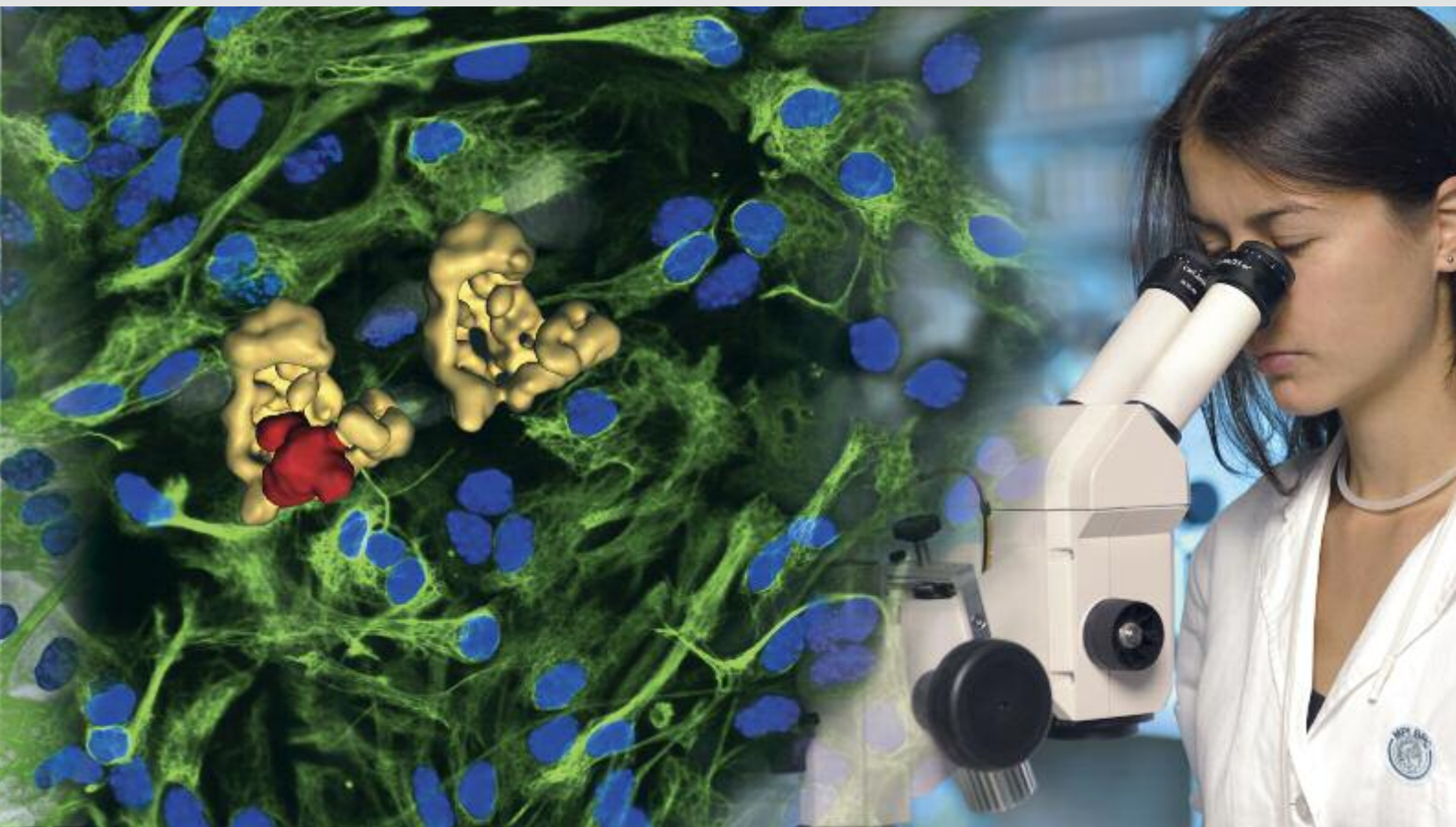




MAX-PLANCK-GESELLSCHAFT



Max Planck Institute
for Biophysical Chemistry
Karl Friedrich Bonhoeffer Institute
Göttingen



Preamble

Located in Göttingen, home of one of Germany's foremost universities, the Max Planck Institute for Biophysical Chemistry has for many years been conducting cutting-edge research. As one of the largest institutes of the Max Planck Society, it houses numerous research groups and provides them with an elaborate network of workshops and central facilities. Students and researchers from various disciplines and nations collaborate not only with their colleagues at the institute but also with a large number of experts worldwide to shed light on complex life processes.

The novel insights gained from the basic research conducted here have continuously facilitated innovation and have led to many economically successful licensing agreements and spin-off companies. With this brochure, we would like to invite you to tour our institute and learn about our history and current research activities. We hope it inspires you to take a closer look at our ongoing research and, eventually, we can welcome you to one of our visitor and training programs

*Helmut Grubmüller
Managing Director, October 2009*



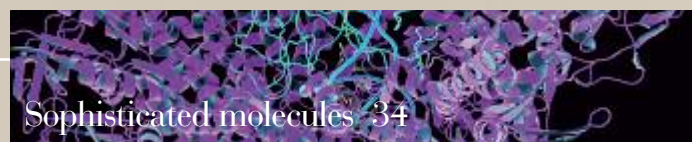
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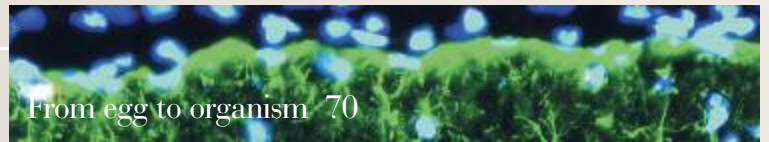
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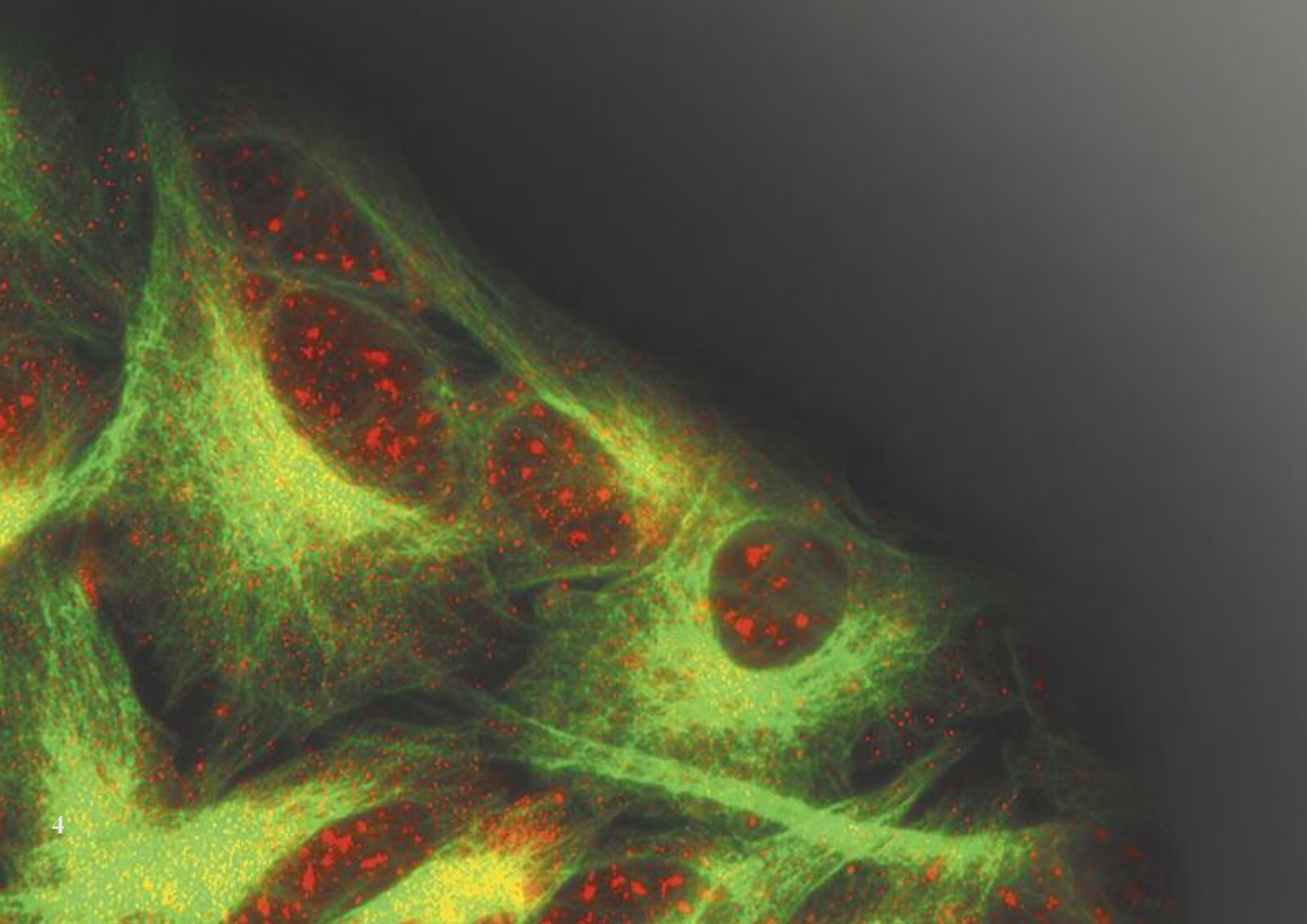
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An expedition into the world of molecules

How do nerve cells talk to each other? How does a complex organism evolve from a single egg cell? How is our »biological clock« controlled? Scientists at the Max Planck Institute for Biophysical Chemistry are on the trail towards unraveling the answers to these, and other, fundamental biological questions. However, observing the molecular mechanisms that control and regulate these vital cellular processes is no easy feat. They occur deep within the nanocosmos of living cells and are therefore invisible to the naked eye. Conventional microscopes can detect bacteria or observe individual body cell. However, what occurs deep within the inner workings of a living cell remains an unsolved mystery.

One focus of the institute's research is the development of special methods that provide a closer look into the world of molecules. Ultra-high resolution fluorescence microscopy, nuclear magnetic resonance spectroscopy, cryo-electron microscopy and computer simulations are just a few of the methods that are successfully used to investigate proteins – the minute nanomachines of living cells. The aim is to unravel the many tricks that proteins play to fulfill their diverse cellular functions as molecular motors, chemical plants or photoelectric cells, for example. Cellular logistics is also carefully managed by proteins. How specific proteins sort and transport different cargos between the various compartments of a cell is one of the topics being explored in greater detail here.

Other scientists are investigating how a cell converts the basic blueprints of proteins into readable





forms, and are revealing how the cellular protein factories – the ribosomes – function. Proteins can only successfully fulfill their tasks when they are correctly assembled. The molecular mechanisms underlying the quality control for protein production are also being intensively studied at the institute.

Likewise, many phenomena of inanimate nature can be traced back to molecular processes. Many molecules, radicals and atoms in the atmosphere react with each other, for example, after they have been produced and excited by solar radiation. Another group of researchers is focusing on investigating these and other forms of internal molecular dynamics.

At the Max Planck Institute for Biophysical Chemistry, scientists from various disciplines and of different nationalities work together to shed light on complex life processes. Biologists, chemists, medical scientists and physicists collaborate not only with their colleagues at the institute, but also with a large number of renowned experts worldwide.

Accordingly, many different languages can be heard on the campus as people exchange views on projects, ideas and results. When the Max Planck Institute for Dynamics and Self-Organization moves from central Göttingen to the Faßberg site at the end of 2010, more than 1,000 employees will be working on the newly established Max Planck Campus.



Research without constraints

Like all other Max Planck Institutes, the Max Planck Institute for Biophysical Chemistry primarily pursues basic research. Here, researchers follow up on fundamental new ideas. This »uninhibited« research, the excellent working conditions and the outstanding international reputation are the reasons why the Max Planck Institute for Biophysical Chemistry has become a center of attraction for both students and renowned researchers from all over the world.

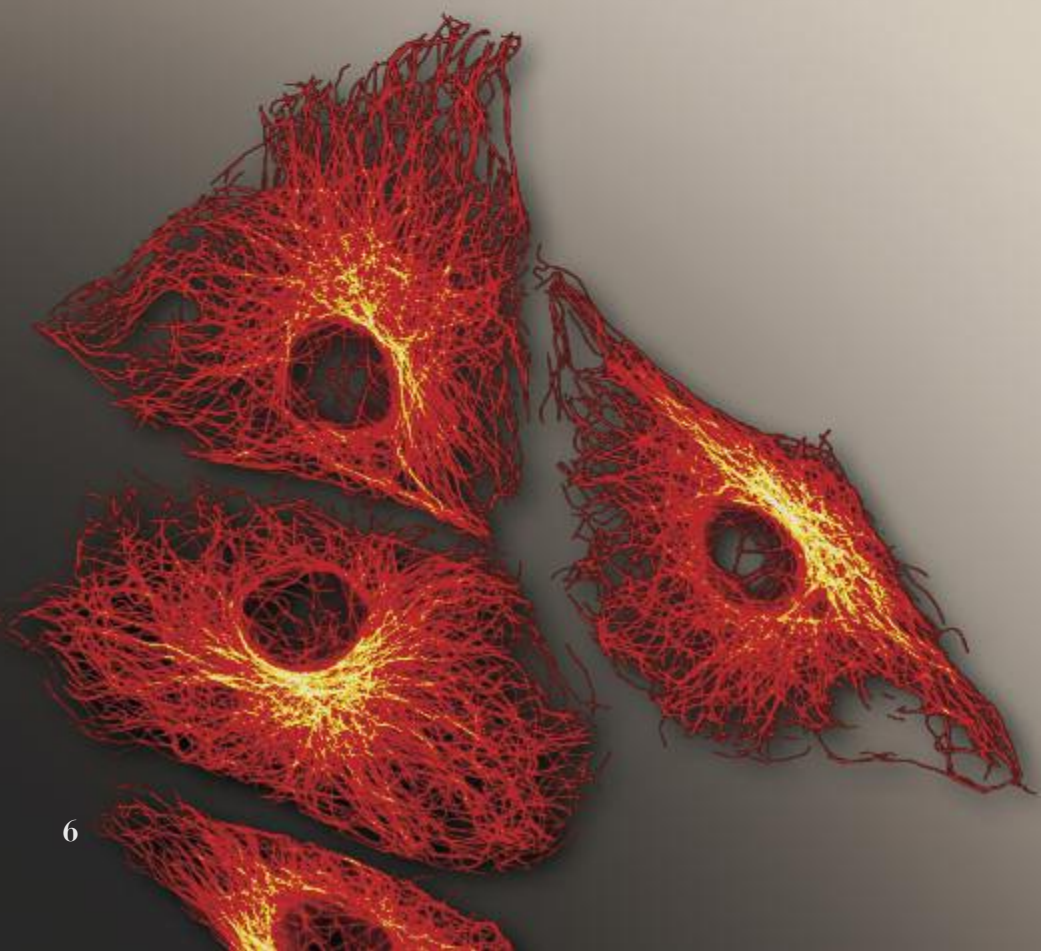
The new findings gained from such scientific research have paved the way for many pioneering applications. For example, a chemical compound called Miltefosine, which was synthesized here, turned out to be a cure for the tropical disease visceral leishmaniasis – also known as kalar-azar. If left untreated, the disease fatality rate can be as high as 100 percent within two years. The World Health Organization (WHO) hopes to use this medicine to control leishmaniasis in the long-term and finally defeat it.

Other researchers have provided groundbreaking ideas for improving optical microscopy to go well beyond current resolution barriers, thus revolutionizing optical microscopy.

Many of the scientists at the institute have received awards and prizes for their work, including the seven recipients of the prestigious Gottfried Wilhelm Leibniz Prize of the German Research Foundation. Currently, two of the 44 Nobel Laureates who studied or worked in Göttingen are conducting their research at the Max Planck Institute for Biophysical Chemistry.

Manfred Eigen was awarded the Nobel Prize for Chemistry in 1967. He succeeded in observing the course of very fast chemical reactions occurring in the range of nanoseconds. He thus broke through a fundamental barrier as, until then, these very fast reaction processes had been considered unmeasurable. His work is of fundamental importance far beyond the scope of chemistry.

Erwin Neher and Bert Sakmann were awarded the 1991 Nobel Prize for Physiology or Medicine. They explored the molecular structures that enable nerve cells to transmit electric signals. In 1976, the two Max Planck researchers developed a method for measuring the incredibly weak electric current that flows for extremely short time periods when single pores open up – the so-called patch clamp





Manfred Eigen received the 1967 Nobel Prize for Chemistry for investigations of »extremely fast chemical reactions, effected by disturbing the equilibrium by means of very short pulses of energy«. Soon afterwards he became Director of the Göttingen Max Planck Institute for Physical Chemistry in the Bunsenstrasse and initiated the founding of the Max Planck Institute for Biophysical Chemistry at the Faßberg site on the outskirts of Göttingen.

technique. Minuscule ion channels – pore-forming proteins – are embedded within the outer membrane of nearly all cell types. They not only transmit the electrical activity of nerve and muscle cells, but also translate physical and chemical sensory stimuli into neuronal signals. Blood cells,

immune cells and liver cells also use ion channels for communication. These »nanomachines« in the membrane are therefore not only involved in nerve cell signaling; they also play a universal role in the »messaging systems« of organisms.



Erwin Neher (left) and Bert Sakmann (right) were awarded the 1991 Nobel Prize for Physiology or Medicine »for their discoveries concerning the function of single ion channels in cells«. This so-called patch clamp technique is now used by research laboratories all over the world. It provides the key to explaining numerous life processes on the cellular level.

Tradition and vision

The Max Planck Institute for Biophysical Chemistry was founded at the Faßberg site on the outskirts of Göttingen on the initiative of Manfred Eigen, and was officially inaugurated in 1971. Its history can be traced back far beyond this date, however, extending back to the former Kaiser Wilhelm Institute for Physical Chemistry in Berlin. In 1949, after the creation of the Max Planck Society, the physicochemist Karl Friedrich Bonhoeffer re-

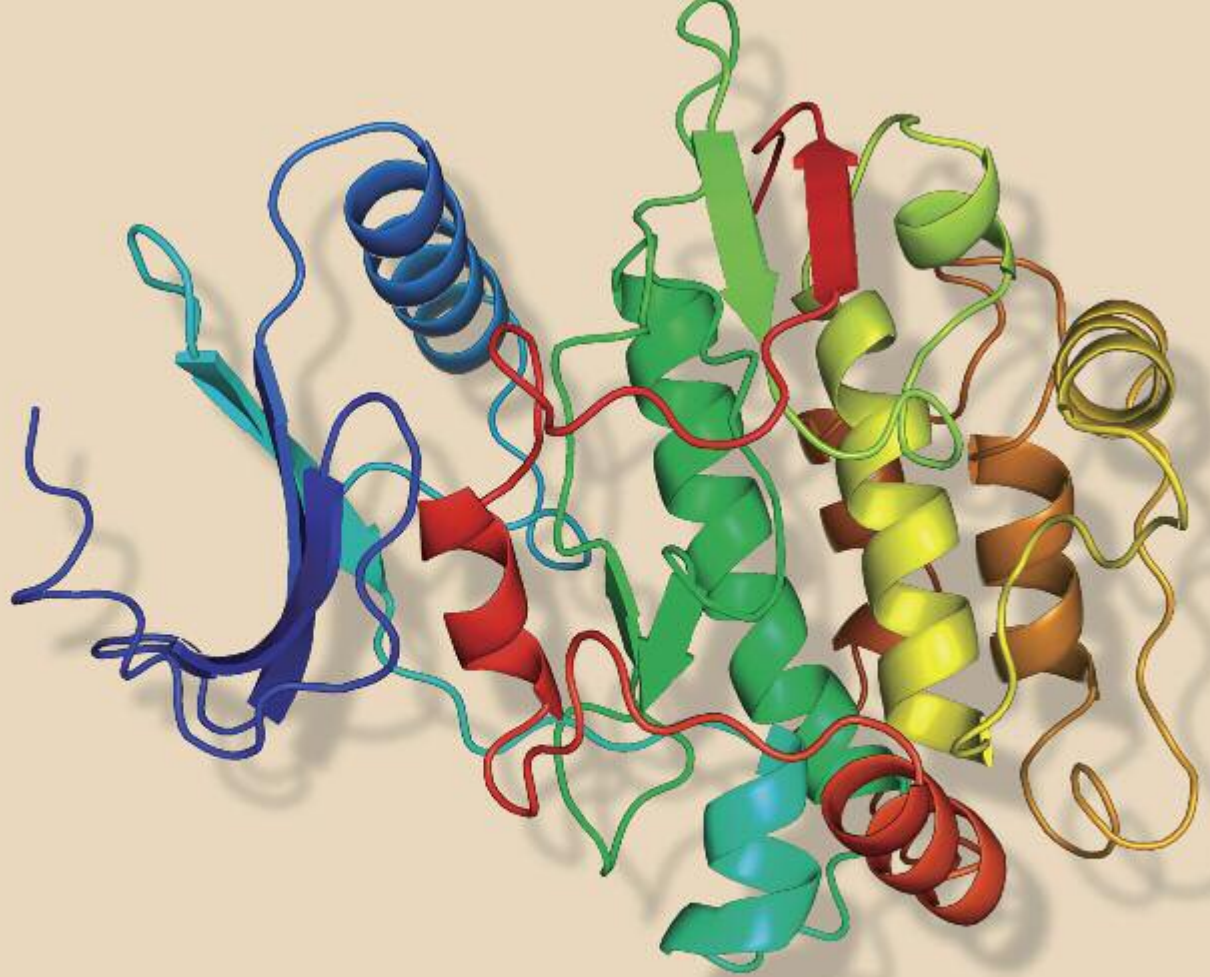


established the Berlin institute as the Max Planck Institute for Physical Chemistry in Göttingen. This institute and the Göttingen Max Planck Institute for Spectroscopy were then merged to form the Max Planck Institute for Biophysical Chemistry.

The focus of the newly founded institute on biological research also has its roots in the work and interests of Karl Friedrich Bonhoeffer. He pursued a strong interdisciplinary approach at a very early stage and applied physical-chemical methods to resolve biological questions – a good reason to name the institute after him.

Manfred Eigen's vision for the newly-established institute was to use biological, chemical and physical methods to study complex life processes: a vision which has played a decisive role in the success of the institute, and which still stands today in the departments and research groups.

Karl Friedrich Bonhoeffer (1899-1957) was the founder and first Director of the Max Planck Institute for Physical Chemistry in Göttingen, which he had re-established in 1949 as the successor institute to the Kaiser Wilhelm Institute for Physical Chemistry in Berlin.



At present, the Max Planck Institute for Biophysical Chemistry comprises eleven departments and 30 research groups with their own research foci. With more than 830 staff members – including 470 scientists – it is not only one of the largest institutes of the Max Planck Society, but is also unique in its interdisciplinarity covering a universal range of research areas.

The Directors of the individual departments are at the same time Scientific Members of the Max Planck Society and decide jointly on the course to be taken by the institute. The current President of the Max Planck Society, Peter Gruss, and the Vice President, Herbert Jäckle, are also Directors here.

The Max Planck Society for the Advancement of Science currently sustains 80 institutes with a broad spectrum of different fields ranging from the humanities and law to the natural sciences and astronomy.

In order to ensure the maintenance of the institute's high quality research, a Scientific Advisory Board of internationally renowned scientists regularly assesses the research accomplished here. A Board of Trustees, comprised of not only scientists but also prominent representatives from business and politics, supports contact with the public at large.



Teaching and learning

Science is based on more than just experience. The future of science depends on the young scientists who drive the research forward. Many researchers at the Max Planck Institute for Biophysical Chemistry teach as professors at the University of Göttingen as well as other universities. They are actively involved in collaborative research centers and graduate schools, and thereby maintain close contact with the students. Many students, on the other hand, come to the institute for their laboratory work during their bachelor and master courses or doctoral studies.

In the international competition for the best young minds, the Max Planck Society and various universities have established a special program of education and training for outstanding students: The International Max Planck Research Schools.

The Max Planck Institutes for Biophysical Chemistry, for Dynamics and Self-Organization and for Experimental Medicine have teamed up with the University of Göttingen to establish the scientific programs *Molecular Biology*, *Neurosciences* and *Physics of Complex and Biological Systems*. The structured education and training, with excellent research and learning conditions, is tailored to prepare especially-talented German and foreign students for their doctoral studies.

The institute has also made a significant contribution to the success of the Georg August University of Göttingen in the national Excellence Initiative with its Göttingen Graduate School for Neurosciences and Molecular Biosciences (GGNB). The GGNB, which is supported by funds from the Excellence Initiative, creates the best possible research and training conditions for doctoral students and supports young scientists with offers of intensive courses and tutoring.

There are also programs for young scientists within the framework of further cooperations between the institute and the University, the Max Planck Institutes for Dynamics and Self-Organization and for Experimental Medicine as well as the German Primate Center. These include:



- the *European Neuroscience Institute (ENI)*, which concentrates on experimental research into functions and diseases of the nervous system,
- the *Göttingen Center for Molecular Physiology of the Brain (CMPB)*, where researchers from various disciplines collaborate in the field of brain research in order to gain a better understanding of the molecular processes and interactions between nerve cells,
- the *Bernstein Center for Computational Neuroscience (BCCN Göttingen)*, where the neuronal basis of our brain activity is investigated with the aid of mathematical models,
- the *Göttingen Microscopy in the Nanometer Range Excellence Cluster*, which develops innovative microscopy methods with a resolution in the nanometer range, making them available on a practical level.



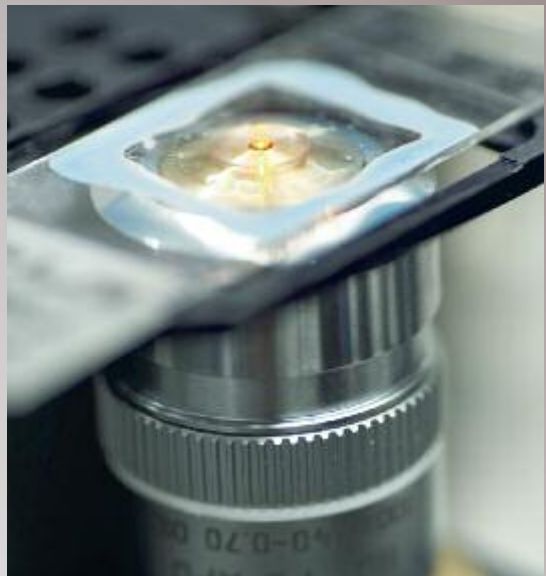
When a new idea ignites something

Whether in the field of medical diagnostics, laser technology or microscopy – the findings of basic research solve many a practical problem that applied research was not able to solve. Such findings are therefore also in high demand in industry.

Many scientists at the institute hold promising patents and founded companies in the area of medical diagnostics and therapy, metrology and environmental technology or ultra-high resolution microscopy, for example.

The newly developed *FLASH* (*Fast Low Angle Shot*) method allows one to take magnetic resonance tomography images 100 times faster. This new technique has revolutionized magnetic resonance tomography and is now routinely used in hospitals worldwide. The *FLASH* patent was one of the most successful patents of the Max Planck Society for a long time.

The RNA interference (RNAi) technique was successfully applied for the first time to mammalian cells at the institute. Using this method, individual genes can be switched to »mute«, thereby enabling their function to be specifically investigated. This technique should make it possible to treat certain hereditary diseases in the future.



The STED microscope developed at the institute allows fluorescence microscopic images with a drastically-improved resolution in comparison to conventional optical microscopes. Minute details inside living cells can thus be observed and even »filmed« live. Since 2007, this ultra-high resolution microscope has also been commercially available.



STED+

Confocal

The revenue from patents and licenses is invested into new projects at the institute. The use of the patents creates new jobs for highly-qualified staff members. In addition, there is a broad spectrum of further cooperative ventures with industrial companies, including pharmaceutical companies and companies that develop industrial measurement technology. Former and present employees of the institute have been involved in the founding of more than a dozen companies.

One of these spin-off companies is DIREVO (now Bayer HealthCare AG), where an automated »Evolution Machine« is used to quickly find and optimize biopharmaceutical active substances.

Another example is Lambda Physik (now Coherent), which specializes in developing lasers that operate with extremely short light pulses. As documented through several patents, the lasers have undergone continuous further development. They are now also used in medicine and research, in addition to printing technology.

The biotechnology company DeveloGen, of which the Max Planck Society itself is a partner, is another successful spin-off from the institute. Two of the institute's scientists, Peter Gruss and Herbert Jäckle, founded the company in 1997. DeveloGen connects research on genetic control processes in the development of different kinds of body tissues with the practical treatment of medical conditions such as obesity and diabetes.



Excellent service for cutting-edge research

What happens if an important building-block is missing – be it in a complicated experiment or in one's own store of knowledge? Workshops and a library are as important for successful research as are well-equipped laboratories.

In order to reveal the processes deep inside living cells, the performance and resolution of experiments and measuring instruments is constantly being improved. The scientists' ideas are put into practice by the experts in the precision mechanics and electronics workshops, whether for the patch clamp technique, for the freezing of biological samples or for ultra-high resolution microscopy.

Some 50 workshop employees construct complicated new devices or optimize existing apparatus for the respective experiments.

The shelves of the Otto Hahn Library hold more than 70,000 volumes of journals and almost 40,000 monographs. Current subscriptions include almost 600 journals. In addition, the Otto Hahn Library offers access to a multitude of electronic catalogues and various databases, as well as the option of an inter-library loan from other specialist libraries. This service is available to both institute members and all interested parties.





Just as indispensable are the employees in the scientific and non-scientific central facilities. In order to provide all researchers at the institute with the best analytical and measurement methods, and to keep them up-to-date, scientists in the central core facilities Electron Microscopy, Innovative Optical Microscopy, Mass Spectrometry and X-ray Crystallography develop new procedures in their own particular fields. Institute members can find help with sample preparation, data acquisition and data analysis.

The IT & Electronics Service team assists in hardware and software problems, and also takes care of the trouble-free storage, archiving and transmission of data internally, as well as to cooperating research facilities within and outside of Göttingen. Colleagues in the reprographic department ensure that photographs and presentation mate-

rials or the internal newsletter, for example, are handled professionally. The EU Liaison Office advises researchers on the writing of applications and supports them in contract negotiations with the EU Commission, as well as with complete project coordination. The workshops, IT & Electronics Service, building services and administration also offer a number of young people qualified training. Between four and six trainees complete their training every year, often with above-average success.

Scientists can only push ahead with their research with commitment if their children are well looked after during the day. Accordingly, the institute offers childcare directly on the institute site since 2005. Some 30 children between one and four years of age are professionally cared for – all day long.

Open doors

At the Max Planck Institute for Biophysical Chemistry anyone who is interested will find that our doors are open. Whether a teacher, pupil, journalist or private individual, everyone is invited to find out about current research projects during guided tours through the institute and individual departments, presentations or discussions.

Since the only way to reach a general audience is through the media, the institute not only issues press releases on current topics but also welcomes journalists to explore deeper by directly contacting experts here. In addition, the institute offers a special program for journalists: the *European Initiative for Communicators of Science* (EICOS). Once a year, up to 14 selected journalists and editors from all over Europe and Israel are invited to gain a close-up view of our research activities. As a result, one finds journalists working side by side with scientists at the bench or in front of the computer. For one week – sometimes longer – they come to grips with a pipette, use a gel chamber and operate a microscope in order to explore how proteins – the

cell's »nanomachines« – perform their tasks. Not only do the journalists obtain insights into the day-to-day workings of a scientist, the direct contact also leads to intensive dialogue that benefits both sides.

The Max Planck Institute for Biophysical Chemistry is also highly committed to outreach activities for pupils and teachers. It invites students and teachers from schools to explore research projects during guided tours and lectures with instructive experiments. The one-week program *Science and Youth* (*Göttinger Woche Wissenschaft & Jugend*), organized by the city of Göttingen every year, provides students an intimate glimpse of the institute's laboratories. During that time, some of the departments and research groups offer special lectures, presentations and lab tours. One can learn something about our »biological clock«, observe proteins »at work«, take a look through a state-of-the-art microscope or experience magnetic resonance tomography first hand. *The Education Outreach Program* (*Schulkontaktprogramm*) pro-





vides students with access to the institute all the year round. The program includes a variety of lectures and lab visits, including experimental demonstrations.

In addition, teachers can expand their knowledge in certain key areas. The institute offers advanced training courses for teachers in cooperation with XLAB – Göttingen Experimental Laboratory for Young People e.V. And if neither textbooks nor researches fail to help – pupils and teachers can pose their questions related to science directly to

the researchers at the institute and XLAB via the online portal *Schools ask Science (Schule fragt die Wissenschaft)*.

And finally, it should not be forgotten that although the main task of the institute is to pursue scientific research it also offers a home for the arts. It hosts regular art exhibitions and, last but not least, participates in the presentation of a scientific lectures series at the *Göttingen Literature Festival (Göttinger Literaturherbst)*.



Emeritus Directors of the institute

Professor Dr. Otto-D. Creutzfeldt †
1971 – 1992 · Neurobiology (left)



Professor Dr. Leo C. M. De Maeyer
1971 – 1996 · Experimental Methods
(middle)



Professor Dr. Manfred Eigen
1971 – 1995 · Biochemical Kinetics ·
(right)



Professor Dr. Dieter Gallwitz
1985 – 2004 · Molecular Genetics (left)

Professor Dr. Manfred Kahlweit
1971 – 1996 · Kinetics of Phase
Transformations (middle)

Professor Dr. Hans Kuhn
1971 – 1984 · Molecular Systems (right)

Professor Dr. Bert Sakmann
1985 – 1988 · Cell Physiology (left)



Professor Dr. Fritz Peter Schäfer
1971 – 1994 · Laser Physics (middle)



Professor Dr. Hans Strehlow
1971 – 1984 · Electrochemistry
and Reaction Kinetics (right)



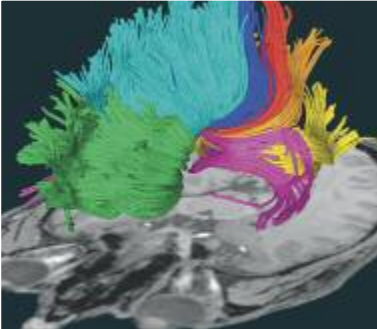
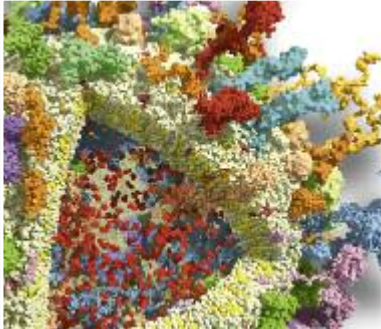
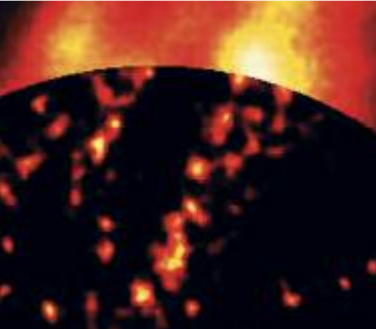
Professor Dr. Klaus Weber
1973 – 2004 · Biochemistry
and Cell Biology (left)

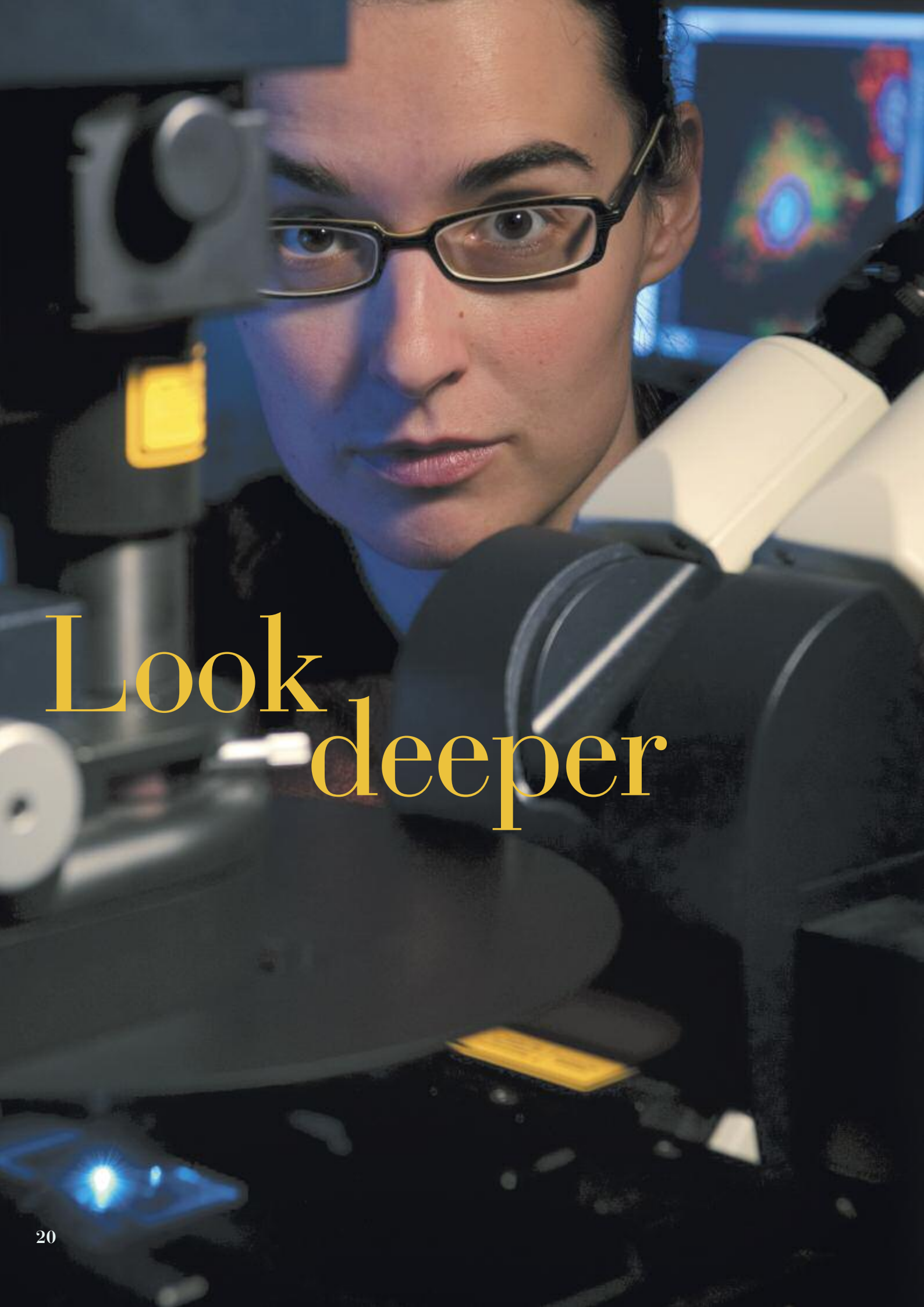
Professor Dr. Albert Weller †
1971 – 1990 · Spectroscopy (middle)

Professor Dr. Victor P. Whittaker
1973 – 1987 · Neurochemistry (right)

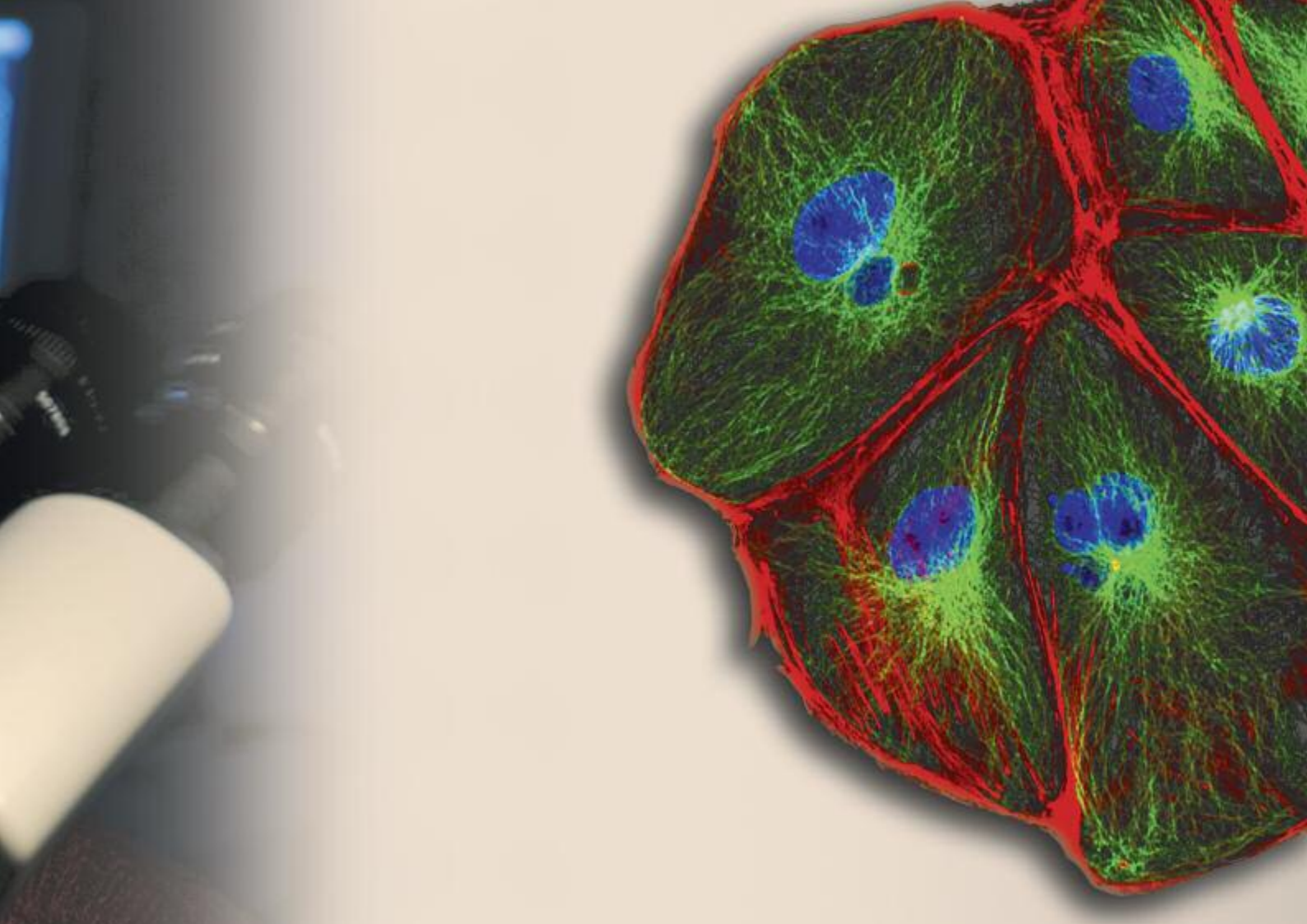


Research in focus





Look deeper



how the unseen becomes visible

Without X-ray structural analysis, Francis Crick and James Watson would not have discovered that DNA – our prime genetic carrier – has a double helix structure. And how would have Robert Koch, also a Nobel Laureate, detected the anthrax bacillus without a good microscope at his disposal? Top scientific achievements require high-end equipment. Therefore, it is no wonder that many of the institute's scientists are involved in methodical innovations. New spectroscopic and microscopic methods are needed, for example, in order to determine structural details at the single molecule level as well as to explore the dynamics of molecular processes.

NanoBiophotonics

Making the smallest details visible using focused visible light – this is the objective of our ultra high resolution light microscopes, in recent years coined nanoscopes. Conventional microscopes reach their resolution limits when two similar objects are closer than 0.2 micrometers (1/5000 of a millimeter) to each other because the diffraction of light blurs them to a single image feature. Even the best microscope lenses cannot change this. Therefore, anyone who desires to image at nanometer or even molecular dimensions must resort to electron or scanning probe microscopy. However, the interior of living cells can only be observed with focused visible light. Fluorescence microscopy, where the molecules (proteins, lipids, nuclei acids) of interest are highlighted by tagging them with specific fluorescent molecules (fluorophores), is the most important light microscopy modality in the life sciences. But like any other light microscopy, standard fluorescence microscopy is also limited by diffraction.

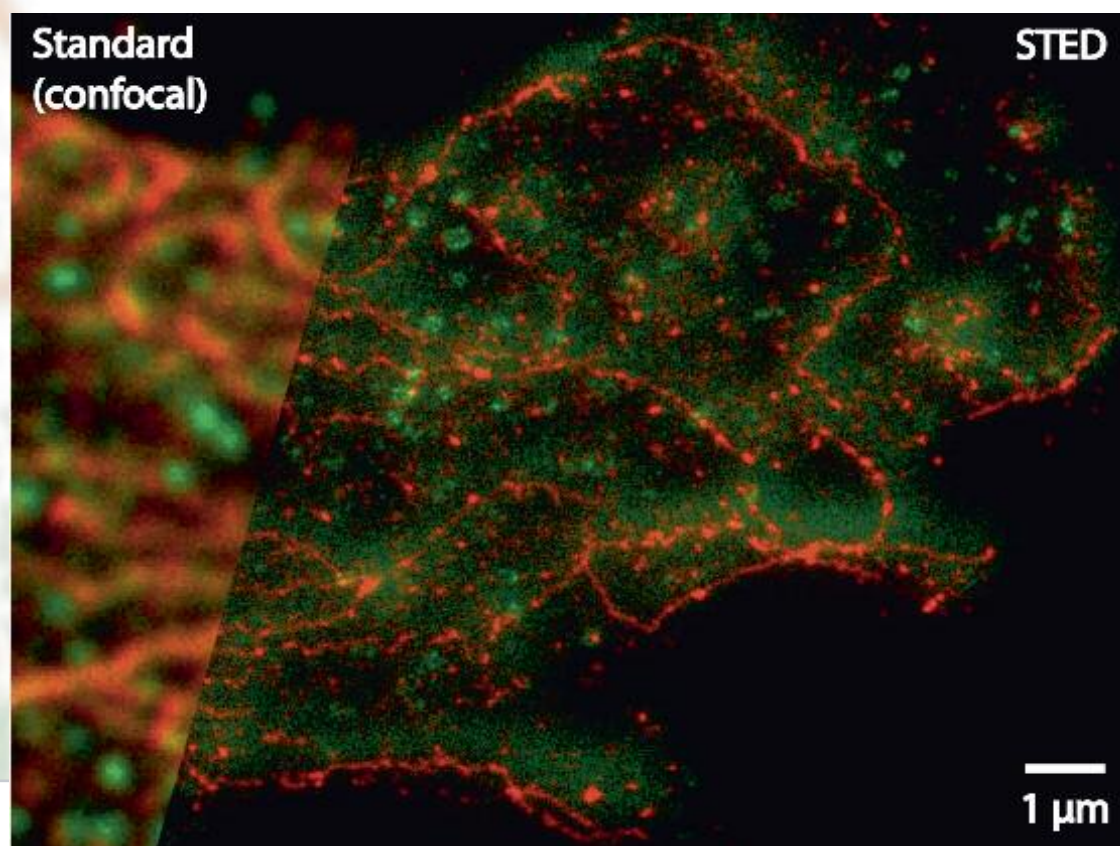
Switching fluorescence off and on by light

In order to outsmart the resolution limiting role of diffraction, we ensure that the adjacent (inseparable) mo-

lecules or groups of molecules emit their fluorescence successively. To this end, we use transitions between fluorophore states that switch or modulate the fluorescence of adjacent molecules for a brief period of time. Switching adjacent molecules consecutively off and on makes them readily distinguishable.

Stimulated Emission Depletion (STED) microscopy, developed by our group, is the first focused light-microscopy method which is no longer fundamentally limited by diffraction. In this approach the focal spot of the fluorescence excitation beam is accompanied by a doughnut-shaped »STED beam« that switches off fluorophores at the spot periphery, by effectively confining them to the ground state. In contrast, molecules at the doughnut center can dwell in the fluorescence »on« state and fluoresce freely. The resolution is typically improved by up to ten times compared with conventional microscopes, meaning that labelled protein complexes with distances of only 15 to 50 nanometers can be discerned.

As the brightness of the STED beam is increased, the spot in which molecules can fluoresce is further reduced in size. As a consequence, the resolution of the system can be increased, in principle, to mol-



Two-color STED image of a glioblastoma, the most frequent malignant brain tumor in adults. Clathrin protein is green; β -tubulin protein is stained red. In contrast to the blurred classical image (left), the STED image (right) shows considerably finer structures. (Picture and sample: J. Bückers, D. Wildanger, L. Kastrup, R. Medda)

ecular dimensions. Combined with fast beam scanning even rapid processes, such as the diffusion of synaptic vesicles inside a neuron, have been followed with high resolution.

Using a (meta)stable switch

Switching fluorescence can also be performed in a different manner. In particular, switching fluorophores between metastable (long-lived) states allows one to overcome the diffraction resolution limit with low levels of light. In a method called RESOLFT, one switches these fluorophores with a spatially structured beam as in STED, but as has been shown more recently, switching individual fluorophores randomly in space is very effective for producing images with resolution on the nanometer scale. In this approach (called STORM, PALM, GSDIM) only one molecule in the diffraction area is »on«, but at an unknown, random position. The adjacent molecules indeed lie within the diffraction spot, but are »off« for the period of detection and therefore do not disturb the registration of the single fluorophore. Imaging the fluorescence signal on a camera allows one to calculate the position of that »on« state fluorophore with an accuracy that is far beyond the resolution

limit. This procedure is repeated again until each molecule has been registered.

In our variant of this approach, called GSDIM, we switch ordinary fluorophores between a bright and a dark state differing in electron spin, thus making this method applicable for a wide range of fluorophores.

Ingeniously combined

Another goal of our research is the development of innovative optical configurations. In the *4Pi microscope*, two objectives are directed at one point so that the wavefronts of the two lenses improve focusing jointly. As a result, a sharpening of the focal light spot by three to seven times is achieved along the longitudinal axis of the microscope.

If one combines the 4Pi microscope with the STED or single molecule switching method, objects which are barely 20-30 nanometers apart can be distinguished in 3D. Until a few years ago this was considered difficult to obtain in practice. In principle even higher resolution is possible: down to the size range of the molecules themselves. Such »optical nanoscopes« are expected to provide completely new insights into nanostructured transparent materials, such as polymers, and especially the inner workings of living cells.



Prof. Dr. Stefan W. Hell

received his PhD in physics at the University of Heidelberg in 1990 and worked from 1991 to 1993 at the European Laboratory for Molecular Biology in Heidelberg. From 1993 to 1996, he did research at the Universities of Turku (Finland) and Oxford (Great Britain). In 1997, he went to the Max Planck Institute for Biophysical Chemistry as head of a Junior Research Group. Since 2002, he has headed the Department of NanoBiophotonics. Stefan Hell has received many awards for his research, among them the Prize of the International Commission for Optics (2000), the Helmholtz Prize (2001), the 10th German Future Prize of the Federal President (2006) and the Julius Springer Prize (2007). In 2008, he received the Leibniz Prize as well as the State Prize of Lower Saxony. In 2009, he was awarded the Otto Hahn Prize of Physics.

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The STED microscopy (circular inset image) provides approximately ten times sharper details of filament structures within a nerve cell compared to a conventional light microscope (outer image). (Picture: G. Donnert, S. W. Hell)

S. W. Hell: Far-field optical nanoscopy. *Science* 316, 1153-1158 (2007).

S. W. Hell: Nanoskopie mit fokussiertem Licht. *Physik Journal* 6, 47-53 (2007).



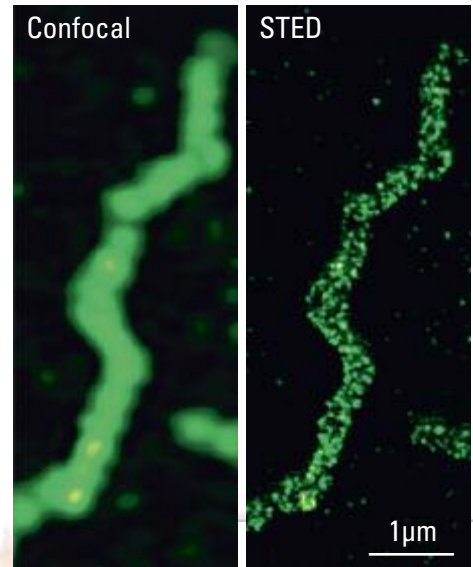
Structure and Dynamics of Mitochondria

Dr. Stefan Jakobs studied biology in Kaiserslautern and Manchester (UK) and carried out his PhD at the Max Planck Institute for Plant Breeding Research in Cologne. Subsequently, he first worked as a postdoctoral fellow in Cologne, then in the Department of Nano-Biophotonics at the Max Planck Institute for Biophysical Chemistry. Since 2005, he heads the Structure and Dynamics of Mitochondria Research Group. Stefan Jakobs completed his habilitation at the University of Göttingen in 2007.

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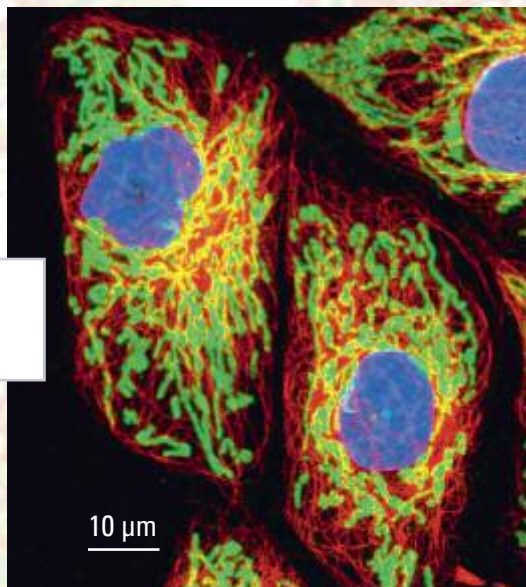
Mitochondria are the »power plants« of the cell. They provide the chemical energy required to keep cellular metabolism moving. When the mitochondria do not function properly, the consequences are correspondingly fatal. Defective mitochondria can lead to disorders such as cancer, Parkinson's or Alzheimer's.

But how are the mitochondria constructed in detail, and which molecular mechanisms lie behind this architecture? Mitochondria are so nanoscopically small that their internal structure could previously only be examined with electron microscopes. However, in this context, cells must be first chemically fixed and cut into ultra-thin slices, which then are examined individually. We therefore know correspondingly little about what occurs in the mitochondria of living cells.



Different protein complexes – in this case the »TOM complex« – accumulate in specific regions of the mitochondrial outer membrane. Left: image made with conventional laser scanning fluorescence microscopy, right: image made with STED microscopy.

Mammalian cells in which mitochondria are labeled green, the microtubule cytoskeleton red, and the cell nucleus blue.



I. E. Suppanz, C. A. Wurm, D. Wenzel, S. Jakobs: The m-AAA protease processes cytochrome c peroxidase preferentially at the inner boundary membrane of mitochondria. *Mol. Biol. Cell* 20, 572-580 (2009).

R. Schmidt, C. A. Wurm, A. Punge, A. Egner, S. Jakobs, S. W. Hell: Mitochondrial cristae revealed with focused light. *Nano Lett.* 9, 2508-2510 (2009).

M. Andresen, A. C. Stiel, J. Fölling, D. Wenzel, A. Schönle, A. Egner, C. Eggeling, S. W. Hell, S. Jakobs: Photoswitchable fluorescent proteins enable monochromatic multilabel imaging and dual color fluorescence nanoscopy. *Nature Biotechnol.* 26, 1035-1040 (2008).

M. Andresen, A. C. Stiel, S. Trowitzsch, G. Weber, C. Eggeling, M. C. Wahl, S. W. Hell, S. Jakobs: Structural basis for reversible photoswitching in Dronpa. *Proc. Natl. Acad. Sci. USA* 104, 13005-13009 (2007).

With light microscopes, even fully intact cells can be examined. However, even with the best conventional microscopes, the spatial resolution is not nearly high enough to examine the interior of these power plants more closely. Consequently, we use new light-microscopy methods, such as *Stimulated Emission Depletion (STED) microscopy*, which increase the optical resolution many times.

A glimpse into the interior of the cellular power plant

To this end, selected proteins are labeled with dyes or fluorescent proteins in order to be able to subsequently localize them in the mitochondria. In this manner, we have, for example, discovered that some protein complexes are concentrated in a specific part of the mitochondrial inner membrane. At present we are exploring the functional meaning of this special localization.

In a second research focus we are striving to improve our molecular tools. We are investigating and developing fluorescent proteins which can be selectively switched »on« and »off« with light flashes. As a result of their particular properties, such photochromic proteins provide completely new possibilities for exploring the inner workings of cells and mitochondria.

Biomolecular Spectroscopy and Single Molecule Detection

In order to investigate the nanocosmos of the cell, scientists develop increasingly sophisticated tools and techniques. Our research group is specialized in examining biomolecules that are usually of high interest for biologically oriented groups at the institute using advanced spectroscopic and microscopic methods and in further improving these techniques.

For example, we study how nerve cells can release extremely rapidly chemical messenger substances in order to transmit signals to other nerve cells. Packed in »messenger packages« – the vesicles – these messenger substances wait inside the nerve cells. When an electrical nerve stimulus indicates that a message is to be transmitted, several synaptic vesicles fuse with the cell membrane and release their contents into the surroundings so that an adjacent nerve cell can immediately recognize the stimulus. These vesicles are only 30 to 60 nanometer (millionths of a millimeter) in size. Despite this we are able to even study individual vesicles in the test tube at the moment when they fuse with artificial membranes. We achieve this by means of highly sensitive microscope techniques which are able to resolve even single fluorescing marker molecules attached to the membranes.

Ultra short laser flashes shed light on photosynthesis

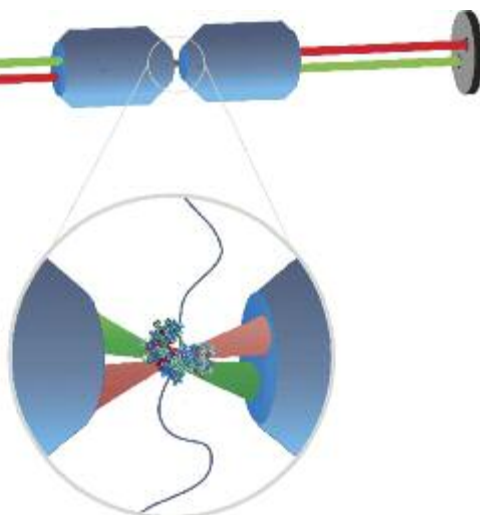
In a further focus we investigate how the solar energy is captured and transformed into chemical energy during photosynthesis. This



An exactly tuned interaction of the energy fluxes between chlorophyll and carotenoid molecules allows the photosynthetic apparatus to utilize nearly every light quantum for electron transfer processes that are finally used to generate biochemically stored energy.

transformation occurs, in part, so rapidly that even state-of-the-art oscilloscopes cannot resolve this time scale. We therefore work with ultra short laser flashes with durations in the order of femtoseconds (10^{-15} seconds), that is a thousand-millionth of a millionth of a second. These are time scales, in which even light itself only travels distances that are shorter than the diameter of a human hair. In the long-term we would like to develop artificial photosynthesis systems which transform solar energy into chemically stored energy and thus have the potential to help resolve the current global problems in energy supply.

In the Biomolecular Spectroscopy and Single Molecule Detection Research Group confocal microscope methods with which even individual biomolecules can be examined are used and developed.



Prof. Dr. Peter Jomo Walla studied chemistry at the Universities of Heidelberg and Göttingen, and subsequently moved to the Max Planck Institute for Biophysical Chemistry to complete his PhD. After research stays at the CNRS in Bordeaux (France) and the University of California at Berkeley (USA) he was initially Department Head at the DIREVO Biotech AG (today: Bayer HealthCare AG) in Cologne. In 2003, he received an Emmy Noether Fellowship that allowed him to establish a Junior Research Group at the institute. Since 2007, he is a full university professor at the Technical University of Brunswick and continues to head the Research Group Biomolecular Spectroscopy and Single Molecule Detection at the Max Planck Institute for Biophysical Chemistry. Peter Jomo Walla received a Research Prize of the Fund of the German Chemical Industry for selected junior professors in chemistry (2003) and the Young Investigators Award of the Gordon Conference on Photosynthesis (2000) for his research.

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P. J. Walla: Modern biophysical chemistry. Wiley-VCH Weinheim, Februar 2009.

S. Bode, C. C. Quentmeier, P.-N. Liao, N. Hafz, T. Barros, L. Wilk, F. Bittner, P. J. Walla: On the regulation of photosynthesis by excitonic interactions between carotenoids and chlorophylls. Proc. Natl. Acad. Sci. 106, 12311-12316 (2009).

P. J. Walla, P. A. Linden, C.-P. Hsu, G. D. Scholes, G. R. Fleming: Femtosecond dynamics of the forbidden carotenoid S1 state in light-harvesting complexes of purple bacteria observed after two-photon excitation. Proc. Natl. Acad. Sci. 97, 10808-10813 (2000).



Laboratory of Cellular Dynamics

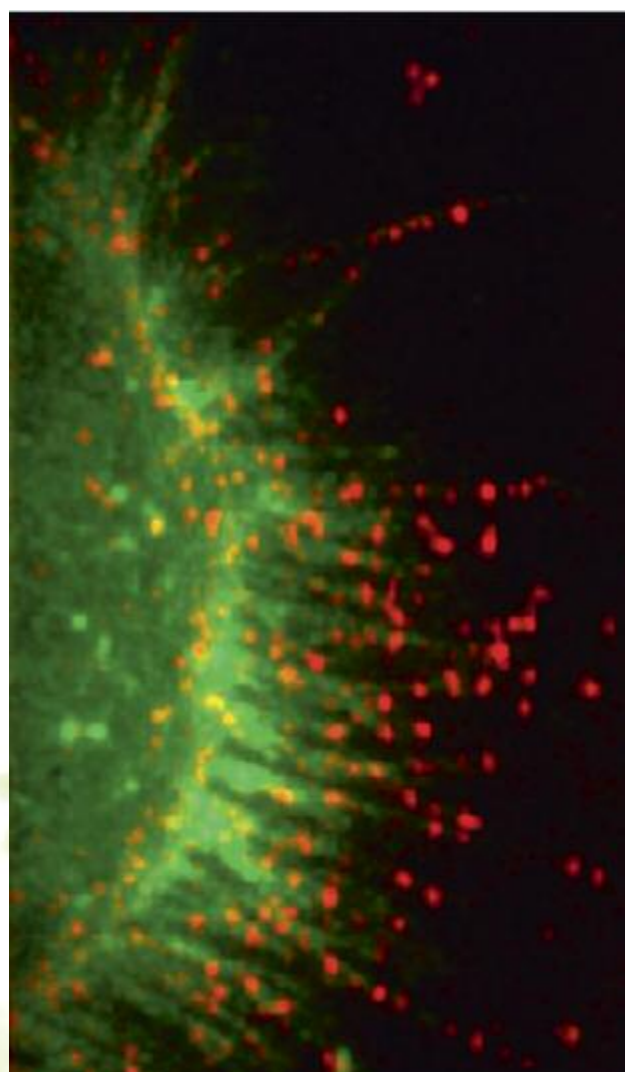
We concentrate our research activities on two major areas. The first has to do with the molecular mechanisms of signal transduction controlled by external growth factors in normal and tumor cells. The second focus is on the molecular mechanisms underlying the pathogenesis of Parkinson's disease (PD). Characteristic of this and other related neurodegenerative diseases, for example Alzheimer's disease (AD), is the appearance of protein aggregates in and around neurons of affected areas in the brain. In PD, the protein in question is α -synuclein. Unfortunately, how the so-called amyloid aggregates

are formed and how they exert their toxic effects is largely unknown. Answers to these questions are required before we can rationally design drugs to inhibit or reverse the progress of PD and AD. We approach this challenge with molecular and cellular biological approaches and biophysical techniques that can be applied in vitro as well as in studies of cells and tissues.

Tracking molecules in living cells

For the cellular studies, we develop and utilize specific biosensors based on fluorescent semiconductor nanocrystals (Quantum Dots), noble metal clusters (Nanodots), and organic compounds. These probes are introduced into biomolecules by chemical or cell expression techniques. In parallel, we have developed a *Programmable Array Microscope* (PAM) that permits high-speed and sensitive imaging of living cells with high spatial, temporal, and spectral resolution. Thus, the location and involvement of probes on and within cells can be followed in real-time, down to the level of individual nanoparticles. In this manner, we have discovered a new mode of receptor transport on mammalian cells.

Donna Arndt-Jovin is responsible for the cell biological research focusing on growth factors and chromatin-related functions in the cell nucleus. The basic research on signaling is being extended to use in the operating room, for example, for detecting brain tumor (glioma) cells, with the Quantum Dot ligands.



Human epithelial tumor cells: Quantum dots (red) carrying the epidermal growth factor (EGF) bind to the cell surface receptor, itself labeled with a green fluorescent protein. The image was acquired with a new microscope (Programmable Array Microscope) developed at the institute for high-speed optically-sectioned imaging of living cells (G. M. Hagen, K. A. Lidke, B. Rieger, W. Caarls, D. J. Arndt-Jovin, T. M. Jovin: *Dynamics of membrane receptors: single molecule tracking of quantum dot liganded epidermal growth factor*. In: Yanagida, T, Ishii, Y, Eds. *Single Molecule Dynamics in Life Sciences*. Orlando: Wiley. pp. 117-130 (2008)).

Dr. Thomas M. Jovin was awarded an MD in 1964 by the Johns Hopkins Medical School (USA). He became a scientific member of the Max Planck Society in 1969 and functioned as director and chairman of the Department of Molecular Biology at the Max Planck Institute for Biophysical Chemistry until 2007. Since then, as an Emeritus Director, he has headed the Laboratory of Cellular Dynamics at the institute and an associated unit at the University of Buenos Aires (Argentina). Thomas Jovin has received honorary degrees from the Limburg University (Belgium) and the University Medical School, Debrecen (Hungary). He is an honorary professor of the University of Buenos Aires, a member of the European Molecular Biology Organization (EMBO), and an honorary member of the Hungarian Academy of Science.

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M. Gralle Botelho, X. Wang, D. J. Arndt-Jovin, D. Becker, T.M. Jovin: Induction of terminal differentiation in Melanoma cells on downregulation of β -amyloid Precursor Protein. *J. Invest. Dermatol.* | doi:10.1038/jid.2009.296 (2009).

M. S. Celej, W. Caarls, A. P. Demchenko, T. M. Jovin: A triple emission fluorescent probe reveals distinctive amyloid fibrillar polymorphism of wild-type α -synuclein and its familial Parkinson's disease mutants. *Biochemistry* 48, 7465-7472 (2009).

E. A. Jares-Erijman, T. M. Jovin: Reflections on FRET imaging: formalism, probes, and implementation. In: »FRET and FLIM Imaging Techniques«. T. Gadella, Jr. ed. S. 475-517. Elsevier (2009).

M. J. Roberti, M. Morgan, G. Menéndez, L. Pietrasanta, T. M. Jovin, E. A. Jares-Erijman: Quantum dots as ultrasensitive nanoactuators and sensors of amyloid aggregation in live cells. *J. Am. Chem. Soc.* 131, 8102-8107 (2009).

Biochemical Kinetics

A lack of perfection during the reproduction process – that is the main reason for the fascinating variety of life forms on earth. Although DNA, our genetic information carrier, is an extremely stable macromolecule, errors sometimes do occur when information is copied and duplicated. Since not all of these errors are corrected and repaired, new variants emerge all the time. These variants form the basis for the evolution of organisms. All living species are subject to these transformations.

»Evolution machines«

We can define evolution as a natural phenomenon, but we may also re-enact it in a controlled setting to meet our own needs. In these experiments, bacteria, viruses, or single nucleic acid molecules are encouraged to reproduce, and then selected according to specific criteria. For this purpose, we have de-

veloped special equipment that can process many thousand samples simultaneously. In this way, we can study basic mechanisms of evolution, e.g. the tricks that HIV and other cunning pathogens use to outsmart our immune system. Moreover, such »evolution machines« can help developing the molecular agents needed for novel drugs.

These different applications have one crucial aspect in common: In all of these cases, tiny quantities of substances need to be analyzed. This amounts to »finding a needle in the haystack«. It also goes for diagnostic applications, such as the early diagnosis of BSE. Special spectroscopic methods enable us to detect even single molecules. This has led to the development of evolutionary biotechnology that permits the recognition of single biomolecules in a specific distribution as well as the optimization of their function.



The pictures show the automated evolution and screening device of DIREVO Biosystems AG / Cologne (now: Bayer HealthCare AG) viewed from the front (above) and back (left). The biotech company grew out of the Department of Biochemical Kinetics at the Max Planck Institute for Biophysical Chemistry.



Prof. Dr. Manfred Eigen received his doctorate from the University of Göttingen in 1951, where he subsequently worked at the Institute of Physical Chemistry. After joining the Max Planck Institute for Physical Chemistry in 1953, he became Director of its Chemical Kinetics Department in 1964. On the initiative of Manfred Eigen, this institute became part of today's Max Planck Institute for Biophysical Chemistry in 1971. Even after his retirement in 1995, he has been active as Emeritus Director of the Department of Biochemical Kinetics to the present day. In 1967, Manfred Eigen was awarded the Nobel Prize for Chemistry for his research on ultra-fast chemical reactions in solutions.

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A. Koltermann, U. Ketting, J. Bieschke, T. Winkler, M. Eigen: Rapid assay processing by integration of dual-color fluorescence crosscorrelation spectroscopy: High throughput screening for enzyme activity. *Proc. Natl. Acad. Sci. USA* 95, 1421-1426 (1998).

M. Eigen, B. Lindemann, M. Tietze, R. Winkler-Oswatitsch, A. Dress, A. von Haeseler: How old is the genetic code? Statistical geometry of tRNA provides an answer. *Science* 244, 673-679 (1989).

M. Eigen: *Stufen zum Leben. Die frühe Evolution im Visier der Molekularbiologie*. R. Piper-Verlag, München (1987).

M. Eigen: Selforganization of matter and evolution of biological macromolecules. *Naturwissenschaften* 58, 465-523 (1971).

M. Eigen: Proton transfer acid-base catalysis, and enzymatic hydrolysis. I. Elementary processes. *Angewandte Chemie Int. Ed.* 3, 1-19 (1964).



Electron Spin Resonance Spectroscopy

Regardless of whether one is considering simple water or complicated proteins, electrons usually occur pairwise in molecules. By means of their spin – a form of angular momentum – electrons generate a microscopic magnetic field. However, since they rotate in opposite directions, their magnetic effects cancel each other. As a consequence, we are only interested in unpaired electrons, which serve as highly sensitive probes in our experiments. These so-called »paramagnetic centers« can provide us with information on how complex biomolecules change their structures while they are fulfilling their specific function. With the methods of electron spin resonance (ESR)(electron paramagnetic resonance (EPR)) spectroscopy we can observe biomolecules under nearly natural conditions and learn about how they act in the living cell.

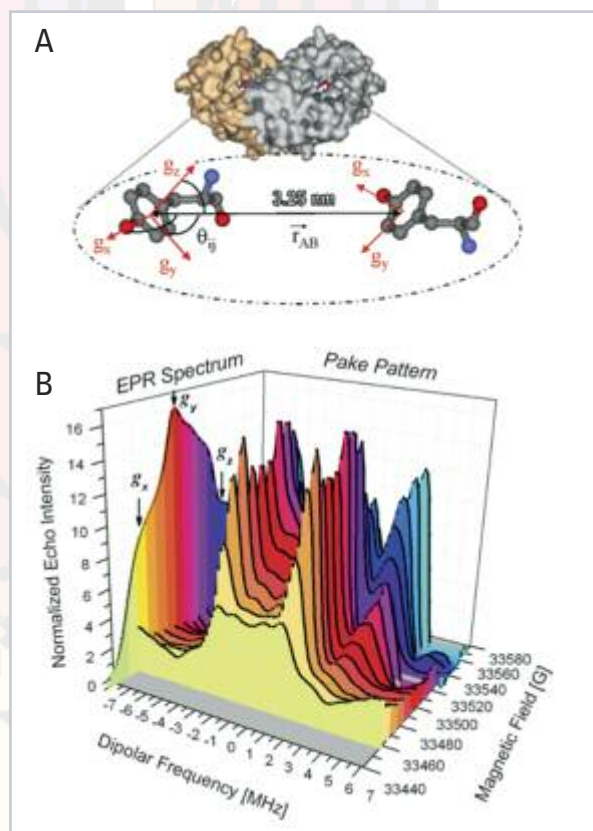
Observing the interior of proteins

We develop techniques to excite a paramagnetic center or simultaneously excite several paramagnetic centers with micro-

waves or radio frequency radiation in order to manipulate their magnetic interactions. In this manner, we can not only measure the distances between the active centers of a protein down to the nanometer range, but also learn about their orientation in the molecule. In addition, we use detection frequencies in the millimeter range, which not only require polarizing superconducting magnets, but also a more complex microwave technology. Therefore, in our research group, biophysical investigations are conducted hand-in-hand with methodological and technical developments.

Paramagnetic centers are involved in many metabolic processes. Representative examples are provided in the process of the photosynthesis or the respiratory chain, but also in proteins such as the enzyme ribonucleotide reductase (RNR). From bacteria up to humans RNR catalyzes the last step in the formation of the individual building blocks of deoxyribonucleic acid (DNA). In this process, paramagnetic states are generated as a result of the transfer of electrons. With the aid of different ESR techniques, we have

succeeded in elucidating several intermediate steps in the catalytic cycle. While paramagnetic centers occur naturally in proteins such as RNR, they have to be inserted artificially into other proteins. To achieve this, we introduce spin labels at selective positions of the proteins. Following this protocol, we have started to investigate the structure of nucleic acids and membrane proteins in collaboration with other research groups of the institute.



A) Structure of the R2 dimer complex from mouse ribonucleotide reductase and orientation of the active tyrosyl radicals as determined by high frequency EPR spectroscopy (Bennati et al., 2008). B) Typical magnetic field dependent dipolar spectra of two interacting radicals at high frequency. The dipolar frequencies observed are a function of the irradiated molecular orientations in the EPR spectrum.

Dr. Marina Bennati received her PhD in experimental physics at the University of Stuttgart in 1995. She subsequently worked at the Massachusetts Institute of Technology (MIT) and the MIT/Harvard Center for Magnetic Resonance in Cambridge (USA). In 2001, she moved to the University of Frankfurt am Main, where she habilitated in 2006. She has been leading the Electron Spin Resonance Spectroscopy Research Group at the Max Planck Institute for Biophysical Chemistry since 2007. In 2002, Marina Bennati received the Young Investigator Award of the International EPR Society for her research.

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V. Demysenkov, D. Biglino, W. Lubitz, T. Prisner, M. Bennati: Structure of the tyrosyl biradical of mouse R2 ribonucleotide reductase from high-field PELDOR. *Angew. Chem. Int. Ed.* 47, 1224-1227 (2008).

M. R. Seyedsayamdost, C. T. Y. Chan, V. Mugnaini, J. Stubbe, M. Bennati: PELDOR spectroscopy with DOPA- β 2 and NH $_2$ - α 2s: Distance measurements between residues involved in the radical propagation pathway of *E. coli* ribonucleotide reductase. *J. Am. Chem. Soc.* 129, 15748-15749 (2007).

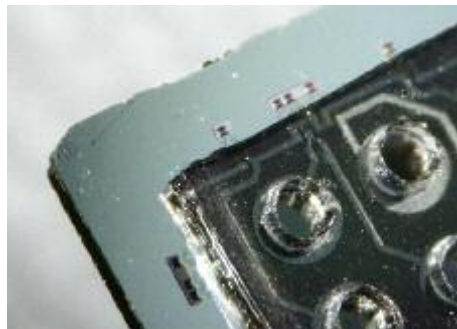
V. Demysenkov, T. Prisner, J. Stubbe, M. Bennati: High-field pulsed electron-electron double resonance spectroscopy to determine the orientation of the tyrosyl radicals in ribonucleotide reductase. *Proc. Nat. Acad. Sci.* 103, 13386-13390 (2006).

Biological Micro- and Nanotechnology

Cells are microscopically small. Yet, they are gigantic compared to the tiny molecular machines which drive the complex biological processes within them. Many such components are a few ten to hundred nanometers (millionths of a millimeter) in size. Even simple parameters, such as the size, mass or number of such nanoparticles, provide scientists with important information. They can help us to better understand cellular processes or to recognize pathological changes. But how is it possible to count or weigh, for example, a group of proteins which are so infinitesimally small? Conventional methods used in the macroscopic world are of no help here. New micro- and nanotechnological procedures, which we develop in our group, may help to address this challenge.

Weighing with a »tuning fork«

In order, for example, to weigh individual nanoparticles, we are developing so-called micromechanical resonators – a kind of miniature »tuning fork«, smaller than the diameter of a hair. A trick is required to be able to examine biological objects in solution with them. The resonator is located in a vacuum and can oscillate practically unattenuated. Inside it there is a channel which is only a few micrometers wide. If a particle passes through the channel, the resonance fre-



Micro- and nanofluidic systems enable complex biological experiments in extremely small channels. Very small objects such as cells can be examined in them individually and under well controlled conditions.

quency – i.e. is the characteristic tone for this tuning fork – changes. From this alternation in »pitch« we calculate the mass with a deviation of a millionth of a billionth of a gram. In this manner we can, for example, weigh a single bacterium with an accuracy of approximately one percent.

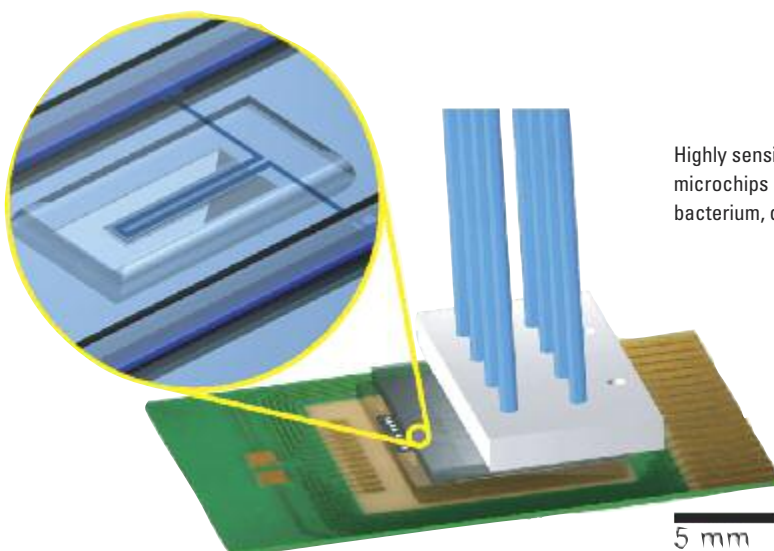
With such physical measurements we aim to ultimately penetrate into the regime of individual molecules. To achieve this, we are bundling the forces of different fields of research and utilize knowledge from biology, chemistry, engineering and physics. They all contribute to the new methods and can also, in turn, profit from their application.



Dr. Thomas Burg studied physics at the Swiss Federal Institute of Technology (ETH) Zurich (Switzerland) and received his PhD in 2005 at the Massachusetts Institute of Technology (MIT) in Cambridge (USA). From 2005 to 2008, he conducted postdoctoral research in the Department of Biological Engineering at MIT. Since 2009, he has headed the Max Planck Research Group Biological Micro- and Nanotechnology at the Max Planck Institute for Biophysical Chemistry.

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Highly sensitive sensors which are integrated on microchips can detect minute particles, such as a single bacterium, directly on the basis of its mass.

T. P. Burg, M. Godin, W. Shen, G. Carlson, J. S. Foster, K. Babcock, S. R. Manalis: Weighing of biomolecules, single cells, and single nanoparticles in fluid. *Nature* **446**, 1066-1069 (2007).

T. P. Burg, S. R. Manalis: Suspended microchannel resonators for biomolecular detection. *Applied Physics Letters* **83**, 2698-2700 (2003).



Spectroscopy and Photochemical Kinetics

Many phenomena of nature can be traced back to molecular processes. For example, molecules, radicals and atoms react with one another in the atmosphere after they have been excited or produced by solar radiation. Amazingly enough, these processes are very similar to those which occur in fire and in internal combustion engines. Even in the formation of new stars in interstellar molecular clouds, such processes play an important role. What's more, elementary photobiological processes, such as photosynthesis, follow similar basic principles in their intra- and intermolecular dynamics.

In order to investigate such molecular processes, we initiate them by means of photochemical activation. Molecules absorb light and achieve highly active states as a result of the added energy, or active particles, such as atoms or radicals, are formed which trigger a chain of reactions. We investigate the temporal course of these reactions and the subsequent, frequently very rapid, processes by analyzing the very specific absorption of light by the molecules with spectroscopic methods.

On the basis of their respective spectra, we are even able to distinguish molecules which only differ in their energy state.

Investigation in the femtosecond range

With the aid of modern optical methods the concentrations of molecules and their energy states can be followed with a temporal resolution down to the femtosecond range – this is the time scale for the movement of atoms. In this manner, even the »ultrafast« inter- and intramolecular dynamics becomes directly »visible«. We analyze the theory behind these processes with the methods of quantum chemistry, reaction dynamics and molecular statistics.

At present we are increasingly focusing on reactions of electrons and molecular ions in so-called plasmas, i.e. the gaseous state of matter in which not only electrically neutral particles, but also charged particles, exist next to one another. This state can be detected on the earth in the ionosphere or in electrical discharges such as lightning. In outer space the majority of all matter exists in this state.

With these results we develop theoretical models, which are useful in many areas: from astrochemistry and atmospheric chemistry, plasma- and photochemistry to combustion chemistry. Even large-scale industrial processes can be optimized with them.

Prof. Dr. Jürgen Troe received his PhD at the University of Göttingen in 1965 and completed his habilitation there in physical chemistry. In 1971, he was appointed as professor at the Swiss Federal Institute of Technology in Lausanne (Switzerland). In 1975, he returned to the University of Göttingen as Director at the Institute for Physical Chemistry. From 1990 to 2008, he additionally headed the Department of Spectroscopy and Photochemical Kinetics at the Max Planck Institute for Biophysical Chemistry, where he continues with his research in the framework of an emeritus group. In addition, since 2009, he works at the University of Göttingen as a »Lower Saxony Research Professor«. Jürgen Troe is an honorary professor at the Swiss Federal Institute of Technology in Lausanne, an honorary doctor of the Universities of Bordeaux and Karlsruhe, and an honorary member of the German Bunsen Society.

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E. I. Dashevskaya, I. Litvin, E. E. Nikitin, J. Troe: Modelling low-energy electron-molecule capture processes. Phys. Chem. Chem. Phys. 10, 1270 (2008).

Ch. Müller, J. Schroeder, J. Troe: Intramolecular hydrogen bonding in 1,8-dihydroxyanthraquinone, 1-aminoanthraquinone, and 9-hydroxyphenalenone studied by picosecond time-resolved fluorescence. J. Phys. Chem. B 110, 19820 (2006).

Ch. Müller, M. Klöppel-Riech, F. Schröder, J. Schroeder, J. Troe: Fluorescence and REMPI spectroscopy of jet-cooled isolated 2-phenylindene in the S₁. J. Phys. Chem. A 110, 5017 (2006).



Reaction Dynamics

The erosion of rocks occurs over a period of many centuries. But as slowly as the underlying chemical reactions apparently take place – at the level of atoms and molecules they occur inconceivably rapidly: in fractions of picoseconds, i.e. the millionth part of a millionth of a second, molecules change their structure, chemical bonds are dissolved or reformed because atoms in molecules move so rapidly.

In the process, the molecules pass through different energy states. These processes are driven, on the one hand, by the transport of energy within the molecule, but on the other hand, energy also moves from molecule to molecule, for example when the reactants interact with the surrounding solvent.

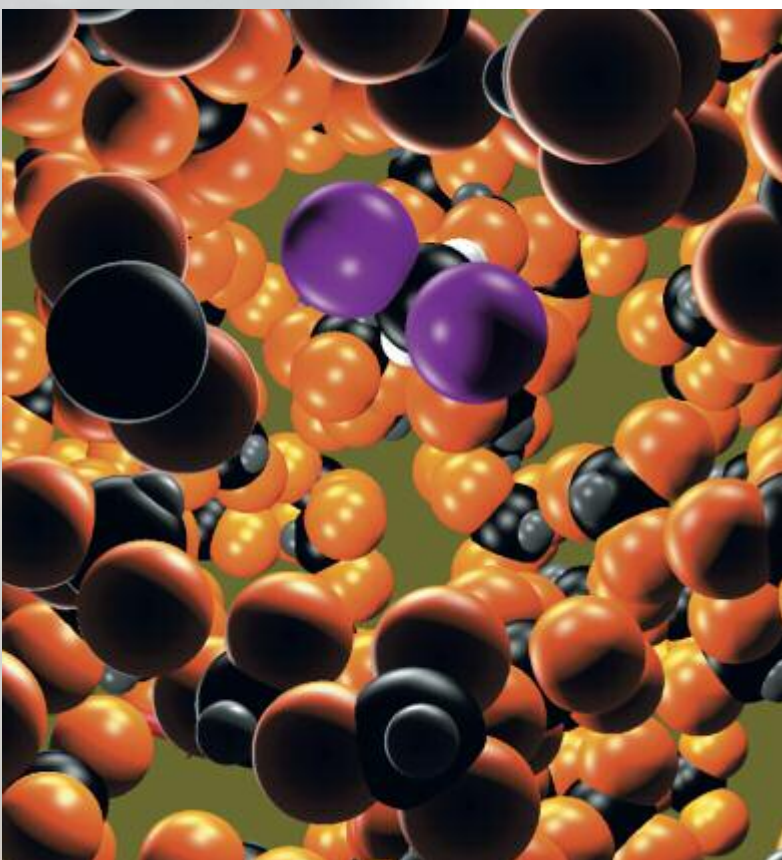
On the time scale of atomic movements

We desire to make the individual steps of these molecular processes visible and un-

derstand them in detail. To achieve this we use laser spectroscopy. It allows us to identify the substances participating in a chemical reaction based on their typical light spectra as if every substance had its own particular rainbow pattern. We also characterize the concentrations, energy contents and other properties of these substances at each point in time in a reaction using this method.

The dynamics of chemical reactions in gases, liquids and supercritical fluids is at the focus of our interest. By changing macroscopic parameters such as temperature, density or viscosity, we alter the strength of interactions between molecules and the frequency of energy-transferring impacts. In this manner we untangle the different, superimposed factors which influence the course of a chemical reaction.

With our experimental methods we can penetrate down to the time scale of the movements of atoms in molecules. In this way, the intermolecular dynamics becomes directly »visible«.



Diiodomethane in chloroform – snapshots of a molecular dynamic computer simulation (iodine atoms are violet, chlorine atoms are orange in color).



Prof. Dr. Dirk Schwarzer received his PhD in physical chemistry in 1989 at the University of Göttingen. After a research stay at the University of Chicago (USA), he moved in 1990 to the Max Planck Institute for Biophysical Chemistry as head of a research group in the Department of Spectroscopy and Photochemical Kinetics. Since 2006, he has headed the Reaction Dynamics Research Group there. Dirk Schwarzer habilitated in physical chemistry at the University of Göttingen in 1999 and has taught there as an adjunct professor since 2002.

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T. Schäfer, D. Schwarzer, J. Lindner, P. Vöhringer: ND-stretching vibrational energy relaxation of NH₂D in liquid-to-supercritical ammonia studied by femtosecond midinfrared spectroscopy. J. Chem. Phys. 128, 064502 (2008).

C. Reichardt, J. Schroeder, D. Schwarzer: Femtosecond IR spectroscopy of peroxy carbonate photodecomposition: S₁-lifetime determines decarboxylation rate. J. Phys. Chem. A, 111, 10111 (2007).



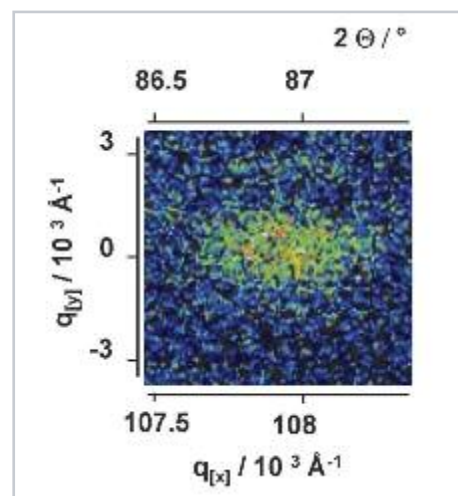
Structural Dynamics of (Bio)Chemical Processes

How swift is a molecule and how agile is it? What happens when its freedom of movement is restricted, for example, because it is locked in a crystalline structure? How do the individual atoms move when a molecule interacts with another one, i.e. when a chemical reaction occurs? These are the questions we try to answer in our Structural Dynamics Research Group.

When we observe atomic and molecular structures, we use X-ray flashes, which only last 10^{-14} seconds – i.e. one hundred trillionth of a second. These extremely short flashes are deflected by the molecules and then detected (ultra-fast X-ray diffraction). In this manner, individual atoms can be localized, namely with an accuracy of one hundred-millionth of a millimeter. Since the investigated molecules are one hundred to one thousand times larger than this, we can thus follow every movement in molecules and in chemical reactions very exactly and on extremely rapid time scales.

Choreography of the molecules

In addition, we observe the residual X-ray flash after a fraction of the light quanta has been captured by the obstructing molecules (ultra-fast X-ray spectroscopy). In this manner, we can determine the movements with which individual atoms of the investi-



Molecular movements during a chemical reaction are examined with soft X-radiation of the fourth generation (free electron laser): Ultra-fast X-ray diffraction on lipids provides a typical diffraction pattern.

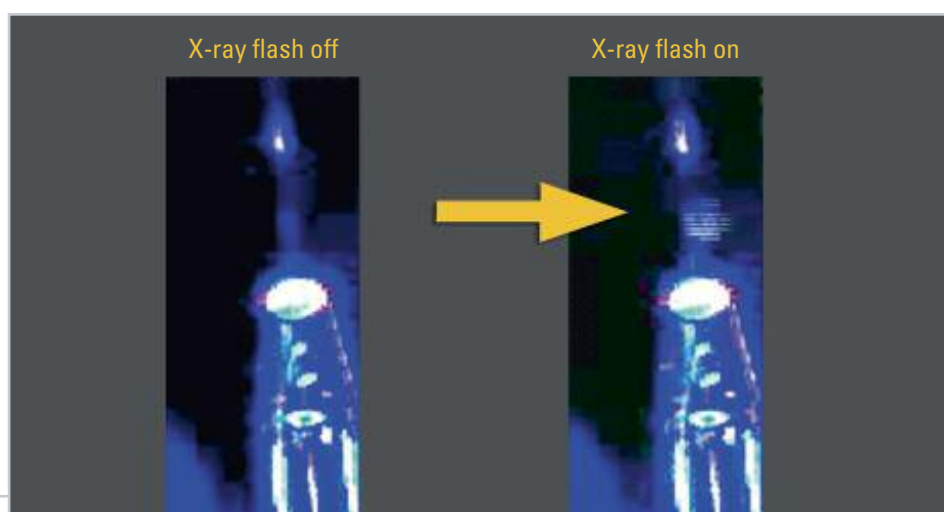
gated molecules participate in the choreography of a chemical reaction. With these methods we hope to understand chemical processes so exactly that we can manipulate them in a targeted manner in order, for example, to develop materials which transform electrical energy into light energy more efficiently. Such light-active materials can be used, for example, in photovoltaic facilities and biopower plants.

Dr. Simone Techert received her PhD at the University of Göttingen in 1997. From 1998 to 2000, she worked as a postdoctoral researcher and scientist at the European Synchrotron Radiation Source in Grenoble (France) and went from there to the Scripps Research Institute in La Jolla, (California, USA). From 2001 to 2004, she headed an Emmy Noether Junior Research Group at the Max Planck Institute for Biophysical Chemistry. In 2005, she habilitated in physical chemistry and worked as a scientist at the Stanford Synchrotron Radiation Light Source in Stanford, California. Since 2006, she has headed the Structural Dynamics of (Bio)Chemical Processes Research Group at the institute. She is a member of the Advanced Study Group of the Max Planck Society at the Center of Free Electron Laser Science, Hamburg. Simone Techert's work has been honored with several prizes such as the ESRF-PdP Prize (1999), the Roentgen Prize (2005), and the Karl Winnacker Fellowship (2006).

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A. Debnarova, S. Techert: *Ab initio* treatment of time-resolved X-ray scattering. *J. Chem. Phys.* 125, 224101 (2006).

A. M. Lindenberg, et al.: Atomic-scale visualization of inertial dynamics. *Science* 308, 392-394 (2005).



X-radiation and water chemistry – when extremely short X-ray flashes impinge on water, free electrons are created in a trillionth of a second.

Laser-induced Chemistry

The sun sends high-energy light to the earth and induces many processes, which can be of thermal or photochemical nature. We investigate the interaction and coupling of high-energy radiation – in particular laser radiation – with or into materials. In this way, we can understand how this energy acts on surfaces, molecules and atoms, and how the desired induced effects can be used for precise and selective chemical processing of advanced materials.

In a controlled manner, any material can gently and with high precision be removed by pulsed laser ablation – with materials ranging from soft organic, such as the cornea of the human eye, all the way to hard metals used in industrial applications, and even diamond.

Growth of sophisticated 3-dimensional filigree structures

Lasers can also be used to create small filigree three-dimensional material structures

made, for example, of ceramics or/and metals. To achieve this, a laser beam is directed onto a substrate surface, which is in direct contact to a suitable gas mixture containing a gaseous precursor compound. This precursor compound is decomposed by the laser's energy leading to deposition of, for example, aluminum or/and aluminum oxide. As a consequence, fine aluminum structures or aluminumoxide wires and rings can grow into the gaseous environment. In this way, the desired structures are laser-written just like a drawing pen creates lines on – however flat – paper. In this manner, ring electrode systems for small cages can be formed. Inside these cages scientists can store and investigate small objects, including cells, freely suspended in aqueous solution, when small high frequency repelling electric fields are applied to those electrodes. Cells are not affected by nearby surfaces.



Small three-dimensional structures can be created by laser-direct-write from the gas phase. Metallic aluminum (left) and aluminum-oxide (above) structures are created with a writing speed in the range of 50-100 micrometers per second. The precursor gas contains dimethylethylamine-alane alone (left) or with added oxygen (above). The compound gently breaks up into the stable gaseous amine $(\text{CH}_3)_2\text{C}_2\text{H}_5\text{N}$ and AlH_3 alane, forming the desired aluminum (left) or aluminumoxide (above) and stable hydrogen H_2 gas.



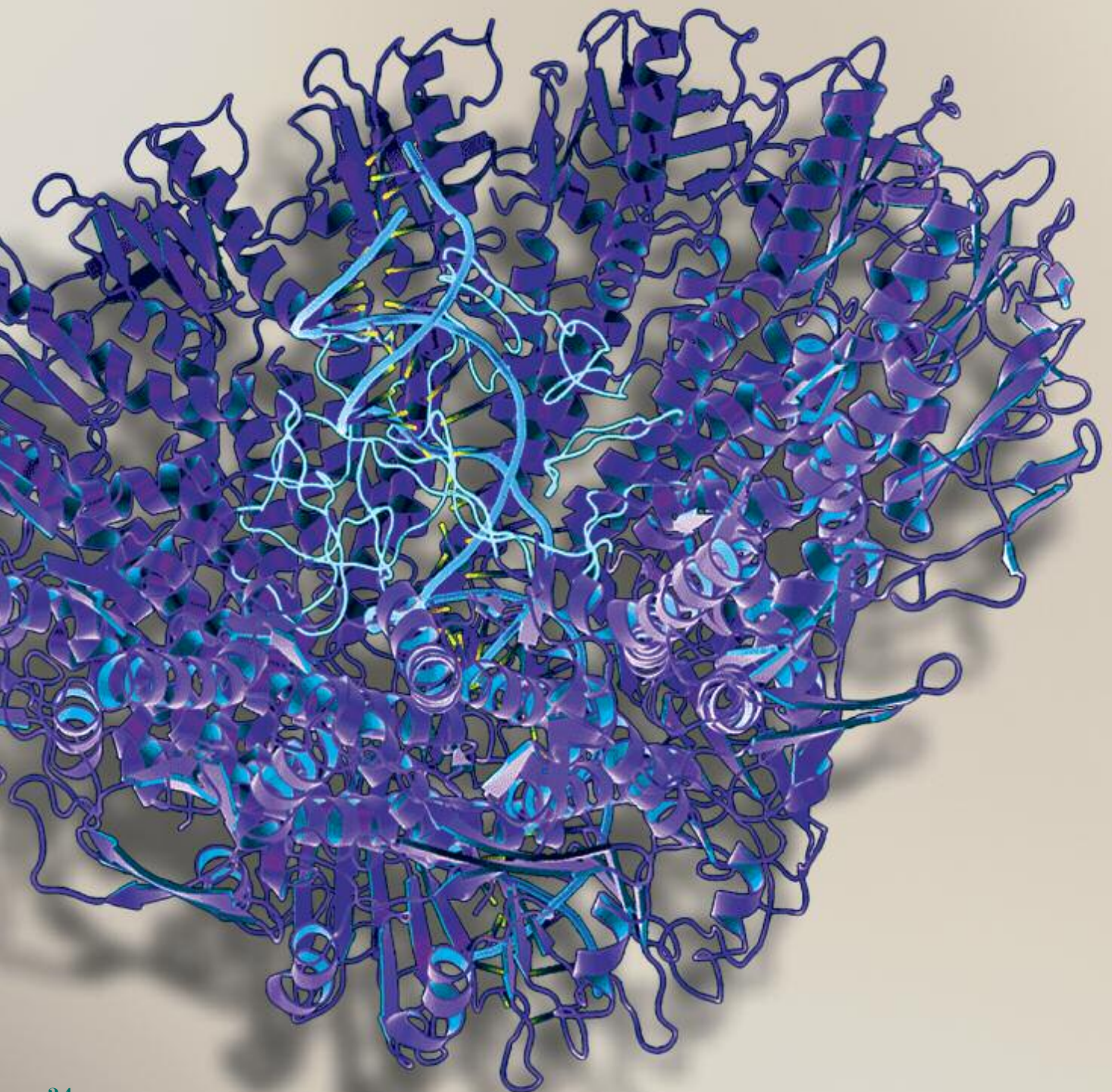
Prof. Dr. Michael Stuke studied physics at the Universities of Marburg, Heidelberg and the Technical University Munich. In 1977, he received his PhD in physical chemistry with a dissertation on laser isotope separation, for which he was awarded the Otto Hahn Medal of the Max Planck Society. Since 1981, he heads the Laser Chemistry Research Group at the Max Planck Institute for Biophysical Chemistry.

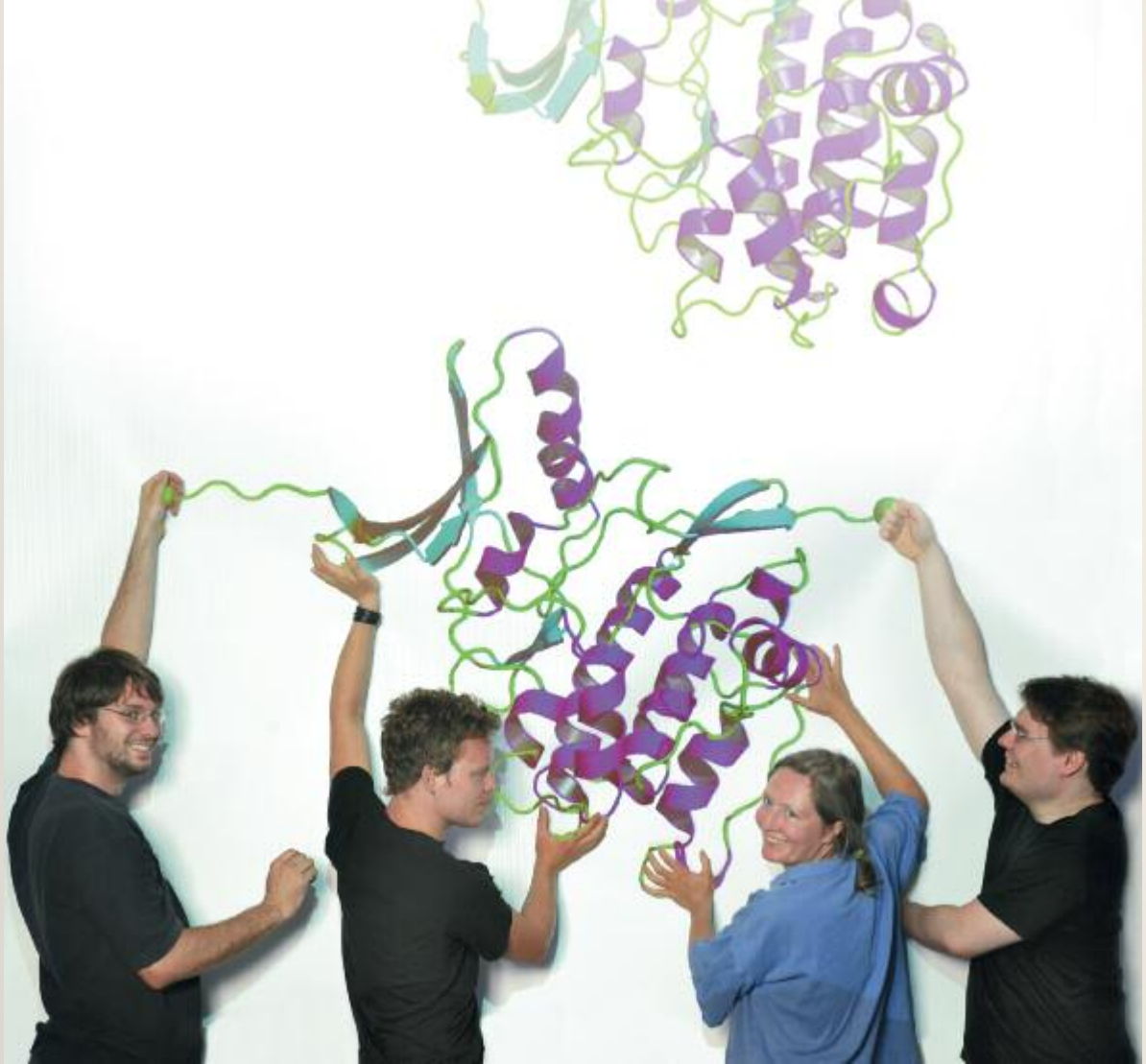
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M. Stuke, K. Mueller, T. Mueller et al.:
Direct-writing of three-dimensional
structures using laser-based processes.
MRS BULLETIN 32, 32-39 (2007),
invited paper.

Sophisticated molecules

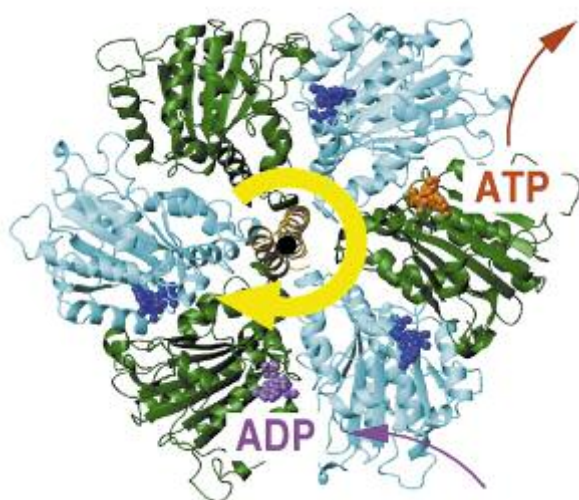




how structure is related to function

The right protein for every purpose – the human body has hundreds of thousands available. Just think of our immune system with its vast selection of antibodies, which protect us from viruses and disease-causing bacteria. However, for many if not most proteins, the exact biological function has not been determined. Even less is known about their specific functional details. There is much to be discovered. On top of this, the scientists focus on yet another category of macromolecules: Nucleic acids do not only carry genetic messages, they also function as catalysts – for instance, in the production of proteins.

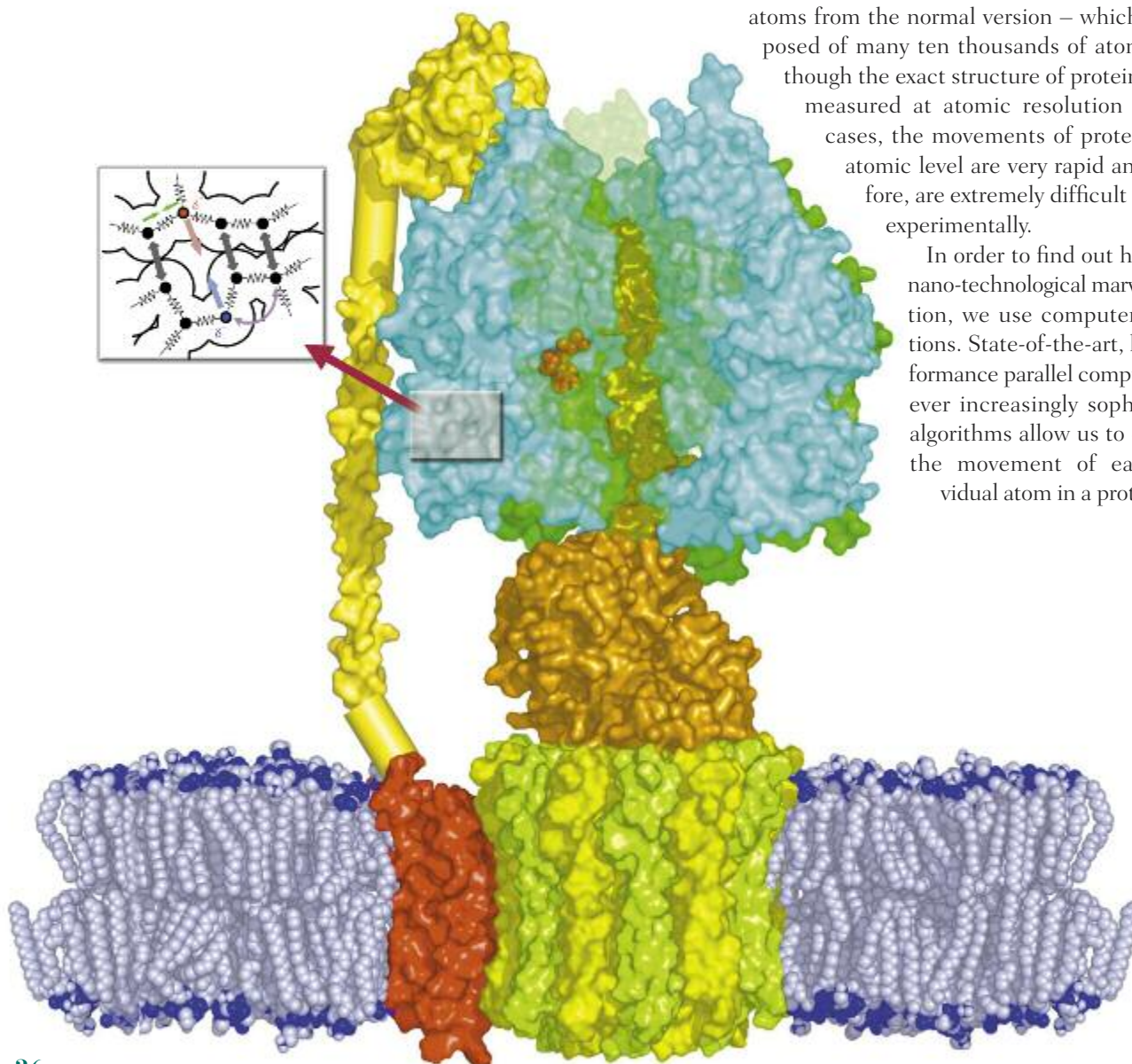
Theoretical and Computational Biophysics

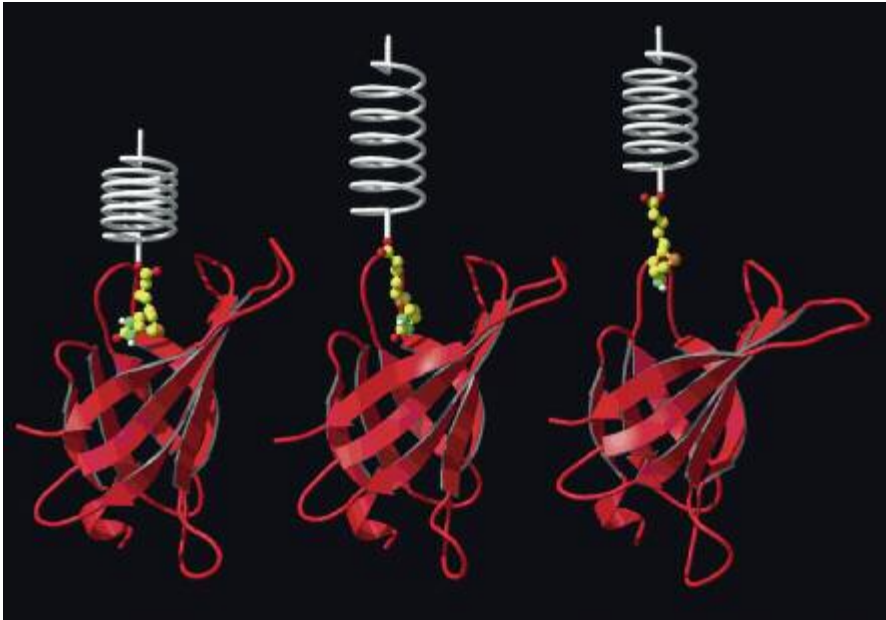


Highly specialized proteins perform and control essentially all processes in the human body. They transport cellular cargo, receive and transmit signals, convert energy, facilitate chemical reactions or ensure growth and movement. These molecules can undoubtedly be characterized as the biochemical »nanomachines« of the cell; they developed in the course of a thousand million years of evolution. Similar to man-made machines, in many cases it is the motions of individual parts of a protein, which implement its function. Accordingly, the internal protein dynamics is extremely well-orchestrated. In many cases the movement of individual atoms is decisive.

No wonder that minute construction errors can have fatal consequences. Some hereditary diseases, for example sickle cell anemia, arise because a specific protein differs by only a few atoms from the normal version – which is composed of many ten thousands of atoms. Even though the exact structure of proteins can be measured at atomic resolution in many cases, the movements of proteins at an atomic level are very rapid and, therefore, are extremely difficult to access experimentally.

In order to find out how these nano-technological marvels function, we use computer simulations. State-of-the-art, high-performance parallel computers and ever increasingly sophisticated algorithms allow us to calculate the movement of each individual atom in a protein com-





Forces often play an important role in molecular nanomachines, but are extremely difficult to access by measurement. Our computer simulations thereby aid in understanding how proteins react to forces. The image shows a vitamin molecule which is being pulled out of a receptor binding pocket – the required force can be measured using an atomic force microscope; the simulation reveals the underlying mechanism.

plex with sufficient precision. To understand in detail the complex processes of life on the basis of the known physical laws, we cooperate closely with experimental research groups.

Proteins at work – the smallest motor in the world

A particularly impressive example of a protein at work is the molecular motor ATP synthase. This protein complex of only twenty nanometers (millionth of a millimeter) in size works in the »power plants« of cells and supplies the required energy for most processes in the body. With the aid of this protein machine, the human body transforms approximately 75 kilograms of the energy storage molecule ATP daily, and even more in peak physical activities. In fact, the similarity between ATP

synthase and an Otto engine is astonishing: In both cases there are force strokes, a turning »crankshaft« and moving »cylinders«. The decisive difference is the efficiency: Whereas the Otto engine achieves only a fraction of the thermodynamical efficiency limit, ATP synthase reaches nearly 100 percent. By means of computer simulations, we have been able to resolve how this energy transfer occurs in detail. The simulations revealed true »nano-mechanics«. The rotary movement of the shaft is translated into an atomically coordinated movement at the synthesis site such that the ATP molecule is synthesized through elaborate assembly.

◀ As a nanomachine *par excellence* ATP synthase produces the universal »fuel« ATP in the human body. The complete machine is just 20 millionth of a millimeter in size. In this computer-generated snapshot an ATP molecule (red) has just been assembled in the top part (cyan/green). The energy required for this is transmitted by a rapidly rotating »crankshaft« (orange, yellow arrow), which in turn is driven by a bottom part in the mitochondrial membrane (green/ yellow). In a manner similar to an electric motor, this bottom part is driven by an electric current, which flows across the membrane along the bottom part and the red stator. In order to examine the nanomachine at work, the computer calculates the forces that act on every single atom, from which the detailed motion of every atom is derived. From these data, a super-resolution video sequence is obtained revealing the tricks of nature.



Prof. Dr. Helmut Grubmüller received his PhD in theoretical physics at the Technical University of Munich in 1994. From 1994 to 1998, he worked as a research assistant at the Ludwig Maximilians University in Munich. In 1998, he moved to the Max Planck Institute for Biophysical Chemistry as research group head. He has headed the Department of Theoretical and Computational Biophysics there since 2003. Helmut Grubmüller is also honorary professor of physics at the University of Göttingen.

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M. Zink, H. Grubmüller: Mechanical properties of the icosahedral shell of southern bean mosaic virus: a molecular dynamics study. *Biophys J.* 96, 1350-1363 (2009).

L. V. Schäfer, E. M. Müller, H. E. Gaub, H. Grubmüller: Elastic properties of photoswitchable azobenzene polymers from molecular dynamics simulations. *Angew. Chemie Int. Ed.* 46, 2232-2237 (2007).

F. Gräter, J. Shen, H. Jiang, M. Gautel, H. Grubmüller: Mechanically induced titin kinase activation studied by force-probe molecular dynamics simulations. *Biophys. J.* 88, 790-804 (2005).

R. Böckmann, H. Grubmüller: Nano-seconds molecular dynamics simulation of primary mechanical energy transfer steps in ATP synthase. *Nature Struct. Biol.* 9, 198-202 (2002).

M. Rief, H. Grubmüller: Kraftspektroskopie von einzelnen Biomolekülen. *Physikalische Blätter*, 55-61, Feb., (2001).



Computational Biomolecular Dynamics

Prof. Dr. Bert de Groot received his PhD in biophysical chemistry at the University of Groningen (The Netherlands) in 1999. From 1999 to 2003, he worked as a post-doctoral fellow in Helmut Grubmüller's Theoretical Molecular Biophysics Research Group at the Max Planck Institute for Biophysical Chemistry. He heads the Computational Biomolecular Dynamics Group there since 2004. Bert de Groot is an adjunct professor of physics at the University of Göttingen since 2009.

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O. F. Lange, N. A. Lakomek, C. Fares, G. F. Schröder, K. F. A. Walter, S. Becker, J. Meiler, H. Grubmüller, C. Griesinger, B. L. de Groot: Recognition dynamics up to microseconds revealed from RDC derived ubiquitin ensemble in solution. *Science* 320, 1471-1475 (2008).

J. S. Hub, B. L. de Groot: Mechanism of selectivity in aquaporins and aquaglyceroporins. *Proc. Nat. Acad. Sci.* 105, 1198-1203 (2008).

D. Seeliger, J. Haas, B. L. de Groot: Geometry-based sampling of conformational transitions in proteins. *Structure* 15, 1482-1492 (2007).

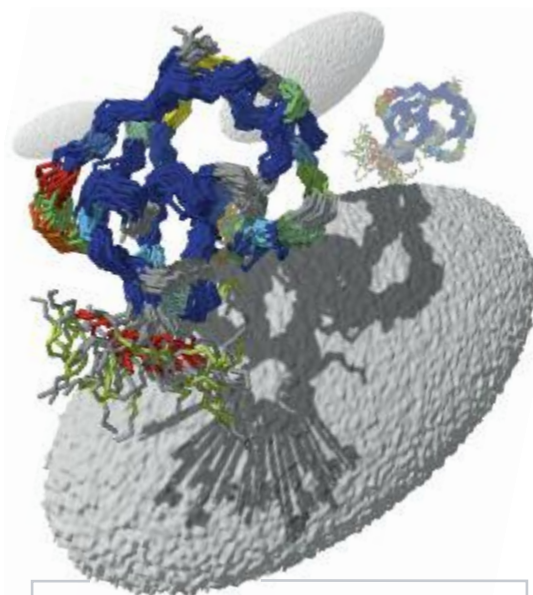
The function of a machine can be much more easily understood if we can observe it in action. The same is true for the tiny machines in our cells – the proteins. Billions of these nanomachines enable, control or support nearly all the processes occurring in our body. Accordingly, the consequences are frequently severe when proteins do not function properly. Many diseases are caused by such dysfunctions.

Which interactions give rise to aggregation of proteins and thus cause disorders such as Alzheimer's or Parkinson's disease? How do cells regulate the influx and efflux of molecules such as water, ions and nutrients? How does molecular recognition function? These are some of the questions we investigate in our research group.

In order to understand the function and dysfunction of proteins, it is usually insufficient to know their blueprints and their three-dimensional shape. Many proteins fulfill their respective task only by means of well-orchestrated movements. Our objective is to understand protein dynamics at the molecular level and to unravel the mechanisms underlying such dynamics.

Water channels, which also filter

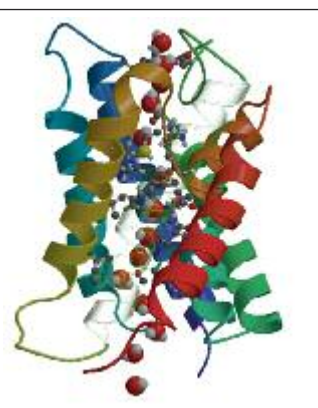
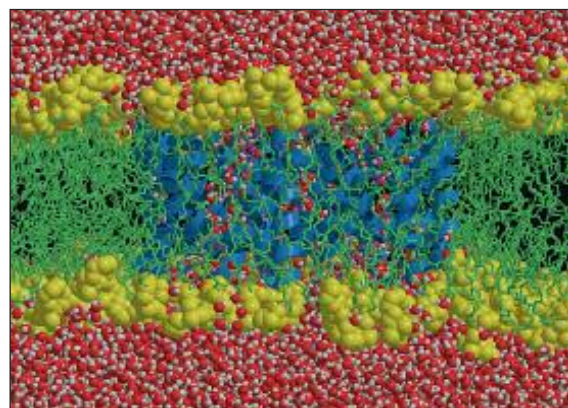
One class of proteins investigated in the group are aquaporins. They form pores in the cell membrane, which function as perfect, highly selective water filters. Only water molecules can pass through; ions and larger molecules are blocked. By means of molecular dynamics simulations we were able to res-



Molecular recognition by ubiquitin. Since ubiquitin can rapidly assume other shapes, it recognizes many different binding partners. One can imagine it to be like a key ring with which different locks can be opened.

ve the mechanism which allows aquaporins to selectively filter in such a way; an electrostatic barrier effectively prevents any ion from passing through.

In addition, we have a true multitalent among the proteins under investigation: ubiquitin. It is part of a sophisticated recycling system in the cell which marks certain proteins as cellular »trash«. But how does ubiquitin manage to recognize and bind to a multitude of different partner molecules? With the aid of molecular dynamics calculations and experiments in cooperation with the Department of NMR-based Structural Biology we were able to demonstrate that ubiquitin is surprisingly mobile. Like a Swiss army knife it continuously changes its shape, extremely rapidly – within a millionth of a second – until it accidentally fits its partner.



Water flow through an aquaporin channel. Left: Water (red/white) does not pass through the lipid membrane (green/yellow), but rather through special pores, which aquaporin forms in the membrane (blue). Right: Path of the water molecules through the aquaporin channel. Specific interactions allow the passage of water, but prevent larger molecules or ions from passing through in an uncontrolled manner. Even tiny protons (hydrogen ions) are excluded.

Structural Investigations of Protein Complexes



Dr. Stefan Becker received his PhD in 1991 at the University of Saarland in chemistry and subsequently went to the C.H. Best Institute in Toronto (Canada). In 1995, he moved to the Grenoble Outstation of the European Molecular Biology Laboratory. Since 1999, Stefan Becker has headed the Molecular Biology Project Group in the Department of NMR-based Structural Biology at the Max Planck Institute for Biophysical Chemistry.

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M. Sevana et al.: A ligand-induced switch in the periplasmic domain of sensor histidine kinase CitA. J. Mol. Biol. 377, 512-523 (2008).

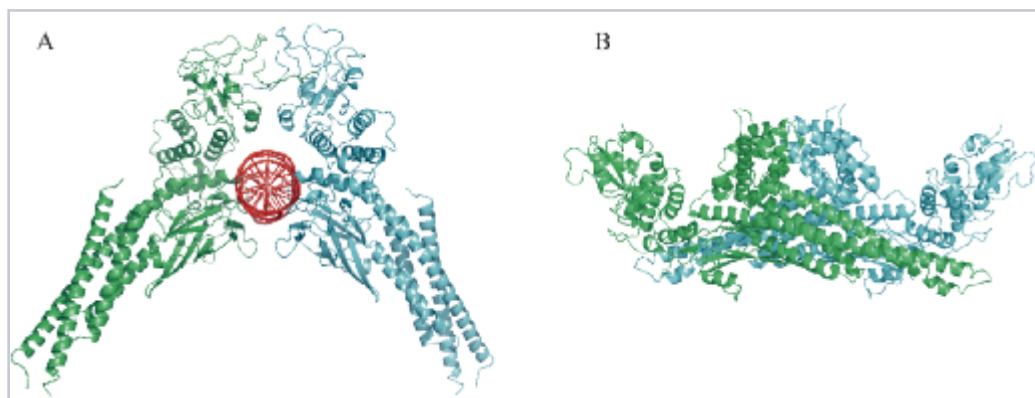
The adage »the communication must be right« also applies to cells. To survive, bacterial cells must, exactly like our body's cells, react to an entire spectrum of stimuli in their environment. However, the stimuli still have a barrier to cross before they can enter the cell: the cell membrane. It shields the cells inner workings effectively from the external environment. However, there is a connection from the outside to the inside: Receiving antennas in the membrane – the sensor proteins – acquire the stimuli and conduct them across the membrane. In the process, they enter into close contact with their communication partners. Sensor proteins are switched on and off depending on the cell's requirements. But, how can they be switched from »on« to »off«?

To find the answer to this question, we grow crystals of such sensor proteins – a sometimes tedious procedure. Then we send high-energy X-ray radiation through such a crystal and thus acquire a complicated diffraction pattern, which provides a large amount of information on the spatial structure of the sensor protein. Admittedly, this method has a disadvantage. In the crystal the protein has practically no freedom of movement. In order to understand dynamic processes such as the switching processes, supplementary nuclear magnetic resonance (NMR) measurements are therefore required because this procedure allows us to examine molecules in solution – with much freedom of movement.

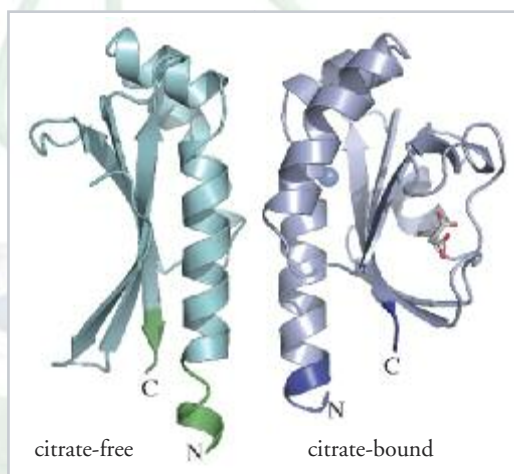
What enables proteins to switch?

Structural changes are of central importance for the »on« and »off« states. The differences between the active and inactive state can be small, as for example in the histidine kinase CitA. This protein is vital for bacteria because it allows them to react to changes in their environment. Our investigations show that when citrate binds to the region of the protein which protrudes outward, the protein contracts at this location. This contraction is ultimately crucial for the transmission of the signal into the cell's interior.

In the case of the STAT proteins, on the other hand, the structural alterations are great. STAT proteins transmit signals from the cell membrane into the interior of the cell nucleus and change the activity of certain genes. Admittedly, in this case always two STAT proteins bind to each other regardless of whether the STAT is active or inactive. However, the binding occurs in very different ways, sometimes arranged parallel to each other and sometimes antiparallel.



In the active form (A) the two STAT molecules are arranged parallel to each other and bind like a nutcracker to the DNA (red structure in central position). However, in the inactive form (B) the STATs are arranged antiparallel to each other; the structure resembles a boat, where the interaction with the DNA cannot take place any more.



Only if citrate binds to the part of the histidine kinase CitA, which extends out of the cell surface, does the molecule contract. As a result the transmembrane part of the histidine kinase is pulled »upwards«. This movement is the signal that is relayed into the cell's interior and triggers the activation of the intracellular histidine kinase domain.

D. Neculai, A. M. Neculai, S. Verrier, K. Straub, K. Klumpp, E. Pfitzner, S. Becker: Structure of the unphosphorylated STAT5a dimer. J. Biol. Chem. 280, 40782-40787 (2005).

S. Becker, B. Groner, C.W. Müller: Three-dimensional structure of the Stat3β homodimer bound to DNA. Nature 394, 145-151 (1998).

NMR-based Structural Biology

For our molecular inventory, the spatial structure of a molecule is just as important as its chemical composition. The lethal consequences of misfolding are evidenced by such incurable pathological conditions as Alzheimer's, Parkinson's or Creutzfeldt-Jakob diseases. In all three diseases, misfolded protein molecules accumulate in brain cells and destroy them. Only properly shaped (folded) proteins and nucleic acids can fulfill their biological function. In this context, we are interested in the question as to which structural details are crucial for proper function applied *in vitro* as well as in studies of cells and tissues.

Getting to the core of the matter

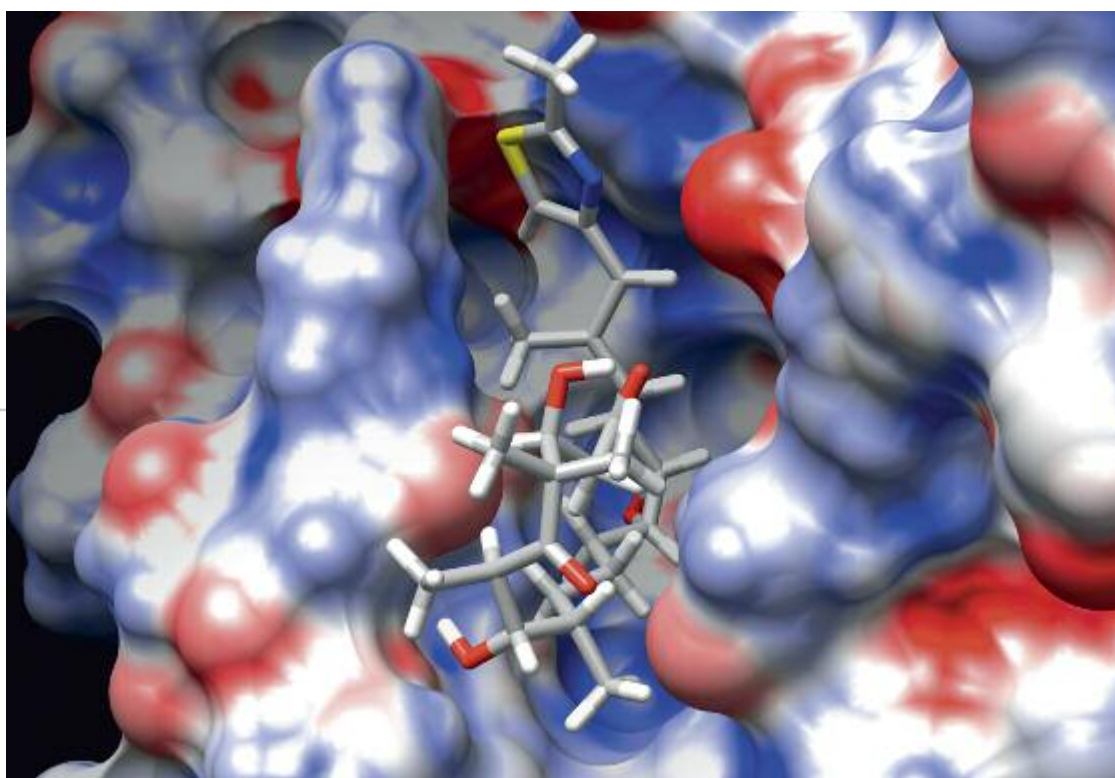
Our method of choice is nuclear magnetic resonance (NMR) spectroscopy. NMR spectroscopy uses a common property of most atomic nuclei, namely that they exhibit magnetic properties. They can be considered to be a kind of electrically charged top which is attempting to orient itself to an external magnetic field. Because of this property, nuclei can absorb electromagnetic radiation that has a certain energy and frequency ($E = h\nu$). The frequency which is absorbed depends on the atomic nucleus' chemical environment. In a mol-

ecule with many differently placed atomic nuclei, a corresponding number of different frequencies is required. As a consequence, an NMR spectrum results, which contains detailed information about the arrangement of the individual atom nuclei and thus about the atoms' location in three-dimensional space.

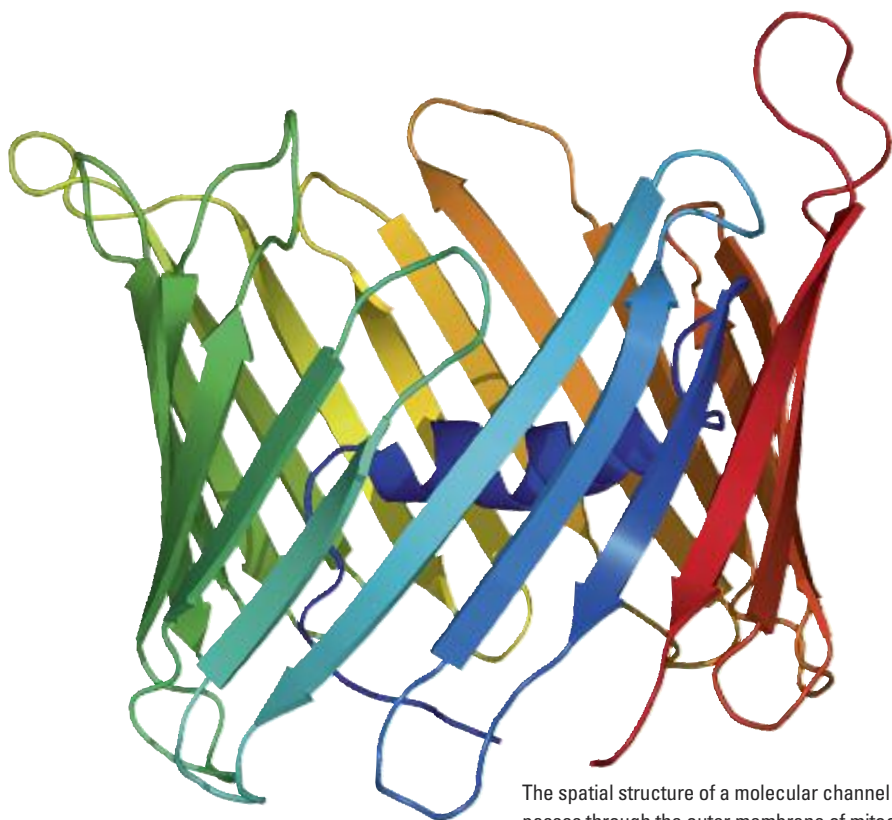
However, the interpretation of this information is an art in itself. And the larger the molecule under investigation is, the more difficult this task becomes. The use of so-called triple resonance experiments, which provide three-dimensional spectra, is required. In protein molecules which comprise more than two hundred amino acids – the building blocks of all proteins – even this type of NMR spectroscopy reaches its limit. However, we are attempting to push these limits even further.

Pushing the limits

In this context, we incorporate isotopes into the molecules. We replace, for example, some of the normal nitrogen with a variant possessing a heavier atomic nucleus (an isotope). We can then make either one isotope or the other visible. In this manner, proteins which are really too large for NMR spectroscopy can be analyzed section by section. However, the first problem to be solved



Spatial reconstruction of a tubulin molecule. NMR spectroscopy shows how the anti-cancer drug penetrates into this structure.



The spatial structure of a molecular channel which passes through the outer membrane of mitochondria – the »power plants« of cells. Via such channels, the mitochondria provide chemical energy in the form of adenosine triphosphate, in the human body this amounts to approximately 75 kg daily.

is how to produce such proteins in adequate numbers. Bacteria which have been equipped with the gene in question have proved helpful. If they are fed with amino acids which contain a specific isotope, they assemble the protein according to our wishes – if everything functions properly.

Electrons, which have a much larger magnetic moment than nuclei, can also be attached to molecules. They provide additional information, which we used, for example, to analyze the spatial structure of one of the most frequent human membrane proteins. In addition, we wish to use electrons for signal amplification using so-called Dynamic Nuclear Polarization (DNP).

Dynamic molecules

NMR spectroscopy can also be used to observe proteins in action. In solution or embedded in a membrane, they can move freely in a manner similar to that in their natural environment during the measurement. When they change their spatial structure in the process, this frequently occurs extremely rapidly. A new procedure enables us to measure the movements of proteins which occur in a time window of between one thou-

sand-millionth and a millionth of a second. In this manner, we obtain new knowledge of how proteins recognize one another and what makes them maneuverable.

Focus on drug molecules

We can now exactly examine how small molecules act on large proteins. Thus, for example, it is possible to show how an active substance used in the chemotherapy of cancer attaches to the protein tubulin. The microtubuli, long nano-tubes which the cell uses to maintain its shape, are made of tubulin. When assembly and reassembly of this lattice does not function, the cells die as soon as they attempt to reproduce. That affects cancer cells severely because they normally reproduce continuously. A small molecule which we prepared can stop diseases such as Alzheimer's, Parkinson's and Creutzfeldt-Jakob – at least in our animal model. We are currently studying the spatial structure of this extremely promising molecule in a complex with its target molecule.



Prof. Dr. Christian Griesinger

studied chemistry and physics at the University of Frankfurt/Main, where he received his PhD in organic chemistry in 1986. From 1986 to 1989, he was a postdoctoral fellow at the Laboratory for Physical Chemistry at the ETH in Zurich (Switzerland). In 1990, he accepted a professorship at the Institute for Organic Chemistry at the University of Frankfurt, and since 1999, he has headed the Department of NMR-based Structural Biology at the Max Planck Institute for Biophysical Chemistry. Christian Griesinger has been awarded a number of renowned prizes, among them the Sommerfeld prize (1997), the Leibniz prize of the DFG (1998) and the Bayer prize (2003). He is member of the Akademie der Wissenschaften zu Göttingen and the German Academy of Sciences Leopoldina. He received an ERC Advanced Grant in 2008.

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O. Lange, N. A. Lakomek, C. Farès, G. Schröder, S. Becker, J. Meiler, H. Grubmüller, C. Griesinger, B. de Groot: Recognition dynamics up to microseconds revealed from an RDC-derived ubiquitin ensemble in solution. *Science* 320, 1471-1475 (2008).

M. Reese, D. Lennartz, T. Marquardsen, P. Höfer, A. Tavernier, P. Carl, T. Schippmann, M. Bennati, T. Carlomagno, F. Engelke, C. Griesinger: Construction of a liquid-state NMR DNP shuttle spectrometer: first experimental results and evaluation of optimal performance characteristics. *Appl. Magn. Reson.* 34, 301-311 (2008).

M. Bayrhuber, T. Meins, M. Habeck, S. Becker, K. Giller, S. Villingier, C. Vornrhein, C. Griesinger, M. Zweckstetter, K. Zeth: Structure of the human voltage-dependent anion channel. *Proc. Natl. Acad. Sci. USA* 105, 15370-5 (2008).



Structure Determination of Proteins Using NMR

Form follows function – this platitude for good product design is very frequently also perfectly implemented in proteins. Depending on their task, they have a very specific shape. The amino acid chains of a muscle protein fold themselves into a different spatial structure than those in a tunnel protein, which allows substances to pass through a membrane.

Proteins out of shape

It thus appears even more surprising that particularly a large part of the human genome contains plans for proteins which are present in the cell in nearly an unfolded form. Biologists term these proteins »intrinsically disordered« or »natively unfolded«. They play an important role in various cellular processes, for example, when a gene is read, when a cell divides, or when signals are transmitted.

It is not exactly easy to investigate this unusual protein group because such proteins are very flexible and mobile. This hinders work using imaging methods as the acquired images – as in photography – are out of focus. Only with nuclear magnetic resonance spectroscopy are we able

to obtain information with atomic resolution. Importantly, high mobility is highly beneficial in the cell. It allows such disordered proteins to bind to many different partners. Additionally, the cell can regulate these proteins efficiently, for example, by attaching small phosphate groups.

In our group we use magnetic resonance to study how these proteins change their form when they recognize other proteins and bind to them or when something goes wrong with their own folding. We investigate the consequences of such misfoldings of proteins such as α -synuclein, tau und amyloid- β , which play a central role in the Parkinson's and Alzheimer's diseases. In Parkinson's and Alzheimer's patients misfolded tau and amyloid- β can be detected in the brain tissue as »protein clumps«. But why are these improperly folded proteins so detrimental? According to our research results, a possible cause is that improperly folded tau protein binds much more weakly to microtubule proteins – the cell's »transport rails«. As a consequence, the material transport inside the nerve cell is disrupted, and nerve cell endings no longer grow properly.

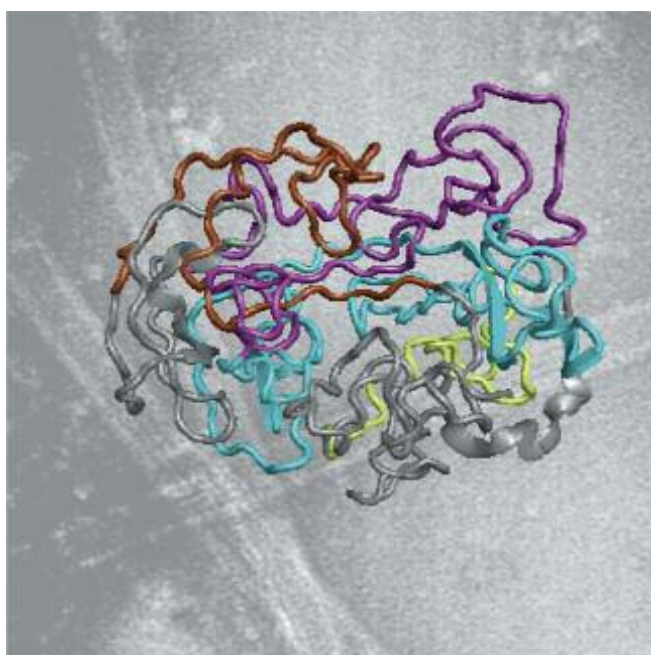
Prof. Dr. Markus Zweckstetter studied physics at the Ludwig Maximilians University in Munich. He did his PhD under the supervision of Tad Holak at the Max Planck Institute for Biochemistry in Martinsried and received his PhD from the Technical University of Munich. Subsequently, from 1999 to 2001, he worked as a postdoctoral fellow in Dr. Adrian Bax's group at the National Institutes of Health in Bethesda (Maryland, USA). Since 2001, he has headed the Research Group Protein Structure Determination Using NMR at the Max Planck Institute for Biophysical Chemistry. Markus Zweckstetter has taught as honorary professor of biology at the University of Göttingen since 2008.

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D. P. Karpinar, M. B. Balija, S. Kügler, F. Opazo, N. Rezaei-Ghaleh, N. Wender, H. Y. Kim, G. Taschenberger, B. H. Falkenburger, H. Heise, A. Kumar, D. Riedel, L. Fichtner, A. Voigt, G. H. Braus, K. Giller, S. Becker, A. Herzig, M. Baldus, H. Jäckle, S. Eimer, J. B. Schulz, C. Griesinger, M. Zweckstetter: Pre-fibrillar alpha-synuclein variants with impaired beta-structure increase neurotoxicity in Parkinson's disease models. *EMBO J.* | doi:10.1038/emboj.2009.257 (2009).

M. D. Mukrasch, S. Bibow, J. Korukottu, S. Jegannathan, J. Biernat, C. Griesinger, E. Mandelkow, M. Zweckstetter: Structural polymorphism of 441-residue tau at single residue resolution. *PLoS Biol.* 17, e34 (2009).

M. Bayrhuber, T. Meins, M. Habeck, S. Becker, K. Giller, S. Villinger, C. Vonrhein, C. Griesinger, M. Zweckstetter, K. Zeth: Structure of the human voltage-dependent anion channel. *Proc. Natl. Acad. Sci. USA* 105, 15370-15375 (2008).



Structure of the tau protein. In the background, an electron microscopic image of the microtubuli, the binding partner of the tau protein, can be seen.

Solid-State NMR Spectroscopy

Life without proteins is inconceivable. They manage and perform the majority of those tasks which ensure the survival of bacteria as well as plants and animals. All proteins are constructed of amino acids, hundreds – sometimes even thousands – of which must be correctly strung together. However, only when they have folded to form their correct three-dimensional structure can they fulfill their respective function in the cell. But, if they are incorrectly folded, they can cause great damage.

Indeed, two incorrectly folded proteins can be detected in the brain tissues of Alzheimer's patients. Amyloid plaques (protein clumps) are found scattered diffusely among nerve cells in the cerebral cortex and other regions of the brain. Inside the nerve cells, there are tau fibrils which have agglutinated to form clusters. In interaction with genetic factors, the latter contribute to the disruption of the nerve cell metabolism and the communication between nerve cells. The nerve cells atrophy and ultimately die.

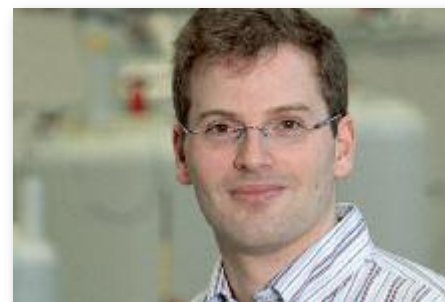
Why do the individual molecules accumulate in this way and why can the cell not degrade them? To find answers to these questions, we are attempting to determine

the structure of individual proteins in the tau tangles.

Rotating molecules

However, conventional methods of structure determination do not function in this case because the plaques and tangles are simply too large and additionally insoluble in water. They are thus neither appropriate for liquid-state nuclear magnetic resonance (NMR) spectroscopy nor for X-ray structure analysis. We therefore use a special form of NMR spectroscopy to investigate these heavy, water-insoluble protein aggregates: solid-state NMR spectroscopy. And we combine this with a trick: As the molecules themselves do not turn rapidly enough, we rotate our entire sample up to 65,000 times per second. With the aid of radio waves we can then examine the structure of the tau fibrils in the strong magnetic field of the NMR spectrometers.

However, we are also interested in the structure of other proteins which are tightly anchored in the cell membrane. For example, how does the structure of the membrane proteins change when other molecules, for example medicinal products, bind to them.



Dr. Adam Lange studied physics in Göttingen and received his PhD in 2006 at the Max Planck Institute for Biophysical Chemistry in cooperation with the National Institutes of Health in Bethesda (Maryland, USA). Subsequently, he moved to the Swiss Federal Institute of Technology (ETH) Zurich with an EMBO Fellowship. Since 2008, he has headed the Solid-State NMR Spectroscopy Research Group at the Max Planck Institute for Biophysical Chemistry. In 2006, Adam Lange was awarded the Otto Hahn Medal of the Max Planck Society and the R. R. Ernst Prize of the German Chemical Society.

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The superconducting magnet generates a magnetic field which is approximately 400,000 times as strong as the earth's magnetic field.

A. Lange, I. Scholz, T. Manolikas, M. Ernst, B. H. Meier: Low-power cross polarization in fast magic-angle spinning NMR experiments. *Chem. Phys. Lett.* 468, 100-105 (2009).

C. Wäsmer, A. Lange, H. Van Melckebeke, A. B. Siemer, R. Riek, B. H. Meier: Amyloid fibrils of the HET-s(218-289) prion form a β -solenoid with a triangular hydrophobic core. *Science* 319, 1523-1526 (2008).

A. Lange, K. Giller, S. Hornig, M. F. Martin-Eauclaire, O. Pongs, S. Becker, M. Baldus: Toxin-induced conformational changes in a potassium channel revealed by solid-state NMR. *Nature* 440, 959-962 (2006).



Systems Biology of Motor Proteins

Dr. Martin Kollmar received his PhD in chemistry at the Max Planck Institute for Medical Research in Heidelberg in 2002. He has been head of the Systems Biology of Motor Proteins Project Group at the Max Planck Institute for Biophysical Chemistry since that time. Martin Kollmar received a Liebig Fellowship in 2002 and was awarded the Sophie Bernthsen Prize in 1998.

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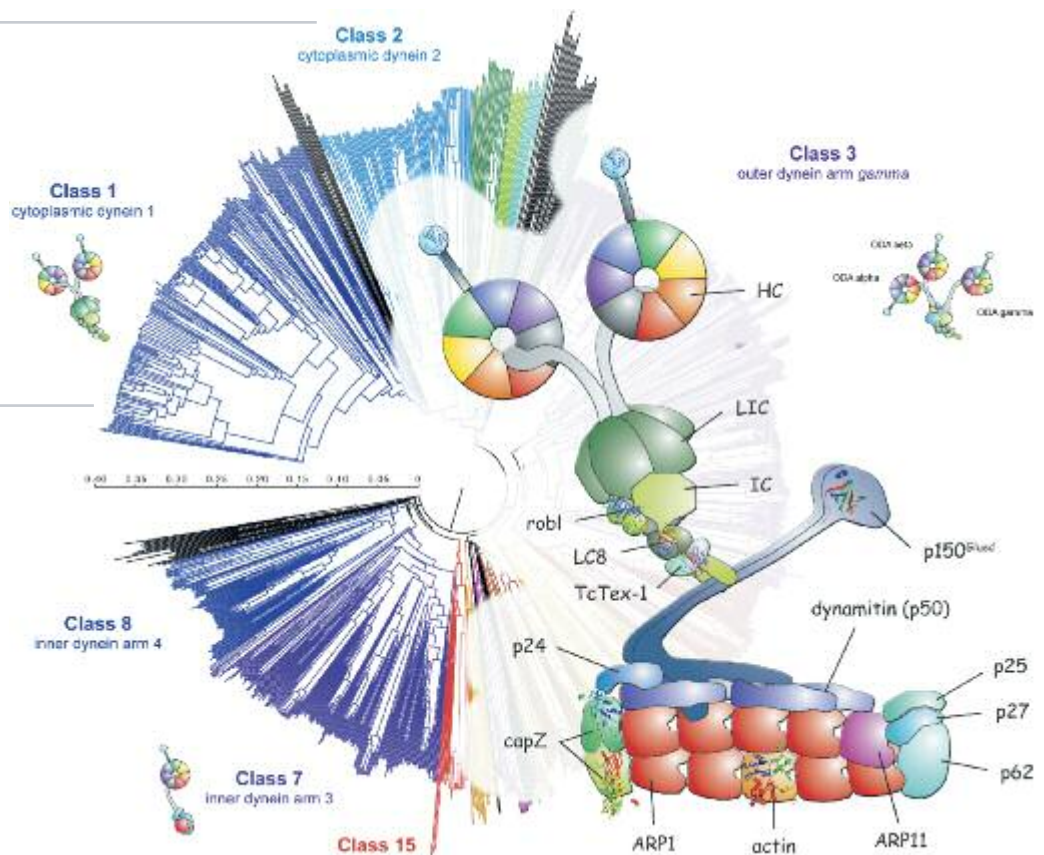
The cells of all organisms, whether animals or plants, have an active inner life. They have a molecular inventory – their motor proteins – with which they can selectively move diverse cell components. Even in the movement of muscles, during cell division or intracellular logistics, such motor proteins are indispensable. We desire to find out how they function and how different motor proteins collaborate in the cell as a complex network. To achieve this, we determine the spectrum of motor proteins which different organisms have at their disposal.

In this context, we can draw upon a continuously increasing number of completely sequenced genomes. To identify motor protein genes, we compare the DNA sequences from the genomes of many different organisms, from mouse and human down to fly, round worm and yeast. However, just because the DNA sequence of the organisms is known does not mean that the genes coded in it are known – far from it. Therefore, we are developing special tools for screening databases and selectively detecting motor protein genes.

Giant in the realm of proteins

The mode of operation of motor proteins can only be understood if we are familiar with the details of their spatial structure. We are currently studying the protein complex comprising dynein und dynactin. It is involved in both the transport of different cell components and cell division, in which the chromosomes must be sorted and correctly distributed between the daughter cells. Dynactin coordinates and regulates the cellular cargo transport, whereas dynein supplies the energy required in this process. However, how this molecular machine transforms chemical energy into mechanical work is still largely unresolved. This is not surprising because cellular dynein is a true giant among proteins. It is approximately twenty times larger than the red blood pigment hemoglobin and thus poorly accessible for structural investigations. As a consequence, it is a particular challenge to decipher its spatial structure.

Foreground: schematic diagram of the organization of the subunits in the cytoplasmic dynein/dynactin complex. Known structures of subunits or fragments thereof are superimposed on the schematic diagram. Background: Phylogenetic tree of the dyneins, in which the different dynein forms are marked in color.

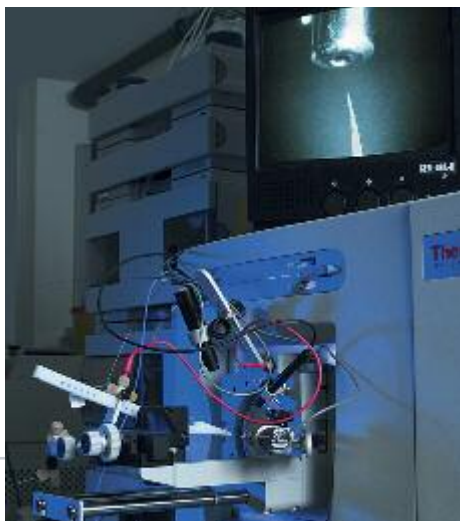


F. Odronitz, H. Pillmann, O. Keller, S. Waack, M. Kollmar: WebScipio: An online tool for the determination of gene structures using protein sequences. *BMC Genomics* 9, 422 (2008).

F. Odronitz, M. Kollmar: Drawing the tree of eukaryotic life based on the analysis of 2269 manually annotated myosins from 328 species. *Genome Biology* 8, R196 (2007).

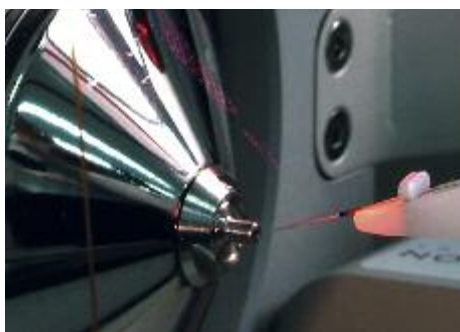
F. Odronitz, M. Hellkamp, K. Kollmar: diArk – a resource for eukaryotic genome research. *BMC Genomics* 8, 103 (2007).

Bioanalytical Mass Spectrometry



State-of-the-art Fourier-transform (FT) electrospray-ionization mass spectrometer for the quantitative analysis of molecules. Tiny quantities of proteins and their fragments are separated by liquid chromatography and sprayed directly into the mass spectrometer. The screen shows the position of the spray nozzle (capillary needle made of silica glass) from which the molecules emerge in front of the opening of the mass spectrometer.

How much does a molecule weigh? Mass spectrometry is used to determine exactly the mass – and thus the weight – of molecules. The state-of-the-art »bioanalytical« mass spectrometry of proteins has become a fundamental analysis technique in the life sciences. As a result of new methods in bioanalytical mass spectrometry, we can quantify the proteins in different cells and developmental stages of an organism, for how cells develop and how organisms age is also reflected in their protein patterns. We determine and compare the protein patterns of protein complexes and cellular compartments, but also entire cells and



Ion source of an electrospray-ionization mass spectrometer. Between the nozzle (1/100 of a millimeter) and the opening of the mass spectrometer a voltage of 1-2 kV is applied. In this way, tiny droplets of liquid containing the molecules to be investigated are sprayed into the mass spectrometer, like a jet of liquid from a scent-spray. Once inside the mass spectrometer, the solvent evaporates, leaving charged molecules, the exact mass of which can then be determined.

tissues, in cooperation with other research groups. The differences which we observe in this context not only help us to understand cellular processes. They also allow conclusions as to what is occurring in a cell when it differentiates from a progenitor cell (for example in bone marrow) to a highly specialized immune cell. Not least, we are interested in how such cellular processes change in certain diseases.

Manageable protein fragments

To achieve this we do not analyze the intact proteins. On the contrary, we initially cleave the proteins, which we have isolated from a cell, into smaller, more manageable protein fragments (peptides). Subsequently, we determine exactly not only their mass, but also the sequence of their individual building blocks, the amino acids. As soon as we know the mass and amino-acid sequence of one or more of these peptides, we can reliably identify the corresponding intact protein in databases and determine its quantity.

The properties of proteins change with the sequence and degree of modification of their amino acids. This must be taken into consideration in the analysis. Thus, proteins that are components of cell membranes and possess, for example, sugar residues require a different analysis procedure compared with those that bear phosphate residues and can switch special genes »on« and »off«. A further objective of our research group is therefore to improve the existing analytical methods and to develop new procedures that will provide even more detailed insight into the diverse protein inventory of cells.



Dr. Henning Urlaub studied biochemistry at the Free University of Berlin and received his PhD at the Max Delbrück Center for Molecular Medicine in Berlin. From 1997 to 2004, he conducted research as a postdoctoral fellow in Reinhard Lührmann's laboratories in Marburg and Göttingen, a period that included for several research stays at the European Molecular Biology Laboratory (EMBL) in Heidelberg. He has headed the Bioanalytical Mass Spectrometry Research Group at the Max Planck Institute for Biophysical Chemistry since 2004. Henning Urlaub has organized the series of Summer Schools on »Proteomic Basics«, held annually since 2004. He is a member of the Executive Board of the German Society for Proteome Research (DGPF).

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T. Oellerich, M. Gronborg, K. Neumann, H. H. Hsiao, H. Urlaub, J. Wienands: SLP-65 phosphorylation dynamics reveals a functional basis for signal integration by receptor-proximal adaptor proteins. *Mol. Cell. Proteomics* 8, 1738-1750 (2009).

L. Weimann, J. Hoeck, T. Ivacevic, T. Ohrt, J. Muetze, P. Schwill, E. Kremmer, V. Benes, H. Urlaub, G. Meister: Importin 8 is a gene silencing factor that targets argonaute proteins to distinct mRNAs. *Cell* 136, 496-507 (2009).

E. Meulmeester, M. Kunze, H. H. Hsiao, H. Urlaub, F. Melchior: Mechanism and consequences for paralog-specific sumoylation of ubiquitin-specific protease 25. *Mol. Cell* 30, 610-619 (2008).

C. Lenz, E. Kuehn-Hoelsken, H. Urlaub: Detection of protein-RNA crosslinks by nanoLC-ESI-MS/MS using precursor ion scanning and multiple reaction monitoring (MRM) experiments. *J. Am. Soc. Mass Spectrometry* 18, 869-881 (2007).



Enzyme Biochemistry

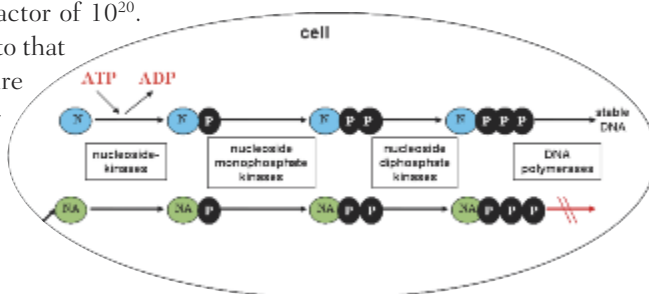
Without enzymes all life would abruptly come to a halt. These bio-catalysts enable a wealth of chemical processes which could otherwise not occur in our body. They accelerate chemical reactions to an incomprehensible extent, sometimes as much as a factor of 10^{20} .

In order to boost reactions to that extent, the enzymes require chemical energy in an appropriate form. Most enzymes work with a small molecule named adenosine triphosphate (ATP), the universal currency of energy supply of the cell. On average a human being forms and consumes about 75 kilograms of this molecule daily.

The primary focus of our work is on enzymes which can activate the ineffective precursors of a medication. In chemotherapy of some cancers and virus infections the patients are not given the active substance itself but rather a chemical precursor, termed pro-drug. In the human body, in a best case scenario only in the diseased cells, the medication then develops the desired destructive effect. In this process, enzymes which attach phosphate groups to components of the genetic material DNA frequently come into play. However, these enzymes accept – more or less readily – similar molecules which are administered to the patient as pro-drugs.

Curbing viruses and tumors

When three phosphate groups have been attached to these »false« building blocks, they are also incorporated into new DNA molecules. However, since they do not fit optimally, the struc-



▲ In human cells there are enzymes from the kinase family which attach phosphate groups to nucleosides (N) – the building blocks of DNA – in three steps. Nucleoside analogues (NA), which are administered as medicine, are also readily accepted, but their incorporation destroys the DNA structure.

ture of the DNA becomes unstable, or the chain of building blocks breaks off prematurely. In this manner, the false DNA building blocks slow down viral reproduction or hinder tumor growth.

We already know which molecular structures are formed when the pro-drugs come into contact with these enzymes. This helps us to understand why the ease of phosphate group attachment varies for different pharmaceuticals. On this basis, we want to construct enzyme variants which activate certain pro-drugs even better and more selectively. In this context, we are particularly interested in the structural mirror images of normal DNA building blocks in living cells.

Dr. Manfred Konrad studied biology, mathematics and chemistry, and received his PhD from the University of Heidelberg in 1981. From 1978 to 1979, he conducted research as a DAAD Fellow at the Boston University, School of Medicine (USA). In 1982, he went to the University of Bristol (United Kingdom) as an EMBO Fellow. After having worked for the Gödecke Company (Freiburg), he initially went to the University of Marburg and then came to the Max Planck Institute for Biophysical Chemistry. Manfred Konrad has headed the Enzyme Biochemistry Research Group there since 2005.

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E. Sabini, S. Hazra, S. Ort, M. Konrad, A. Lavie: Structural basis for substrate promiscuity of deoxycytidine kinase. *J. Mol. Biol.* 378, 607-621 (2008).

B. M. Wöhrl, L. Loubière, R. Brundiers, R. S. Goody, D. Klatzmann, M. Konrad: Expressing engineered thymidylate kinase variants in human cells to improve AZT phosphorylation and human immunodeficiency virus inhibition. *J. Gen. Virol.* 86, 757-764 (2005).

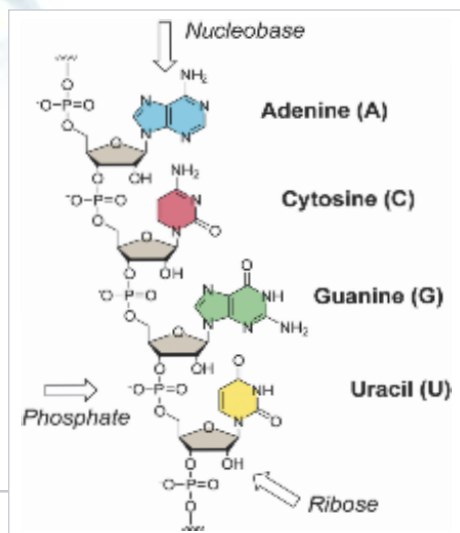
C. Monnerjahn, M. Konrad: Modulated nucleoside kinases as tools to improve the activation of therapeutic nucleoside analogues. *Chem. Bio. Chem.* 4, 143-146 (2003).

Both enantiomers are welcome

The enzyme deoxycytidine kinase attaches phosphate groups to the DNA building block deoxycytidine. In this context, image (D-deoxycytidine, D-dC) and mirror image (L-deoxycytidine, L-dC) are equally well accepted.

Nucleic Acid Chemistry

Nucleic acids are essential building blocks of all organisms. The nucleic acids DNA and RNA store and transmit genetic information. However, this does



The primary structure of ribonucleic acid (RNA). Chemical modifications can be added to the nucleobases, the sugar unit (ribose) or the phosphate backbone.

not nearly exhaust their functional repertoire. Nucleic acids also perform tasks which scientists have only known from proteins for a long time. For example, just like proteins RNAs can act as catalysts (ribozymes) and accelerate various chemical reactions in nature. RNAs also regulate the activities of genes by means of riboswitches or RNA interference.

DNA and RNA enzymes

We want to understand how RNA functions as an enzyme or a gene regulator. To achieve this we must first chemically modify the RNA. Thus, we furnish it for example with chemical markers, which we can site-specifically attach to the nucleic acid. We analyze the properties of this modified RNA in cooperation with other research groups in the institute.

It has only been known for a few years that DNA also has catalytic ability. These DNA catalysts (deoxyribozymes) have put our knowledge about catalytic nucleic acids on a new foundation. Today scientists already use DNA enzymes in research and are investigating their possible use as medicine or as alternative biosensors.

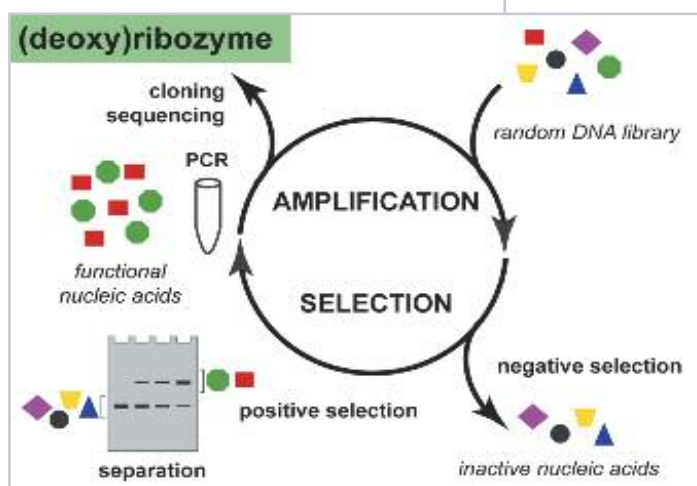
In our group we develop DNA enzymes with which we can manipulate and chemically modify RNA in a desired manner. However, to construct such custom-tailored tools out of deoxyribozymes, we must have very detailed knowledge of how they function. Which of the thousands of atoms in a DNA enzyme participate in the catalysis? Where does the driving force required to accelerate a reaction come from? In order to answer these questions, we analyze the molecular details of the structure, function and mechanisms of DNA catalysts with an entire arsenal of methods from chemistry, biochemistry and biophysics.



Dr. Claudia Höbartner studied technical chemistry at the Technical University of Vienna (Austria) and the Swiss Federal Institute of Technology (ETH) Zurich (Switzerland). She received her PhD in organic chemistry from the Leopold Franzens University of Innsbruck in 2004. From 2005 to 2007, she did research as an Erwin Schrödinger Postdoctoral Fellow of the Austrian Science Fund (FWF) at the University of Illinois at Urbana-Champaign (USA). In 2007, she returned to the University of Innsbruck – funded by the Herta and Paul Amirian Program of the Austrian Federal Ministry for Science and Research. She has headed the Max Planck Research Group Nucleic Acid Chemistry at the Max Planck Institute for Biophysical Chemistry since 2008.

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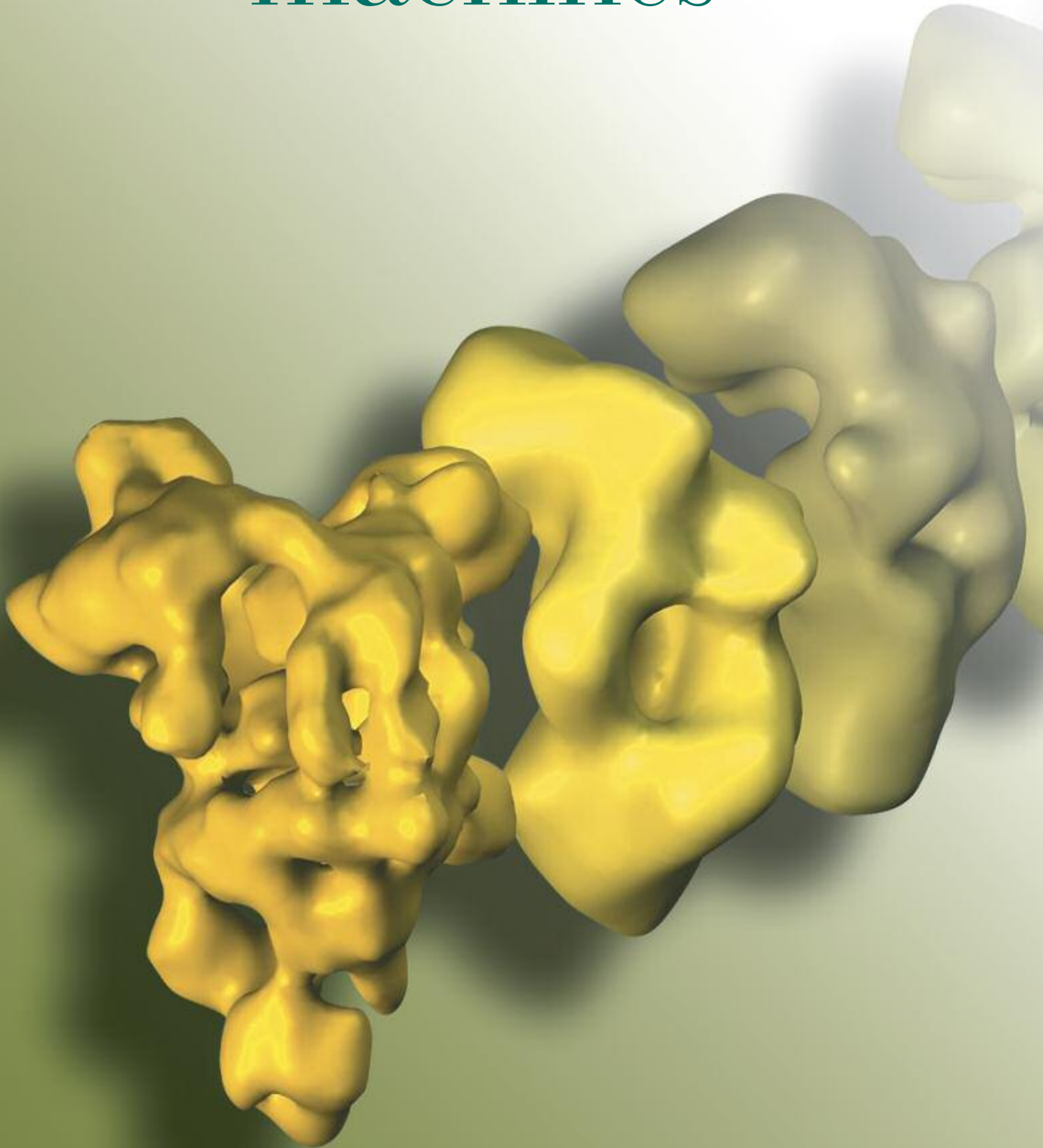


In vitro selection is the procedure with which catalytic nucleic acids are found in the laboratory. In this method, a library of different nucleic acids is repeatedly sorted and amplified until a (deoxy)ribozyme with the desired properties is obtained.

R. Rieder, C. Höbartner, R. Micura: Enzymatic ligation strategies for the preparation of purine riboswitches with site-specific chemical modifications. In: Riboswitches (Ed. A. Serganov). *Methods Mol. Biol.* 540, 15-24 (2009).

C. Höbartner, S. K. Silverman: Recent advances in DNA catalysis. *Biopolymers* 87, 279-292 (2007).

Cellular machines





how molecules divide up the work

Yeast fungi are organisms that consist of a single cell. But many body cells in multi-cellular organisms, such as mice and humans, function autonomously as well. In order for the cells to reliably fulfill their various functions, a large number of different proteins – the cell's nanomachines – must smoothly work together. Which molecular machines are at work in different cell types, how do they function in detail, and how do they interact? These are some of the questions that several departments and research groups address by employing biochemical, molecular genetic, microscopic and fluorescence spectroscopic methods.

Cellular Biochemistry

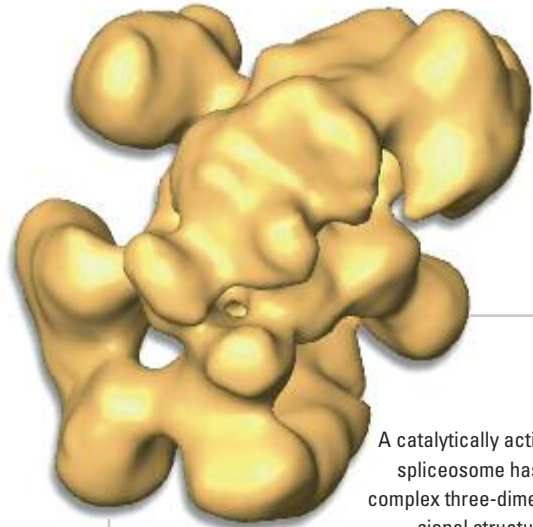
Regardless of whether muscle, skin or liver, in every tissue there is an abundance of diverse proteins. The blueprints for all of these protein molecules are present in encoded form in the genes found in the cell nucleus.

In order to be able to produce proteins according to these blueprints, a gene is initially transcribed into a precursor messenger ribonucleic acid (pre-mRNA). However, this precursor form (draft version) cannot be immediately utilized for protein production, because the blueprint for a protein is not normally present in one piece, but rather in several segments – the exons. Between these exons there are regions that have to be excised from the precursor version – the introns. Only after this operational step (termed splicing) has occurred are all of the required exons contiguously connected in a ready-to-use messenger RNA.

This appears to be unnecessarily complicated, but it has a decisive advantage: Different exons can be selected and assembled to form different messenger RNAs as required. As a consequence, a single gene can provide the blueprints for many different proteins. This process, which is termed »alternative splicing«, explains how human beings can produce more than 100,000 different proteins from a rather modest complement of approximately 25,000 genes.

Cutting to measure

In order to transform the precursor version of a messenger RNA into a functional end product, splicing must occur very precisely. Thus, it is no wonder that this process is performed by a very complicated molecular machine, the spliceosome. This

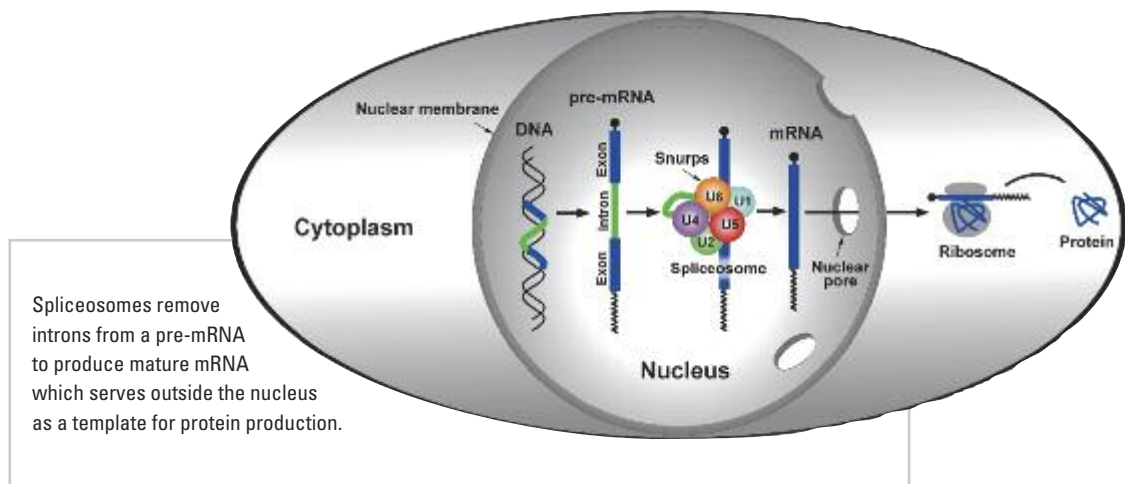


A catalytically active spliceosome has a complex three-dimensional structure.

machine is comprised of more than 150 proteins and five small RNA molecules (the snRNAs U1, U2, U4, U5 und U6). Many of these spliceosomal proteins are not scattered around the cell nucleus in an unordered manner, but form precisely organized complexes. Thus, for example, approximately 50 of these proteins associate with the snRNAs to form RNA-protein particles. These so-called snRNPs (pronounced »snurps«) bind to the pre-mRNA as pre-fabricated complexes and are the main subunits of the spliceosome.

A molecular editing table

The spliceosome is assembled on site for each splicing event. To achieve this, snRNPs and other helper proteins are successively assembled on the pre-mRNA. Each of these five RNA-protein particles performs specific tasks. Thus, for example, the



beginning and end of an intron must be recognized and brought closer to each other in order to immediately splice out the intron and couple the two exons originally separated by the intron. The molecular »scissors«, which excises the intron, is successively activated during this process. In the course of splicing a brisk coming and going of snRNAs and proteins occurs; the timing of these molecular exchanges is exactly controlled. Presumably, this complex procedure ensures the exact excision of an intron and hence error-free assembly instructions for each protein.

Our objective is to record the dramatic structural dynamics of the spliceosome to generate a movie of the splicing process. Concomitantly, we would like to understand how the spliceosome's molecular scissors – its catalytic center – is assembled and would like to observe this pair of scissors during the excision of an intron. To achieve this, we have stopped the spliceosome at different operational steps, isolated it in these states and analyzed its components.

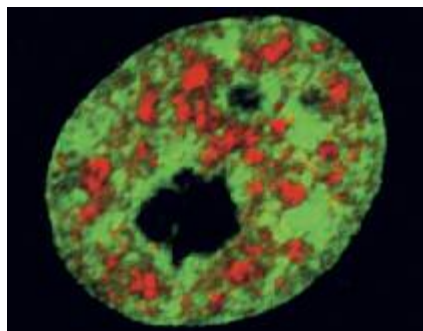
In addition, we are able to reassemble biologically active spliceosomes from isolated components. By selectively removing or modifying individual components, we can observe how these manipulations affect the spliceosome.

In order to understand this fascinating molecular machine's mode of operation in detail, we use an interdisciplinary approach. In addition to biochemical and

biophysical methods, we primarily use high-resolution electron microscopy and X-ray crystal structure analysis. They provide us with three-dimensional models of individual snRNPs and entire spliceosomes, as well as details of the participating macromolecules.

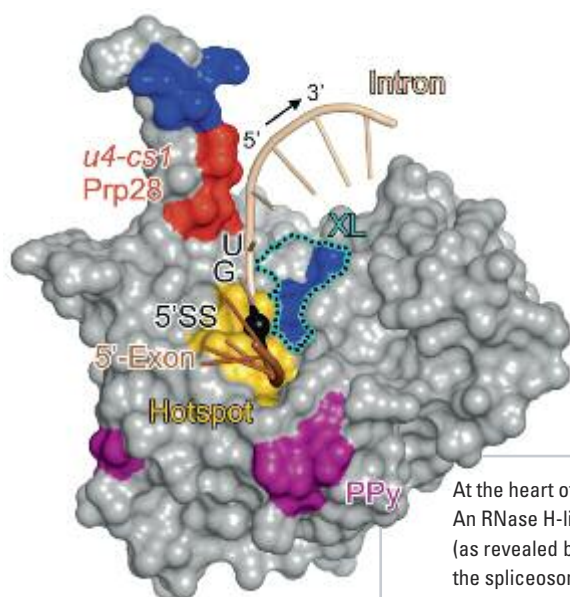
Errors with grave consequences

The molecular analysis of the spliceosome, which is the focus of our interdisciplinary approach, will not only pro-



Active spliceosomes (stained red) are only present in distinct regions of the cell nucleus.

vide information about the causes of molecular disorders that result from errors in the splicing of messenger RNA, but will also allow new therapeutic approaches for the treatment for such diseases. New estimates suggest that more than 20 percent of human genetic diseases are the result of mutations that impair the function of spliceosomes.



At the heart of the spliceosome: An RNase H-like domain in the Prp8 protein (as revealed by X-ray crystallography) suggests that the spliceosome is an RNP enzyme.



Prof. Dr. Reinhard Lührmann

received his PhD in chemistry at the University of Münster in 1975. He went from there to the Max Planck Institute for Molecular Genetics in Berlin where he headed an independent research group at the Otto Warburg Laboratory from 1981 to 1988. In 1982, he acquired his professorship qualifications (habilitation) in molecular biology and biochemistry at the Free University of Berlin. In 1988, he accepted a professorship for physiological chemistry and molecular biology with the School of Medicine at the University of Marburg. He has headed the Department of Cellular Biochemistry at the Max Planck Institute for Biophysical Chemistry since 1999. Reinhard Lührmann has been awarded numerous scientific prizes, among them the Max Planck Research Prize (1990), the Leibniz Prize (1996), the Feldberg Prize (2002), and the Ernst Jung Prize for Medicine (2003). He is an honorary professor at the Universities of Göttingen and Marburg.

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M. C. Wahl, C. L. Will, R. Lührmann: The spliceosome: design principles of a dynamic RNP machine. *Cell* 136, 701-718 (2009).

S. Bessonov, M. Anokhina, C. L. Will, H. Urlaub, R. Lührmann: Isolation of an active step 1 spliceosome and composition of its RNP core. *Nature* 452, 846-850 (2008).

H. Stark, R. Lührmann: Electron cryo-microscopy of spliceosomal components. *Ann. Rev. Biophys. Biomol. Structure* 35, 435-457 (2006).



Prof. Dr. Holger Stark studied biochemistry at the Free University Berlin and received his PhD at the Fritz Haber Institute in Berlin in 1997. Subsequently, he did research at Imperial College London (United Kingdom) and, from 1998 to 1999, was a group leader at the University of Marburg. In 2000, he relocated to the Max Planck Institute for Biophysical Chemistry as research group head. He has been a professor for molecular electron cryo-microscopy at the University of Göttingen since 2008. Holger Stark has received many awards for his research, among them the Otto Hahn Medal of the Max Planck Society (1997), the Advancement Award of the German Society for Electron Microscopy (1998) as well as the BioFuture Prize (2005).

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F. Herzog, I. Primorac, P. Dube, P. Lenart, B. Sander, K. Mechtler, H. Stark, J. M. Peters: Structure of the anaphase promoting complex/cyclosome interacting with a mitotic checkpoint complex. *Science* 323, 1477-1481 (2009).

A. Chari, M. M. Golas, M. Klingenhager, N. Neuenkirchen, B. Sander, C. Englbrecht, A. Sickmann, H. Stark, U. Fischer: An assembly chaperone collaborates with the SMN complex to generate spliceosomal SnRNPs. *Cell* 135, 497-509 (2008).

B. Kastner, N. Fischer, M. M. Golas, B. Sander, P. Dube, D. Boehringer, K. Hartmuth, J. Deckert, F. Hauer, E. Wolf, H. Uchtenhagen, H. Urlaub, F. Herzog, J. M. Peters, D. Poerschke, R. Luhrmann, H. Stark: GraFix: sample preparation for single-particle electron cryomicroscopy. *Nat. Methods* 5, 53-55 (2008).

B. Sander, M. M. Golas, E. M. Makarov, H. Brahm, B. Kastner, R. Luhrmann, H. Stark: Organization of core spliceosomal components U5 snRNA loop I and U4/U6 Di-snRNP within U4/U6.U5 Tri-snRNP as revealed by electron cryo-microscopy. *Mol. Cell* 24, 267 (2006).

3D Electron Cryo-Microscopy

The cell keeps its metabolism running with molecular machines. Frequently, the latter are very complex structures comprising a large number of different components. In order to observe these machines directly in action in the nanocosmos of the cell scientists must however expend a great deal of effort.

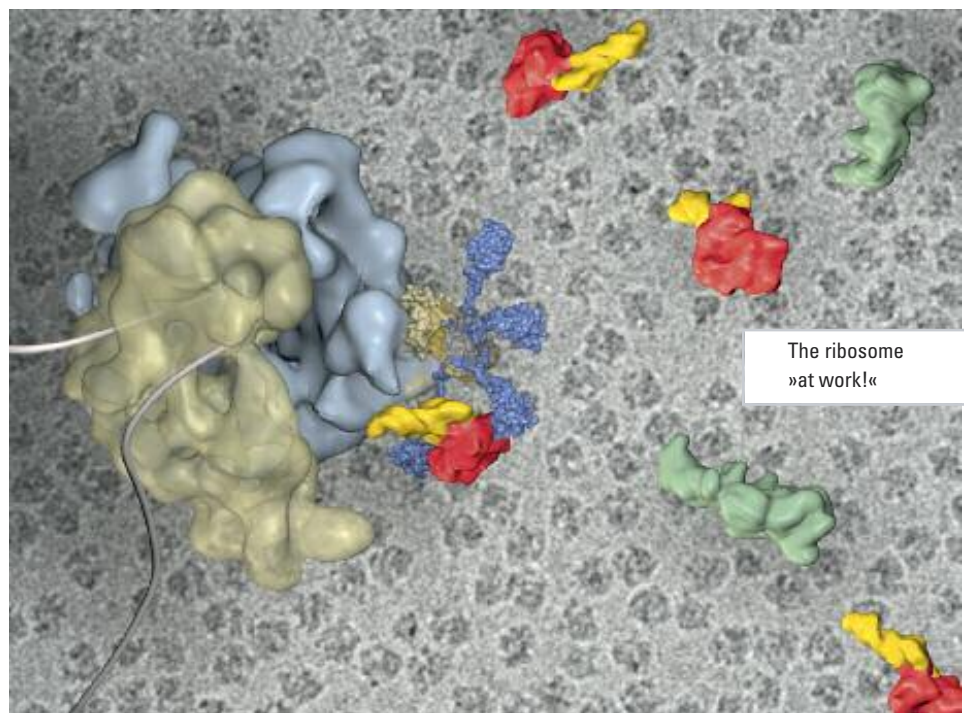
Snapshots in a state of shock

In our group we investigate macromolecules in a shock-frozen state with the aid of the electron cryo-microscope. That sounds perhaps paradoxical, but the molecular machinery can be stopped in very different operational steps by means of flash freezing. The electron microscope provides us with a complete series of images of a single macromolecule with these samples from different spatial perspectives and at different points in time. From these individual images we ultimately assemble the three-dimensional structure with the aid of special computer programs. They show us what the molecular machine looks like and how it changes during their functional cycle – and does so in 3D.

We apply this technique to a large number of different molecular machines, which

are located at important switching points of cellular information processing. These machines frequently comprise not only proteins but rather are complex associations of proteins and nucleic acids. For example, we investigate how the cell's protein factory – the ribosome – produces proteins by reading out the genetic information. At present we are also observing spliceosomes. They go into action after the blueprints for proteins have been copied into the draft version of a messenger RNA. Spliceosomes cut the unnecessary parts out of the messenger RNA and thus convert the blueprints into a legible form. In addition, we are studying a vital protein complex, which plays an important role during cell division: the so-called Anaphase Promoting Complex. It ensures that the genetic information is correctly distributed to the two daughter cells.

With the aid of electron cryo-microscopy we can observe the spatial structure and movements of such different molecular machines directly at work. In this manner, we learn to understand their functioning in detail.



Ribosome Dynamics

Label for membrane proteins

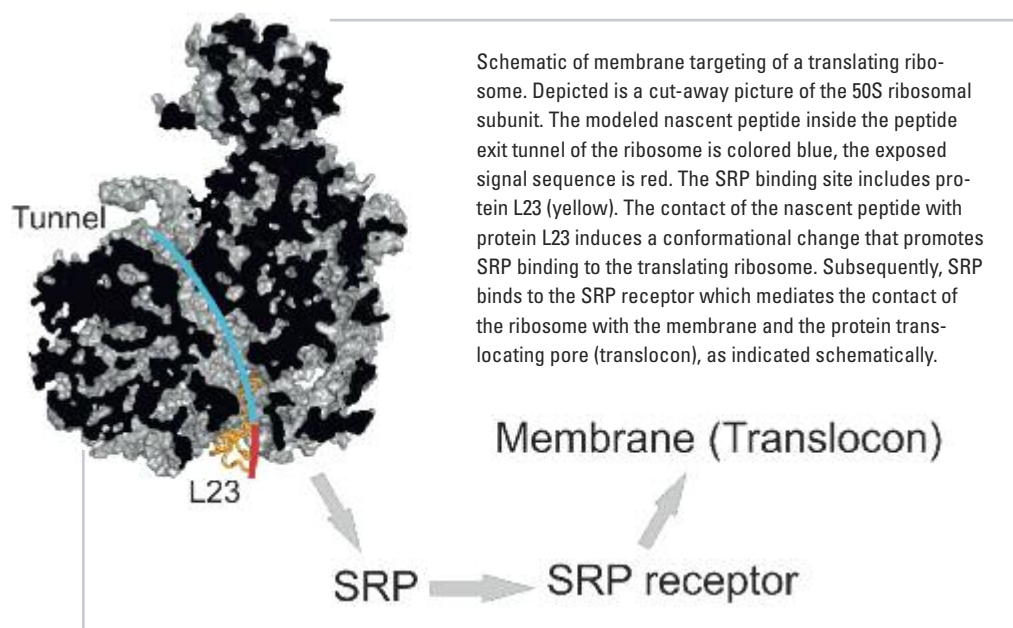
All living cells are enclosed in a membrane, which separates the cell's interior from its external environment. The membrane comprises a double layer of fat-like molecules, in which many proteins are embedded; the latter fulfill very specific functions. Some of them function as »receiving antennae« for the reception of signals. Others serve as channels, which only allow certain substances to pass into the cell. But how are such proteins inserted into the cell membrane?

For the most part their incorporation into the cell membrane occurs while the protein is being assembled on the ribosome. Ribosomes, which assemble membrane proteins, must therefore be directed to the

membrane. Interactions of the ribosome with the signal recognition particle and the helper proteins ultimately result in the developing protein being integrated in the membrane during the further course of synthesis. The molecular events occurring in this process are one focus of our research.

Ribosomes in motion

In a second project we are investigating, in cooperation with Marina Rodnina and Holger Stark, the molecular movements of molecules on the ribosome during protein synthesis. The energy required for this is stored in the energy-storage molecule GTP and is released when GTP is cleaved. In this context, the task of the elongation factor G is to transfer this energy selec-



cell membrane. But how is a membrane protein recognized and how are the ribosomes that are involved in the assembly recruited?

When a membrane protein is assembled on ribosomes, a specific label (»signal sequence«) is incorporated, mostly in the early part of the protein. This signal is recognized by a ribonucleic acid-protein complex – the signal recognition particle (SRP) – which directs the ribosome assembling the membrane protein to the cell

tively to the ribosome. We aim to find out how these movements on the ribosome are initiated: directly, by means of a mechanical coupling comparable to the shaft in a motor, or indirectly, via a mechanism which uses the spontaneous movement of the molecules – the Brownian motion. In order to reach this goal, we are combining physical biochemistry methods, including rapid kinetics and single-molecule approaches as well as electron cryo-microscopy.



Prof. Dr. Wolfgang Wintermeyer received his PhD in chemistry at the Ludwig Maximilians University of Munich. Subsequent to his habilitation in 1979, he conducted research on a Heisenberg Fellowship from the German Research Foundation at the University of Munich, at the Karolinska Institutet in Stockholm (Sweden) and at the Massachusetts Institute of Technology in Cambridge (Massachusetts, USA). From 1987 until he became an emeritus professor in 2009, he held the Chair for Molecular Biology at the Private University of Witten/Herdecke. From 1991 until 2007, he was also the Dean of the Faculty of Life Sciences there. Since 2009, Wolfgang Wintermeyer has been a Max Planck Fellow and head of the Ribosome Dynamics Research Group in the Department of Physical Chemistry at the Max Planck Institute for Biophysical Chemistry.

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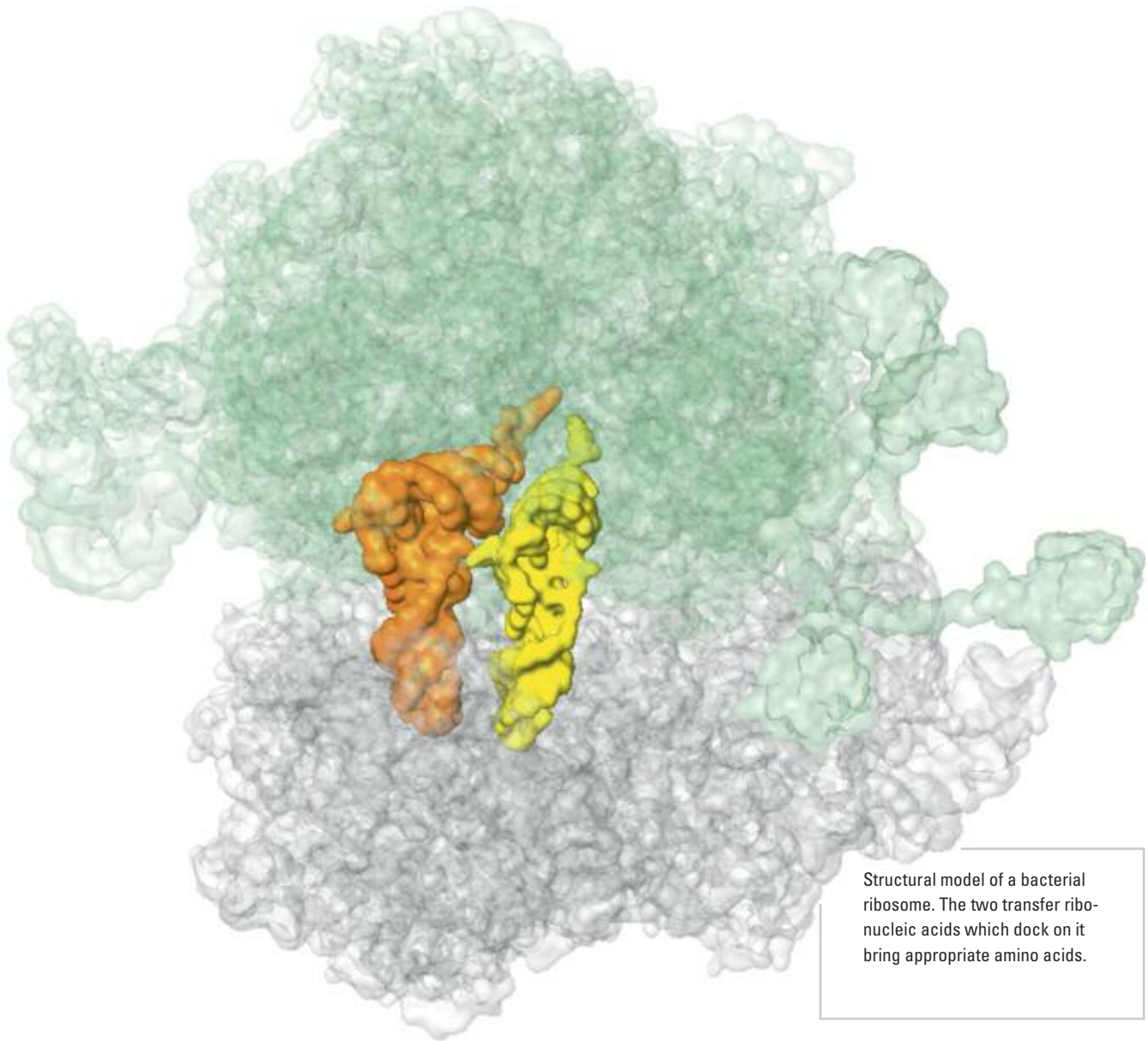
T. Bornemann, J. Jöckel, M. V. Rodnina, W. Wintermeyer: Signal sequence-independent membrane targeting of ribosomes containing short nascent peptides within the exit tunnel. *Nat. Struct. Mol. Biol.* 15, 494-499 (2008).

A. Savelsbergh, V. I. Katunin, D. Mohr, F. Peske, M. V. Rodnina, W. Wintermeyer: An elongation factor G-induced ribosome rearrangement precedes tRNA-mRNA translocation. *Mol. Cell* 11, 1517-1523 (2003).

Physical Biochemistry

In the cell nothing occurs without proteins. They maintain the cell's shape, provide for mobility, for transport and for communication, and finally they are involved in all constructive, degradative, reorganization processes. Proteins are produced according to blueprints, which are stored in the genetic material, deoxyribonucleic acid (DNA). Complex molecular machines (ribosomes) are responsible for putting together the individual building blocks of

the proteins – the amino acids – in the sequence dictated by the blueprint. Composed of more than fifty protein components and three to four ribonucleic acid molecules, ribosomes, with dimensions of 10 to 20 nanometers, are approximately as large as the smallest viruses. In order to observe these molecular complexes at work, we use different biophysical methods such as fluorescence spectroscopy and fast kinetic techniques.



Structural model of a bacterial ribosome. The two transfer ribonucleic acids which dock on it bring appropriate amino acids.

Precision work

Even when hundreds, sometimes thousands, of amino acids are linked to each other, every one of them is important. A single false building block can render the protein non-functional. In the worst case a defective protein can even cause damage. How ribosomes manage to keep the error frequency so astonishingly low is therefore of particular interest to us. It is already known that ribosomes do not normally react if they are confronted with an incorrect building block. Only when the correct building block docks on, does this trigger a structural change, which ultimately results in the linking of the amino acids. The ribosome brings two amino acids into such a position that they are readily connected. Thus, the ribosome is a catalyst which accelerates the linking of amino acids by ten million times. We are currently attempting to find out which molecular processes play a role in inducing the structural change and how the catalytic mechanism functions in detail.

Programmed »errors«

Normally, the production of a protein goes according to plan. A blueprint made of ribonucleic acid dictates exactly which of the twenty standard amino acids have to be linked in which sequence. Errors rarely occur, but they are occasion-

ally even necessary to obtain the desired protein. Only when the ribosome seemingly makes an error, can it indeed incorporate special amino acids, such as selenocysteine, which do not belong to the standard repertoire of the amino acids. But which mechanisms allow such exceptions to the rule? When we understand that, we hope to simultaneously better understand how errors are normally avoided. Conceivably, one day such knowledge may also be used in a medical context.

Continuously restructured

While the ribosome is progressively assembling a protein, it changes its three-dimensional structure. In this process, specific protein factors come into play. For example, the elongation factor G uses chemical energy to bring about a profound restructuring of the ribosome, similar to the manner in which motor proteins in muscle cells transform chemical energy into mechanical work. Together with Wolfgang Wintermeyer's research group we are studying this process with biochemical and biophysical methods. In co-operation with Holger Stark's research group we attempt to visualize these structural changes of the ribosome using electron cryo-microscopy.



Prof. Marina Rodnina studied biology in Kiev and received her PhD there in 1989. Subsequently, she went to the University of Witten/Herdecke on a research fellowship from the Alexander von Humboldt Foundation. She worked there as a research assistant from 1992 to 1997. After her habilitation in 1997, she was appointed university professor there and, from 2000 to 2009, she held the Chair of Physical Biochemistry. Marina Rodnina has headed the Department of Physical Biochemistry at the Max Planck Institute for Biophysical Chemistry as a Director since 2008. She has also been a member of the German Academy of Natural Scientists Leopoldina in Halle since that year.

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P. Milon, A. L. Konevega, C. O. Gualerzi, M. V. Rodnina: Kinetic checkpoint at a late step in translation initiation. *Mol. Cell* 30, 712-720 (2008).

A. L. Konevega, N. Fischer, Y. P. Semenkov, H. Stark, W. Wintermeyer, M. V. Rodnina: Spontaneous reverse movement of tRNA-mRNA through the ribosome. *Nat. Struct. Mol. Biol.* 14, 318-324 (2007).

M. Diaconu, U. Kothe, F. Schliinzen, N. Fischer, J. Harms, A. G. Tonevitski, H. Stark, M. V. Rodnina, M. C. Wahl: Structural basis for the function of the ribosomal L7/L12 stalk in factor binding and activation of GTP hydrolysis. *Cell* 121, 991-1004 (2005).

Talkative nerve cells



how signals are transmitted

To catch a ball, recognize danger in time, remember something or solve an arithmetical problem – our nervous system stores experiences from earliest childhood, controls complicated movements and creates our consciousness, all seemingly without effort. The human nervous system consists of approximately one hundred billion nerve cells, and each of these individual nerve cells can make contact with thousands of neighboring cells. Tiny membrane storage vesicles release special messenger substances that affect the behavior of adjacent cells. The researchers aim to shed light on the molecular processes that give nerve cells their ability to collect and process information – including such complex brain functions as learning and memory.

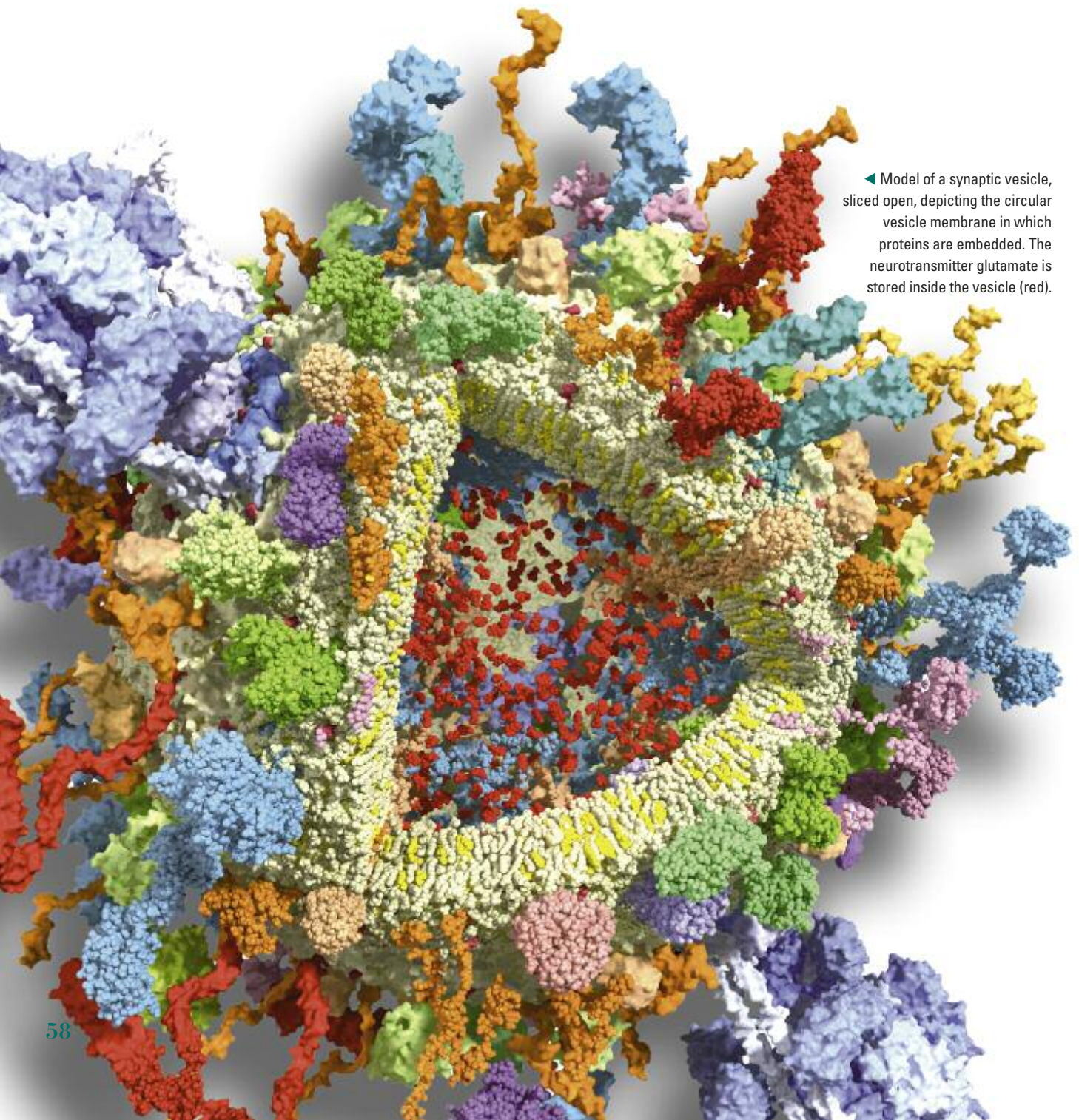
Neurobiology

Nerve cells (or neurons) are communication specialists. They receive and process signals and transmit them to recipient cells such as muscle cells or other neurons. Transmission is mediated by specialized signaling molecules, the neurotransmitters. Within nerve endings, neurotransmitters are stored in small membrane-enclosed containers termed synaptic vesicles. If electrical signals indicate that a message is to be sent, some of the synaptic vesicles fuse with the cell membrane and release their content, which in turn is recognized by the receiving cell.

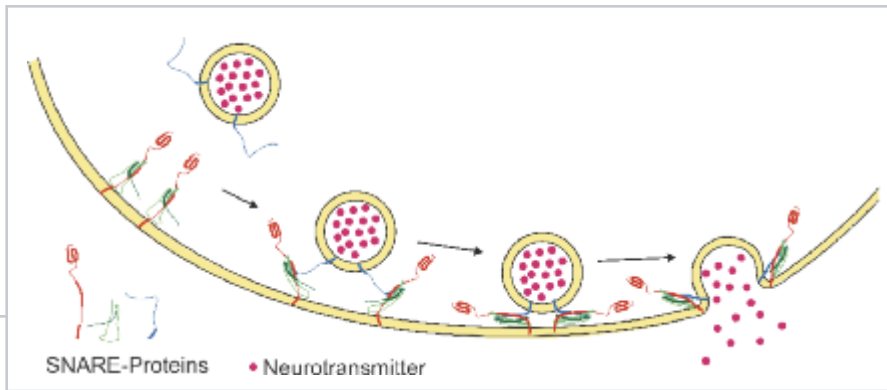
Sociable SNARES

The molecular makeup of synaptic vesicles has recently been unraveled. They contain a fascinating array of proteins that execute all the jobs that the vesicles must perform. Synaptic vesicles not only need to sequester and store neurotransmitters, but they also respond to signals and undergo fusion with the plasma membrane. Fusion is mediated by a group of specialized proteins termed SNAREs.

Together with other research groups we want to find out how exactly the SNAREs bring about



◀ Model of a synaptic vesicle, sliced open, depicting the circular vesicle membrane in which proteins are embedded. The neurotransmitter glutamate is stored inside the vesicle (red).



The fusion of membranes is mediated by SNARE proteins (blue, red, and green).



Prof. Dr. Reinhard Jahn studied biology and chemistry, and obtained his PhD at the University of Göttingen in 1981. Between 1983 and 1986, he worked at the Rockefeller University (USA), and thereafter at the Max Planck Institute for Psychiatry (today: Neurobiology) in Munich. In 1991, he became professor at Yale University in New Haven (Connecticut, USA). In 1997, he was appointed head of the Department of Neurobiology at the Max Planck Institute for Biophysical Chemistry. He is an adjunct professor of biology at the University of Göttingen, and he is the Dean of the Göttingen Graduate School for Neurosciences and Molecular Biosciences (GGNB). Reinhard Jahn has received numerous awards including the Max Planck Research Prize (1990), the Leibniz Prize of the German Research Foundation (2000), and the Ernst Jung Prize for Medicine (2006).

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membrane fusion. Indeed, we already have a very good idea of how they achieve this goal: If SNAREs that are attached to the vesicle and the plasma membrane meet, they become entangled and change their conformation. As result, they exert a pulling force that pushes the membranes against each other until they fuse. This process, which is controlled by many additional proteins, can be reconstituted in the test tube. In our research, we want to find out how these proteins work together to achieve the precision and speed of synaptic vesicle fusion.

SNAREs are not only needed for neurotransmitter release. They are also involved in the countless membrane fusion reactions that occur in every cell of our body. Biological membranes turn over continuously. They shed small vesicles that are then transported to other membranes where they fuse. We want to understand how these fusion reactions are controlled. After all, vesicles only fuse with their specific target membrane, and they only fuse if they are told to do so. Synaptic vesicles, for instance, only fuse if they need to transmit a signal. One of our objectives is to find out what these fusions have in common and how they differ from each other.

Hans Dieter Schmitt and colleagues also work on SNAREs. As model organism they use baker's yeast because it is

easy to introduce or delete individual genes in this unicellular organism. Their main interest is focused on intracellular transport vesicles. These vesicles have to be covered by a protein coat in order to form and to detach from the precursor membrane. Intriguingly, upon arrival at their destination, help by the SNAREs in the target membrane is needed to get rid of the coat. Only after the coat proteins are shed, can the SNAREs do their job and fuse the membranes.

Karin Kühnel studies a destructive process that also involves membranes. If cells are starving they begin to digest parts of themselves. To this end, they form membrane vesicles that randomly enclose parts of the cell's interior. The vesicle content is in turn delivered to the cell's »stomach«, the lysosome, where it is digested. This process, termed autophagocytosis, not only helps cells to survive periods of starvation but also participates in routine clean-ups. For instance, damaged or aggregated cell components are disposed of by this mechanism. The research group is primarily interested in determining the three-dimensional structures of the participating proteins.

Research group:

Dr. Karin Kühnel
 Dr. Hans Dieter Schmitt

A. Stein, A. Radhakrishnan, D. Riedel, D. Fasshauer, R. Jahn: Synaptotagmin activates membrane fusion through a Ca²⁺-dependent trans-interaction with phospholipids. *Nature Struct. Mol. Biol.* 14, 904-911 (2007).

D. Zwilling, A. Cypionka, W. Pohl, D. Fasshauer, P. J. Walla, M. C. Wahl, R. Jahn: Early endosomal SNAREs form a structurally conserved SNARE complex and fuse liposomes with multiple topologies. *EMBO J.* 26, 9-18 (2007).

S. Takamori, M. Holt, K. Stenius, E. A. Lemke, M. Grønborg, D. Riedel, H. Urlaub, S. Schenck, B. Brügger, P. Ringler, S. A. Müller, B. Rammner, F. Gräter, J. S. Hub, B. L. De Groot, G. Mieskes, Y. Moriyama, J. Klingauf, H. Grubmüller, J. Heuser, F. Wieland, R. Jahn: Molecular anatomy of a trafficking organelle. *Cell* 127, 831-846 (2006).



Structural Biochemistry

Dr. Dirk Fasshauer studied biology and received his doctorate at the University of Göttingen in 1994. He conducted research at Yale University, New Haven (Connecticut, USA) from 1995 to 1997 and moved from there to the Max Planck Institute for Biophysical Chemistry. He has headed the Structural Biochemistry Research Group in the Department of Neurobiology there since 2002.

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T. H. Klopper, C. N. Kienle, D. Fasshauer: SNAREing the basis of multicellularity: Consequences of protein family expansion during evolution. *Mol. Biol. Evol.* 25, 2055-2068 (2008).

P. Burkhardt, D. A. Hattendorf, W. Weis, D. Fasshauer: Munc18a controls SNARE assembly through its interaction with the syntaxin N-peptide. *EMBO J.* 27, 923-933 (2008).

A. Pobbati, A. Stein, D. Fasshauer: N- to C-terminal SNARE complex assembly promotes rapid membrane fusion. *Science* 313, 673-676 (2006).

The cells of our bodies – as also those of other animals, plants, fungi and many unicellular organisms – have a much more complex structure than the cells of bacteria. In our complex eukaryotic cells there are many separate reaction compartments in which different tasks are performed. The cell manufactures proteins in the so-called endoplasmatic reticulum, a particularly large branched area. It digests material foreign to the cell in the much smaller lysosomes. A characteristic set of molecules performs specific functions in each membrane-enclosed compartment.

In order for these tasks to be smoothly carried out, it is essential for cell survival that these molecules are available in sufficient quantities and at the correct time. They are therefore distributed to the different areas by a complex transport system.

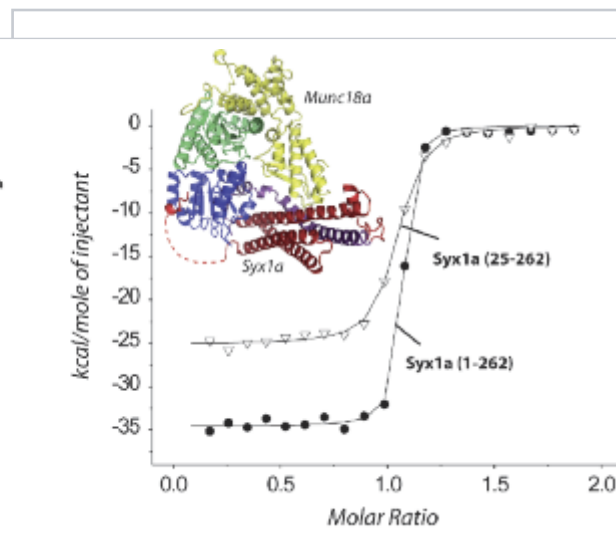
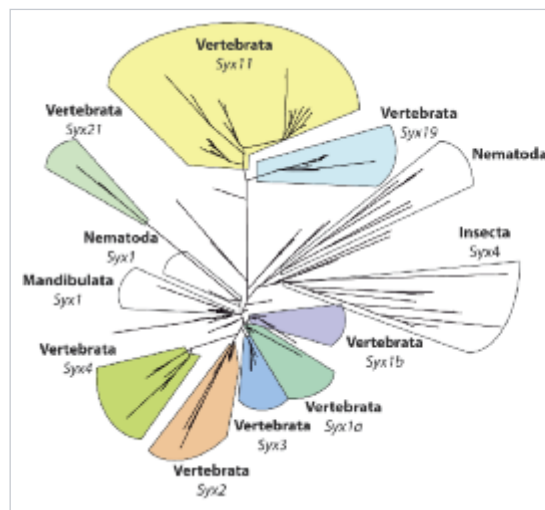
Transport in vesicles

In this context the molecules are transported in small, membrane-enclosed sacks, the vesicles, from compartment to compartment. When a cargo is ready, the vesicle buds off the membrane of one compartment, the donor organelle. Then the loaded vesicle migrates through the main cell

space, the cytosol, and ultimately merges with the membrane of the target compartment, in which the molecule is required.

When the transport vesicle fuses with the membrane, so-called SNARE proteins play the main role. This is a group of relatively small membrane-bound proteins. To date it has been assumed that a stable complex is formed between the membranes of the transport vesicle and the target compartment in a zipper-like process.

We are investigating exactly what happens when the SNARE proteins form this complex. We also want to know which other factors control and catalyze this process and how the stable complex is disassembled into its components when the process has been completed. Ultimately, we are interested in the evolutionary development of this transport system because it has been shown that the molecular machines involved in the basic process of the transport process can be found in all eukaryotic cells. This is an indication of their common descent, which we are studying. Therefore, we combine not only structural and biophysical approaches, but also incorporate the phylogenetic relationships between the protein machines.



Phylogenetic tree of the secretory syntaxin proteins of animals (left). Using isothermal titration calorimetry it is possible to determine the strong tendency of the two proteins Munc18a and syntaxin 1a to bind to each other. This complex forms during the release of the messenger substances from synaptic vesicles (right).

Biophysics of Synaptic Transmission

When a nerve cell transmits a signal to the next one, this signal is normally transformed twice. The »transmitting« cell translates its electrical signal into a chemical one, and the »receiving« cell, in turn, transforms the chemical signal into an electrical one. Why does this have to be so complicated?

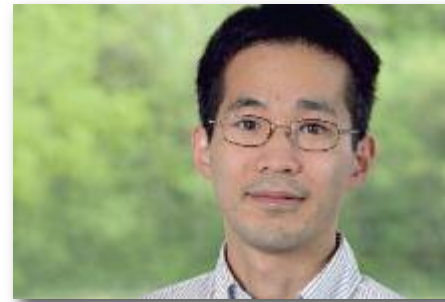
The chemical communication via neurotransmitters offers a decisive advantage. In contrast to electrical signals neurotransmitters can not only activate, but also inhibit the next nerve cell. Additionally, the necessary contact locations between the nerve cells – the synapses – are remarkably adaptable. Depending on the position, they can transmit electrical signals in attenuated form, but they can also amplify them. This synaptic dynamics is of extreme importance for information processing in the brain. We would like to know what the underlying molecular mechanisms are.

To this end, we compare various synapse types from different areas of the brain, for example, from the cerebellum, the cerebral cortex and the brainstem. We investigate

these synapses by combining electrophysiological methods with imaging procedures and molecular biological techniques. As we now know, the recycling of the synaptic vesicles filled with chemical messengers on the sides of the transmitting nerve cell contributes decisively to the diversity of synaptic dynamics among different synapse types.

Determining the rules of interconnectivity

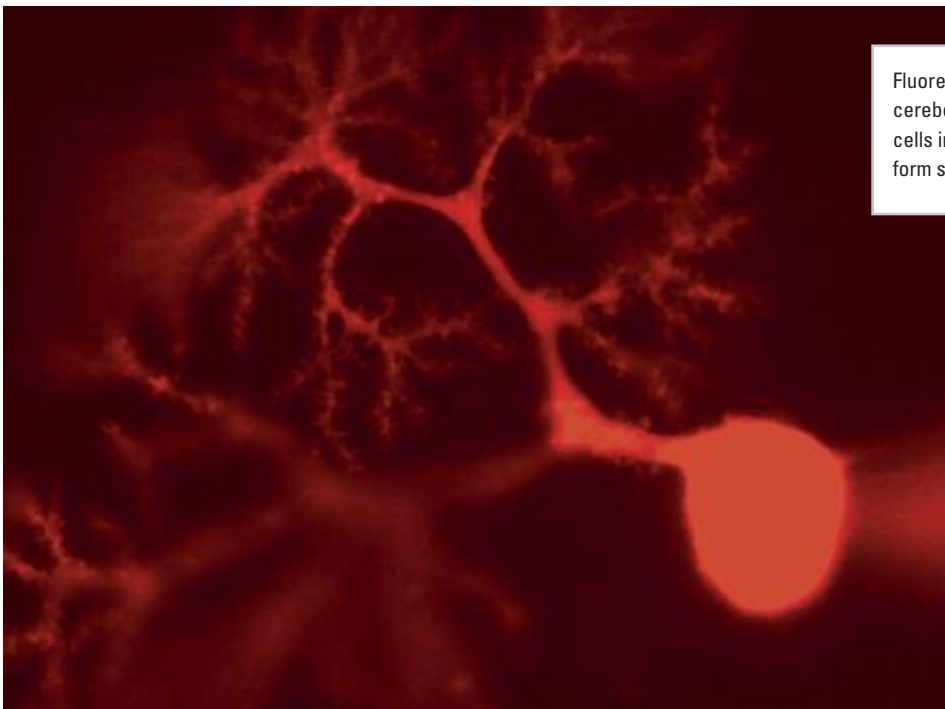
But, which role do the dynamic properties of the synapses play when the nerve cells congregate to form complex neuronal networks in our brain? It may be possible that nerve cells connect completely randomly with different synapse types. But these interconnectivities could also follow very specific rules. It may, for example, be the case that a group of neurons only connects each other to facilitating synapses. We are on the track of the »connectivity rules« which the nerve cells have to follow in the neuronal connectivity between the cerebral cortex and the brainstem.



Dr. Takeshi Sakaba received his PhD in psychology at the University of Tokyo (Japan) in 1998. From 1998 to 2006, he did research as a postdoctoral fellow under Erwin Neher in the Department of Membrane Biophysics at the Max Planck Institute for Biophysical Chemistry. Since 2006, he has headed the Max Planck Research Group Biophysics of Synaptic Transmission there.

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Fluorescence image of a Purkinje cell in the cerebellum; many nerve endings of other nerve cells impinge on its process (the dendrites) and form synapses there.

N. Hosoi, M. Holt, T. Sakaba: Calcium dependence of exo- and endocytotic coupling at a glutamatergic synapse. *Neuron* 63, 216-229 (2009).

T. Sakaba: Two Ca-dependent steps controlling synaptic vesicle fusion and replenishment at the cerebellar basket cell terminal. *Neuron* 57, 406-419 (2008).

K. Wädel, E. Neher, T. Sakaba: The coupling between synaptic vesicles and Ca^{2+} channels determines fast neurotransmitter release. *Neuron* 53, 563-575 (2007).

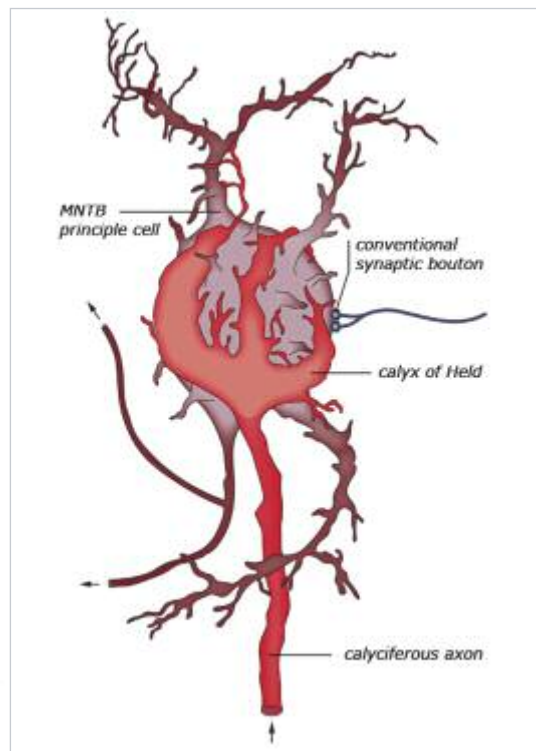
Membrane Biophysics

Many vital processes rely on the exchange of signals between sub-cellular structures, but also between our body's cells. In this context biological membranes occupy a key position because they not only compartmentalize tissues and organs, but also provide for the communication between the compartments. We use biophysical and molecular approaches to study the relevant signaling mechanisms. In this endeavor we increasingly rely on fluorescent molecules in order to label specific proteins, which participate in the signal flow.

Flexible circuits in the brain

Synapses are the contact points for information flow in our brain, where signals are exchanged between individual neurons. Contrary to the circuit elements in electronic computers where connections are hard-wired, synaptic strength – the electrical response of the receiving neuron to a nerve impulse in the transmitting one – is not constant, but is use-dependent. It varies depending on the information being processed. Each type of synapse has its own »personality« with regard to this so-called »synaptic plasticity«. Changes, which are caused by short, intensive bursts of activity may be only transient or else persist for hours and days. Neuroscientists consider the very long-term changes as the basis of learning and memory. Likewise, the short-term changes in synaptic strength have an important role in the second-by-second information processing, for instance during adaptation of sensory signals.

The research projects of the department concentrate on short-term plasticity and its molecular and physiological mechanisms. A nerve impulse causes the liberation of a signal substance from the nerve ending of the transmitting neuron. The immediate trigger for the release of this »neurotransmitter« is an increase in the presynaptic concentration of calcium ions. This causes the fusion of storage vesicles, which contain the neurotransmitter, with the cell membrane. Thereby neurotransmitter is released into a narrow gap between the transmitting and the receiving neuron. But calcium ions can do even more. They promote the delivery of new vesicles, which hold neurotransmitter ready for release. Synapse strength then depends on how many synaptic vesicles the transmitting cell uses per nerve impulse and how rapidly they can be replenished. In addition to calcium ions, other signal substances, for example cyclic AMP, are involved in the regulation of replenishment. Short-term synaptic plasticity is thus the result of numerous processes, which intertwine.



The calyx of Held is an exceptionally large nerve ending, which makes contact with a compact postsynaptic nerve cell body. It is an important connection in the auditory pathway and is particularly well suited for biophysical investigations because it is accessible for electrophysiological measurements (patch-clamp) due to its size.

How is it possible that one and the same signaling substance – calcium ions – can control several processes in different ways? The answer resides in quantitative detail, as we could show by biophysical investigations: The triggering of neurotransmitter release by calcium is a highly cooperative process, which sets in only at rather high calcium concentrations, but then accelerates greatly. The re-supply of vesicles accelerates linearly with increased calcium, reaching adequate rates already below the threshold, at which release commences. Depending on the calcium level, either one or the other one of these processes will be preferentially activated.

Allowing contacts to mature

The calyx of Held synapse, an important synaptic junction in the mammalian auditory pathway, has been at the focus of our research for a long time. Its cup-shaped synaptic terminal is so generously dimensioned that it can be easily manipulated. Holger Taschenberger was able to show that, as is the case

with the majority of other synapses in our brain, the calyx undergoes a functional maturation process. More mature synapses use their supply of synaptic vesicles more sparingly. In comparison with a younger synapse, a single nerve impulse releases a much smaller fraction of the available vesicles. As a result, the synaptic strength decreases much less rapidly during repeated stimulation. In addition, mature synapses optimize their use of calcium ions. They require less calcium influx for triggering vesicle fusion because the calcium channels there are located closer to those vesicles, which are ready to release their contents. On the other hand, these synapses produce proteins, which bind calcium ions such that their concentration is rapidly reduced to a basal level.

Viral gene expression of synaptic proteins

The calyx of Held is ideally suited for biophysical studies on synaptic transmission in brain slices. However, it was not previously possible to selectively change the relevant proteins in this preparation. Therefore, Samuel Young developed custom-tailored high expression recombinant adenoviruses and a method to deliver these recombinant viruses into the nerve cells. The synaptic calcium sensor synaptotagmin and a variant carrying site-specific mutations were cloned into the viral ge-

nome. After infection of the nerve cells with such viruses, it was possible to observe how the modified calcium sensor affected the synaptic function.

Colorful proteins

How do the different signal processes at the nerve ending interact? In order to make this machinery visible, it is necessary to simultaneously display the distribution and temporal changes of as many signal carriers as possible. Today, molecular biology provides excellent tools for selectively labeling proteins with multi-color fluorescent tags. When analyzing spectrally resolved images of multiply labeled preparations, one has to separate the individual contributions of labels, which are present at a given pixel. To achieve this, we developed an algorithm of so-called »blind source separation«, which allows determination of both the spectral characteristics of the dyes involved and their individual contributions to the overall image.

Internal Research Groups:

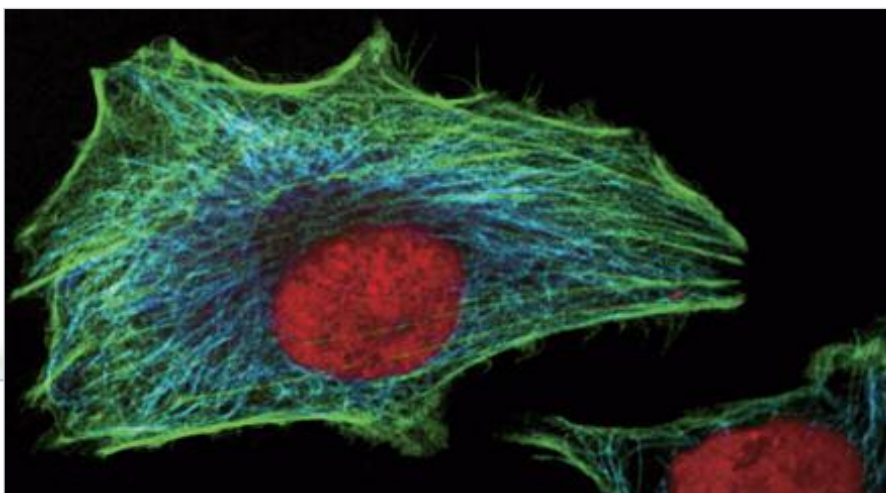
Dr. Meike Pedersen
Dr. Holger Taschenberger
Dr. Samuel Young



Prof. Dr. Erwin Neher studied physics in Munich and at the University of Wisconsin (USA). He redirected his interests to biophysics and performed a PhD project at the Max Planck Institute for Psychiatry (now: Max Planck Institute for Neurobiology) in Munich. He joined the Max Planck Institute for Biophysical Chemistry in 1972, worked for a year at Yale University in New Haven (Connecticut, USA) and has headed the Department of Membrane Biophysics at the institute since 1983. Erwin Neher is a honorary professor at the University of Göttingen. For his research on ionic currents flowing through individual membrane pores he received the Nobel Prize for Medicine and Physiology jointly with Bert Sakmann in 1991.

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Triply labeled fibroblast cells. The proteins of the cytoskeleton and the nucleic acids of these cells are marked with fluorescent dyes (actin: green; tubulin: blue; nucleic acids: red). The spectral analysis of their fluorescence allows separation of the three components on the basis of a single image acquisition (in cooperation within the DFG Research Center Molecular Physiology of the Brain).

R. A. Neher, M. Mitkovski, F. Kirchhoff, E. Neher, F. J. Theis, A. Zeug: Blind source separation techniques for the decomposition of multiply labeled fluorescence images. *Biophys. J.* 96, 1-10 (2009).

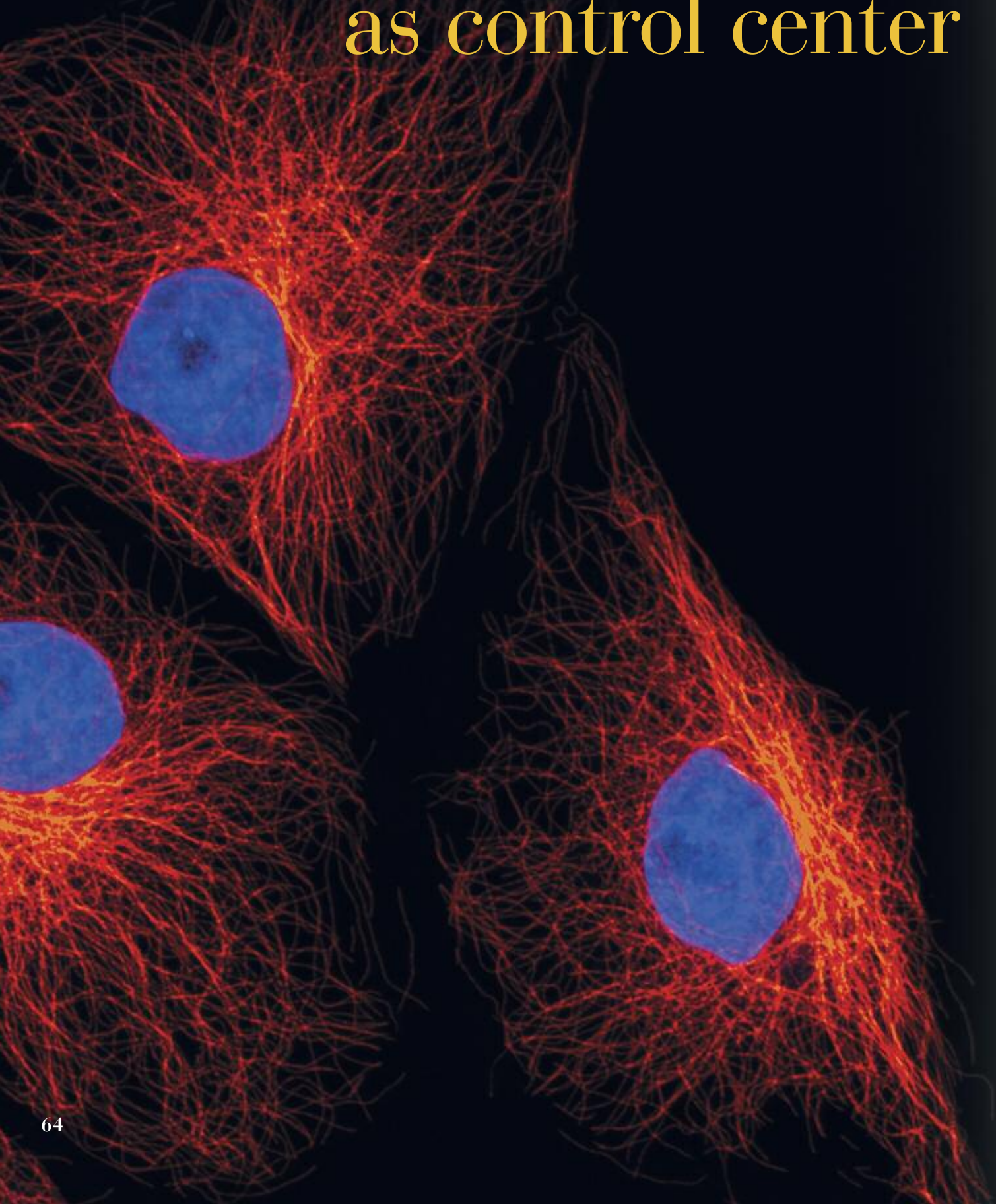
E. Neher, T. Sakaba: Multiple roles of calcium ions in the regulation of neurotransmitter release. *Neuron* 59, 861-872 (2008).

J. Włodarczyk, A. Woehler, F. Kobe, E. Pomimaskin, A. Zeug, E. Neher: Analysis of FRET-signals in the presence of free donors and acceptors. *Biophys. J.* 94, 986-1000 (2008).

E. Neher: A comparison between exocytic control mechanisms in adrenal chromaffin cells and a glutamatergic synapse. *Pflügers Arch.* 453, 261-268 (2006).

T. Sakaba, A. Stein, R. Jahn, E. Neher: Distinct kinetic changes in neurotransmitter release after SNARE-protein cleavage. *Science* 309, 491-494 (2005).

The cell nucleus as control center





active information storage with input and output control

Communication must work: this also applies to living cells. In order for the cells to fulfill their duties, they require communication paths between their individual compartments – from packaging and sorting stations via the power plants to the cell nucleus. Minute pores in the nuclear envelope serve as vital transport and communication channels that control the delivery of molecular cargos between the cell nucleus and other cellular compartments. These nuclear pores are highly selective gates: While small molecules usually pass unhindered, larger ones rely on a cellular shuttle service for their transport. Scientists at the institute are attempting to reveal the underlying highly complex processes on the molecular level.

Cellular Logistics



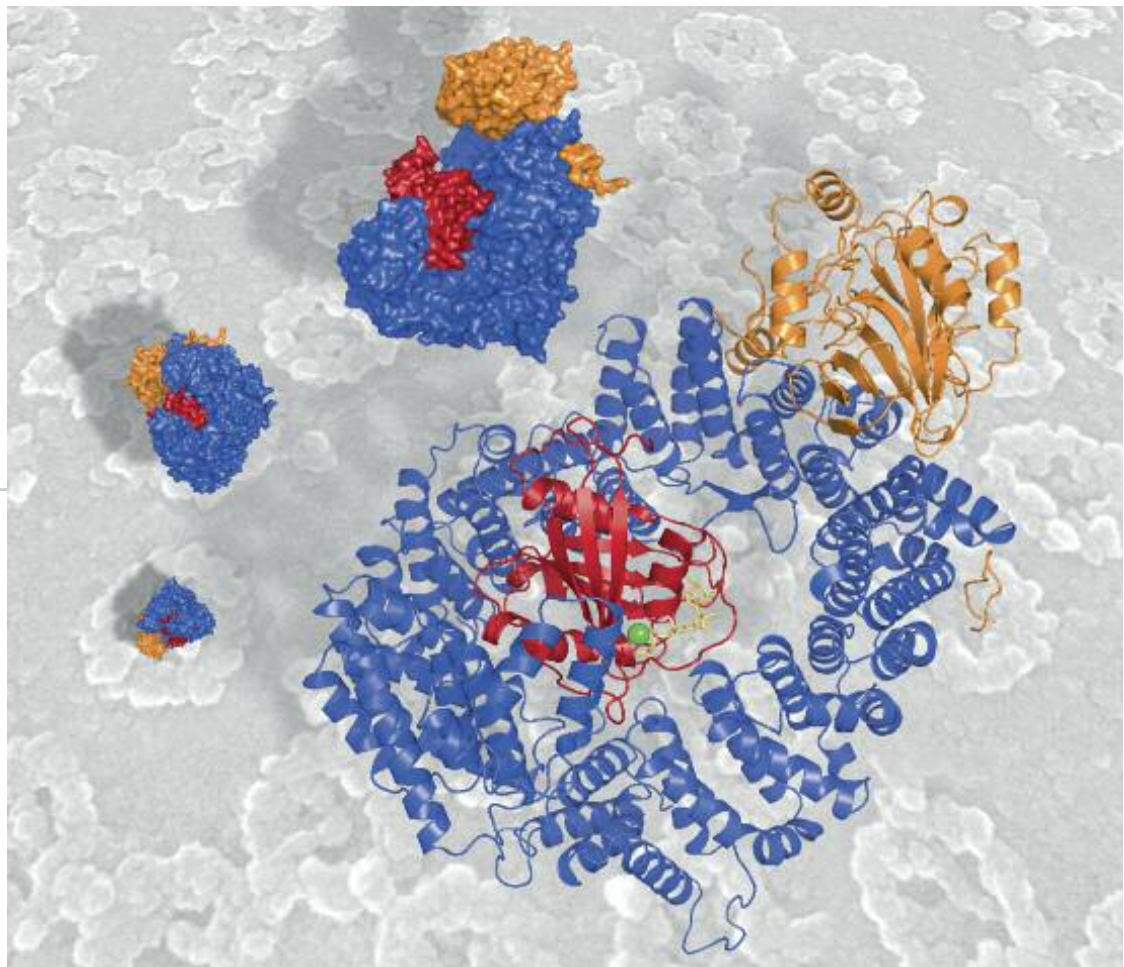
Recipe for success – division of labor

Eukaryotic life forms, such as plants or animals, are characterized by a division of labor in their cells. The cell nucleus focuses on the administration of the genome, mitochondria on supplying energy to the cell, whereas the so-called cytosol has specialized in protein synthesis. The advantages of this organization can be impressively summarized by the fact that only eukaryotes have evolved to complex, multicellular organisms. But this also has its price and must be maintained by a sophisticated logistic system. Cell nuclei lack protein synthesis and must therefore import all required enzymes and structural proteins from the cytosol. In return, nuclei produce and export decisive components of the protein synthesis machinery (for example ribosomes) to the cytosol and thus enable cytosolic protein synthesis.

Gates and transporters

The cell nucleus is enclosed in two membranes that are impermeable for proteins and other macromolecules. An exchange of material can therefore not occur directly through these membranes. Instead, so-called nuclear pore complexes are embedded in the nuclear envelope. One can imagine them as highly selective gates, which make up the stationary part of an entire transport machinery system.

The mobile part of this transport machinery is comprised of nuclear import receptors (importins) and exportins. Although the nuclear pores appear tightly closed to the majority of macromolecules above a certain size limit, importins and exportins have the privilege of being able to pass nearly unimpeded through the permeability barrier of nuclear pores. In this context, the decisive point is

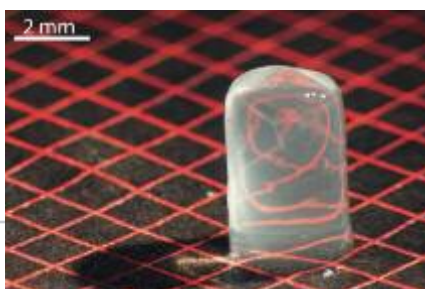


An export complex at atomic resolution. The exportin CRM1 (blue) with its cargo molecule snurportin (orange) and Ran (red) bound. Ran is a molecular switch, which determines the direction into which the cargo is carried (in this case from the cell nucleus to the cytosol). CRM1 exports, for example, the ribosomes mentioned above as well as hundreds of regulatory factors out of the cell nucleus. Viruses such as HIV misuse CRM1 to export their genetic material from the nucleus to the cytoplasm, where it is packed in viral particles. In the background, a scanning electron microscopic image of nuclear pore complexes – the giant transport channels in the nuclear envelope – is shown.

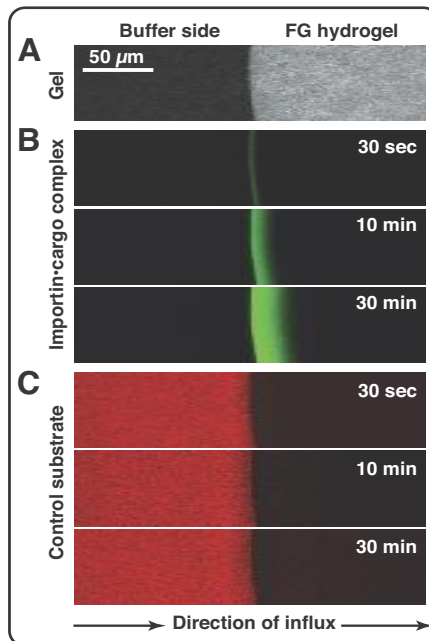
that they can also carry cargo or passengers on their passage through the pores. Not every passenger is allowed on board; instead, importins and exportins recognize with molecular precision which molecule is to be imported into the nucleus and which is to be exported. The mechanisms of these recognition processes are the focus of our research.

How does the sorting unit of nuclear pores work?

Nuclear pores are extremely effective sorting machines and each of them can transport up to 1000 cargo complexes per second. Nuclear pores have an extremely complex structure, and each of them comprises about 700 protein molecules or approximately 20 million individual atoms. In order to comprehend the functional principle of such a complex system, it has to be reduced to essentials. As a decisive step in this direction, we were recently able to reconstitute the nuclear pore's permeability barrier in a test tube. It consists of so-called »FG repeats« and forms an »intelligent« hydrogel with amazing material properties. It suppresses the passage of »normal« macromolecules, but allows an up to 20,000 times faster influx of



The permeability barrier of the nuclear pore is a hydrogel, i.e. an elastic solid, which consists primarily of water, similar to gummy bears or the vitreous body of the eye. The translucent red pattern of lines on the background provides an impression of the transparency of the object. Since the hydrogel comprises FG repeats, it is termed FG hydrogel. The *in vitro* reconstituted FG hydrogel shown here is several millimeters in size. In contrast, the barriers of the nuclear pore measure only about 50 nanometers.



Permeability properties of an FG hydrogel. A) An optical section through a fluorescence-labeled FG hydrogel. Light-colored areas correspond to the gel; dark ones to the surrounding buffer. B) The same region, imaged in another fluorescence channel, shows the influx of a green fluorescent importin-cargo complex at three different points in time. The complex penetrates rapidly into the gel, accumulates there 100 to 1000-fold and moves so rapidly into the gel that it could traverse a nuclear pore within 10 milliseconds. C) A red fluorescent control substrate in comparison. It does not bind the importin and therefore cannot penetrate into the gel.

the same molecules when they are bound to an appropriate importin or exportin. The efficiency of the influx of importins and exportins into this gel reaches the limits of what is physically possible and is only limited by the speed of transport to the barrier. We are currently intensively investigating the chemical and physical bases of this unique phenomenon. We not only expect to gain a deep understanding of a process that is essential for eukaryotic life, but also impulses for the development of new materials.



Prof. Dr. Dirk Görlich studied biochemistry in Halle (Saale) and received his PhD at the Humboldt University in Berlin in 1993. After a two-year research period at the Wellcome/CRC Institute in Cambridge (United Kingdom), he was first appointed head of a research group in 1996 and then, in 2001, professor for molecular biology at the Center for Molecular Biology of the University of Heidelberg (ZMBH). Since 2007, he has been heading the Department of Cellular Logistics at the Max Planck Institute for Biophysical Chemistry. Dirk Görlich has received numerous scientific awards, among them the Karl Lohmann Prize of the German Society for Biological Chemistry (1993), the Heinz Maier Leibnitz Prize (1997), the EMBO Gold Medal (1997), and the Alfried Krupp Promotion Prize (2001).

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T. Monecke, T. Güttler, P. Neumann, A. Dickmanns, D. Görlich, R. Ficner: Crystal structure of the nuclear export receptor CRM1 in complex with snurportin1 and RanGTP. *Science* 324, 1087-1091 (2009).
S. Frey, D. Görlich: A saturated FG-repeat hydrogel can reproduce the permeability properties of nuclear pore complexes. *Cell* 130, 512-523 (2007).
S. Frey, R. P. Richter, D. Görlich: FG-rich repeats of nuclear pore proteins form a three-dimensional meshwork with hydrogel-like properties. *Science* 314, 815-817 (2006).



Dr. Volker Cordes received his PhD in cell biology at the University of Heidelberg in 1992. From 1992 to 1997, he worked as a research associate at the Bio-center of the University of Würzburg and at the German Cancer Research Center in Heidelberg. In 1997, he relocated to the Institute for Cell and Molecular Biology at the Karolinska Institutet in Stockholm (Sweden). There he was initially head of a junior research group, and after his habilitation, he became a university lecturer. In 2004, he returned to Germany as head of a research group at the University of Heidelberg. In 2007, he moved to the Max Planck Institute for Biophysical Chemistry, where he has headed the Nuclear Architecture Research Group ever since.

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Nuclear Architecture

Higher vertebrates, such as the human being, are comprised of more than 200 different cell types. With only a few exceptions, these cell types all have a nucleus, which is the cell's control center and which contains the hereditary information stored in the chromosomes. This genetic material is separated from the rest of the cell by a membrane jacket, the nuclear envelope. To still allow extremely diverse molecules to be exchanged between the nucleus and the cytoplasm, the nuclear envelope is traversed by many nuclear pores, through which the entire cargo transport takes place.

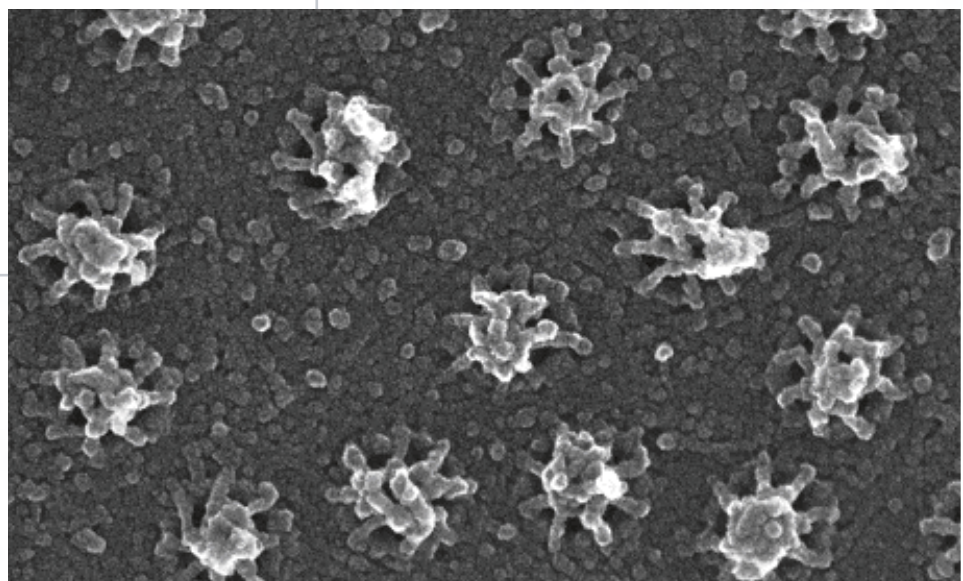
Even though the cell nucleus is normally depicted as a sphere, it can assume very different shapes depending on the cell type. In many cell types it is actually spherical or ellipsoid, whereas in others it can be tubular, lobed or even segmented. The architecture in the interior of such nuclei sometimes also appears just as different. Why this is the case and which molecules play a role in such nuclear organization is still largely unclear. Nor is much known as to whether the nuclear architecture influences the function of a cell type.

Nuclear basket made of thread-like proteins

To be able to answer these questions, we are attempting to identify the proteins that are involved in the construction and infrastructure of the cell nucleus. The starting point of our investigations are thread-like building blocks, which are anchored to the inner side of the nuclear pores and form structures which are reminiscent of round baskets. We have discovered that a major component of these nuclear baskets is a large rod-shaped protein, which we have been able to detect at the nuclear periphery in many cell types. There it also functions as a docking site for other nuclear components, which can differ depending on the cell type.

At least in some of these cell types, this rod-shaped protein plays an important role in the distribution of the genetic material in the proximity of the nuclear envelope. There it contributes substantially to the internal architecture of the cell nucleus. However, the nuclear basket and this protein probably also have other functions, which we desire to elucidate.

Scanning electron microscopic image of the inner side of a cleaned oocyte nuclear envelope from the clawed frog *Xenopus laevis*. Thread-like structures, similar to round baskets, are located directly over the nuclear pores. The diameter of such nuclear baskets is approximately 120 nanometers (millionth of a millimeter).



Chromatin Biochemistry

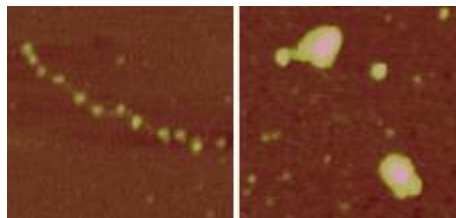
Everyone is familiar with the structural image of DNA: The carrier of hereditary information looks like a rope ladder that has been twisted to form a double helix. In living cells it is stored in the cell nucleus, the »command center« of the cell. However, the DNA does not occur there as isolated molecules. In conjunction with specific proteins it forms chromatin. At regular intervals short segments of the DNA are wound around a complex of histone proteins, forming the nucleosome. These constitute the fundamental repetitive unit of chromatin. While all nucleosomes are essentially assembled in the same manner, there are fine molecular differences which are primarily due to chemical alterations in the histone proteins. These alterations are termed post-translational histone modifications. Today we assume that the cell uses these alterations in nucleosomes as signals or markers in order to recognize and define different regions of chromatin. Apparently they play an important role in cellular hereditary.

Not only the product of our genes

Hereditary differences between individual cells and entire organisms are not only the result of different gene sequences, but are also due to the same hereditary information being read differently. Deviating characters

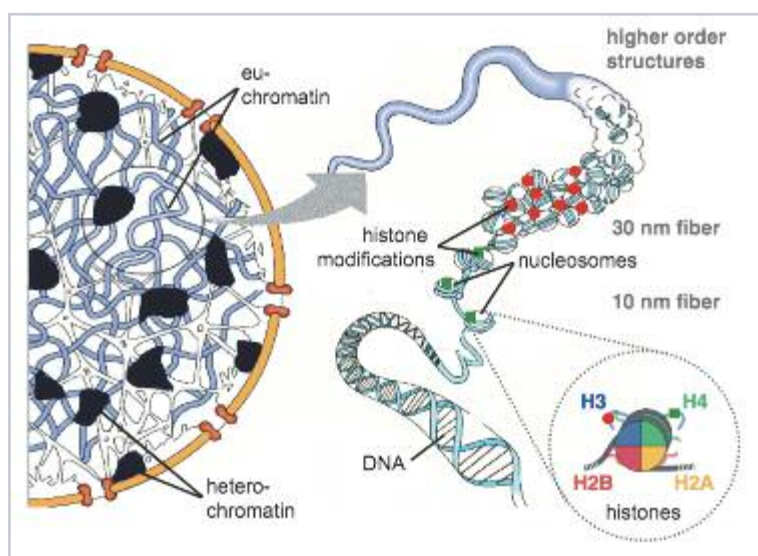
arise, for example, because the same gene is more active in certain cells of an organism than in others. At the chromatin level the cell executes this control over the differential reading of genes via stable patterns of histone modifications. These so-called epigenetic effects play, for example, a role in cell differentiation, in the development of the embryo, but also when cells undergo cancerous changes.

We want to find out in detail how these histone modifications influence the organization and dynamics of chromatin and to



Chromatin segments are present in different states, which are controlled by post-translational histone modifications. The images were taken with an atomic force microscope.

understand how they control the read-out of the genome. In order to achieve this we combine experimental approaches from different disciplines such as biochemistry, biophysics, cell biology, and molecular biology.



Organization of chromatin.



Dr. Wolfgang Fischle studied biochemistry at the University of Tübingen. Thereafter he went to the University of California at San Francisco (USA) as a scholar of the Boehringer Ingelheim Foundation and received his PhD in the field of biochemistry in 2002. As a postdoctoral fellow on a fellowship of the Damon Runyon Cancer Research Foundation he worked at the University of Virginia at Charlottesville and at the Rockefeller University in New York (USA) from 2002 to 2005. He heads the Max Planck Research Group Chromatin Biochemistry at the institute since 2006. Wolfgang Fischle received a fellowship of the European Network of Excellence »Epigenome« in 2006.

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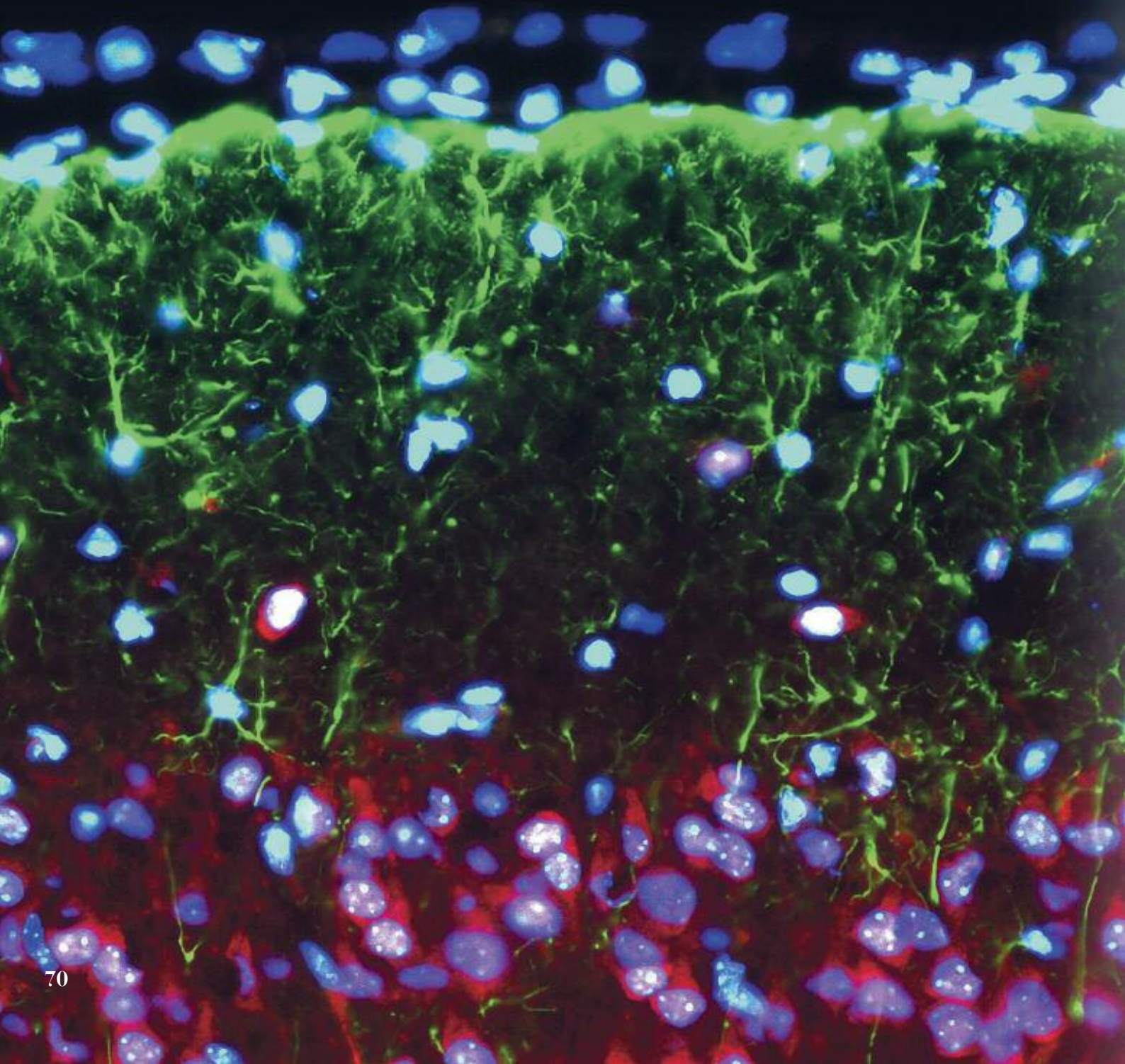
K. Zhang, K. Mosch, W. Fischle, S. I. Grewal: Roles of the Ctr4 methyltransferase complex in nucleation, spreading and maintenance of heterochromatin. *Nat. Struct. Mol. Biol.* 15, 381-388 (2008).

W. Fischle, H. Franz, S. A. Jacobs, C. D. Allis, S. Khorasanizadeh: Specificity of the CDY family of chromodomains for lysine-methylated ARKS/T motifs. *J. Biol. Chem.* 283, 19626-19635 (2008).

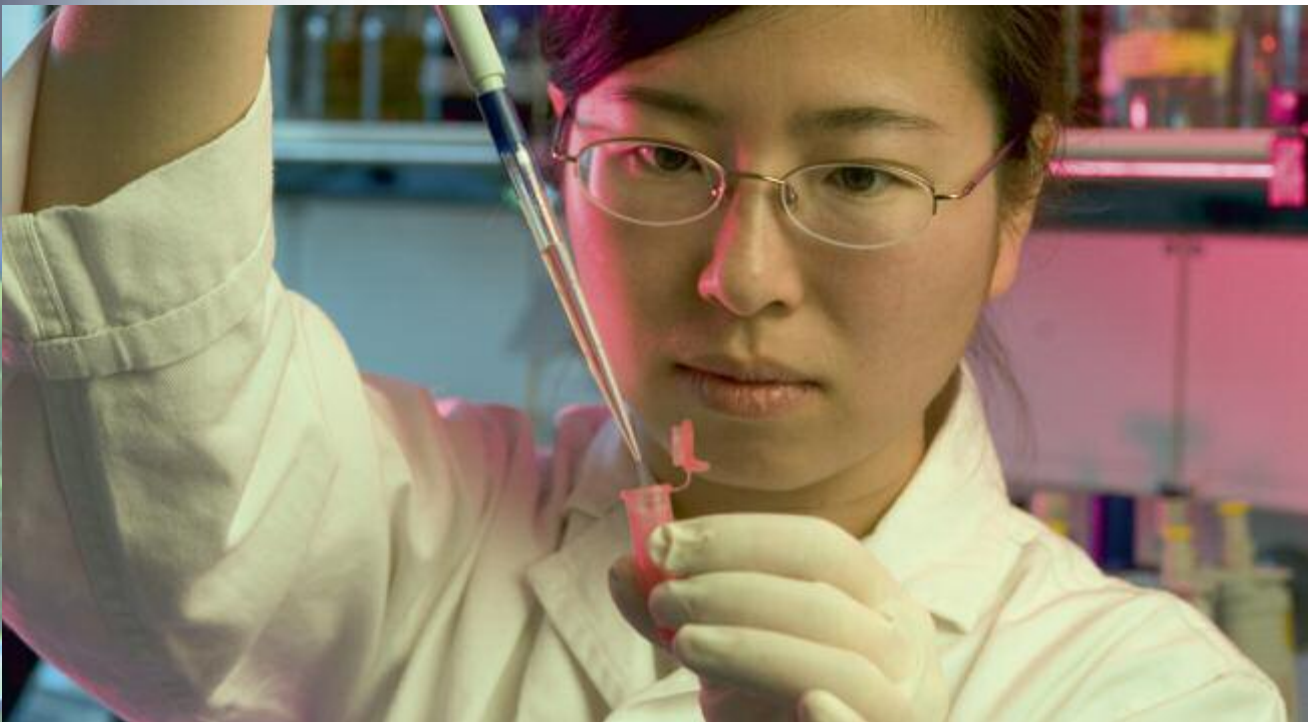
W. Fischle: Talk is cheap – Crosstalk in the establishment, maintenance, and readout of chromatin marks. *Genes Dev.* 22, 3375-3382 (2008).

From egg to organism

how living beings are formed and controlled



Heart and brain, liver and lung— all parts of our body can be traced back to a single egg cell. But exactly how does the egg cell evolve into so many different cell types? And how do the cells in the embryo form complex organs that interact successfully? The researchers unravel these enigmatic processes on the molecular level, in flies and also in mice. Despite their obvious anatomical differences their embryonic development follows fairly similar rules. Thus, the results obtained from this research permit us to gain insights into

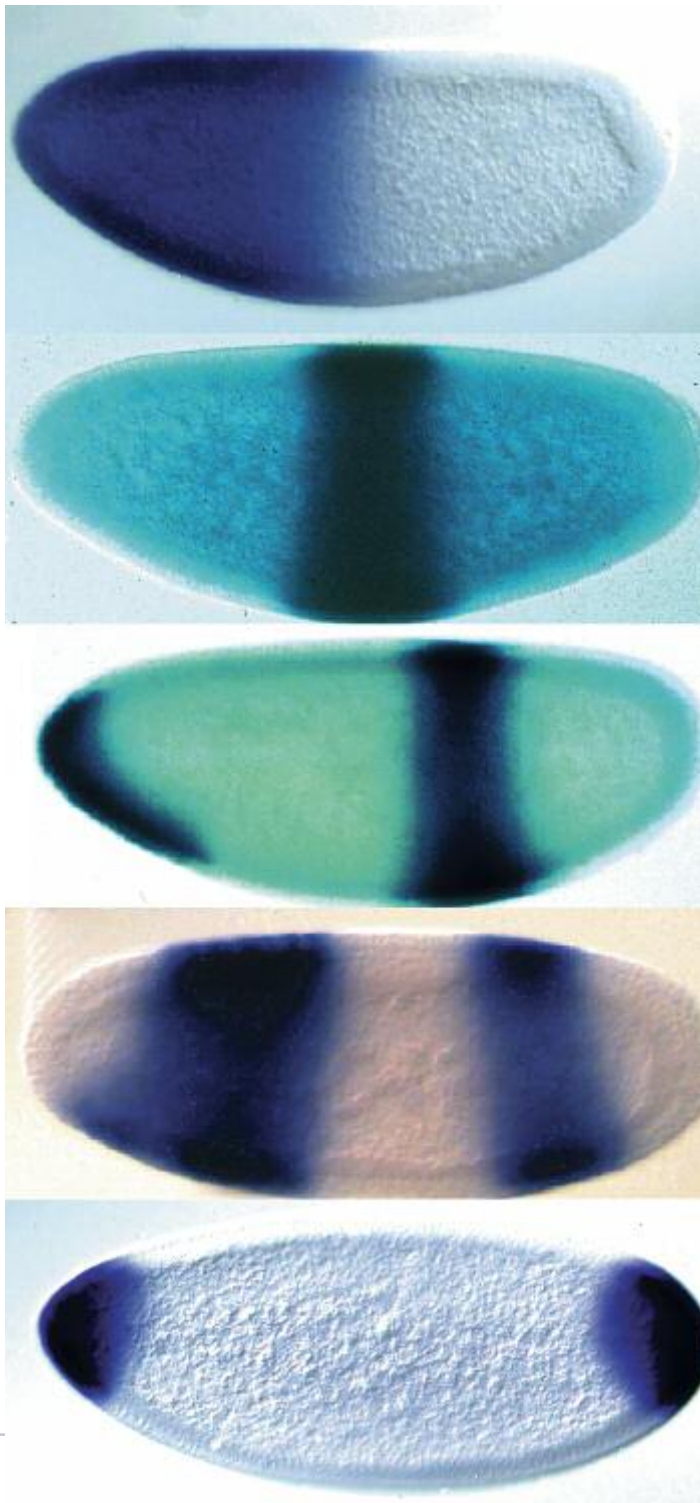


the earliest stage of human life. These findings help us to better understand and treat diseases that can be traced back to malformations at this early stage of life.

A large portion of our life is spent asleep – but why is this? How is our »biological clock« controlled, causing us to become tired in the evening and awake again in the morning? Scientists at the institute are also investigating these fascinating questions.

A »live broadcast« of a beating heart or »images of thinking« – last but not least, the researchers continuously improve imaging methods like magnetic resonance tomography in order to provide detailed insights into the inner life of human beings and animals.

Molecular Developmental Biology



All five fruit fly embryos are at the same developmental stage. However, by using a special staining technique the products of different genes have been made visible. They show that a fruit fly embryo, which is only two hours old, is already divided into specific areas (blue) and – although not yet morphologically visible – already exhibits segmental units.

The fruit fly *Drosophila* is a very popular scientific research object for good reasons. Undemanding and immensely prolific, despite its diminutive size, it is a very complex organism – absolutely comparable to a mammal. As is the case in all animals this fly develops from a single, small egg cell. But how does a complex body with extremely diverse cell types and organs develop from this one cell? To answer this great biological riddle, we delve deeply into the molecular control mechanisms which regulate such developmental processes from egg to fly. Frequently, the control factors which we found in the fly are present in similar form in the human genome. They are not some special achievement of flies, but rather a common genetic legacy of all animals. *Drosophila*'s genetic inventory is correspondingly informative in medical questions: When developmental processes derail in humans, genes and entire control systems, which we have already known in the fly for a long time, are presumably often disturbed.

Early settings of the course

The body structure of the fly has already been determined before the egg cell is fertilized. The female flies not only supply their eggs with nutrients, they also provide proteins and their blueprints, which intervene as control factors in development. These factors are asymmetrically distributed in the egg and consequently stipulate the body axes. In the process they activate a gene cascade which subdivides the embryo into increasingly smaller segments. As in an architect's blueprints, the basic structural plan of the body with its segments and organs, which only become visible much later, are nearly invisibly sketched, and the areas in which body parts develop are stipulated. In this context, communication processes between the cells play a role. They define the respective developmental fate of a cell at exact positions in the body via the interaction of signal substances and corresponding receptor molecules.

Additional facets

Alf Herzig and his research group focus on very special cells: the so-called stem cells. These cells divide in the same manner as their genetically identical sister cells, but do not initially develop into a specific cell type. Indeed, that is not even desired because stem cells are the body's silent reserves. They subsequently develop into exactly those cells which the organism has lost due to cell



▲ The fruit fly
Drosophila melanogaster.

death. But how does the organism prevent these cells from differentiating simultaneously with the other cells? Apparently, the genes of the stem cells are specially packaged and stored in the cell nucleus. Consequently, Herzig's team studies the packaging characteristics – i.e., the histone modifications – and the position of the genes in the cell nucleus, and compares them with adjacent cells which differentiate.

Gerd Vorbrüggen's research group is concerned with a specialist among the differentiated cells, a cell which everyone is familiar with: the muscle cell. The scientists investigate how muscle cells are formed and how they unerringly position themselves into an exactly stipulated overall pattern in the body.

In order for muscles to be able to work they need energy. Ronald Kühnlein, Mathias Beller and Ralf Pflanz's research groups are working on the ques-

tion of how the fly controls its energy budget. They want to understand how an organism knows how much energy it must store in the form of fat deposits to cover its energy requirements even in times of famine. These projects will help us to better understand human obesity, which in the meantime with its consequences such as cardiovascular diseases, diabetes and certain types of cancer has become a worldwide health problem. These researchers expect the fly, as a biomedical model, to make a contribution to the diagnosis of and to new therapies for obesity in the long-term.

Internal groups:

Dr. Mathias Beller, Dr. Alf Herzig, Dr. Ronald Kühnlein, Dr. Ralf Pflanz, Dr. Gerd Vorbrüggen; the research group of Dr. Reinhard Schuh is associated with the department.



The fruit fly embryo shortly before larva formation.



Prof. Dr. Herbert Jäckle received his PhD in biology from the University of Freiburg in 1977. He subsequently worked at the University of Texas at Austin (USA), the European Molecular Biology Laboratory in Heidelberg, and the Max Planck Institute of Developmental Biology in Tübingen. In 1987, he became a university professor for genetics at the Ludwig Maximilians University in Munich. He has headed the Department of Molecular Developmental Biology at the Max Planck Institute for Biophysical Chemistry since 1991. In 2002, he became the Vice-President of the Max Planck Society. Herbert Jäckle has received numerous renowned science awards, among them the Leibniz Prize in 1986, the Feldberg Prize in 1990, the Otto Bayer Prize in 1992, the Louis Jeantet Prize and the German Future Prize in 1999. He has taught as an honorary professor at the University of Göttingen since 1993.

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M. Beller, C. Sztalryd, N. Southall, M. Bell, H. Jäckle, D. S. Auld, B. Oliver: COPI complex is a regulator of lipid homeostasis. *PloS Biology* 6, 2530-2549 (2008).

S. Grönke, A. Mildner, S. Fellert, N. Tennagels, S. Petry, G. Müller, H. Jäckle, R. P. Kühnlein: Brummer lipase is an evolutionary conserved fat storage regulator in *Drosophila*. *Cell Metab.* 1, 323-330 (2005).

R. Rivera-Pomar, H. Jäckle: From gradients to stripes in *Drosophila* embryogenesis: filling in the gaps. *Trends Genet.* 12, 478-483 (1996).



Prof. Dr. Michael Kessel received his PhD at the University of Kiel in Biology in 1981. From 1981 to 1983, he worked at the National Cancer Institute in Bethesda (Maryland, USA) and from 1983 to 1986 at the Center for Molecular Biology in Heidelberg. Since 1986, he has been a member of the Department of Molecular Cell Biology at the Max Planck Institute for Biophysical Chemistry and has headed the Developmental Biology Research Group there since 1992. Concurrently, Michael Kessel also teaches as a senior lecturer on the Biological Faculty of the University of Göttingen.

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L. Luo, Y. Uerlings, N. Happel, N. S. Asli, H. Knoetgen, M. Kessel: Regulation of geminin functions by cell cycle dependent nuclear-cytoplasmic shuttling. *Mol. Cell. Biol.* 27, 4737-4744 (2007).
M. Pitulescu, M. Kessel, L. Luo: The regulation of embryonic patterning and DNA replication by Geminin. *Cell. Mol. Life Sci.* 62, 1425-1433 (2005).
L. Luo, X. Yang, Y. Takihara, H. Knoetgen, M. Kessel: The cell-cycle regulator geminin inhibits Hox function through direct and polycomb-mediated interactions. *Nature* 427, 749-753 (2004).

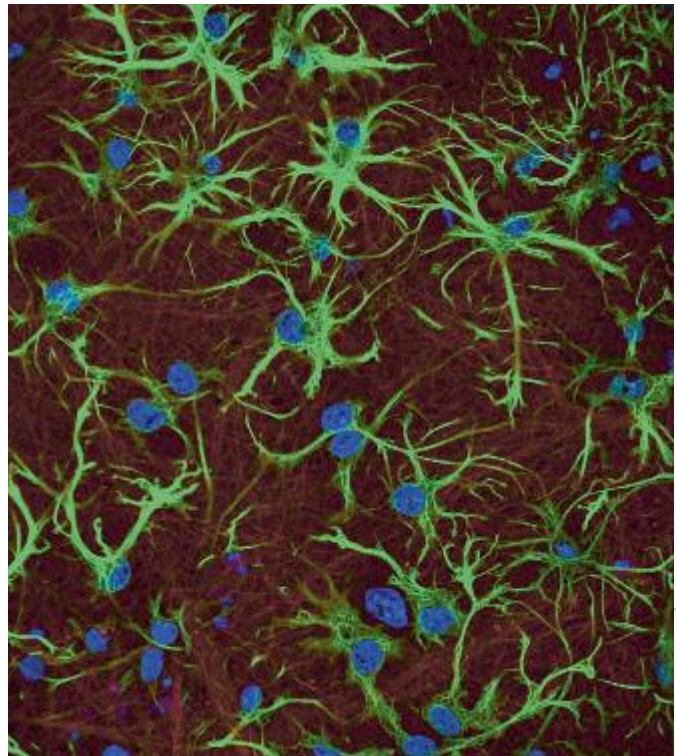
Developmental Biology

When a complex organism develops from a single egg cell, even experienced biologists are amazed time and again. In humans, as in mice, the blueprints of development are encoded in the genes, which are passed on from the parents to their offspring. With each cell division the embryo approaches its goal: to become a completely developed organism. On the way to this objective, the same cycle repeats itself time and time again. First, the genetic material is duplicated, and then it is distributed among the developing daughter cells.

During embryogenesis, and tightly coupled to the cell divisions, a multitude of molecular switches determining cell fates are set. Thus, for example, a decision may be taken as to whether a cell keeps many developmental options open or whether its prospective destiny has already been determined. Whether or not it will lie in the forward or rear part of the body, or whether it will have a career as a muscle or a nerve cell may also be decided. When the final state has been reached, the cell cycle is terminated and the fully-differentiated cell concentrates completely on its specialized task, for example signal transmission in the nervous system. However, many organs also retain cells which further divide and in the process renew themselves. These stem cells, which act as the body's silent reserve, can deliver the necessary replenishment of differentiated cells and ensure that the cell repertoire is maintained.

Gene »on«, gene »off«

We want to understand how the two elementary gear systems, cell division and cell differentiation, interact. Which molecular



Neural stem cells which have differentiated into so-called astrocytes (green) are obtained from the embryonic mouse brain. Cell nuclei are stained blue.

mechanisms are the bases of these processes, which genes are involved, and how are their functions coordinated? We investigate these questions using the mouse as our model. Although this rodent appears completely different from a human being, their organs and tissues are similar, and for an impressive 99 percent of the mouse genes there are similar sequences in the human genome. To plumb the depths of these mechanisms at a molecular level, we search for genes which are involved in both processes. In order to get on their track we attempt to activate or switch them off with the precisely targeting instruments of genetic engineering. Based on the consequences of such experiments we can determine which role a gene plays under normal conditions. With the information obtained, we want to contribute to a molecular understanding of mammalian development.

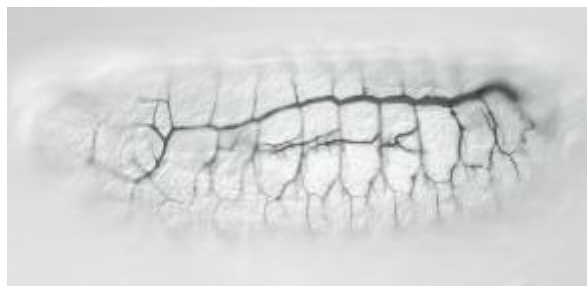
Molecular Organogenesis

We breathe in and out approximately 20,000 times a day without thinking about it. Each time the respiratory air flows through the delicate tube system of our lungs, which contain five to six liters of air, and exchange approximately half a liter of air with every breath. Like the crown of a tree, the system transitions into increasingly finer branches down to the alveoli where oxygen migrates into the circulatory system

Our research group wants to understand how the installation of these highly branched pathways for the respiratory air occurs. However, the examination of the underlying molecular mechanisms is very difficult and time-consuming to examine in mammals. As a consequence, we investigate our complex questions on biology's model organism par excellence: the fruit fly *Drosophila melanogaster*.

Drying out the respiratory system

The fly and the human being are more similar in many aspects than one might think. From a total of 13,600 *Drosophila* genes approximately 7,000 genes are also present in similar form in the human genome. The fruit fly indeed has no lungs, but instead a system of arboreally branched tubular pathways for the respiratory air, the trachea. In



The lung of the insects, the tracheal system, permeates the entire embryo of the fly.

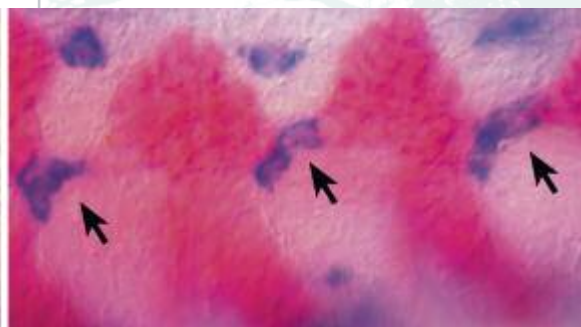
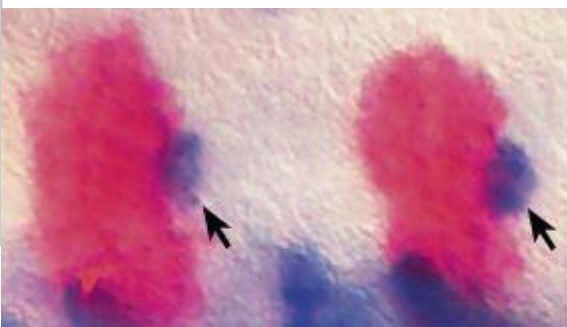
the meantime we know that the installation of this system of tubes is very similarly organized to the development of the lungs. A number of closely related steps during embryonic development ensure that the tubes branch at the proper locations and that they are not too narrow or too wide at the end.

Common to both, flies and mammals, is also that they have respiratory tubes that are initially filled with liquid during the developmental phase. Therefore, they must be dried out at the proper time, otherwise the developing organism will display severe respiratory problems. In babies born prematurely, for example, there is danger of the respiratory distress syndrome (RDS). Even in adult humans liquid in the lungs can result in life-threatening edemas.

Among the 7,000 genes that are similar in humans and flies, we have discovered 20 genes which ensure that the tubes develop properly and are dried out at the right time. Now, we want to determine the molecular mechanisms in which these genes are embedded and whether they have kept their functions across the species boundaries.

The cross-linkage of tracheal cell groups (red marking) is enabled by closely associated bridge cells (blue marking and designated by arrows).

Right: During development, the bridge cells (blue; arrow) stretch and connect the tracheal cell groups (red). The tracheal cells migrate along the bridge cell and thus form a connecting network.



Prof. Dr. Reinhard Schuh

received his PhD at the Eberhard Karls University, Tübingen, in biology in 1986. From 1986 to 1988, he worked as a scientific staff member at the Max Planck Institute for Developmental Biology in Tübingen and from 1988 to 1991 as lecturer at the Institute for Genetics and Microbiology at the Ludwig Maximilians University in Munich. Since 1992, he has been a member in the Department of Molecular Developmental Biology at the Max Planck Institute for Biophysical Chemistry. He has headed the Molecular Organogenesis Research Group there since 2005. Reinhard Schuh also teaches as an adjunct professor on the Biological Faculty of the University of Göttingen.

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M. Behr, C. Wingen, C. Wolf, R. Schuh, M. Hoch: Wurst is essential for airway clearance and respiratory-tube size control. *Nature Cell Biol.* 9, 847-853 (2007).

C. Krause, C. Wolf, J. Hemphälä, C. Samakovlis, R. Schuh: Distinct functions of the leucine-rich repeat transmembrane proteins capricious and tartan in the *Drosophila* tracheal morphogenesis. *Dev. Biol.* 296, 253-264 (2006).

M. Behr, D. Riedel, R. Schuh: The claudin-like megatrachea is essential in septate junctions for the epithelial barrier function in *Drosophila*. *Dev. Cell* 5, 611-620 (2003).

C. Wolf, R. Schuh: Single mesodermal cells guide outgrowth of ectodermal tubular structures in *Drosophila*. *Genes Dev.* 14, 2140-2145 (2000).



Prof. Dr. Ahmed Mansouri

received his PhD at the Technical University of Brunswick in chemistry in 1978. Subsequently, he did research as a postdoctoral fellow in the Institute for Human Genetics at the University of Göttingen, in the Friedrich Miescher Laboratory of the Max Planck Society in Tübingen and at the Max Planck Institute for Immunobiology in Freiburg. In 1989, he became a staff scientist at the Max Planck Institute for Biophysical Chemistry in the Department of Molecular Cell Biology. In 1999, he habilitated in the Medical Faculty of the University of Göttingen and since 2002, he has been head of the Molecular Cell Differentiation Research Group. Ahmed Mansouri has held the professorship of the Dr. Helmut Storz Foundation at the University Medical School in Göttingen since 2005.

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Molecular Cell Differentiation



Whether heart or kidney, pancreas or brain – the organs in our body are the equivalents of small factories, in which specialized »units« perform specific tasks. In the pancreas there are primarily two cell types which share the work. While the majority of them produces digestive juices, the smaller cell group produces hormones such as insulin, which regulates the blood sugar level. The mesencephalon (mid-brain) also comprises highly specialized cells, for example nerve cells, which produce the messenger substance dopamine.

But as different as the cellular specialists are – they all evolve during the development of an organ from nearly identical progenitor cells. In our group we are studying the underlying mechanisms behind this process.

We already know that certain genes control the maturation of an organ and thus determine the subsequent fate of the cells. These control genes provide the blueprint for specific proteins, so-called transcription factors. These factors selectively switch on genetic programs or suppress them and thus transform progenitor cells into cells with specific characteristics. This has been shown by tests in which these genes were inactivated. For example, without the con-

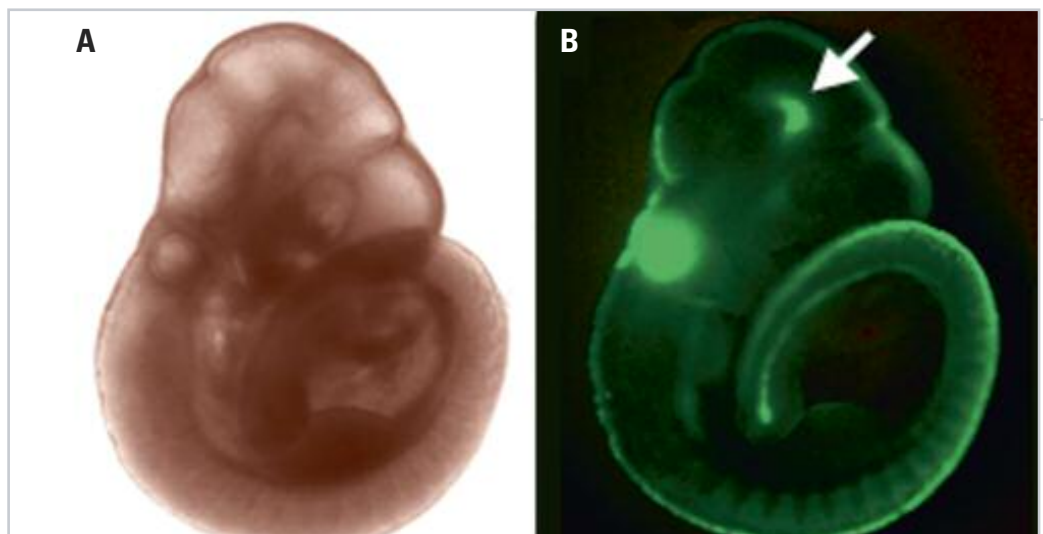
trol gene Pax4, no insulin-producing cells develop in the pancreas. Other factors cause cells to produce glucagon, insulin's antagonist. The situation in the mid-brain is very similar. There, for example, the factor *lmx1a* activates the characteristics of a specific group of nerve cells, which should produce the messenger substance dopamine. In order for cells with different tasks to be formed in the correct proportions in an organ, the respective factors interact with each other and thus tare out the required balance.

Mouse research for human beings

We investigate the maturation of an organ in mice because we can very easily modify this small rodent genetically and can thus selectively examine the role of the participating factors. The information obtained in our research is also of fundamental importance for human medicine. It can be used to generate dopamine-producing cells from human embryonic stem cells – the cells which die in the mid-brain of Parkinson's patients. Dopamine-producing cells are not limited to being cultured in a culture dish to test the efficacy of potential medicines. In the future they may also be used for stem cell therapies.

P. Collombat, X. Xu, P. Ravassard, B. Sosa-Pineda, S. Dussaud, N. Billestrup, O. D. Madsen, P. Serup, H. Heimberg, A. Mansouri: The ectopic expression of Pax4 in the mouse pancreas converts progenitor cells into α - and subsequently β -cells. *Cell* 138, 449-462 (2009).

R. Dressel, J. Schindehütte, T. Kuhlmann, L. Elsner, P. Novota, P. C. Baier, A. Schillert, H. Bickeböller, T. Herrmann, C. Trenkwalder, W. Paulus, A. Mansouri: The tumorigenicity of mouse embryonic stem cells and in vitro differentiated neuronal cells is controlled by the recipients' immune response. *PLoS ONE* 3:e2622 (2008).



In the mouse embryo (A) the activity of the *lmx1a* factor can be made visible with the aid of a reporter (B). The *lmx1a* factor determines the fate of the labeled cells in the mid-brain (arrow). With this factor they become nerve cells which specialize in the production of dopamine.

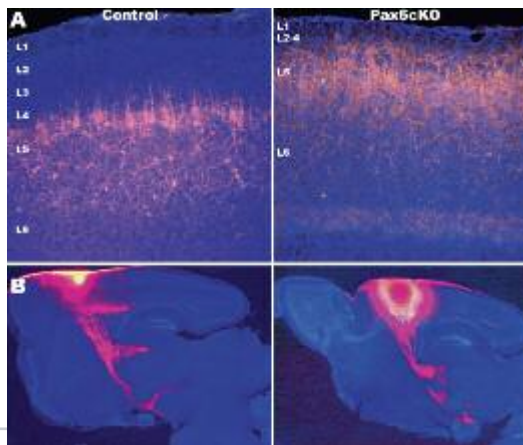
Molecular Developmental Neurobiology

The human brain is both fascinating and puzzling. Hundreds of billions of neurons and many more glia cells are linked to form a complex network in it. Despite the vast number and variety of cells, the mammalian brain develops from astonishingly few original cells, the stem cells. We are investigating how this occurs in the developing cerebral cortex, the outer region of the mammalian cerebrum, in which environmental stimuli converge, motion sequences are planned and initiated, and perceptions and cognitions develop.

The complexity of the cerebral cortex arises only in the course of its development. Completely developed, it comprises six cellular layers and many functionally diverse areas, each with its own responsibilities. The nerve cells of the different layers are born at particular developmental stages; they have a typical morphology, function, and make connections with diverse regions of the brain and spinal cord. These processes are controlled by the expression of specific genes in the progenitors and/or nerve cells. In addition, the combinatorial expression of sets of transcription factors in the progenitors develops a sort of map within the germinative zone which anticipates the subsequent functional arealization.

We study the molecular mechanisms which control the development of the cerebral cortex in mouse models. The transcription factor Pax6, for example, plays a decisive role in the development of brain and eye. If the *Pax6* gene is inactive, fewer nerve cells form in the cortex, the

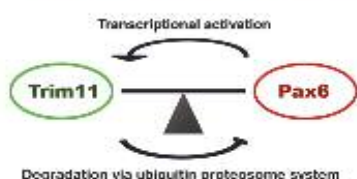
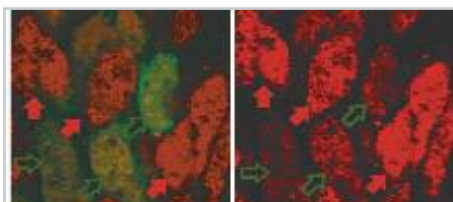
layers and functional areas exhibit abnormal development, and *Pax6*-deficient mice show cognitive disabilities. Defect in the human *PAX6* causes similar cortical abnormalities and behavioral deficiency (aniridia syndrome). We investigate how Pax6, together with its interacting partners and target genes, regulates the differentiation



A) If *Pax6* is not active, more deep layer (L6, L5) but almost no upper-layer (L4 to L2) neurons are produced in the cortex, due to a premature stop of the progenitor division that depletes the progenitor pool.

B) Although the motor area is distinctly shrunken in mice with an inactive *Pax6* gene, major axonal trajectories are preserved.

of cortical progenitors in the developing brain. In the adult brain only limited neurogenesis occurs. Given the neurogenic properties of *Pax6*, we are attempting to understand whether activation of this gene or its partners in the normal or damaged brain – for example, under inadequate blood supply – could promote generation of more neuronal cells for eventual repair.



Autoregulatory feedback loop between *Trim11* and *Pax6* maintains the Pax6 dosage at a physiological level necessary to assure normal neurogenesis.



Dr. Anastassia Stoykova received her PhD in neurochemistry at the Institute for Molecular Biology of the Bulgarian Academy of Sciences in Sofia (Bulgaria).

From 1973 to 1989, she worked at the Regeneration Research Laboratory and the Institute for Molecular Biology, interrupted by a research stay as an Alexander von Humboldt Fellow at the Göttingen Max Planck Institutes for Experimental Medicine (1980-1981) and for Biophysical Chemistry (1988-1989). After her habilitation in 1989, she worked as a research assistant professor at the Institute for Molecular Biology, Sofia. Subsequently, she returned to Göttingen and worked as a staff scientist in the Department of Molecular Cell Biology at the Max Planck Institute for Biophysical Chemistry (1992-2002). In 2002, she became a group leader in the Molecular Cell Biology Department. Since 2008, she has headed the Molecular Developmental Neurobiology Research Group in the scope of the Minerva Program at the institute.

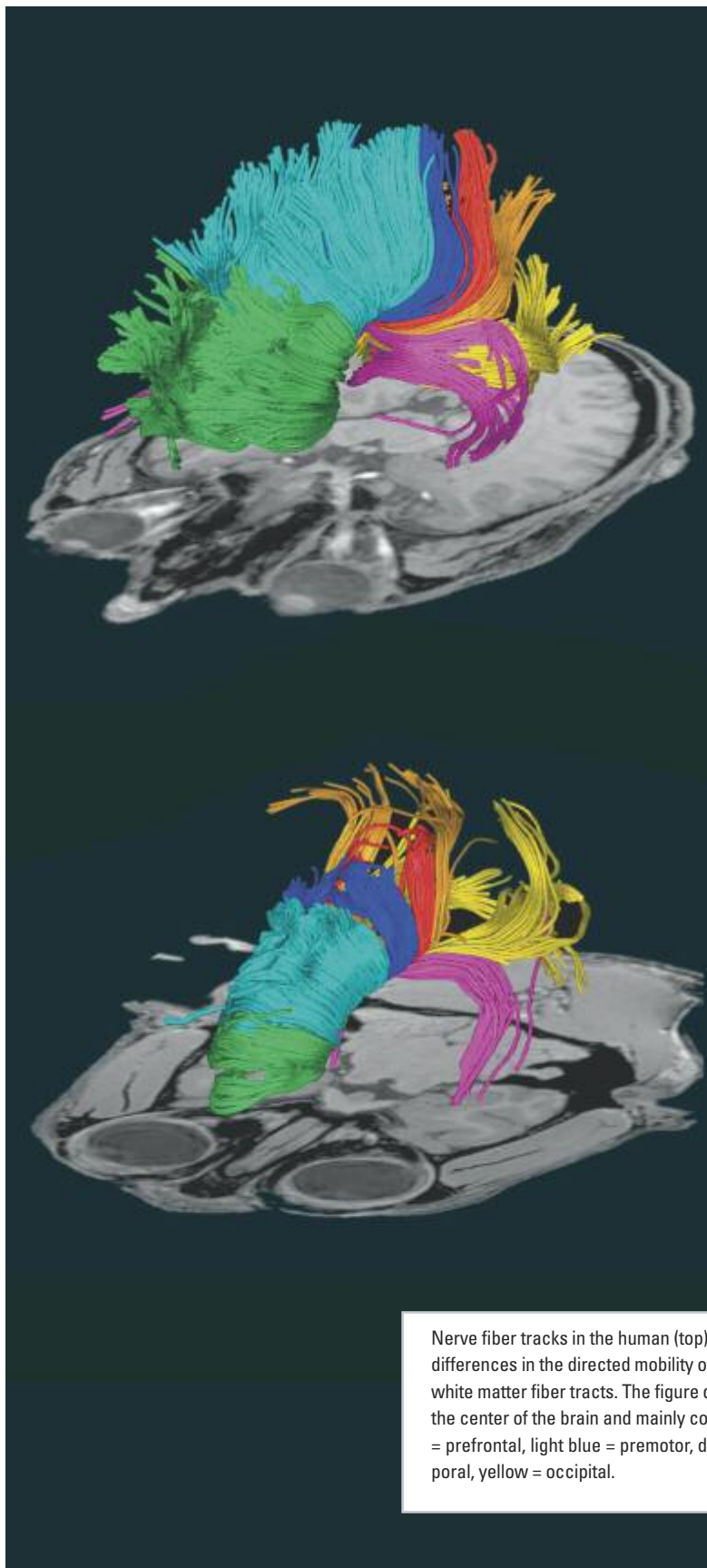
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T. C. Tuoc, K. Radyushkin, A. B. Tonchev, M. C. Piñon, R. Ashery-Padan, Z. Molnar, M. S. Davidoff, A. Stoykova: Selective cortical layering abnormalities and behavioral deficits in cortex-specific Pax6 knock-out mice. *J. Neurosci.* 29, 8335-8349 (2009).

T. C. Tuoc, A. Stoykova: Trim11 modulates the function of neurogenic transcription factor Pax6 through ubiquitin proteasome system. *Genes Dev.* 22, 1972-1986 (2008).

G. M. Fimia, A. Stoykova, A. Romagnoli, L. Giunta, S. Di Bartolomeo, R. Nardacci, M. Corazzari, C. Fuoco, A. Ucar, P. Schwartz, P. Gruss, M. Piecentini, K. Chowdhury, F. Cecconi: AMBRA1 regulates autophagy and development of the nervous system. *Nature* 447, 1121-1125 (2007).



The visualization of structures and functions by imaging plays an increasingly important role in biological and medical research. In this respect magnetic resonance imaging (MRI) offers detailed and completely noninvasive insights into the body of animals and humans. MRI is therefore widely used in diagnostic imaging, but also emerges as an indispensable research tool in the biomedical sciences. It allows us to link advances in molecular biology and genetics to anatomical, biochemical, and physiologic properties in the intact organism. Thus, MRI contributes to a translation of new biological findings in animals into future diagnostic methods and therapies for humans.

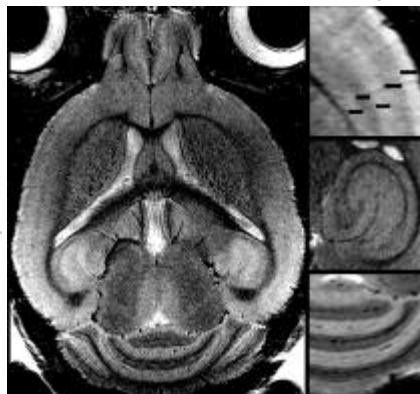
The primary aim of our team is the further development of MRI methods and applications. As early as in the mid-eighties we invented a new principle for the acquisition of rapid images (FLASH) that revolutionized the scientific potential and clinical impact of MRI. Currently, we work on non-Cartesian strategies for encoding the spatial information as well as on novel mathematical approaches for image reconstruction. Such techniques represent major advances for a variety of applications including »parallel« MRI, which relies on the simultaneous data acquisition with multiple receive coils, and »real-time« MRI. As another example, we have made great progress by combining radial encodings with the FLASH technique. Such applications resulted in the recording of high-quality MRI movies in real time, which acquire, reconstruct, and visualize organ motions such as the beating heart without any detectable delay.

Access to the »thinking« brain

A second major focus addresses neuroimaging techniques that allow us to investigate the central nervous system in a more specific manner beyond the mere imaging of anatomy. For example, by

Nerve fiber tracks in the human (top) and rhesus monkey brain (bottom). MRI measurements that probe differences in the directed mobility of water molecules in brain tissue allow for a reconstruction of white matter fiber tracts. The figure depicts major fiber pathways, which cross the corpus callosum in the center of the brain and mainly connect cortical areas of similar function in both hemispheres. Green = prefrontal, light blue = premotor, dark blue = motor, red = sensory, orange =parietal, magenta = temporal, yellow = occipital.

High-resolution MRI of the mouse brain. A resolution of 30 micrometers identifies cellular layers in the cortex and subcortical regions of an anesthetized mouse. Contrasts are mainly due to differences in cellular density and degree of myelination. The inserts depict enlarged sections of the cortex (top), hippocampus (middle), and cerebellum (bottom).

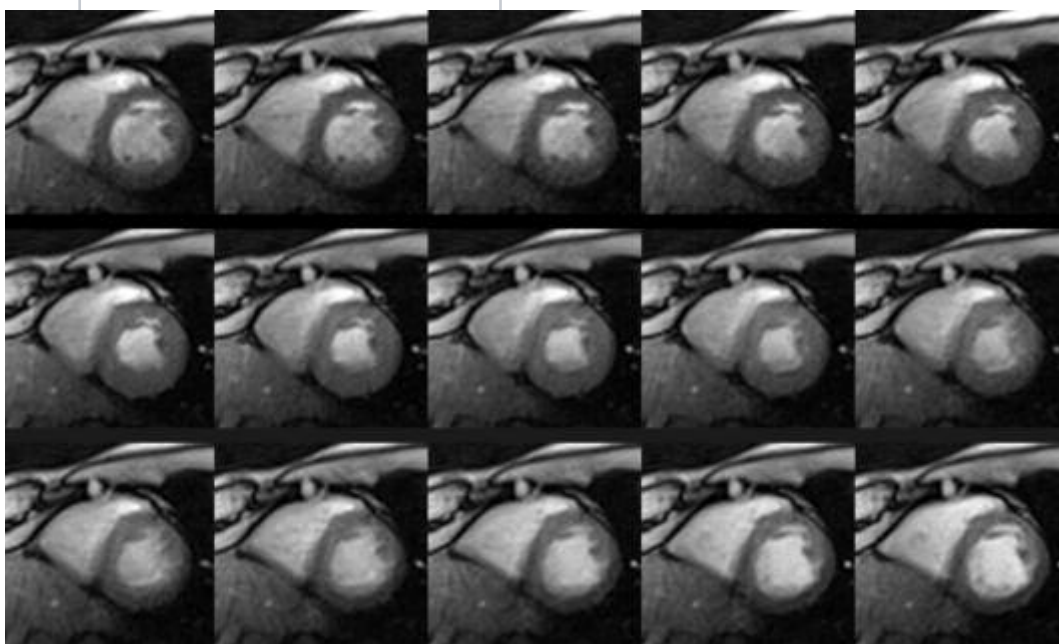


exploiting the directional mobility of water molecules in gray and white matter, we can virtually reconstruct the three-dimensional pathways of nerve fiber bundles in the brain. In this manner we redefined the topography of fibers passing through the central white matter structure (corpus callosum) of the human and rhesus monkey brain. Other MRI sequences provide access to the »thinking« brain. They are sensitive to changes of the intravascular concentration of paramagnetic deoxyhemoglobin which in turn reflect changes in neural activity. Applications range from information processing in

primary sensory systems to cognition and even extend to the establishment of a method for immediate functional feedback (neurofeedback), so that subjects can learn how to control the activity of selected systems of their own brain.

A third important part of our research is devoted to MRI of animals, which mainly refers to brain studies of genetically modified mice. In collaboration with other researchers we examine models of human brain disorders such as neurodegenerative diseases and multiple sclerosis. A benefit of MRI is the fact that we can look at disease progression and response to treatment in individual animals. Recent studies of the mouse brain at very high spatial resolution complement histological analyses with tissue characterizations in living animals.

Real-time MRI of the human heart. Radial FLASH techniques allow us to visualize the beating heart during free breathing and without synchronization to the electrocardiogram. The figure depicts 15 consecutive images (top left to bottom right) with a temporal separation of 30 milliseconds (corresponding to a frame rate of 33 images per second). The 450 millisecond movie section covers the systolic phase of a single heartbeat, which is characterized by a thickening and contraction of the myocardial wall.



Prof. Dr. Jens Frahm received his PhD in physical chemistry from the University of Göttingen in 1977. The same year, he became a scientific assistant at the Max Planck Institute for Biophysical Chemistry, where he subsequently headed his own independent research group from 1982 to 1992 funded by major grants of the Ministry for Research and Technology. Since 1993, Jens Frahm has been Director of the Biomedizinische NMR Forschungs GmbH, a non-profit company financed by the Max Planck Society from royalties of the group's patents. In 1994, he obtained his habilitation from the University of Göttingen, where he has been teaching as an adjunct professor since 1997. In 1991, Jens Frahm was awarded the Gold Medal of the Society for Magnetic Resonance in Medicine, followed by the Karl Heinz Beckurts Award (1993), the State Award of Lower Saxony (1996), and the Sobek Research Award (2005). In 2005, he was elected as an Ordinary Member of the Academy of Sciences at Göttingen.

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S. Boretius, T. Michaelis, R. Tammer, A. Tonchev, R. Ashery-Padan, J. Frahm, A. Stoykova: In vivo MRI of altered brain anatomy and fiber connectivity in adult Pax6 deficient mice. *Cerebr. Cortex*, doi: 10.1093/cercor/bhp057 (2009).

M. Uecker, T. Hohage, K. T. Block, J. Frahm: Image reconstruction by regularized nonlinear inversion – Applications to parallel imaging. *Magn. Reson. Med.* 60, 674-682 (2008).

S. Hofer, J. Frahm: Topography of the human corpus callosum revisited – Comprehensive fiber tractography using magnetic resonance diffusion tensor imaging. *NeuroImage* 32, 989-994 (2006).



Gene Expression and Signaling

Dr. (Univ. Kiev) Halyna Shcherbata studied biology at the National University of Lviv (Ukraine) and received her PhD from the Institute of Plant Physiology and Genetics of the National Academy of Sciences of Ukraine in Kiev in 1996. From 2000 to 2003, she was assistant professor at the Department of Genetics and Biotechnology at the National University of Lviv, followed by a postdoctoral stay at the Department of Biochemistry, University of Washington (Seattle, USA). Halyna Shcherbata has been head of the Max Planck Research Group Gene Expression and Signaling at the Max Planck Institute for Biophysical Chemistry since 2008.

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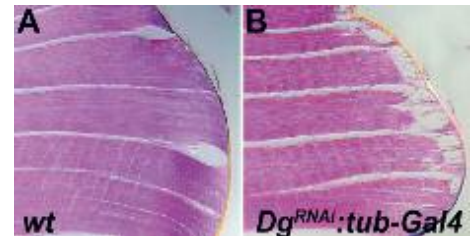
Muscular dystrophy in the fruit fly

In Western Europe more than 70,000 people suffer from severe forms of muscular dystrophy. Those patients have progressive muscle weakening and loss and so far no cure exists to treat these fatal disorders. *Drosophila's* easy-to-manipulate genetic system, relatively short life cycle, low cost, and biological complexity make the fruit fly a perfect system to investigate these hereditary muscle diseases. Previously we have developed a *Drosophila* model for studying muscular dystrophies and shown that the phenotypes caused by *Drosophila* Dystroglycan and Dystrophin mutations are remarkably similar to phenotypes observed in human muscular dystrophy patients. Mutant flies show decreased mobility, age-dependent muscle degeneration and brain defects.

With the aid of our fly model we investigate the molecular components and signaling pathways which regulate these genes. This should provide a better understanding of the causes of muscular dystrophy and could contribute to the development of new therapeutic approaches in the future.

Stem cells as a silent reserve

Stem cells are one of our additional research focuses. There are two types of stem cells: embryonic stem cells and adult stem cells. Adult stem cells form the body's reserve to supply the tissues with the required replenishment of new cells. To prevent stem cells from becoming depleted, they divide asymmetrically into another stem cell and



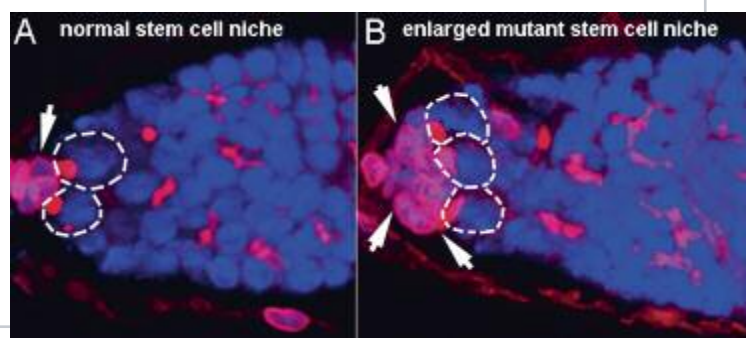
▲ *Drosophila* develops an age-dependent muscular dystrophy phenotype that was used to screen for modifiers. (A) Architecture of wild type transverse muscle section. (B) *Dg* mutants show severe muscle dystrophy.

a daughter that differentiates. Stem cells do not work autonomously in their tissues, but rather they are regulated by signals from their surroundings – their stem cell niche. Only in its special niche can a stem cell regenerate itself. We are interested to find out how the stem cell division and maintenance is controlled and more specifically how microRNAs – miRNAs – are involved in the process. Since *Drosophila* has good markers for stem cells and niche cells, we are able to use genetic screens in order to discover novel genes, which interact with miRNAs in the process of stem cell self-renewal and cell to cell communication in the adult stem cell niche. Given that miRNAs regulate gene expression and enter readily into cells, they have a potential as therapeutics in regenerative medicine and cancer therapies.

H. R. Shcherbata, A. S. Yatsenko, L. Patterson, V. D. Sood, U. Nudel, D. Yaffe, D. Baker, H. Ruohola-Baker: Dissecting muscle and neuronal disorders in a *Drosophila* model of muscular dystrophy. *EMBO J.* 26, 481-493 (2007).

H. R. Shcherbata, E. J. Ward, K. A. Fischer, J. Y. Yu, S. H. Reynolds, C. H. Chen, P. Xu, B. A. Hay, H. Ruohola-Baker: Stage-specific differences in the requirements for germline stem cell maintenance in the *Drosophila* ovary. *Cell Stem Cell* 1, 698-709 (2007).

(A) *Drosophila* adult germline stem cells (outlined with white lines) and their stem cell niche (marked with arrows, pink).
 (B) Mutant enlarged stem cell niche can host more stem cells.



Sleep and Waking

Sleep and waking are part of the life of every animal and every human being. Why we are awake appears obvious. But why do we sleep? One moves less and is barely aware while asleep. Anyone who sleeps is more vulnerable. So why do animals enter such a hazardous state?

Without sleep we feel tired and are inefficient. Today researchers believe that sleep is not only important for energy conservation but also for the nervous system, which has time to regenerate. But what is regenerated in the nerve cells during sleep?

Our research group is trying to find out what happens in nerve cells during sleep. We want to know how a nervous system falls asleep and wakes up, how it knows that it is tired and has to sleep, and we would like to understand the essential functions of sleep, which make it impossible for animals not to sleep.

The sleeping worm

We study sleep and waking on one of the simplest animal models that has a sleep-like state: the round worm (nematode) *Caenorhabditis elegans*. In most animals, which are exposed to sunlight, the sleep-wake rhythm is coordinated with the external day-night cycle. In contrast, *C. elegans* is a ground-dwelling animal. Its sleep-wake rhythm is controlled by an exactly predetermined internal developmental program. *C. elegans* larvae experience exactly four distinctive sleep waking cycles during their development. After each sleep phase the animals moult.

A great advantage for our investigations is the simple nervous system of *C. elegans*. Because the animals are transparent, we can observe and manipulate the nervous system in intact animals both during sleep and waking.

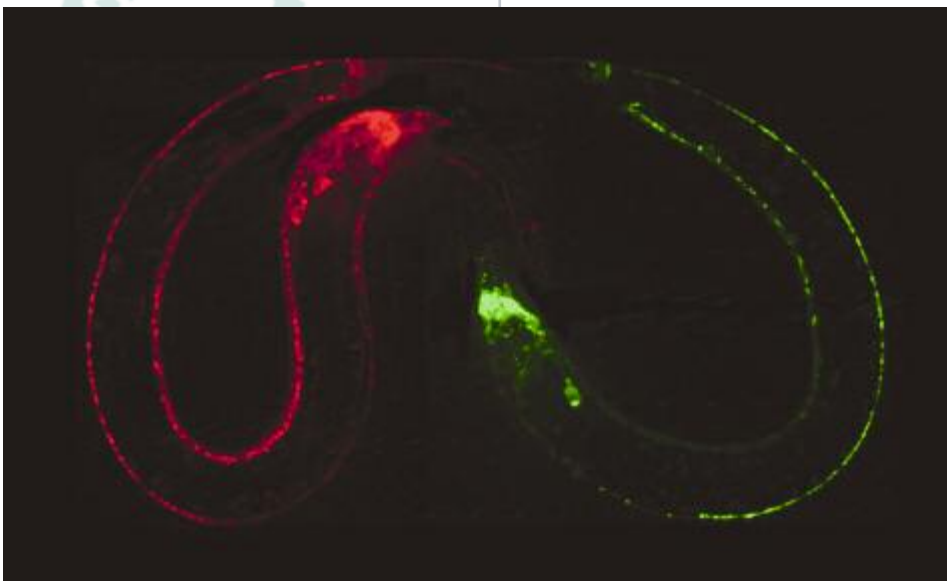
We will subsequently test the results from our worm sleep studies in more complex animal models and also in human beings in order to learn about the differences and similarities.



Dr. Henrik Bringmann studied biology in Göttingen and Heidelberg and received his PhD at the Max Planck Institute for Cell Biology and Genetics in Dresden in 2007. Subsequently, he worked as a postdoctoral fellow at the Laboratory of Molecular Biology in Cambridge (United Kingdom). Since 2009, he has been heading the Sleep and Waking Max Planck Research Group at the Max Planck Institute for Biophysical Chemistry. Henrik Bringmann was awarded the Otto Hahn Medal of the Max Planck Society in 2008.

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Photomontage of two pictures of the same worm. The photograph shows connections between nerve cells (the synapses) during waking (red) and sleep (green).

H. Bringmann: Mechanical and genetic separation of aster- and midzone-positioned cytokinesis. *Biochem. Soc. Trans.* 36, 381-383 (2008).

H. Bringmann, C. R. Cowan, J. Kong, A. A. Hyman: LET-99, GOA-1/GPA-16, and GPR-1/2 are required for aster-positioned cytokinesis. *Curr. Biol.* 17, 185-191 (2007).

H. Bringmann, A. A. Hyman: A cytokinesis furrow is positioned by two consecutive signals. *Nature* 436, 731-734 (2005).

H. Bringmann, G. Skiniotis, A. Spilker, S. Kandels-Lewis, I. Vernos, T. Surrey: A kinesin-like motor inhibits microtubule dynamic instability. *Science* 303, 1519-1522 (2004).

Genes and Behavior

Anyone who has flown across several time zones knows the feeling in the first days thereafter. During the day one is lamed by leaden fatigue, and at night one tosses and turns, wide awake in bed – a clear case of jetlag. Our circadian clock requires a few days until it has re-adjusted to our shifted circadian rhythm. But it works: After a few days we again »tick« synchronously with the outside world. The circadian clock has a period of nearly 24 hours, hence the term »circadian« which means approximately one day (derived from Latin *circa*, »around«, and *dies*, »day«).

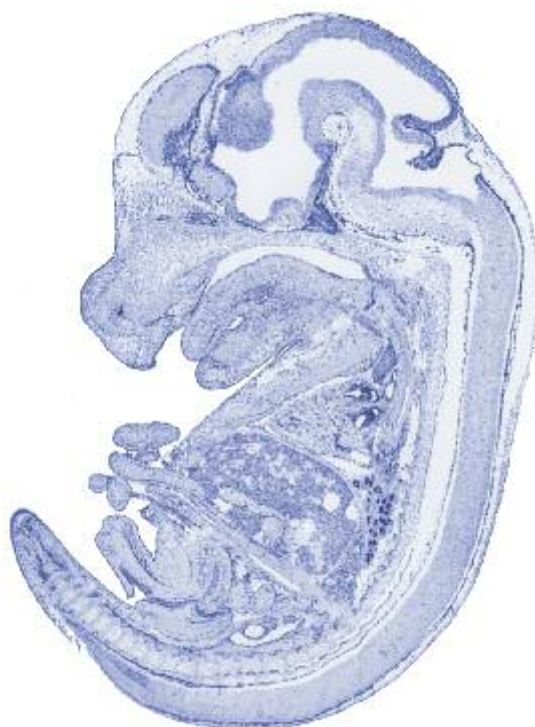
The problems which occur in jetlag are a vivid example of how external influences disturb our circadian clock. In this context, it is really correct to speak of many clocks for the physiological processes in our body – up to our behavior – are coordinated by an entire network of molecular »zeitgebers«. The individual organs each accommodate their own peripheral oscillators, which are under the central control of the circadian pacemaker in the hypothalamus, the suprachiasmatic nucleus (SCN).

The circadian clocks of the organs adapt to altered external influences at different rates. In this context, the adrenal gland's clock plays a decisive role, as we were able to show in experiments with mice. This organ normally secretes the hormone cortisol and thereby has a decisive influence on the clocks of other organs. If one inhibits cortisol synthesis, the body adapts more rapidly to the new time zone. This knowledge opens a pathway to hormone therapy of jetlag.

An atlas of the clock genes

We obtain insights of this type by analyzing the genes relevant to the circadian clock. An extensive, unique source of clock gene candidates are the atlases of gene activity in the brain (www.brain-map.org, www.gene-atlas.org), which we helped to develop. In order to construct these molecular maps, we developed high-resolution, automated techniques. In this manner, we can identify genes

► In the Down syndrome three copies of the *PCP4* and *Col18a1* genes, in addition to many others, are present. *PCP4* (left) is effective in the nervous system, whereas *Col18a1* – a collagen gene (right) – is active in the connective tissue of the mouse embryo.





genes influence the development of the brain in the embryo – the second large research focus in our department. We concentrate primarily on those genes which coordinate the growth and structure of the cerebral cortex.

An example is the *Esco2* gene, which is active for a brief period in the stem cells of the cerebral cortex and which regulates the cohesion of the chromosomes during cell division. If this gene is removed from the growth zone of the cerebral cortex of mice, the animals are born without this part of the brain.

People, in whom the *Esco2* gene has mutated, suffer from a severe hereditary disease, which is termed the Roberts syndrom. We use our *Esco2* mutants to study both the molecular processes of cell division and the cause of the Roberts syndrom.

which, for example, are activated in a circadian fashion in the SCN, the body's master clock. We then remove such candidate genes from the SCN of mice and determine by means of running wheel and molecular experiments whether the circadian clock is defective.

The expression atlas also provides us with new insights into the metabolome, the totality of all molecules involved in the metabolism of an organism. Many of these molecules exhibit concentration fluctuations in the course of a day. In the meantime we know, for example, the activity pattern of all 360 *solute carrier* (SLC) genes. They contain the blueprints for the SLC proteins, important pore proteins in biological membranes. They transport small molecules such as nutrients, vitamins, hormones and minerals, in and out – between cells and within cells. If we know when which genes are active, we can construct a high-resolution atlas of substance exchange throughout the entire mammalian organism with a particular emphasis on the circadian aspects of metabolism.

Developmental genes under the magnifying glass

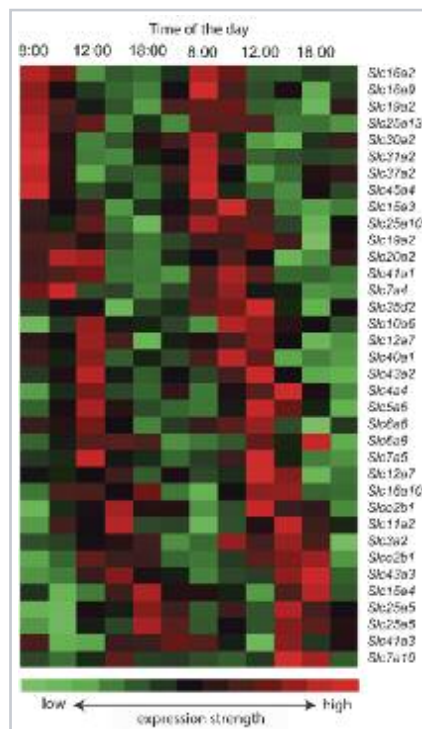
Our atlases of gene expression (www.genepaint.org, www.eurexpress.org) are also an important resource for investigating how



Prof. Dr. Gregor Eichele studied chemistry and structural biology and received his doctorate in 1980 at the University of Basel (Switzerland). Subsequently, he did research in the field of developmental biology from 1981 to 1984 as a postdoctoral fellow at the University of California at San Francisco (USA). From 1985 to 1990, he was a faculty member at the Harvard University School of Medicine in Boston (USA) and worked at the Baylor College of Medicine (Houston, USA) from 1991 to 1998. In 1997, he became a scientific member of the Max Planck Society and, in late 1998, he became a Director at the Max Planck Institute for Experimental Endocrinology. Gregor Eichele has been a Director at the institute and head of the Department of Genes and Behavior since 2006. For his research he has received numerous awards, among them the Friedrich Miescher Prize, the McKnight Neuroscience Development Award in 1991 as well as the Innovation Award in Functional Genomics in 2000.

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In the figure the activity strength (green to red) of the 36 *solute carrier* genes (SLCs) in the adrenal gland of the mouse is shown. The earlier in the day an SLC reaches its activity maximum (red), the closer it is to the top of the diagram. It is easy to see that even in a SLC subset of merely 36 genes there is a definite preference for peak expression at a specific time of day.

V. Jakubcakova, H. Oster, F. Tamanini, C. Cadenas, M. Leitges, G. T. van der Horst, G. Eichele: Light entrainment of the mammalian circadian clock by a PRKCA-dependent posttranslational mechanism. *Neuron* 54, 831-843 (2007).

E. S. Lein, M. J. Hawrylycz, N. Ao, et al.: Genome-wide atlas of gene expression in the adult mouse brain. *Nature* 445, 168-176 (2007).

A. Visel, J. Carson, J. Oldekamp, M. Warnecke, V. Jakubcakova, X. Zhou, C. A. Shaw, G. Alvarez-Bolado, G. Eichele: Regulatory pathway analysis by high-throughput in situ hybridization. *PLoS Genet.* 3, 1867-1883 (2007).

J. P. Carson, T. Ju, H. C. Lu, C. Thaller, M. Xu, S. L. Pallas, M. C. Crair, J. Warren, W. Chiu, G. Eichele: A digital atlas to characterize the mouse brain transcriptome. *PLoS Comput. Biol.* 1, e41 (2005).



Circadian Rhythms

Dr. Henrik Oster received his PhD in biochemistry from the University of Fribourg (Switzerland) in 2002. From 2002 to 2007, he worked as a postdoctoral fellow at the Max Planck Institute for Experimental Endocrinology in Hanover and at the Wellcome Trust Centre for Human Genetics at the University of Oxford (United Kingdom). In 2007, he received an Emmy Noether Grant from the German Research Foundation. Since then, he has headed the Circadian Rhythms Research Group at the Max Planck Institute for Biophysical Chemistry. Henrik Oster received the Faculty Prize of the University of Fribourg in 2002 and was awarded the Otto Hahn Medal of the Max Planck Society in 2003.

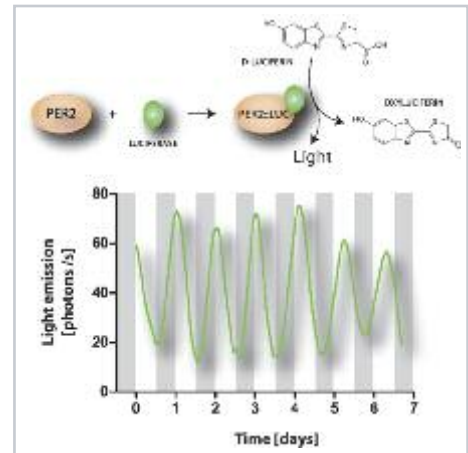
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Life on earth is shaped by multiple environmental cycles. One of the most influential of these is the succession of day and night. In most species – from prokaryotes to humans – internal timekeepers (so-called circadian clocks) that anticipate these daily events and fine-tune physiology to the varying demands of activity and rest have evolved. Circadian clocks are genetically encoded and, thus, do not cease functioning in the absence of external time cues such as the sun. Our clock makes us feel tired in the evening, wakes us again every morning, and regulates the secretion of various hormones with a 24 hour rhythm.

Our group is interested in the molecular mechanisms behind these phenomena. For this purpose, we have generated mice in which we have specifically disrupted some of those genes that regulate circadian time-keeping – which, by the way, are remarkably similar in mice and men. We study the changes in daily rhythms in these mice, with a focus on sleep-wake regulation and the interaction between internal clocks and the uptake/metabolism of food.

Visualizing the ticking of the clock

By providing our mice with a running wheel we can very precisely measure their daily activity rhythms. In some animals, we have genetically tagged the clock with the firefly's light-producing protein. By measuring light emission we can now follow the molecular »ticking« of the circadian clock in living tissue and observe how it reacts to different external stimuli.



Top: When we couple the clock protein PER2 to the light-producing enzyme luciferase we can visualize the rhythm of the internal clock by monitoring the light signal produced by the oxidation of the luciferin molecule. Bottom: Light emission rhythm from liver slice of a mouse bearing the PER2-luciferase fusion protein.

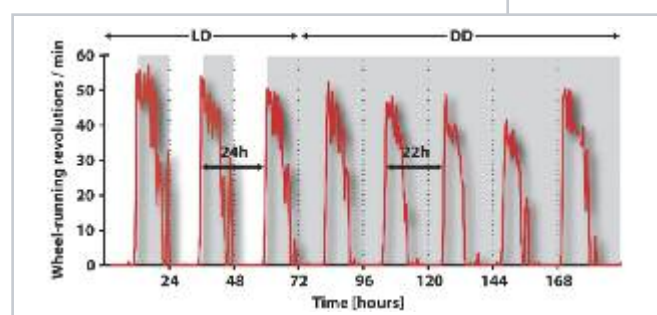
We hope that our findings will lead to new approaches for the treatment of »rhythm diseases« such as sleep disruption, winter depression, and the so-called »night eating syndrome«.



D. Lupi, H. Oster, S. Thompson, R. G. Foster: The acute light-induction of sleep is mediated by OPN4-based photoreception. *Nat. Neurosci.* 11, 1068-1073 (2008).

H. Oster, S. Damerow, S. Kiessling, V. Jakubcakova, D. Abraham, J. Tian, M. W. Hoffmann, G. Eichele: The circadian rhythm of glucocorticoids is regulated by a gating mechanism residing in the adrenal cortical clock. *Cell Metab.* 4, 163-173 (2006).

H. Oster, S. Baeriswyl, G. T. van der Horst, U. Albrecht: Loss of circadian rhythmicity in aging mPer1-/-mCry2-/- mutant mice. *Genes Dev.* 17, 1366-1379 (2003).



Wheel-running activity of a *Cry1* mutant mouse under light/dark (LD) and constant darkness (DD) conditions. In DD, the activity rhythm shows a period length of ~22 hours, whereas in LD the internal clock is synchronized to the external light cycle of 24 hours.

The institute at a glance

Institute staff members:

837, including 473 scientists

Managing Director:

Professor Dr. Helmut Grubmüller (2009 – 2010)

Assistant to the Managing Director:

Eva-Maria Hölscher

Head of administration:

Manfred Messerschmidt

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Cellular Logistics (*Professor Dr. Dirk Görlich*)

Genes and Behavior (*Professor Dr. Gregor Eichele*)

Membrane Biophysics (*Professor Dr. Erwin Neher*)

Molecular Cell Biology (*Professor Dr. Peter Gruss*;
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... by car

Autobahn A7 Kassel-Hannover, Göttingen Nord Exit. Continue straight on at the first traffic light following the B27 in the direction of Braunlage. After about 2 km turn left in the direction of Nikolausberg (signposted). The third road junction on the left-hand side leads onto the institute's campus.

... by plane

Via the Frankfurt or Hannover airports.

From the Frankfurt Airport, direct trains to Göttingen leave every two hours at the long-distance train station. Alternatively, from the regional train station, take the train or urban railway (S-Bahn) to the Central Station (Frankfurt/Hbf) (trains to Göttingen at half hour intervals). Travel time with a car to Göttingen is approximately 3 hours.

... by train

Take a taxi from the Göttingen train station to the institute or the city bus number 8 or 13 in the direction of downtown. Transfer to bus number 5 in the direction of Nikolausberg at one of the first four bus stops. The »Am Faßberg« bus stop is located across the street from the institute site.

From the Hannover Airport, take the urban railway (S-Bahn) to the Hannover Central Station. From there take an ICE or an IC train to Göttingen (travel time approximately 30 minutes). Travel time with a car to Göttingen is approximately 1.5 hours.



Pictures

Page 5 (bottom, left): Medial nucleus of the trapezoid body (MNTB) in the brain injected with GFP adenovirus (green). The vesicular glutamate transporter VGLUT1 is stained red. (Meike Pedersen, Department of Membrane Biophysics)

Page 5 (bottom, second from left): Structure of bacteriorhodopsin, a protein used by archaea which acts as a proton pump. (Helmut Grubmüller, Department of Theoretical and Computational Biophysics)

Page 5 (bottom, second from right): Neural stem cells originating from the embryonic brain of mice which have differentiated into star-shaped glial cells, so-called astrocytes (green). Cell nuclei are stained blue. (Michael Kessel, Research Group Developmental Biology)

Page 5 (bottom, right): The insuline hormone in two different schematizations. (Helmut Grubmüller, Department of Computational and Theoretical Biophysics)



Page 6: Confocal fluorescence micrograph showing the tubulin cytoskeleton of rat kangaroo cells. (Christian Wurm, Stefan Jakobs; Research Group Mitochondrial Structure and Dynamics)

Page 7 (bottom, right): Max Planck Institute for Neurobiology

Page 8 (left): Archives of the Max Planck Society

Page 9 (top): Spatial structure of the molecular force sensor titin kinase. (Helmut Grubmüller, Department of Theoretical and Computational Biophysics)

Page 9 (bottom): Dirk Bockelmann, Department of NMR-based Structural Biology

Page 12 (top): Deutscher Zukunftspreis, Ansgar Pudenz

Page 13: Filament structures within a nerve cell. The STED microscope provides much sharper details compared to a conventional microscope. (Stefan W. Hell, Department of NanoBiophotonics)

Page 21: Confocal fluorescence micrograph showing the actin cytoskeleton (red), the tubulin cytoskeleton (green) and the cell nucleus (blue) of rat kangaroo cells. (Christian Wurm, Stefan Jakobs; Research Group Mitochondrial Structure and Dynamics)

Page 30: Federico Neri, fotolia.com

Page 33: Inter-Stilist, fotolia.com

Page 34: The head-tail connector of the phi29 DNA polymerase. The blue part in the middle represents DNA. The connector is involved in DNA packaging during virus reproduction, where virus DNA is pushed into an empty virus shell. (Matthias Popp; Department of Theoretical and Computational Biophysics)

Page 48/49: Three-dimensional structure of the largest subunit of the spliceosome, the »tri-snRNP« (left) and four more structures of its flexible component (»U5 snRNP«). (Holger Stark, former Research Group 3D Electron Cryo-Microscopy)

Page 54/55 (top): Bacterial cells. (Irochka, fotolia.com)

Page 56/57: Hundreds of billions of neurons are linked to form a complex network in our brain. (Sebastian Kaulitzki, fotolia.com)

Page 64: Confocal fluorescence micrograph showing the tubulin cytoskeleton (red) and the cell nucleus (blue) of rat kangaroo cells. (Christian Wurm, Stefan Jakobs; Research Group Mitochondrial Structure and Dynamics)

Page 65: Alexander Egner, Facility for Innovative Light Microscopy

Page 70/71: A section through the mouse brain shows star-shaped glial cells (astrocytes, green) in the upper layer and neurons in the lower layer (red). Cell nuclei are stained blue. (Michael Kessel, Research Group Developmental Biology)

Page 76/77: Emilia Stasiak, fotolia.com

Page 82/83: FinePix, fotolia.com (old clock)

Page 84: Kyslynsky, fotolia.com

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