstrate that the latter was the case, using a method called *isothermal titration calorimetry*. The technique monitors and compares the temperature of two cells: one a control, and one which is subjected to experiments. In this case the researchers injected penetratin into the experimental cell. They monitored its interactions with lipid molecules by measuring the heat released upon injecting lipid vesicles into the cell. The instrument is so precise that it can distinguish between different types of chemical reactions based on the heat released or consumed in the reaction cell. The study showed that penetratin was not entering on its own – suggesting that endocytosis plays a key role in the cellular uptake of the peptide.

“Calorimetry is very good for tracking the movement of substances into the cell,” Sandro says, “but it is not easy to use, the results are difficult to analyze – and most researchers simply don't have access to it.”

Searching for a simpler method, Sandro and his colleagues began using *fluorescence spectrometry* to watch the passage of proteins through membranes. “This approach monitors lipid vesicles with ultraviolet radiation and measures light instead of heat,” he says. “It can be used with any molecule whose fluorescence properties change when it comes in contact with the cell membrane.”

The method uses ultrasound (sound that is inaudible to the human ear) to force the compound of interest into the interior of lipid vesicles. The vesicles are then diluted to “loosen up” the interactions between the compound and the lipid molecules. If the compound can cross the lipid membrane and leave the vesicles, its fluorescence properties change since it is no longer in contact with the membrane. If, however, the compound is not able to get out of the vesicles, it will remain bound to the membrane, and its fluorescence properties won't change. The technique passed a proof of principle test by confirming the results of the earlier calorimetry studies of penetratin, and the laboratory is now using it to study other molecules.

■ ■ ■



A new delivery vehicle for drugs will need a key – a cell-penetrating peptide like penetratin – ideally, one giving access to many different types of cells. Whether a vesicle can dock onto a cell and deliver its cargo depends on the proteins and lipids on its surface. But having the right ingredients isn't always enough; they also need to be put together the right way.

“One of the simplest sorts of transport vehicles that people use to try to introduce substances into the cell is a small fat droplet called a *micelle*,” Sandro says. “This is a small greasy drop that self-assembles because of its chemistry. It's the same phenomenon that you see when you put oil into water – it forms a drop. That's because each oil molecule has a hydrophobic tail that wants to avoid contact with water and a hydrophilic side; the oil molecules cluster together and turn the hydrophobic tails inward, away from the water, toward the inside of the droplet.”

Even when accompanied by a cell-penetrating protein like penetratin, micelles don't always work well as transporters, he says. Micelles assemble as a single layer, and the cell membrane is a double layer of lipids. A membrane protein in a single-layer micelle probably won't fold or behave the way it would in its normal environment of a double-membrane layer.

Some proteins dock onto lipids and are able to assemble membranes around themselves. The best-case scenario would be to have a delivery protein that did this, as well. If it could collect a high number of lipids, they might form a double layer. After a search, Margitta Dathe and colleagues at the FMP decided to work with a small molecule called A2. Another group had discovered that attaching this peptide to a vesicle caused it to be absorbed by capillary cells in the brains of rats. Sandro wondered whether A2 could be prompted to assemble lipids around itself.

That's where the bribe came in; something could be added to A2 to make it more likely to build vesicles. “We knew from the literature of a way to do this that might work,” he says. “If you create an artificial mole-

cule by combining a small protein and fatty acids, it tends to accumulate a lot more lipid molecules – maybe enough to make a double-layered membrane. So we modified A2, giving it binding sites that lipids could dock onto, and another element that should serve as a binding site for *proteoglycans*. These proteins are found in membranes everywhere and when they bind to their partner molecules, they cause them to be taken up into cells.”

In the test tube, the new hybrid A2 molecules bound to each other in clusters. Isothermal titration calorimetry revealed that the groups contained a “record-setting” number of lipids, compared to other hybrid molecules. A positive sign, Sandro says, because it made it more likely that delivery vehicles with double-layered membranes would form. Next it was time to see how cells would respond to the peptide. The scientists “doped” vesicles with A2 and watched what happened using the laser scanning microscope and other techniques.

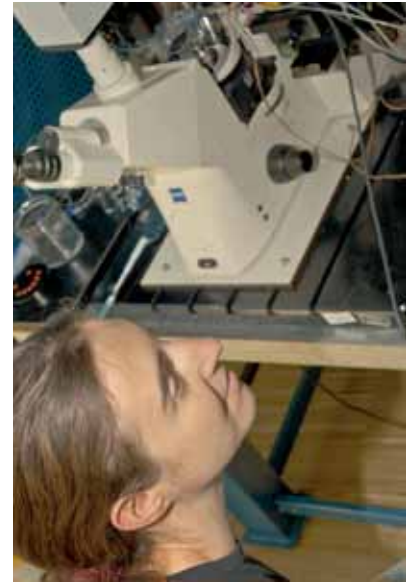
They discovered that vesicles with A2 readily docked onto cells and were carried through the membrane very efficiently; once inside, they dissolved slowly. This hints that in addition to being better at entering cells, vesicles with A2 might protect a drug long enough for it to be effective.

All in all, the new version of A2 seems to work well as a “bribe” to get cells to take up drugs and other substances. “The A2 vesicles and micelles seem to be a significant improvement over some other types of delivery vehicles,” Sandro says. “It suggests that you might be able to add A2 to other transporters and improve their characteristics. We're trying that now. We also hope to use the vesicles to solve other kinds of problems – for example, to insert complex probes that can monitor cellular processes.” ■

The electrician's toolbox

A systematic look at the movement of ions across membranes





In a classic science fiction story by Ray Bradbury, a time traveler accidentally steps on a prehistoric butterfly, then returns to his own era to find that human history has taken an entirely different course. Events over time are intricately connected, the story implies, so even the smallest changes can have a huge impact on the future.

Often when scientists remove a gene from an organism, something similar happens: there is a disruption of a great network of finely-tuned relationships that have evolved over billions of years. The genome of every species is living history, a library-like record of the successes and failures of evolution stretching back to the beginning of life. Like cities standing on millennia of archeological layers, each new species builds on the foundations of its parents. Just as the character of a city cannot be appreciated without digging into its historical layers, genes are rarely understood until scientists know where they came from and how related molecules function in various tissues in the body. This is especially true in trying to understand why a defect in a molecule causes disease.

For nearly 20 years Thomas Jentsch has been conducting a systematic study of an ancient and now widespread family of molecules called *chloride channels*. When they first evolved in one-celled organisms, their function was to sense changes in the environment and help the cell survive by adjusting its chemistry. With the arrival of multicellular life, they allowed cells to communicate with each other and coordinate the activities of the body. Dozens of types of chloride channels are known. Thomas' work has focused on an important subset of these molecules, called *CLCs*, and other ion channels and transporters.

"The human genome contains nine CLC chloride channels and transporters, all copies of a single ancestral gene," Thomas says. His laboratory found the first example over a decade ago and has systematically uncovered many more. "The extra copies arose because of errors that were made as DNA was replicated, and over time they have evolved different functions. Many of these molecules float in the plasma membrane on the fringes of the cell, where they act as doormen. Their job is to control the flow of negatively-charged chloride atoms (ions) through the membrane. Others are lodged in membranes within the cells, for instance in bubble-like compartments called *vesicles* that take up material from the exterior and break it down."

The movement of ions across the cell membrane is crucial to a wide range of cellular processes, for example moving salt and water into and out of the cell, or coordinating the activity of muscles and neurons. One job of "internal" CLCs is to recycle cellular waste.

But scientists still don't have a full view of the functions of channels and transporters in the body's tissues. The main method of finding out has been to delete genes one-by-one in model organisms like the mouse and then to closely watch the animals' development and behavior. "Another approach has been to guess that a CLC gene might be mutated in certain human disorders," Thomas says. "If we find CLC mutations in certain types of patients, then diseases can also provide powerful clues toward the normal function of the protein."

Over the last two decades Thomas has taken on the CLCs one-by-one to uncover their functions. Some of these projects have yielded insights into disease and have promising therapeutic implications. Thomas' experiments have shown that removing one channel leads to deafness; when another is deleted, kidney stones form; yet another is needed to maintain the normal density of bones, and others seem to be connected to conditions like epilepsy.

Understanding why a chloride channel or transporter has its effects requires first getting a look at its cellular functions, but that is no easy task. It requires a sophisticated array of techniques – from structural investigations of the building plans of the molecules, to their roles in mouse cells and human diseases. Thomas' new home at the Timoféeff-Ressovsky building on the Berlin-Buch campus is an ideal place to put together an interdisciplinary team. Some methods have been imported from his last post at the Center for Molecular Neurobiology at the University of Hamburg. Others are available at the FMP. He can also draw on resources from the Max Delbrück Center for Molecular Medicine, his second employer.



Like many of Buch's scientists, Thomas has interests that extend to art and culture. His office is adorned with cult objects he obtained in Papua Niugini: carvings of the heads of crocodiles and other creatures. They all have a triangular shape, which reflects their source: originally, each was an ornament carved into the prow of a boat.

“Our cells contain over 60 different genes that encode channels to transport potassium ions. Why should there be so many molecules that have about the same function?”

When a boat falls into disuse, Thomas says, the carvings are removed. They look over his shoulder with fierce expressions as he talks about his 20 years of research into membrane proteins.

“Our cells contain an astounding variety of channels for the transport of ions,” he says. “There are over 60 different genes that encode channels to transport potassium ions. Why should there be so many molecules that have about the same function? The diverse roles of some of them have been discovered through studies of knock-out animals, or through examination of tissues of patients suffering from diseases, but a great number remain puzzles. I believe that as they are investigated, we will uncover many more connections to disease.”

In the late 1980s, when Thomas began studying the molecules, this work was in its infancy. Scientists knew that chloride channels had to exist but had not yet identified any genes encoding them. A good place to look, Thomas thought, was one of the world’s oddest fish, the *Torpedo* – also known as the *electric ray* for its ability to stun prey with a powerful electric charge from its fins. The creature has intrigued scientists since the ancient Greeks. An early Roman physician, Scribonius Largus, even used the fish in treatments for gout and headaches, either by having patients stand on one to receive a shock, or immersing parts of their body in water containing several of the creatures. Later doctors who read his works began thinking of other ways that electricity could be used to treat neurological conditions or other health problems.

Thomas’ interest stemmed from the fact that *Torpedo* cells require an unusually high number of chloride channels to produce the massive electric discharge, which can reach levels of 100 volts, with currents up to one ampere. Even so, attempts to isolate CLCs from the cells had failed. “To find a protein you needed a molecule that would bind to it quite specifically, and we hadn’t found one for the chloride channels,” he says.

“So we took another approach; we decided to clone *Torpedo* RNAs that might contain the channel and put them into *Xenopus* egg cells – a frog which is widely used as a model organism. The genes of these eggs are inactive, but all the machinery is there to transform RNAs into proteins. The cell used the *Torpedo* RNAs to make proteins. By watching its electrical behavior, we could tell whether we had imported a molecule that functioned as a channel.”

This could be checked using a *voltage clamp* technique. In this method an electric current is “injected” into the egg and scientists measure the output – changes of voltage over the egg’s membrane. If the cell has produced additional chloride channels because of molecules from the electric fish, the charge will leak out through them, and more current needs to be injected to reach the same voltage. The measurements allowed Thomas and his colleagues in Hamburg to identify the first CLC and pin down specific regions of the protein that were responsible for opening the channel and moving ions. Over the next decade, Thomas and his laboratory identified several new CLCs and began systematically removing them from mice to study their functions. The work has revealed numerous connections between defects in the channels and disease. In several cases, the scientists first discovered patients who carried mutations in the genes, and then later created mouse models of the diseases.

A lack of CIC-1 leads to a condition known as *inherited myotonia*, in which the muscles don’t relax after voluntary activity like shaking someone’s hand or squinting in bright sunlight. The condition was first described in 1876 by Danish physician Julius Thomsen, who suffered from it himself, as did several members of his family. Over a century later, Thomas and his colleagues traced the disease to the chloride channel gene and even identified the specific mutation that caused the disease in Dr. Thomsen himself.

“We think that by allowing chloride ions to flow into the cell, CIC-1 helps muscles relax and ‘reset’ so that they are ready for the next stimulation,” he says. “Without the molecule, external potassium levels build up and cells begin to stimulate themselves. This leads to a sort of seizure in which muscles no longer relax.”

It wasn’t hard to imagine something similar happening in other tissues. Thomas was intrigued by a report from a clinical group which had discovered mutations in CIC-2, found in the brain and other organs, in a family of human patients suffering from epilepsy. Was the loss of channel proteins causing an overstimulation of neurons, leading to brain seizures? He was skeptical. A couple of years earlier, the lab had developed a strain of mouse without CIC-2. Although mice can suffer from epilepsy, this was not the case in the knock-out mice. Instead, the animals experienced degeneration of the testes and the retina.

Judith Blanz, a student in Thomas’ group at Hamburg, decided to conduct a more careful examination of the

nervous system of mice lacking this chloride channel. She discovered that while individual neurons still had their normal forms, the animals' brains and spines developed severe defects. "They took on a sponge-like appearance because of holes that formed in the white matter of the brain," Thomas says. "The only real behavioral difference was related to blindness, caused by the retinal degeneration we had noticed in the previous study. And the mice responded somewhat more slowly to stimuli, caused by holes in the white matter. Signals in the brain were not being transmitted as quickly."

The sponge-like gaps in the brain were familiar; they also appear in a human disease called *leukodystrophy*. Researchers had already found several genes for different forms of human leukodystrophy, but some families suffering from the disease didn't have mutations in any of these genes. So there was reason to believe that other, unknown genes might be involved as well; CIC-2 might be one culprit. The scientists obtained DNA from a group of 150 leukodystrophy patients and began looking for a pattern of mutations. "We didn't find them," Thomas says. "That was disappointing; on the other hand, the damage in the brains of humans who suffer from this condition is mostly more severe than what we observe in the mice. We may have picked the wrong subset of patients; we don't know of human patients where the symptoms are combined with degeneration of the retina. Such a case would more closely resemble what we see in the mouse. We are currently collaborating with groups in human genetics to try to find such patients."

A mutation's effects on health are often strange and unpredictable. While looking at a potassium channel called KCNQ2, the scientists found mutations which cause epilepsy in human newborns. The discovery suggested that KCNQ proteins would make a good target for anti-epileptic drugs. Later it was found that a known substance, currently in phase III clinical trials for use in treatments of epilepsy, was specifically targeting KCNQ potassium channels. These KCNQ proteins are now being used by pharmaceutical companies to develop more specific anti-epileptic drugs. It's another example of the beneficial side-effects of basic research, he says: finding a gene responsible for a very rare genetic condition can sometimes lead to therapies for much more common diseases.

Another serendipitous discovery arose from studies of mice that lacked CIC-7. "Removing this gene leads to unusually thick and brittle bones in mice," Thomas says.



"We discovered that some humans who suffer from a rare disease called *osteopetrosis*, also characterized by thick bones, carry a defective form of the gene. One aspect of this study is that it may give us hints about how to treat a much more common disease – *osteoporosis*, in which bones become too thin, particularly in elderly women."

What's the connection between thick bones in mice and thin ones in aging humans? "CIC-7 is active in *osteoclasts*, cells which degrade bones," he explains. "You wouldn't want that degradation to happen in elderly people, especially women. A drug that partially inhibited CIC-7 might help preserve bone density as a treatment for osteoporosis."



Recently the lab has extended its attention to a family of molecules called *KCCs* that transport both chloride and potassium in a coupled way. "We call these molecules *co-transporters*, and they were first discovered in studies of red blood cells," Thomas says. "As these cells are squeezed through tiny capillaries, and have to go through cycles of oxygenation which are associated with fluxes of salt and water, they have to precisely regulate their volume. At least two types of *KCCs* help carry out this job in the membrane of red blood cells." A channel is relatively passive – allowing ions to pass "downstream", for example by shipping negatively charged particles into an area where the charge is positive. But transporters like *KCCs* are able to push chloride ions "uphill" – by moving them at the same time as potassium ions.

Jentsch group in front of the Timoféeff-Ressovsky building.



Thomas and his colleagues from Hamburg developed strains of mice lacking KCC1 and KCC3, the two types of potassium-chloride transporters found in red blood cells. Taking away KCC3, or both molecules, reduces the transport of chloride and potassium ions out of the cell.

In general, he says, the KCCs are mainly responsible for the outward passage of chloride and fluid from the cell. Seth Alper of the Harvard Medical School, who has been collaborating with Thomas on the KCC project, showed that there is too much transport of potassium and chloride out of the cell during sickle cell anemia. In this disease, red blood cells lose too much volume and take on a deflated, sickled shape. The cells then become wedged in tiny capillaries, obstructing the flow of blood and causing irreversible damage to organs. In sickle cell anemia a mutation causes hemoglobin proteins to form clumps that do not dissolve inside the cell when things get too crowded – and that happens when red blood cells can't control their volume properly.

Seth has done a great deal of work on the relationship between hemoglobin, cell volume, and the symptoms of

the disease. "Clumps form when there is a high concentration of mutant hemoglobin in the cell," Seth says. "This somehow causes cells to take on their odd, sickle shape. If we could prevent the aggregations, we could stop their harmful effects. That means keeping the concentration of hemoglobin low, and having enough water in the cell is an important part of the process. If KCC channels are too active, the cell loses ions and water. If it didn't happen, we think the course of the disease would be greatly slowed."

It sounded good, but proving the hypothesis would require studying KCCs in the blood more carefully. Thomas and his colleagues from Hamburg developed strains of mice lacking KCC1 and KCC3, the two types of potassium-chloride transporters found in red blood cells. Removing KCC1 had no obvious effects on the

mice. But taking away KCC3, or both molecules, reduces the transport of chloride and potassium ions out of the cell.

Would this offer the animals protection from sickle-cell disease? The scientists crossed the mice with another strain which has defects in hemoglobin and develops a condition very much like human sickle-cell anemia. "On the whole," Thomas says, "the blood cells of these animals had more volume, and there was less ion transport. But it didn't help the cells that were most dehydrated and deformed. One hypothesis is that this subpopulation of cells lose their water a different way, earlier in the process by which red blood differentiates. Maybe a combination of inhibiting KCCs and other ion transporters like potassium channels could help."

■ ■ ■

Currently Thomas is most excited about his work with chloride transporters at work inside the cell, including other members of the CLC family. "These molecules are found in membranes along the *endocytotic* pathway," Thomas says. "That's a route used by the cell to absorb molecules from the outside, to capture toxins and other foreign substances, and to recycle proteins to the outer membrane or to degrade them."

Substances are wrapped in membranes so that they can be processed. These membrane bubbles are absorbed by internal cellular compartments that contain high levels of acid and enzymes that break things down. Raising the acid level means bringing high numbers of protons into the compartments. Vesicles are equipped with proton pump molecules that do this, but they can't do the whole job – once the acidity reaches a certain level, the pump can no longer push protons "upstream" into the compartment. "The solution is to neutralize the positive charge going in by importing an equal negative charge," Thomas says. "That happens through the CLC transporter, and it allows the vesicles to continue to increase their acidity."

"A whole branch of the CLC family – five molecules in humans – are found mostly in internal membranes, and we didn't know what they were doing," he says. "In 2005, we helped to show that CIC-4 and CIC-5 were also acting as coupled transporters, and not as channels – inside the cell."

Here, too, there were links to disease: ten years ago, Raj Thakker in London and Thomas had found that mutations that inactivate CIC-5 cause Dent's disease, an inherited human kidney stone disorder. Nils Piwon, a graduate student in Thomas' lab, then generated a mouse without CIC-5 and found that the animals' kidneys were severely impaired in their ability to take up

proteins, which then entered the urine. The scientists developed a hypothesis to explain why: without the CIC-5 chloride transporter, kidney cells are unable to absorb another small protein, a hormone called PTH. It therefore accumulates at too high levels in the primary urine, triggering abnormal signals in the cell that limit the reabsorption of phosphate. Through a complex series of signaling events, this also increases vitamin D levels. This molecule, along with PTH, is the main hormone in regulating body calcium. Patients with Dent's disease take in too much calcium from food, which the body has to get rid of through the kidney. The combination of high calcium and high phosphate in the urine then leads to precipitation of calcium crystals that finally build up to the terribly painful kidney stones.

Thomas lists other diseases linked to internal CLCs: neurodegeneration associated with CIC-3, lysosomal storage disease (CIC-6) and now osteopetrosis (CIC-7). You can tell that he isn't finished, that he's somewhere in the middle of the roadmap that he designed 20 years ago. It's still a good plan and he talks about it eagerly. Behind him the lab is filling with new methods, postdocs, students, and technicians. There is still an immense amount to do with ion channels. He's building a laboratory for the next 20 years. ■



Bad origami

The way 40 amino acids are folded makes the difference between life and death



Even with step-by-step instructions, it's hard enough to fold a sheet of paper into an elegant origami crane. But suppose you had to figure out how to do it by unfolding a finished one. That would give you an idea of how Bernd Reif feels when he looks at *amyloid plaques*. These clumps of protein fragments accumulate between brain cells to cause Alzheimer's and other degenerative diseases of the nervous system. In trying to figure out how they form, the situation is like that of the crane: even knowing a lot about the starting material (paper) and seeing the finished product (a paper bird) doesn't explain how to get from one to the other.

"Amyloid plaques accumulate between nerve cells, leading to cell death and the symptoms of Alzheimer's," Bernd says. "But we understand very little about what causes those effects. We think that protein fragments assemble into amyloids in an orderly way, going through many steps in which they take on different structures. Some of those structures are more toxic than others, although they are made of the same subunits. Finding out what makes the plaques so dangerous will probably require getting a step-by-step look at how the protein fragments assemble."

Bernd's laboratory at the FMP is trying to uncover some of the basic principles underlying Alzheimer's, Huntington's Diseases, prion diseases, and other neurodegenerative conditions that are caused by accumulations of proteins. They all begin when a protein needed by the healthy body begins to behave in an unusual way. In the case of Alzheimer's disease, brain cells produce protein fragments all the time, but normally the body can get rid of them.

"The same small bits of protein – 40 amino acids – are sometimes completely harmless, sometimes fatal," Bernd says. "If you watch them bind to each other in the test tube, you get a variety of forms: single fragments that don't accumulate, or stringy fibers, or clumps that are not very organized. What you get depends on the experimental conditions."

The plaques may assemble differently in the test tube than in the brain, and even under controlled conditions it has been difficult to get a glimpse of the stages. The battle, he says, has been to find or invent methods to get a look at single stages of how protein fragments interact with each other and other molecules found in the clumps. Call it a folding manual.



If *beta-amyloid* isn't yet a household word, it probably will be soon. More and more families are confronting Alzheimer's disease, the most common age-related neurodegenerative disease in the world. Beta-amyloid, a fragment of a protein, is thought to be the major culprit in the development of the disease.

Beta-amyloid begins as part of a much larger molecule called APP (for *amyloid precursor protein*) which can be found in high numbers in the membranes of nerve cells. What it normally does there is still not completely understood. Mice born without the molecule develop smaller brains, problems of body coordination, and impaired memory and learning, but scientists do not know how APP contributes to these processes in healthy animals or humans.

Like most proteins, APP is processed by other molecules in several ways. First it is cut by an enzyme called *β-secretase*, or *BACE*. Another protein called *gamma-secretase* cleaves the chain for a second time, releasing the beta-amyloid fragment. This is a normal, life-long process that undoubtedly has a role in the healthy brain, but the fragment's function is not yet known. Normally it is broken down and disposed of, but with age our cells and molecules behave differently. Later in life the fragments accumulate to form fibers and plaques in the space between cells.

"There are a few theories about why the aggregations are dangerous," Bernd says. "One is that they create holes in cell membranes that allow charged calcium atoms to pass through. Those have to be carefully regulated for the cell to behave properly and survive. Another idea is that plaques stabilize or produce free radicals, highly reactive atoms that are dangerous because they can modify a wide range of molecules. Whatever the answer is, beta-amyloid only does this in a specific type of cluster, or what we call an oligomer. That's simply a complex in which more than one copy of the same molecule is bound together. For example, if copies assemble in oligomers of six or eight they might not be dangerous, whereas groups of ten could be fatal. Determining which of these forms is the deadly one has been extremely challenging."

And amyloid plaques contain more than beta-amyloid. Recently another laboratory discovered that a *chaperone* protein called *αB-crystalline* could bind to the fragments in the test tube as well as in living cells. Chaperones are "assistants" whose normal job is to help keep proteins



A tiny region of beta-amyloid permits two or more copies to bind to each other. The same region serves as the binding site for α B-crystalline. In fact, α B-crystalline binds much more strongly to beta-amyloids than the fragments bind to each other.

folded in their proper forms – if they become unfolded, the cell usually destroys them. Folding gives a protein the shape it needs to interact with other molecules and snap into “molecular machines.” A protein begins as a long string; folding happens because of chemical interactions between the amino acids that make it up. Sometimes it needs help to achieve or maintain the right form, which is where chaperones come in. Experiments showed that the brains of some Alzheimer patients contained chaperones, including α B-crystalline, in abnormal places.

“That’s interesting because α B-crystalline is disturbed in people who suffer from Down Syndrome,” Bernd says, “and nearly all of them develop Alzheimer’s disease. They develop it early, at around 40 years of age, which is a fairly dramatic acceleration of the disease process.”

Experiments in the test tube revealed that α B-crystalline blocks the formation of amyloid fibers. Fragments can still bind to each other in the molecule’s presence, but they don’t take on the long, orderly shape of fibers. Instead, copies of beta-amyloid cluster together, in

groups of twos (dimers), threes (trimers), or higher combinations. Some remain alone.

“It is unclear why only one oligomeric structure should be toxic,” Bernd says. “To find out, we’ll have to watch the process of assembly. And that has been a huge technical challenge. What is very interesting is that the combination of beta-amyloid with α B-crystalline is more deadly to cells than beta-amyloid on its own. Maybe if fibers could form, the situation wouldn’t be so dangerous; α B-crystalline is keeping that from happening.”

Were fibers a transition state – were they involved at all in the development of disease symptoms? Wondering why α B-crystalline increased the deadliness of beta-amyloid, Bernd’s lab applied several innovative techniques to study the nature of the contact between the two molecules and its relationship to the formation of fibers and plaques. The first question to answer had to do with basic architecture: what regions of beta-amyloids were linked to each other, and which regions could bind to α B-crystalline? Next the scientists wanted to know how many copies of beta-amyloid could be found linked within a single cluster, and whether this changed because of the presence of α B-crystalline. If changes occurred, they might happen in several steps. Sometimes in origami, a fold is made and then undone, so that the crease can serve another function.

Saravanakumar Narayanan, a PhD student from India, used a combination of methods to find some of the answers. First, beta-amyloid and α B-crystalline were mixed together at changing concentrations. Presumably

a small number of copies of α B-crystalline would have different effects on the fragment than a high number. As the amounts changed, the structures were examined by NMR, which provided an atom-by-atom view of some regions of the molecules.

The experiments showed Saravanakumar and his colleagues the docking sites of the two molecules. A tiny region of beta-amyloid permits two or more copies to bind to each other. The same region serves as the binding site for α B-crystalline. In fact, α B-crystalline binds much more strongly to beta-amyloids than the fragments bind to each other. "That is extremely interesting," Bernd says, "because it suggests a way by which α B-crystalline can change the groupings and the function of beta-amyloid fragments. Without α B-crystalline, there is nothing to keep the fragment from forming large groups, including structures like fibers. When the molecule is present, however, it competes for the binding site. Fewer copies of beta-amyloid will bind."

The findings closely echo what the group found while investigating another protein, *Sup35*, using similar methods. *Sup35* is a yeast prion protein, similar to the human protein that causes prion diseases (like Mad Cow Disease). Both yeast and human prion proteins have functions in healthy cells that they can no longer perform when they have accumulated in clumps. Saravanakumar and Bernd discovered that a chaperone protein called *Hsp104*, known to interact with *Sup35*, links to clusters of *Sup35* molecules and tosses out some of them, leading to smaller clusters.

α B-crystalline might be doing something similar, but the scientists needed to know if it was changing the number of beta-amyloid molecules attached to each other. While investigating the yeast prions, the scientists had developed a method to filter different sizes of molecules by placing them in a dialysis bag and allowing them to leak out. Such bags are used to filter the blood of people with kidney diseases; they are full of holes so tiny that they trap large molecules while allowing small ones to pass through. By using bags with holes of different sizes, the scientists could test amounts of *Sup35* molecules that were detached from *Sup35* fibrils. They could also track *Hsp104*'s effects on the size of these groups.

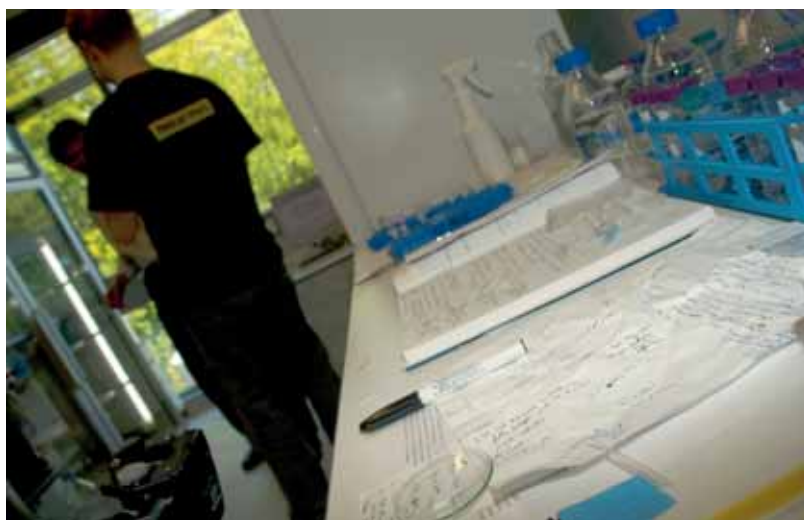
Similar experiments revealed that α B-crystalline binds weakly and only for a very short time to single copies of beta-amyloid. But it binds very strongly to multiple copies of the fragment that have formed fibers or are in

the process of doing so. "When that happens, it causes a structural change," Bernd says. "We believe that α B-crystalline shifts the preference of the fragments – instead of linking up into long, stringy fibers containing thousands of copies of the fragment, they collect in smaller clusters."

The association between the molecules has an additional effect: one of the amino acid subunits of beta-amyloid becomes *oxidized*. This means that the molecule picks up additional electrons, changing its overall charge and the way it interacts with other molecules. That, Bernd says, might help explain why amyloid plaques are so deadly.

"Oxidation seems to destabilize fibers so that they can dissolve," he says. "That might also explain why this form of beta-amyloid is toxic to neurons, because it produces highly reactive forms of oxygen."

To test the idea, the scientists used a probe that could measure α B-crystalline's oxidizing activity. They put α B-



crystalline into a mixture of single molecules called *GSH*. On its own, the oxygen that is dissolved in the solution provides an electron that oxidizes *GSH*. Adding another protein may slow down the process, because it also undergoes oxidation and captures some of the electrons that would otherwise be used by *GSH*. That's what happened when Bernd and his colleagues mixed the two types of molecules. Mixing *GSH* with beta-amyloid fragments, however, had almost no effect. This meant that the fragments alone weren't producing the oxidizing reactions.

To “turn up the heat,” Bernd and his colleagues added copper to the mixture of GSH and α B-crystalline. “Copper doesn’t change the oxidation state of GSH on its own,” Bernd says, “but in combination with α B-crystalline, it triggers much faster reactions. Our interpretation is that copper binds tightly to α B-crystalline and pushes its oxidizing activity into higher gear.”



We still have a long way to go in understanding the molecular processes that lead to Alzheimer’s disease, Bernd says. “We’re asking questions that tax all of our current methods. Finding answers has required combining techniques in new ways.”

“Methods development is dry compared to some of the science stories, and it is sometimes difficult to publish interdisciplinary papers where the focus is on methods. But the beta-amyloid studies are a good example of how techniques have to be adapted or used in creative new ways to solve the kinds of questions we are asking.”

For example, the laboratory would like to get a look at how molecules bind to beta-amyloid in the fiber form. NMR, the technique of choice at the FMP for getting structural information, works best with molecules that float freely in solution. It applies a strong magnetic field to protein samples. This alters the energy levels in the nucleus of every atom in the sample in a way which is slightly different for each proton in a protein. The slight energy difference allows scientists to determine the identity of each proton – in other words, to tell what type of atom it belongs to. When the strength of the magnetic field is lowered, or “relaxed”, they can probe the distances between protons, permitting the creation of atom-by-atom maps and thus a structure of the protein. But for the method to work, molecules need to swim around freely, which they can’t do if they are locked into a fiber.

In addition, Bernd says, one can try to get a lot of molecules in the sample to align themselves in the same direction. He knew that some of his colleagues had been using magnetic fields to force fibers into a more organized structure, allowing them to obtain higher-resolution images using X-rays. Maybe the same thing could be done using the powerful magnetic field of the NMR machine.

“The amino acid subunits of proteins have different magnetic properties,” Bernd says. “Think of a fibril as a shoestring that several small magnetic beads have been strung onto – representing the most magnetic subunits of the protein. If you lay the string alongside an iron pipe, it will attach itself and hold on. This is an analogy of how the protein fiber can be oriented in a certain way by the magnetic field. Now if other proteins come along and dock on, they can only attach themselves in certain ways. Those constraints give us enough information to see some of the details.”

It often takes months or years to get such ideas to work, Bernd says, and in many institutes scientists don’t have the freedom. “Methods development is dry compared to some of the science stories,” he says, “and it is sometimes difficult to publish interdisciplinary papers where the focus is on methods. But the beta-amyloid studies are a good example of how techniques have to be adapted or used in creative new ways to solve the kinds of questions we are asking right now. Walter Rosenthal, the FMP’s director, has been very supportive of this approach.”



With method developments and a wide range of scientific interests, Bernd clearly has his hands full, but he recently took on another major project with the creation of a new graduate school in Molecular Biophysics at the FMP. The program has now been launched in collaboration with universities and other institutions. The Leibniz Association has financed the creation of several of graduate schools, on a competitive basis, over the past few years. Walter Rosenthal says that Bernd’s proposal to create a department devoted to biophysics – specifically the investigation of protein interactions – was successful for several reasons.

“In the first place the topic fills a gap in the landscape of German academic programs,” Walter says. “And it was a very strong proposal. Bernd developed the concept, convinced all of us and the partner institutes, and wrote the grant application.”



Reif group at the new NMR spectrometer

Currently there are 15 students enrolled in the program, about half doing their work in Berlin and the rest at other institutions. Each receives a healthy, competitive stipend – to which some expectations are attached. The students present their work once a year in an annual report, and participate in two major “block” courses whose purpose is to give them a broader background than students normally receive.

“Biophysical techniques are acquiring a growing role in the investigation of biological processes,” Bernd says. “We’re also in the midst of a very exciting time in which new methods are being invented all the time. Young scientists will need a very wide exposure to the palette of methods that are available and should think creatively about new ones.”

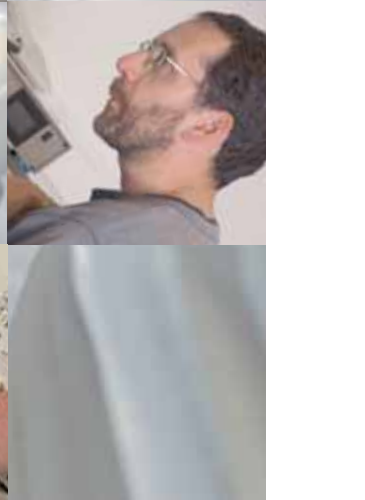
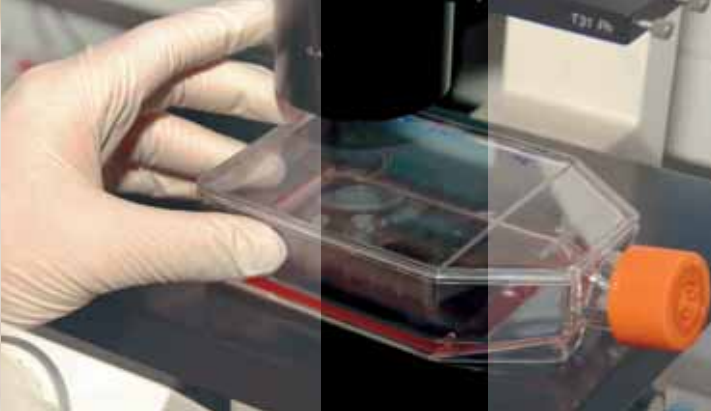
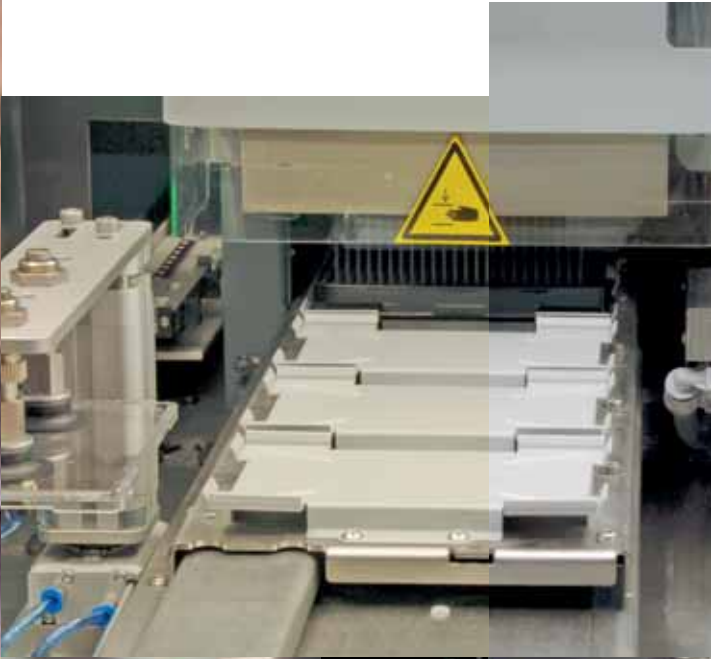
The proposal captures the best experts in particular areas from the region, Bernd says. It will draw on campus expertise in X-ray crystallography, NMR, and signal transduction; institutes within the Charité will contribute in

areas such as electron microscopy and biochemistry; the Institute for Experimental Physics and other departments of the Freie Universität will offer spectroscopy and other types of expertise, and the Humboldt-Universität adds expertise in membrane biophysics and several other fields. The University of Potsdam is another partner in the areas of CD spectroscopy and thermodynamics.

“In putting together this program, we’ve collected a wide range of techniques practiced by groups that have a lot of experience working together,” Bernd says. “Scientific themes will include the biophysics of protein aggregations, such as our work on Alzheimer’s disease; signal transduction in processes like visual perception; the molecular machines that build proteins, and viral infections. The questions in all of these fields are extensive and require a combination of approaches. Our plan is that each student in the program will be mentored by two group leaders, to increase interdisciplinarity.” ■

A kiss, interrupted

Learning to block interactions between proteins



It's probably dangerous to compare a pair of proteins to a sculpture, especially one that has been called a "sensual masterpiece," a "mixture of idealism and eroticism" – Auguste Rodin's kissing lovers sit on a rock that is only half-hewn from the marble block; above, their glossy bodies entwine to bring their lips together. But the analogy might be helpful in imagining the encounters between molecules that drive processes within cells. Proteins also kiss, sometimes in brief encounters that last for picoseconds. They enjoy a fleeting contact that changes one or both molecules, transmitting information, loading each other with energy, or carrying out other types of transformations. Sometimes there is only a small peck on the cheek, precisely and neatly delivered. In other cases, the molecules fold and wrap around each other.

Many of the encounters in the cell are the pecking type; they take place between a tiny partner such as a hormone and a much larger protein. Others involve whole-body embraces between two proteins, huge surfaces that scientists have had trouble understanding and manipulating. Achieving this understanding is important, says Hartmut Oschkinat, if researchers want to find drugs that control interactions between proteins.

"Such interactions are crucial in most cellular processes," Hartmut says. "Finding ways to inhibit them is usually very challenging. In the first place, these surfaces are large and flat, and it's hard for a small molecule to get a grip – the way that you can't free-climb on a surface that's too smooth. And there are very few natural small molecules that bind to these surfaces, which would also give you a place to start in designing new drugs."

The active components of drugs are often small molecules that have been extracted from much larger substances and then purified, leaving only the pair of lips – only what is needed to deliver a kiss or block someone else's attempt to do so. Often they snap into a pocket or a groove in one of the cell's proteins, taking the place of another molecule that is somehow linked to the symptoms of a disease. If researchers find such a pocket, it may not be hard to find or design something else that fits. That doesn't mean it will be easy to turn their invention into a drug, for a variety of reasons. But finding an inhibitor that blocks the interaction of modules is much harder.

That's not to say that it can't be done; some existing drugs work on larger surfaces. Taxol, which is widely



used to fight lung and breast cancer, was isolated from the bark of Pacific yew trees growing near the Mount St Helens volcano in Washington. It latches onto a protein called *tubulin* and prevents this molecule from binding to its partners, thus triggering a cellular self-destruct program called *apoptosis*. Another drug, cyclosporine A, was derived from a fungus that thrives in the soil in Norway. It has been widely used for over 20 years as an immune system suppressor to reduce inflammations and prevent organ rejections. It works by blocking interactions between proteins in T cells, reducing their ability to properly stimulate an immune response.

"These examples show that in principle, protein-protein interactions can be inhibited, and the molecules that do so might make powerful drugs," Hartmut says. "But we lack a lot of basic knowledge about the interactions. I thought that if we could find the right pair of proteins, it might give us a good model system to use to get a handle on the problem."

■ ■ ■

The publication of the human DNA sequence and other complete genomes has given scientists a full catalogue of proteins and other molecules that can be produced by our cells. Scientists already knew that certain types of patterns could be found over and over again; the same structures appeared in many different proteins. They usually arose through errors that happened as DNA was copied, creating duplicates of genes or parts of them. These were preserved through evolution, gradually taking on different functions. One structure, called a *PDZ domain*, has now been found in 250 proteins.



Jens Peter von Kries in his office.

"This is one of the most important modules in cells that govern interactions between proteins," Hartmut says. "It helps organize networks of signaling proteins; it participates in the delivery of parts to 'molecular machines,' and one of its most important jobs is to control events at the cell membrane. We began looking at this module because of its importance, and also because of a feature found on its surface. PDZ domains contain a shallow groove – not a real pocket, but something that an inhibitor might get a grip on."

Mangesh Joshi, a PhD student in the group, took on the project and focused on a protein called *AF6*, which contains a PDZ module. One reason for the choice was the role of *AF6* in the development of a disease called chronic myeloid leukemia. This type of cancer arises through a flaw in another protein, called *BCR*, which binds to the PDZ domain in *AF6*. Recently Yunyu Shi's laboratory at the University of Science and Technology in Hefei, China, discovered that *BCR* docks onto just the surface groove that Mangesh intended to look at.

Each PDZ domain docks onto a specific module in one other protein. Ten years ago, Hartmut's group helped figure out how PDZs recognized the right targets: they latch onto a loose, tail region of a domain in the partner molecule. Because the amino acid recipe of each tail is slightly different, it has different binding properties. Evolution has tailored specific tails to fit the groove of a particular PDZ.

"Because of what we know about how these molecules bind, it seemed likely that plugging the groove with another molecule might block access of the normal partner," Mangesh says. "But to show that to be the case, we had to find an inhibitor."

■ ■ ■

Since 2002 the FMP has been creating a "library" of molecules – ranging from natural substances to small artificial peptides – that can be used in projects like the one planned by Hartmut and Mangesh. The library is housed in a high-tech screening facility in the newly constructed Genomics center, a sleek, black building on the east edge of the campus. The building was opened in 2006 by the FMP and their campus neighbor, the Max-Delbrück Center for Molecular Medicine.

Jens Peter von Kries heads the facility. His office has large windows that look over the campus, which he knows well; from 1995-2000 he was a scientist in the group of Walter Birchmeier at the MDC. He spent the

next two years of his career as the scientific head of the screening unit for a Berlin pharmaceutical company before being recruited by the FMP to set up a similar facility. Today his foot is in a temporary cast from a football accident that happened while he played with his son. He places it on his desk like a paperweight as he talks about the mission of the facility.

"Our aim is to help scientists search for inhibitors for specific molecules and processes," Jens says. "The point may be to find a lead compound that can be developed into a drug – or the goal may be to develop a new tool to study a cellular process."

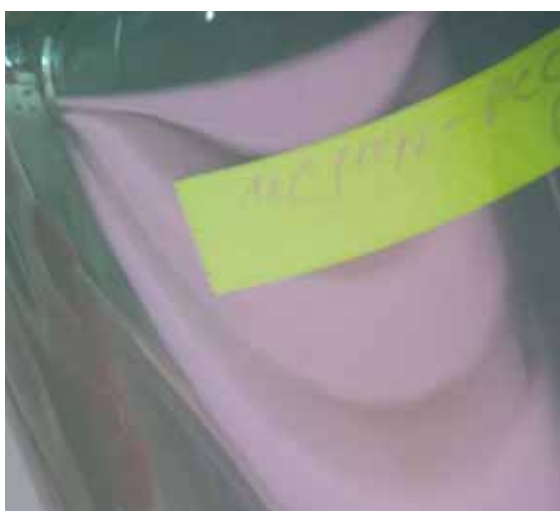
Sometimes, as in the case of Mangesh's project, the role of the facility is simply to open its library for scientists to use. Mangesh had decided to carry out the screen itself in another part of the institute, using the FMP's NMR machines. Hartmut's group has helped turn NMR into the institute's major tool used in studying protein structures and other applications.

"There were two reasons to do the screen with NMR," Mangesh says. "First, because of the nature of the PDZ binding region, we anticipated that our first 'hits' might bind quite weakly. NMR is good at detecting those. Secondly, it would give us a detailed look at the structures of the molecules as they interacted. That would be important for the second stage of the project: taking a substance and rebuilding it so that it would bind better."

At other times the screening facility takes a much more active role in projects, offering a full range of screening services and assistance in chemistry for in-house researchers and visitors. Compounds from the library are incubated with samples, either purified proteins or live cells, and then examined to see if there have been effects. All of this work used to be done by hand, but the entire process has been automated. The day of our tour, final tests were being run on a new automated screening machine, so massive that it takes up half of a lab. Packed inside are robots that handle samples in a "hotel", driving their containers to a central corridor where they encounter substances from a large library of chemical compounds.

The FMP's library currently includes about 50,000 substances, mostly purchased from other collections. "Some major pharmaceutical companies have collections of millions of substances," Jens says. "Obviously the FMP couldn't afford to build a library of that size, so we had to be selective. That was an intensive process – making

The PDZ domain is one of the most important modules in cells that govern interactions between proteins. It helps organize networks of signaling proteins; it participates in the delivery of parts to molecular machines, and one of its most important jobs is to control events at the cell membrane.



decisions on the best representatives of different classes of molecules, so that we would have a good spectrum of substances to work with."

FMP Director Walter Rosenthal says the screening unit is necessary to help the FMP and the wider research community carry out better research as well as fill in some of the gaps in the drug discovery process. "The point is to take a tool that is commonly used in the pharmaceutical industry and make it accessible to academia," he says. "The goal is not to compete with pharmaceutical companies which are much better equipped to 'try everything' in the search for new drugs. Our perspective is that academic researchers need access to a screening platform, the way they have access to other mass technologies. Here we've built a platform – unique to



Monika Beerbaum and
Martin Zieger of the
Solution NMR group.

Academic researchers need access to a screening platform, the way they have access to other mass technologies. Here we've built a platform – unique to Germany and Europe – that they can use; we're making it as open as possible.

Germany and Europe – that they can use; we're making it as open as possible. Your everyday scientist hasn't had that kind of access because the price of having a screen done may be prohibitive, or a major pharmaceutical company may simply not be interested in a particular project. Academic researchers may need an inhibitor simply to study a process that they're interested in, which is a much too basic problem for most companies."

If a screen turns up a hit, researchers can turn to the FMP's chemical groups for help in improving it. The institute has recently added new groups such as that of Jörg Rademann to strengthen its chemistry program. "The farther we make it along the process of trying, testing, and improving an inhibitor that works in a disease process," Jens says, "the more likely it is that a company will take interest and move it into development and clinical trials."

There have already been some interesting success stories. One project of the unit involves *Mycobacterium tuberculosis* (Mtb), the organism that causes the dreaded lung disease, and is a good example of how modern molecular science is being applied to infectious diseases. The incidence of tuberculosis is on the rise – an estimated one-third of the world's population is infected, although only a small percentage show symptoms – and about 20 percent of the bacteria have developed resistance to the most common anti-tuberculosis drugs. This situation has prompted the formation of national and international research campaigns aimed at finding new ways to fight the disease.

Tuberculosis projects across the world have been looking for good drug targets among the molecules in the tuberculosis bacterium. The ideal target is a protein from the invader that has unique characteristics – so that it can be blocked without damaging something important going

on in the cell. Recently Jens and Larissa Podust, then at Vanderbilt University, Nashville, and now at the University of California, San Francisco, carried out a screen to find inhibitors for a protein called *CYP51*. This molecule is found in all kinds of cells, ranging from bacteria to humans, where one of its jobs is to synthesize cholesterol. This is needed to create membranes, and without it the bacteria cannot survive. The version of *CYP51* found in bacteria has unique features that might make a good drug target. Another advantage is that when the protein binds to another molecule, there is a change that can be observed with a spectrometer, which gave the scientists a good method to observe the results of the screen.

High-throughput experiments yielded three hits, including a known antifungal drug called *EPBA* that locked onto the bacterial protein strongly and very specifically – not disturbing other molecules. *EPBA* is used widely as a preservative in cosmetics. The scientists discovered that it strongly resembled the structure of another molecule, called *BSPPA*, which they also tested; the second compound behaved exactly the same way. "Larissa Podust went to the synchrotron at the Argonne National Laboratory in Illinois to get detailed structural pictures of how both of these molecules dock onto *CYP51* using X-rays," Jens says. "The images show us how the compounds might be adapted to target other types of *CYP51*s. We're hopeful that *EPBA* can be used as a structural scaffold to spin off specific new substances to do that. The fact that the molecule has already been through clinical trials in another context – as an antifungal drug – could dramatically speed up its development into an anti-tuberculosis therapy."

The collaboration with a lab in the United States shows that the facility is ready to take on long-distance partnerships in interesting projects. Jens is already working closely with a network in Scandinavia called ChemBioNet, and other collaborations are underway with Oxford University.

One ongoing project involves a group at the University of Oslo, Norway, that works on specialized types of stem cells. Since the screen involves live cells, they had to be shipped to Berlin from Scandinavia. It's not as far as Illinois, but in this case, distance proved to be a problem.

"Jo Waaler tried to send the cells three times using express shipping services," Jens says. "Each time they got hung up somewhere at the border, and the cells



A probe head from the NMR

died. Finally Jo gave up, packed the cells in a rental car, and drove them down himself. All the way from Oslo.”

This time the cells – and the project – survived.

■ ■ ■

When Mangesh began his screen on the AF6 PDZ module, no synthetic binding partners were known. He began working with a subset of the FMP’s collection, 5,000 small molecules particularly suited for screening with NMR. This included representatives of all the basic kinds of molecules in the collection, including the types most frequently found in drugs.

“There are three critical stages for the design of inhibitors in drug discovery,” Mangesh says. “First you try to find lead compounds that show moderate activity toward the target. Then you go to the test tube and try to optimize the compound. Here a knowledge of the structure of the molecule bound to its target is extremely helpful, because it points out regions that are crucial to the interaction. Finally, the system has to be put to work in cells and organisms.”

The screen turned up three different types of compounds that bound to the PDZ domain of AF6. The scientists chose the one with the strongest effect, *2-thioxo-4-thiazolidinone*, for further work. Carolyn Vargas, another student in Hartmut’s group, began systematically synthesizing and purifying new spin-off compounds from it, with help from Volker Hagen, head of the Synthetic Organic Chemistry group at the FMP. The structural pictures from the NMR screen revealed specific points that might help strengthen the bonds between the compound and AF6.

After some work, Carolyn and Volker had come up with a new substance with the awe-inspiring name *(2R,5R)-2-sulfanyl-5-[4-(trifluoromethyl)benzyl]-1,3-thiazol-4-one*. It’s not the kind of thing you would want to have to say in the lab every day, or repeat on the telephone, so the compound was nicknamed *7i*. Further NMR experiments showed that *7i* was about as good as binding to the PDZ domain as AF6’s natural partners in the cell.

“Although the AF6 PDZ itself is highly similar to other PDZ domains,” Carolyn says, “we were in for a surprise when we saw how it bound to the new compound. Somehow *7i* had docked itself into a subpocket of the PDZ domain that had never been seen before, in any structural picture of a PDZ. We think this means that as *7i* and the PDZ come into contact, the

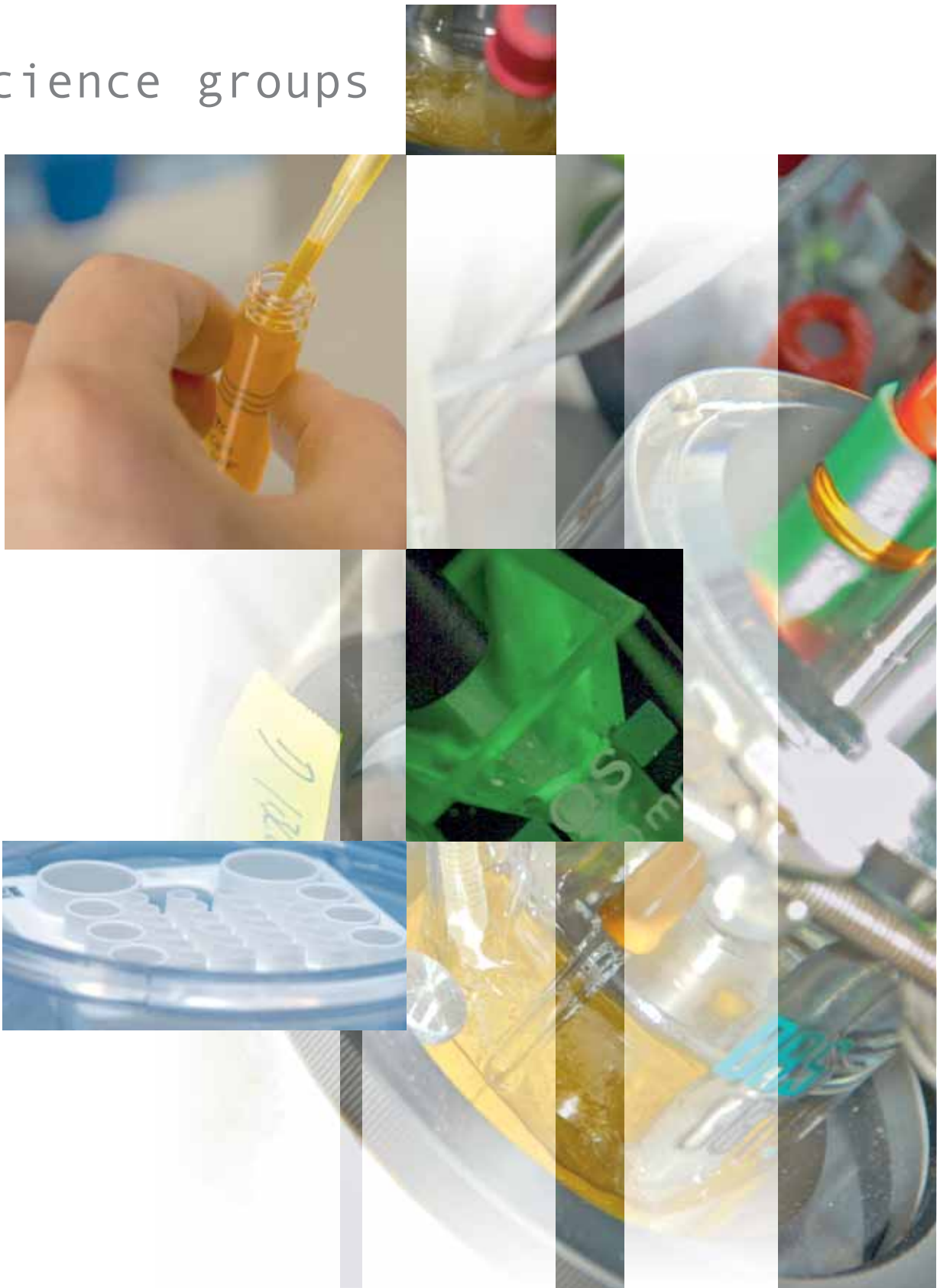


domain undergoes rearrangements that create this new pocket.”

No statue could ever do that – not even one as sensual as Rodin’s carving of a kiss – but proteins are anything but static objects. Their highly dynamic nature sometimes makes it hard to design a new drug, or predict how it will affect a protein. But in the case of AF6, the structural change might simplify things.

“When we originally thought about finding a way to make drugs to disturb interactions between proteins,” Hartmut says, “we chose the PDZ domain because of its shallow groove. Now it appears that a class of molecules like *7i* can create new pockets that allow them to get a better grip. This makes PDZs even more promising candidates for manipulations by drugs – especially if it turns out that these structural rearrangements are a general characteristic of PDZ domains. That’s likely to be the case; if so, it may give us a new strategy to design drugs that can influence a huge range of important cellular molecules.” ■

Science groups





Group Leader:
Hartmut Oschkinat

Protein Structure

Aims

Structural biology provides a rational basis for pharmacological work, given that high-resolution structures may be used as „rulers“ in pharmacology, molecular biology, cell biology and molecular medicine. We want to provide structural information on protein-protein interactions, and from heterogeneous cellular environments like the cell membrane.

NMR on heterogeneous systems

A living cell is a heterogeneous, highly compartmentalized entity in which proteins interact with partners in aqueous and lipid phases, or with immobilized components. These interactions are often modeled in-vitro by bringing together the soluble parts in a suitable buffer system. In this way, a homogenous system is created that can be subjected to crystallization or solution NMR for further studies. However, there are cases where the natural environment cannot be mimicked satisfactorily. Cellular crowding is one example; another is when membrane proteins require certain interactions with lipids for stability and function; it is also difficult to prepare actin fibers, microtubules, the dystrophin/utrophin network and specialized complexes attached to them while maintaining satisfactory conditions for the structural studies. A particularly important aspect of these systems is the dynamic nature of the formation and destruction of protein-protein interactions, and the heterogeneity of the components. As a method for obtaining high-resolution structural information in such situations we develop and apply magic-angle-spinning (MAS) solid-state NMR. As a first example for the power of the method, the structure of the basic unit of the protofibrils formed by the CA150 WW domain was determined.

Inhibition of protein-protein interactions

Despite their central role in most regulatory processes and disease mechanisms, protein-protein interactions (PPIs) remain a largely unconquered ground for drug discovery and the generation of chemical tools. In many cases, interactions are mediated by protein interaction domains like Src homology 2 (SH2), Src homology 3 (SH3), WW, and postsynaptic density/Discs large/zona occludens-1 (PDZ). PDZ domains may be considered „drugable“ because of a shallow ridge on their surface which is, however, not a proper cavity. They are hence good test cases for the development of PPI inhibitors.

The protein AF6 (**ALL-1** fusion partner on chromosome 6), also known as s-afadin, contains one type II PDZ domain (abbreviated: AF6 PDZ), two N-terminal Ras-

association (RA) domains, one forkhead-association (FHA) domain, and one dilute (DIL) domain. When the C-terminal valine of full-length Bcr is mutated to Ala, binding to full-length AF6 is abrogated in various cell-based assays.

We identified novel, low-molecular-weight ligands for the AF6 PDZ domain by NMR-based screening and chemical synthesis. These compounds are active in competition assays and represent building blocks for the design of tight-binding competitors. Furthermore, we have determined the solution structure of AF6 PDZ in complex with the ligand of highest affinity. The synthetic ligand induces the formation of a hydrophobic subpocket in AF6 PDZ absent in published structures of both apo and peptide-bound PDZ domains. This unexpected ligand-subpocket interaction redefines the protein's drugability and opens the door to small-molecule modulators for the entire family of PDZ domains.

Internal collaborations

The primary method we use to investigate structures is NMR, which is particularly well suited to study weak interactions between targets and ligands and for structural investigations of proteins, DNA and RNA in heterogeneous environments. The group is thus linked to the FMP's chemical biology efforts, with the development of ligands that inhibit protein-peptide interactions, to the peptide chemistry department, and to the other NMR groups.



We have determined the solution structure of AF6 PDZ in complex with the ligand of highest affinity. The synthetic ligand induces the formation of a hydrophobic subpocket in AF6 PDZ absent in published structures of both apo and peptide-bound PDZ domains. This unexpected ligand-subpocket interaction redefines the protein's drugability and opens the door to small-molecule modulators for the entire family of PDZ domains.

Selected publications*

Ball LJ, Kühne R, Schneider-Mergener J, **Oschkinat H** (2005) Recognition of proline rich motifs (PRMs) by protein-protein interaction domains. *Angew Chem Int Ed* 44, 2852-2869.

Fossi M, **Castellani F**, Nilges M, **Oschkinat H**, **van Rossum JB** (2005) SOLARIA: a protocol for automated cross-peak assignment and structure calculation for solid-state magic-angle spinning NMR. *Angew Chem Int Ed* 44, 6151-6154.

Hiller M, **Krabben L**, Vinothkumar KR, **Castellani F**, **van Rossum BJ**, Kühlbrandt W, **Oschkinat H** (2005) Solid-state MAS NMR of outer-membrane protein G from *Escherichia coli*. *Chembiochem* 6, 1-7.

Pires JR, Parthier C, Aido-Machado R, **Wiedemann U**, Otte L, Böhm G, Rudolph R, **Oschkinat H** (2005) Structural basis for APPTPPPLPP peptide recognition by the FBP11WW1 domain. *J Mol Biol* 348, 399-408.

Ferguson N, **Becker J**, Tidow H, **Tremmel S**, Sharpe TD, **Krause G**, **Flinders J**, Petrovich M, Berriman J, **Oschkinat H**, Fersht AR (2006) General structural motifs of amyloid protofilaments. *Proc Natl Acad Sci USA* 103, 16248-16253.

Joshi M, **Vargas C**, Moelling K, **Boisguerin P**, **Diehl A**, **Schmieder P**, **Krause G**, **Hagen V**, Schade M, **Oschkinat H** (2006) Making protein-protein interactions drugable: discovery and 3D structure of low-molecular-weight ligands complexed with the AF6 PDZ domain. *Angew Chem Int Ed* 45, 3790-3795.

* FMP authors in bold, group members underlined.

Group Leader:
Hartmut Oschkinat

Protein Structure

Members of the group

Dr. Prisca Boisguerin*
Dr. Christoph Brockmann*
Dr. Elizabeth Dowler*
Dr. Michele Fossi*
Dr. Victoria Ann Higman*
Dr. Ludwig Krabben*
Dr. Silke Radetzki***
Dr. Barth-Jan van Rossum

Inventions¹

Kühne R, Oschkinat H, Brockmann C, Schmalz HG, Zaminer J
"P2-Helix-Analoga als biologische Wirkstoffe"
Priority establishing patent application: 25.09.2006
Number of pending applications: 2

Labudde D, Leitner D, Schubert M, Winter R, Oschkinat H, Schmieder P
"Vorrichtung und Verfahren zur Zuordnung der NMR-Signale von Polypeptiden"
Priority establishing patent application: 11.09.2001
Number of patents granted: 1 (14.08.2003)
Rights ceased in 2006

Labudde D
"Verfahren zur Ermittlung von Verschiebungen der Hirnareale durch Tumorbildung und deren Darstellung auf einem Bildschirm"
Priority establishing patent application: 09.03.2000
Number of patents granted: 2 (22.07.2004/09.05.2006)
Rights ceased in 2006

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft
"Bestimmung der Raumstrukturen von Rezeptor-gebundenen Agonisten und Antagonisten mittels Festkörper-NMR-Spektroskopie" (Sub-project of the Collaborative Research Center "Struktur und Funktion membranständiger Rezeptoren")
SFB 449 TP B1 (Hartmut Oschkinat)

Deutsche Forschungsgemeinschaft
"Schlüsselreaktionen der biologischen Wasserstoffaktivierung am Beispiel der [NiFe]-Hydrogenasen" (Sub-project of the Collaborative Research Center "Protein-Kofaktor-Wechselwirkungen in biologischen Prozessen")
SFB 498 TP C1 (Hartmut Oschkinat, Bärbel Friedrich/Humboldt-Universität zu Berlin)

Deutsche Forschungsgemeinschaft
"Theoriegestützte NMR-spektroskopische Analyse von Protein-Ligand-Wechselwirkungen unter Verwendung von Peptidbibliotheken" (Sub-project of the Research Unit "Optimierte molekulare Bibliotheken zum Studium biologischer Erkennungsprozesse")
FOR 299/2-1 TP2 (Hartmut Oschkinat)

Deutsche Forschungsgemeinschaft
"Analyse von essentiellen Wechselwirkungen in β -sheet-Strukturen am Beispiel der WW-Domäne mittels Einbau nicht natürlicher Aminosäuren" (Sub-project of the Research Unit "Bildung und Stabilität von β -Faltblättern")
FOR 475 (Hartmut Oschkinat, Jens Schneider-Mergener/Jerini AG, Rudolf Volkmer-Engert/Charité – Universitätsmedizin Berlin, Uli Koert/University of Marburg)

Deutsche Forschungsgemeinschaft
"Modulation of PDZ-domain-mediated protein-protein interactions" (Sub-project of the Research Unit "Interfering with intracellular protein-protein interactions – probing protein functions with small molecules")
FOR 806 TP 5 (Hartmut Oschkinat, Gerd Krause, Jörg Rademann)

Deutsche Forschungsgemeinschaft
"Struktur, Stabilität und Spezifikation von nichtkatalytischen Proteindomänen und deren Verwendung als Werkzeuge für das Design einer stabilen minimalen Beta-Faltblattstruktur und das Verständnis von pathologischen Prozessen" (Sub-project of the Research Unit "Optimierte molekulare Bibliotheken zum Studium biologischer Erkennungsprozesse")
FOR 299/2-2 TP2 (Michael Beyermann, Hartmut Oschkinat, Michael Bienert)

Deutsche Forschungsgemeinschaft
"Struktur und Mechanismus der 3,4-Dihydroxy-2-butanon-4-phosphat-Synthase"
OS 106/4-2 (Hartmut Oschkinat)

Deutsche Forschungsgemeinschaft
"Analyse von Wechselwirkungen in β -Faltblattstrukturen ausgesuchter WW-Domänen durch den Einbau synthetischer Dipeptidisoistere"
OS 106/5-4 and 5-5 (Hartmut Oschkinat)

Federal Ministry of Education and Research
"Festkörper-NMR-Spektroskopie" (Sub-project of the collaborative project "Proteomweite Analyse membrangebundener Proteine – ProAMP")
0312890G (Hartmut Oschkinat)

Federal Ministry of Education and Research
"Screening von Substanzbibliotheken und Struktur-basiertes Wirkstoffdesign" (Sub-project of the "Strukturproteomik-Konsortium Hamburg: Hochdurchsatz-Strukturanalyse von *Mycobacterium-tuberculosis*-Zielproteinen und ihrer Ligandenkomplexe zur Suche nach Wirkstoffen")
0312992J (Jens Peter von Kries, Hartmut Oschkinat)

European Community (6th Framework Programme)
Sub-project of the Coordinated Action NMR-LIFE: "Focusing NMR on the machinery of life"
LSHG-CT-2005-018758 (Hartmut Oschkinat)

Land Berlin
"Optimierung eines Hochzell-dichte-Fermentationssystems zur kosteneffektiven Markierung von Proteinen für NMR-gestützte Strukturaufklärung" ("Programm zur Förderung von Forschung, Innovationen und Technologien – ProFIT")
IBB 10134687 (Annette Diehl)

Dr. Annette Diehl (technical assistance)
Sebastian Fiedler (scientist)
Nadja El Fassi (visiting scientist)
Paul Gooley (visiting scientist)
Meesook Sung (visiting scientist)
Johanna Becker (doctoral student)**
Katjana Daskalow (doctoral student)**/*
Feng Ge (doctoral student)**/*
Matthias Hiller (doctoral student)**

Jan Henrik Holtmann (doctoral student)**
Stefan Jehle (doctoral student)**
Mangesh Joshi (doctoral student)
Christian Köhler (doctoral student)**
Vivien Lange (doctoral student)**
Nikolaj Schröder (doctoral student)**/*
Carolyn Vargas (doctoral student)**
Ingo Scholz (doctoral student)**/*
Michael Soukenik (doctoral student)**/*

Janet Zapke (doctoral student)**/*
Stefan Bartsch (technical assistance)
Heide Evers (technical assistance)
Lieselotte Handel (technical assistance)**
Martina Leidert (technical assistance)**
Karola Marsch (technical assistance)**/*
Kristina Rehbein (technical assistance)
Anke Wellmann (student)

Volkswagenstiftung

“ Understanding and exploiting conformational effects on interaction of binding small molecules to the colchicine binding site of tubulin”

VW I/80 851 (Hartmut Oschkinat)

Human Frontier Science Program (HFSP)

“ Strukturuntersuchungen an fibrillären Systemen mit der Lösungs-NMR-Spektroskopie”

HFSP fellowship (Hartmut Oschkinat)

Rhein Biotech GmbH

“ Grundlagenforschung zur Optimierung der Bindungseigenschaften von Proteinen und ihrer Antigenpräsentation”

Rhein Bio – OS1 (Hartmut Oschkinat)

Cambridge Isotope Laboratories

“ Development of isotope labelled media for protein expression in bacteria, yeast, insects, and higher organisms”

Cambridge Iso – OS1 (Hartmut Oschkinat)

Schering AG

“ Durchführung von kernresonanzpetroskopischen Messungen”

Schering – OS1 (Hartmut Oschkinat)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Peter Schmieder

Solution NMR

Aims

The group Solution NMR uses the full repertoire of solution state NMR techniques in conjunction with a variety of labeling patterns to address questions of biological and pharmacological importance. These range from the development of new techniques for solution state NMR in order to elucidate the constitution and configuration of biologically active peptides to the determination of the three-dimensional structure of peptides and proteins.

Structural investigation of the light sensing module of bacterial phytochromes

For the understanding of the mechanism of light triggered signal transduction in phytochrome proteins, knowledge of the chromophore binding pocket at atomic resolution is of essential importance. We use solution state NMR spectroscopy to determine the structure of the binding pocket, using the sensory domain of Cph1, the phytochrome from *Synechocystis* PCC6803 (amino acids 1 to 514, named Cph1 Δ 2) as a representative system. We have established the labeling of the chromophore with ^{13}C and ^{15}N and use full deuteration of the protein in conjunction with reverse labeling to obtain structural information. We have already determined the protonation state of the chromophore in both photo-states and obtained information on its dynamics and exchange behavior. A homology model of the binding pocket has been created in cooperation with Ronald Kühne based on a photochemically disabled, but structurally related protein, allowing to deduce certain aspects of the conformation of the chromophore in the binding pocket.

Photo-switchable ligand molecules for PDZ domains

A variety of biological processes are triggered by the absorption of light, two prominent examples being rhodopsin and phytochrome. In both proteins, light absorption causes an E/Z isomerisation of a double bond in the cofactor, which subsequently leads to conformational changes in the protein and to the transduction of a signal. This concept – to use double bond isomerisation to induce structural changes and, subsequently, a biological effect – has been copied from nature and applied in the laboratory using several different applications and a variety of photosensitive compounds. Since the conformation of peptides is of essential importance for their function, the photomodulation of the conformation of cyclic peptides is particularly attractive for the design of a model system for photoregulation of peptide function within the context of signal transduction. In

cooperation with the group of Karola Rück-Braun from the TU Berlin, we apply the concept of photomodulation of peptide structure to peptide ligands of two protein domains that act as protein adaptor modules in a variety of biological processes, namely the SH2 domain of Grb2 and the PDZ domain of synthrophin. We are in the process of determining the three-dimensional structure of photoswitchable cyclic peptides in both states of the photoswitch using NMR spectroscopy. The first structures of PDZ-binding peptides have already been determined.

Structure and function of antimicrobial agents

A rapidly spreading bacterial resistance to existing drugs and a decline in the development of new antibiotics present a significant threat to human health. The identification of new antibacterial and antifungal agents is, therefore, of considerable importance. The elucidation of their mechanism of action would provide a sound basis for the further development of new generation of therapeutic agents. In cooperation with the group of Hans von Döhren at the TU Berlin, we have determined the constitution of two cyclic depsipeptides, Hassalidin A and B, that exhibit antifungal activity. The peptide is the first peptide of cyanobacterial origin that contains a lipid chain as well as a carbohydrate moiety. We are currently working on the synthesis of the peptide to define the stereochemistry and on determining the three-dimensional structure in various media. We have also determined the three-dimensional structure of several cyclic, cationic antimicrobial peptides bound to detergent micelles by NMR spectroscopy. This allowed us to determine that the presence of a sufficient number of indole and guanidinium groups arranged on a scaffold in the proper way should be sufficient to achieve antimicrobial activity. As a first step in further investigations, simple non-peptidic compounds were synthesised that showed the same antimicrobial activity as the original peptide.

Internal collaborations

The group Solution NMR has a major responsibility in the maintenance of the NMR facility at the FMP and is involved in almost all work that uses solution state NMR, for example with the groups of Oschkinat, Reif, Freund, Kühne, Beyermann, Hagen, and Rademann.

Research collaborations are being carried out with the groups of **Gerd Krause** (structural aspects of tight junction proteins), **Michael Beyermann** (photoswitchable ligands of the PDZ domain) and **Margitta Dathe** (structures of antimicrobial peptides).

Members of the group

Christian Appelt (doctoral student)**/*
Janina Hahn (doctoral student)**
Tolga Helmbrecht (doctoral student)**/*
Marco Röben (doctoral student)**/*
Sabine Seedorff (doctoral student)**/*
Holger Strauss (doctoral student)**/*
Brigitte Schlegel (technical assistance)
Monika Beerbaum (student)

Zerrin Fidan (student)*
Martin Zieger (student)*

Selected publications*

Appelt C, **Wessolowski A**, **Söderhäll JA**, **Dathe M**, **Schmieder P** (2005) Structure of antimicrobial, cationic hexapeptide cyclo(RRWWRP) and its analogues in solution and bound to detergent micelles. *ChemBiochem* 6, 1654-1662.

Neuhof T, **Schmieder P**, Preussel K, Dieckmann R, Pham H, Bartl F, von Döhren H (2005) Hassallidin, a glycosylated lipopeptide with antifungal activity from the cyanobacterium *Hassallia sp.* *J Nat Prod* 68, 695-700.

Strauss H, Hughes J, **Schmieder P** (2005) Heteronuclear solution-state NMR studies of the chromophore in cyanobacterial phytochrome. *Biochemistry* 44, 8244-8250.

Neuhof T, **Schmieder P**, Seibold M, Preussel K, Döhren H. von (2006) Hassallidin B – Second antifungal member of the Hassallidin family. *Bioorg Med Chem Lett* 16, 4220-4222.

Rohmer T, **Strauss H**, Hughes J, Groot H. de, Gärtner W, **Schmieder P**, Matysik J (2006) 15N MAS NMR studies of Cph1 phytochrome: chromophore dynamics and intramolecular signal transduction. *J Chem Phys B* 110, 20580-20585.

FMP authors in bold, group members underlined.

Inventions¹

Schmieder P, Neuhof T, Dieckmann R, von Döhren H, Seibold M, Preußel K
"Bioaktive glykosylierte Lipopeptide aus Cyanobakterien"
Priority establishing patent application: 02.03.2005
Rights ceased in 2007
Shared invention of TU Berlin and FMP

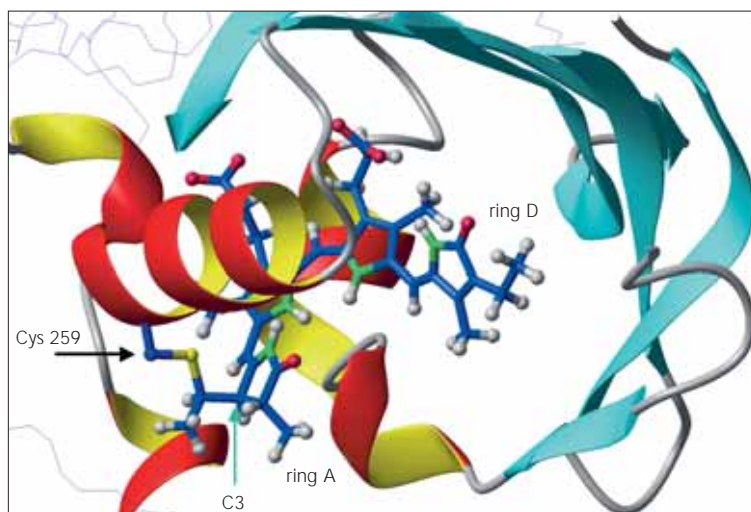
Labudde D, **Leitner D**, **Schubert M**, **Winter R**, **Oschkinat H**, **Schmieder P**
"Vorrichtung und Verfahren zur Zuordnung der NMR-Signale von Polypeptiden"
Priority establishing patent application: 11.09.2001
Number of patents granted: 1 (14.08.2003)

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft
"NMR-spektroskopische Untersuchungen von lichtreduzierten Strukturveränderungen in Protein-Chromophorkomplexen" (Sub-project of the Collaborative Research Center "Protein-Kofaktor-Wechselwirkungen in biologischen Prozessen")
SFB 498 TP B6 (Peter Schmieder)

Federal Ministry of Education and Research
"Durchführung von ³¹P-NMR-Untersuchungen am DRX600"
02WF0469/ IGB (UA) (Peter Schmieder)



Model of the chromophore binding pocket of the phytochromes Cph1 from *Synechocystis* PCC6803 based on the X-ray structure of a bacterial phytochrome. Based on the stereochemistry at C3 known from earlier degradation experiments the conformation of the chromophore can be determined as ZZZssa.

Volkswagenstiftung

"Control of protein-protein-interactions through conformational changes induced by light: photoswitchable ligand molecules for PDZ domains"
VW I/80 771 (Peter Schmieder)

Berlin-Chemie AG

"NMR-spektroskopische Untersuchungen"
Berlin Chemie – SC1 (Peter Schmieder)

Hans-Fischer Association

"Struktur-Untersuchungen an Phycocyanobilin mittels NMR-Spektroskopie"
HFG Schmieder (Peter Schmieder)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Gerd Krause

Structural Bioinformatics

Aims

The group is focused on sequence-structural analysis of proteins using structural bioinformatics combined with functional studies of changed sequence(s) to reveal sequence- and structure-function relationships of proteins. The main aim is the rational discovery of molecular mechanisms and sites for protein-protein interactions and protein-ligand interaction. Locations for potential pharmacological intervention are narrowed down to the amino acid and atom levels by predicting functional sensitive residues or atoms that are subsequently experimentally evaluated, such as by site directed mutagenesis or peptide mapping.

Molecular Activation Mechanism of 7TM-Receptor

Several Structural Determinants Identified

A web accessible resource system for sequence structure function analysis (www.fmp-berlin.de/ssfa) at Glyco-protein hormone receptors was developed using functional data from 900 mutations. A semi-quantitative analysis allows discriminations of molecular and structural determinants that are responsible for different functionalities in the activation mechanisms. Utilizing constitutive active pathogenic mutants, we have analyzed conformational changes which are necessary for the 7TM receptor activation. Using a multidisciplinary strategy, we narrowed down intramolecular interaction sites between the ecto- and serpentine domains of the thyroid stimulating hormone receptor (TSHR). Combining comparative modelling, site directed second site mutations, we identified specific residues that are thought to be members of a TSH receptor internal signal transmitter (cooperation with R. Paschke, University of Leipzig).

Detection of binding site for small ligand at TSHR

No small ligand has been found for TSHR, whereas the hormone binds solely at the extracellular domain. The known small ligand of CG/LHR activates TSHR only weakly. Utilizing the rare sequence differences between the close homologues, the non hormone allosteric binding site was identified in the transmembrane domain. Chimera mutants increase ligand activity at TSHR. This provides the base for rational design also of a TSHR specific small ligand and opens new perspectives of therapeutic treatment of thyroid diseases (cooperation with S. Neumann and M. Gershengorn, NIH Bethesda).

Protein Domain Interaction Specificities and Interference

The molecular mechanisms of selective recognition of protein domain subtype interactions, such as for PDZ domains, are the focus of our studies. Bioinformatic studies of binding pockets at different PDZ domains led to the quantitative specificity profile for each position of the binding pocket as a function of ligand sequence-dependent affinity contributions. Our models supported experimentally-determined dissociation constants and, hence, allowed us to design super binding peptides which were confirmed experimentally (<http://www.fmp-berlin.de/nmr/pdz>). This specificity profile is utilized to generate structure-function relationships for optimization of initial competitive inhibitors of PDZ domain interactions, which were identified by NMR screening of compound libraries.

Interaction Sites of Junctional Proteins

Junctional proteins serve to connect and seal the contact sites in between endothelial cells. The general objective of this project is to understand the intermolecular formation of tight junctions (TJ). To provide support for the intracellular recruiting mechanisms at TJ and Adherens Junctions (AJ), we determined how the TJ protein, occludin, and the AJ protein, α -catenin, interact with the junctional recruiting protein, Zonula Occludens-1 (ZO-1). Structural bioinformatic and molecular homology studies led us to the hypothesis that occludin and α -catenin share a common structure and, subsequently, a common interaction mechanism with ZO-1, which was experimentally confirmed in collaboration with O. Huber, Charité Berlin.

Internal collaborations

Bioinformatic sequence-structure-function studies narrowing down and identifying sites of proteins (amino acid, atom level) that are essentially involved in intra- or intermolecular mechanisms of proteins, followed by experimental probes for pharmacological intervention with several groups, especially:

- I. Blasig: Interactions of tight junction proteins
- P. Schmieder: Structures of tight junction proteins
- R. Schülein: GPCR signalling mechanisms
- E. Klußmann: AKAP-peptide/Rli interaction patterns
- H. Oschkinat, J. Rademann: Interference of protein-protein interaction

Coordination intergrated FMP project: Identification of ligands and binding sites for 7TMR

Members of the group

Maren Claus (doctoral student)^{***}

Gunnar Kleinau (doctoral student)^{**}

Jens Lättig (doctoral student)^{***}

Sebastian Müller (doctoral student)

Urs Wiedemann (doctoral student)^{***}

Mara Brehm (student)^{*}

Selected publications*

Müller SL, Portwich M, **Schmidt A**, **Utebergenov DI**, Huber O, **Blasig IE**, **Krause G** (2005) The tight junction protein occludin and the adherens junction protein α -catenin share a common interaction mechanism with ZO-1. *J Biol Chem* 280, 3747-3756.

Tunaru S, **Lättig J**, Kero J, **Krause G**, Offermanns S (2005) Characterization of determinants of ligand binding in the nicotinic acid receptor GPR109A (HM74A/PUMA-G). *Mol Pharmacol* 68, 1271-1280.

Ferguson N, **Becker J**, Tidow H, **Tremmel S**, Sharpe TD, **Krause G**, **Flinders J**, Petrovich M, Berriman J, **Oschkinat H**, Fersht AR (2006) General structural motifs of amyloid protofilaments. *Proc Natl Acad Sci USA* 103, 16248-16253.

Jäschke H, Neumann S, Moore S, Thomas C, Colson AO, Constanzi S, **Kleinau G**, Jiang JK, Paschke R, Raaka BM, **Krause G**, Gershengorn M (2006) A low molecular weight agonist signals by binding to the transmembrane domain of TSHR and LHCGR. *J Biol Chem* 281, 9841-9844.

Joshi M, **Vargas C**, Moelling K, **Boisguerin P**, **Diehl A**, **Schmieder P**, **Krause G**, **Hagen V**, Schade M, **Oschkinat H** (2006) Making protein-protein interactions drugable: discovery and 3D structure of low-molecular-weight ligands complexed with the AF6 PDZ domain. *Angew Chem Int Ed* 45, 3790-3795.

Kleinau G, **Brehm M**, **Wiedemann U**, **Labudde D**, Leser U, **Krause G** (2007) Implications for molecular mechanisms of glycoprotein hormone receptors using a new sequence-structure-function analysis resource. *Mol Endocrinol* 21, 574-580.

Kleinau G, **Claus M**, Jaeschke H, Mueller S, Neumann S, Paschke R, **Krause G** (2007) Contacts between extracellular loop two and transmembrane helix six determine basal and hormone induced activity of the thyroid stimulating hormone receptor. *J Biol Chem* 282, 518-525.

FMP authors in bold, group members underlined.

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

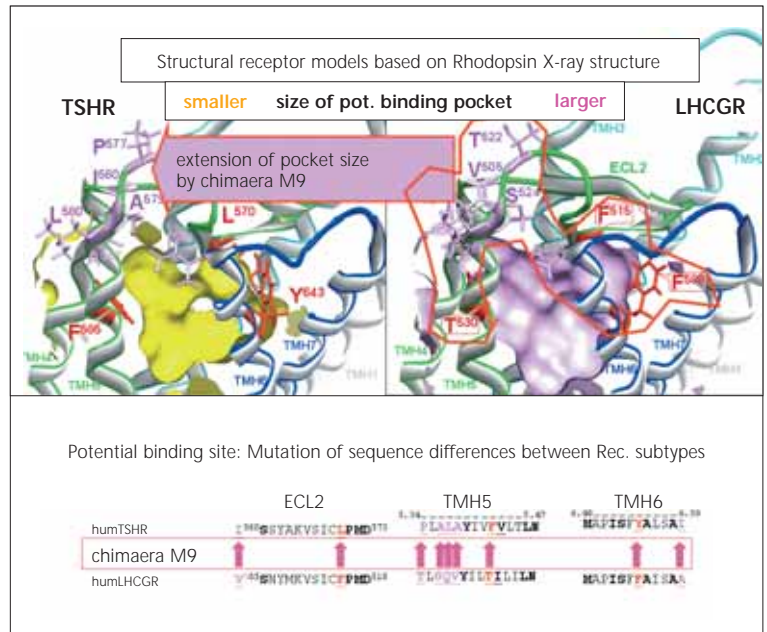
"Modulation of PDZ-domain-mediated protein-protein interactions" (Sub-project of the Research Unit "Interfering with intracellular protein-protein interactions – probing protein functions with small molecules")

FOR 806 TP 5 (Hartmut Oschkinat, Gerd Krause, Jörg Rademann)

Deutsche Forschungsgemeinschaft

"Molekulare und strukturelle Muster parazellulärer Poren durch Subtyp-abhängige Wechselwirkungen in tight junctions" (Sub-project of the Research Unit "Structure and function of tight junctions")

FOR 721 TP 6 (KR 1273/3-1) (Gerd Krause, Peter Schmieder)



Binding pocket for a LMW ligand at CGLHR and TSHR identified in the transmembrane core. Location and dimension is identified by modelling driven mutations of differing residues (Chimaera M9), which line (red) and cover (magenta) the cleft. They are less bulky in CGLHR than in TSHR subsequently the pocket dimension is larger (violet) or smaller (yellow). M9 mutant extended the the binding pocket size and increased LMW ligand activity (Jäschke et al. 2006, *J Biol Chem* 281, 9841-9844).

Deutsche Forschungsgemeinschaft

"Identifizierung eines rezeptorinternen endogenen stillen Transmitters (RIT) im TSH-Rezeptor"

KR 1273/1-1 and 1-2 (Gerd Krause)

Deutsche Forschungsgemeinschaft

"Differenzierung molekularer Determinanten der G Protein Selektivität des TSH-Rezeptors"

KR 1273/2-1 (Gerd Krause)

Deutsche Forschungsgemeinschaft

"Wechselwirkungen von Blut-Hirnschranken-Proteinen und deren Regulation"

BL 308/6-3 and 6-4 (Ingolf Blasig, Gerd Krause)

National Institute of Health (NIH, USA)

"Predoctoral fellowship"

NIH Kleinau (Gunnar Kleinau)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Ronald Kühne

Drug Design

Aims

The research of the Drug Design group focuses on the design of low molecular weight ligands that bind to target proteins which have been either structurally examined within the bio-structural department at the institute or modelled using homology modelling methods. The special expertise of the group includes a wide range of molecular modelling techniques, including protein modelling, two-dimensional- and three-dimensional-QSAR methods to study series of small molecules, virtual screening, molecular dynamics-supported docking and NMR-spectroscopy structure calculations. During the past year, we have enhanced our expertise in computational chemistry, especially in developing methods to estimate drug likeness and the application of database mining.

GPCR ligand design

Model-based design of ligands for G protein-coupled receptors (GPCR) is one of the key areas of our research, specifically on two GPCRs of the human reproductive axis. Compounds targeting these receptors are used for treatment of sex-hormone dependent disorders like (e. g., endometriosis, prostate and breast cancer, uterus myoma, benign prostate hyperplasia, and precocious puberty) and can be used as well for the control of human fertility. The GPCRs are modelled using the bovine rhodopsin X-ray structure as a template and refined using simulated annealing and molecular dynamics simulations in implicit as well as explicit lipid/water environments. These models were exploited in virtual screening and lead optimisation using simulated annealing and pharmacophore mapping, the results of which were used to develop new competitive and non-competitive receptor antagonists. One of the compounds is now in pre-clinical phase. Group members are named on three patents that were filed by our cooperation partners (Zentaris AG and Bayer Schering Pharma).

Proline mediated protein-protein interaction

ProlineRichMotif (PRM) recognition domains (PRD) are highly abundant. Currently, six distinct families of PRM-binding modules (Src-homology 3 (SH3) domains, WW domains, EVH1 (Ena/VASP homology 1) domains, GYF domain (also known as CD2-binding domain), UEV (ubiquitin E2 variant) domains and profilin) are known. Occurring in many multicomponent signalling complexes, such PRDs recognise proline residues by means of stacked aromatic amino acid residues on their surface. PRDs are involved in the modulation of cytoskeleton dynamics, regulation of actin assembly, activation of T-cells, and replication of viruses like HIV or Ebola. High-resolution complex structures for all six domain families

and PRM-containing peptides are available. We designed an organic building block which constitutes a conformationally restricted mimic of vicinal prolines in a poly-proline II (PPII) helix (a frequently occurring secondary structure of at least four successive prolines within a protein). We found that the peptides containing our synthetic PPII-mimic were able to bind to the human VASP-EVH1 domain with a similar dissociation constant to that of the 'wild type' peptide (see fig. 1). Based on our PRM mimic, we want to design new domain-specific ligands interfering PRD-mediated protein-protein interactions. This project is part of the DFG Research Unit entitled "Interfering with intracellular protein-protein interaction – probing protein functions with small molecules".

Development of small molecular 'MHC-loading enhancers' (MLE)

Class I and class II MHC molecules are proteins encoded by the major histocompatibility complex (MHC). They function as peptide receptors that display antigens on the cell surface for surveillance by T-cells. Upon recognition, these antigens can trigger the destruction of the cell – a quality that made them the focus of experimental tumour immune therapies. While exogenously added peptides can activate tumour specific T-cells very efficiently, their efficacy is severely reduced by the low number of MHC molecules accessible for loading. Using a combined strategy of screening, calculation of quantitative structure activity relationships (QSAR), homology modelling, and docking techniques, we found small molecules that are able to generate peptide-receptive MHC molecules. These small molecules open the binding site of human class II MHC molecules by specific interactions with a defined pocket (see fig. 2). The project is funded by the BMBF (FKZ 01GU0514).

Ligand design and computational chemistry within the ChemBioNet

The aim of the ChemBioNet initiative is to bundle academic groups which are interested in the synthesis and biological characterisation of low molecular weight ligands. An important step in developing this initiative is to a comprehensive library of biologically active compounds available for study with respect to binding to variable targets. The design of such a library is challenging, as the expected wide range of biological targets does not allow strategies commonly used for developing focused libraries. The library should contain drug-like and chemically diverse compounds. Therefore, we have developed a new strategy which allows us to select compounds consisting of drug-like scaffolds with high structural diversity and high distribution according to biologically

Members of the group

Dr. Frank Eisenmenger
Dr. Michael Lisurek^{*,†}
Dr. Bernd Rupp^{*}
Dr. Anna Schrey^{*}
Dr. Arvid Söderhäll^{*}
Dr. Jörg Wichard
Robert Opitz (doctoral student)^{*}
Stefan Hübel (system administration)

important physico-chemical properties. The new screening library is now used within the screening projects of our institute. Three projects (i.e., inhibition of SARS protease, inhibition of a tyrosine phosphatase of *M. tuberculosis*, and inhibition of CYP51) were selected for QSAR and modelling studies to optimise the screening hits.

Internal collaborations

The Drug Design group belongs to the Structural Biology section and is closely networked with the Chemical Biology section. Its expertise in the modelling of protein-ligand interaction, homology modelling of proteins (e. g. G protein-coupled receptors), NMR structure calculation, ligand design, and design of screening libraries has led to numerous internal collaborations. The main topics are the design of small ligands to modulate poly-proline mediated protein-protein interactions (AGs Oschkinat, Freund, Beyermann), library design and structure-activity relationships of screening results (AGs Rademann, v. Kries) and the design of MHC-loading enhancers (Freund, v. Kries).

Selected publications^{*}

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Ball LJ, Kühne R, Schneider-Mergener J, Oschkinat H (2005) Recognition of proline rich motifs (PRMs) by protein-protein interaction domains. *Angew Chem Int Ed* 44, 2852-2869.

Söderhäll A, Polymeropoulos EE, Paulini K, Günther E, Kühne R (2005) Antagonist and agonist binding models of the human gonadotropin-releasing hormone receptor. *Biochem Biophys Res Commun* 333, 568-582.

Rothfuss A, Steger-Hartmann T, Heinrich N, **Wichard J** (2006) Computational prediction of the chromosome-damaging potential of chemicals. *Chem Res Toxicol* 19, 1313-1319.

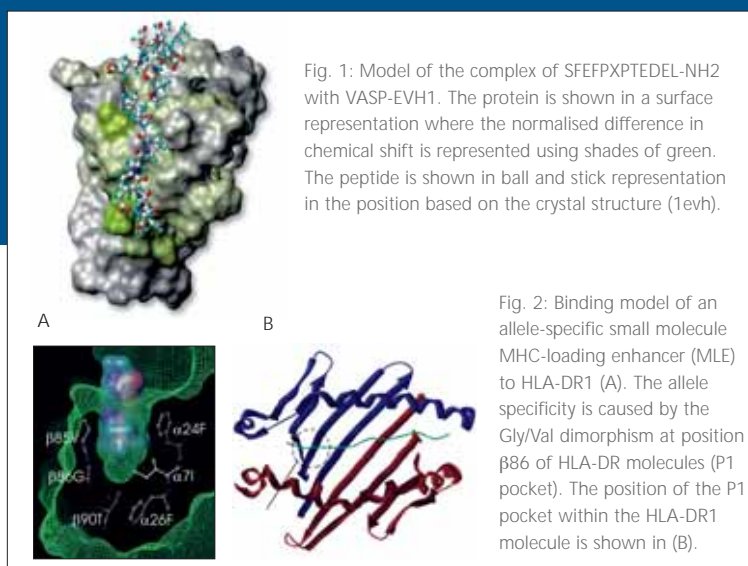
Höpner S, Dickhaut K, Hofstätter M, Krämer H, Rückerl D, **Söderhäll JA, Gupta S, Marin-Esteban V, Kühne R, Freund C, Jung G, Falk K, Röttschke O** (2006) Small organic compounds enhance antigen-loading of class II MHC proteins by targeting the polymorphic P1 pocket. *J Biol Chem* 281, 38535-38542.

Schroeder S, **Schrey AK, Knoll A, Reiß P, Ziemer B, Koert U** (2006) Tetrahydropyran-amino acids: novel building blocks for Gramicidin-hybrid ion channels. *EurJOC* 12, 2766-2776.

[#] FMP authors in bold, group members underlined.

Inventions¹

Wortmann L, Menzenbach B, Cleve A, **Schrey A, Kühne R, Langer G, Muhn P, Koppitz M, Kosemund D**
"Neue Acyltryptophanole als Antagonisten des FSH-Rezeptors"
Rights vested in Schering AG



Wortmann L, Kosemund D, Menzenbach B, Koppitz M, **Schrey A, Muhn P, Kühne R**
"1,2-Diarylacetylen-substituierte Acyltryptophanole"
Rights vested in Schering AG

Kühne R, Oschkinat H, Brockmann C, Schmalz HG, Zaminer J
"P2-Helix-Analoga als biologische Wirkstoffe"
Priority establishing patent application: 25.09.2006
Number of pending applications: 2

Söderhäll A, Kühne R, et al.
"Neue, peptidomimetische, oral verfügbare LHRH-Antagonisten mit Tetrahydrocarbozol-Grundkörper"
Priority establishing patent application: 13.07.2004
Rights sold in 2005

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

"Design and synthesis of low molecular weight prolin-rich motif (PRM) mimetics recognized by PRM binding domains" (Sub-project of the Research Unit "Interfering with intracellular protein-protein interactions – probing protein functions with small molecules")
FOR 806 TP 3 (Ronald Kühne, H.-G. Schmalz/University of Cologne)

Federal Ministry of Education and Research

"Computational Core facility: Compound library optimization, structure based ligand design and NMR-supported mechanistic modelling of MHC" (Sub-project of the collaborative project "MHC-loading enhancers: Molekülmodellierung und Computerchemie, Screening und strukturelle und biophysikalische Untersuchungen")
01GU0514 – KÜ (Ronald Kühne)

Conaris AG

"Feasibility studies on the IL-6/sIL-6R/gp130 complex (the TARGET) to identify structural elements within the TARGET"
Conaris – KÜ1 (Ronald Kühne)

Schering AG

"Zusammenarbeit im Bereich des Modellings und des Ligandendesigns für den putativen 7-Transmembranrezeptor HE-6 und den Melanocortin-1 Rezeptor (MC-1R)"
Schering – KÜ2 (Ronald Kühne)

Zentaris AG

"Auffindung von niedermolekularen Verbindungen als potentielle GnRH-Rezeptor Antagonisten"
Zentaris – KÜ1 (Ronald Kühne)

^{*}part of period reported
^{**}part time
yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Bernd Reif

Solid-State NMR

Aims

Our group is interested in identifying, at atomic resolution, the mechanisms which lead to protein aggregation. We use a combination of solution and solid-state NMR methodology to address this question. In particular, we study the interactions between the molecular chaperone Hsp104 and the prion protein Sup35p in yeast, and between Alzheimer's disease (AD) beta-amyloid peptide with various small heat shock proteins (sHSP) which are found to colocalize in the plaques of AD patients. In order to determine the structure of aggregation-inhibitors of the Alzheimer's disease beta-amyloid peptide, we make use of the spontaneous orientation of the amyloid fibrils in the magnetic field and the residual dipolar couplings (RDCs) induced this way which allow to restrain the structure of the inhibitor in the bound state. This approach is potentially applicable for the development of new therapeutic or diagnostic ligands in the treatment of Alzheimer's disease. In addition, we develop novel MAS Solid-State NMR techniques which allow high sensitivity detection and characterization of dynamics in the solid-state.

Conformation of Peptide Inhibitors Binding to Alzheimer's Disease β -Amyloid Fibrils

AD is the most common form of age-related neurodegenerative disorder and is characterised by the accumulation of insoluble fibrillar aggregates of β -amyloid peptides (A β) into plaques. These contain several other components, including the sHSP α B-crystallin. *In vitro* experiments have shown that in the presence of α B-crystallin, A β fibril formation is suppressed. However, instead of protecting the cell from the neurotoxicity of A β , α B-crystallin leads to increased neurotoxicity. This has led to the hypothesis that the disease-causing form is not the fibrillar species but, rather, an oligomeric A β . In the absence of α B-crystallin, we could show that A β ¹⁻⁴⁰ undergoes a chemical exchange between a monomeric, soluble state and an oligomeric, aggregated state under physiological conditions. By NMR spectroscopy, we identified the chemical groups that are involved in interactions between monomeric and oligomeric A β . We observed that α B-crystallin competes efficiently for A β monomer-monomer interactions. Copper enhances α B-crystallin oligomerisation. At the same time, interactions between α B-crystallin and A β are abolished and A β /A β interactions are promoted. On the basis of our results, we suggest that α B-crystallin is involved in copper-regulated signalling.

MAS Solid-State Methods Development

Sensitivity and resolution impose major limitations on the use of MAS solid-state NMR spectroscopy experiments in higher-molecular-weight samples like membrane proteins. Currently, protons are not routinely employed for detection because of their inherently broad resonance lines. We previously suggested the use of perdeuterated proteins in which exchangeable deuterons are back-exchanged with protons in order to efficiently scale down dipolar interactions. We showed that this approach allows highly sensitive ¹H detection, the determination of long-range ¹H-¹H distances, as well as the localisation of mobile water molecules in the protein structure. In addition, we demonstrated that dynamic information for uniformly ²H, ¹³C, ¹⁵N isotopically enriched, crystalline proteins can be obtained by MAS solid-state NMR spectroscopy.

Solid-State NMR Studies of Membrane Proteins

We have recently carried out a project to structurally characterize the *E. coli* multidrug resistance transporter (EmrE), which plays a role in the development of resistance to antibiotics. This system can serve as a paradigm for antibiotic resistance and the loss of effectiveness of anti-tumor agents.

Internal Collaborations

Structure Determination of AKAP/PKA (Rosenthal/Klußmann)
Influence of D-enantiomer amino acids on the misfolding properties of β -amyloid (Beyermann/Bienert/Keller)
MAS Solid-State NMR Methods Developments (Oschkinat)
Solution-State NMR (Schmieder)
Modelling of A β inhibitor structures (G. Krause)

Members of the group

Dr. Katja Fälber^{*}
Dr. Mangesh Joshi^{*}
Vipin Agarwal (doctoral student)^{**}
Zhongjing Chen (doctoral student)^{*}
Veniamin Chevelkov (doctoral student)^{**}
Muralidhar Dasari (doctoral student)^{**}
Tomas Jacso (doctoral student)^{**}
Rasmus Linser (doctoral student)^{**/*}

Uwe Fink (technical assistance)
Stefan Bibow (student)
Po Wang (student)^{*}

Selected publications[#]

Hologne M, Faelber K, Diehl A, Reif B (2005) Characterization of dynamics of perdeuterated proteins by MAS solid-state NMR. *J Am Chem Soc* 127, 11208-11209.

Narayanan S, Reif B (2005) Characterization of chemical exchange between soluble and aggregated states of beta-amyloid by solution state NMR upon variation of the salt conditions. *Biochemistry* 44, 1444-1452.

Agarwal V, Diehl A, Skrynnikov N, Reif B (2006) High resolution proton detected protoncarbon correlation spectra of a protein in MAS solid-state NMR spectroscopy. *J Am Chem Soc* 128, 12620-12621.

Chevelkov V, Rehbein K, Diehl A, Reif B (2006) Ultra-high resolution in solid-state NMR at high levels of deuteration. *Angew Chem Int Ed* 45, 3878-3881.

Narayanan S, Kamps B, Boelens W, Reif B (2006) α B-crystalline competes with Alzheimer's disease beta-amyloid peptide for peptide-peptide interactions and induces oxidation of Abeta-Met35. *FEBS Lett* 580, 5941-5946.

[#] FMP authors in bold, group members underlined.

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

" Physiologische Bedeutung membranständiger Formen des Amyloid-Vorläuferproteins (APP) und strukturbasierte Vorhersage für die Bindung von Substrat und Inhibitoren an Sekretasen" (Sub-project of the Collaborative Research Center " Struktur und Funktion membranständiger Rezeptoren")

SFB 449 TP B15 (Bernd Reif, Gerd Multhaup/Freie Universität Berlin)

Deutsche Forschungsgemeinschaft

" Structural characterization of chaperone modulated protein aggregation in *S. cerevisiae*" Sub-project of the Collaborative Research Center " Von Molekülen zu Modulen: Organisation und Dynamik zellulärer Funktionseinheiten"

SFB 740 TP B02 (Bernd Reif)

Deutsche Forschungsgemeinschaft

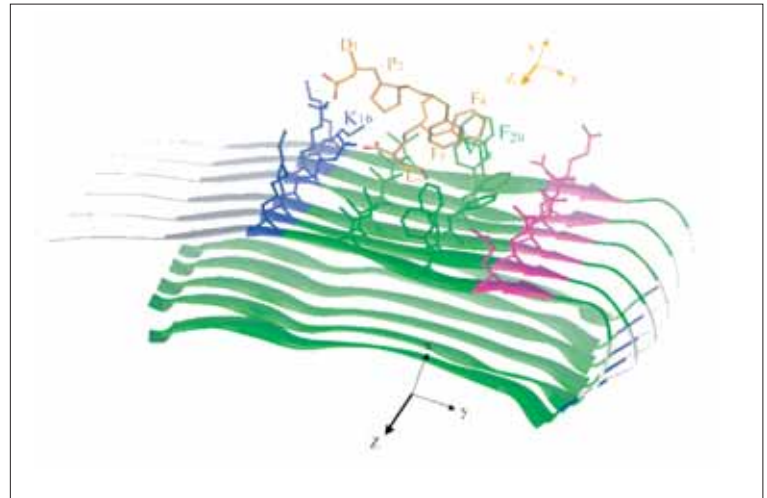
" Strukturelle und kinetische Charakterisierung von Intermediaten der Fehlfaltung und Aggregation von Proteinen" (Sub-project of the Research Unit " Bildung und Stabilität von β -Faltblättern")

FOR 475 (RE 1435/5-2) (Bernd Reif)

Deutsche Forschungsgemeinschaft

" Strukturelle und kinetische Charakterisierung von Intermediaten der Fehlfaltung und Aggregation von Proteinen" (Sub-project of the Research Unit " Bildung und Stabilität von β -Faltblättern")

FOR 475 (RE 1435/5-2V) (Bernd Reif)



Model of iA β 5 bound to β -amyloid fibrils

Deutsche Forschungsgemeinschaft

" Entwicklung NMR-spektroskopischer Methoden zwischen Flüssigkeit und Festkörper. Strukturuntersuchungen an orientierten Biomakromolekülen"

RE 1435/2-2 (Bernd Reif)

Deutsche Forschungsgemeinschaft

" Strukturelle Charakterisierung des Multidrug-Transporters EmrE mittels MAS Festkörper-NMR-Spektroskopie"

RE 1435/3-1 and 3-2 (Bernd Reif)

Land Berlin

" Neuartige Inhibitoren krankheitsrelevanter Proteine" (" Programm zur Förderung von Forschung, Innovationen und Technologien – ProFIT")

IBB 10134759 (Bernd Reif, Christian Freund)

^{*}part of period reported

^{**}part time

^{yellow} Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Christian Freund

Protein Engineering

Aims

Our group is interested in the molecular interactions that govern the assembly of receptor-proximal protein complexes in immune cells. Our primary focus is on scaffolding proteins that mediate the adhesion and cytokine production of T cells. We employ NMR spectroscopy, protein biochemistry, fluorescence microscopy and screening of biomolecular libraries as research tools. We use protein engineering approaches to implement random and structure-based mutagenesis as strategies to derive biologically relevant information. Interfering with these interactions by small molecules and peptides is used as a strategy to infer the function of the individual domains and to test their suitability as potential drug targets in a cellular setting.

GYF domains

GYF domains are small, versatile adapter domains that recognize proline-rich sequences (PRS) in the T cell adhesion molecule CD2 and other proteins by a conserved set of aromatic amino acid side-chains. We have characterized essential structural features of GYF-domain-mediated interactions and will continue with this work. Another project will be to analyze PRS recognition across domain borders. Small molecule interference with GYF domain mediated protein interactions will be probed in collaboration with the Medicinal Chemistry group, the Screening Unit and the Drug Design group at the FMP.

Scaffolding proteins in inside-out signaling

ADAP is a critical component of the intracellular protein complex that regulates communication between the T cell receptor and integrins. As a scaffolding protein, ADAP aids in the coordinated assembly of other adapter proteins, kinases, phosphatases, and small G proteins at the plasma membrane. ADAP itself becomes tyrosine phosphorylated by Fyn tyrosine kinase upon T cell stimulation and is then bound by the SH2 domains of the SLP-76 adapter protein and Fyn kinase. We have determined the three-dimensional structure of the folded domains of ADAP which represent a helically extended variant of the SH3 fold. hSH3 domains were further identified as lipid binding modules. One of the two hSH3 domains of ADAP contains a vicinal disulfide bridge prone to act as a redox switch in T cell signalling. Tandem-tagged expression of ADAP in mammalian cell lines is now aimed at identifying lipid- and redox-dependent components of the multiprotein complex.

The modulation of MHC:peptide interactions by MLE's

Certain small molecules are able to catalyse the exchange of peptides bound to Major Histocompatibility Complex (MHC) class II molecules. Thus, these MHC loading enhancers (MLEs) are able to recover inactive MHC molecules on the surface of antigen presenting cells and to dramatically alter T cell responses. Exploiting this idea offers new ways for tumour immune therapies and vaccinations. As part of a BMBF-consortium investigating MLE:peptide:MHC interactions, we are probing the mechanism of MLE action using NMR spectroscopy and other biophysical methods in order to set a rational basis for the design of second generation MLE compounds.

Internal collaborations

The combination of proteomics approaches, screening of libraries of organic compounds and NMR spectroscopic investigations has led to a network of mutual collaborations: H. Oschkinat/P. Schmieder (NMR investigations of PRS recognition domains)

E. Krause (Differential regulation of scaffolding proteins by tyrosine phosphorylation in T cells).

M. Beyermann: Design and improvement of peptide inhibitors of GYF domains

J. Rademann: Development of a fluorescence polarization-based assay for the screening of cyclophilin A and for GYF domains.

R. Kühne: Molecular modeling of protein:inhibitor complexes and development of novel compounds with improved properties for immunological interference.

(Phage display technology is provided as a useful technique by our lab that allows the identification of peptide inhibitors of protein: protein interactions.)

Selected publications[#]

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Kofler M, **Motzny K**, **Beyermann M**, **Freund C** (2005) Novel interaction partners of the CD2BP2GYF domain. *J Biol Chem* 280, 33397-33402.

Kofler M, **Motzny K**, **Freund C** (2005) GYF domain proteomics reveals interaction sites in known and novel target proteins. *Mol Cell Proteomics* 4, 1797-1811.

Heuer K, **Sylvester M**, Kliche S, Pusch R, **Thiemke K**, Schraven B, **Freund C** (2006) Lipid-binding hSH3 domains in immune cell adapter proteins. *J Mol Biol* 361, 94-104.

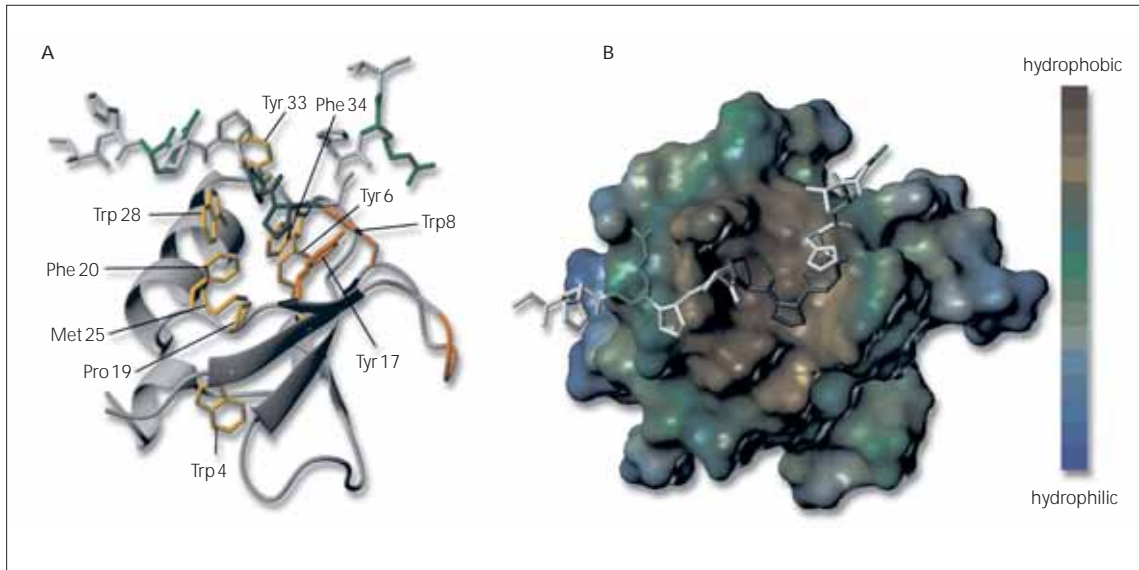
Zimmermann J, **Kühne R**, **Sylvester M**, **Freund C** (2007) Redox-regulated conformational changes in an SH3 domain. *Biochemistry* 46, 6971-6977.

[#] FMP authors in bold, group members underlined.

Members of the group

Dr. Nele Alder-Baerens*
Dr. Katja Heuer*
Dr. Kirill Piotukh
Dr. Jana Sticht*
Dr. Jürgen Zimmermann*
Matthias Heinze (doctoral student)**
Michael Kofler (doctoral student)
Sebastian König (doctoral student)*

Roland Lehmann (doctoral student)*
Marc Sylvester (doctoral student)**
Kathrin Motzny (technical assistance)**
Katharina Thiemke (technical assistance)
Daniela Kosslick (student)*
Andreas Schlundt (student)*



(A) Complex structure of the GYF domain of CD2BP2 with the CD2 peptide SHRPPPPGHRV. GYF domain side chains of conserved residues and residues involved in ligand binding are shown in yellow/orange, while interacting residues of the ligand are depicted in green.

(B) Lipophilic surface potential of CD2BP2-GYF as calculated with the program Sybyl (Tripos Inc., St. Louis, MO). The ligand binding surface of CD2BP2-GYF in complex with the CD2 peptide is shown. The surface is colour coded according to the lipophilic potential, ranging from brown for hydrophobic to blue for hydrophilic surface areas. The ligand is coloured as in (A).

Inventions¹

Freund C, Zimmermann J

“Verfahren zum Redox-Potential-abhängigen Nachweis von Targetmolekülen durch wechselwirkende Polypeptide“

Priority establishing patent application: 25.02.2005
Number of pending applications: 2

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

“Regulatory phosphorylation of scaffolding proteins of the inside-out-signalling complex” (Sub-project of the Collaborative Research Center “Von Molekülen zu Modulen: Organisation und Dynamik zellulärer Funktionseinheiten”)

SFB 740 (FR 1325/4-1) (Christian Freund)

Deutsche Forschungsgemeinschaft

“Analysis and inhibition of GYF domain-mediated protein interactions” (Sub-project of the Research Unit “Interfering with intracellular protein-protein interactions – probing protein functions with small molecules”)

FOR 806 TP 4 (FR 1325/6-1) (Christian Freund)

Federal Ministry of Education and Research

“Struktur-Funktionsbeziehung wichtiger T-Zell-Proteine und Design von Agonisten und Antagonisten der T-Zell vermittelten Immunantwort” (BioFuture laureate)

0311879 (Biofuture) (Christian Freund)

Federal Ministry of Education and Research

“Structural and biophysical investigations of MHC-ligand interactions” (Sub-project of the collaborative project: “MHC-loading enhancers: Molekülmodellierung und Computerchemie, Screening und strukturelle und biophysikalische Untersuchungen”)

01GU0514 – FR (Christian Freund)

Land Berlin

“Entwicklung Oxidations-empfindlicher Biomarker”

IBB 10130236 (Christian Freund)

Land Berlin

“Neuartige Inhibitoren krankheitsrelevanter Proteine”

(“Programm zur Förderung von Forschung, Innovationen und Technologien – ProFIT”)

IBB 10134759 (Bernd Reif, Christian Freund)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Ralf Schüle

Protein Trafficking

Aims

Integral membrane proteins must attain their proper sub-cellular locations. Cells possess a complex transport system, the secretory pathway, to deliver them. The secretory pathway starts with the insertion of the proteins into the membrane of the endoplasmic reticulum. Proteins are folded and correct folding is monitored by a quality control system (QCS). Only correctly folded proteins are allowed to enter the vesicular transport via the ER/Golgi intermediate compartment (ERGIC) and the Golgi apparatus to the plasma membrane. Mutations in the genes of membrane proteins frequently lead to misfolded proteins that fail to pass the QCS. The consequence is disease. Our group studies how the cell carries out quality control on mutant membrane proteins. We hope to identify new drug targets among the proteins involved in QCS. We are also studying how the ER insertion mechanisms of G protein-coupled receptors influence receptor densities at the plasma membrane. Another new project will carry out trafficking studies using tags with switchable fluorescence.

Quality control mechanisms of membrane proteins in post-ER compartments and identification of new drug targets influencing protein transport

Using NDI-causing, transport-defective V_2 Rs, we have shown that a quality control system is also present in the ER/Golgi intermediate compartment (ERGIC), i.e. outside the ER. Some mutant receptors are retained exclusively in the ER whereas others reach the ERGIC and cycle back. We are now characterizing the properties of the mutants which escape from the ER and identifying the proteins involved. First results indicate that the retention mechanism may depend on the location of the mutation within the protein. In coming years, we also intend to use automatic screening microscopes to identify new substances influencing protein transport.

Influence of the ER insertion mechanisms of G protein-coupled receptors on the establishment of specific receptor densities at the cell surface

Some GPCRs contain additional cleavable signal peptides mediating ER insertion. In the case of the CRF receptor subtypes, we have shown that these peptides influence receptor densities at the plasma membrane. The CRF₁ receptor possesses a cleaved signal peptide which strongly promotes ER insertion and thus leads to a high receptor density. In contrast, the CRF_{2(a)} receptor possesses an uncleaved pseudo signal peptide allowing only very low receptor expression.

We now study whether the signal of the CRF_{2(a)} receptor functions as a pseudo signal peptide in every cell type and physiological condition. Moreover, we are characterizing the influence of the pseudo signal peptide on the internalization process. The CRF receptors play a role in depression and anxiety disorders. The study of how defined CRF receptor densities are established may also be of clinical relevance.

Use of tags with switchable fluorescence for trafficking studies

Protein trafficking has been widely studied using fusions with the green fluorescent protein (GFP). For many experiments, however, it would be desirable to have tags whose fluorescence can be converted once the protein has reached a particular compartment. We show that fusions with the Kaede protein from the coral *Trachyphyllia geoffroy* may be useful for these kind of studies.

Internal collaborations

The identification of new drug targets plays a key role in the FMP concept. Our studies are performed in cooperation with many FMP groups:

Structural Bioinformatics: Prediction and modelling of folding/transport-defective vasopressin V_2 receptors (V_2 Rs)

Cellular Imaging: Application of the Kaede protein

Peptide Synthesis: Studies on structure, function and localization of corticotropin releasing factor (CRF) receptors

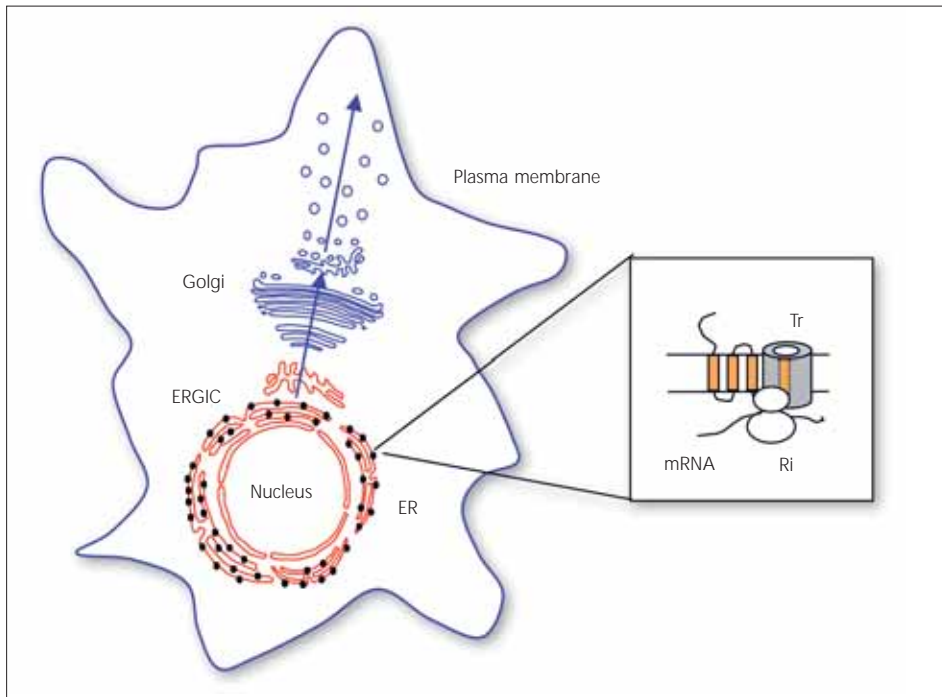
Mass Spectrometry: Identification of protein ligands of the V_2 R

Screening Unit: Influence of small molecules on the transport of membrane proteins (under construction)

Members of the group

Dr. Ute Donalies*
Dr. Gisela Papsdorf (technical assistance)**
Dr. Claudia Rutz (technical assistance)**
Morad Oueslati (doctoral student)*
Antje Schmidt (doctoral student)*
Eva Schönenberger (doctoral student)**/*
Katharina Schulz (doctoral student)
Susanne Vogelbein (doctoral student)**

Dagmar Michl (technical assistance)**/*



Transport of integral membrane proteins along the secretory pathway. In the beginning, proteins are integrated into the ER membrane by the translocon complex (Tr). This process takes place while ribosomal (Ri) translation is in progress. Correctly folded proteins are transported via the ERGIC and the Golgi apparatus to the plasma membrane.

Selected publications*

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Oueslati M, Hermosilla R, Schönenberger E, Oorschot V, **Beyermann M, Wiesner B, Schmidt A**, Klumperman J, **Rosenthal W, Schülein R** (2007) Rescue of a nephrogenic diabetes insipidus-causing vasopressin V₂ receptor mutant by cell-penetrating peptides. *J Biol Chem* 282, 20676-20685.

FMP authors in bold, group members underlined.

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

"Strukturelle und funktionelle Charakterisierung von Transportsignalen des Vasopressin-V₂-Rezeptors und ihrer Proteinliganden" (Sub-project of the Collaborative Research Center "Struktur und Funktion membranständiger Rezeptoren") SFB 449 TP A3 (Ralf Schülein, Walter Rosenthal)

Deutsche Forschungsgemeinschaft

"ER-Insertion und Qualitätskontrolle von G-Protein-gekoppelten Rezeptoren" (Sub-project of the Collaborative Research Center "Zelluläre Signalerkennung und Umsetzung") SFB 366 TP A11 (Ralf Schülein, Walter Rosenthal)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Enno Klußmann*

Anchored Signalling

Aims

The main goal of our group is to identify and characterize A kinase anchoring proteins (AKAPs) involved in vasopressin (AVP)-dependent water reabsorption and cardiac myocyte contractility. We are investigating AKAPs and their partners in search of new drug targets, and generating molecular tools capable of modulating AKAP-dependent protein-protein interactions. Another aim is to clarify the role of the cytoskeleton in the AVP-induced AQP2 shuttle

A kinase anchoring proteins (AKAPs) orchestrate cellular signalling

While cells have a large number of receptors for external stimuli, they possess only a limited number of intracellular signal transduction pathways and second messengers such as cAMP and Ca^{2+} . The compartmentalisation of signalling pathways and selective protein-protein interactions within pathways helps explain how each stimulus evokes a specific cellular response. Spatially distinct, tethered populations of protein kinase A (PKA) read gradients of cAMP locally, permitting the phosphorylation of nearby substrates. The tethering of PKA to cellular compartments is facilitated by its direct interaction with A kinase anchoring proteins (AKAPs). Several AKAPs bind additional signalling molecules, thus acting as scaffolds for multi-protein complexes that propagate and integrate a broad range of cellular events.

We are using the AVP-mediated water reabsorption in renal collecting duct principal cells as a model of compartmentalised cAMP/PKA signalling processes. Upon activation through AVP, PKA phosphorylates the water channel aquaporin-2 (AQP2) located on intracellular vesicles, triggering the redistribution of AQP2 to the plasma membrane. We discovered that the anchoring of PKA to AKAPs is a prerequisite for this translocation. Yet many proteins involved in compartmentalised cAMP-dependent signalling remain to be identified and characterized. We have identified a new splice variant of AKAP18, AKAP18 δ , which appears to anchor PKA in close proximity to AQP2 on intracellular vesicles. Phosphodiesterases of the PDE4 family degrade cAMP. We detected the isoform PDE4D on AQP2-bearing vesicles. PDE4D directly interacts with AKAP18 δ and is involved in the regulation of the AVP-dependent redistribution of AQP2 to the plasma membrane. In addition, we are currently investigating the roles of AKAP Ht31 and its rat orthologue Rt31, which we identified, in the AVP-induced redistribution of AQP2.

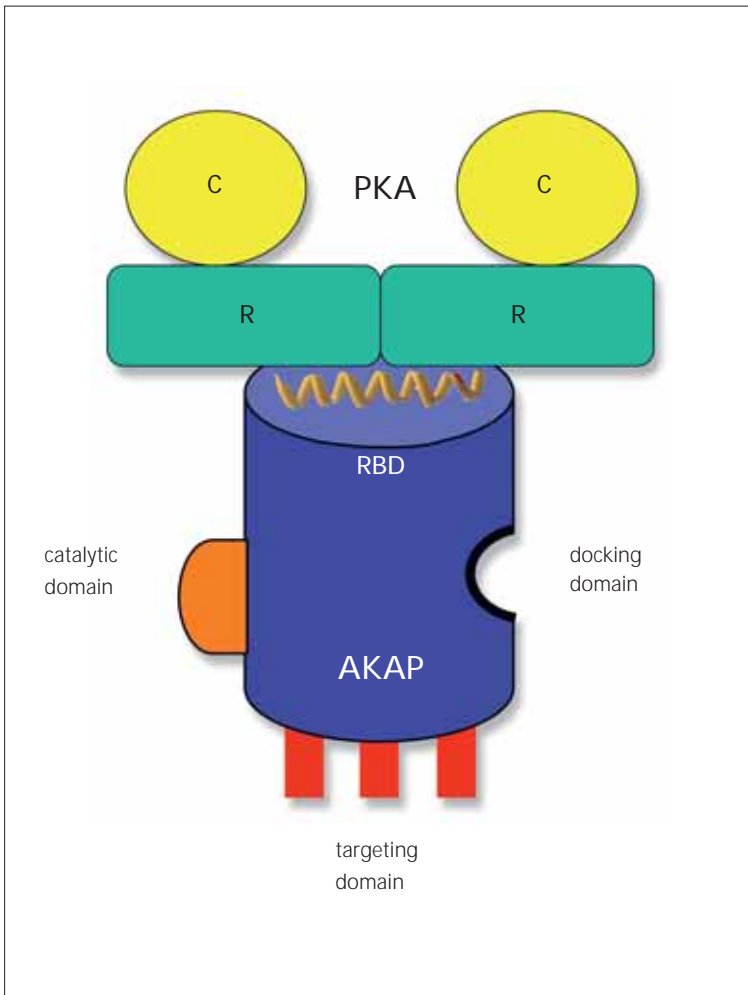
Pharmacological interference with AKAP functions

AKAPs are essential in many other cAMP-controlled processes (e. g. cardiac myocyte contractility and insulin secretion). Disregulation of such processes leads to diseases including nephrogenic diabetes insipidus, water retention, hypertension, and heart disease. We developed AKAP18 δ -derived, high affinity peptide disruptors of AKAP-PKA interactions. Such peptides prevent the AVP-induced redistribution of AQP2 to the plasma membrane and prevent β -adrenoceptor-evoked increases in L-type Ca^{2+} channel currents in cardiac myocytes, an effect resembling that of beta-blockers. Pharmacological interference with AKAPs may, therefore, prove to be a suitable concept for the treatment of diseases. In the future, we aim to develop small molecules that disrupt interactions between AKAPs and their protein partners. The disrupting agents will be utilised for functional studies of AKAPs in physiologically relevant processes including the AVP-induced redistribution of AQP2 to the plasma membrane, cardiac myocyte contraction, and renin and proton secretion. We also plan to utilise these agents to evaluate the potential of AKAPs as drug targets in animal models.

The role of the cytoskeleton in the vasopressin-induced AQP2 shuttle

The AVP-induced redistribution of AQP2 to the plasma membrane involves microtubules and F-actin, and a further interest of the group is to elucidate the mechanisms by which the cytoskeleton participates in the shuttle's regulation. We have shown that inhibition of the small GTPase Rho and the resulting reduction of F-actin are prerequisites for the AQP2 shuttle. A future goal is to elucidate the signalling cascade downstream of Rho that regulates F-actin and, thereby, the cellular localisation of AQP2. A further goal is to visualise and mechanistically explain the movement of AQP2-bearing vesicles along microtubules and F-actin in live IMCD cells. Recently, we found that the motor protein myosin Vb transports AQP2-bearing vesicles to the plasma membrane. This involves the myosin Vb-binding proteins Rab11 and Rab11-FIP2.

*Position financed by the Charité.



Schematic illustration of an A kinase anchoring protein (AKAP) with different domains. The presence of a structurally conserved binding domain for the regulatory subunits of PKA (RBD, regulatory subunit binding domain) is the unifying characteristic of all AKAPs. The targeting domain, which tethers the AKAP to cellular compartments, and docking domains, which bind further signaling molecules (e. g. phosphatases, other kinases, or phosphodiesterases) are specific for individual AKAPs. Catalytic domains (with e. g. RhoGEF-activity) have been identified in only a few AKAPs.

Internal collaborations

Identification and characterisation of AKAPs

Eduard Stefan, Christian Hundsruker, Katja Santamaria, Viola Popara, Dorothea Lorenz, Andrea Geelhaar, Anita Neumann

Inhibition of AKAP-dependent protein interactions

Marta Szaszak, Frank Christian, Christian Hundsruker, Dorothea Lorenz, Andrea Geelhaar, Anita Neumann

The role of the cytoskeleton in the AVP-induced AQP2 shuttle

Pavel Nedvetsky, Andrea Geelhaar, Anita Neumann

Group Leader:
Enno Klußmann

Anchored Signalling

Selected publications[#]

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Tamma G, **Klußmann E**, Oehlke J, Krause E, **Rosenthal W**, Svelto M, Valenti G (2005) Actin remodelling requires ERM function to facilitate AQP2 apical targeting. *J Cell Sci* 118, 3623-3630.

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Lygren B, Carlson CR, **Santamaria K**, Lissandron V, **McSorley T**, **Litzenberg J**, **Lorenz D**, **Wiesner B**, **Rosenthal W**, Zaccolo M, Tasken K, **Klußmann E** (2007) AKAP-complex regulates Ca²⁺ reuptake into heart sarcoplasmic reticulum. *EMBO Rep* (in press).

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Sachs BD, Baillie GS, McCall JR, Passino M, Schachtrup C, Wallace DA, Dunlop AJ, MacKenzie KF, **Klußmann E**, Lynch MJ, Sikorski SL, Nuriel T, Tsigelny I, Zhang J, Houslay MD, Chao MV, Akassoglou K (2007) p75 Neurotrophin receptor regulates tissue fibrosis through inhibition of plasminogen activation via a PDE4/cAMP/PKA pathway. *J Cell Biol* 18, 1119-1132.

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[#] FMP authors in bold, group members underlined.

Inventions¹

Rosenthal W, **Klußmann E**, Carlson C, Lygren B, Tasken K
"New A-kinase anchoring proteins in the heart as potential drug targets"
Priority establishing patent application: 05.09.2005
Shared invention of University of Oslo and FMP
Number of pending applications: 2

Rosenthal W, **Klußmann E**, **Christian F**, **Rademann J**, **Meyer S**
"Niedermolekulare Substanzen zur Hemmung der Interaktion zwischen Proteinkinase A und Proteinkinase A-Ankerprotein"
Priority establishing patent application: 18.05.2005
Number of pending applications: 2

Rosenthal W, **Klußmann E**, **Hundsrucker C**
"Peptide zur Inhibition der Interaktion von Proteinkinase A und Proteinkinase A-Ankerprotein"
Priority establishing patent application: 29.06.2004
Number of pending applications: 2

Rosenthal W, **Klußmann E**, **Oksche A**
"Neue Spleißvariante eines Proteinkinase A-Ankerproteins und Verwendung dieser"
Priority establishing patent application: 07.02.2003
Number of patents granted: 1 (16.08.2006)
Number of pending applications: 5

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft
"Defining cellular functions of the A kinase-anchoring protein AKAP18 by pharmacological ablation of its interaction sites" (Sub-project of the Research Unit "Interfering with intracellular protein-protein interactions – probing protein functions with small molecules")
FOR 806 TP 1 (KL 1415/4-1) (Enno Klußmann, Walter Rosenthal, Rudolf Volkmer/Charité – Universitätsmedizin Berlin)

Deutsche Forschungsgemeinschaft
"The role of the cytoskeleton in the vasopressin-induced aquaporin-2 shuttle in renal collecting duct principal cells" (Sub-project of the Research Unit "Epithelial mechanisms of renal volume regulation")
FOR 667 TP 3 (KL 1415/3-1) (Enno Klußmann, Walter Rosenthal)

Deutsche Forschungsgemeinschaft
"Die Rolle des Zytoskeletts bei der Vasopressin-induzierten Translokation von Aquaporin-2 in die Plasmamembran renaler Hauptzellen"
KL 1415/1-1 (Enno Klußmann, Walter Rosenthal)

Deutsche Forschungsgemeinschaft
"Biologische Funktionen des Proteinkinase-A-Ankerproteins AKAP18"
KL 1415/2-2 (Enno Klußmann, Walter Rosenthal)

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Members of the group

Dr. Volker Henn*	Frank Christian (doctoral student)**/*	Marion Holweck (technical assistance)*
Dr. Anne Höner (coordination thera cAMP)**/*	Christian Hundsrucker (doctoral student)**	Anita Neumann (technical assistance)*
Dr. Theresa McSorley*	Viola Popara (doctoral student)**	Marcin Teodorczyk (student)*
Dr. Pavel Nedvetsky	Katja Santamaria (doctoral student)**	
Dr. Marta Szaszak*	Philipp Skroblin (doctoral student)**/*	
Dr. Anna Vossenkämper*	Eduard Stefan (doctoral student)**/*	
Dr. Gisela Papsdorf (technical assistance)**	Andrea Geelhaar (technical assistance)	
	Michael Gomoll (technical assistance)*	

Deutsche Forschungsgemeinschaft

" 1st International meeting on Anchored cAMP Signalling Pathways, Berlin-Buch"

4851/170/05 (Enno Klußmann, Walter Rosenthal)

European Community (6th Framework Programme)

STREP thera-cAMP: " Identification of therapeutic molecules to target compartmentalized cAMP signalling networks in human disease"

LSH-2005-1.2.5-3 (Enno Klußmann – Coordinator)

European Community (5th Framework Programme)

" Anchored cAMP signalling – implications for treatment of human disease"

QLK3-CT-2002-02149 (Enno Klußmann, Walter Rosenthal)

German Academic Exchange Service (Vigoni Programme)

" Die Rolle der ERM-Proteine Ezrin, Radixin und Moesin bei der Vasopressin-vermittelten Wasserrückresorption in renalen Hauptzellen"

PPP-Italien (Enno Klußmann, Walter Rosenthal)

Charité – Universitätsmedizin Berlin

" 1st International meeting on Anchored cAMP Signalling Pathways, Berlin-Buch"

AKAP-Meeting WR (Enno Klußmann, Walter Rosenthal)

Cotech, Schering AG, Biaffin, BioLog, Geyer, TSB Berlin

" 1st International meeting on Anchored cAMP Signalling Pathways, Berlin-Buch"

AKAP-Meeting BM (Björn Maul, Enno Klußmann)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.

Group Leader:
Alexander Oksche,
Charité

G Protein-coupled Receptors

This liaison group (Charité) closed on April 30, 2006

G protein-coupled receptors (GPCRs) are involved in many biological processes including the control of blood pressure and salt excretion. Dysregulation of GPCRs is associated with cardiovascular disease (e. g., hypertension, coronary heart disease, and cerebral ischemia), but the molecular mechanisms are poorly understood. One of our main goals is to study the signalling cascades initiated by GPCRs which play a pivotal role in the regulation of vascular tone and remodelling.

Signalling pathways of the endothelin B receptor in vascular smooth muscle cells

Endothelin-1 acts on endothelin A (ET_A) and endothelin B (ET_B) receptors. ET_A receptors are predominantly expressed in vascular smooth muscle cells (VSMCs) mediating vasoconstriction whereas ET_B receptors are mainly expressed in endothelial cells and elicit vasodilatation. ET_B receptors are up-regulated in VSMCs of arteriosclerotic lesions yet their functional role remains elusive. Recently we showed that the ET_B receptor undergoes an agonist-induced N-terminal proteolysis by a metalloprotease, resulting in a non-glycosylated, truncated ET_B receptor.

VSMCs expressing the wild-type or a mutant D2-64 ET_B receptor differ in their ability to mediate ERK1/2 activation. Results of our studies suggest that biphasic ERK1/2 activation via the ET_B receptor contributes to differentiation in VSMCs and depends on a G_i/bg/metalloprotease-induced transactivation of the EGF receptor.

Characterisation of rat and human urotensin receptors

The role of the recently identified urotensin (UT) receptor for vascular regulation is poorly defined. Stimulation of the UT receptor leads to a long-lasting vasoconstriction of rat aortic ring preparations. Re-stimulation after 60 minutes causes the same vasoconstriction as the initial administration. We discovered that UT receptors expressed in human embryonic kidney (HEK) cells are internalised rapidly but recycled quantitatively within 60 minutes. Immunofluorescence studies further demonstrated that agonist-bound UT receptors were internalised and sorted to recycling and sorting endosomes but did not appear in late endosomes or lysosomes. Live cell imaging of HEK cells co-expressing UT receptors with arrestin3-GFP demonstrated that application of fluorescent Ull resulted in a transient recruitment of arrestin3-GFP. During endocytosis of UT receptors, arrestin3 was not co-localised. Internalisation of UT receptor-GFP fusion proteins was also found in mouse embryonic

fibroblasts (MEF) lacking endogenous arrestin expression (*arr2^{-/-}arr3^{-/-}*) which was comparable to that seen in MEF cells with endogenous arrestin expression. The data demonstrate that UT receptors internalise arrestin-independently and recycle quantitatively. The continuous externalisation of UT receptors provides the basis for repetitive and lasting Ull-mediated vasoconstriction.

Pharmacochaperone-mediated restoration of cell surface expression of mutant vasopressin V₂ receptors

Water homeostasis in mammals is regulated through vasopressin, acting on the vasopressin V₂ receptor (V₂R) expressed in the renal collecting duct. In X-linked nephrogenic diabetes insipidus (NDI), the kidney shows a resistance to the action of vasopressin, caused by inactivating mutations of the human V₂R (hV₂R) gene. Most of the encoded hV₂R mutants are retained within the ER and not transported to the cell surface.

To clarify the mechanisms of antagonist-promoted restoration of cell surface expression, we investigated the predominantly ER-retained murine V₂ receptor (mV₂R). Laser scanning microscopy (LSM) of transiently transfected HEK293 cells revealed that wild-type mV₂R.GFP and mutant hV₂R.GFPs were predominantly located within the ER. In live cell imaging analysis, as well as in immunoblot and cell surface biotinylation experiments, we found that both a membrane-permeable V₂ receptor-selective antagonist and a V_{1a} receptor-selective antagonist restored cell surface delivery of the mV₂R. The half maximal concentrations (EC₅₀) required for cell surface delivery were very similar to the K_d values of the two antagonists for the mV₂R, suggesting that pharmacochaperones act directly on the ER-retained receptor and not indirectly, e. g. by interactions with molecular chaperones. Thus, pharmacochaperones interact directly with the ER-retained receptor via the binding cleft.

Members of the group²

Dr. Evelina Grantcharova-Angelova^{*}
Berit Bohnkamp (doctoral student)^{*}
Solveig Großmann (doctoral student)^{** / *}
Anja Löffler (doctoral student)^{** / *}

Selected publications[#]

de Mattia F, Savelkoul PJ, Kamsteeg EJ, Konings IB, van der Sluijs P, Mallmann R, **Oksche A**, Deen PM (2005) Lack of arginine vasopressin-induced phosphorylation of aquaporin-2 mutant AQP2-R254L explains dominant nephrogenic diabetes insipidus. *J Am Soc Nephrol* 16, 2872-2880.

Giebing G, Tölle M, **Jürgensen J**, **Eichhorst J**, **Furkert J**, **Beyermann M**, Neuschäfer-Rube F, **Rosenthal W**, Zidek W, van der Giet M, **Oksche A** (2005) Arrestin-independent internalization and recycling of the urotensin receptor contribute to long-lasting urotensin II-mediated vasoconstriction. *Circ Res* 97, 707-715.

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Endres-Becker J, Heppenstall PA, Mousa SA, Labuz D, **Oksche A**, Schafer M, Stein C, Zollner C (2007) μ -opioid receptor activation modulates transient receptor potential vanilloid 1 (TRPV1) currents in sensory neurons in a model of inflammatory pain. *Mol Pharmacol* 71, 12-18.

Jankowski V, Vanholder R, van der Giet M, Tölle M, Karadogan S, Gobom J, **Furkert J**, **Oksche A**, **Krause E**, Tran TN, Tepel M, Schuchardt M, Schlüter H, Wiedon A, **Beyermann M**, Bader M, Todiras M, Zidek W, Jankowski J (2007) Mass-spectrometric identification of a novel angiotensin peptide in human plasma. *Arterioscler Thromb Vasc Biol* 27, 297-302.

Niendorf S, **Oksche A**, **Kisser A**, Lohler J, Prinz M, Schorle H, Feller S, Lewitzky M, **Horak I**, **Knobeloch KP** (2007) Essential role of ubiquitin-specific protease 8 for receptor tyrosine kinase stability and endocytic trafficking *in vivo*. *Mol Cell Biol* 27, 5029-5039.

[#] FMP authors in bold, group members underlined.

Inventions¹

Rosenthal W, **Klußmann E**, **Oksche A**

“Neue Spleißvariante eines Proteinkinase A-Ankerproteins und Verwendung dieser”

Priority establishing patent application: 07.02.2003

Number of patents granted: 1 (16.08.2006)

Number of pending applications: 5

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

“Rolle der Endothelin-B-Rezeptor-vermittelten Signaltransduktion des Wasserhaushalts” (Sub-project of the Graduate School “Zelluläre Signalerkennung und Umsetzung”)

GK 276 (Alexander Oksche, Walter Rosenthal)

Deutsche Forschungsgemeinschaft

“Nachweis von Protein-Protein-Interaktionen an Endothelinrezeptoren und deren Bedeutung für die Rezeptorfunktion” (Sub-project of the Graduate School “Vaskuläre Regulationsmechanismen”)

GK 865 (Alexander Oksche, Walter Rosenthal)

^{*}part of period reported

^{**}part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Burkhard Wiesner

Cellular Imaging

Aims

Our group uses the full repertoire of light microscopy techniques (confocal laser scanning microscopy for 3D, two-photon excitation, uncaging processes, FCS, FRAP, FRET, FLIM, cellular uptake of peptides, colocalisation studies, intracellular ion concentrations, etc.) in conjunction with the electron microscopic and electrophysiological techniques to address questions of biological and pharmacological importance. The expertise of group members is in high demand by other groups, so we have been evolving into a sort of core facility that collaborates on a wide range of projects.

Applications of new caged compounds

Caged compounds are photolabile inactive derivatives of biological active substances, from which the active biomolecule, e. g. an intracellular transmitter, is rapidly liberated by UV light. The group has demonstrated the application of novel caged substances (caged cAMP, caged cGMP, caged ATP) and used them successfully to study cyclic nucleotide-gated ion channels. Furthermore, by using the fluorescence properties of the released photoproduct, the suitability of a method for quantifying the released amount of cAMP after photolysis of BCMACM-caged cAMP was shown in inner medullary collecting duct

Degradation of V_2 vasopressin receptor mutants

Degradation of intracellular proteins is necessary to maintain cellular homeostasis. There are two main degradative pathways in the cells: lysosomes and the ubiquitin-proteasome system. We study the degradation pathways of the human V_2 vasopressin receptor (V_2R) and mutant V_2Rs . Microscopic and biochemical analysis of the mutants showed that the different mutants localize to different compartments of the secretory pathway: some mutants are retained in the ER exclusively, others reach the ER-Golgi intermediate compartment (ERGIC) or are transported to the Golgi. In our interest are especially the mutants in ERGIC and Golgi, is there a special degradation pathway or are they substrate for the ER-associated degradation?

Motility of proteins in cellular structures

Fluorescence recovery after photobleaching (FRAP) is a popular method that utilizes changes in the recovery of fluorescence after local bleaching events to measure the dynamics of 2D or 3D molecular mobility e. g. diffusion or transport of fluorescence-labelled molecules in mem-

branes or inside living cells. In most cases it is sufficient to determine the accurate half- and final recovery time to describe the differences between proteins or protein mutants. In the modification of the data analysis we address the following: the quantitative determination of the immobile fraction, quantitative calculation of protein interactions, the determination of the time course of the binding of different proteins.

Protein-protein interaction (dimerisation)

Fluorescence resonance energy transfer (FRET) is the non-radiative transfer of photon energy from a donor fluorophore to an acceptor fluorophore when both are located within close proximity of 1-10 nm. Another method for investigations of protein-protein interactions is Fluorescence lifetime imaging microscopy (FLIM). Using both biophysical methods (FRET and FLIM) and using some model constructs (vectors) we will examine whether G protein-coupled receptors form dimers and/or monomeric proteins at the plasma membrane of living cells.

Internal collaborations

The group members have experience in microscopic techniques and single-cell techniques which have led to good collaborations with many groups. Thus, the group is developing into more a core facility. Thereby, we are seen as an equal research partner. The identification of new drug targets plays a key role in the concept of the FMP. Therefore, investigations are performed in cooperation with many groups at the institute:

Protein Trafficking: studies of colocalisation, protein-protein interactions, translocation of proteins

Anchored Signalling: protein-protein interactions, translocation of proteins, intracellular ion concentrations

Molecular Cell Physiology: studies of colocalisation, protein-protein interactions

Cellular Signal Processing: translocation of proteins, motility of proteins in cellular structures

Peptide Transport: cellular uptake of peptides

Peptide Biochemistry: protein-protein interactions, cellular uptake of peptides

Synthetic Organic Biochemistry: applications of new caged compounds

Biophysics: water permeability of cell membranes

Members of the group

Dr. Dorothea Lorenz

Katja Lautz (doctoral student)**/†

Ursula Brandt (technical assistance)*

Jenny Eichhorst (technical assistance)

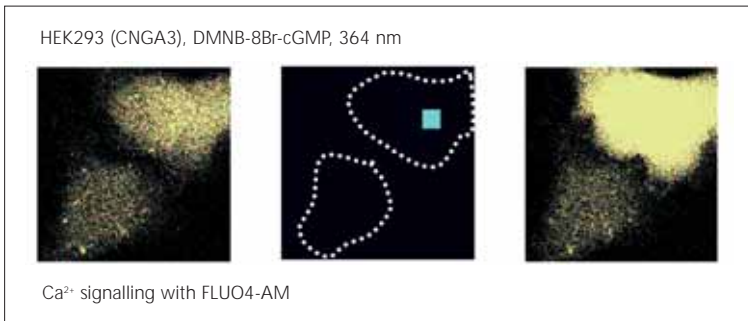
Brunhilde Oczko (technical assistance)

Martina Ringling (technical assistance)**

Anke Bielicke (student)*

Marleen Neumann (student)*

Antje Schmidt (student)*



By using the fluorescence properties of the released photoproduct, the suitability of a method for quantifying the released amount of cAMP after photolysis of BCMACM-caged cAMP was shown in inner medullary collecting duct (IMCD of rats) and human embryonic kidney (HEK) cells (patch clamped cells).

Selected publications*

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Hagen H, Dekowski B, Nache V, Schmidt R, Geißler D, Lorenz D, Eichhorst J, Keller S, Kaneko H, Benndorf K, Wiesner B (2005) Coumarinylmethyl esters for ultrafast release of high concentrations of cyclic nucleotides upon one- and two-photon photolysis. *Angew Chem Int Ed* 44, 7887-7891.

Kraetke O, Wiesner B, Eichhorst J, Furkert J, Bienert M, Beyermann M (2005) Dimerization of corticotropin-releasing factor receptor type 1 is not coupled to ligand binding. *J Recept Signal Transduct Res* 25, 251-276.

Lödige I, Marg A, Wiesner B, Malecova B, Oelschläger T, Vinkemeier U (2005) Nuclear export determines the cytokine sensitivity of STAT transcription factors. *J Biol Chem* 280, 43087-43099.

Bulwin GC, Heinemann T, Bugge V, Winter M, Lohan A, Schlawinsky M, Schulze A, Walter S, Sabat R, **Schülein R, Wiesner B, Veh RW, Lohler J, Blumberg RS, Volk HD, Utku N** (2006) TIRC7 inhibits T cell proliferation by modulation of CTLA-4 expression. *J Immunol* 177, 6833-6841.

McSorley T, Stefan E, Henn V, Wiesner B, Baillie GS, Houslay MD, Rosenthal W, Klussmann E (2006) Spatial organization of AKAP18 and PDE4 isoforms in renal collecting duct principal cells. *Eur J Cell Biol* 85, 673-678.

Rutz C, Renner A, Aiken M, Schulz K, Beyerman M, Wiesner B, Rosenthal W, Schülein R (2006) The corticotropin-releasing factor receptor type 2a contains an N-terminal pseudo signal peptide. *J Biol Chem* 281, 24910-24921.

FMP authors in bold, group members underlined.

Inventions¹

Hagen V, Kaupp UB, Bendig, Wiesner B

“ Neue photolabile Coumarinylmethylester von cyclischen Nucleotiden, Verfahren zu deren Herstellung und ihre Verwendung”

Priority establishing patent application: 20.04.2000

Number of patents granted: 4 (24.08.2005)

Number of pending applications: 2

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

“ Degradations-Mechanismen des humanen Vasopressin-V₂-Rezeptors und einiger von Patienten mit X-chromosomalem nephrogenem Diabetes insipidus isolierten V₂-Rezeptormutanten”

HE 4486/1-1 and 1-2 (Burkhard Wiesner, Ricardo Hermsilla/Charité – Universitätsmedizin Berlin)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Ingolf E. Blasig

Molecular Cell Physiology

Aims

This group focuses on the elucidation of structure, function, and manipulation of cell-cell contacts. The major objective is to explore tight junctions (TJ) in barrier-forming endothelial and epithelial cells under normal and pathological conditions to disclose the neuropathophysiological mechanisms underlying stroke, lesional epilepsy, and other conditions to find better therapies. In addition, the development of new strategies specifically modulating the blood-brain barrier (BBB) may lead to improved drug delivery. The tightness of the BBB is determined by transmembrane proteins which constantly seal the intercellular cleft. We are concentrating on the oligomerisation, scaffolding, and regulation of TJ proteins.

Oligomerisation of claudins

In the BBB, claudin-5 tightens the barrier for pharmacologically relevant molecules with a molecular weight smaller than 800 Da. However, the molecular interaction mechanism and regulation are unknown. For the first time, we demonstrated self-association of claudin-5 in cell-cell contacts of intact cells using fluorescence resonance energy transfer (FRET), a prerequisite for explaining the strand formation mechanism (Blasig et al. 2006, *Cell Mol Life Sci* 63, 505-514). We also identified the amino acid motif and mechanism of interaction.

Functional investigations are in progress to verify the effect of mutations on the BBB as well as to develop small molecules (in collaboration with J. P. v. Kries, Screening Unit, FMP) to modulate the barrier tightness for pharmacological use.

Scaffolding function of ZO-1

Studies of ZO-1 (scaffolding cell contact and cytoskeleton proteins) demonstrated that the SH3-domain, hinge-region, and GuK-domains interact as a common functional unit which self-associates. We found that occludin's cytosolic coiled-coil domain (acidic helices) interacts on the unit (basic helices) and leads to dimerisation. We devised a general dimerisation concept of transmembrane TJ proteins via dimerising ZO-1. We identified proteins that preferentially bind to the hinge region of ZO-1, suggesting that this region acts as adaptor for potential regulators such as occludin, adherens junction protein-catenin, G-proteins, G protein regulators, Ca²⁺-related ahnak, or two subunits of an L-type Ca²⁺-channel.

A common regulatory mechanism of forming associates is implicated which represents a general molecular feature regulating ZO-1's scaffolding function. While these

studies must be supplemented with functional assays, they offer new perspectives for the specific modulation of TJ.

Regulation of tight junctions

We discovered a regulatory antagonism of the tightness of TJ by isoforms of protein kinase C (PKC).

Conventional, Ca²⁺ dependent PKC blocked TJ assembly and tightness, whereas novel PKC, independent of Ca²⁺, promoted both. We plan to identify the sites and pathological relevance of the phosphorylations in order to understand the molecular mechanism and to develop new pharmacological approaches. Clinical studies were continued to confirm our findings that oxidative stress may injure the BBB as well as to optimise treatment. For mood disorders accompanied by depression, we found that opening of the BBB as well as selected antipsychotics reduced the disturbances.

Outlook

We plan a reconstitution of functional TJ at the protein, cellular, and animal levels. We will develop a common model of TJ assembly showing how ZO-1 dimers form higher oligomers to promote strand formation and to regulate the BBB. Crystallisation studies of interacting proteins will be expanded to include new TJ-proteins. Regulatory aspects will be further studied such as the direct influence of G proteins. Moreover, protein-protein binding assays will be developed to screen for pharmacologically relevant agents in the FMP compound library. In this way, new therapeutic approaches to improve the delivery of antiepileptic agents to overcome the BBB in multidrug resistant epilepsy may be found.

Internal collaborations

Our interdisciplinary approach to studying the structure, function, and interactions of cell contact proteins has led to numerous collaborations:

Spectroscopic analyses are performed with peptide interactions, mass spectrometry, and the Protein Structure group to develop structure, function, interaction models with structural bioinformatics.

Functional studies of the distribution, interaction of TJ proteins, and paracellular tightness with living cells to prove physiological relevance.

Biophysical (SPR), chemical (peptide mapping) methods, and confocal microscopy (FRET) are used in collaboration (Department of Peptide Chemistry and Biochemistry, Screening Unit, Cellular Imaging group) to characterize binding between TJ proteins.

We screen and design substances to modulate TJ in with the Screening Unit and Peptide Synthesis group.

Members of the group

Dr. Reiner Haseloff

Dr. Jörg Piontek

Dr. Christine Rückert

Olaf Kostbahn (scientist)**/*

Ariane Schuster (scientist)**/*

Manjot Singh Bal (doctoral student)**/*

Victor Manuel Castro Villela (doctoral student)*

Dörte Lohrberg (doctoral student)**

Kerstin Mikoteit (doctoral student)**/*

Christian Piehl (doctoral student)**/*

Juliane Walter (doctoral student)**

Lars Winkler (doctoral student)**

Sandra Bittmann (technical assistance)*

Barbara Eilemann (technical assistance)

Gislinde Hartmann (technical assistance)**/*

Corinna Gagell (student)*

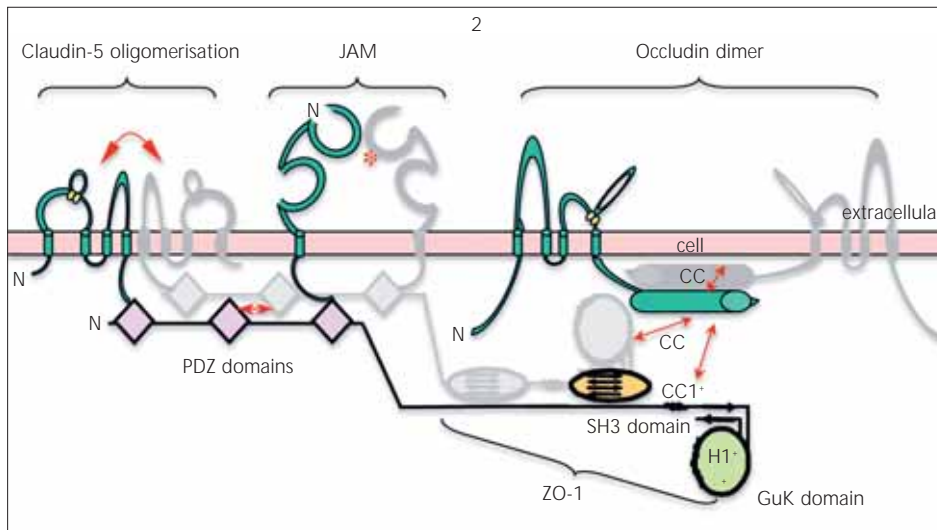
Christian Niehage (student)*

Cletus Timah Sali (student)*

Martin Voß (student)*

Constanze Wolf (student)*

Nikolaj Zuleger (student)*



Oligomerisation concept of TJ proteins

Selected publications*

Müller SL, Portwich M, **Schmidt A**, **Utepergenov DI**, Huber O, **Blasig IE**, **Krause G** (2005) The tight junction protein occludin and the adherens junction protein alpha-catenin share a common interaction mechanism with ZO1. *J Biol Chem* 280, 3747-3756.

Zassler B, **Blasig IE**, Humpel C (2005) Protein delivery of caspase-3 induces cell death in malignant C6 glioma and brain capillary endothelial cells. *J Neurooncol* 71, 127-134.

Andreeva AY, **Piontek J**, **Blasig IE**, **Utepergenov DI** (2006) Assembly of tight junction is regulated by the antagonism of conventional and novel protein kinase C isoforms. *Int J Biochem Cell Biol* 38, 222-233.

Blasig IE, **Winkler L**, **Lassowski B**, **Mueller SL**, **Zuleger N**, **Krause E**, **Krause G**, Gast K, Kolbe M, **Piontek J** (2006) On the self-association potential of transmembrane tight junction proteins. *Cell Mol Life Sci* 63, 505-514.

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Sury MD, Frese-Schaper M, Muhlemann MK, Schulthess FT, **Blasig IE**, Tauber MG, Shaw SG, Christen S (2006) Evidence that N-acetylcysteine inhibits TNF-alpha-induced cerebrovascular endothelin-1 upregulation via inhibition of mitogen- and stress-activated protein kinase. *Free Radic Biol Med* 41, 1372-1383.

FMP authors in bold, group members underlined.

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

"Lokalisation und Phosphorylierungsmuster von Occludin" (Sub-project of the Graduate School "Schadensmechanismus im Nervensystem – Einsatz von bildgebenden Verfahren") GK 238/3 (Ingolf Blasig)

Deutsche Forschungsgemeinschaft

"Wechselwirkungen von Blut-Hirnschranken-Proteinen und deren Regulation" BL 308/6-3 and 6-4 (Ingolf Blasig, Gerd Krause)

Deutsche Forschungsgemeinschaft

"Struktur und Funktion extrazellulärer Loops von Blut-Hirnschranken-Proteinen" BL 308/7-1 (Ingolf Blasig)

Deutsche Forschungsgemeinschaft

"Dimerisierungskonzept transmembranaler Blut-Hirnschranken-Proteine" BL 308/7-3 (Ingolf Blasig)

Federal Ministry of Education and Research

"Molecular Fingerprinting of the Blood-Brain Barrier in Hypoxia – Targeting Brain Vessels to Treat Stroke" (Joint project with the Institute of Biological Sciences NRC, Canada) 01 SF 0201/6 (Reiner Haseloff, Ingolf Blasig)

European Community

"Free radical-induced protein expression in brain endothelial cells" (Marie Curie Grant) HPMT-CT-2001-00399 (Gerty Schreibelt)

Land Berlin

"Modulation der Blut-Hirnschranke zur Behandlung von ZNS-Erkrankungen" ("Programm zur Förderung von Forschung, Innovationen und Technologien – ProFIT") IBB 10134694 (Ingolf Blasig)



Biochemical Neurobiology

Group Leader:
Wolf-Eberhard Siems

Aims

Our group investigates the biochemical, pharmaceutical, and molecular aspects of membrane-bound peptidases. The research is focused on angiotensin-converting enzyme (ACE), neutral endopeptidases (NEP) and some related enzymes. ACE and NEP cleave a broad spectrum of endogenous substrates and play consequently important roles in the regulation of several body functions. We study the biochemical and functional properties of these enzymes as related to alcohol consumption, obesity, neuronal disorders and heart diseases.

ACE: Voluntary alcohol consumption and new molecular functions

The aim our experiments was understand the role of ACE, its substrates, and inhibitors in voluntary alcohol consumption. We e. g. characterized the role of central angiotensin II (AngII) in alcohol intake by using transgenic rats which express an antisense RNA against angiotensinogen in the central nervous system (CNS). These animals with sharply reduced AngII levels in the CNS consumed markedly less alcohol when compared to their wildtypes littermates. While Spirapril, an ACE inhibitor which passes the blood-brain barrier, did not alter the voluntary alcohol consumption in the transgenic rats, it significantly reduced the alcohol intake in wild-type rats. Finally, studies with knockout mice have proven that the effect of AngII on alcohol consumption is mediated by the angiotensin receptor AT1 and that a dopaminergic transmission is involved in AngII-controlled alcohol preference. Our results indicate that a distinct drug-mediated control of the central renin-angiotensin system could provide a new basis for the treatment of alcoholism.

Further studies are directed on the molecular functions of the two catalytic domains of ACE. Using stable transfected CHO cells with domain-selective forms of ACE (N- or C-domain), we try to clarify (i) unexpected endopeptidolytic actions of ACE, (ii) receptor-like processes after interaction of the transfected cells with several ACE substrates and inhibitors and (iii) ACE actions on adjacent membranal proteins (e. g. GPI-anchored proteins).

NEP: natriuretic peptide degradation, obesity and learning

We studied the catabolism of the cardioprotective, strongly related natriuretic peptides ANP, BNP, and CNP. The main enzyme degrading ANP and CNP is NEP. BNP is also quickly degraded by the kidney and lung membranes but found to be NEP-resistant. Together with the

Structural Bioinformatics group of our institute we developed a hypothesis for the interaction of NEP with these peptides based on a three-dimensional model. This provides a rational explanation for the phenomenon that BNP is resistant to NEP. Using membranes of NEP-deficient mice (NEP^{-/-}), we discovered that the kidney endopeptidase meprin A initially degrades BNP by truncation of the N-terminal part of the peptide. According to our model, this truncation enables the subsequent inactivation by NEP. These discoveries open new possibilities for the potentiation of the cardioprotective action of BNP and related compounds.

We are also interested in the in vivo relevance of the NEP deficiency. For example, we observed that NEP^{-/-} mice develop a late-onset obesity. NMR-spectroscopy studies showed that the higher body weight in NEP^{-/-} mice is exclusively due to an accumulation of fat. The molecular bases of NEP-related obesity are presently being investigated. In contrast to several other genetically modified animals expressing obese phenotypes, the alterations in NEP^{-/-} mice affect a great number of orexigenic substrates (substrates that increase the appetite). Therefore, this may be an animal model of the typical, polyfactorial human obesity.

Furthermore, NEP belongs to the group of enzymes which catabolize the β -amyloid peptides ($A\beta$), the so-called "Alzheimer peptides". We demonstrated that the murine form of $A\beta$ is also degraded by NEP. Consequently, $A\beta$ levels are strongly elevated in the brain of NEP^{-/-} mice. Surprisingly, very old NEP^{-/-} mice had even a better performance in memory and learning than their wildtype littermates (e. g. in a Morris-water maze). This was confirmed in LTP (long-term potentiation) experiments and demonstrates the contradictory action of NEP: while NEP improves the brain function by degradation of the plaque forming $A\beta$ -peptides, it apparently also degrades other peptides which protect or even improve learning processes.

Internal collaborations

Several other FMP groups intensively deal with selective potentiation of peptidases.

The work described here was carried out in collaborations with the Structural Bioinformatics group of G. Krause (establishing and verifying of a model of the catalytic centre of NEP), the group for Cellular Imaging of B. Wiesner and Mass Spectrometry of E. Krause (discovery of ACE-related CPM-activation) and J. Furkert (ELISA-development for murine $A\beta$, Galanin, GLP-1).

Members of the group

Dr. Winfried Krause^{***}

Nils Dietrich (doctoral student)^{*}

Florian Gembardt (doctoral student)

Kristin Pankow (doctoral student)^{**}

Xiaoou Sun (doctoral student)^{**}

Marlis Grunow (technical assistance)^{*}

Bettina Kahlich (technical assistance)

Tordis Borowski (student)^{*}

Selected publications*

Maul B, Krause W, Pankow K, Becker M, Gembardt F,

Alenina N, Walther T, Bader M, **Siems WE** (2005) Central angiotensin II controls alcohol consumption via its AT1 receptor. *FASEB J* 19, 1474-1481.

Faber F, **Gembardt F, Sun X,** Mizutani S, **Siems WE,** Walther T (2006) Lack of angiotensin II conversion to angiotensin III increases water but not alcohol consumption in aminopeptidase A-deficient mice. *Regul Pept* 136, 130-137.

Sommer WH, Rimondini R, Marquitz M, Lidström J, **Siems WE,** Bader M, Heilig M (2007) Plasticity and impact of the central renin-angiotensin system during development of ethanol dependence. *J Mol Med* (in press).

Pankow K, Wang Y, **Gembardt F, Krause E, Sun X, Krause G,** Schultheiss HP, **Siems WE,** Walther T (2007) Successive action of meprin A and neprilysin catabolizes B-type natriuretic peptide. *Circ Res* (in press).

Walther T, Tschöpe C, Sterner-Kock A, Westermann D, Heringer-Walther S, Riad A, Nikolic A, Wang Y, Ebermann L, **Siems WE,** Bader M, Dörner A (2007) Accelerated mitochondrial adenosine diphosphate/adenosine triphosphate transport improves hypertension-induced heart disease. *Circulation* 115, 333-344.

FMP authors in bold, group members underlined.

Inventions¹

Siems WE, Walther T, Melzig MF

"Verwendung von NEP-assoziierten Molekülen zur Behandlung von nichtimmunogenen-nichthypertensiven Zivilisationskrankheiten"

Priority establishing patent application: 12.03.2003

Rights ceased in 2006

Shared invention of Freie Universität Berlin, Charité and FMP

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

"Neuropeptidasen und Alkoholkonsum – Untersuchungen an transgenen und knockout-Tieren"

SI 483/3-2 (Wolf-Eberhard Siems, Björn Maul)

European Community (6th Framework Programme)

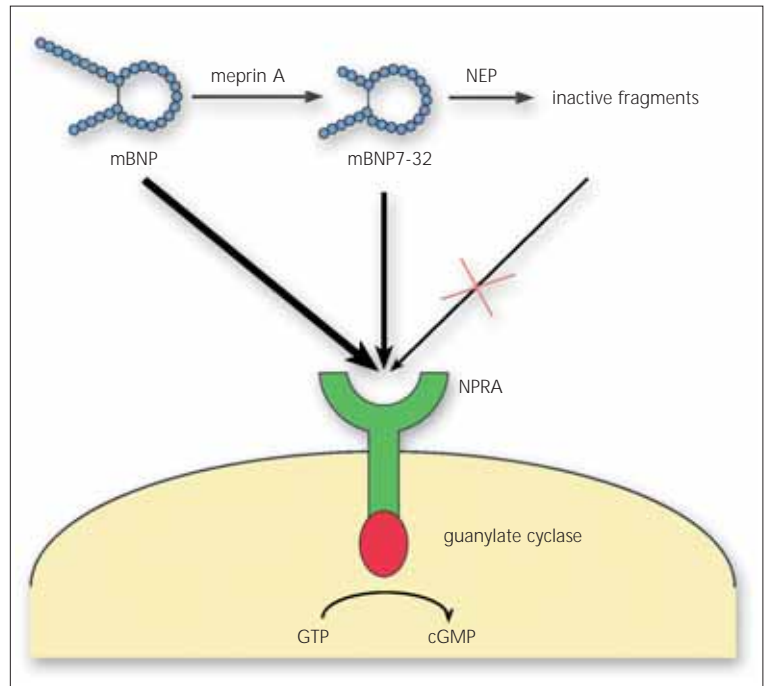
"Impact of genotype on fetal programming responses to dietary protein supplementation – cardiovascular descriptors, energy metabolism and appetite" (Sub-project of the Integrated Project EARNEST: "Early Nutrition Programming – long term efficacy and safety trials and integrated epidemiological, genetic, animal, consumer and economic research")

FOOD-CT-2005-007036 (Wolf-Eberhard Siems, Cornelia Metges/FBN Dummerstorf)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Cooperative catabolism of BNP

ASTA Medica

"Untersuchung der Beziehungen zwischen ACE-Hemmung durch Spirapril und der Substanz P"

ASTA Medica – SI1 (Wolf-Eberhard Siems)

Charité – Universitätsmedizin Berlin

"Catabolism of natriuretic peptides, involved enzymes and the biological activity of products after limited peptidolysis"

Charité – SI (Wolf-Eberhard Siems)



Department Leader:
Thomas Jentsch

Physiology and Pathology of Ion Transport

This joint group of MDC and FMP started to work on the campus in September 2006.

Aims

We aim to understand ion transport processes from the molecular level (structure-function analysis) to the sub-cellular and cellular level (e. g. role in endosomes) up to the level of the organism. The latter aspects are largely tackled by investigating the phenotypes of respective *knock-out* and *knock-in* mice, and by an analysis of corresponding human diseases (channelopathies).

We focus on three molecular classes of ion transport proteins: CLC chloride channels and transporters, KCNQ potassium channels, and KCC potassium-chloride cotransporters. Two of our most important research areas concern the role of vesicular pH and chloride in the endosomal/lysosomal system, and the regulation of neuronal cytoplasmic chloride and its impact on neuronal function and development. As the ion transport proteins under study are expressed in a wide range of tissues, we analyze their function in many organs (brain, inner ear, eye, kidney, pancreas, bone, testis, etc.).

CLC chloride channels and transporters

Proteins of the CLC gene family, discovered by us in 1990, reside in the plasma membrane and intracellular vesicles. Surprisingly, several (or all) vesicular CLCs are Cl⁻/H⁺-exchangers. We have generated KO mouse models for most CLCs and have identified corresponding human diseases, yielding insights into their diverse physiological functions. We have also identified two ancillary β -subunits (barttin and Ostm1), mutations in which also cause human disease.

Current projects focus on the role of vesicular CLCs in determining endosomal/lysosomal pH and Cl⁻ and the impact on vesicular function and trafficking; structure-function analysis to understand the difference between Cl⁻ channels and Cl⁻/H⁺-exchangers; conditional and multiple KO mice to unravel the importance of CLCs for various cellular functions and their redundancy; investigation of CLC trafficking and identification of sorting signals; identification and role of associated proteins.

KCNQ potassium channels

We cloned and characterised the K⁺ channels KCNQ2-5, have shown that mutations in KCNQ2 and 3 cause neonatal epilepsy and mutations in KCNQ4 a form of dominant deafness (DFNA2). KCNQ2-5 mediate highly regulated, M-type currents that are important for the regulation of neuronal excitability. We have recently generated mouse models for KCNQ4 and KCNQ5 (other mouse models are in progress). Both KCNQ4 KO mice, as well as mice carrying a dominant negative mutation

we have previously identified in human deafness, develop deafness (the dominant negative with a slower time course). This is due to a selective degeneration of sensory outer hair cells.

We are currently analysing a dominant negative KCNQ5 KI mouse, investigating the role of KCNQs in other organs, and are generating new KCNQ mouse models.

KCC K-Cl cotransporter

We have disrupted (in mice) all four isoforms of electroneutral K-Cl cotransporters, leading to interesting pathologies for KCC2-4. The disruption of KCC3 and KCC4 leads to deafness: thus, together with our KCNQ4 mice and our unpublished barttin KO mice, we have now four mouse models for deafness and ion homeostasis in the inner ear.

K-Cl cotransport, in particular KCC2, plays a crucial role in establishing the inhibitory response to GABA and glycine, with the early excitatory response believed to be important for neuronal development. The double KO of KCC1 and KCC3 impaired the regulation of red blood cells and partially alleviated the erythrocyte pathology of a sickle cell anemia mouse model.

Current projects focus on the role of KCC4 in other tissues, generating and analyzing tissue-specific double KOs using newly generated floxed mice, and a detailed analysis of the role of KCC2 in defined brain regions using conditional KOs. In addition, we are looking at the roles of other Cl transporters in regulating intraneuronal Cl concentration and thereby neuronal excitability.

Internal collaborations

As we have only recently joined the FMP (August 2006), we have just begun to develop intramural collaborations. Our interest in structure and function will benefit from the FMP's strength in structural biology and molecular modeling. Several proteins under study are interesting drug targets (e. g. ClC-7/Ostm1 for osteoporosis, KCNQ2/3/4 for epilepsy and pain, KCC for sickle cell anemia), suggesting collaborations with the Screening Unit. The organic chemistry that is well represented at the FMP, as well as the top imaging facilities, will provide excellent opportunities to jointly investigate vesicular pH and Cl with novel indicator molecules, or change the cellular environment with novel caged compounds. We are collaborating with J. Rademann to develop chloride indicators that may be taken up by endocytosis. We collaborate with E. Krause to identify by mass spectroscopy proteins binding to CLC transporters. Together with B. Wiesner, we are establishing the FLIM technology at the FMP in order to measure vesicular chloride concentrations. Our diverse mouse models and expertise in various organ systems will significantly bolster the strength of the FMP in systems biology and pharmacology. As our group belongs to both the FMP

Members of the group

Dr. Muriel Auberson
 Dr. Jens Fuhrmann
 Dr. Ioana Neagoe
 Dr. Gaia Novarino
 Dr. Vanessa Plans
 Dr. Tobias Stauber
 Dr. Vitya Vardanyan
 Dr. Stefanie Weinert

Dr. Anselm Zdebik
 Dr. Rubén Vicente Garcia (visiting scientist)
 Maren-Rebecca Knoke (doctoral student)**
 Philipp Lange (doctoral student)**
 Carsten Pfeffer (doctoral student)**
 Patricia Preston (doctoral student)**
 Gesa Rickheit (doctoral student)**
 Lena Wartosch (doctoral student)**

Alexander Fast (technical assistance)
 Petra Göritz (technical assistance)**
 Nicole Krönke (technical assistance)
 Ina Lauterbach (technical assistance)
 Rainer Leben (technical assistance)
 Janet Liebold (technical assistance)
 Silke Zillmann (technical assistance)
 Pia Philippi (secretary)

All members, except for the head of the group and Petra Göritz, are employed at the MDC, but financed by both organisations equally.

and the MDC (joint appointment), we will also have a role in increasing the synergy between both institutions.

Selected publications[#]

Kasper D, Planells-Cases R, **Fuhrmann JC**, Scheel O, Zeitz O, Ruether K, Schmitt A, Poët M, Steinfeld R, Schweizer M., Kornak U, **Jentsch TJ** (2005) Loss of the chloride channel CIC-7 leads to lysosomal storage disease and neurodegeneration. EMBO J. 24, 1079-1091.

Scheel O, **Zdebik AA**, Lourdel S, **Jentsch TJ** (2005) Voltage-dependent electrogenic chloride-proton exchange by endosomal CLC proteins. Nature 436, 424-427.

Kharkovets T, Dedek K, Maier H, Schweizer M, Khimich D, Nouvian R, **Vardanyan V**, Leuwer R, Moser T, **Jentsch TJ** (2006) Mice with altered KCNQ4 K⁺ channels implicate sensory outer hair cells in human progressive deafness. EMBO J 25, 642-652.

Lange PF, **Wartosch L**, **Jentsch TJ**, **Fuhrmann JC** (2006) CIC-7 requires Ostm1 as a β -subunit to support bone resorption and lysosomal function. Nature 440, 220-223.

Poët M, Kornak U, Schweizer M, **Zdebik AA**, Scheel O, Hoelzer S, Wurst W, Schmitt A, **Fuhrmann JC**, Planells-Cases R, Mole SE, Hübner CA, **Jentsch TJ** (2006) Lysosomal storage disease upon disruption of the neuronal chloride transport protein CIC-6. Proc Natl Acad Sci USA 103, 13854-13859.

Rust MB, Alper SL, Rudhard Y, Shmukler BE, **Vicente R**, Brugnara C, Trudel M, **Jentsch TJ**, Hübner CA (2007) Disruption of erythroid KCl-cotransporters alters erythrocyte volume and partially rescues erythrocyte dehydration in SAD mice. J Clin Invest 117, 1708-1717.

[#] FMP authors in bold, group members underlined.

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

"Protein modules involved in vesicular acidification and trafficking: focus of CIC-6" (Sub-project of the Collaborative Research Center "Von Molekülen zu Modulen: Organisation und Dynamik zellulärer Funktionseinheiten")
 SFB 740 TP C05 (Thomas Jentsch)

Deutsche Forschungsgemeinschaft

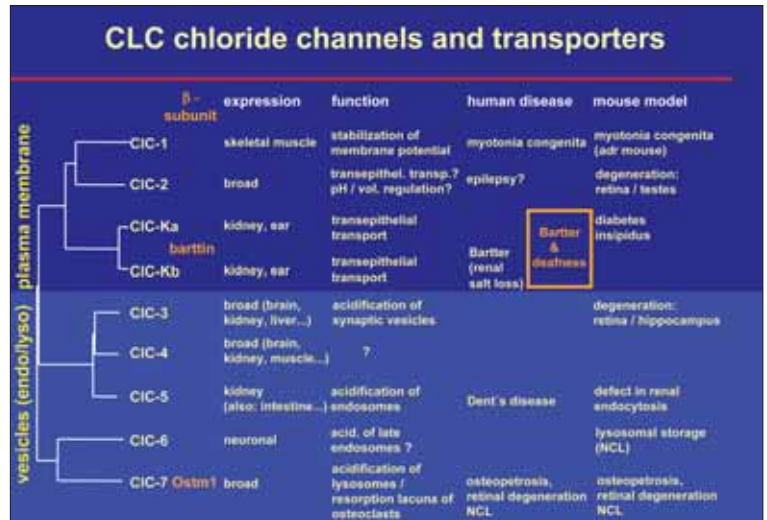
"Dissektion der Rolle von CIC-5 bei der renalen Endozytose und tubulären Transportprozessen" (Sub-project of the Research Unit "Epitheliale Mechanismen der renalen Volumenregulation")
 FOR 667 (JE 164/6-1) (Thomas Jentsch)

Deutsche Forschungsgemeinschaft

"Untersuchung der physiologischen Rolle der K-Cl-Kotransporter mit transgenen Mausmodellen"
 JE 164/4-4 (Thomas Jentsch)

Deutsche Forschungsgemeinschaft

"Der CIC-7/Ostm1 Chloridtransporter in Lysosomen und Osteoklasten"
 JE 164/7-1 (Thomas Jentsch)



Proteins of the CLC gene family, discovered by us in 1990, reside in the plasma membrane and intracellular vesicles. Surprisingly, several (or all) vesicular CLCs are Cl⁻/H⁺-exchangers. We have generated KO mouse models for most CLCs and have identified corresponding human diseases, yielding insights into their diverse physiological functions. We have also identified two ancillary β -subunits (barttin and Ostm1), mutations in which also cause human disease.

Deutsche Forschungsgemeinschaft

"Strukturelle Grundlagen und physiologische Funktion des Cl⁻/H⁺ Gegen-austausches bestimmter CLC Chloridtransportproteine"
 ZD 58/1-1 (Thomas Jentsch, Anselm Zdebik)

Federal Ministry of Education and Research

"Systematische Gen-Identifikation und funktionelle Analysen bei häufigen neurologischen Erkrankungen" (Sub-project in the "NGFN2-Neuronetz")
 01GS0664 (Thomas Jentsch)

European Community (6th Framework Programme)

Sub-project of the Integrated Project EuReGene: "European Renal Genome Project"
 LSHG-CT-2004-005085 (Thomas Jentsch)

European Community (6th Framework Programme)

Sub-project of the STREP NEUROKCNOPATHIES: "Cell biology of rare monogenic neurological disorders involving KCNQ channels"
 LSHM-CT-2004-503038 (Thomas Jentsch)

European Community (6th Framework Programme)

Sub-project of the Integrated Project EuroHear: "Advances in hearing science: from functional genomics to therapies"
 LSHG-CT-2004-512063 (Thomas Jentsch)

Fondation Louis Jeantet de Médecine

"Prix Louis Jeantet"
 Prix Louis Jeantet (Thomas Jentsch)

*part of period reported **part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Klaus-Peter
Knobeloch

Cytokine Signaling

Aims

Protein modification by ubiquitin and ubiquitin like (UBL) molecules represents an important regulatory mechanism controlling a wide variety of biological processes including growth, protein interaction and immune response. We use targeted mutagenesis in the mouse to analyze the biologic functions of distinct components of the ubiquitin system directly in the context of the whole organism aiming to identify novel targets for therapeutic intervention.

Functions of ISG15

Interferon Stimulated Gene 15 (ISG15) is a 15kD ubiquitin-like protein, identified as a product of an Interferon-stimulated gene in humans. ISG15 expression is strongly induced in many cell types by IFNs, viral infection, bacterial endotoxins, dsRNA, and genotoxic stress. Speculations on the biological significance of ISG15 were further amplified by analysis of mice lacking UBP43/USP18, a protease that was described to specifically remove conjugated ISG15 from substrates. Mice with a targeted deletion of UBP43 (UBP43^{-/-}) show elevated levels of ISG15 conjugates and develop brain cell injuries that peak in the development of a hydrocephalus and early death. In order to directly address the role of ISG15 and ISG15 modification in a loss of function approach, we have generated ISG15 deficient mice. The mice are healthy without any obvious defect and analyses did not reveal any evidence for defective STAT1 and IFN signalling or altered responses against VSV or Lymphocyte Choriomeningitis Virus (LCMV). Our work unequivocally showed that ISG15 is not essential for STAT1 signalling and immune responses against LCMV and VSV. To clarify the role of ISG15 in the phenotype of UBP43^{-/-} mice, we applied a rescue approach by generating ISG15^{-/-}UBP43^{-/-} double knockout mice. As the lack of ISG15 is insufficient to revert the phenotype of UBP43^{-/-} animals, we were able to demonstrate that the biological relevant function of UBP43 is different from that of an ISG15 protease and that the substrate specificity needs to be reexamined.

Conditional inactivation reveals an essential role for Ubiquitin isopeptidase 8 (USP8/UBPy) in endosomal transport and receptor tyrosine kinases stability

Ubiquitin can be removed from target proteins by deubiquitinating enzymes that act in analogy to phosphatases in protein phosphorylation by counteracting functional consequences mediated by ubiquitin con-

jugation. UBPy is a Ubiquitin isopeptidase that accumulates upon growth stimulation. Protein levels of UBPy decrease when cells undergo growth arrest by contact inhibition, suggesting a possible role in the control of mammalian cell proliferation. UBPy was also shown to bind to STAM2 which, together with HRS, forms a complex that plays an important role in endocytic trafficking. Recently, the Epidermal growth factor receptor (EGFR) was reported to directly interact with UBPy. However the biological function and physiological relevance of UBPy *in vivo* is still unclear.

To analyze the functional role of UBPy within the context of the whole organism, we generated mice that allow the conditional inactivation of UBPy using cre loxP mediated gene targeting. Adult mice with induced deletion of UBPy die due to severe liver failure. We could show that protein levels of receptor tyrosine kinase receptors (RTKs, e. g., EGFR, c-Met and ERBB3) are severely reduced or absent in UBPy^{-/-} hepatocytes. Thus, inhibition of UBPy by small molecules might be a suitable strategy to down-regulate RTKs and interfere with aberrant proliferation. To elucidate the molecular mechanisms in detail, a cell line that allows effective inducible inactivation of UBPy was generated. Deletion of UBPy leads to enhanced ubiquitination of distinct structures colocalising with an early endosomal marker, loss of the integrity of the HRS/STAM complex, and endosomal enlargement. Our results reveal a fundamental role of UBPy in endosome trafficking.

Internal collaborations

Target identification in the ubiquitin/UBL system;

Functional analysis using KO and transgenic mice for target validation;

Combination of *in vitro* and biological assays as a strategy to identify specific inhibitors of deubiquitinating enzymes;

With J. P. von Kries, Screening Unit: Screen to identify inhibitors of UBPy using a fluorescence based assay

With U. Vinkemeier, Cellular Signal Processing: Generation of three different STAT-1 knock-in mouse models

With C. Freund, Protein Engineering: Collaboration to identify the role of UBPy in proximal T-cell signaling

With E. Krause, Mass Spectroscopy: Identification of UBPy interaction partners and substrates

Members of the group

Agnes Kisser (doctoral student)**

Sandra Niendorf (doctoral student)

Melanie Benedict (technical assistance)

Marcus Wietstruk (technical assistance)

Stefanie Manthey (student)

Selected publications*

Bundschuh K, **Knobloch KP**, Ullrich M, Schinke T, Amling M, Engelhardt CM, Renne T, Walter U, Schuh K (2005) Gene disruption of *spred-2* causes dwarfism. *J Biol Chem* 280, 28572-28580.

Knobloch KP, Utermohlen O, **Kisser A**, Prinz M, **Horak I** (2005) Reexamination of the role of ubiquitin-like modifier ISG15 in the phenotype of UBP43-deficient mice. *Mol Cell Biol* 24, 11030-11034.

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Osswald C, Baumgarten K, Stumpel F, Gorboulev V, Akimjanova M, **Knobloch KP**, **Horak I**, Kluge R, Joost HG, Koepsell H (2005) Mice without the regulator gene *Rsc1A1* exhibit increased Na⁺-D-glucose cotransport in small intestine and develop obesity. *Mol Cell Biol* 25, 78-87.

Lenschow DJ, Lai C, Frias-Staheli N, Giannakopoulos NV, Lutz A, Wolff T, **Osiak A**, Levine B, Schmidt RE, Garcia-Sastre A, Leib DA, Pekosz A, **Knobloch KP**, **Horak I**, Virgin HW (2007) IFN-stimulated gene 15 functions as a critical antiviral molecule against influenza, herpes, and Sindbis viruses. *Proc Natl Acad Sci USA* 104, 1371-1376.

Niendorf S, **Oksche A**, **Kisser A**, Löhler J, Prinz M, **Horak I**, **Knobloch KP** (2007) Essential role of Ubiquitin specific protease 8 (UBPy) for receptor tyrosine kinase stability and endocytic trafficking *in vivo*. *Mol Cell Biol* 27, 5029-5039.

FMP authors in bold, group members underlined.

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

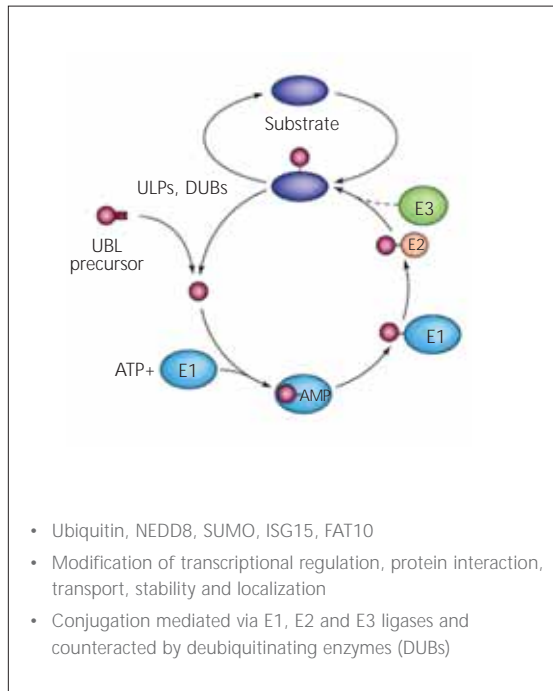
"Untersuchungen zur Funktion des Interferon-stimulierten Genes 15 (ISG15)"

KN 590/1-1 and 1-2 (Klaus-Peter Knobloch)

Deutsche Forschungsgemeinschaft

"Analyse der Funktion der Ubiquitin Isopeptidase (USP8) *in vivo*"

KN 590/2-1 (Klaus-Peter Knobloch)



Posttranslational modification by ubiquitin and ubiquitin like molecules (UBLs)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Dirk Carstanjen

Molecular Myelopoiesis

Aims

Myelopoiesis is the tightly regulated genesis of billions of blood cells each day governed by multiple transcription factors. Failure of this developmental program leads to myeloid malignancies or bone marrow failure. Our group aims at gaining insight into the genetic regulation of normal and malignant myeloid (i.e. bone marrow derived) blood cell development. To this end, we are currently analysing the contribution of three different transcription factors with major functions in myelopoiesis: *Icsbp (Irf-8)*, *Klf4*, and *Stat5*.

We have observed novel functions for each of those transcription factors in mice *in vivo* and we are currently studying the molecular mechanisms underlying those observations.

Icsbp – a transcription factor fine tuning the myeloid development program

Deficiency of *Icsbp* (Interferon Consensus Sequence Binding Protein) not only leads to an immune deficiency due to a loss of important interferon functions but, surprisingly, also results in a myeloproliferative disorder resembling human chronic myeloid leukaemia. The reason for this phenomenon is as of yet unknown. We have recently shown that haploinsufficiency of Neurofibromin 1 (*Nf1*), the gene mutated in man leading to von Recklinghausens disease, synergizes with loss of *Icsbp* in the induction of leukemias. In contrast to the common view, loss of heterozygosity (LOH) of *Nf1* is not mandatory for disease progression but segregates with other genetic abnormalities and leukemic phenotypes. We therefore identified a novel combination of tumor suppressor genes which, when both are mutated will lead to leukemia.

We also ascribe a novel physiological function for innate immunity to *Icsbp*. *Icsbp* not only affects neutrophilic granulocyte development but expression of this transcription factor is mandatory for eosinophilic granulocyte development, a cell that is both crucial for immunity against parasites but also responsible for several allergic diseases including human asthma.

Our current research aims at elucidating the molecular mechanism as to how *Icsbp* controls myeloid and, in particular, eosinophil development. These findings will broaden our understanding of the regulation of myeloid development which ultimately might lead to a more thorough understanding of the physiology and pathophysiology of the myeloid immune system.

Klf4 – novel functions in myelopoiesis

Klf4 gained our interest as a transcription factor aberrantly expressed in *Icsbp* deficient myeloid progenitor cells. *Klf4* is a protein of the Krueppel-family of zinc-finger proteins and has an ambiguous function in oncology. While in colon carcinoma this protein suppresses tumour growth and is lost in some primary carcinomas, in breast carcinoma *Klf4* is often overexpressed and acts as an oncogene. *Klf4* is required for normal development of the skin and the intestinal endothelium but no specific role of *Klf4* in myelopoiesis has been described to date.

We observed two novel functions of *Klf4*. First, expression of *Klf4* induces macrophage maturation and loss of *Klf4* in mice through gene targeting leads to selective loss of inflammatory monocytes. These cells have gained major interest recently as they may play a pivotal role in innate immunity against parasites and other pathogens. On the other hand, inflammatory monocytes may be important for the propagation of several auto-immune diseases as Multiple Sclerosis, Rheumatoid Arthritis or Arteriosclerosis. Currently, straight forward animal models for the function of inflammatory monocytes are lacking. Therefore, we have established for the first time mutant mice with selective loss of this important immune cell population.

Second, a zinc-finger deletional mutant of *Klf4* acts as a novel myeloid oncogene. Expression of this mutant induced a block in myeloid maturation, enhanced colony formation of myeloid progenitor cells, and induction of a self renewal gene expression signature. Deletional mutants of *Klf4* created in our laboratory identified different protein domains required for macrophage/monocyte differentiation, regulation of proliferation or myeloid differentiation blockade.

Our research ascribes novel functions to this transcription factor and aims to delineate the molecular mechanisms as to how *Klf4* regulates normal and malignant hematopoiesis.

Stat5 – investigating structure function relationship in a potentially ‘druggable’ transcription factor

Stat5a and *b* are two crucial transcription factors relaying signals from cytokine receptors towards the nucleus. We have established an *in vivo* bone transplantation model whereby over expression of *Stat5a* leads to myeloproliferation. In close collaboration with the Cellular Signal Processing group (U. Vinkemeier & T. Meyer), we introduced rationally designed mutations into the cDNA of *Stat5a* that will lead to predictable functional defects. This system permits the study of the structure-function

Members of the group

Dr. Martina Alken*

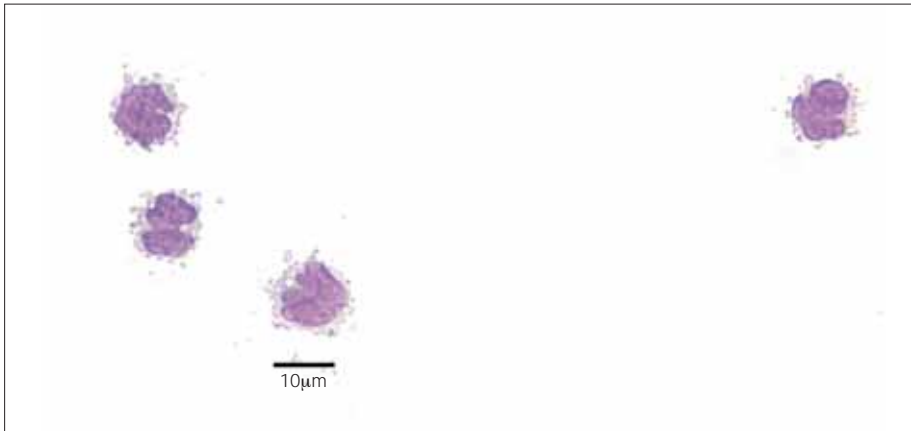
Dr. Rosel Blasig**

Jessica Königsmann (doctoral student)**

Maja Milanovic (doctoral student)

Janet Klemm (technical assistance)*

Didier Nana Kouegoua (student)



May-Gruenwald staining of inflammatory monocytes isolated from the peripheral blood of mice.

relationship of the Stat5a protein in a relevant oncogenic *in vivo* model. Following the fate of mice transplanted with bone marrow cells overexpressing dimerisation- and tetramerisation-deficient Stat5a mutant proteins, we are studying the contribution of different oligomerised Stat5a species in target gene activation. C-terminal proteolytic processing is equally important for Stat5a function and is currently being investigated using the same approach. To explore the pharmacological potential of STAT5 inhibition, we utilized recombinant STAT5 expressed in *E. coli* and are currently screening the FMP compound library in order to identify substances inhibiting activation of STAT5 in a joint effort with J. Rademann (Medicinal Chemistry), A. Diehl (NMR) und J. P. von Kries (Screening Unit).

Internal collaborations

Development of a specific *STAT5a* and *b* inhibitor in a joint effort with J. Rademann (Medicinal Chemistry), A. Diehl (NMR) und J. P. von Kries (Screening Unit) as a novel pharmaceutical agent to treat myeloid malignancies.

Selected publications*

Carstanjen D, Dutt P, Moritz T (2001) Heparin inhibits retrovirus binding to fibronectin as well as retrovirus gene transfer on fibronectin fragments. *J Virol* 75, 6218-6222.

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Terszowski G, Waskow C, Conradt P, Lenze D, **Koenigsmann J**, **Carstanjen D**, **Horak I**, Rodewald HR (2005) Prospective isolation and global gene expression analysis of the erythrocyte colony-forming unit (CFU-E). *Blood* 105, 1937-1945.

FMP authors in bold, group members underlined.

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

"Die Rolle des Adaptor-Proteins Disabled-2 bei der Zytokinsignalvermittlung in hämatopoetischen Zellen"
CA 306/1-1 and 1-2 (Dirk Carstanjen)

Deutsche Forschungsgemeinschaft

"Gene in Entwicklung und Funktion von myeloiden Zellen"
HO 493/12-2 (Ivan Horak)

Land Berlin

"Entwicklung eines spezifischen STAT5 Inhibitors" ("Programm zur Förderung von Forschung, Innovationen und Technologien – ProFIT")
IBB 10134292 (Dirk Carstanjen, Jörg Rademann)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Uwe Vinkemeier

Cellular Signal Processing

This group closed in June, 2007, upon an external appointment of the group leader.

Aims

Our group aims to understand how cytokines such as interferons and interleukines signal to the nucleus and regulate gene expression. We hope to understand the roles these signalling pathways play in the modulation of the immune system and how they are perturbed in disease and cancer.

Nucleocytoplasmic Transport

Cytokines and growth factors signal via membrane bound receptors and a family of signal transducers termed STAT proteins, which receive signals directly at the receptors at the cell membrane and relay them to the nucleus, where STATs function as DNA binding transcription factors. We are investigating the molecular mechanisms that govern the nucleocytoplasmic trafficking of STAT proteins. We were the first to identify transport signals for a STAT protein and to discover the constitutive carrier-independent nucleocytoplasmic shuttling of STATs. Our work has amounted to a substantially revised model of STAT signaling wherein the STATs constitutively shuttle between cytoplasm and nucleus. Our current research is exploring the means by which cells modulate nucleocytoplasmic flux rates. We have developed an assay to directly observe the nuclear export of STAT proteins in cytokine stimulated cells. In combination with automated microscopy to screen small molecule libraries this approach may yield substances that intercept with cytokine signaling in an entirely novel manner.

Interferon Resistance

The interferons have potent growth restraining activities that are an important facet also of the antiviral activity of these cytokines. Consequently, the interferons are the medication of choice in the treatment of melanomas and hepatitis virus infections. Yet, for unknown reasons, cells may turn interferon insensitive, resulting in facilitated tumor growth or loss of virus protection. One model proposed that arginine methylation of STAT1 enhances its DNA binding by reducing association with the specific inhibitor PIAS1, thus intensifying the growth restraining activities of the interferons. This finding may explain the interferon (IFN) insensitivity of cancer cells that accumulate high levels of the methyltransferase inhibitor methylthioadenosine (MTA). However, our work has contradicted this model and provided alternative explanations for the effects of MTA on the transcriptional activity of STAT1. Our analyses indicated that SUMO, a small ubiquitin-like protein, controls the activity of STAT1. Interestingly, the PIAS1 protein has SUMO ligase activity,

and we are exploring the links between interferon resistance, PIAS1, and the SUMOylation of STAT1 and STAT3.

Target Gene Finding and Discrimination

How DNA binding transcription factors localize short nucleotide sequences in an ocean of DNA remains poorly understood. We are exploring the contributions both of protein-DNA and protein-protein interactions for target gene finding of STATs. Particularly the highly conserved aminoterminal domain is at the center of our attention. This small domain of 130 residues mediates cooperative DNA binding, tyrosine dephosphorylation, nuclear transport, and receptor recognition. Based on its crystal structure, we can rationally manipulate this intriguing protein interaction module. We are using in vitro biochemistry (e. g. analytical ultracentrifugation, phosphatase assays), live cell fluorescence imaging (FRET, FIAsh, FRAP), and site-directed gene targeting of the mouse to dissect the various functional surfaces from the molecular to the level of the whole organism.

The Nuclear Lamina

The nuclear lamina is a meshwork of intermediate filaments that underlies the inner surface of the inner nuclear membrane. Integral membrane proteins interact with the building blocks of the lamina, the lamins (the product of the *LMNA* gene). Mutations in the *LMNA* splice variants lamin A and lamin C cause a number of inherited diseases that can affect striated muscle, fat and nerve tissue. Many of the mutant lamin proteins cause fairly tissue specific disorders, making inherited disorders of the nuclear lamina some of the most intriguing puzzles in cell biology. Patients with partial lipodystrophy syndromes (Dunnigan-type) have mutations in exon 8 of *LMNA*. The respective mutations would be expected to perturb the binding of a specific partner. Partial lipodystrophy is characterized by gradually disappearing subcutaneous adipose tissue, insulin resistance and considerable muscular hypertrophy. Strikingly, the same combination of defects are reported in animals that are defective in the cytokine myostatin. However, there are no defects found in myostatin expression or the myostatin protein in patients with Dunnigan-type partial lipodystrophy. Preliminary work indicates aberrant downstream signaling in patient material. We are therefore examining whether mutations of the nuclear lamina are associated with defective nucleocytoplasmic transport of cytokine-responsive signal transducers.

Members of the group

Dr. Andreas Begitt

Dr. Andreas Marg

Dr. Thomas Meyer

Regis Cartier (doctoral student)*

Mathias Droescher (doctoral student)*

Tim Kolmsee (doctoral student)**/*

Inga Lödige (doctoral student)

Torsten Meißner (doctoral student)**

Petra Schütz (doctoral student)*

Susanne Tech (doctoral student)**/*

Nikola Wentz (doctoral student)**

Sebastian Winsel (doctoral student)**/*

Mandy Kummerow (technical assistance)*

Melanie Lange (technical assistance)*

Stephanie Meyer (technical assistance)

Claudia Reichling (technical assistance)*

Marleen van Rossum (technical assistance)**/*

Christine Neumann (student)*

Manuela Peucker (student)*

Rita Sachse (student)*

Edda Schulz (student)*

Francis Wolfram (student)*

Internal collaborations

Our research topics complement the work of the functionally oriented groups that pursue signaling research at the FMP (W. Rosenthal, T. Jentsch), with particularly close ties to the immunologically oriented groups of I. Horak, D. Castanjen, K.-P. Knobloch and C. Freund. Our spectrum of methods makes full use of the wide range of methodologies employed at the FMP. It includes techniques provided by the structurally and chemically oriented groups, e. g. analytical ultracentrifugation, protein isolation, peptide synthesis, and mass spectrometry. In addition, it covers fluorescence microscopy and mouse genetics, as well as the possibility to use high-throughput microscopical analyses to study the influence of small molecules on the nucleocytoplasmic transport of STAT transcription factors. Together with B. Wiesner and outside collaborators we are currently establishing computational methods in order to model signaling events.

Selected publications[†]

Lödige J, Marg A, Wiesner B, Malecova B, Oelschlager T, Vinkemeier U (2005) Nuclear export determines the cytokine sensitivity of STAT transcription factors. *J Biol Chem* 280, 43087-43099.

Vinkemeier U, Meyer T (2005) Antiviral activity of oligomerization-deficient STAT1. *Genome Inform Ser Workshop Genome Inform* 16, 44-48.

Meyer T, Begitt A, Vinkemeier U (2007) Green fluorescent protein-tagging reduces the nucleocytoplasmic shuttling specifically of unphosphorylated STAT1. *FEBS J* 274, 815-826.

FMP authors in bold, group members underlined.

Inventions[†]

Vinkemeier U

“Neue Nucleus-Export-Signalpeptide (NES), sie enthaltende Fusionsproteine sowie deren Verwendung”

Priority establishing patent application: 14.07.2000

Rights ceased in 2005

Vinkemeier U, Meyer T

“A single residue modulates Tyrosine Dephosphorylation, oligomerization, and nuclear accumulation of Stat Transcription factors”

Priority establishing patent application: 10.02.2004

Rights ceased in 2006

Vinkemeier U, Meyer T

“Verfahren zur Detektion von nukleocytoplasmatischen Transportprozessen”

Priority establishing patent application: 20.09.2004

Number of pending applications: 1

Vinkemeier U, Meyer T, Marg A

“Hyperactive Stat molecules and their use in assays employing gene activation”

Priority establishing patent application: 17.09.2004

Rights ceased in 2006

Meyer T, Vinkemeier U

“Verwendung von dimer-spezifischen Nuclear-Lokalisations-Signalpeptiden (dsNLS) abgeleitet aus der STAT-DNA Bindungsdomäne”

Priority establishing patent application: 17.01.2002

Number of pending applications: 2

[†]Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

“Konstitutiver nukleocytoplasmatischer Transport von STAT1 und die Regulation der Transkription”

VI 218/2-1, 2-2 and 2-3 (Uwe Vinkemeier)

Deutsche Forschungsgemeinschaft

“Untersuchung des Einflusses der Tyrosin-Dephosphorylierung auf die Zielgenfindung des Transkriptionsfaktors STAT1”

VI 218/3-1 and 3-2 (Uwe Vinkemeier)

Deutsche Forschungsgemeinschaft

“Analyse einer konservierten Protein-Interaktionsdomäne: Wie 125 Aminosäuren die Tetramerisierung, Dephosphorylierung, PIAS-Bindung und den Kernimport des Transkriptionsfaktors STAT1 koordinieren”

VI 218/4-1 and 4-2 (Uwe Vinkemeier)

Federal Ministry of Education and Research

“Molekulare Grundlagen der zellulären Signalverarbeitung” (BioFuture laureate)

0311872 (Biofuture) (Uwe Vinkemeier)

Avontec GmbH

“Analyse der DNS-Bindung von rekombinantem humanem STAT1 und STAT3, Einfluss des GAS-Oligonukleotids auf Kernimport von STAT1”

Avontec – VI1 (Uwe Vinkemeier)

Thyssen Foundation

“Gestörte Weiterleitung von Zytokinsignalen bei Muskel-erkrankungen infolge von Mutationen des Lamin A/C Gens” 20.06.0.047 (Uwe Vinkemeier, Simone Spuler/Charité – Universitätsmedizin Berlin)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Michael Beyermann

Peptide Synthesis

Aims

A molecular understanding of the interactions between membrane-spanning receptors and their ligands may lead to a new concept for the development of ligands which are more selective with respect to subtype receptor binding and/or receptor signalling. Our group is interested in the molecular basis of interactions between peptide ligands and their receptors, in particular G protein-coupled receptors (GPCRs) class B. Since these biologically important receptors are functional only when embedded in a membrane, it is difficult to obtain direct structural information on interaction with ligands by spectroscopic methods. By modifying the ligand or receptor structure we obtain information on specific domains and their role for receptor binding, activation and signalling. Improvements in chemical methods of peptide synthesis and the combination of chemical and enzymatic techniques with molecular methods of protein synthesis permits us to study complex molecules, including constructs of the extracellular receptor domains which could serve as baits for ligand libraries.

Mechanism of ligand-receptor interactions

CRF receptors are involved in mediating anxiety and depressive disorders and other stress-associated pathologies. These receptors are activated by polypeptides (Urocortins, Sauvagine, Urotensin-I, CRF) that are the subject of structure-activity relationship studies. We have demonstrated, for the first time, agonist-directed signalling for the Corticotropin-Releasing Factor (CRF) receptor and are now trying to elucidate the molecular basis for this selectivity.

Results of ongoing studies (in collaboration with H. Berger) show that the signalling-selectivity of CRF₁ receptors for distinct G protein pathways is controlled by an N-terminal signalling domain of Urocortin-1, and that appropriate modifications resulted in signalling-selective ligands. Single amino acid replacements within this domain, but not in other parts of the polypeptide, provided analogues which simultaneously exhibited both full G_s activation and complete inhibition of G_i coupling. This demonstrates that by segregating functional domains, peptides offer a way for the rational design of signalling-selective receptor ligands.

Combination of chemical & enzymatic methods and protein expression for protein synthesis

The incorporation of depsipeptide units into difficult-to-synthesise peptides was shown to strongly improve both the quality and yield of the products. A simple method

for the preparation of thiol esters for chemical ligation has been developed, and the application of enzyme-catalysed ligation using Sortase A has been optimised. Only by applying these methods was the first preparation of a protein complex consisting of all four extracellular receptor domains achieved. This complex behaved, indeed, like an artificial receptor, showing similar relative binding behaviour for natural ligands as the wild-type receptor. Such an artificial receptor now enables further investigations, including ligand fishing from peptide libraries and structural analysis of ligands bound.

Internal collaborations

Our work on ligand-dependent signalling and the role of the ectodomains of Corticotropin-Releasing Factor receptors directly contributes to a main research topic of the FMP, the activation and signalling of G protein-coupled receptors. In this area, we collaborate with R. Schüle (intracellular transport of CRF receptors), B. Wiesner (receptor dimerization) and G. Krause (bioinformatics/modelling). Our capability to prepare difficult sequences provides a basis of research in other groups (B. Reif/amyloid peptides, S. Keller/membrane domains, H. Oschkinat/FBP-28 WW domain). Moreover, peptides play a crucial role in many other FMP projects (C. Freund, I. Blasig, E. Krause, M. Dathe, R. Kühne) and as tools used in the screening unit (J. P. v. Kries). In joint projects we are investigating new light-triggered protecting groups (with V. Hagen) and switch-units (P. Schmieder/K. Rück-Braun (TU Berlin).

Selected publications*

Klose J, Fechner K, Beyermann M, Krause E, Wendt N, Bienert M, Rudolph R, Rothemund S (2005) Impact of N-terminal domains for corticotropin-releasing factor (CRF) receptor ligand interactions. *Biochemistry* 44, 1614-1623.

Kraetke O, Holeran B, Berger H, Escher E, Bienert M, Beyermann M (2005) Photoaffinity cross-linking of the corticotropin-releasing factor receptor type 1 with photoreactive urocortin analogues. *Biochemistry* 44, 15569-15577.

Pätzel M, **Pritz S**, Liebscher J

Alpha-heteroatom-substituted alkanamides. In: *Science of synthesis: Houben-Weyl methods of molecular transformations* (Ed.: Weinreb S), 21, Thieme Verlag 2005, Stuttgart.

Berger H, Heinrich N, Wietfeld D, Bienert M, Beyermann M (2006) Evidence that corticotropin-releasing factor receptor type 1 couples to G_s- and G_i-proteins through different conformations of its J-domain. *Brit J Pharmacol* 149, 942-947.

Coin I, Dölling R, Krause E, Bienert M, Beyermann M, Sferdean CD, Carpino LA (2006) The depsipeptide methodology for solid phase peptide synthesis: circumventing side reactions and development of an automated technique via depsipeptide units. *J Org Chem* 71, 6171-6177.

Beyermann M, Heinrich N, Fechner K, Zhang W, Kraetke O, Bienert M, Berger H (2007) Achieving signalling selectivity of

Members of the group

Dr. Nadjeschda Heinrich

Dr. Oliver Krätke

Dr. Jana Klose (visiting scientist)**/'

Benoit Briand (doctoral student)**/'

Irene Coin (doctoral student)**

Christian Hoppmann (doctoral student)**/'

Stephan Pritz (doctoral student)**

Sandra Tremmel (doctoral student)**/'

Annerose Klose (technical assistance)

Dagmar Krause (technical assistance)

Dagmar Michl (technical assistance)**/'

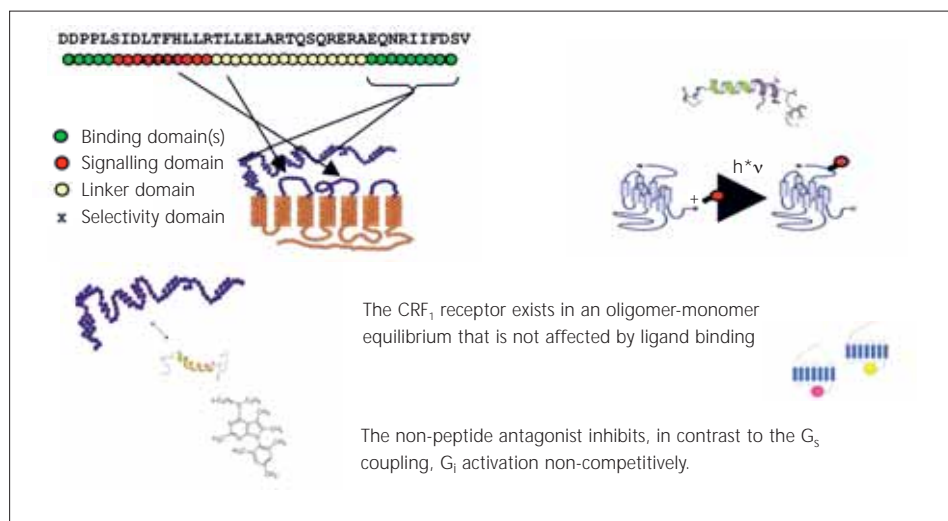
Barbara Pisarz (technical assistance)**

Bernhard Schmikale (technical assistance)

Christian Femmer (student)*

Magnus Krüger (student)*

Wei Zhang (student)*



The CRF₁ receptor in HEK 293 cells shows agonist-evoked activation of G_s and G_i proteins which is directed by a signalling domain. Specific substitutions of the signalling domain result in ago-antagonists that, at the same time, activate G_s but inhibit the coupling of G_i proteins by a single receptor.

ligands for the corticotropin-releasing factor type 1 receptor by modifying the agonist's signalling domain. *Brit J Pharmacol* 151, 851-859.

Pritz S, Wolf Y, Kraetke O, Klose J, Bienert M,

Beyermann M (2007) Synthesis of biologically active peptide nucleic acid-peptide conjugates by sortase-mediated ligation. *J Org Chem* 72, 3909-3912.

FMP authors in bold, group members underlined.

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

"Synthesis, optimisation, and screening of small molecule libraries targeting protein-protein interactions" (Sub-project of the Research Unit "Interfering with intracellular protein-protein interactions – probing protein functions with small molecules") FOR 806 TP Z1 (RA 895/5-1) (Jörg Rademann, Michael Beyermann, Jens Peter von Kries)

Deutsche Forschungsgemeinschaft

"Studium der Ligand-Erkennungsregion von CRH-Rezeptoren mit Peptid- und nicht peptidischen Bibliotheken" (Sub-project of the Research Unit "Optimierte molekulare Bibliotheken zum Studium biologischer Erkennungsprozesse") FOR 299/2-1 TP7 (Michael Bienert, Jens Schneider-Mergener/Jerini AG)

Deutsche Forschungsgemeinschaft

"Struktur, Stabilität und Spezifikation von nichtkatalytischen Proteindomänen und deren Verwendung als Werkzeuge für das Design einer stabilen minimalen Beta-Faltblattstruktur und das Verständnis von pathologischen Prozessen" (Sub-project of the Research Unit "Optimierte molekulare Bibliotheken zum Studium biologischer Erkennungsprozesse") FOR 299/2-2 TP2 (Michael Beyermann, Hartmut Oschkinat, Michael Bienert)

Deutsche Forschungsgemeinschaft

"Studium der Ligand-Erkennungsregion von CRH-Rezeptoren mit Peptid- und nicht peptidischen Bibliotheken" (Sub-project of the Research Unit "Optimierte molekulare Bibliotheken zum Studium biologischer Erkennungsprozesse") FOR 299/2-2 TP7 (Michael Bienert, Jens Schneider-Mergener/Jerini AG)

Deutsche Forschungsgemeinschaft

"Synthese, Reinigung und Charakterisierung schwieriger Peptide über ihre Depsipeptidanaloga" BE 1434/5-2 (Michael Beyermann)

Deutsche Forschungsgemeinschaft

"Orthogonale photolabile SH-Schutzgruppen in der Proteinsynthese" HA 2694/3-1 (Volker Hagen, Michael Beyermann)

Deutsche Forschungsgemeinschaft

"Strukturelle Untersuchungen früher Phasen der Fehlfaltung des Proteins beta2-Mikroglobulin – ein Beitrag zum Verständnis der Entstehung von Amyloidosen" NA 226/12-2 (Michael Beyermann, Dieter Naumann/Robert-Koch-Institut)

Volkswagenstiftung

"Control of protein-protein-interactions through conformational changes induced by light: photoswitchable ligand molecules for PDZ domains" VW I/80 771 (Michael Beyermann)

Schering AG

"Zusammenarbeit im Bereich des Modellings und des Liganden-Designs für den putativen 7-Transmembranrezeptor HE-6 und den Melanocortin-1 Rezeptor (MC-1R)" Schering – KU1/Bienert (Michael Bienert)

* part of period reported

** part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leaders:
Margitta Dathe
Johannes Oehlke

Peptide-Lipid Interaction / Peptide Transport

Aims

The modulation of protein functions, one of the main goals of research at the FMP, requires intracellular delivery of interacting molecules. Our research is focused on the elucidation of the structural requirements of peptides as uptake-promoting and targeting tools for attached cargos and lipid-based carriers.

Furthermore, we are interested in the role of the lipid matrix in mediating transmembrane transport and in modulating selectivity of membrane lytic peptides.

Structural requirements for the cargo delivery ability of peptides

Our strategy has been to undertake a comparative investigation of cellular uptake and biological activity of a model cargo (PNA) conjugated with cell-penetrating peptides and derivatives thereof showing systematically altered structural properties. The cellular uptake was studied by means of confocal laser scanning microscopy and by use of capillary electrophoresis combined with laser-induced fluorescence detection (CE-LIF) and FACS analysis. The biological activity was studied by means of the "Kole splice-correction-assay." Surprisingly, no significant effects of the structural changes of the peptide-tags could be found irrespective of the cell type used. These findings suggest very limited possibilities in cell selective targeting by altering the structural properties of the delivery peptides. More pronounced structural effects became apparent after energy depletion suggesting the presence of active export pumps and, thereby, offering a promising target for approaching cell selective delivery.

Peptide-modified liposomal and micellar carriers

The highly cationic tandem dimer A2, which is derived from the low-density-lipoprotein-receptor (LDLr)-binding domain of apolipoprotein E (apoE), was used as a vector to induce drug carrier transport across the blood-brain barrier (BBB). In addition to covalent coupling of A2 to liposomes, the physicochemical properties favoured the dipalmitoylated derivative, P2A2, as a highly promising candidate for the development of liposomal as well as micellar nanocarriers stable at pharmacologically relevant time scales. The cell matrix component, heparan sulfate proteoglycan, plays a decisive role in the efficient uptake of the liposomal carrier into BBB-forming capillary endothelial cells. An LDL-receptor-mediated targeting effect could not be detected. Different uptake patterns of the two particulate systems in studies with inhibitors of specific endocytotic pathways pointed to distinct differences in the transport routes, a subject of future investigation.

Small antimicrobial peptides

Studies of the structural and functional principles of small antimicrobial peptides suggested that the activity-determining structural motif of R- and W-rich hexapeptides is amphipathicity. This biophysical characteristic is based on cyclisation-induced clustering of aromatic side chains and the positioning of the charged residues in the plane of the back bone ring. The peptides act by permeabilising the bacterial membrane. However, their high selectivity for gram negative bacteria seems to be related to interactions with specific components of the outer wall of bacteria. The constrained, amphipathic, and enzymatically resistant hexapeptides will serve in future application-oriented studies as lead compounds for the development of additives in sperm conservation and *in vitro* fertilisation.

Detergent-resistant membrane domains

Because of the physiological and pathological implications of so-called lipid rafts, the relation between the latter and detergent-resistant membrane domains has been of utmost interest. The classical three-stage model of membrane solubilisation fails to describe the phenomenon of detergent-resistant membranes. We have established a quantitative model analogous to the three-stage model that comprises three components (two lipids and one detergent) in four phases (liquid-ordered and liquid-disordered membranes, micelles, and detergent in aqueous solution). The results demonstrate that the abundance and composition of different domains may vary substantially upon addition of detergent, implying that detergent-resistant membrane domains must not be identified with lipid rafts thought to be functional *in vivo*.

Internal collaborations

Discovery of a cell-penetrating synthetic helical-amphipathic peptide enhancing the activity of intracellularly interacting oligonucleotides and peptides (with M. Beyermann, E. Krause, G. Papsdorf, B. Wiesner, R. Schüle, E. Klußmann, A. Oksche).

Penetratin does not translocate cross pure lipid bilayers. (with P. Pohl and B. Wiesner).

Generation of ApoE-derived vector peptides and liposomal membrane-translocating carriers (with M. Beyermann, E. Krause, H. Strauss).

Basic insights into structural requirements of high activity and selectivity of small antimicrobial peptides (with M. Beyermann and P. Schmieder).

Application-oriented collaborative project between basic research (FMP, IZW), industry (Biosyntan) and potential users in reproduction biology (IFN).

Members of the group

Mojtaba Bagheri (doctoral student)^{***}
Anshul Bhardwaj (doctoral student)^{***}
Christof Junkes (doctoral student)^{***}
Sandro Keller (doctoral student)^{*}
Eik Leupold (doctoral student)^{**}
Yvonne Wolf (doctoral student)^{**}
Heike Nikolenko (technical assistance)
Gabriela Vogelreiter (technical assistance)

Karin Welfle (technical assistance)^{*}
Gerdi Hölzl (student)
Franziska Schumann (student)

Selected publications[#]

Bárány-Wallje E, **Keller S**, Serowy S, **Geibel S**, Pohl P, **Bienert M**, **Dathe M** (2005) A critical reassessment of penetratin translocation across lipid membranes. *Biophys J* 89, 2513-2521.

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Pritz S, Pätzelt M, Szeimies G, **Dathe M**, **Bienert M** (2007) Synthesis of a chiral amino acid with bicyclo[1.1.1]pentane moiety and its incorporation into linear and cyclic antimicrobial peptides. *Org Biomol Chem* 5, 1789-1794.

[#] FMP authors in bold, group members underlined.

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

"Hirntargeting mittels oberflächenmodifizierter Nanosuspensionen und Apolipoprotein E-Peptid beladener Trägersysteme" (Sub-project of the Research Unit "Innovative Arzneistoffe und Trägersysteme – Integrative Optimierung zur Behandlung entzündlicher und hyperproliferativer Erkrankungen")

FOR 463 TP 7B (DA 324/5-1) (Margitta Dathe)

Deutsche Forschungsgemeinschaft

"Hirntargeting mittels oberflächenmodifizierter Nanosuspensionen und Apolipoprotein E-Peptid beladener Trägersysteme" (Sub-project of the Research Unit "Innovative Arzneistoffe und Trägersysteme – Integrative Optimierung zur Behandlung entzündlicher und hyperproliferativer Erkrankungen")

FOR 463 TP 7B (DA 324/5-2) (Margitta Dathe)

Deutsche Forschungsgemeinschaft

"Interaktion amphipathischer Peptide mit Membranen: Vermittlung einer Rezeptor-abhängigen Wirkstoffaufnahme in Hirnendothelzellen und Modulation selektiver zellzytischer Prozesse"

DA 324/4-2 (Margitta Dathe)

Deutsche Forschungsgemeinschaft

"Hirntargeting mittels oberflächenmodifizierter Nanosuspensionen und Apolipoprotein E-Peptid-beladener Trägersysteme"

DA 324/5-3 (Margitta Dathe)

Federal Ministry of Economics and Technology

"Biophysikalische und zellbiologische Untersuchungen zu den strukturellen Grundlagen der Aktivität und bakteriellen Selektivität kleiner Peptide" (Sub-project of the Collaborative Project: "Antimikrobielle Peptide zur Ablösung konventioneller Antibiotika und ihre Anwendung in der Fortpflanzungsbiologie")

AiF KF0376001MD6 (Margitta Dathe, Stephanie Speckl/ZW, Rudolf Dolling/Biosyntan GmbH)

Land Berlin

"Biomaterialien: Untersuchungen zur antimikrobiellen Aktivität peptidbeladener Oberflächen" ("Programm zur Förderung von Forschung, Innovationen und Technologien – PROFIT")

IBB 10134769 (Margitta Dathe)

European Community (5th Framework Programme)

"Interaction of cell-penetrating peptides with artificial lipid membranes and peptide translocation through lipid layers" (Sub-project of the STREP "Target specific delivery systems for gene therapy based on cell penetrating peptides")

QLK3-CT-2002-01989 (Margitta Dathe, Johannes Oehlke, Michael Bienert)

German Academic Exchange Service

"Untersuchungen zur intrazellulären Verteilung und biologischen Aktivität von PNA-Peptid-Konjugaten"

A/06/07030 (Johannes Oehlke)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Eberhard Krause

Mass Spectrometry

Aims

Mass spectrometry has increasingly become an important method for studying protein functions and protein-protein interactions in the field of cellular biology. Methodological studies are aimed to improve the MS-based peptide and protein analysis. These advanced methods and techniques enable us to identify specific proteins belonging to molecular networks in cells and to elucidate functionally important post-translational protein modifications.

Phosphorylation of signalling proteins linked to function

Our work in mass spectrometry methods includes attempts to improve phosphopeptide analysis. A phospho-specific enrichment strategy was developed for comprehensive detection of phosphopeptides in phosphoproteomics. To define the potential and limitations, a systematic study of the intrinsic properties of peptides that affect the specific binding of phosphopeptides and nonspecific binding of nonphosphorylated peptides has been undertaken using well-characterized synthetic mixtures consisting of peptides of different charge and hydrophobicity. In summary, our results clearly demonstrate the high selectivity of titanium dioxide for phosphorylated sequences. Based on methods which we developed, a variety of novel phosphorylation sites of signaling proteins have been characterised. For example, we analyzed the phosphorylation sites of cAMP phosphodiesterase-4D3. Thus, it could be shown that oxidative stress employs phosphatidylinositol 3-kinase and ERK signalling pathways to activate PDE4D3 through multi-site phosphorylation at Ser239 and Ser579 (cooperation with M. D. Housley, University of Glasgow).

Structural changes of cGMP-gated channel upon ligand binding

Cysteines may constitute important reporters of structural changes promising further insight into protein domains that undergo rearrangements induced by ligand binding or protein-protein interactions. We studied the cysteine accessibility of the native rod CNG channel in the absence and presence of the ligand 8Br-cGMP by mass spectrometry. Different classes of cysteines were found: (a) cysteines that were accessible to thiol-specific reagents independent of ligand binding, (b) cysteines that were only accessible in the nonliganded state, (c) cysteines that were only accessible in the ligand-bound state, and (d) cysteines that were not accessible to chem-

ical modification independent of the state of ligand binding. The cysteine accessibility of the native rod cyclic nucleotide-gated channel varies markedly upon ligand binding, thus indicating major structural rearrangements, which are of functional importance for channel activation (cooperation with P. J. Bauer, Institute of Biological Information Processing, Jülich).

Differential protein expression in brain capillary endothelial cells induced by oxidative stress

Cerebral ischemia causes functional alteration of the blood-brain barrier, formed by brain capillary endothelial cells (BCEC). Changes in protein expression and activity of selected differentially expressed enzymes were investigated in BCEC subjected to hypoxia and posthypoxic reoxygenation. A proteomic study provides evidence of an adaptive response of the highly specified brain capillary endothelium to hypoxia and posthypoxic reoxygenation exhibited in a coordinated up-regulation of enzymes and proteins involved in glycolysis, mitochondrial and ER function, stress response, and cytoskeletal reorganization. The observed changes in protein expression profiles and enzyme activities translate into functional alterations and structural and morphological changes necessary for cell adaptation and survival after oxygen withdrawal (cooperation with R. F. Haseloff, FMP).

Quantitative proteomics of synaptic membranes

Using stable isotope labelling methods (SILAC and enzymatic ¹⁸O-labelling) in combination with advanced nanoLC-tandem mass spectrometry, we are developing new proteomics strategies for analysis of protein pools in the low femtomole range. These methods permit the detection and quantification of proteins that are expressed at low levels, offering a more comprehensive analysis of complex cellular responses. Synaptic vesicles (SVs) in the central nervous system upon stimulation undergo rapid calcium-triggered exocytotic cycling. Quantitative proteomics analysis of detergent-resistant membranes (DRMs) isolated from rat brain synapses by LC-MS/MS identified 122 proteins which can be classified as cholesterol-dependent DRM or DRM-associated proteins, many of which with proven or hypothesized functions in exocytotic vesicle cycling including clathrin, the clathrin adaptor complex AP-2, and a variety of SV proteins. synaptic membrane lysates. The data suggest that lipid microdomains may act as spatial coordinators for exocytotic vesicle cycling at synapses (collaboration with V. Haucke, Institute of Chemistry and Biochemistry, FU Berlin).

Members of the group

Dr. Cornelia Czupalla*
Dr. Christoph Gelhaus*
Dr. Sandra Körbel*
Dr. Karin Lemke (technical assistance)**/*
Dr. Michael Schümann (technical assistance)
Pablo Sotelo Torres (visiting scientist)*
Jungyong Jia (visiting scientist)*
Clementine Klemm (doctoral student)**/*

Sabine Lange (doctoral student)**/*
Kristin Keller (technical assistance)*
Stephanie Lamer (technical assistance)
Heidemarie Lerch (technical assistance)**/*
Heike Stephanowitz (technical assistance)*
Isabella Gravenstein (student)
Jan Gropengießer (student)
Sebastian Otto (student)

Internal collaborations

Ongoing collaborative projects with the Anchored Signalling, Protein Trafficking, Protein Engineering, and Molecular Cell Physiology groups focus on quantitative proteomic analysis of phosphorylation-mediated protein-protein interactions, e. g. for identification of AKAP18 interaction proteins and for phosphorylation analysis of ADAP proteins (Integrated FMP project: A proteomic strategy for the characterisation of phosphorylation-mediated protein-protein interaction). In addition, we support various research groups by the analysis of synthesized peptides and recombinant proteins, by elucidation of the structure of naturally occurring peptidic compounds, localization of disulfide bonds, and the structure of cross-linking products.

Selected publications*

Bauer PJ, **Krause E** (2005) Accessibility of cysteines in the native rod cGMP-gated channel. *Biochemistry* 44, 1624-1634.

Körbel S, **Schümann M**, Bittorf T, **Krause E** (2005) Relative quantification of erythropoietin receptor-dependent phosphoproteins using in-gel ¹⁸O labeling and tandem mass spectrometry. *Rapid Commun Mass Spectrom* 19, 2259-2271.

Baust T, **Czupalla C**, **Krause E**, Bourel-Bonnet L, Hoflack B (2006) Proteomic analysis of AP-1A coats selectively assembled on liposomes. *Proc Natl Acad Sci USA* 103, 3159-3164.

Jia J, **Lamer S**, **Schümann M**, Schmidt M, **Krause E**, Haucke V (2006) Quantitative proteomic analysis of detergent-resistant membranes from chemical synapses: Evidence for cholesterol as spatial organizer of synaptic vesicle cycling. *Mol Cell Proteomics* 5, 2060-2071.

Klemm C, **Otto S**, **Wolf C**, **Haseloff RF**, **Beyermann M**, **Krause E** (2006) Evaluation of the titanium dioxide approach for MS analysis of phosphopeptides. *J Mass Spectrom* 41, 1623-1632.

Strünker T, Weyand I, Bönigk W, Van Q, Loogen A, Brown JE, Kashikar N, **Hagen V**, **Krause E**, Kaupp UB (2006) A K⁺-selective cGMP-gated ion channel controls chemosensation of sperm. *Nature Cell Biol* 8, 1149-1154.

FMP authors in bold, group members underlined.

Grants/Externally-funded projects

Federal Ministry of Education and Research

"Identifizierung von Proteinen über In-Gel-Verdau, MS und Datenanalyse"

BfG 0313139 (UA) (Eberhard Krause)

German Academic Exchange Service

"Analysis of NSP5 phosphorylation during Rotavirus infection"

A/06/00510 (Pablo Sotelo)

Biosyntan GmbH

"Massenspektrometrische Charakterisierung von Fluoreszenzmarkierten Glyko- und Phosphopeptiden"

Biosyntan – EK1 (Eberhard Krause)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Volker Hagen

Synthetic Organic Biochemistry

Aims

The core activity of our group is research in organic chemistry whereby we aim to design and to synthesise new reagents and tools for investigation of biological problems. At present, our main goal is the development of so-called caged compounds. Caged compounds are photolabile inactive derivatives of biomolecules from which the biologically active molecules are generated rapidly using UV/Vis or IR light. Caging and uncaging of biomolecules are techniques for studies of the mechanisms and the kinetics of complex cellular processes and their resolution in time and space.

Design and characterisation of novel caging groups

In the past few years, we have developed a series of substituted coumarinylmethyl moieties as novel photoremovable protecting groups for caging of phosphates, sulfates, carboxylates, amines, alcohols, phenols, thioalcohols, and thiols. The novel protecting groups show long wavelength absorptivities (up to 430 nm), high photoefficiencies, and are sensitive to two-photon excitation (740–770 nm). Some derivatives are highly soluble in aqueous buffer.

We studied the mechanism and the kinetics of the photocleavage of a series of coumarinylmethyl-caged biomolecules. In this context, we developed in collaboration with J. Bendig (HU Berlin) und R. Schmidt (University Frankfurt/Main) a fluorescence spectroscopic method for the determination of the rate constants of steps of the photolysis pathways and found that the photocleavage occurs within a few nanoseconds. Thus, coumarinylmethyl esters which directly release the biomolecules by photolysis belong to the fastest phototriggers known. Additionally, we found for the first time that it is possible to quantify biomolecule release inside cells from coumarinylmethyl caged compounds using fluorescence spectroscopy.

Novel caged biomolecules

Using the novel coumarinylmethyl protecting groups, we synthesised strongly improved caged versions of cyclic nucleoside monophosphates (cNMPs). Their usefulness was demonstrated in physiological studies of different types of cyclic nucleotide-gated ion channels. Some of the important results include the clarification of the mechanism of the activation of olfactory-type cNMP-gated channels (K. Benndorf, University of Jena), and the qualification of the models of the cellular and physiological reactions underlying chemotaxis in sperm (U. B. Kaupp, Forschungszentrum Jülich).

We were also concerned with the development and application of caged protons, caged amino acids, and caged peptides. In collaboration with S. Frings (University of Heidelberg), we developed potent caged vanilloid receptor agonists as tools for kinetic examinations of TRPV1 channels in somatosensory neurons. Together with S. Cambridge (MPI, Munich), we are continuing to synthesise and characterise new caged activators of gene expression as tools to control the temporal and spatial expression of selected genes.

Development of orthogonal photolabile SH protecting groups and their application to problems in peptide chemistry

We are currently working on a project (in collaboration with M. Beyermann, FMP) dealing with the application of different photolabile protecting groups to problems in peptide chemistry. For instance, we are examining the wavelength-controlled orthogonal photolysis of different photolabile SH protecting groups and their use in the chemosynthesis of proteins.

Internal collaborations

Our group supports the FMP's aims in molecular pharmacology through the development of caged biomolecules as powerful tools for investigating cellular signaling dynamics. In collaboration with the Cellular Imaging group we look for photoactivatable protecting groups showing biologically useful profiles for one- and two-photon photolysis and apply novel caged compounds to study gating of ion channels using laser scanning microscopy. In collaboration with the Biophysics group we are concerned with the development and use of caged protons. With the Peptide Synthesis group we are working on a project dealing with the application of photolabile protecting groups to problems in peptide chemistry. Furthermore, we support the synthesis of ligands for the AF6 PDZ domain (cooperation with the Protein Structure group).

Members of the group

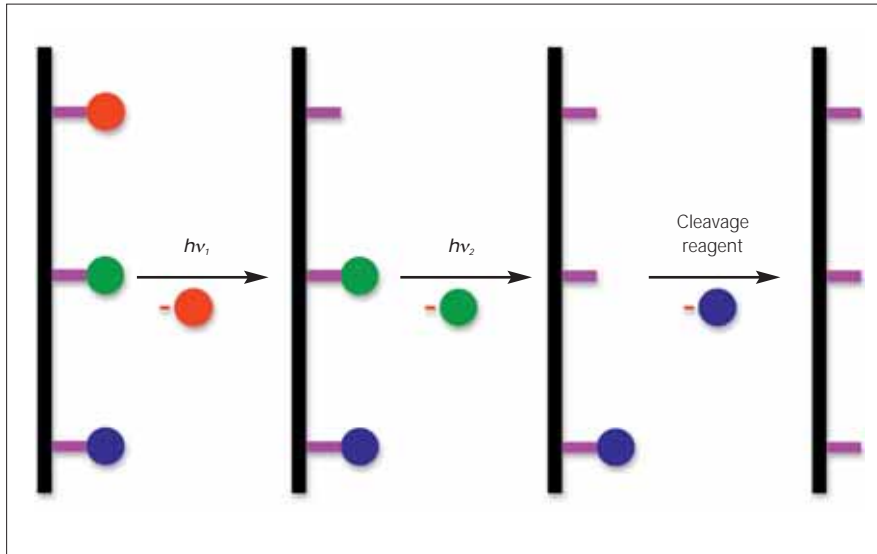
Dr. Daniel Geißler*

Nico Kotzur (doctoral student)**

Ralf Lechler (doctoral student)**/*

Brigitte Dekowski (technical assistance)

Janina Schaal (student)*



Orthogonal removal of different SH protecting groups from a model peptide containing several cysteines

Selected publications*

Böhmer M, Van Q, Weyand I, **Hagen V**, **Beyermann M**, Matsumoto M, Hoshi M, Hildebrand E, Kaupp UB (2005) Ca²⁺ spikes in the flagellum control chemotactic behavior of sperm. EMBO J 24, 2741-2752.

Geißler D, Antonenko YN, Schmidt R, **Keller S**, **Krylova OO**, **Wiesner B**, Bendig J, **Pohl P**, **Hagen V** (2005) Novel (coumarin 4-yl)methyl esters as highly efficient ultrafast phototriggers for protons and their application to membrane surface acidification. Angew Chem Int Ed 44, 1195-1198.

Hagen V, **Dekowski B**, Nache V, Schmidt R, **Geißler D**, **Lorenz D**, **Eichhorst J**, **Keller S**, Kaneko H, Benndorf K, Wiesner B (2005) Coumarinylmethyl esters for ultrafast release of high concentrations of cyclic nucleotides upon one- and two-photon photolysis. Angew Chem Int Ed 44, 7887-7891.

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Strünker T, Weyand I, Bönigk W, Van Q, Loogen A, Brown JE, Kashikar N, **Hagen V**, **Krause E**, Kaupp UB (2006) A K⁺-selective cGMP-gated ion channel controls chemosensation of sperm. Nature Cell Biol 8, 1149-1154.

FMP authors in bold, group members underlined.

Inventions¹

Hagen V, Bauer PJ

“Als Verknüpfungsreagenzien einsetzbare dimaleinimidosubstituierte Dihydroxyalkane und Verfahren zu deren Herstellung”

Priority establishing patent application: 13.09.1995

Number of patents granted: 4 (15.06.1999/02.05.2003)

Hagen V, Kaupp UB, Bendig, **Wiesner B**

“Neue photolabile Coumarinylmethylester von cyclischen Nucleotiden, Verfahren zu deren Herstellung und ihre Verwendung”

Priority establishing patent application: 20.04.2000

Number of patents granted: 4 (24.08.2005)

Number of pending applications: 2

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

“Orthogonale photolabile SH-Schutzgruppen in der Proteinsynthese”

HA 2694/3-1 (Volker Hagen, Michael Beyermann)

Land Berlin

“Entwicklung neuer photoaktivierbarer Derivate von Biomolekülen als Werkzeuge für zeit- und orts aufgelöste Untersuchungen der Mechanismen schneller zellulärer Prozesse”

(“Programm zur Förderung von Forschung, Innovationen und Technologien – ProFIT”)

IBB 10134688 (Volker Hagen)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Group Leader:
Jörg Rademann

Medicinal Chemistry

Aims

The group identifies and optimizes small molecules as specific biological effectors for studying protein structure, protein function, cell biology, and as potential starting points for pharmacological intervention. For this purpose, we develop strategies in the areas of synthetic organic chemistry, library design, and bioassays.

Chemical Biology with small molecules: Library synthesis, bioassays and hit evaluation

In order to identify small molecules as powerful and specific biological switches, chemical libraries are designed and composed. For the synthesis of proprietary, focused libraries, we develop and apply methods of combinatorial chemistry conducted in solution, with polymer reagents, and on solid phase. In most cases, library syntheses are operated in parallel with medium throughput. Synthetic expertise in the group is mainly developed for major heterocyclic hit classes and in the areas of peptidomimetics and carbohydrate derivatives.

Assay development is conducted for proteases, phosphatases and protein-protein interactions. We have devised the synthesis of fluorophore-labelled libraries for use in fluorescence polarisation-based detection in-vitro as well as for imaging application in cells and in-vivo. Compounds have been evaluated on 384 microtiter plate format, in mobility shift assays (Lab-Chip system) and in cellular test systems. For targeting multivalent binding sites and proteins, we have presented a concept for the preparation of multivalent ligands that should enable us to investigate the multivalency of a binding site in biological systems as well as to significantly increase the affinity of monovalent ligands. Recently, we have developed a concept for the discovery of binding fragments by dynamic ligation screening. The concept allows the site-directed detection of low affinity fragments in a high throughput format without prior synthesis and its systematic optimisation in iterative cycles. So far it has been applied for the discovery of inhibitory fragments binding to SARS CoV main protease (see features, p. 38), currently the concept is extended on other target classes.

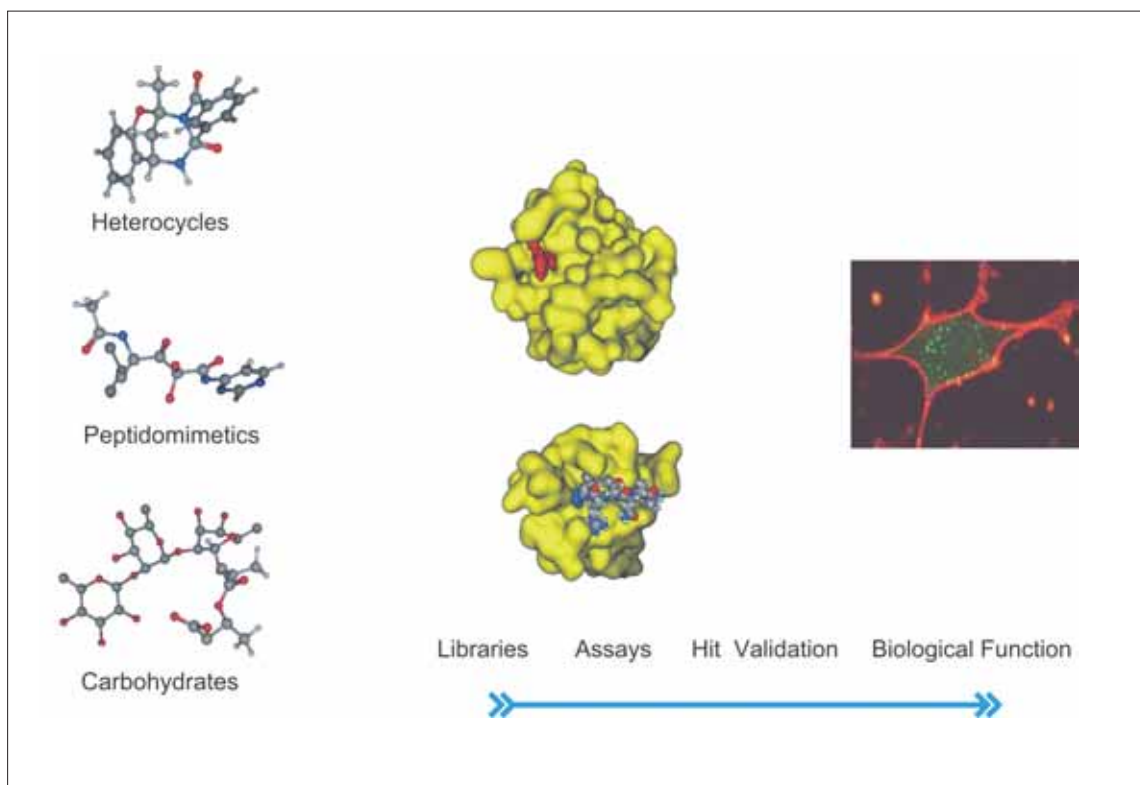
For the generation of biologically relevant hits, proper hit evaluation procedures are essential. Therefore, we have devised procedures for a systematic validation of hits including the use of analytical methodology, reaction and compound databases as well as biochemical and biophysical characterization tools. The procedures are routinely used for hits arising from the groups' own projects. As a service this expertise is offered to other groups from the institute and external collaborators using the

screening unit in order to improve the hit quality. Several groups have accepted this offer.

Internal collaborations: targeting protein-protein interactions

We work on the translation of knowledge collected in the areas of structural biology and cell biology into pharmacologically relevant "drug-like" molecules. Our major collaborative activity is in the area of protein-protein interactions. Protein-protein interactions are commonly considered as "difficult" targets for pharmacological intervention but are crucial for understanding and manipulating signalling networks in cells. As several groups at the institute are interested in protein-protein interactions, we have initiated and coordinate the crosslinking project "Identification of small-molecule inhibitors for protein-protein interactions via fluorescence-based assays". To achieve this goal, we have established methodology for the set-up of bioassays based on the reduction of protein-protein interactions to peptide protein interactions. We can generate libraries of fluorophore-labelled molecules that serve as probes in fluorescent polarisation assays in order to identify competitive small molecule binders. Thermal shift assays have been used for secondary hit validation.

The same fluorophore-labelled ligands can be used in cellular assays to localise binding partners. Moreover, the immobilisation of the identified ligand on beads will be used for the affinity-based fishing of those proteins interacting with the small molecule ligand directly from cell lysates. Fished proteins will be identified in collaboration with the Mass Spectrometry group. Major internal collaborations are with the Screening Unit, the Drug Design groups and the groups Anchored Signalling (AKAP-PKA), Protein Structure (PDZ), Protein Engineering (GYF), and Molecular Myelopoiesis (STAT-5).



Novel, bioactive molecules prepared by chemical synthesis are powerful tools to understand structure and functions in biological systems.

Recent highlights:

Growth of SARS CoV inhibited *in vivo*

We have developed reversible inhibitors of this viral enzyme employing our concept of dynamic ligation screening. Via two cycles of optimization a peptide aldehyde inhibitor has been transformed into a non-peptidic inhibitor which was highly active in an enzymatic *in vitro* assay. Finally, the hit molecules were tested in human cells infected by the SARS virus and were efficient in killing the virus.

Understanding the role of *Mycobacterium tuberculosis* protein tyrosine phosphatase:

In collaboration with the Screening Unit the group has identified a novel class of highly active protein tyrosine phosphatase inhibitors. The hit structure was modified by library synthesis. Structure-activity studies and hit evaluation revealed a unique mode of action for the hit molecules. The heterocyclic core is reduced by cellular reductants generating intermediates which react with molecular oxygen. By this reaction, the active inhibitory species are formed in a process termed as physiological arming. *In-vivo* studies with mycobacteria growing within mouse macrophages showed that the growth of bacteria in the host cells is shut down at inhibitor concentrations which are completely non-toxic to either the mycobacteria and the human cells. The finding indicates the essential role of protein tyrosine phosphatase in mycobacterial infection.

Group Leader:
Jörg Rademann

Medicinal Chemistry

Selected publications[#]

Weik S, **Rademann J** (2003) A phosphorane as supported acylation equivalent. Linker reagents for smooth and versatile C-C-coupling reactions. *Angew Chem Int Ed* 42, 2491-2494.

Barth M, Fischer R, Brock R, **Rademann J** (2005) Reversible cross-linking of hyperbranched polymers: a strategy for the combinatorial decoration of multivalent scaffolds. *Angew Chem Int Ed* 44, 1560-1563.

Bauer J, **Rademann J** (2005) Hydrophobically assisted switching phase synthesis: the flexible combination of solid-phase and solution-phase reactions employed for oligosaccharide preparation. *J Am Chem Soc* 127, 7296-7297.

Al-Gharabli SI, **Ali Shah ST**, **Weik S**, **Schmidt M**, Mesters JR, Kuhn S, Klebe G, Hilgenfeld R, **Rademann J** (2006) An efficient method for the synthesis of peptide aldehyde libraries employed in the discovery of SARS coronavirus main protease (SARS-CoV Mpro) inhibitors. *ChemBiochem* 7, 1084-1055.

Bauer J, Brandenburg K, Zähringer U, **Rademann J** (2006) Chemical synthesis of a glycolipid library by a solid phase strategy allows to elucidate the structural specificity of immune stimulation by rhamnolipids. *Chem Eur J* 12, 7116-7124.

Weik S, Luksch T, Evers A, Böttcher A, Sotriffer CA, Hasilik A, Löffler HG, Klebe G, **Rademann J** (2006) The potential of P1 site alterations in peptidomimetic protease inhibitors as suggested by virtual screening and explored by the use of C-C-coupling reagents. *Chemmedchem* 1, 445-457.

Schmidt M, **Isidro-Llobet A**, **Lisurek M**, Hilgenfeld R, **Rademann J** (2007) Dynamic ligation screening: a method for the site-directed discovery of low affinity inhibitory fragments, submitted for publication.

[#] FMP authors in bold, group members underlined.

Inventions¹

Hellmuth K, Birchmeier W, **Rademann J**, Grosskopf S
"Shp-2 inhibitors, pharmaceutical compositions comprising them and their use for treating phosphatase-mediated diseases"

Priority establishing patent application: 01.06.2005

Number of pending applications: 1

(Shared invention of FMP and MDC)

Rademann J

"Polymerverbindung aufgebaut aus linearen Polymerketten"

Priority establishing patent application: 10.04.2002

Number of pending applications: 2

Rademann J, Weik S et al.

"Substituted ethane-1,2-diamines for the treatment of Alzheimer's disease"

Priority establishing patent application: 22.05.2004

Rights: Boehringer Ingelheim International GmbH

Rosenthal W, Klußmann E, Christian F, Rademann J, Meyer S

"Niedermolekulare Substanzen zur Hemmung der Interaktion zwischen Proteinkinase A und Proteinkinase A-Ankerprotein"

Priority establishing patent application: 18.05.2005

Number of pending applications: 2

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

"Synthesis, optimisation, and screening of small molecule libraries targeting protein-protein interactions" (Sub-project of the Research Unit "Interfering with intracellular protein-protein interactions – probing protein functions with small molecules")

FOR 806 TP Z1 (RA 895/5-1) (Jörg Rademann, Michael Beyermann, Jens Peter von Kries)

Deutsche Forschungsgemeinschaft

"Modulation of PDZ-domain-mediated protein-protein interactions" (Sub-project of the Research Unit "Interfering with intracellular protein-protein interactions – probing protein functions with small molecules")

FOR 806 TP 5 (Hartmut Oschkinat, Gerd Krause, Jörg Rademann)

Deutsche Forschungsgemeinschaft

"Reagent linkers for the synthesis of protease inhibitors"

RA 895/2-4 (Jörg Rademann)

Deutsche Forschungsgemeinschaft

"Reaktive Intermediate in polymeren Gelen, ihre Anwendung in parallelen Synthesen von Proteaseinhibitoren sowie deren biochemische Evaluierung"

RA 895/2-5 (Jörg Rademann)

Members of the group

Dr. Samer Al-Gharabli*

Dr. Samuel Beligny*

Dr. Pradeep Kumar (visiting scientist)*

Dr. Liudmila Perepelitchenko
(technical assistance)*

Gunthard Stübs (scientist)*

Jörg Bauer (doctoral student)**/*

Adeeb El-Dahshan (doctoral student)**

Stefanie Grosskopf (doctoral student)**/*

Alberto Isidro-Llobet (doctoral student)*

Sina Meyer (doctoral student)**/*

Richard Bunnag von Briesen Raz
(doctoral student)**/*

Martin Richter (doctoral student)**/*

Marco Schmidt (doctoral student)**/*

Viviane Uryga-Polowy (doctoral student)**

Swantje Behnken (student)*

Roland Kersten (student)*

Franziska Meier (student)*

Johannes Preidl (student)*

Deutsche Forschungsgemeinschaft

" Diversitätsorientierte Synthese von Rhamnolipiden"

RA 895/3-1 (Jörg Rademann)

Deutsche Forschungsgemeinschaft

" Multifunctional peptide polymers for the efficient modulation
of intracellular signal transduction"

RA 895/4-1 (Jörg Rademann)

Deutsche Forschungsgemeinschaft

" Ansbuchfinanzierung SFB 765"

Start-up SFB 765 (Jörg Rademann)

European Community (6th Framework Programme)

Sub-project of the Integrated Project MolDiag-Paca: " Novel
molecular diagnostic tools for the prevention and diagnosis of
pancreatic cancer"

PL018771 (Jörg Rademann, Jens Peter von Kries)

Alexander von Humboldt Foundation

" Sponsoring for a research stay"

3-8121/INI/1033153 (Pradeep Kumar)

Boehringer Ingelheim Pharma

" Exclusive synthesis of aspartate protease inhibitors"

Boehringer Ingelheim – RA2 (Jörg Rademann)

Fonds der Chemischen Industrie

" Förderung der chemierelevanten Grundlagenforschung"

FCI – 166647 (Jörg Rademann)

Freie Universität Berlin

" Einwerbepremie Humboldt-Stipendium"

Einwerbepremie Kumar (Pradeep Kumar)

Generalitat de Catalunya

" Synthese von SARS CoV Inhibitoren"

Katalonien (Albert Isidro-Llobet)

Land Berlin

" Entwicklung eines spezifischen STAT5 Inhibitors" (" Programm
zur Förderung von Forschung, Innovationen und Technologien –
ProFIT")

IBB 10134292 (Dirk Carstanjen, Jörg Rademann)

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.



Screening Unit

Group Leader:
Jens Peter von Kries

Aims

The Screening Unit has been established at the FMP to serve as an open platform for screening projects, particularly for academic users. It draws on the FMP collections of ca. 50,000 compounds. The Unit works to establish and improve new screening technologies such as automated microscopes and the LabChip mobility shift HTS. The group manages the central compound collection of ChemBioNet and is involved in library design with the drug design and modelling group of the FMP.

Mycobacterium tuberculosis enzyme inhibitor screens

Infection with *Mycobacterium tuberculosis* results predominantly in an asymptomatic persistent infection, often referred to as latency. Recent molecular genetic approaches have yielded *Mycobacterium* proteins and lipids important for virulence and persistence.

The "Structural Proteomics Consortium" selected proteins from *Mycobacterium tuberculosis* on the basis of gene ablation experiments (in cooperation with MPI für Infektionsbiologie, Berlin) resulting in reduced infectiveness. The structures of selected proteins are elucidated at the European Molecular Biology Laboratory (EMBL) Hamburg and screened for small molecule inhibitors at the Screening Unit in Berlin and by Combinature (NMR-screening, Berlin).

Since autumn 2004, the Screening Unit has performed 40 primary screens for small molecule inhibitors of protein interactions or enzyme functions. The projects included assay set up for high-throughput screening (HTS) of the 20,000 compounds of the FMP fragment library and about 17,000 compounds of the ChemBioNet collection (bringing the number of compounds in all FMP libraries to about 50,000), hit analysis by structural similarity clustering, and validation of concentration-dependent mode of action. For selected projects, virtual screens and models for binding of inhibitors were developed by the computational chemist of the unit. Target proteins have come from *M. tuberculosis*, *Trypanosoma brucei* and *cruzi*, and human enzymes for specificity profiling of bioactive compounds in collaboration with Oxford University.

Two primary screens using the *Mycobacterium* 3-isopropylmalate dehydrogenase and *Mycobacterium* sterol 14 α -demethylase resulted in the identification of inhibitors with IC₅₀-values in the micro molar range. One of the CYP51 antagonists inhibits growth of tuberculosis bacteria in human macrophages. This compound has already been co-crystallised for analysis of substrate

recognition mechanisms in the catalytic site of the enzyme. IPMDH inhibitors have been identified by HTS with 20,000 compounds of the FMP library and by virtual screening of 35,000 compounds. Other *Mycobacterium* enzymes screened focus on phospho-tyrosine and inositolphosphate-phosphatases involved in cellular signalling pathways of the bacteria or of the infected host cells. Specificity profiling of bioactive compounds against related human enzymes (collaborations with the group of W. Birchmeier and M. Sundström, MDC Berlin and Oxford-University) and co-crystallisation trials have begun. These projects may result in the identification of molecules which disable *Mycobacterium tuberculosis* to evade the organism's immune defense. The compounds may also be of use in the development of drugs against tuberculosis infections in partnership with pharmaceutical companies.

MHC-loading enhancers (collaboration with O. Röttschke, MDC Berlin-Buch)

MHCs function as peptide receptors which display antigens on the cell surface for surveillance by T cells. Upon recognition, these antigens can trigger the destruction of the cell, which raised the possibility of their use in experimental tumour immune therapies. In principle, exogenously added peptides can activate tumour specific T cells very efficiently, but their efficacy is severely reduced by the low number of MHC molecules actually accessible for loading. The group of O. Röttschke (MDC Berlin-Buch) recently discovered small molecules from the FMP library that are able to generate peptide-receptive MHC molecules. Increased loading efficiency translates directly into a dramatic enhancement of the T cell response.

The Screening Unit has already supported primary screening and validation of small molecule modulators of one specific MHC-subtype. In the next phase of this BMBF-funded project, other MHC-subtype screens and activity optimisation trials will be supported by the Screening Unit in cooperation with the Drug Design group.

Internal collaborations

MLE, enhancer of antigen presentation (R. Kühne, C. Freund; BMBF)

Library design for ChemBioNet (R. Kühne)

Novel antagonists for *Mycobacterium* (H. Oshkinat; BMBF)

Interfering with UBP_y deubiquitylation (K. P. Knobloch)

Pharmacological interference with AKAP (E. Klußmann)

Diagnostic tools for pancreatic tumors (J. Rademann; EU)

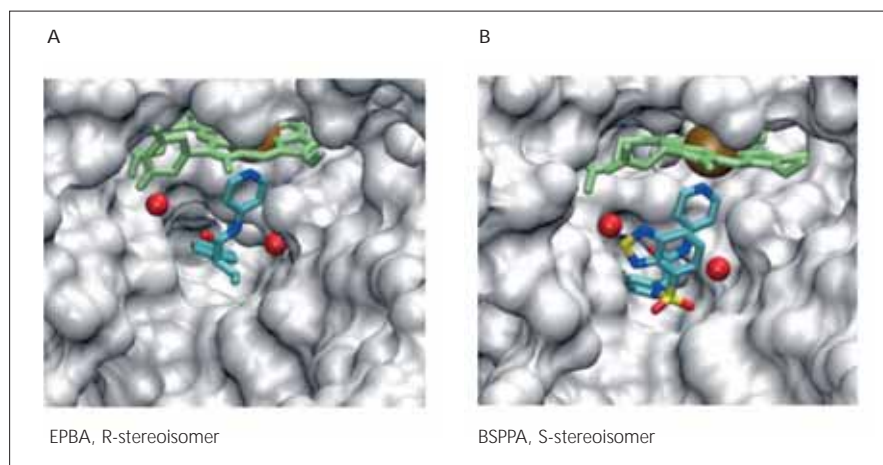
Members of the group

Dr. Michael Lisurek^{***1*}

Angelika Ehrlich (technical assistance)

Christoph Erdmann (technical assistance)

Franziska Hinterleitner (technical assistance)*



Binding of EPBA and BSPPA in the MtCYP51 active site. EPBA (A) and BSPPA (B) bound in the active site of MtCYP51, which is represented by a space filled model. Both compounds (cyan) and the heme edge (green) are clearly seen through the active site opening created by a bend of the I helix and an open conformation of the BC-loop. The ethyl, in A, and benzothiadiazol sulfonamide, in B, groups protrude into the bulk solvent through an open space of the active site entrance. In A, heme propionate side chain is shown in two alternative conformations. In B, benzothiadiazole ring of BSPPA flips over to make stacking contacts with the pyridine ring of the same molecule and the Y76 side chain. Some contacts are also made with the heme propionate chain, which apparently stabilizes the latter in a single conformation. Images were generated using VMD software.

Selected publications*

Manger M, Scheck M, Prinz H, **von Kries JP**, Langer T, Saxene K, Schwalbe H, Fürstner A, **Rademann J**, Waldmann H (2005) Discovery of *Mycobacterium tuberculosis* protein tyrosine phosphatase A (MtpA) inhibitors based on natural products and a fragment-based approach. *Chembiochem* 6, 1749-1753.

Singh RK, Kefala G, Janowski R, Mueller-Dieckmann C, **von Kries JP**, Weiss MS (2005) The high-resolution structure of LeuB (Rv2995c) from *Mycobacterium tuberculosis*. *J Mol Biol* 346, 1-11.

Eswaran J, **von Kries JP**, Marsden B, Longman E, Debreczeni JE, Ugochukwu E, Turnbull A, Lee WH, Knapp S, Barr AJ (2006) Crystal structures and inhibitor identification for PTPN5, PTPRR and PTPN7 – a family of human MAP-kinase specific protein tyrosine phosphates. *Biochem J* 395, 483-491.

Mueller-Dieckmann C, Kernstock S, **Lisurek M**, **von Kries JP**, Haag F, Weiss MS, Koch-Nolte F (2006) The structure of human ADP-ribosylhydrolase 3 (ARH3) provides insights into the reversibility of protein ADP-ribosylation. *Proc Natl Acad Sci USA* 103, 15026-15031.

FMP authors in bold, group members underlined.

Inventions¹

von Kries JP, Birchmeier W

"Agents for treating human diseases, especially treating tumors such as colonic cancers and melanomas or for regenerating tissue"

Priority establishing patent application: 16.09.1999

Number of pending applications: 1

Rights: MDC

¹Inventors' declaration and/or priority establishing patent application during the period reported. FMP inventors in bold. As at 31.12.2006

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.

Grants/Externally-funded projects

Deutsche Forschungsgemeinschaft

"Synthesis, optimisation, and screening of small molecule libraries targeting protein-protein interactions" (Sub-project of the Research Unit "Interfering with intracellular protein-protein interactions – probing protein functions with small molecules") FOR 806 TP Z1 (RA 895/5-1) (Jörg Rademann, Michael Beyermann, Jens Peter von Kries)

Federal Ministry of Education and Research

"Screening Unit: Assay development screening for lead identification and optimization" (Sub-project of the collaborative project: "MHC-loading enhancers: Molekülmodellierung und Computerchemie, Screening und strukturelle und biophysikalische Untersuchungen") 01GU0514 – KR (Jens Peter von Kries)

Federal Ministry of Education and Research

"Screening von Substanzbibliotheken und Struktur-basiertes Wirkstoffdesign" (Sub-project of the collaborative project "Strukturproteomik-Konsortium Hamburg: Hochdurchsatz-Strukturanalyse von *Mycobacterium-tuberculosis*-Zielproteinen und ihrer Ligandenkomplexe zur Suche nach Wirkstoffen") 0312992J (Jens Peter von Kries, Hartmut Oschkinat)

European Community (6th Framework Programme)

Sub-project of the Integrated Project MolDiag-Paca: "Novel molecular diagnostic tools for the prevention and diagnosis of pancreatic cancer" PL018771 (Jörg Rademann, Jens Peter von Kries)

Land Berlin

"Ein automatisiertes Mikroskop zum Ausbau der Screening-Plattform des Netzwerks für Wirkstoffentwicklung in Berlin/Brandenburg" IBB 10132986 (Jens Peter von Kries)



Group Leader:
Sandro Keller

Biophysics of Membrane Proteins

Aims

Our group is applying thermodynamic approaches to elucidate the complex interplay between proteins and lipids in bilayer membranes and detergent micelles. Highly specific contacts between transmembrane helices control the folding, oligomerisation, and intramembrane substrate binding of membrane proteins. We hope to achieve a quantitative understanding of the principles underlying the behavior of membrane proteins and how changes contribute to their functions.

Thermodynamics of intramembrane protein folding

We are using calorimetric and spectroscopic methods to characterise the thermodynamics of protein folding and protein-protein interactions in lipid membranes and detergent micelles. As a first step in this direction, the assembly of the simple bacterial membrane protein *Mistic* is assessed by systematically quantifying the interactions among four synthetic peptides corresponding to the protein's four transmembrane domains.

This work focuses on the following questions:

- Which forces determine intramolecular interactions in membrane proteins?
- Can we derive the topology of membrane proteins from interhelical affinities?
- Which forces drive intermolecular interactions in the membrane environment?

Domain formation in complex membranes

"Lipid rafts" are under intense investigation because of their physiological and pathological implications. We are interested in protein-induced domains that are too small to be visualised microscopically and hope to understand: Using novel microcalorimetric techniques and confocal laser scanning microscopy, we are examining the properties of peptide- and protein-dependent lipid clusters. Poly(L-lysine) peptides of different chain lengths are useful model systems for studying the influence of proteins on the following questions:

- How large and how stable are these protein-tethered membrane domains?
- How is the aggregational state of one leaflet coupled to that of another leaflet?
- To which extent can peptides and proteins modulate membrane curvature?

Internal collaborations

Membrane proteins are outstandingly important drug targets and play an eminent role in most research themes represented at the FMP. Our approach relies on the availability of large amounts of well-characterised synthetic peptides and therefore requires close collaborations with groups focussing on protein expression and purification (A. Diehl), Peptide Synthesis (M. Beyermann), Mass Spectrometry (E. Krause), and Peptide Lipid Interaction (M. Dathe). Furthermore, the biophysical methods we provide for characterising molecular interactions complement the expertise available at the Department of NMR-Supported Structural Biology (H. Oschkinat), the Solid-State NMR group (B. Reif), the Protein Engineering group (C. Freund), and the Synthetic Organic Chemistry group (V. Hagen).

Selected publications*

Bárány-Wallje E, **Keller S**, Serowy S, Geibel S, Pohl P, **Bienert M**, **Dathe M** (2005) A critical reassessment of penetratin translocation across lipid membranes. *Biophys J* 89, 2513-2521.

Keller S, Sauer I, Strauss H, Gast K, **Dathe M**, **Bienert M** (2005) Membrane-mimetic nanocarriers formed by a dipalmitoylated cell-penetrating peptide. *Angew Chem Int Ed* 44, 5252-5255.

Keller S, Tsamaloukas A, Heerklotz H (2005) A quantitative model describing the selective solubilization of membrane domains. *J Am Chem Soc* 127, 11469-11476.

Cambridge SB, **Geißler D**, **Keller S**, Cürten B (2006) A caged doxycycline analogue for photoactivated gene expression. *Angew Chem Int Ed* 45, 2229-2231.

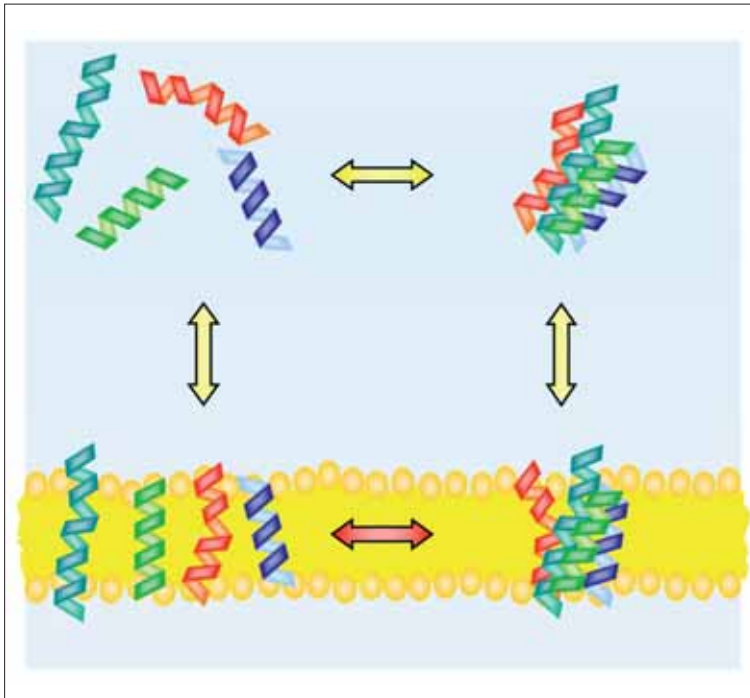
Keller S, Heerklotz H, Blume A (2006) Monitoring lipid membrane translocation of sodium dodecyl sulfate by isothermal titration calorimetry. *J Am Chem Soc* 128, 1279-1286.

Keller S, Heerklotz H, **Jahnke N**, Blume A (2006) Thermodynamics of lipid membrane solubilization by sodium dodecyl sulfate. *Biophys J* 90, 4509-4521.

* FMP authors in bold, group members underlined.

Members of the group*

Natalie Bordag (doctoral student)**
Monika Georgi (technical assistance)
Maiko Thiele (technical assistance)**
Anja Wasmund (technical assistance)**
Anja Sieber (student)



MistC is a small bacterial membrane protein consisting of four transmembrane helices. This protein lends itself particularly well to thermodynamic investigations because it can fold autonomously in aqueous solutions, lipid membranes, and detergent micelles, thus allowing for a direct comparison among the folding processes in these environments.

*This group started in 2006.

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.

Microdialysis Service (Regina Richter)

Aims

The group uses reverse microdialysis in combination with advanced mass spectrometric techniques (collaborating with the Mass Spectrometry group) in order to study the clearance of neuropeptides in the brain of conscious rats and mice close to real-time. The methodology is available for external groups as well as for in-house researchers.

We are also applying this established technology to our own research projects. We aim to understand the mechanisms of *in vivo* processing of brain neuropeptides related to cardiovascular dysfunction, senescence and, ultimately, neurodegenerative processes such as Alzheimer's disease (AD). Our interests are focused on strategies leading to reduced levels of amyloid- β peptides (A β), the major component of AD-related plaques and peptides of the renin angiotensin system (RAS). To address these specific questions of peptide processing, experiments with transgenic and gene-targeted mouse models have been performed.

NEP and IDE: major proteases involved in the A β clearance

Evidence suggests that excessive A β accumulation is not in all AD cases associated with increased A β production. Multiple proteases such as neutral endopeptidase (NEP) and insulin-degrading enzyme (IDE) have recently been identified to degrade extracellular A β , however, *in vivo* studies are rare and differ from *in vitro* reports.

For NEP, we identified a primary cleavage site at position 33/34 (Gly-Leu) and found a N-terminal directed ladder-like degradation in the hippocampus of rats and mice. Both were successfully blocked by the NEP inhibitors phosphoramidon and thiorphan. The particular role of IDE in A β degradation was explored in studies on conscious NEP knockout mice (in collaboration with H.B. Hersh, University of Kentucky, Lexington, USA). These studies revealed a cleavage pattern that was dominated by short N-terminal fragments while the C-terminal region remained intact. Next, the clearance of A β peptides will be studied in greater detail using IDE knockout mouse models.

Role of ADAM 10 proteinase in the clearance of A β

There is *in vitro* evidence that members of a disintegrin and metalloproteinase (ADAM) family may generate A β peptides in cell lines by a cleavage mechanism relevant to α -secretase activity in the amyloid precursor protein (APP). The main cleavage site of the ADAM 10 species was found to be between the peptide bond Lys₁₆ and Leu₁₇ of the A β chain yielding the p3 peptide A β ₁₇₋₄₂. Thus, it has been speculated that ADAM 10 activity may also prevent amyloid plaque formation. Recently, we characterized the processing of A β peptides in ADAM 10 overexpressing mice versus wildtype mice (in collaboration with F. Fahrenholz, University of Mainz).

While the degrading activity in wildtype resulted in the postulated (1-16) and (17-33) fragments, the cleavage of the A β ₁₋₄₀ chain in ADAM 10 mice was focused only on the N-terminal region. These findings suggest that efficient α -secretase activity *in vivo* may be restricted to the cleavage of the APP molecule. To verify these results, investigations with a dominant negative mutant (ADAM-dn) expressing reduced α -secretase activity and long-term cerebral administration of specific inhibitors using ALZET microinfusion pumps are underway.

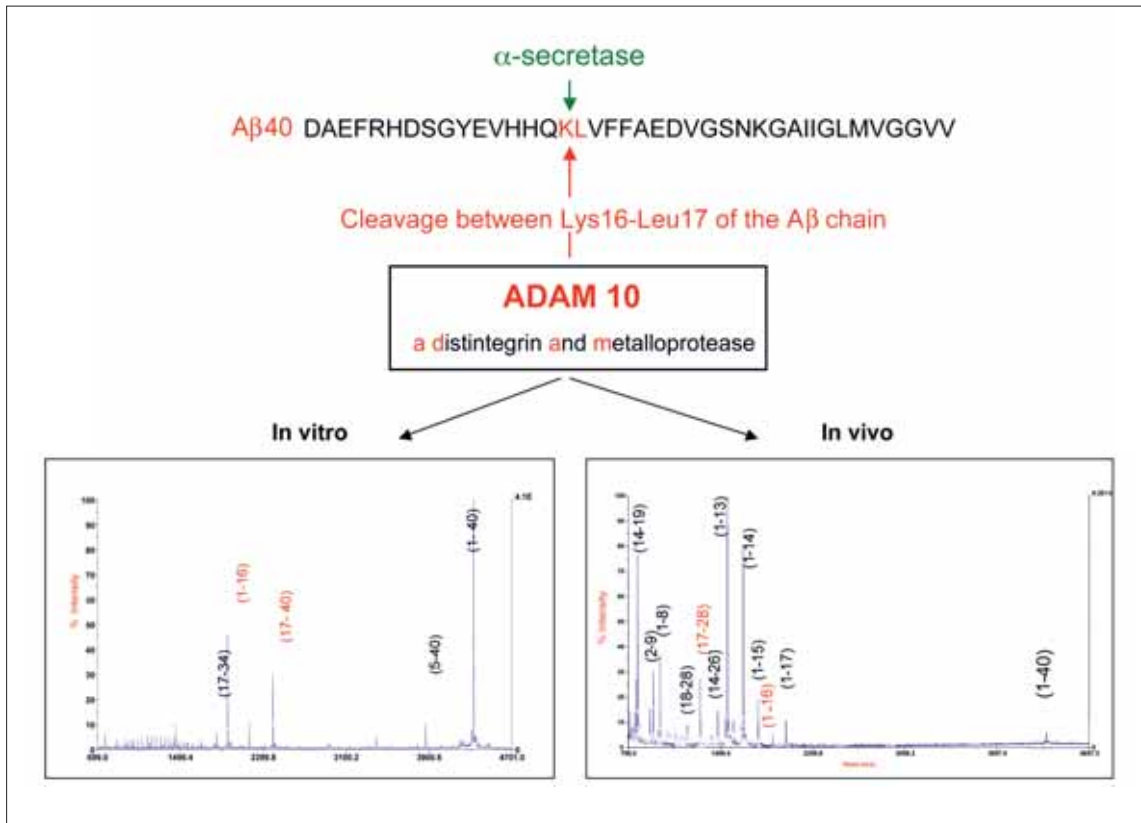
Metabolic pathways of angiotensin peptides

The exploration of the enzymatic pathways of Angiotensin I (Ang I) revealed the formation of numerous bioactive angiotensin peptides. In particular, the metabolic product angiotensin (1-7) [Ang-(1-7)] is thought to mimic and oppose the multiple actions of angiotensin II. Differential metabolic pathways and proteases, such as angiotensin-converting enzyme (ACE), its homologue ACE2 and NEP are involved in the formation of angiotensin (1-7) [Ang-(1-7)]. The metabolic pathways and the role of substrate availability are, however, not fully understood.

Using a NEP knockout mouse model and protease inhibitors we tested the hypothesis that lack of NEP or substrate would diminish or even prevent the formation of Ang-(1-7). We found the N-terminal angiotensin fragments (1-9), (1-8), (1-7), as well as numerous C-terminal fragments of differing intensity in NEP-knockout and wildtype mice. After pretreatment with the ACE inhibitors, the formation of the fragment (1-8), one major source of (1-7), seemed to be diminished. Nevertheless, substantial signals of the fragments (1-7) and (1-9) were detected suggesting a more central role for both the enzymes ACE and ACE 2 than for NEP in the formation of Ang-(1-7).

Members of the group

Oliver Klein (technical assistance)**
Nadine Scharek (technical assistance)**/*
Christian Wolff (technical assistance)**/*



MALDI mass spectra show cleavage sites of Aβ(1-40) peptide fragments obtained from an *in vitro* preparation (left) and an ADAM 10 overexpressing mouse *in vivo* (right). The peptide was infused into the hippocampus of the animal at a very low flow rate.

Selected publications*

Richter RM, Kraus M, Wolff C, Schuchardt S (2005) Impact of structural formation of amyloid β-peptides on the *in vivo* enzymatic degradation in rat hippocampus. Soc Neurosci Abstr 325.11.

Richter RM, Wolff C, Schuchardt S (2006) *In vivo* processing of bioactive angiotensin peptides in the mouse brain: focus on Angiotensin (1-7). Soc Neurosci Abstr 453.9.

Richter RM, Wolff C, Schuchardt S (2006) Role of NEP in the formation of Angiotensin(1-7) – a microdialysis study in NEP knockout Mice. Neuropeptides 40 (Abstracts from the Summer Neuropeptide Conference, Miami Beach, USA), 154-155.

FMP authors in bold, group members underlined.

*part of period reported

**part time

yellow Position funded externally (3rd-party funds) for at least part of the reporting period.

Administrative and Technical Services



Directorate

Prof. Walter Rosenthal (Director)
Dr. Björn Maul (Scientific coordination, public relations)
Dr. Anne Höner (EU-liasion officer)^{***}
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Thomas Jahn (Network administration)
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Alexander Heyne (Student)
Björn Schümann (Student)

^{*}part of period reported

^{**}part time

Offices

Andrea Steuer (Department of NMR-supported Structural Biology)
Alexandra Kiesling (Department of Molecular Genetics)
Marianne Dreißigacker (Department of Peptide Chemistry and Biochemistry)
Pia Philippi (Department of Physiology and Pathology of Ion Transport)

DNA Sequencing Service

Dr. Erhard Klauschenz^{**}
Barbara Mohs (Technical assistance)^{*}

Animal Housing

Dr. Regina Richter (Head)^{*}
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Safety officers

Dr. Jens Furkert^{*}
Hans-Ulrich Heyne^{***}

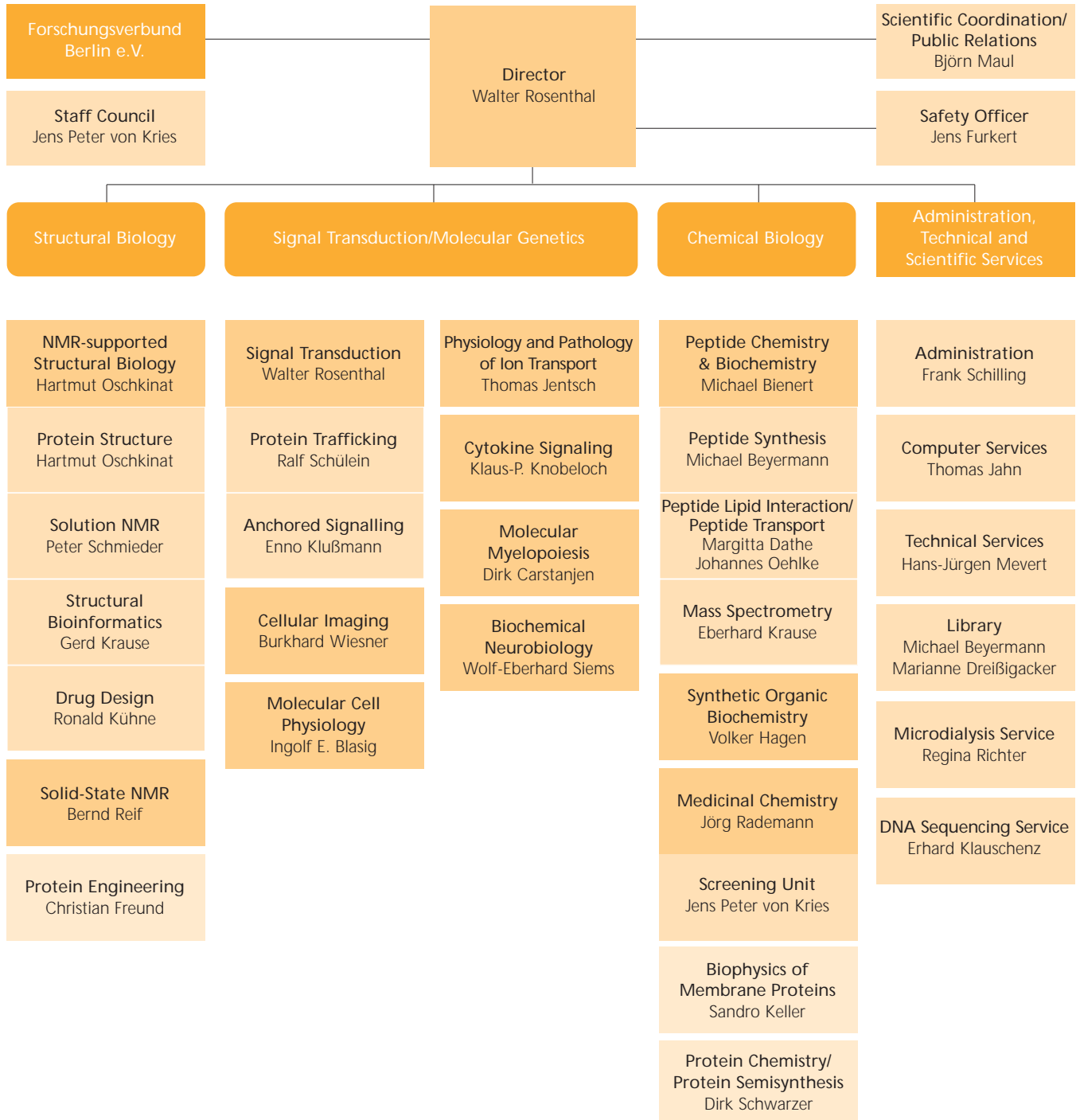
Technical Service

Hans-Jürgen Mevert
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Holger Panzer
Michael Uschner
Stephanie Wendt
Roy Wolschke

Library

Dr. Michael Beyermann^{**}
Renate Peters (Librarian)^{***}
Marianne Dreißigacker^{**}

Structure of the FMP



- (round corners): sections
- departments and independent groups
- scientific groups associated with departments or with independent groups
- junior research groups (temporary)

As at August 2007



□ **Common Facilities**

- A 8 Gate House with Café Max and apartments
- A 9 Reception gate
- A 13 Life Science Learning Lab; CampusInfoCenter
- A 14 Cafeteria

Guesthouses of the MDC

- B 54 Hans-Gummel-House
- B 61 Salvadore-Luria-House with kindergarten

■ **Research**

Max Delbrück Center for Molecular Medicine (MDC)

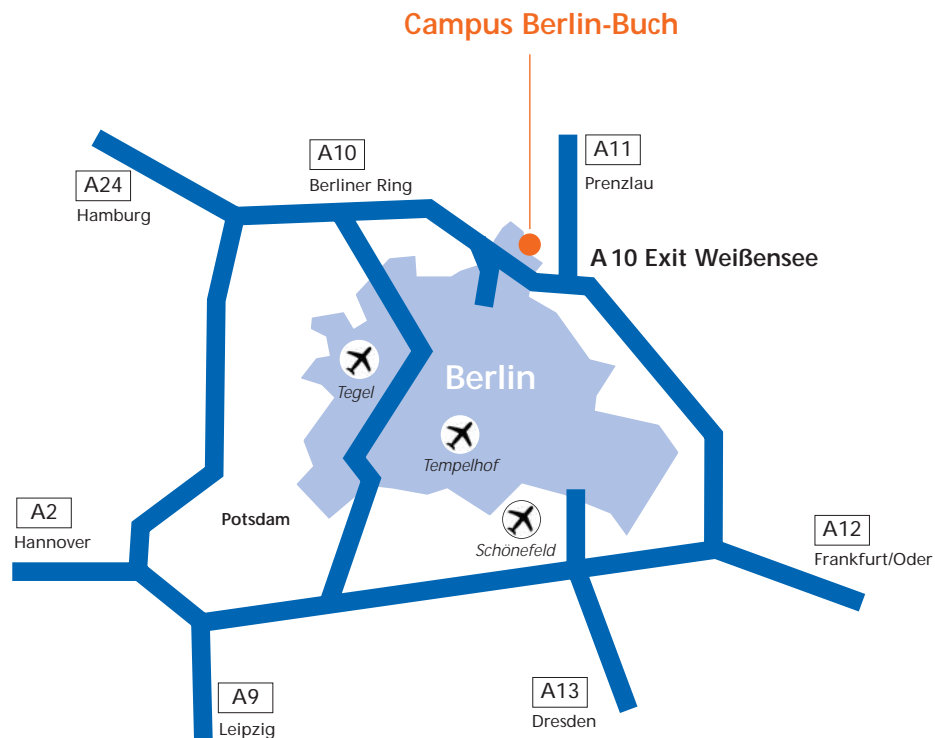
- C31.1-3 Max-Delbrück-House
- C 27 Walter-Friedrich-House
- C 83 Max-Delbrück-Communications Center (MDC.C)
- C 84 Hermann-von-Helmholtz-House
- C 87 Timoféeff-Ressovsky-House
- C 71
- B 63 } Research services
- B 64 }
- A 10 Library
- Leibniz-Institut für Molekulare Pharmakologie (FMP)**
- C 81 FMP

■ **Clinics**

- B42-53 Robert-Rössle-Clinic

■ **Companies**

- A 15 car workshop, EZAG, Charles River, WISAG
- B 55 **Oskar-u.-Cécile-vogt-House:**
BBB-post office, Patent lawyer Dr. Baumbach, ConGen, E.R.D.E., Höppner, GHZ & S, Human TECAN, Dr. Scherrer, ART-CHEM, Roboklon Fresenius, GM Food, Quintiles
- B 64 epo
- D16/23 Eckert & Ziegler, MEMOD, Eurotope, Glycotope, BEBIG, Eckert Consult, Isotope Products
- D 79 **Erwin-Negelein-House:**
GLYCOTOPE, Bavarian Nordic (domicile H31.1), BioTeZ, Isotope Products, celares, FILT
- D 80 **Otto-Warburg-House:**
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- D 85 **Arnold-Graffi-House:**
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Imprint:

Leibniz-Institut für Molekulare Pharmakologie

Campus Berlin-Buch
 Robert-Rössle-Str. 10
 13125 Berlin
 Germany

phone: +49-(0)30-94793-102
 fax: +49-(0)30-94793-109
 e-mail: maul@fmp-berlin.de

The Leibniz-Institut für Molekulare Pharmakologie is administratively organised within the Forschungsverbund Berlin e.V., along with seven other research institutions, and is a member of the Leibniz Association. The FMP is funded equally by the Federal Government and the Senat of Berlin.

Research Report 2005 2006

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