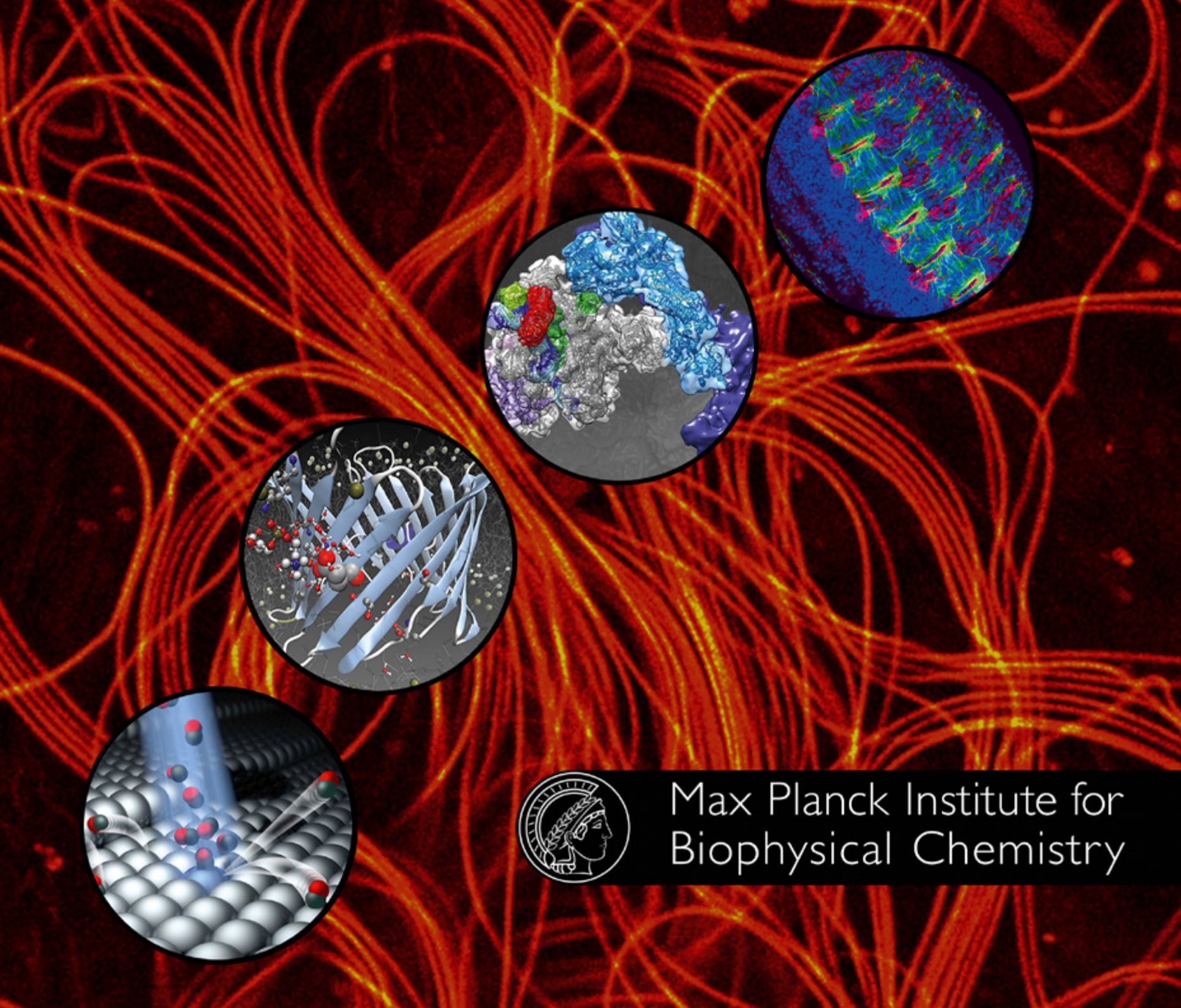
Max Planck Institute for
Biophysical Chemistry

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Content

Cellular machines 52

From egg to organism 80

Communication and logistics 68

An expedition into the world of molecules

How do nerve cells communicate with each other? How does a complex organism evolve from a single fertilized 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 not an 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 cells. 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. The patch-clamp method allowing to measure ion currents at cell membranes (Nobel Prize for Physiology or Medicine 1991 to Erwin Neher and Bert Sakmann), ultra-high resolution fluorescence microscopy on the nanometer scale (Nobel Prize for Chemistry 2014 to Stefan W. Hell), nuclear magnetic resonance spectroscopy, cryo-electron microscopy, or computer simulations are just a few of the methods that are successfully used to investigate proteins.

Tricks of nature

The goal 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.

The scientists are further investigating how a cell converts the basic blueprints of proteins into a readable format, and are revealing the roles that cellular nanomachines – DNA polymerases, spliceosomes, and ribosomes – play in these processes.

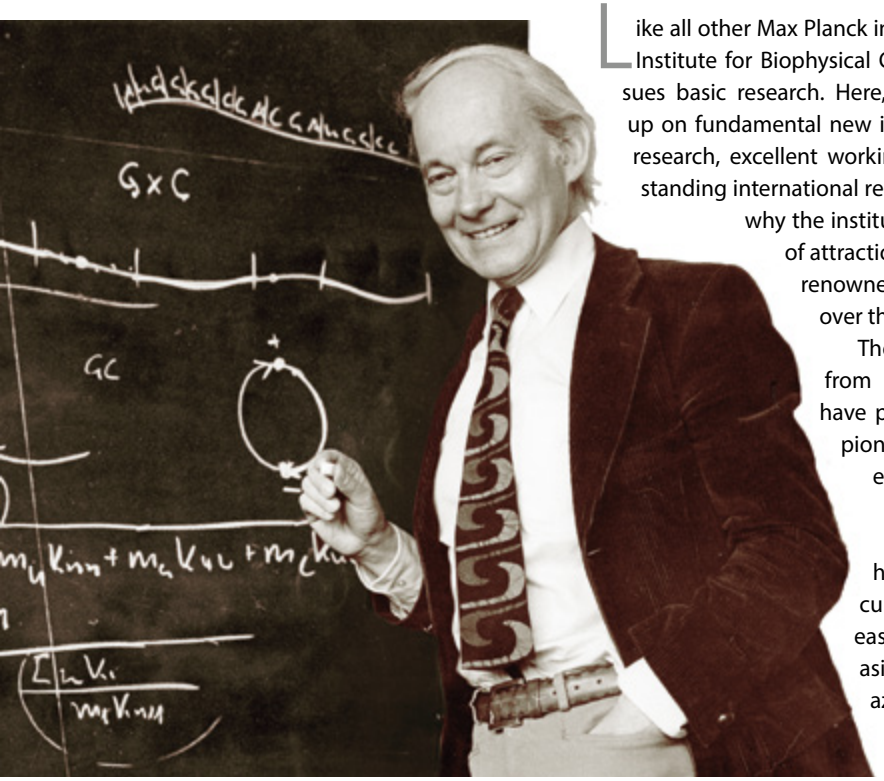
Nanomachines also function in cellular logistics. How specific proteins sort and transport different cargo between the various compartments of a cell is one of the topics explored in greater detail.

Moreover, researchers elucidate how protein aggregates damage living cells and which role these protein clumps play in neurodegenerative diseases. Scientists are further interested in how genetic defective regulation can lead to obesity and metabolic disorders and how – focusing on phenomena of inanimate nature – energy conversion processes at surfaces are controlled.

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

Accordingly, as they exchange views on projects, ideas, and results, many different languages can be heard on the Max Planck Campus, which comprises the Max Planck Institute for Dynamics and Self-Organization and the *Gesellschaft für wissenschaftliche Datenverarbeitung Göttingen* (GWDG) as well.

Research without constraints



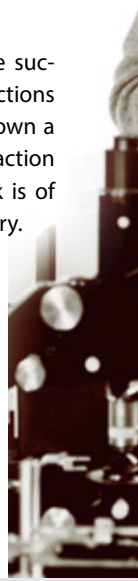
Like all other Max Planck institutes, the Max Planck Institute for Biophysical Chemistry primarily pursues basic research. Here, the researchers follow up on fundamental new ideas. This «uninhibited» research, excellent working conditions, and outstanding international reputation are the reasons why the institute 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, the chemical compound 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, this disease almost always leads to death within two years. The World Health Organization hopes to use this medicine to control leishmaniasis in the long-term and to finally defeat it.

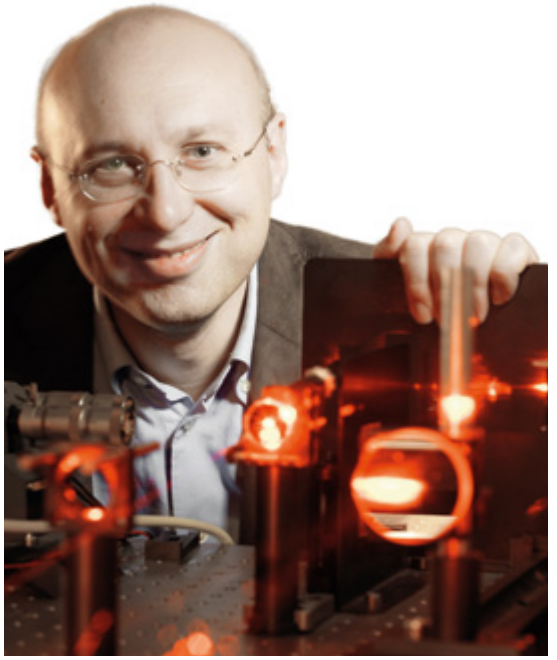
Other researchers have provided ground-breaking ideas for revolutionizing magnetic resonance imaging and optical microscopy. Thanks to these new methods, processes in our body such as beating of the heart or blood flow can even be studied in real-time.

Many of the scientists at the institute have received awards and prizes for their work, including the 13 recipients of the prestigious Leibniz Prize of the German Research Foundation. The highest scientific honor, the Nobel Prize, has been awarded three times for research carried out at the Max Planck Institute for Biophysical Chemistry – one in every generation of researchers:

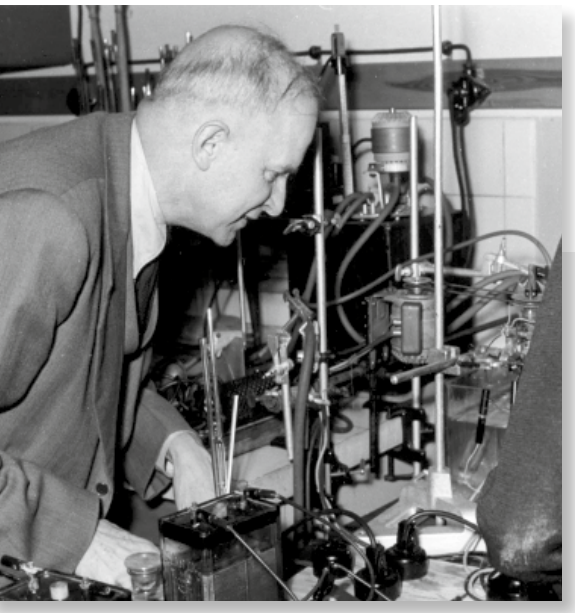
Manfred Eigen
was awarded the Nobel Prize for Chemistry in 1967. He succeeded in observing the course of very fast chemical reactions occurring in the range of nanoseconds. He thus broke down a fundamental barrier as, until then, these very fast reaction processes had been considered unmeasurable. His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



Erwin Neher and Bert Sakmann
were the recipients of 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 periods of time when single ion channels open up – the so-called patch-clamp technique. Miniscule 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 crucial role in the messaging systems of organisms.



Stefan W. Hell
was awarded the Nobel Prize for Chemistry in 2014 for his pioneering work in the field of ultra-high resolution fluorescence microscopy. With his invention of STED (stimulated emission depletion) microscopy and related processes, he revolutionized light microscopy. Conventional light microscopes already reach their resolution limit when two objects are closer than 200 nanometers (one nanometer is a millionth of a millimeter) from each other because the diffraction of light blurs them into a single image feature. This limit, discovered about 130 years ago by Ernst Abbe, had been considered an insurmountable hurdle. Stefan Hell was the first to radically overcome the resolution limit of light microscopes – with a completely new concept. STED microscopy, invented and developed by him to application readiness, is the first focused light-microscopy method which is no longer limited by diffraction. It allows an up to ten times better image resolution in living cells and makes structures visible that are much smaller than 200 nanometers. By applying this method, biologists and physicians can look deeper into the nanocosmos of living cells than ever before.



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. It was inaugurated in 1971, but its history can be traced back far beyond this date, extending back to the former Kaiser Wilhelm Institute for Physical Chemistry in Berlin. In 1949, after creation of the Max Planck Society, the physical chemist Karl Friedrich Bonhoeffer (photo) 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.

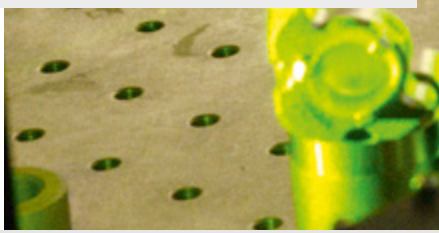
The focus of the newly founded institute on biological research also has its roots in the work and interests of Karl Friedrich Bonhoeffer. He pursued a strong interdisciplinary approach at a very early stage and applied physical-chemical methods to answer biological questions – a good reason to name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

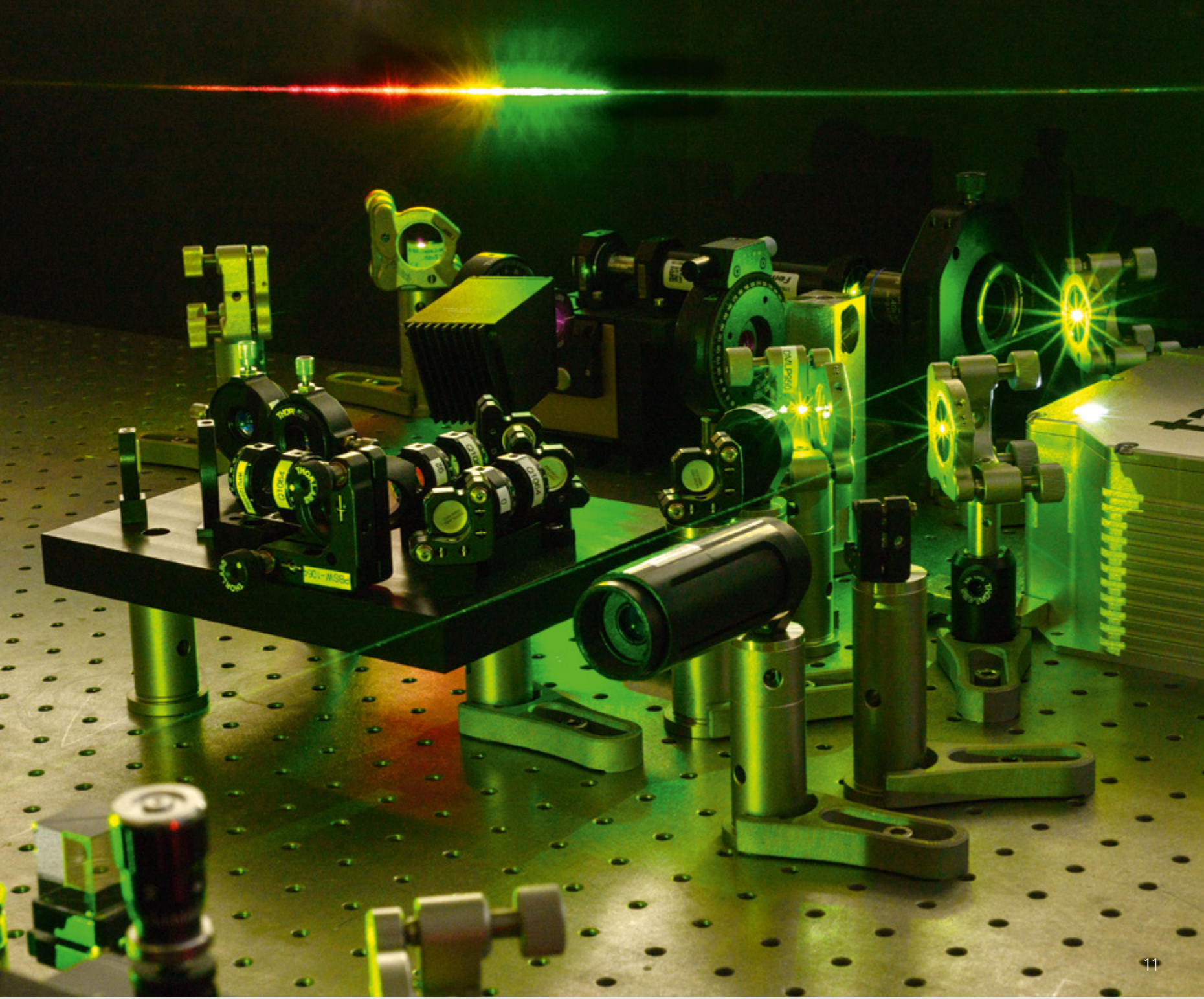
Manfred Eigen's vision for the newly-established institute was to find answers to seemingly unsolvable scientific questions through inter- and multidisciplinary research and to apply the novel findings for the benefit of mankind: A vision which has played a decisive role in the institute's success, and which still stands today in the departments and research groups.

At present, the Max Planck Institute for Biophysical Chemistry comprises 13 departments and 20 research groups, each with its own focus. With more than 800 staff members – including about 400 scientists – it is not only one of the largest institutes of the Max Planck Society, but is also unique in its inter- and multidisciplinarity covering a wide 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.

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 performed here. A Board of Trustees, comprising not only scientists but also prominent representatives from business and politics, ensures good contact with the general public.





Teaching and learning

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

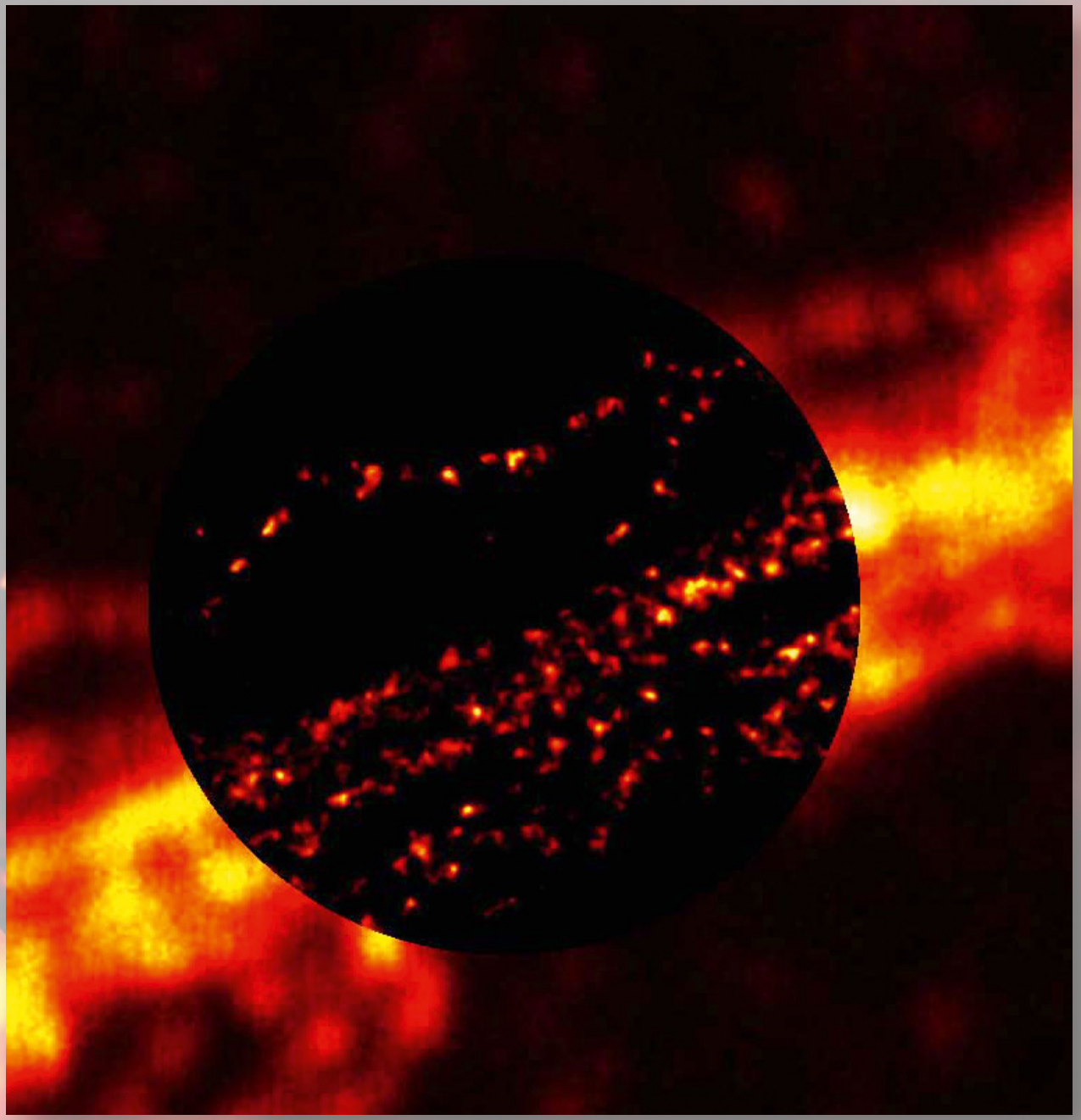
In the international competition for the best young minds, the Max Planck Society and various universities have established a special program of education and training for outstanding students: the *International Max Planck Research Schools* (IMPRS). The Max Planck Institutes for Biophysical Chemistry, for Dynamics and Self-Organization, and for Experimental Medicine have teamed up with the University of Göttingen to establish the International Max Planck Research Schools *Molecular Biology, Neurosciences,* and *Physics of Complex and Biological Systems*. A new program for *Genome Science* is in the developmental phase. 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.

Additionally, the institute and the IMPRS are involved in the *Göttingen Graduate School for Neurosciences, Biophysics, and Molecular Biosciences* (GGNB), which contributed significantly to the success of the University of Göttingen in the national Excellence Initiative.

Structured doctoral studies

The award-winning GGNB offers intensive courses and tutoring and paved the way for structured doctoral studies in Germany. There are also programs for young scientists within the framework of further cooperation 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 *Bernstein Center for Computational Neuroscience* (BCCN Göttingen), which investigates the neuronal basis of our brain activity with mathematical models,
- the *European Neuroscience Institute* (ENI), which concentrates on experimental research into functions and diseases of the nervous system,
- the Cluster of Excellence and DFG Research Center *Nanoscale Microscopy and Molecular Physiology of the Brain* (CNMPB), 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. Furthermore, the researchers aim at developing microscopy methods with resolution in the nanometer range and making them available on a practical level.



When a new idea ignites

Whether in the field of nuclear magnetic resonance (NMR) tomography, laser technology, or microscopy – the findings of basic research solve many a practical problem that applied research was not able to overcome. Such findings are therefore also of economic importance.

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.

Highly successful techniques

The newly developed FLASH (fast low-angle shot) method allows one to take NMR images 100 times faster. It has revolutionized NMR tomography and is now routinely used in hospitals worldwide with over 100 million examinations per year. The FLASH patent is the most successful patent of the Max Planck Society. A further acceleration of the NMR imaging now even makes it possible to generate movies from inside our body in real-time.

The RNA interference (RNAi) technique was successfully applied to mammalian cells for the first time at the institute. Using this method, individual genes can be switched to «mute», facilitating the investigation of their function. This technique might contribute to the treatment of certain hereditary diseases in the future.

DNA analysis from minimal traces of skin scales, root of a hair, blood, or saliva may be done with the help of the short tandem repeats (STR) technology, in whose development the scientists at the Max Planck Institute for Biophysical Chemistry had a substantial part. The method is applied for paternity tests. It is also used in criminological investigations to assign samples to specific individuals.

Scientists as company founders

The revenue from patents and licenses is invested into new projects at the Max Planck Institute for Biophysical Chemistry. 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, which include pharmaceutical companies and those that develop industrial measurement technology.

Moreover, former institute's employees have been involved in the founding of more than a dozen companies. One of these spin-offs is *DIREVO Biotech* (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 ultraviolet lasers that operate with extremely short light pulses. The lasers are continuously undergoing further development and are nowadays also used in medicine and research, in addition to printing technology.

The biotechnology companies *Evotec and DeveloGen* (today also *Evotec*), are two more successful spin-offs of the institute. They connect 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.

The enterprise *Abberior Instruments* was also co-founded by employees of the institute. It specializes in ultra-high resolution microscopes and translates newest findings into innovative research equipment.

Open doors

At the Max Planck Institute for Biophysical Chemistry anyone interested will find that our doors are open. During guided tours through the institute or single labs, during presentations and discussions, everyone – be it a teacher, pupil, journalist, or interested person – can find out more about current research.

Teachers can also register with their school classes for visits to get to know our research during lectures and experimental demonstrations.

Moreover, every year in April, pupils are given the opportunity to become active themselves in our labs and workshops during the *Zukunftstag für Jungen und Mädchen* (Future Day for Boys and Girls) – up to 80 children visit the institute on that day. Teachers are also invited to deepen their knowledge on specific topics. In cooperation with the *XLAB – Göttinger Experimentallabor für Junge Leute e.V.* the institute offers training for teachers.

The Max Planck Institute for Biophysical Chemistry further takes part in the *Nacht des Wissens* (Science Night) where the scientific institutions at Göttingen Campus welcome the general public. Even more can be discovered at the Open Day – here, the entire institute presents itself to the general public with a wide spectrum of offers reaching from informative talks to fascinating experimental stations and a rally for children.

Together with four other Göttingen Max Planck Institutes, the *Göttinger Literaturherbst GmbH*, and the Göttingen State and University Library, the institute furthermore participates in the *Göttinger Literaturherbst* – a literature festival – with a series of scientific lectures. Here, renowned researchers from all over the world present their latest results and discuss current topics with the audience.

Anyone interested can always stay up-to-date with the institute's magazine *MPIbpc News*. It reports on research news, awards, events, and much more in ten issues per year. However, a larger audience can only be reached via the free media. We therefore not only publish press releases on current topics but also put journalists in touch with experts for enquiries and questions.

Last but not least, it should be noted that beside the science, there is also room for culture at the institute – such as the art exhibitions regularly hosted in the foyer.


Looking
deeper

How the unseen becomes measurable and visible

Without X-ray structural analysis, Francis Crick and James Watson would not have discovered that DNA, our prime genetic carrier, comes along as a double helix. And how would Robert Koch have detected the anthrax bacillus without a good microscope at his disposal? Top scientific achievements require high-end equipment. New spectroscopic and microscopic methods are needed, for example, to determine structural details at the single molecule level as well as to explore the dynamics of molecular or even atomic processes. Therefore, it is not surprising that many of the institute's scientists work on methodological innovations, constantly pushing the boundaries of what is possible.

NanoBiophotonics

Stefan W. Hell
received his PhD in physics at the University of Heidelberg in 1990 and worked from 1991 to 1993 at the European Molecular Biology Laboratory (EMBL) in Heidelberg. From 1993 to 1996, he carried out research at the Universities of Turku (Finland) and Oxford (Great Britain). Subsequently, he obtained his habilitation at the University of Heidelberg. In 1997, he went to the Max Planck Institute for Biophysical Chemistry as head of the *High-Resolution Optical Microscopy* Research Group, where he has headed the Department of *NanoBiophotonics* since 2002. Stefan W. Hell has received many national and international awards for his research, among them the Kavli Prize in Nanoscience (2014) and the Nobel Prize in Chemistry (2014).

Contact
stefan.hell@mpibpc.mpg.de
hell@nanoscopy.de
www.mpibpc.mpg.de/hell
www3.mpibpc.mpg.de/groups/hell

Making the smallest details visible using focused visible light – this is the objective of our ultrahigh-resolution light microscopes, known in recent years as nanoscopes. Conventional microscopes reach their resolution limits when two similar objects are closer than 0.2 micrometers (1/5,000 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, nucleic 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

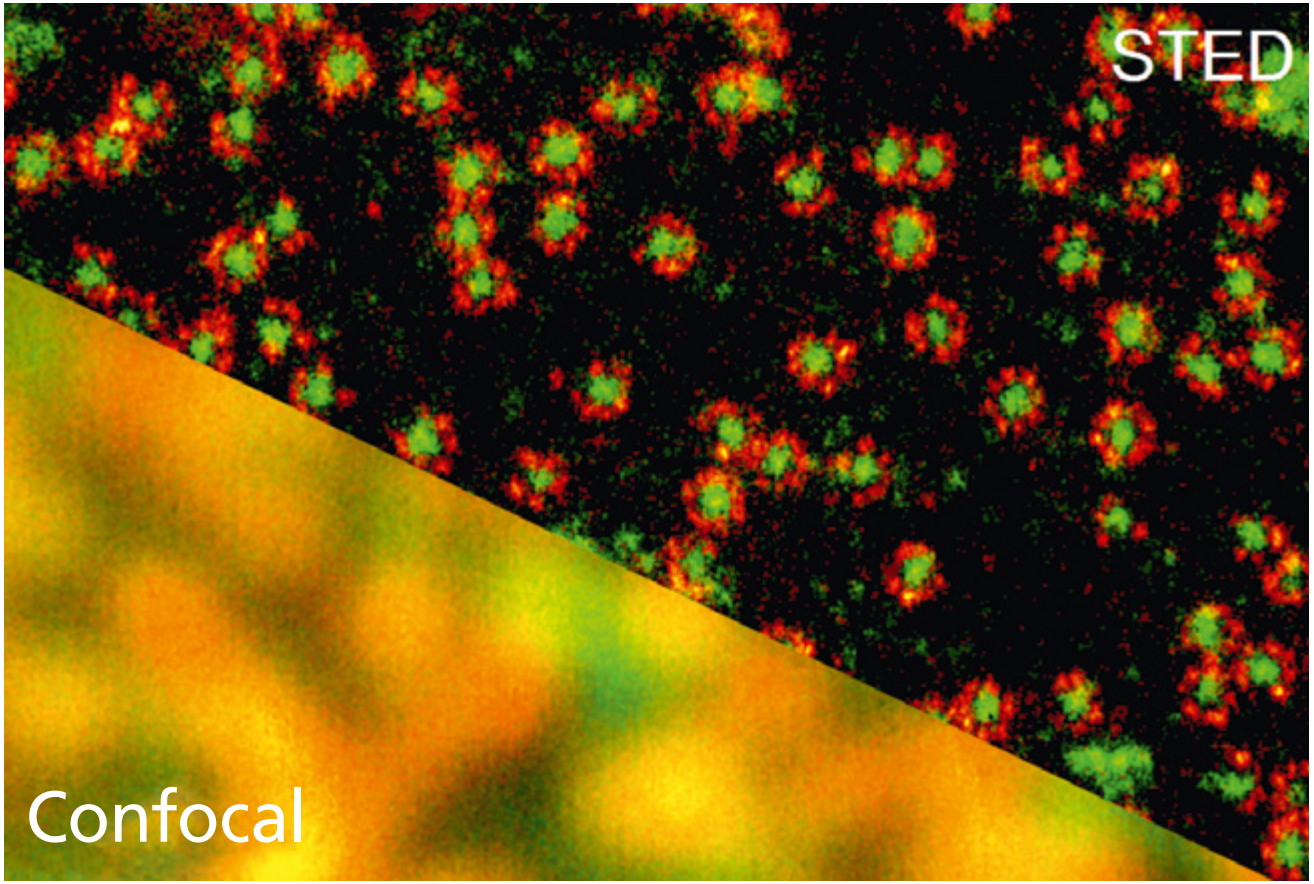
In order to outsmart the resolution-limiting role of diffraction, we ensure that the adjacent molecules or groups of molecules emit their fluorescence successively. To this end, we use transitions between fluorophore states that switch or modulate the fluorescence of adjacent molecules for a brief period 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 labeled protein complexes with distances down to 20 nanometers can be discerned.

As the brightness of the STED beam is increased, the region in which molecules can fluoresce is further reduced in size. As a consequence, the resolution of the system can be increased, in principle, to molecular 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 doughnut beam, similarly as in the STED method; 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 switched 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



◀ Comparison of high-end diffraction-limited conventional microscopy (Confocal, bottom left) to STED nanoscopy (top). Protein superstructures, called nuclear pore complexes, in the membrane of an intact cell nucleus are shown. The eight-fold symmetry can be seen in the red channel of the STED recording, where resolution is improved about ten-fold compared to the confocal microscope.

a precision that is far beyond the resolution limit. This procedure is repeated again until each molecule has been registered. In our version of this approach, called GSDIM, we switch ordinary fluorophores between bright and dark states differing in electron spin, thus making this method applicable for a wide range of fluorophores.

Ingeniously combined

Another goal of our research is to develop innovative optical configurations. In the 4Pi microscope, two objectives are directed at one point so that the wave fronts of the two lenses jointly improve the focusing. 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 that are barely 20-30 nanometers apart can be distinguished in three dimensions. 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 transparent nanostructured materials, such as polymers, and especially the inner workings of living cells. In more recent efforts of the department, we have aimed at realizing nanoscopy of very fast dynamics and deeper inside sections of tissues.

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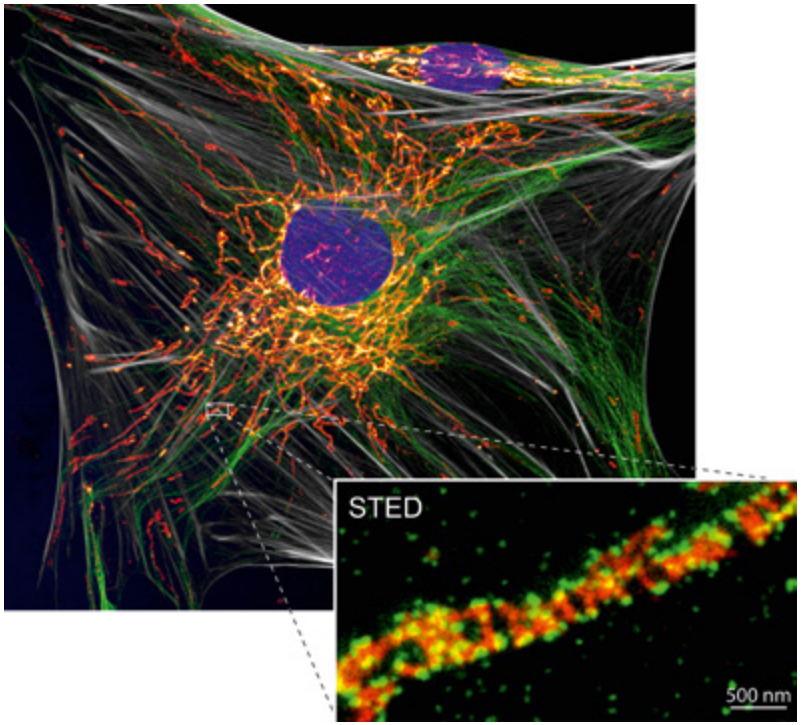
Structure and Dynamics of Mitochondria

Stefan Jakobs
studied biology in Manchester (Great Britain) and Kaiserslautern and received his PhD at the Max Planck Institute for Plant Breeding Research in Cologne in 1999. Subsequently, he first worked in Cologne, then in the Research Group *High-Resolution Microscopy* at the Max Planck Institute for Biophysical Chemistry. Since 2005, he has been leading the Research Group *Structure and Dynamics of Mitochondria* in the Department of *NanoBiophotonics* at the same institute. Stefan Jakobs completed his habilitation at the University of Göttingen in 2007. Since 2010, he has been professor for high resolution microscopy in neurodegenerative diseases at the University Medical Center Göttingen.

Mitochondria are the power plants of the cell. Through the process of cellular respiration, 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 disease.

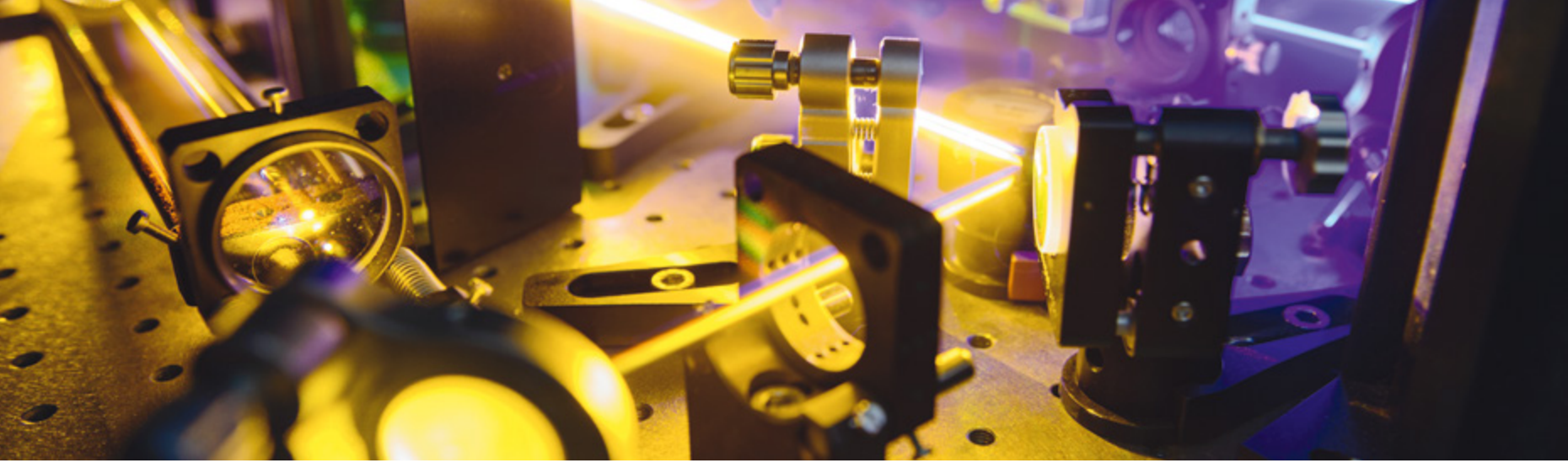
But how are mitochondria constructed in detail, and which molecular mechanisms are behind this architecture? Mitochondria are so nanoscopically small that their internal structure could previously only be examined with electron microscopes. However, for this cells must be chemically fixed and cut into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

In contrast, fully intact cells can be examined by means of light microscopes. However, even with the best conventional microscopes, the spatial resolution is not nearly high enough to examine the interior of the cell's power plants more closely. There-



◀ The overview depicts a human cell in which mitochondria are labeled yellow and red. The nucleus is marked in blue. Furthermore, the actin (white) and microtubule cytoskeleton (green) are visible. In the foreground, a close-up of a single mitochondrion is shown, in which two different proteins are resolved using STED nanoscopy (Mic60, green, and F_1F_0ATPase, red).

Contact
sjakobs@mpibpc.mpg.de
www.mpibpc.mpg.de/jakobs
www.mitoweb.de



▲ Part of a screening set-up with several lasers, mirrors, and filters.

fore, we use new light-microscopic methods, such as stimulated emission depletion (STED), or reversible saturable optical linear fluorescence transitions (RESOLFT) nanoscopy, with which the optical resolution can be dramatically increased.

A glimpse into the interior of the cellular power plant

To this end, we label selected proteins in the cell with dyes or fluorescent proteins. This enables us to subsequently localize the proteins of interest 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 where they influence membrane curvature. Other protein complexes form very large structures that ensure a regular architecture inside of mitochondria – an essential prerequisite for proper cellular growth. In addition to microscopic methods, we also use biochemistry and molecular biology methods to elucidate how such structures are organized and how they support the tiny power plants in their function. We are aiming to understand what consequences it has for the cell when the inner architecture of the mitochondria or the interaction between the different proteins is disturbed.

Novel proteins for nanoscopy

For super-resolution light microscopy, we always require novel and better fluorescent proteins. Hence, we are striving to develop such proteins based on the green fluorescent protein (GFP) in a second research focus. For this, we generate and screen large protein libraries and investigate whether they are suitable for nanoscopic techniques. Our aim is to design fluorescent proteins that can be selectively switched on and off with light flashes. As a result of their particular capabilities, such photochromic proteins provide completely new possibilities for exploring the inner workings of living mitochondria, cells, and even whole tissues.

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* co-corresponding authors

Laboratory of Cellular Dynamics

Thomas M. Jovin
was awarded his M.D. (Doctor of Medicine) in 1964 by the Johns Hopkins Medical School in Baltimore (United States). 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. As an Emeritus Director he has headed the *Laboratory of Cellular Dynamics* at the institute and, until recently, an associated unit at the University of Buenos Aires (Argentina). Thomas M. Jovin has received honorary degrees from the University of Limburg (Belgium) and the University Medical School of Debrecen (Hungary). He is an honorary professor of the University of Buenos Aires and a member of the European Molecular Biology Organization (EMBO).

Contact
tjovin@mpibpc.mpg.de
www.mpibpc.mpg.de/jovin

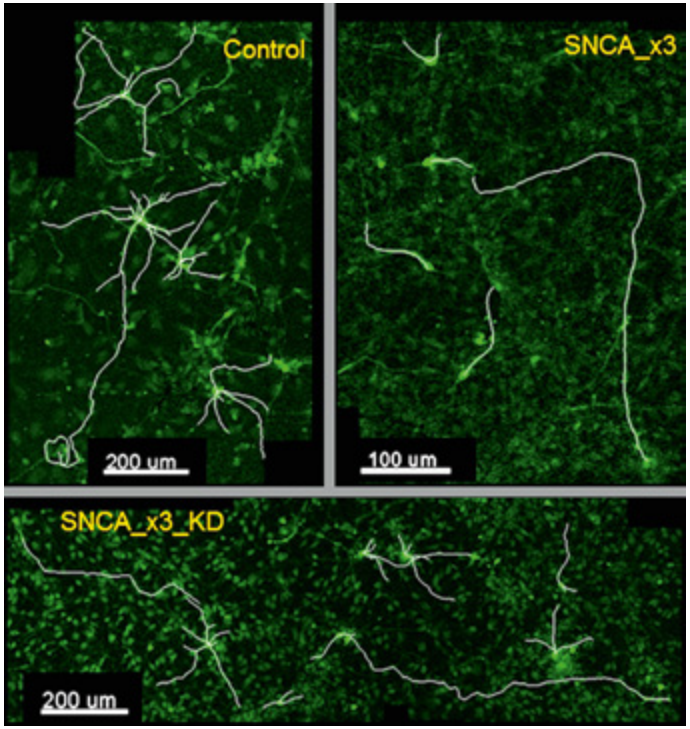
Increased life expectancy leads to a higher risk of developing diseases such as cancer, Alzheimer's, and Parkinson's. Therefore, new possibilities for treating such diseases become increasingly important. In our work, we seek a better understanding of how these diseases arise.

We concentrate our research activities in 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 disorders, for example Alzheimer's disease (AD), is the appearance of protein aggregates – «clumped» proteins – in and around neurons of affected areas, primarily in the brain. In PD, the protein in question is alpha-synuclein.

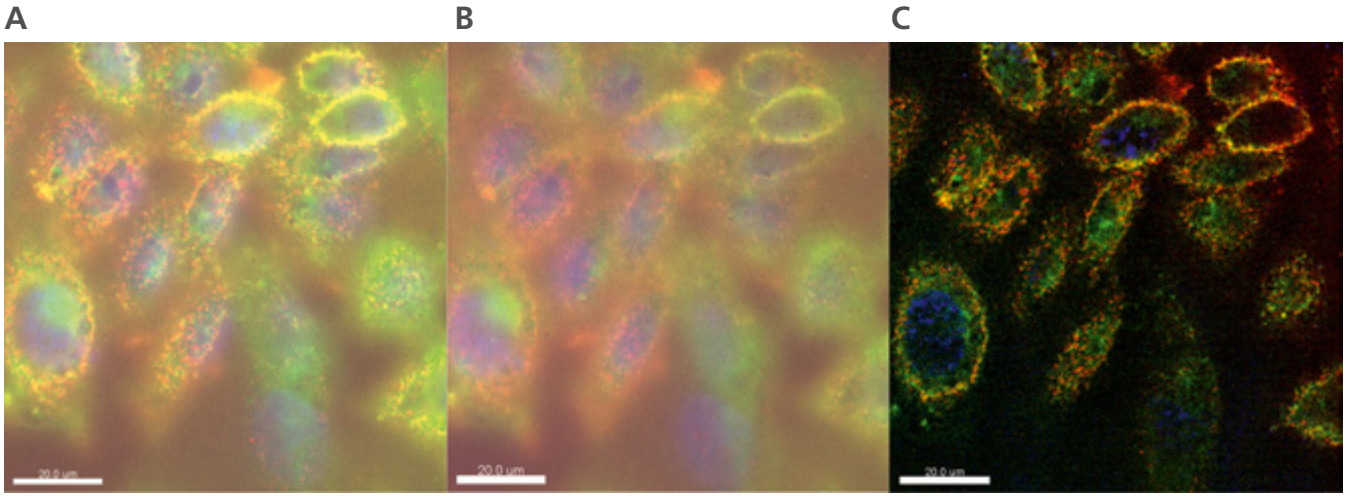
Unfortunately, how the so-called amyloid aggregates are formed and how they and other distinct oligomeric species exert their toxic effects is largely unknown. Answers to these questions are required before one can rationally design drugs to inhibit or reverse the progress of PD and AD. We approach this 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. We employ established laboratory cell lines and induced pluripotent stem cells derived from PD patients.

Tracking molecules in living cells

For the cellular studies, we develop and utilize novel fluorescent probes based on nanoparticles, for example semiconductor nanocrystals (Quantum Dots), and environment-sensitive organic compounds. These sensors are introduced into biomolecules and cells by chemical, physical, and cell expression techniques.



▲ *In vitro* studies help shed light on the mechanism of cell dysfunction in Parkinson's disease. The picture compares induced stem cells from a healthy individual (left, control), from a Parkinson's disease patient with a triplication in the alpha-synuclein gene SNCA (right), and from the same Parkinson's disease patient after SNCA gene expression has been reduced by 2-fold (bottom). Cell lines with SNCA triplication exhibited reduced capacity to differentiate into neurons, decreased neurite outgrowth, and also showed lower electrophysiological neuronal activity compared to lines from healthy individuals. The ability to differentiate into dopaminergic neurons was partially restored by the SNCA gene knockdown. These studies show that higher expression of the SNCA gene leads to dysregulation of genes involved in neuronal development and to higher sensitivity to oxidative stress. (*Oliveira et al.* 2015)



▲ Live multispectral optical sectioning with the fourth generation iPAM: Human epidermoid carcinoma cells expressing the protein ErbB3 bound to mCitrine (yellow) and labeled with Quantum Dot-EGF (625 nanometers, red). The nucleus is stained blue. **A)** Conjugate, in-focus image of the cells. **B)** Non-conjugate, out-of-focus image of the cells. **C)** The final image is produced by subtracting the non-conjugate from the conjugate image. (*de Vries et al.* 2015)

For the application of such probes, we initiated – already in 1997 – a long-term development of a programmable array microscope (PAM) for sensitive, optically-sectioned imaging of living cells with high spatial, temporal, and spectral resolution. The instrument is based on a micromirror array for exciting the fluorescent probes as well as detecting the fluorescent emission in two different channels corresponding to the in-focus and out-of-focus signals. In a parallel effort, a new fluorescence-lifetime imaging microscopy (FLIM) method denoted eeFLIM has been devised using long rather than the conventional ultra-short light pulses.

Donna Arndt-Jovin is responsible for the cell biological research focusing on growth factors and the effects of overexpressed or familial mutant alpha-synuclein on primary neurons and on patient-derived induced pluripotent stem cells. The basic research on signaling is being extended to use in the operating room, for example for detecting and identifying tumor cells in resected tissue as well as in the surgical margins remaining in the organism.

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Electron-Spin Resonance Spectroscopy

Marina Bennati
received her PhD in physics from the University of Stuttgart in 1995. She subsequently worked at the Massachusetts Institute of Technology (MIT) in Cambridge (United States). In 2001, she moved to the University of Frankfurt am Main, where she habilitated in physical chemistry in 2006. She has been leading the Research Group *Electron-Spin Resonance Spectroscopy* at the Max Planck Institute for Biophysical Chemistry since 2007. Furthermore, in 2011, she was appointed professor at the Chemistry Department of the University of Göttingen. She is chair of the DFG priority program *New Frontiers in Sensitivity for EPR Spectroscopy: From Biological Cells to Nano Materials* since 2012. For her scientific contributions she received the Young Investigator Award of the International EPR Society in 2002.

Contact
marina.bennati@mpibpc.mpg.de
www.mpibpc.mpg.de/bennati

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 – they generate a microscopic magnetic field. However, since the spins are oriented in opposite directions, their magnetic effects cancel each other. As a consequence, we are only interested in unpaired electrons, which are magnetically active and serve as highly sensitive probes in our experiments. These so-called paramagnetic centers can provide us with unique information on how complex biomolecules change their structures while they are fulfilling their specific function. With different methods of electron paramagnetic resonance (EPR) spectroscopy we can observe biomolecules under nearly natural conditions or learn about how they act in the living cell.

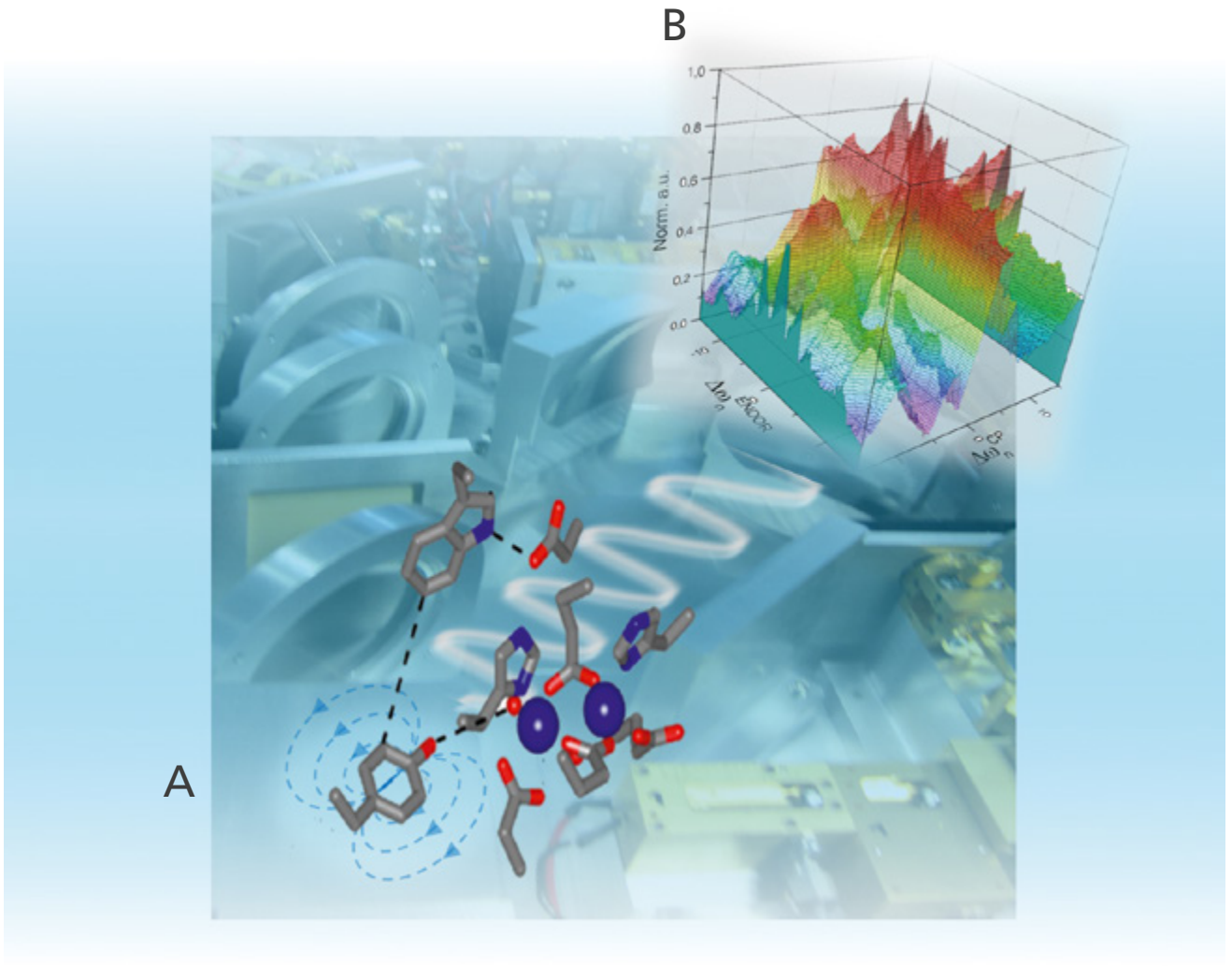
In our group, we develop EPR techniques to simultaneously excite several paramagnetic centers with microwaves or radio frequency in order to manipulate their magnetic interactions. In this manner, we cannot only measure distances between the paramagnetic centers of a protein down to the nanometer range, but also gain important information on their orientations in the biomolecule. Since EPR sensitivity and resolution substantially increase with the applied static magnetic field, we perform our experiments at fields up to ten tesla and excitation wavelengths down to the sub-millimeter range, which is in the regime where sophisticated microwave technology and novel spectrometer designs are required. Therefore, in our group, biophysical investigations are conducted hand-in-hand with methodological and technical developments.

Observing the interior of proteins

Paramagnetic centers are involved in many fundamental biological processes. Representative examples are provided by photosynthesis or the respiratory chain, but also in the biosynthesis of our hereditary material, the DNA. There, enzymes called ribonucleotide reductases (RNRs) play a pivotal role. From bacteria up to humans, the RNR catalyzes the last step in the formation of the individual building blocks, the DNA. In this process, paramagnetic states are generated as a result of the translocation of electrons and protons through and between proteins. With the aid of different EPR 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 investigate the structure of representative classes of biomolecules such as nucleic acids, aggregating proteins, and membrane proteins in collaboration with other research groups of the institute.

Polarization of nuclear spins

In our research we further use EPR in parallel with nuclear magnetic resonance, thereby combining the advantages of both techniques. The magnetic moment of an electron spin is about three orders of magnitude larger than that of protons. If those nuclear spins interact with electron spins, spectra of nuclei can be observed with much higher sensitivity – a feature that can considerably expand the application fields of magnetic resonance. These types of experiments are called electron-nuclear double resonance (ENDOR) if electron spins are detected, or dynamic nuclear polarization (DNP) if nuclear spins are detected. We have been investigating fundamental principles of polarization transfer between electron and nuclear spins and have recently introduced a new concept to allow fast and coherent electron-nuclear polarization transfer. We have been exploring these phenomena also in the liquid state, driven by relaxation mechanisms. We foresee exciting applications in a variety of research fields, ranging from biology to nuclear magnetic resonance imaging (MRI) and material sciences.



▲ The image shows an EPR experiment to observe the active site of an enzyme. Represented are: **A)** the structure of a protein radical in *E. coli* RNR with surrounding amino acids and **B)** a high-frequency (94 gigahertz) electron-nuclear double resonance (ENDOR)-spectrum of protons that are in the vicinity of this radical. Background: View of the microwave bridge for EPR in quasi-optical regime.

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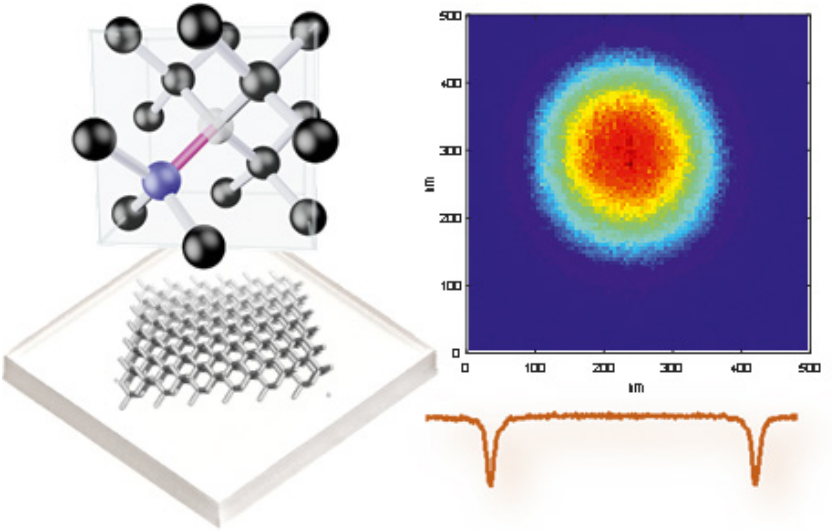
Nanoscale Spin Imaging

Gopalakrishnan Balasubramanian

received his PhD in physics at the Indian Institute of Science, Bangalore (India) in 2005 for studies on electrical and magnetic properties of disordered carbon films. From 2005 to 2006, he carried out research as a postdoctoral fellow at the University of Karlsruhe, investigating the onset of magnetism in low-dimensional materials. Following that, he worked as a postdoctoral researcher at the University of Stuttgart on developing nitrogen-vacancy defects in diamonds for use in quantum sensing and imaging from 2006 to 2010. Since 2011, he has headed the Max Planck Research Group *Nanoscale Spin Imaging* at the Max Planck Institute for Biophysical Chemistry.

Diamonds are brilliant not only as gem stones: Also scientists have become increasingly interested in these precious carbon crystals. Although as a jewel the colorless and flawless variant sparkles brightly, in science the defective diamonds are much more desirable. One such defect is due to impurities in the diamond lattice, for example nitrogen. If a nitrogen atom occupies a vacancy – an empty spot – in the carbon crystal lattice, the diamond gets a characteristic pink color. This defective diamond can be used as a very sensitive and precise quantum sensor. This ability is based on a property of the nitrogen atom's electrons – their so-called spin, which magnetically interacts with nearby atoms and molecules. We can use this «one-atom sensor» to very precisely measure nanoscale magnetic fields, electric fields, spins, charge, temperature, and pressure. The atomic size and exceptional sensitivity of the single nitrogen-vacancy (NV) sensor is a unique combination that outperforms most other sensors and offers possibilities that are beyond the current state-of-the-art technologies.

Our research group is developing new methods and technologies based on this diamond sensor for biophysical and biomedical applications which are beyond the current possibilities. Structural biology has revolutionized life science, yet several molecules of biomedical importance remain unexplored and still pose a challenge to be investigated using current techniques.

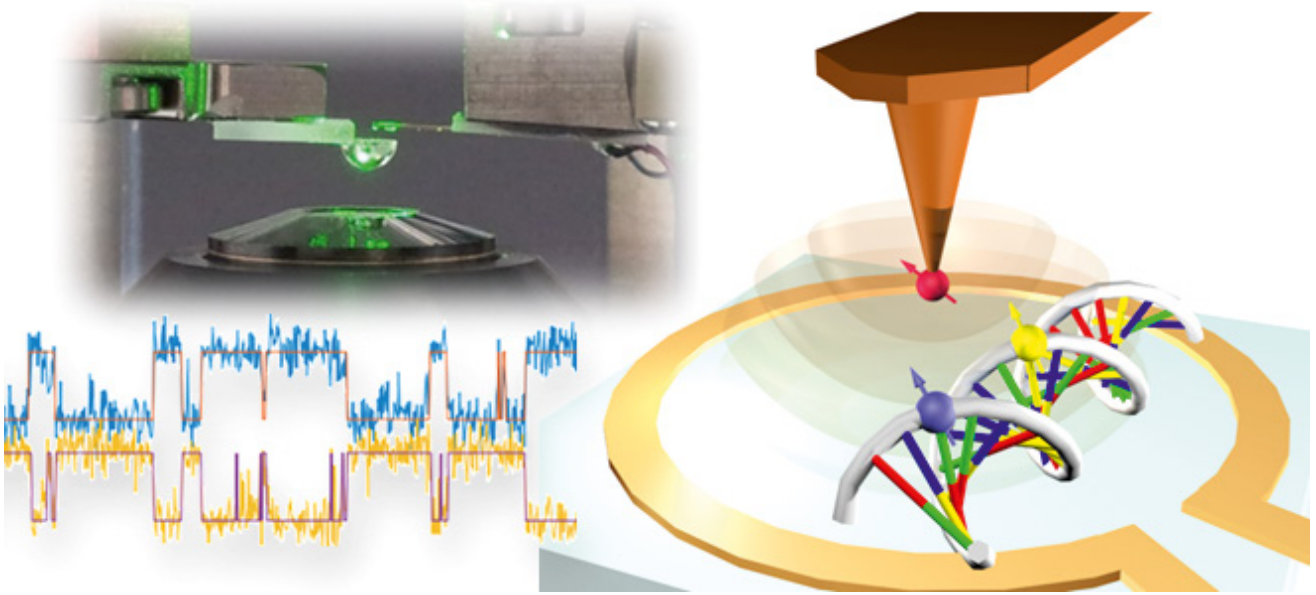


A vacancy in the regular crystal lattice of a diamond can be occupied by nitrogen (blue). This lends properties to the diamond that turn it into an extremely precise sensor – the so-called NV sensor.

Contact

gbalasu@mpibpc.mpg.de

www.mpibpc.mpg.de/balasubramanian



◀ We are developing a microscopy technique using the unique properties of the NV sensor. With this, we can elucidate the three-dimensional structures of single biomolecules (here: DNA).

This necessitates new imaging techniques that could overcome limitations and complement the structural landscape in understanding the molecular mechanism or function of diseases and their cures. To this aim, we are developing a spin microscopy technique that enables imaging single biomolecules.

Images from atomic spin

Atoms, which make up biomolecules, have a spin, which acts like a tiny compass needle that aligns along magnetic field lines. When a biological molecule – for example a protein – is placed in a magnetic field, the spins of its hydrogen atoms orient in this field. If we then subject the molecule to a short electromagnetic pulse, the spins are deflected briefly. We can measure this deflection with the NV sensor. By changing the direction of the magnetic field that is used to align the spins, we record images from different projections or perspectives. We analyze such images using image reconstruction algorithms to obtain the three-dimensional image of a single biomolecule.

The nuclear magnetic resonance (NMR) technique has been used for many years to elucidate the structure of biological molecules. However, until now, scientists could only measure many molecules at the same time, making the result an average, and therefore, minute variations are overlooked. Our group is developing nano-NMR technology that will allow us to create precise three-dimensional images of single molecules and complexes.

Furthermore, we have established interdisciplinary collaborations with other groups to explore unique applications for the NV sensor. Our efforts include developing quantum control strategies for sensing applications as well as techniques for enhancing magnetic resonance signals. Due to the very promising prospect of using diamond sensors, our research aims to contribute new tools and technologies towards the betterment of science and society.

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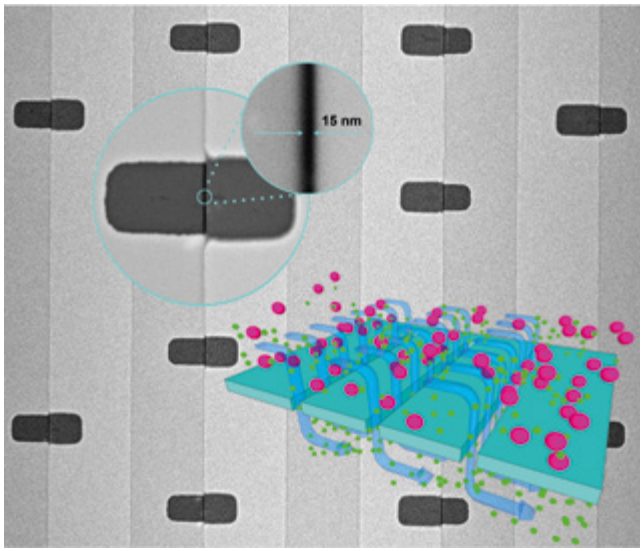
Biological Micro- and Nanotechnology

Thomas Burg

studied physics at the Swiss Federal Institute of Technology (ETH) Zurich (Switzerland) and earned his doctorate in 2005 at the Massachusetts Institute of Technology (MIT) in Cambridge (United States). From 2005 to 2008, he conducted research as a research associate at the MIT Department of *Biological Engineering*. Since 2009, Thomas Burg has been head of the Max Planck Research Group *Biological Micro- and Nanotechnology* at the Max Planck Institute for Biophysical Chemistry.

The microscopic cells that make up all living organisms are amazingly complex. Tiny molecular machines are constantly on the move to create, shape, or recycle intricate structures only nanometers – or millionths of a millimeter – in size. To improve our ability to study these processes experimentally, our group is developing new physical methods with which we can observe, measure, and manipulate the world on the micro- and nanometer scale.

Using biologically inspired artificial nanopores, for example, we are investigating new methods to separate subtly different molecules, and with the help of nanomechanical sensors, we are hoping to better understand why proteins sometimes clump together and give rise to disease. Furthermore, we are interested in new opportunities provided by microsystems technology in the field of microscopy.



▲ Ultra-precise nanofabricated filter membranes are used for experimental studies of complex molecular transport mechanisms in artificial nanopores.

Modern microscopes can render complex three-dimensional cellular structures visible with stunning clarity and detail. However, when any of these structures are changing or moving quickly, it often is impossible to follow the dynamics in real time. This is an especially challenging problem when different methods, such as light and electron microscopy, must be combined in order to understand complex processes. A solution to this problem is simply to flash freeze the object. To avoid damaging delicate structures, however, the water inside cells must be kept from crystallizing. Today, all existing methods to accomplish this have significant shortcomings. Firstly, due to the need for complex sample preparation, researchers today have no way to freeze an object at an exact point in time. Secondly, there is no way to observe the object until just prior to solidification. Both would be important in order to understand which function is carried out by certain structures in the cell, or in what sequence and how quickly a specific process occurs.

At a fundamental level, this challenge is due to the fact that there is no way in nature to extract an arbitrary amount of heat from an object on command. The only way to initiate cooling is to bring the sample into contact with a cold environment, for example by dipping it in liquid nitrogen. To do this, the sample must be removed from the microscope, thus creating a gap of several seconds in the observation.

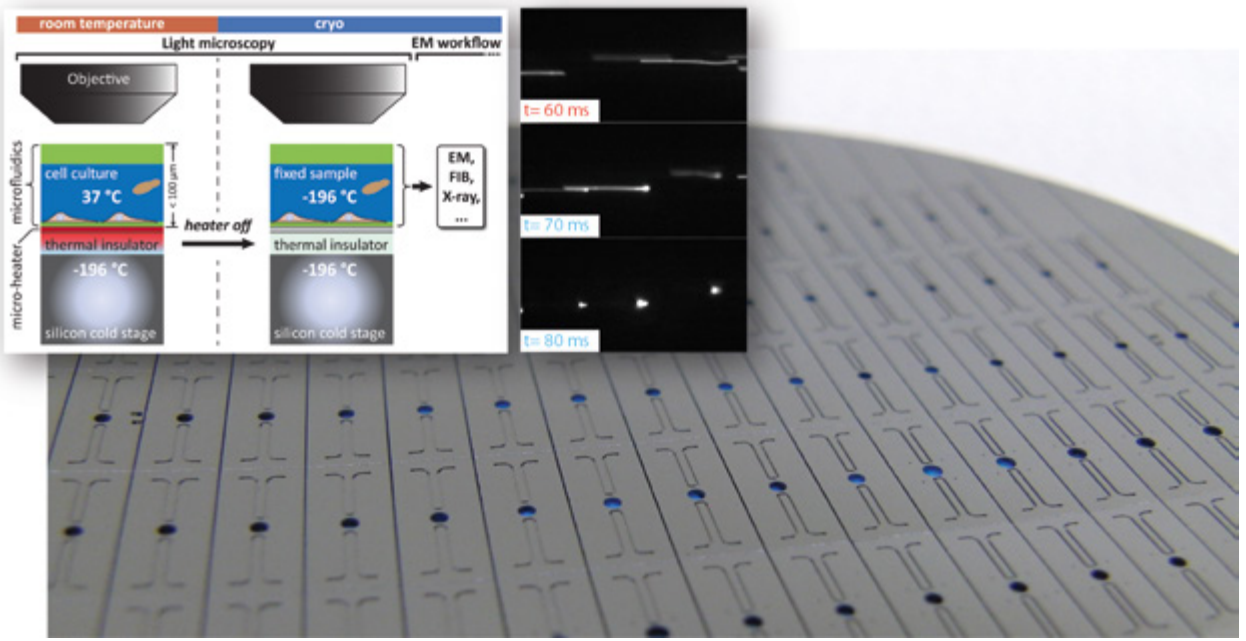
Dynamic processes in real time under the microscope

Microtechnology allows us to bypass this problem with a trick, so that continuous live cell microscopy and cryo-microscopy can be combined seamlessly. For this purpose, we slowly cool the whole environment of the sample with liquid nitrogen, while at the

Contact

tburg@mpibpc.mpg.de

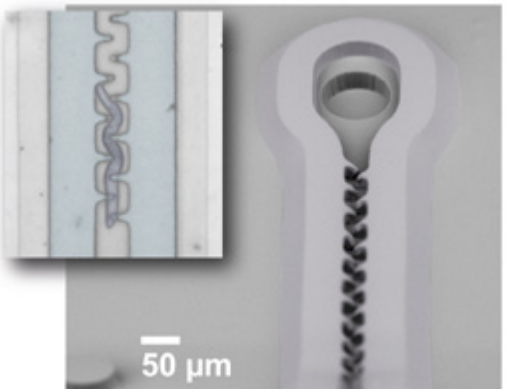
www.mpibpc.mpg.de/burg



◀ Microsystems are used by our laboratory to enable ultra-rapid freezing of biological samples in the light microscope for high-resolution studies at cryogenic temperature.

same time the sample is heated electrically. While the heater is active, a steep temperature gradient between the sample and the cold stage is maintained. The object thus always remains at room temperature, unaware of its chilly environment. At the same time, dynamic processes can be triggered and observed in real time through the light microscope. When the heater is turned off, the fragile temperature gradient collapses rapidly, much like the filament in a light bulb cools rapidly when the current is switched off.

Numerous interesting questions can already be investigated with the above technology. For example, the possibility to heat and cool at controlled rates of more than 100,000 degree Celsius per second is of interest for studying mechanisms that may be relevant for the long-term cold storage of oocytes or sperm. To achieve these rates, the object must be thinner than approximately one hundredth of a millimeter, and the walls that surround it may only measure a few micrometers. This requires novel lithographic manufacturing processes, which our group is developing. Going forward, we expect that this work will help to advance our understanding of the functioning of cells through observations with high resolution in space and time.



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Spectroscopy and Photochemical Kinetics

Jürgen Troe
received his PhD from the University of Göttingen in 1965, where he also completed his habilitation in physical chemistry. In 1971, he was appointed professor at the *École Polytechnique Fédérale* (EPFL) 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 his research with an emeritus group. Since 2009, he also holds a Lower Saxony Professorship at the University of Göttingen. Jürgen Troe is an honorary professor at the EPFL, an honorary doctor of the Universities of Bordeaux (France), Karlsruhe, and Helsinki (Finland), and has received numerous awards, among these the Otto Hahn Award of the German Physical and Chemical Societies and the City of Frankfurt.

Contact
jtroe@gwdg.de / shoff@mpibpc.mpg.de
www.mpibpc.mpg.de/troe
www.uni-pc.gwdg.de/troe

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

In order to investigate such molecular processes, the reactants can be generated by means of photochemical activation or by collisions in hot gases. Molecules absorb light and reach highly active states as a result of the added energy, or active particles are formed which start a series of reactions. We investigate the temporal course of these reactions and the subsequent, frequently 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 differ in nothing but their energy state. As a central part of our work we theoretically model intra- and intermolecular processes by means of classical and quantum-mechanical dynamics. One starts with the calculation of interaction forces and follows the reaction dynamics governed by them.

Reactions of atoms, radicals, electrons, and ions

At present, we are increasingly focusing on reactions of molecular ions in so-called plasmas, that is in the gaseous state of matter in which electrically neutral and charged particles exist in parallel. This state is present on earth in the ionosphere or in electrical discharges such as lightning. In outer space the majority of matter exists in this state.

In addition we are interested in reactions of atoms and radicals generated by sun light and determining the structure of the earth's atmosphere. Such studies help to understand the consequences of air pollution caused by man. The same processes as in the atmosphere – although at much higher temperatures – also play a central role in combustion. Thus, our studies can help to optimize the use of energy and to minimize pollutant emission in technical applications.

The theoretical models following from our research are useful in many areas: from astrochemistry and atmospheric chemistry to plasma- and photochemistry to combustion chemistry. Even large-scale industrial processes can be described with them.

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Structural Dynamics of (Bio)Chemical Processes

Simone Techert
received her PhD in 1997 from the University of Göttingen. After her postdoctoral period at the European Synchrotron Radiation Facility in Grenoble (France), she returned to the Max Planck Institute for Biophysical Chemistry in 2000 with an Emmy Noether junior research group. After her habilitation in physical chemistry in 2005, she directed a Minerva working group until 2011. Since 2012, Simone Techert has been head of the Research Group *Structural Dynamics of (Bio)Chemical Processes* at the Max Planck Institute for Biophysical Chemistry, and she is professor at the University of Göttingen as well as senior scientist at the German Electron Synchrotron in Hamburg. Simone Techert's developments in ultra-fast X-ray science with synchrotron and free electron laser radiation have earned her various prizes, including the ESRF PdP Prize, the X-ray Prize, and a Winnacker fellowship.

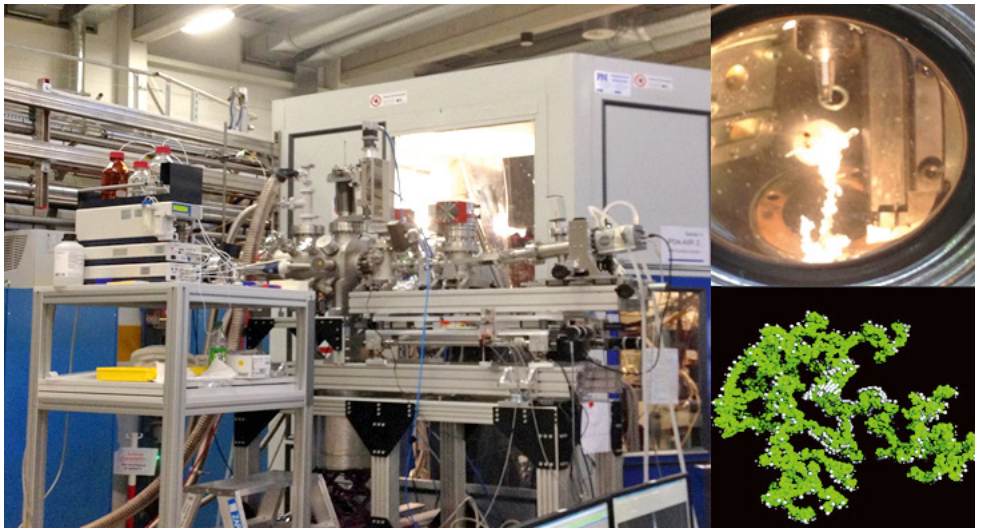
Contact
stecher@mpibpc.mpg.de
www.mpibpc.mpg.de/techert

How quick and how agile is a molecule? How do individual atoms move when one molecule interacts with another in a chemical reaction? What are the elementary time scales? These are the questions we are investigating in our research group.

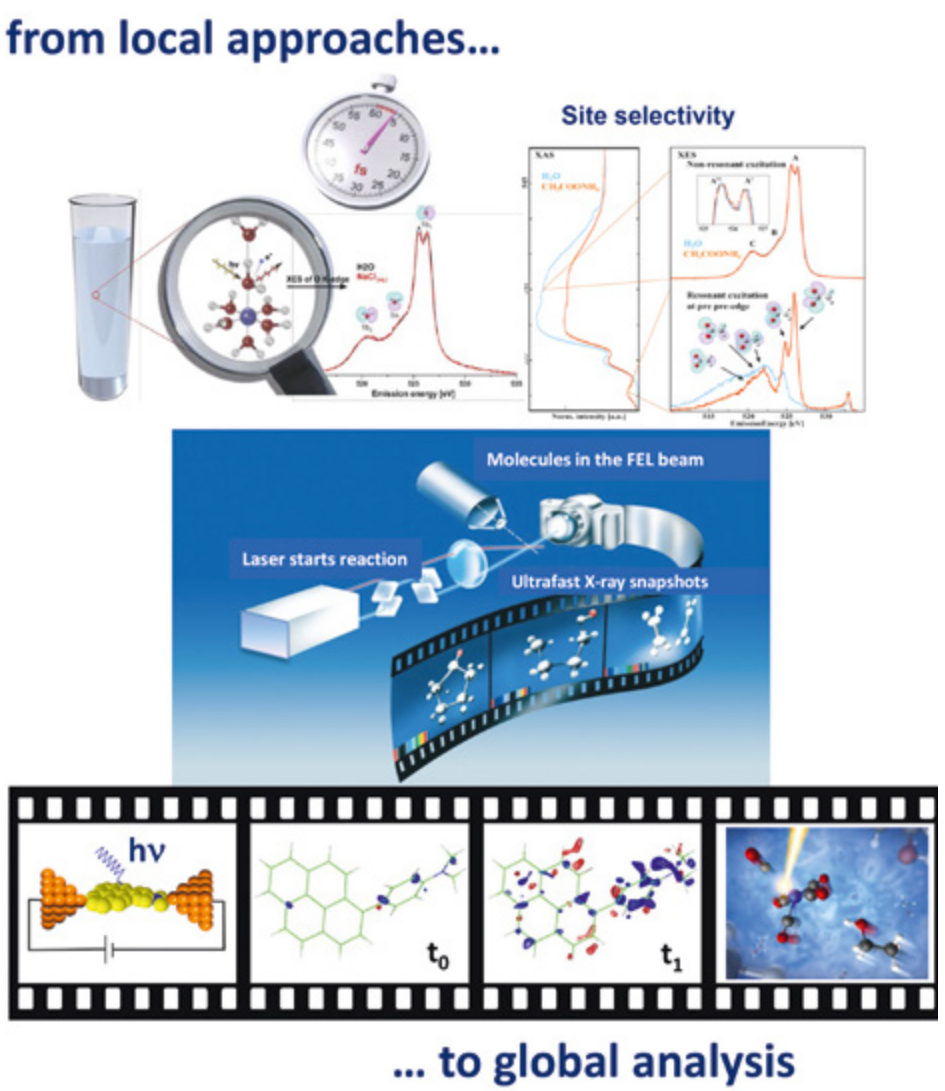
As the smallest units in a molecule, atoms can move within one hundred trillionth of a second. This is also the time period in which the fastest processes occur in a chemical reaction. On the other hand, however, processes in complex systems such as a living cell can extend to minutes or hours. We investigate the extent to which these different time scales depend on each other, and which structural patterns have to be present (or not present) in order to influence these different time scales.

Choreography of molecules

For this purpose, we are developing measuring instruments and methods using optical lasers, high-brilliancy synchrotron X-ray sources, and X-ray lasers, that span the different chemical reaction time scales and allow us to determine in real-time exactly where an atom is at a certain point in time. For example, we use ultra-fast X-ray spectroscopy and ultra-rapid X-ray diffraction to observe what remains of an X-ray flash after a part of the light quanta was collected by the molecules «standing in the way». Thus, we can identify the motions with which single atoms of the molecules under investigation are involved in the choreography of a chemical or biochemical reaction.



◀ Insight into our X-ray laser apparatus: When extremely short X-ray flashes meet with water, chemical reactions occur within a trillionth of a second, which can be used for biochemical analysis purposes.



◀ Ultra-fast X-ray spectroscopy and X-ray diffraction are complementary methods. Combining these, it is possible to monitor ultra-fast bonding changes in chemical reactions very closely. In this way, changes in poorly structured biosystems can be recorded in real-time with atomic resolution.

Based on the very accurate understanding of chemical and biochemical processes gained from this operation, we are developing new materials that convert electrical energy more efficiently into light energy. Such light-active materials are, among other things, used in new types of photovoltaic systems or as biosensors.

In more complex systems, such as living cells, we determine so-called «transient structures», that is, structures that exist only for a very short time. For example, we transfer nucleotides, peptides, or proteins into very short-lived non-equilibrium states by means of external stimuli such as photons, and we demonstrate the rapid change in their structure by combining time-resolved, chemical-analytical measurement techniques with time-resolved X-ray imaging methods.

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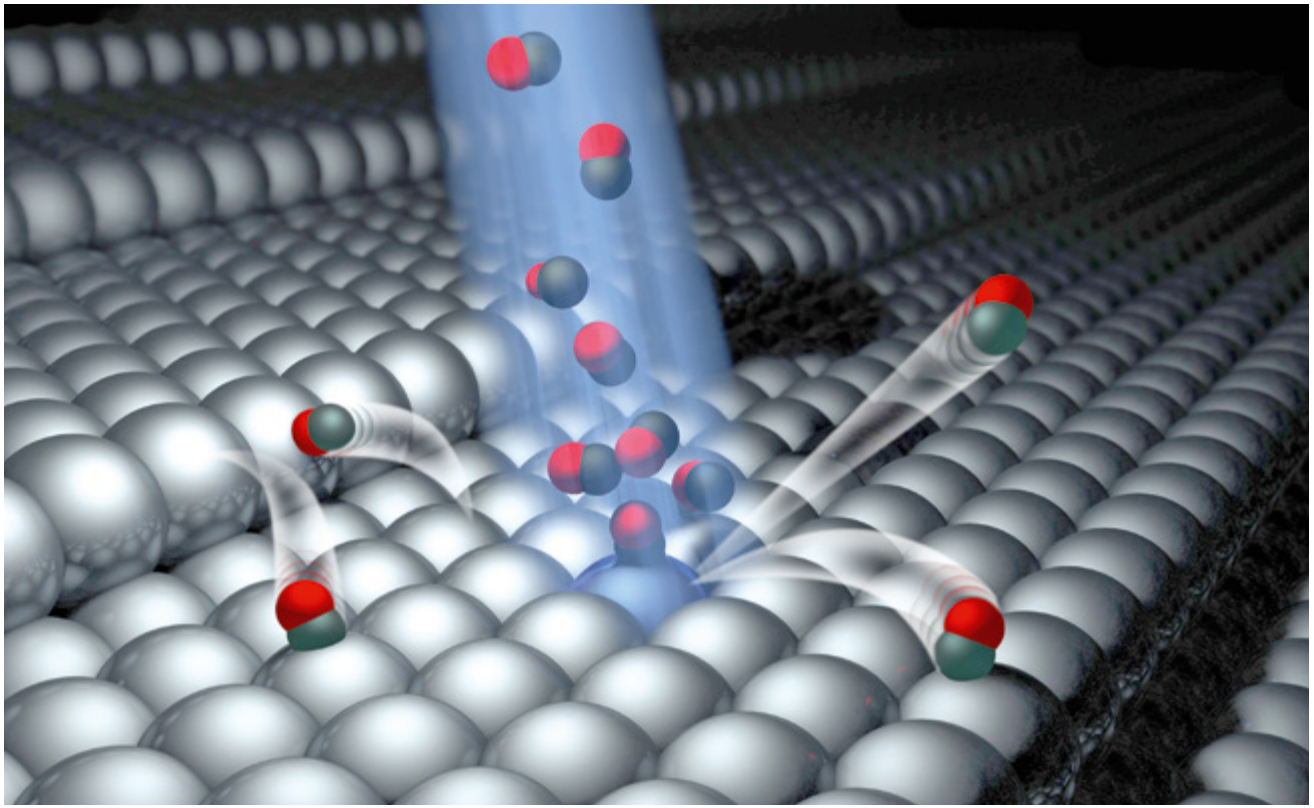
Dynamics at Surfaces

When hearing the term «crash test», most people will have the same picture in mind: A vehicle collides with another vehicle or an obstacle with full force. Such crash tests are used to investigate how a vehicle behaves in a collision and which forces are at work. One can thus identify weak points and potential for improvement.

Crash testing atoms and molecules

But crash tests are not only performed on huge objects like cars. Scientists can make atoms and molecules collide in the laboratory. With such «nano crash tests», it is possible to investigate the physical laws of chemical reactions. Researchers can thus learn more about how energy is stored and converted on an atomic



▲ Chemical reactions at metal surfaces are studied one collision at a time. Using molecular beams, we can dose the surface with molecules in a highly controlled way. We use high performance lasers to detect how fast the molecules are moving before and after the collision and to control the molecule's vibrational motion.

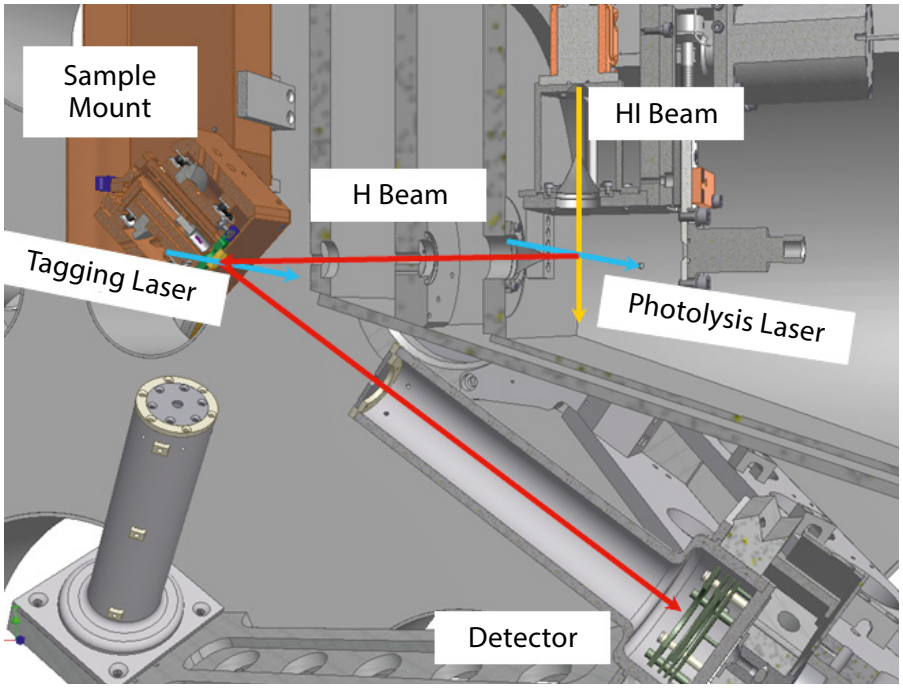
Alec M. Wodtke
received his bachelor's degree in chemistry from the University of Utah (United States) in 1981 and his PhD degree in physical chemistry from the University of California in Berkeley (United States) in 1986. After a two-year postdoctoral research appointment in Göttingen, he served on the faculty of the Department of *Chemistry and Biochemistry* at the University of California, Santa Barbara (United States) from 1988 until 2010. Following a joint proposal of the University of Göttingen and the Max Planck Institute for Biophysical Chemistry, he was awarded the prestigious Alexander von Humboldt Professorship in 2010. Since then, he has been a Professor at the University of Göttingen and Director at the Max Planck Institute for Biophysical Chemistry, where he heads the Department of *Dynamics at Surfaces.*

Contact
alec.wodtke@mpibpc.mpg.de
www.mpibpc.mpg.de/wodtke
www.uni-goettingen.de/en/211983.html

level or how chemical catalysis can be improved. The corresponding field of research is termed chemical dynamics.

In this field, chemical reactions are categorized depending on the relative state of the precursor molecules, which are called reactants: These reactants can exist in the gaseous, liquid, or solid phase. If reactants are in different phases and, moreover, not «thoroughly» mixed, they encounter each other at their interface. This special case is dealt with in so-called surface chemistry.

Our group is particularly interested in what exactly happens when atoms and molecules from the gas phase impinge upon solid surfaces. If you think of these collisions as a crash test, our mission is to report on damage control. To do this, we work according to the guiding principle «follow the energy of the product». When atoms or molecules collide with solid surfaces, other atoms and molecules form as products, which exhibit a different energy than the initial reactants. By monitoring with quantum specificity how the total energy of the reactants is conserved and distributed amongst the interfacial reaction products, we are able to gather valuable information about the crash scene – meaning that we can resolve the circumstances of the collision and answer questions such as: What caused these results? What are the forces at work between the interacting particles? Can we optimize the reaction for a certain purpose? And what are the rate-determining steps?



▲ One of our instruments is used to study the energy transfer of hydrogen (H) atom collisions with solid surfaces. A beam of hydrogen iodide (HI) is dissociated with a photolysis laser beam forming an H atom beam. The H atoms collide with a solid surface held in the sample mount and are thereafter excited with a tagging laser. They fly to the detector where their time of arrival is recorded.

Collisions in ultra-high vacuums

In order to measure as precisely as possible, we need a very complex experimental set-up. It is essential that we control the molecular encounter completely, and this means completely isolating our reactants from other particles and environmental influences. Therefore, we perform our experiments in ultra-high vacuums with about one trillion times less than atmospheric pressure, where no unwanted molecules skitter about, which might interfere with the experiment. Also, we need to carefully clean the surface from atomic «dust». The temperature and morphology of the surface is also carefully probed and controlled.

When everything is set up and ready, the crash test follows. We spray our gas phase molecules into the vacuum using a high-tech version of an automobile fuel injector, called a pulsed molecular beam jet. By controlling the temperature of the jet, we control the speed of the molecular beam. With lasers, we precisely tune the vibration and rotation of the molecules before they strike the surface.

Following the collision, we measure the product molecule's energy. We have recently pioneered a new tool for this: We take pictures of the crash site, so to speak. We use laser beams to «shine light» onto the scattered products and subsequently record their image. From the image data, we can calculate, for instance, the duration of the encounter, or how the energy was redistributed by the impact.

However, many questions we address cannot be answered by experiments alone. Our group has a very powerful theoretical team, which can either simulate our experiments or make calculations *a priori*. This interplay and synergy between theory and experiments has produced quantum leaps forward in the field of surface chemistry.

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Precision Infrared Spectroscopy on Small Molecules

Samuel Meek

studied astrophysics at the University of Oklahoma (United States) and did his PhD at the Fritz Haber Institute of the Max Planck Society in Berlin. After completing his doctoral thesis in 2010, he was a postdoctoral fellow at the Fritz Haber Institute for another two years, followed by two more years as a research assistant at the Max Planck Institute for Quantum Optics in Garching. Since 2014, Samuel Meek has been heading the Otto Hahn Group *Precision Infrared Spectroscopy on Small Molecules* at the Max Planck Institute for Biophysical Chemistry.

Contact

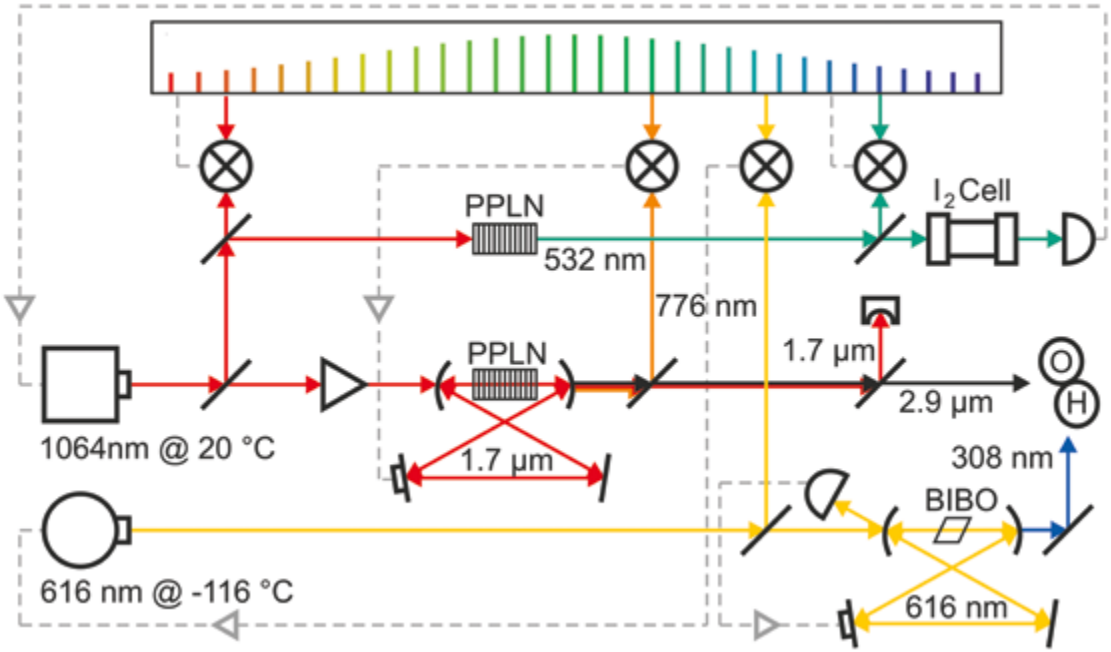
samuel.meek@mpibpc.mpg.de

www.mpibpc.mpg.de/meek

Careful measurements have shown that the proton, the particle at the center of every hydrogen atom, is about 1836.15267389 times heavier than the electron orbiting it. This value seems quite arbitrary, and so far, no theory has been developed that can predict this number. This has led some scientists to speculate that, over time, one of these particles might actually be getting slightly heavier relative to the other. If the ratio were changing, the change must be very slow: Previous measurements have ruled out any change larger than 0.1 parts per trillion per year.

Vibrating molecules

Vibrational oscillations of molecules can provide a sensitive way to detect any changes of the electron-proton mass ratio. Our



▲ A simplified overview of the laser system for the hydroxyl molecule spectroscopy experiment.

group is focused on developing and optimizing laser systems which can measure molecules with extreme precision and possibly detect changes that previous experiments missed.

We have chosen to focus on simple molecules consisting of only two atoms. These molecules only have one way in which they can vibrate, making the pattern of their oscillations simple to understand and to model. In our experiments, we primarily investigate vibrations of the hydroxyl (OH) molecule, consisting of one oxygen and one hydrogen atom. Hydroxyl molecules react quickly with other molecules in the environment, so in order to better study them, we create them in a vacuum chamber, where they can fly freely and undisturbed by other molecules.

The OH molecules in our vacuum chamber will not normally vibrate on their own. In order to cause them to start vibrating, we need to excite them with invisible infrared light from a laser. Laser light has a single, well-defined frequency, and the molecules will only start to vibrate if this frequency exactly matches the frequency of the vibrational oscillation. If the frequency is even just a bit off, the molecules will not be affected by the laser at all. By measuring the exact laser frequency that excites the molecules, we can effectively determine the oscillation frequency of the molecules.

Once excited, the molecules will continue vibrating for many milliseconds, retaining the energy they received from the laser. This can make it difficult to tell whether they have been excited at all. To determine how many molecules have been excited, we introduce a second laser, emitting UV light. The light from this laser causes the molecules to glow, but only if they are vibrationally excited. We can then measure this glow with a sensitive light detector.

A need for extreme precision

How precisely we can determine the vibrational frequency of the molecules is limited by how long we can measure them. Normally, the molecules fly through the vacuum chamber with a velocity of hundreds of meters per second, passing through the laser beam in only a fraction of a millisecond. In order to be able to observe them longer, we make use of a decelerator that slows the molecules using electric fields. Such a device can slow OH molecules to a small fraction of their original velocity, greatly increasing the observation time.

Measuring the energy levels of atoms and molecules more precisely has often helped advance our knowledge of fundamental physics. Nearly 70 years ago, the measurement of the Lamb shift in atomic hydrogen opened up the field of quantum electrodynamics, and in recent years, precise measurements of electronic transitions in hydrogen have indicated that the proton might be smaller than previously thought. Similar measurements on molecules can be used to look for other effects, such as whether fundamental physical constants vary over time, or how the weak nuclear force affects the motions of electrons in a molecule. To see these effects, it will be necessary to construct ever more precise lasers and frequency standards.

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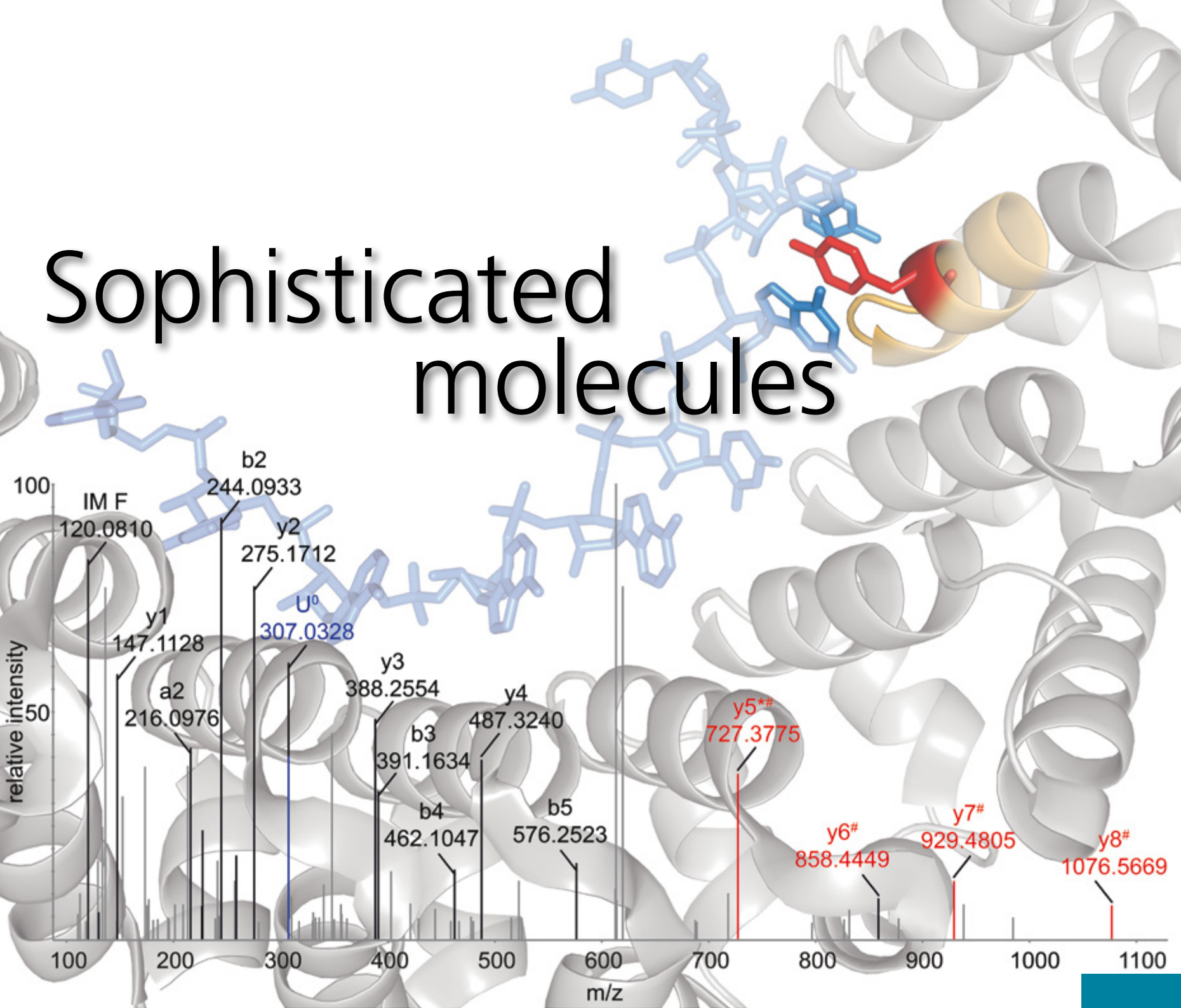
Sophisticated molecules



How structure is related to function

For every purpose the right protein – the human body has hundreds of thousands available. Just take for example our immune system with its vast selection of antibodies, which protect us from pathogens. However, for many proteins the biological task remains elusive. Even less is known about specific functional details: Which forces are at work within and between the proteins? How do the proteins move and change their shape while fulfilling their tasks? Which proteins are present at which point in what amount, and how do they cooperate? To answer these questions, the scientists at the institute apply a wide range of methods, such as nuclear magnetic resonance spectroscopy, mass spectrometry, ultra-high resolution microscopy, or computer simulations.

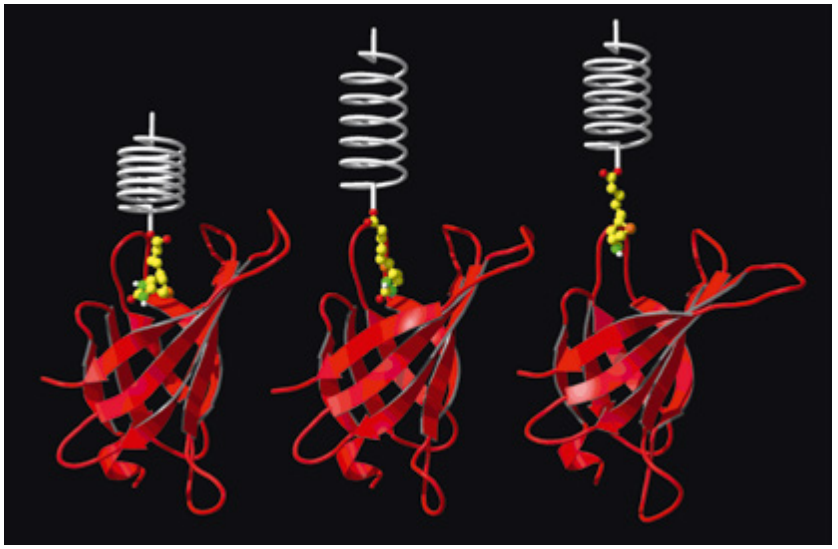
Theoretical and Computational Biophysics

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 Maximilian University in Munich. In 1998, he moved to the Max Planck Institute for Biophysical Chemistry as research group leader. 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.

Practically all processes in the human body are performed and controlled by highly specialized proteins. 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. As is also the case with man-made machines, it is often 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.

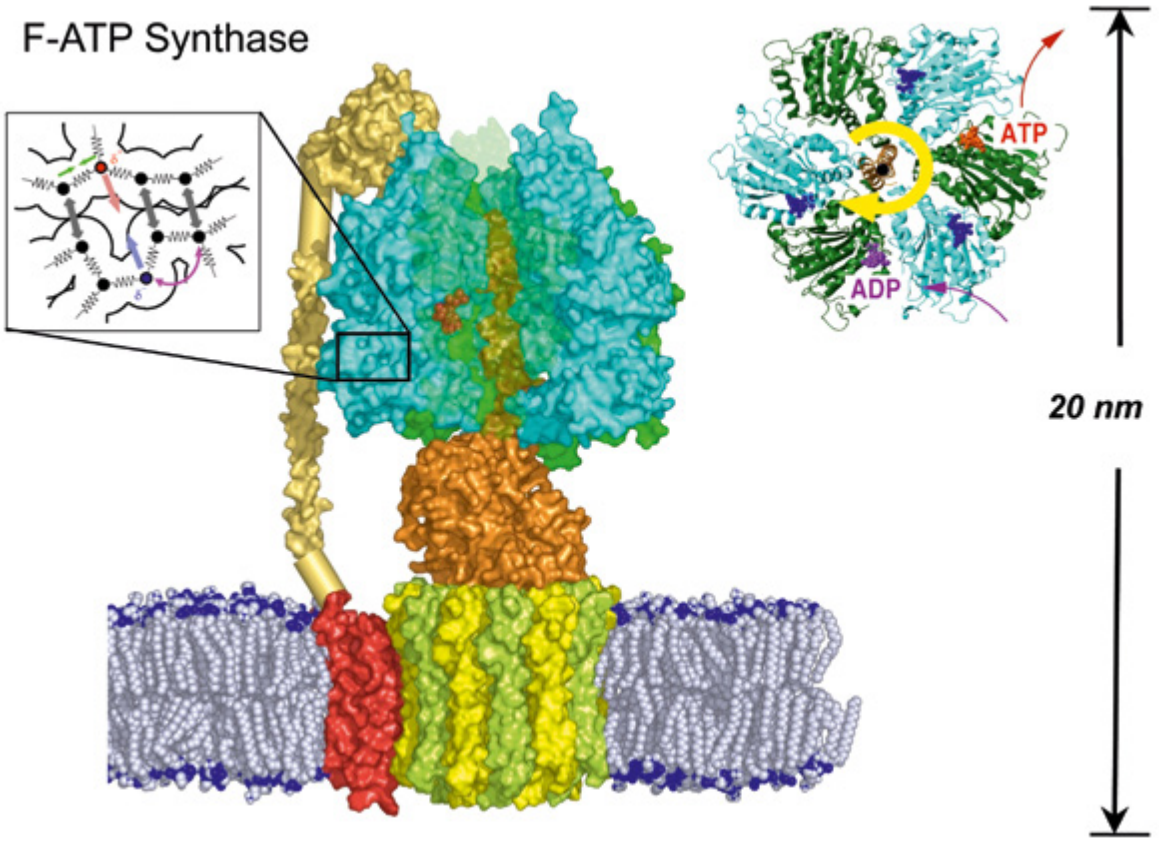
No wonder that minute construction errors can have fatal consequences. Some hereditary diseases, for example sickle cell anemia, are attributable to the fact that a specific protein differs from the normal version by only a few atoms – and that although proteins frequently are composed of many ten thousands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

In order to find out how these nanotechnological marvels function, we use computer simulations. State-of-the-art, high-



◀ Forces often play an important role in molecular nanomachines, but are extremely difficult to measure. Our computer simulations 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 determined using an atomic force microscope; the simulation reveals the underlying mechanism.

Contact
hgrubmu@gwdg.de
www.mpibpc.mpg.de/grubmueller



◀ As a nanomachine par excellence ATP synthase produces the universal «fuel» ATP in the human body. In this snapshot, which we produced using a computer, 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 measuring only 20 nanometers in size 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.

performance parallel computers and increasingly more sophisticated algorithms allow us to calculate the movement of each individual atom in a protein complex with sufficient precision. To understand complex processes of life on the basis of the known physical laws in detail, 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 20 nanometers (millionth of a millimeter) in size works in the power plants of the 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, in peak physical activities even much more.

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. We were able to resolve how this energy transfer occurs in detail by means of computer simulations. 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.

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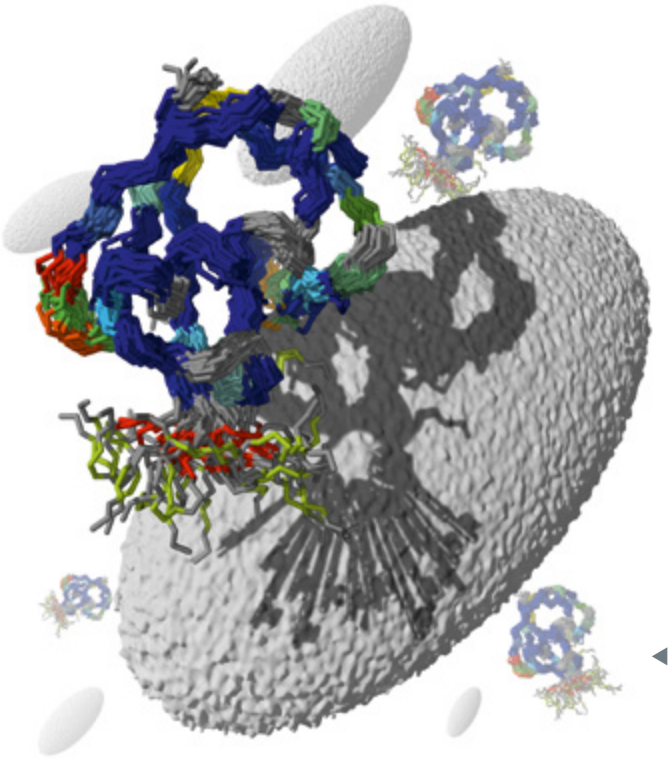
Computational Biomolecular Dynamics

Bert L. 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 postdoctoral fellow in the Research Group *Theoretical Molecular Biophysics* at the Max Planck Institute for Biophysical Chemistry. He has headed the Research Group *Computational Biomolecular Dynamics* at the same institute since 2004. Bert L. de Groot has been an adjunct professor of physics at the University of Göttingen since 2009.

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 bodies. 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 the Research Group *Computational Biomolecular Dynamics*.

To understand the function and dysfunction of proteins, it is usually insufficient to know their three-dimensional structure. 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.



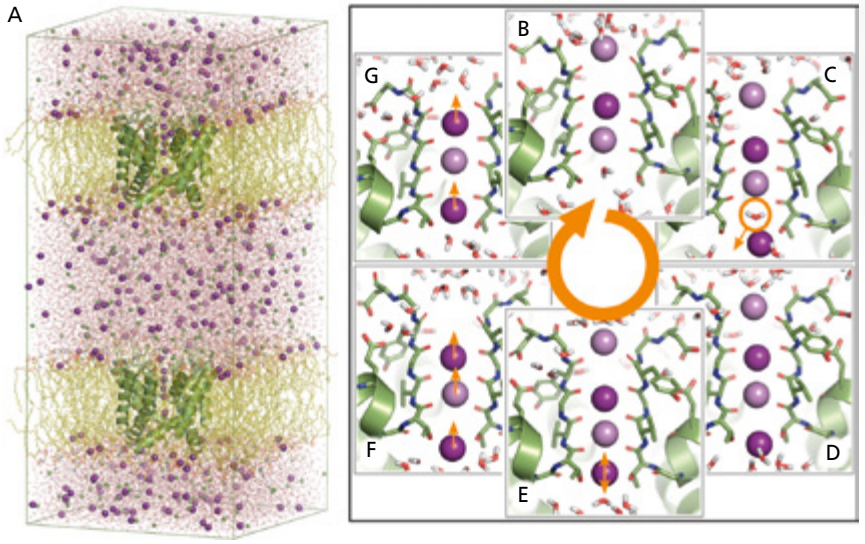
◀ Molecular recognition by ubiquitin. Since ubiquitin can rapidly assume a number of shapes, it recognizes many different binding partners. One can picture it to be like a key ring with which different locks can be opened.

Potassium channels – highly selective ion filters

One class of proteins investigated in the group are ion channels. They form pores in the cell membrane, which function as perfect, highly selective ion filters and allow only specific ions to pass through. This is, for example, the basis of signal transduction in nerve cells. What is the physical basis of such a remarkable selectivity, which simultaneously enables a high efficiency close to the diffusion limit? Molecular dynamics simulations facilitate resolving these questions at the atomic level.

Contact
bgroot@gwdg.de
www.mpibpc.mpg.de/degroot
www3.mpibpc.mpg.de/groups/de_groot

In addition, we are investigating a truly multi-talented protein: 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 incidentally fits its partner.



▲ Permeation mechanism of potassium ions through the selectivity filter of a potassium channel. **A)** A double membrane simulation system is used for computational electrophysiology. **B-G)** An incoming ion displaces a water molecule, resulting in unfavorable ion-ion interactions, allowing the transmembrane potential to drive an upward permeation event.

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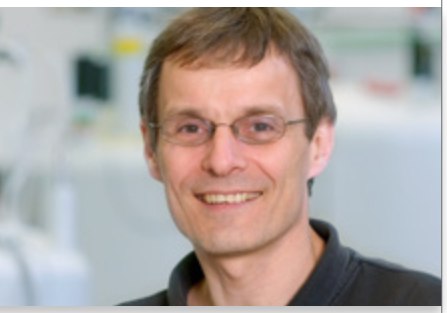


NMR-based Structural Biology

Christian Griesinger
studied chemistry and physics at the University of Frankfurt and received his doctorate in 1986. From 1986 to 1989, he worked as a postdoctoral fellow at the Swiss Federal Institute of Technology (ETH) Zurich (Switzerland). He became professor at the Institute of Organic Chemistry at the University of Frankfurt in 1990. In 1999, the Max Planck Institute for Biophysical Chemistry appointed him as Director, where he has been heading the Department of *NMR-based Structural Biology* ever since. Christian Griesinger has received numerous awards, including the Sommerfeld Prize, the Leibniz Prize, the Bayer Prize, the Ampere Prize, and the Prize of the Korean Magnetic Resonance Society. He is a member of the European Molecular Biology Organization (EMBO) and of several scientific academies. His work on protein dynamics was supported by an ERC Advanced Grant.

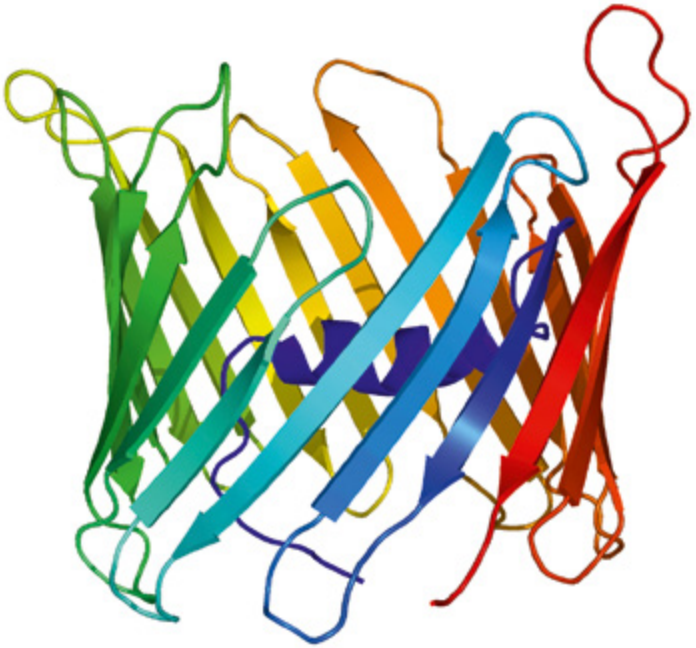
Contact
cigr@nmr.mpibpc.mpg.de
www.mpibpc.mpg.de/griesinger

In the case of molecular «inventories» of the cell, whether proteins or nucleic acids, spatial structure and dynamics are just as important as chemical composition. The fatal consequences which a defect in the shape can cause become evident through hitherto incurable diseases such as Alzheimer's, Parkinson's and Creutzfeldt-Jakob disease. In all three cases, deformed protein molecules accumulate in brain cells and destroy them. However, only if proteins and nucleic acids retain their shape they can fulfill their biological function. We are interested in the question which structural details are important for this and at which time scales the different three-dimensional structures interconvert.

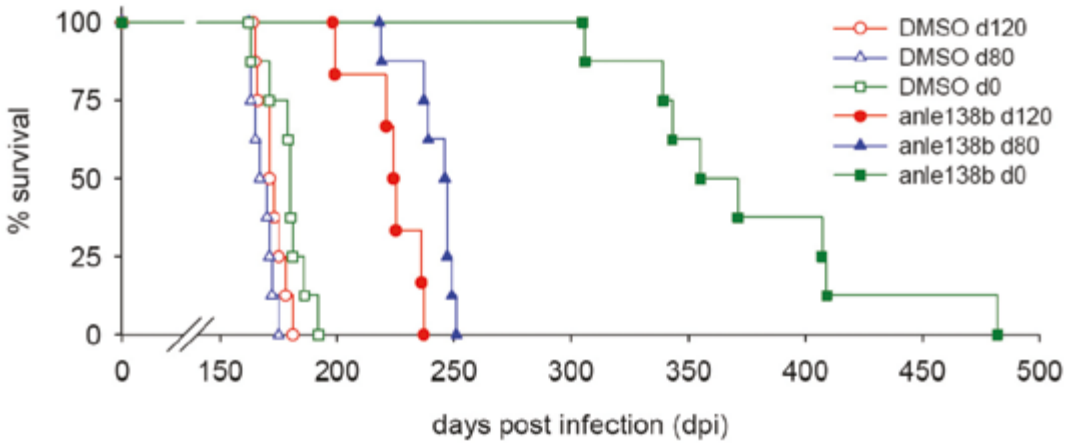
The heart of the matter

Our method of choice is nuclear magnetic resonance (NMR) spectroscopy. NMR makes use of the fact that most atomic nuclei have a magnetic moment. They can be regarded as electrically charged gyroscopes, which try to align themselves with an external magnetic field. Because of this property, the atomic nuclei can absorb electromagnetic radiation of specific energy. The frequency that is absorbed depends on the chemical environment. In a molecule with many atomic nuclei in different positions, a correspondingly large number of different energy portions is required. This results in an NMR spectrum which contains detailed information about the arrangement of the individual atomic nuclei and thus about the atoms' location in three-dimensional space.

However, deciphering this information is an art in itself. And the larger the molecules under investigation, the more difficult this task becomes: To accomplish this, we use so-called triple-resonance experiments, which yield three-dimensional or even higher-dimensional spectra. In the case of protein molecules



▲ Spatial structure of the open form of the molecular channel VDAC, which penetrates the outer membrane of mitochondria – the «power plants» of cells. Via such channels, the mitochondria provide chemical energy in the form of adenosine triphosphate, which accounts for about 75 kilograms daily in the human body. We are currently also pursuing the closed form.



◀ Mice can be used as a model for Creutzfeldt-Jakob disease. If these mice are treated with a compound named anle138b directly after the onset of the disease (d0), from day 80 (d80), or from day 120 (d120) onwards, their survival time on average increases by 200, 70, or 50 days, respectively. We also observe positive effects in Parkinson, Alzheimer, and Diabetes II mouse models.

composed of more than 200 amino acids – the building blocks of all proteins – even this form of NMR spectroscopy reaches its limits. But we are attempting to push these limits even further.

Going beyond the limits

Among other things, we work with magnetically active isotopes of elements that are not otherwise magnetically active. This applies to ordinary nitrogen and carbon, which are selectively replaced by heavier, magnetically active, non-radioactive variants. In the NMR spectrum we can then make either one or the other isotope visible. This way, proteins which are normally too large for NMR spectroscopy can also be analyzed.

In order to be able to use such labeling strategies, which make the spectra simpler and thus interpretable, we must first produce the protein. To accomplish this task, we use genetically modified bacteria that produce the protein in large amounts. Further, electrons, which have a much greater magnetic moment, can be attached to molecules. They provide additional information, which helped to elucidate, for example, the spatial structure and functioning of one of the most common human membrane proteins: the VDAC channel, the cell's «fuel pipeline». These channels in the outer membrane of mitochondria provide the cell with chemical energy in the form of adenosine triphosphate (ATP). We apply this approach also to other vital membrane proteins. Furthermore, we use electrons for signal amplification.

Focus on drugs

We can already investigate very easily how small molecules interact with large proteins with the so-called INPHARMA method. With this technique, medically relevant compounds can be optimized in an early phase. In another project, we work with Armin Giese at the Ludwig Maximilian University (Munich) on a small molecule from our own production, named anle138b, which prevents the above-mentioned misfolding of proteins such as alpha-synuclein (Parkinson's disease), Aβ and Tau (Alzheimer's disease), the prion protein (Creutzfeldt-Jakob disease), and the islet cell amyloid protein (diabetes).

The compound delays diseases such as Alzheimer's, Parkinson's, and Creutzfeldt-Jakob as well as diabetes – at least in the animal model. By using anle138b, the lifetime of a mouse is extended by an average of 200 days when treated with the molecule. We are currently studying the spatial structure of this promising molecule in a complex with its target molecule and are preparing the substance for clinical trials at the company *MODAG*.

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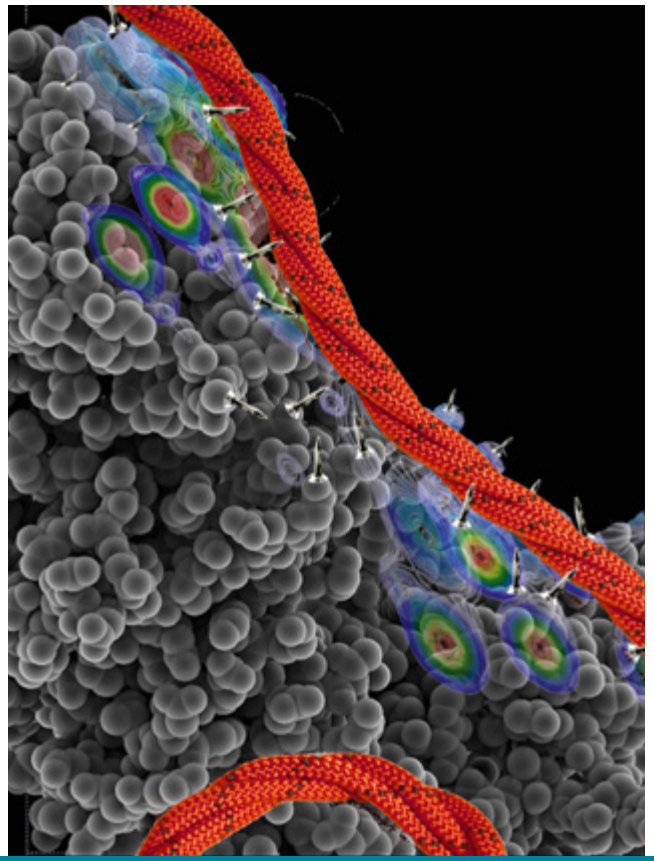
Structure Determination of Proteins Using NMR

Markus Zweckstetter
studied physics at the Ludwig Maximilian University in Munich. He conducted his doctoral research 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 at the National Institutes of Health in Bethesda (United States). In 2001, he established his Research Group *Structure Determination of Proteins Using NM*R at the Max Planck Institute for Biophysical Chemistry. Markus Zweckstetter is also head of the Senior Research Group *Structural Biology in Dementia* at the German Center for Neurodegenerative Diseases and has been teaching as a professor at the University Medical Center Göttingen since 2012.

Contact
mzwecks@mpibpc.mpg.de
www.mpibpc.mpg.de/zweckstetter

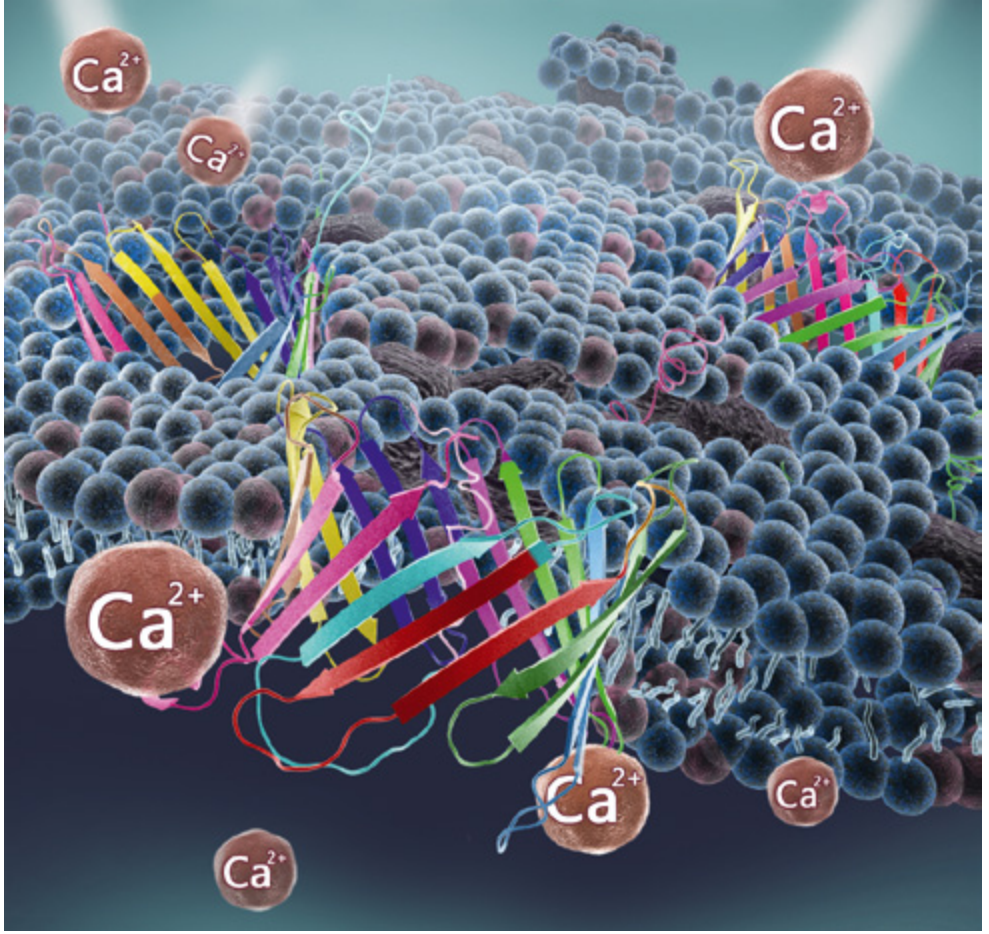
More and more people, particularly the elderly, suffer from neurodegenerative diseases such as Alzheimer's or Parkinson's. In Germany alone, there are 300,000 new cases every year. These diseases often develop because certain proteins do not function properly anymore. This may have various reasons: Often, the shape of these proteins – their three-dimensional structure – is altered. This can have far-reaching consequences: It is the protein's structure that determines whether the protein can bind to other proteins, whether it can be usable as a tool, how well it is soluble, and much more. All these things influence how and to what extent a protein can execute its function in the cell. Therefore, it is important to find out how the three-dimensional structure of a protein is altered in certain neurodegenerative diseases to find new therapeutic approaches.



In order to uncover the structural transformation of proteins, there are few methods more suitable than nuclear magnetic resonance (NMR) spectroscopy. Using NMR spectroscopy, it is possible to determine the structure of even exceptionally «stubborn» proteins. These include proteins embedded in membranes as well as very flexible and dynamic proteins, which, similar to photography with moving motifs, appear blurred when viewed under the microscope. Furthermore, with NMR spectroscopy, one can even analyze so-called «intrinsically unfolded» proteins in atomic detail. These special proteins do not follow the general rule that proteins find their spatial structure by folding in a determined pattern. Instead, in the cell, these proteins switch back and forth between different conformations.

The importance of knowing a protein's structure in detail is well illustrated through Tau. It has been known for a long time that the Tau protein plays a crucial role in a number of neurodegenerative diseases such as Alzheimer's. How precisely Tau contributes to neuronal malfunction, however, has remained elusive. In recent years, using NMR spectroscopy, we discovered

◀ NMR spectroscopy examines how the molecular helper protein Hsp90 binds to the Alzheimer-related protein Tau.



◀ Three-dimensional structure of the mitochondrial membrane protein VDAC.

the diverse structures Tau may take, and how it can be chemically modified and thus regulated by the addition of a phosphate group by the cell, and how it interacts with microtubules – the cell's «transport rails».

Membrane proteins in 3D

Another research area of our group is targeting the structure of mitochondrial membrane proteins. Mitochondria serve as the cell's power plants, supplying it with energy. They differ from other parts of the cell in their unique structure and functional characteristics. Moreover, there is increasing evidence that mitochondria play a central role in age-related neurodegenerative diseases. This makes mitochondria a possible target for drugs to improve the treatment of these diseases. Here, research is focused on mitochondrial membrane proteins, many of which are important for the transport of metabolites and proteins over the mitochondrial membrane.

In recent years, our laboratory determined the three-dimensional structure of two important mitochondrial membrane proteins. One of them, called VDAC, acts as a «gatekeeper» and controls which metabolites may enter and exit the mitochondrion. We were able to show, for the first time, how VDAC changes its shape to fulfill its function as gatekeeper.

The second protein, TSPO, has the task of transporting cholesterol into the mitochondrion. The TSPO structure also shows a small molecule bound to the protein, which serves as a diagnostic marker. This detail is important as TSPO is targeted by pharmaceutical agents in diagnostics and therapy.

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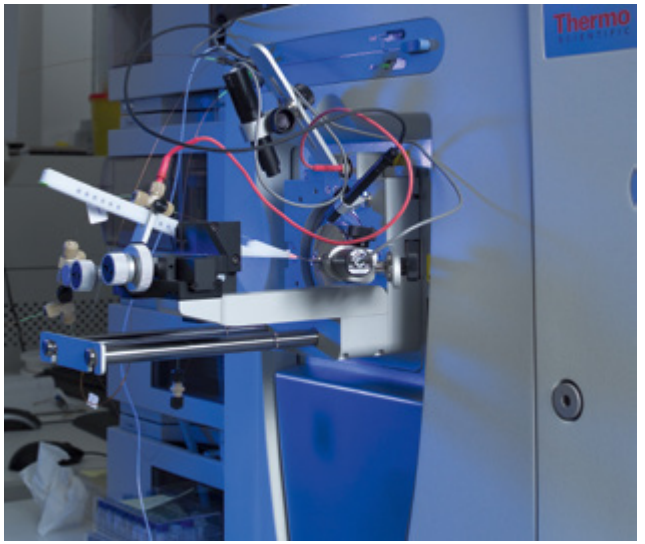
Bioanalytical Mass Spectrometry

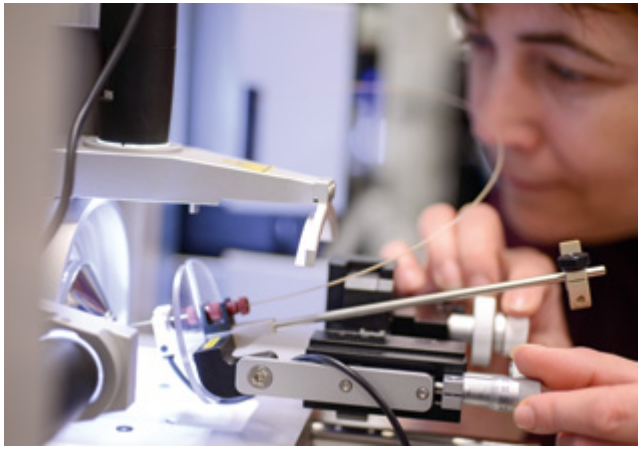
Henning Urlaub
studied biochemistry at the Free University of Berlin and received his PhD at the Max Delbrück Center for Molecular Medicine in Berlin. From 1997 to 2004, he did research as a postdoctoral fellow in Reinhard Lührmann's lab in Marburg and Göttingen. This period included several research stays at the European Molecular Biology Laboratory (EMBL) in Heidelberg. He has headed the Research Group *Bioanalytical Mass Spectrometry* at the Max Planck Institute for Biophysical Chemistry since 2005. Since 2010, he has been professor of bioanalytics at the University Medical Center Göttingen. Henning Urlaub has organized the series of EU-wide Summer Schools on «Proteomic Basics», held annually since 2004.

Contact
henning.urlaub@mpibpc.mpg.de
www.mpibpc.mpg.de/urlaub

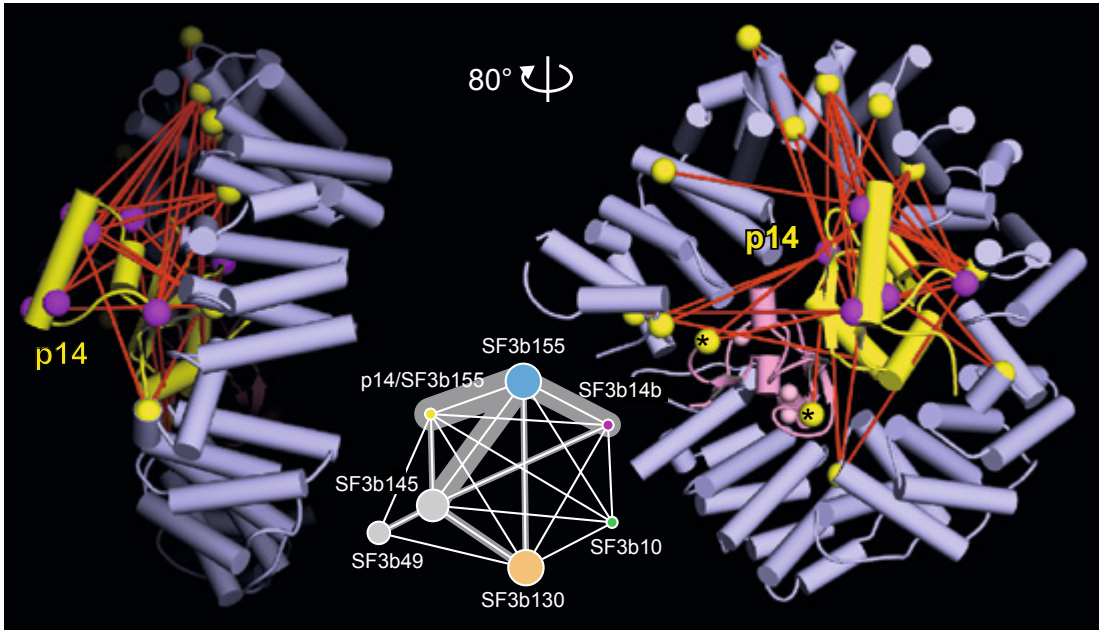
How much does a molecule weigh? Mass spectrometry is used to determine the exact mass – and thus the weight – of molecules. State of the art bioanalytical mass spectrometry of proteins has become a fundamental technique in life sciences. As a result of new methods in bioanalytical mass spectrometry, we can quantify proteins in different cells and developmental stages of an organism. After all, how cells develop and how organisms age is also reflected by their protein patterns. We determine and compare protein patterns of protein complexes and cellular compartments, but also entire cells and tissues. This type of research is generally known as «proteomics». The differences that we observe in this context not only help us understand cellular processes – they also allow conclusions about what occurs in a cell when it develops from a progenitor cell (for example in the bone marrow) to a highly specialized cell such as an immune cell. Moreover, we can investigate how cellular processes change in certain diseases. Beside these «classical» proteomic approaches, we are also interested in how proteins directly interact in the cell with other



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



◀ Adjusting the capillary needle in front of the ion source of an electrospray-ionization mass spectrometer. A voltage of 1,000 to 2,000 volts is applied between the needle (1/100 of a millimeter) and the opening of the mass spectrometer. In this way, tiny droplets of liquid containing the molecules to be investigated are sprayed into the mass spectrometer like a mist of liquid from a perfume bottle. Once inside the mass spectrometer, the solvent evaporates, leaving charged molecules, whose exact masses can then be determined.



◀ Example of how protein cross-links identified by mass spectrometry are used to model the position of a protein (in yellow) relative to another protein (blue) within a protein complex. This is especially useful for proteins that cannot be assessed using other methods such as co-crystallization. The lines connect those parts of the various proteins that were cross-linked and sequenced in the mass spectrometer. The insert shows the overall cross-linking of the entire protein complex with its components. Gray shading indicates cross-links, which are especially frequently found in the mass spectrometer.

proteins, with RNA, or with DNA. To address these questions, we apply various cross-linking techniques. These involve using cross-linking reagents or UV light, both of which permanently connect individual binding partners, thus allowing us to identify them as an entity in the mass spectrometer.

Manageable protein fragments

To achieve the various types of protein identification, we do not usually analyze intact proteins in the mass spectrometer. On the contrary, we initially cleave proteins that have been isolated from cells into smaller, more manageable protein fragments termed peptides. Subsequently, we determine with high precision not only the masses of the peptides, 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 depend on both the sequence and the degree of modification of their amino acids. This must be taken into consideration during analysis. Thus, proteins that are components of cell membranes and carry, for example, bound sugar molecules will require a different procedure for sample preparation and analysis than those that bear phosphate residues (which can switch some genes «on» and «off»). Experiments that determine the modifications of proteins in a quantitative manner are extremely important when one compares normal and altered cells, like for example cancer cells. Differences in protein modification shed light on which biochemical pathways are followed in the respective cell types and which key molecules are influenced in altered cells.

Cellular proteins are not only frequently modified; they are also hardly ever isolated in the cell because they form complexes with other proteins and with biomolecules such as DNA, RNA, or lipids. It is of general interest to acquire knowledge of which proteins interact with which other molecules. We achieve this, as described above for protein modifications, by cleavage of cross-linked proteins and protein complexes, followed by determining the sequences of the still cross-linked peptides in the mass spectrometer. Such studies can be performed on isolated complexes, but also – more challengingly – on cross-linked cellular organelles or even entire cells. Therefore, a further aim of our research group is to improve existing analytical methods and to develop new procedures providing even more detailed insight into cells' diverse protein inventories and their spatial arrangement.

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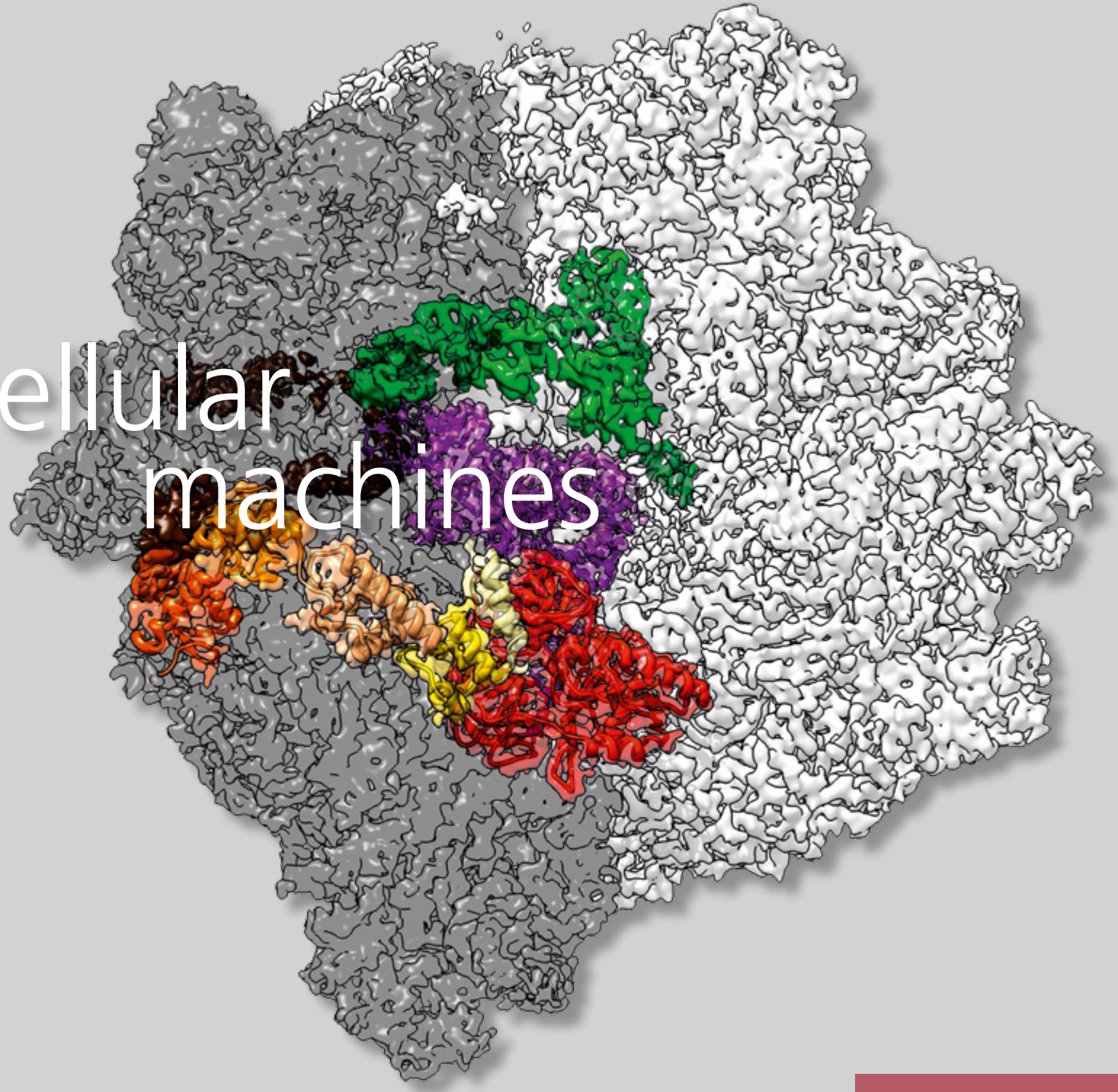
** co-corresponding authors*

Cellular machines



How molecules cooperate

In complex multicellular organisms like the human being, cells share the work: Nerve, immune, or skin cells are specialists in their respective fields. However, also within every individual cell, the division of labor is crucial so that it can reliably fulfill its various functions. For this, a multitude of different molecules must smoothly work together as nanomachines, for example to translate the genetic information into proteins. Which molecular machines are at work here? How do they function in detail? And how is their interaction organized? Such questions are addressed by several departments and research groups by applying biochemical, molecular genetic, microscopic, fluorescence spectroscopic, and computational methods.

Molecular Biology

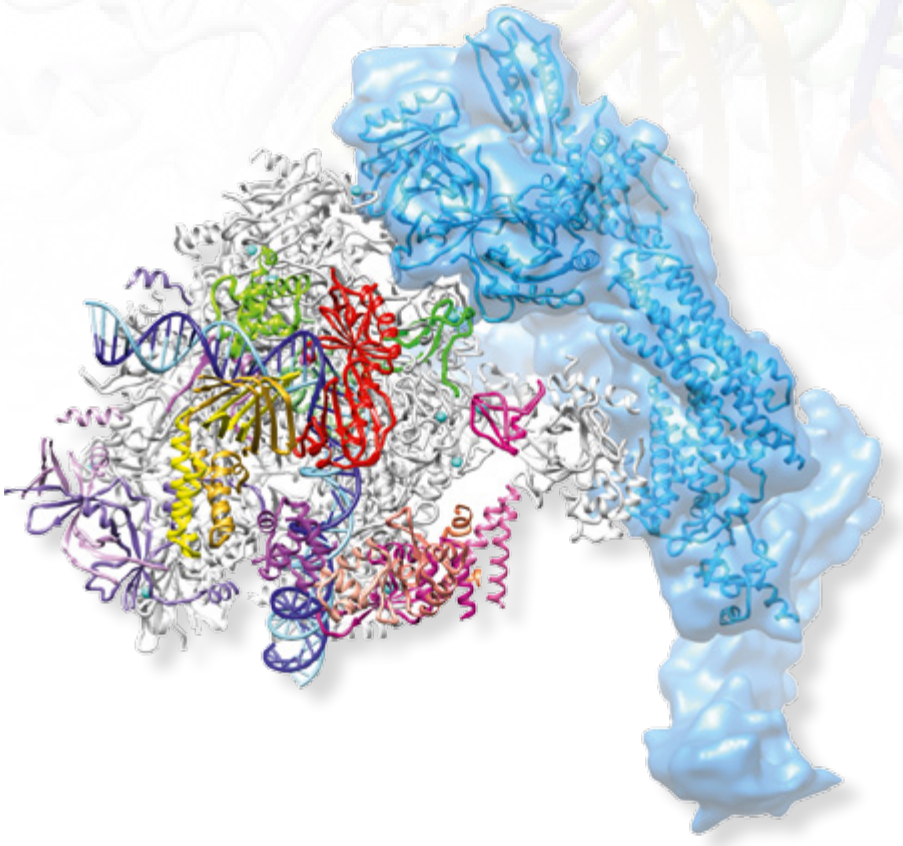
Patrick Cramer
studied chemistry at the Universities of Stuttgart and Heidelberg and was a research student at the Universities of Bristol and Cambridge (Great Britain). He conducted his doctoral research at the European Molecular Biology Laboratory (EMBL) in Grenoble (France) from 1995 to 1998, and was a postdoctoral fellow at Stanford University (United States) from 1999 to 2001. Patrick Cramer then worked as a professor of biochemistry at the Ludwig Maximilian University in Munich from 2001 to 2014. From 2004 to 2013, he also served as Director of the Gene Center Munich. Since 2014, he has been Director at the Max Planck Institute for Biophysical Chemistry. Patrick Cramer received several awards, including the Leibniz Prize and the German Cross of Merit (*Bundesverdienstkreuz*).

Contact
patrick.cramer@mpibpc.mpg.de
www.mpibpc.mpg.de/cramer

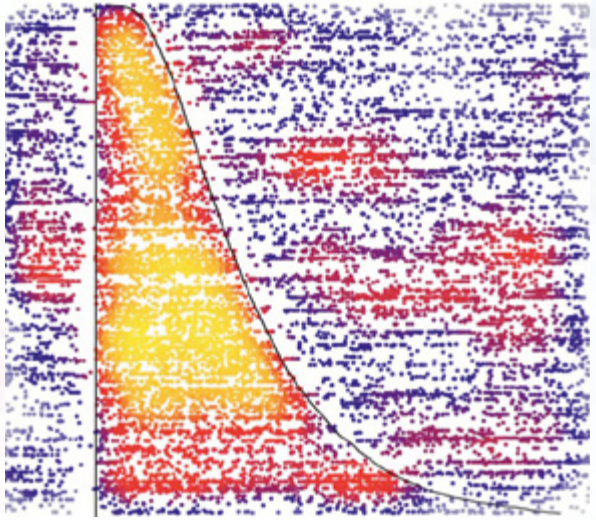
Our body is comprised of billions of cells. But regardless of whether muscle, skin, liver, or nerve cell – they all contain the same genome. It consists of DNA and stores all the information required for the development and maintenance of life. To ensure that each of our cells carries out its dedicated functions, only the required genes are «switched on» and activated.

Genes are activated in a process called gene expression. Our laboratory investigates the first step in this event – the transcription process. During gene transcription, DNA is copied into RNA, which then serves as a blueprint for protein production. The resulting proteins are the «work horses» of living cells and fulfill numerous functions, such as the transport of cargos, energy conversion, signal transduction, or catalysis of enzymatic reactions. The regulation of gene transcription forms the basis of cell growth, differentiation, and organism development. Accordingly, it is not surprising that its deregulation causes severe diseases, including cancer.

Our goal is to understand the molecular mechanisms of gene transcription and the principles of transcriptional regulation in living cells. In one approach, we combine different methods to determine the three-dimensional structures of large protein complexes



▲ The three-dimensional structure of the transcription initiation complex derived from a combination of cryo-electron microscopy and X-ray crystallography.



◀ Binding sites of a protein factor to thousands of RNA transcripts in cells, arranged from short to long RNAs (from top to bottom).

involved in transcription, since shape determines function. This work resulted in a movie depicting many aspects of the transcription process in atomic detail. The main actor in this movie is a molecular nanomachine called RNA polymerase II (Pol II), which transcribes DNA into RNA. The nanomachine, made of twelve protein subunits, cooperates with dozens of accessory proteins to initiate transcription at the beginning of a gene, to elongate the RNA chain as it passes through gene bodies, and to terminate transcription at the end of the gene.

Unravel how genes are switched on

As a recent highlight from our research, we succeeded in determining the structure of the transcription machinery during the initiation phase, when transcription begins. We assembled a complex of 35 proteins with DNA and resolved its architecture by combining cryo-electron microscopy and X-ray crystallography. In the future, we will extend this work to even larger compounds and resolve the three-dimensional structure of unstable transcription intermediates in order to eventually understand the entire process. This work will help us unravel how genes are switched on.

To study how transcription in cells is regulated on a genome-wide level, we also developed and applied techniques based on DNA sequencing and computational approaches. Recent work has enabled us to monitor gene activity in cells and to relate it to the underlying DNA sequence features and the distribution of regulatory protein factors over the genome. A picture emerges from this work about how DNA, RNA, and proteins form functional dynamic networks that regulate cellular life.

As one example, we recently developed a method called transient transcriptome sequencing, which uses metabolic RNA labeling to map the entire range of RNA species in cells, including very short-lived RNAs. In the future, we will study the mechanisms of transcription and its regulation in mammalian cells as well as the deregulation of gene transcription in cancer cells. Eventually, we wish to understand the «regulatory code» of the genome that forms the basis of life.

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Johannes Söding
obtained his PhD in 1996 for work in experimental physics at the Max Planck Institute for Nuclear Physics in Heidelberg. After two years of mainly experimental postdoctoral work on Bose-Einstein condensation of neutral atoms at the *École Normale Supérieure* in Paris (France), he worked for three years as a strategy management consultant with the *Boston Consulting Group*. In 2002, he started his career in bioinformatics as staff scientist at the Max Planck Institute for Developmental Biology in Tübingen and became independent research group leader in 2007 at the Gene Center of the Ludwig Maximilian University in Munich. Since 2014, he leads the Research Group *Quantitative and Computational Biology* at the Max Planck Institute for Biophysical Chemistry.

Quantitative and Computational Biology

It is one of the great enigmas of life how a single fertilized cell can develop into a complex, multicellular organism composed of hundreds of different types of cells. The organism's genome directs the cells to follow individual molecular programs in which the expression of genes into proteins is switched «on» and «off» depending on the exact time and cell type.

Changing research with high-throughput sequencing

Experiments based on determining the sequences of DNA or RNA can now probe with unprecedented breadth and depth these mechanisms by which cells regulate the expression of genes into proteins. The rapid improvement of high-throughput sequencing technology in the last decade is thus boosting the pace of progress in biological research.

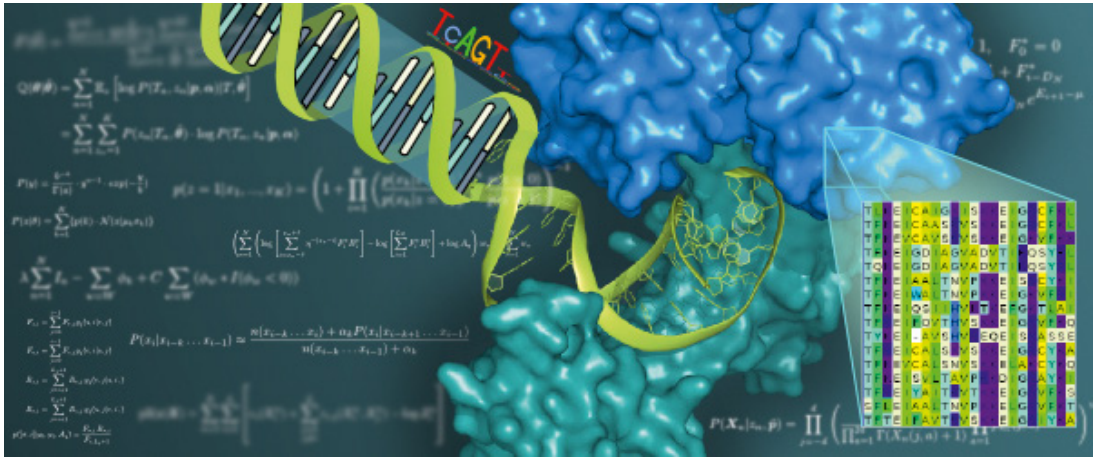
Sequencing technologies are also gaining increasing importance in medical research. In systems medicine, researchers aim to understand the origins of most common diseases by investigating what changes in the genomes of patients predispose to these diseases and what the mechanisms are by which these changes influence disease risk. These insights will help us to develop better drugs to prevent and treat common diseases.

A great advantage of the novel high-throughput, data-driven approach to biological research is that it is unbiased and can lead to unexpected discoveries, as it allows us to ask many questions to the data in little time without the need to formulate concrete hypotheses before the experiment is done.

But the data are often noisier than measurements from conventional, low-throughput methods. Our group develops statistical and computational methods to make better use of the information hidden in these data. In this way we aim to facilitate data-driven approaches to cell and developmental biology, genetics, microbiology, and systems medicine.

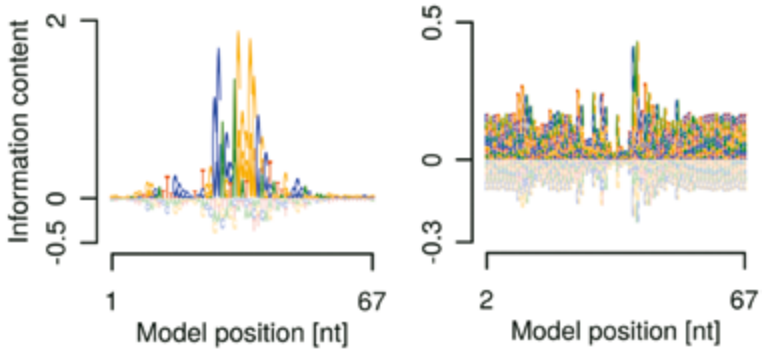
Software tools for biological research

First, our group develops computational methods for predicting the structure, function, and evolution of proteins, the most important building blocks of cells. We develop statistical methods



▲ The group develops statistical and computational methods to analyze data from high-throughput biological experiments investigating transcriptional regulation and methods to predict the structure and function of proteins from their amino acid sequences.

Contact
soeding@mpibpc.mpg.de
www.mpibpc.mpg.de/soeding



◀ The binding site motif models that our motif discovery method *BaMM!motif* learns from regulatory sequences can predict binding strengths of transcription factors (here CTCF) more accurately than the commonly used models (left). The plot on the right shows the information added by learning dependencies between neighboring nucleotides in the binding site sequences.

that enable us to make use of the vast amount of sequence information that is becoming available at an ever-increasing pace. The goal is to provide life scientists with more and more powerful tools in order to guide their experimental work. Our software for the detection of remote common ancestry between proteins based on their sequences (*HHpred*, *HH-suite*) is widely used to predict the function and structure of proteins. Our software *MMseqs* combines high sensitivity to detect related proteins with an extremely high search speed, which is required to analyze the huge datasets of DNA and RNA sequences from environmental probes in the burgeoning field of metagenomics.

▲ Using the tool *HHblits*, we could build a structural model for the human protein Pip49 and, on account of the conservation of the catalytic amino acids (red), predicted it to be a fully functional calcium ion-activated protein kinase.

Understanding the genome's «regulatory code»

Second, we want to help in understanding how the most important level of the regulation of gene expression, namely transcriptional regulation, is encoded in each gene's regulatory regions. We develop computational methods to analyze these regions and to detect regulatory sequence motifs. We also want to predict transcription rates, using probabilistic modeling, statistical physics, and machine learning techniques. We collaborate extensively with experimental groups to elucidate the molecular processes regulating the various steps of transcription.

Probing gene regulatory networks

Third, we have recently begun to develop statistical methods for the derivation of gene regulatory networks, groups of genes that regulate each other and that are at the heart of understanding the molecular programs that the genome encodes. We make use of gene expression measurements of hundreds to thousands of single cells. This new field has the potential to revolutionize developmental biology by providing highly time-resolved time course data of differentiating cells in their natural environments.

We also develop statistical models to predict groups of genes whose deregulation can give rise to common diseases such as coronary artery disease, Parkinson's or Alzheimer's disease. For that purpose we build statistical models to integrate datasets of patients from collaborating groups with publicly available, massive datasets (for example genome-wide association studies and functional genomics datasets).

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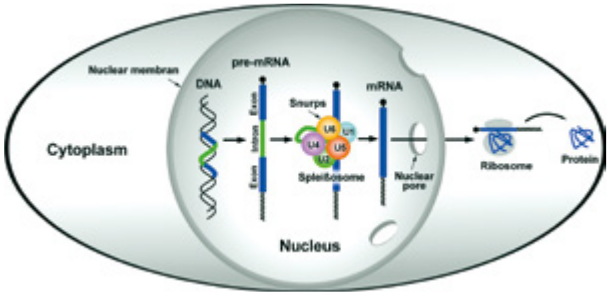
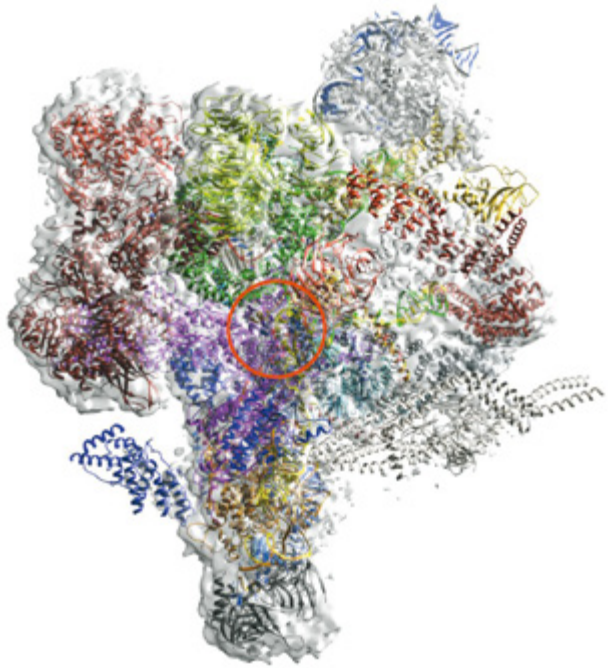
Cellular Biochemistry

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 all of the required exons are contiguously connected in a ready-to-use messenger RNA.



▲ Spliceosomes remove introns from a pre-mRNA to produce mature mRNA which serves as a template for protein production outside the nucleus.



▲ A catalytically active spliceosome has a complex three-dimensional structure. Red circle: position of the catalytic center.

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 machine is comprised of more than 150 proteins and five small RNA molecules (the snRNAs U1, U2, U4, U5, and U6). Many of these spliceosomal proteins are not scattered around the cell nucleus in an unorderly 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 prefabricated complexes and are the main building blocks of the spliceosome.

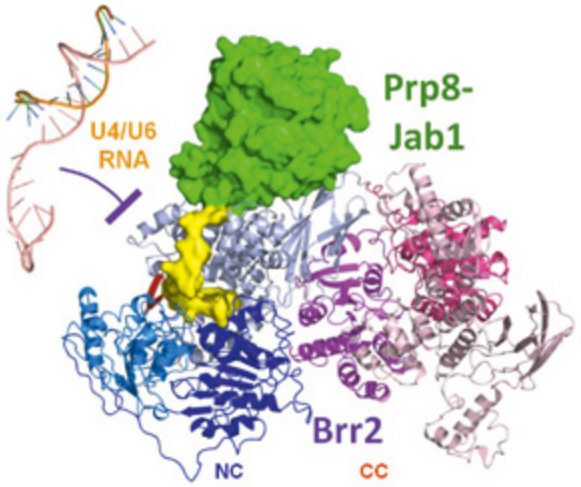
Reinhard Lührmann
received his PhD in chemistry at the University of Münster in 1975. From 1981 to 1988, he headed a research group at the *Otto Warburg Laboratory* at the Max Planck Institute for Molecular Genetics in Berlin. In 1982, he acquired his habilitation in molecular biology and biochemistry at the Free University of Berlin. In 1988, he became professor for physiological chemistry and molecular biology 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 prizes, such as the Leibniz Prize (1996), the Feldberg Prize (2002), the Ernst Jung Prize for Medicine (2003), the Cozzarelli Prize (2006) and the Lifetime Achievement Award of the RNA Society (2014). He is an honorary professor at the University of Göttingen.

Contact
reinhard.luehrmann@mpibpc.mpg.de
www.mpibpc.mpg.de/luehrmann

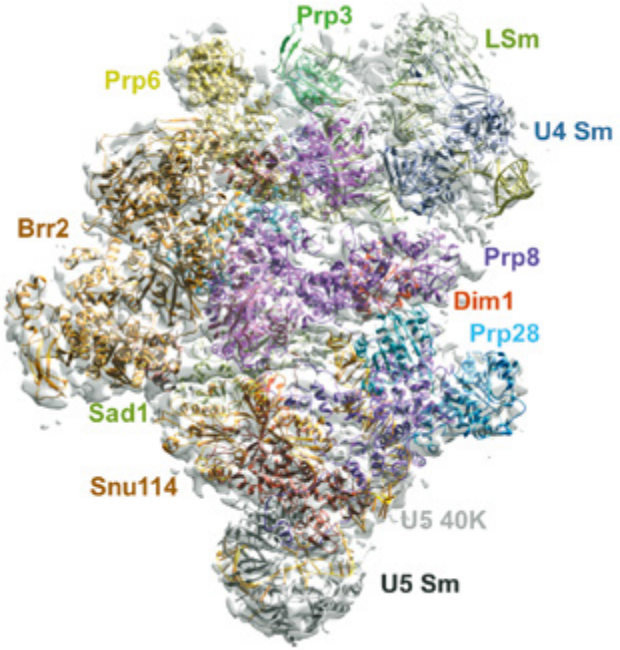
A molecular editing table

The spliceosome is assembled on site for each splicing event. To achieve this, snRNPs and other helper proteins are successively assembled on each pre-mRNA intron. 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 excise the intron, are 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 – are assembled and would like to observe the scissors' action 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.



▲ The enzyme Brr2 plays a key role during the catalytic activation of the spliceosome and is regulated by Prp8's Jab1 domain (X-ray crystal structure of the Brr2-Jab1 complex).



◀ Architecture of the human U4/U6.U5 tri-snRNP, the largest preformed subunit of the spliceosome (cryo-EM structure).

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 provide 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 of such diseases. Recent estimates suggest that more than 20 percent of human genetic diseases are the result of mutations that impair the function of spliceosomes.

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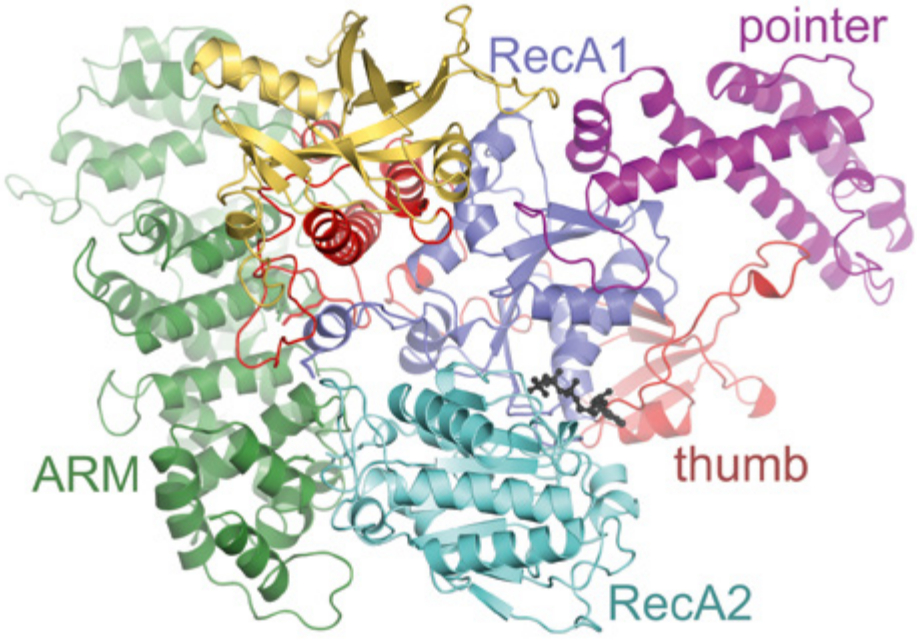
Macromolecular Crystallography

Vladimir Pena
studied biochemistry at the University of Bucharest (Romania) from 1995 to 2000. He remained there until 2001 to work as a research assistant. Subsequently, he joined the European Molecular Biology Laboratory (EMBL) in Heidelberg, where he received his PhD in 2005. He became a post-doctoral fellow at the Max Planck Institute for Biophysical Chemistry in 2006 and established a project group within the Department of *Cellular Biochemistry* at the same institute in 2009. Since 2014, Vladimir Pena has been heading his Research Group *Macromolecular Crystallography* as an independent group leader.

Contact
vlad.pena@mpibpc.mpg.de
www.mpibpc.mpg.de/pena

None of our actions would be possible without enzymes – biocatalysts which allow the numerous biochemical reactions in every living being's body to run properly. Not only proteins but also the nucleic acids RNA and DNA can act as enzymes. One large enzymatic complex in which RNAs play an important role is the spliceosome. This nanomachine is a key component of protein production in our cells – if it makes any mistakes, severe diseases can be the consequence. We are utilizing biochemistry, X-ray crystallography, and, increasingly, electron microscopy to understand how the spliceosome works.



▲ **Figure 1:** Crystal structure of the RNA helicase Aquarius.

RNA and proteins work together in the spliceosome

Proteins are assembled following instructions that are encoded in the genes on our DNA. To synthesize a certain protein, the cell first makes a copy of the gene, the so-called messenger RNA (mRNA). Before the mRNA can be used for protein production, it needs to undergo splicing, a process catalyzed by the spliceosome. Splicing is necessary because the assembly instruction for the protein is not represented in one contiguous piece of mRNA, but in regions called exons, which are intermitted by introns. During splicing, the introns are removed and the exons are joined together. Moreover, either all or just certain exons from one and the same mRNA can be joined to form the final assembly instructions. Hence, the information contained in one gene can be used in a combinatorial way to synthesize different proteins depending on the cell's needs.

The spliceosome needs to be able to react to changes promptly – it is arguably the most complex enzyme of our cells. Within the spliceosome, five distinct small nuclear RNAs (snRNAs called U1, U2, U4, U5, and U6) and about 150 different proteins work together. The snRNAs catalyze the biochemical reactions of splicing, whereas the proteins construct, maintain, and remodel the spliceosome during its work cycle. Our goal

is to understand the structural basis of mRNA splicing. Here, we place particular emphasis on the spliceosome's regulation.

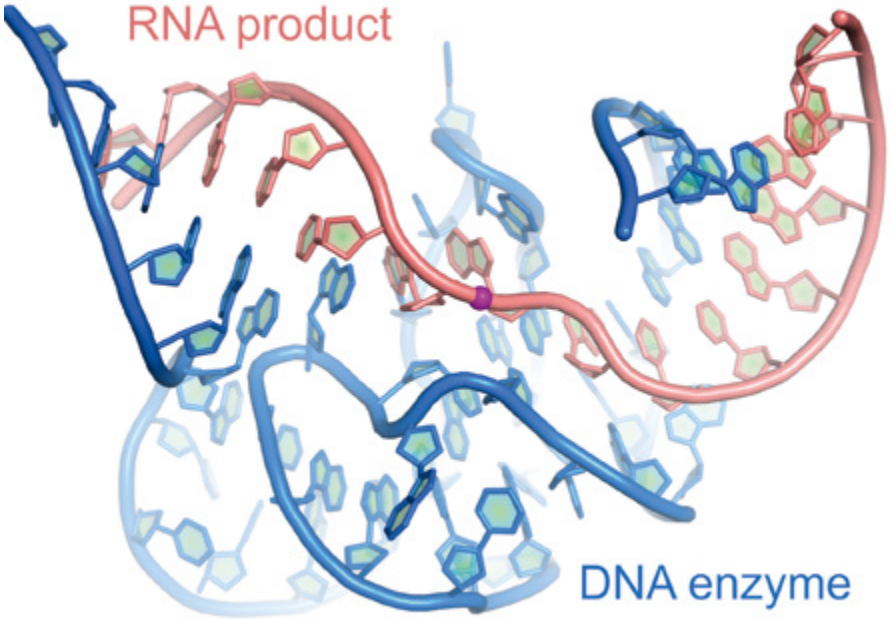
Helicases: the main driving forces of the splicing cycle

In a cyclic pathway, the spliceosome undergoes numerous stages, during which it varies in structure and composition as well as in its internal RNA-RNA and RNA-protein contacts. The driving forces and control mechanisms of these remodeling processes are provided by so-called RNA helicases.

We recently determined the structure of an RNA helicase called Aquarius (Figure 1) and showed that it mediates important interactions within the spliceosome and helps to properly remove the introns and correctly join the exons. Aquarius also maintains the structural integrity of the intron-binding complex and helps to correctly position the exon junction complex. In the future, we would like to elucidate the molecular details of how Aquarius fulfills these tasks.

DNA enzymes

DNA is known mainly for its capacity to encode information in the form of genes. However, twenty years ago, scientists identified DNA molecules that catalyze various chemical reactions. To date, not much is known about how such DNA enzymes function. We have now determined the first crystal structure of a DNA enzyme (Figure 2), in collaboration with the lab of Claudia Höbartner at the University of Göttingen. We plan to determine the structures of further DNA enzymes and to elucidate how they can catalyze reactions. We would like to use this knowledge to synthesize DNA enzymes for scientific applications – they could possibly be of medical importance in the future.



◀ **Figure 2:** Crystal structure of a DNA enzyme (9DB1) catalyzing the ligation of two RNA molecules.

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Physical Biochemistry

▲ Marina Rodnina, Cristina Maracci, and Wolf Holtkamp (from left).

Marina V. Rodnina
studied biology in Kiev (Ukraine) 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 at this university as a research assistant from 1992 to 1998. After her habilitation in 1998, she was appointed university professor there and, from 2000 to 2009, she held the chair of physical biochemistry. She has headed the Department of *Physical Biochemistry* at the Max Planck Institute for Biophysical Chemistry as a Director since 2008. She has been a member of the European Molecular Biology Organization (EMBO) since 2004 and of the Leopoldina – National Academy of Sciences since 2008.

Contact
rodnina@mpibpc.mpg.de
www.mpibpc.mpg.de/rodnina

In the cell, nothing happens without proteins. They maintain the cell's shape, drive biochemical reactions, and provide for transport and communication. In all cells, proteins are produced by molecular machines called ribosomes. The ribosomes interpret the information encoded in the genes in order to produce functional proteins out of their building blocks, the amino acids. This process, termed translation, makes heavy use of the resources of the cell and consumes a large amount of energy. The well-being of cells depends on how many ribosomes they have and how well they work. On one hand, defective translation can result in a variety of diseases, including cancer as well as neurological, immunological, infectious, metabolic, and mitochondrial diseases. On the other hand, drugs that target ribosomes can be used to kill pathogens such as bacteria or fungi.

Our lab is interested in understanding how ribosomes work and how translation is regulated in health and disease. We use a wide range of experimental approaches including biophysical methods such as fluorescence spectroscopy, rapid kinetics, and single-molecule techniques, as well as biochemistry and genetics to study the mechanisms of action of this ancient macromolecular machine.

Precision work

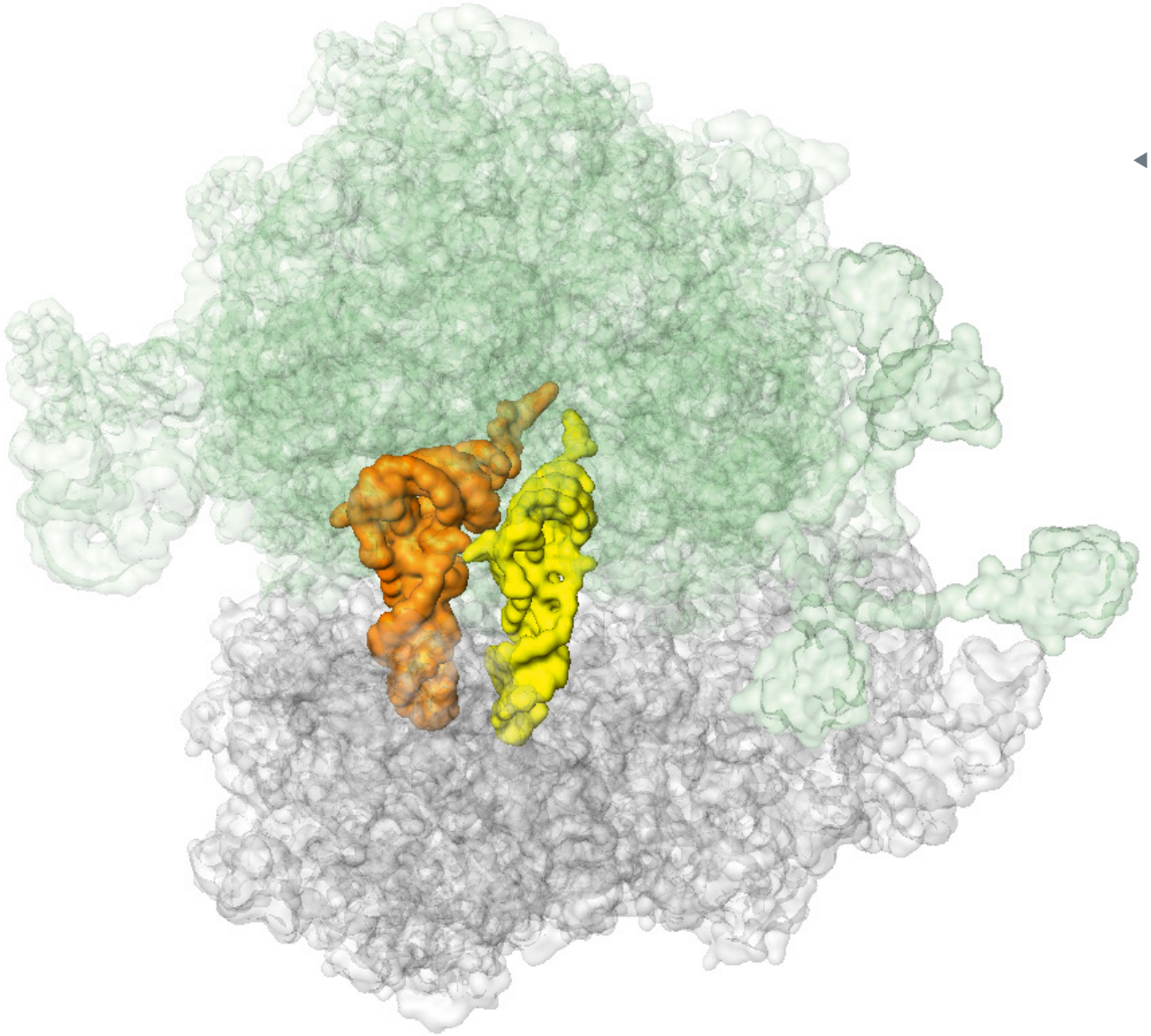
Even when hundreds of amino acids are linked to each other to form a protein, each one of them can be important, as a single incorrect building block may render the protein non-functional. A defective protein can cause damage or increase the cost of quality control and clean-up procedures in the cell. We want to estimate how often the ribosomes make mistakes, how they manage to keep the error frequency manageable, and how they cooperate with other machineries in the cell to get rid of erroneously made products.

Programmed «errors»

Sometimes, the ribosomes make errors on purpose. These programmed errors can enrich cellular diversity by producing two different proteins from one coding sequence. Moreover, the ribosomes can sometimes re-code the message and incorporate special amino acids such as selenocysteine, which do not belong to the repertoire of the 20 standard amino acids. But which mechanisms allow such exceptions to the rule? Once we understand that, we also hope to be able to better understand how errors are normally avoided. Someday, such knowledge could also be used in medicine and biotechnology to synthesize «designer proteins».

A paradigm for a molecular machine

While the ribosome is progressively assembling a protein, it moves along its track, the messenger RNA (mRNA). We want to understand how the ribosome converts thermal and chemical energy into directed movement. What is the molecular choreography of the movement? Each time the ribosome steps along the mRNA, it moves by precisely one codon – the unit on the mRNA that tells the ribosome which amino acid it should add to the protein it is presently producing. How does the ribosome maintain this precise step? Answering these questions will give us a deeper understanding of how molecular motors work and how living cells utilize energy.



◀ Structural model of a bacterial ribosome synthesizing a protein. The two transfer ribonucleic acids (orange and yellow) which dock on it bring appropriate amino acids (not shown).

Defining the final shape of proteins

While proteins are essentially a chain of amino acids, they are not linear in their final form. To fulfill their functions in the cell, proteins have to fold into complex, precisely defined three-dimensional structures. Folding starts while the proteins are still being synthesized, and the ribosome can guide the emerging protein to assume its ultimate shape. We want to understand how the ribosome helps the protein find the correct way to its native shape. Deviations from correct folding can lead to severe diseases. Better understanding the code that regulates protein maturation on the ribosome will help prevent these diseases.

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Ribosome Dynamics

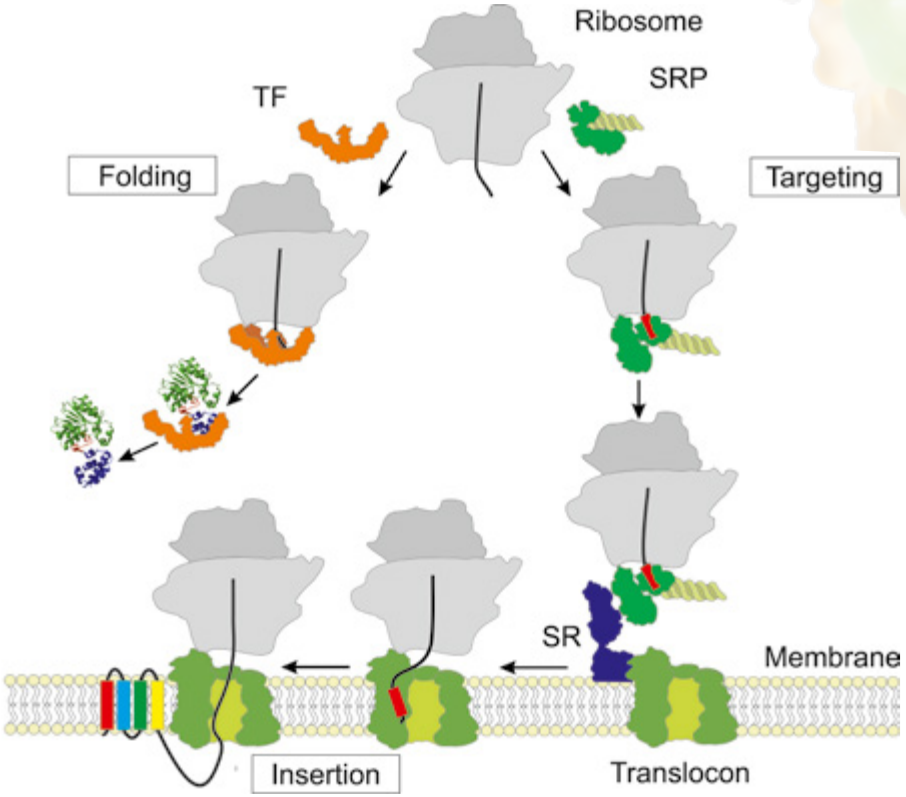
Wolfgang Wintermeyer
received his PhD in chemistry at the Ludwig Maximilian University in Munich. Subsequent to his habilitation in 1979, he did research in Munich supported by a Heisenberg Fellowship from the German Research Foundation, at the *Karolinska Institutet* in Stockholm (Sweden), and at the Massachusetts Institute of Technology (MIT) in Cambridge (United States). From 1987 until 2009, he held the chair for molecular biology at the Private University of Witten/Herdecke and thereafter became an emeritus professor. From 1991 until 2007, he was also Dean of the Faculty of Life Sciences there. Since 2009, he has been a Max Planck Fellow and head of the Research Group *Ribosome Dynamics* in the Department of *Physical Biochemistry* at the Max Planck Institute for Biophysical Chemistry.

Contact
wolfgang.wintermeyer@mpibpc.mpg.de
www.mpibpc.mpg.de/wintermeyer

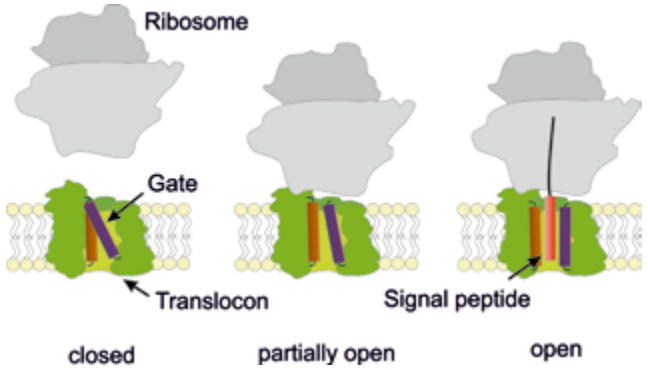
All living cells are surrounded by a membrane, which separates the cell's interior from its external environment. The membrane comprises a double layer of lipids (fat-like molecules), in which many proteins are embedded; the latter fulfill very specific functions. Some of them function as receptors for the reception of signals from the cell´s environment. Others serve as channels which only allow certain substances to pass. Overall, about one third of all proteins of the cell are membrane proteins. 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 from amino acids on the ribosome. Ribosomes which are assembling membrane proteins must therefore be directed to the cell membrane. But how is a membrane protein recognized and how are the ribosomes that are involved in the assembly recruited? We are studying how this complex process is executed and regulated in bacterial cells.

When a membrane protein is assembled on ribosomes, a specific label, the signal sequence, is incorporated, mostly in the early part of the protein. This signal is recognized by a ribonucleic acid-protein



▲ **Figure 1:** Interaction of protein biogenesis factors with the nascent peptide chain on the ribosome. The signal sequence, which is recognized by SRP, is highlighted in red.



◀ **Figure 2:** Translocon opening on binding ribosomes and signal peptide.

complex, the signal recognition particle (SRP). It directs the ribosome, assembling the membrane protein to the cell membrane (Figure 1). Interactions of the ribosome with SRP and helper proteins – among them the SRP receptor (SR) – ultimately result in the nascent protein being integrated in the membrane during the further course of synthesis. Membrane integration takes place in a protein complex located in the membrane, the translocon. The translocon forms a pore through which the newly synthesized membrane protein can enter the membrane. The molecular events occurring in this process form one focus of our research. Using a special fluorescence technique (photo-induced electron transfer, PET) we could show that the translocon opens laterally towards the membrane as soon as a ribosome assembling a membrane protein has docked (Figure 2). We are studying how successive structural elements of the membrane protein are integrated into the membrane and the accompanying topological changes by single-molecule fluorescence analysis.

Competition for interaction with nascent proteins on the ribosome

Besides SRP, which recognizes nascent membrane proteins on the ribosome and directs them to the membrane, there is a number of other factors that bind to nascent proteins (Figure 1). One of those factors is the trigger factor (TF), which helps newly synthesized proteins to avoid misfolding and to assume the correct three-dimensional fold. For some of these factors (for example SRP and TF) we have shown that they can bind concurrently to one ribosome and influence each other's function. We are studying these interactions by applying biophysical techniques as the ones mentioned above.

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Structural Dynamics

Holger Stark
studied biochemistry at the Free University of Berlin and completed his PhD at the Fritz Haber Institute in Berlin in 1997. Subsequently, he performed research at the Imperial College London (Great Britain), and from 1998 to 1999, he was a group leader at the University of Marburg. In 2000, he joined the Max Planck Institute for Biophysical Chemistry as research group leader. He was appointed Director and head of the Department of *Structural Dynamics* at the institute in 2015. He has also been a professor for molecular electron cryo-microscopy at the University of Göttingen since 2007. 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), the BioFuture Prize (2005), and the Ernst Ruska Prize (2013).

Contact
holger.stark@mpibpc.mpg.de
www.mpibpc.mpg.de/stark

Cells keep their metabolism running with the help of molecular machines. Frequently, these are very complex structures, so-called macromolecules, comprising a large number of different components. However, in order to observe these machines directly in action in the «nanocosmos» of the cell, scientists must expend a great deal of effort.

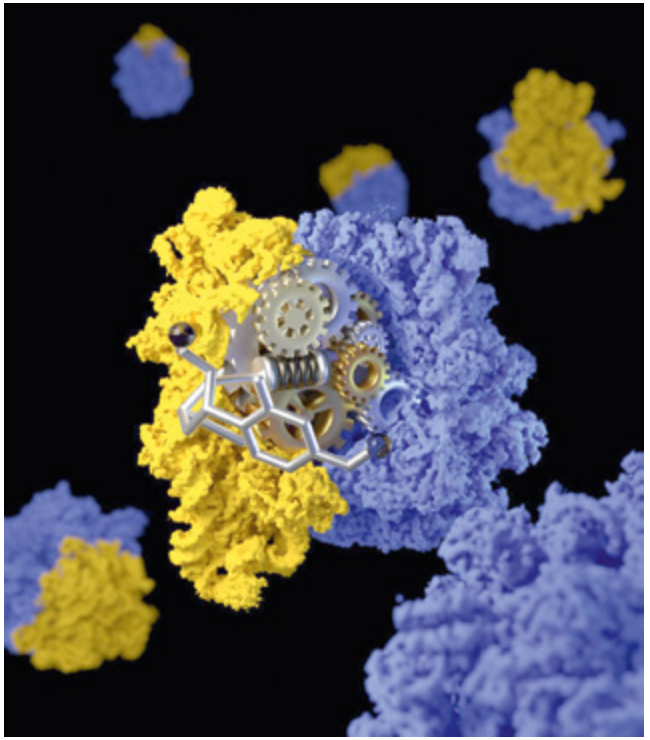
Snapshots in a state of shock

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

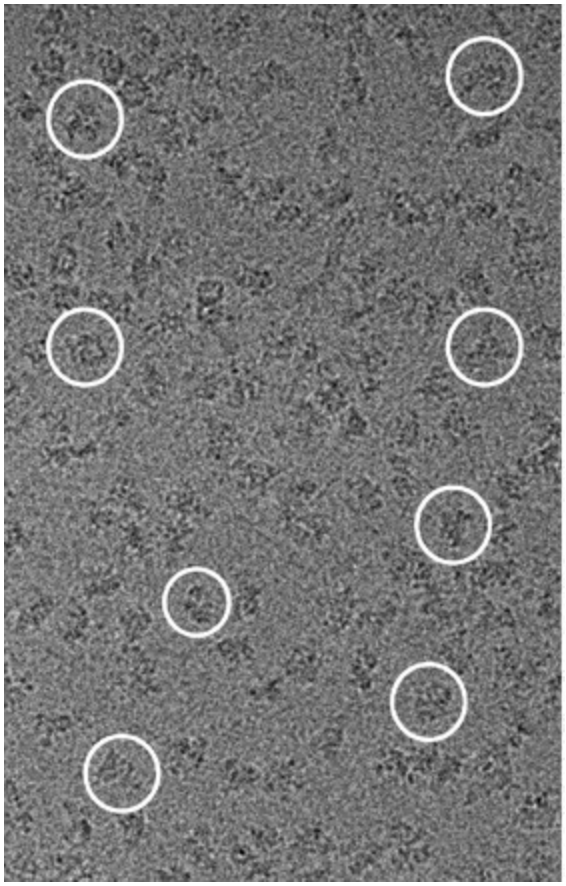
We apply this technique to a large number of different molecular machines, which are located at important switching points of cellular information processing. These machines frequently comprise not only proteins, but rather are complex associations of proteins and nucleic acids.

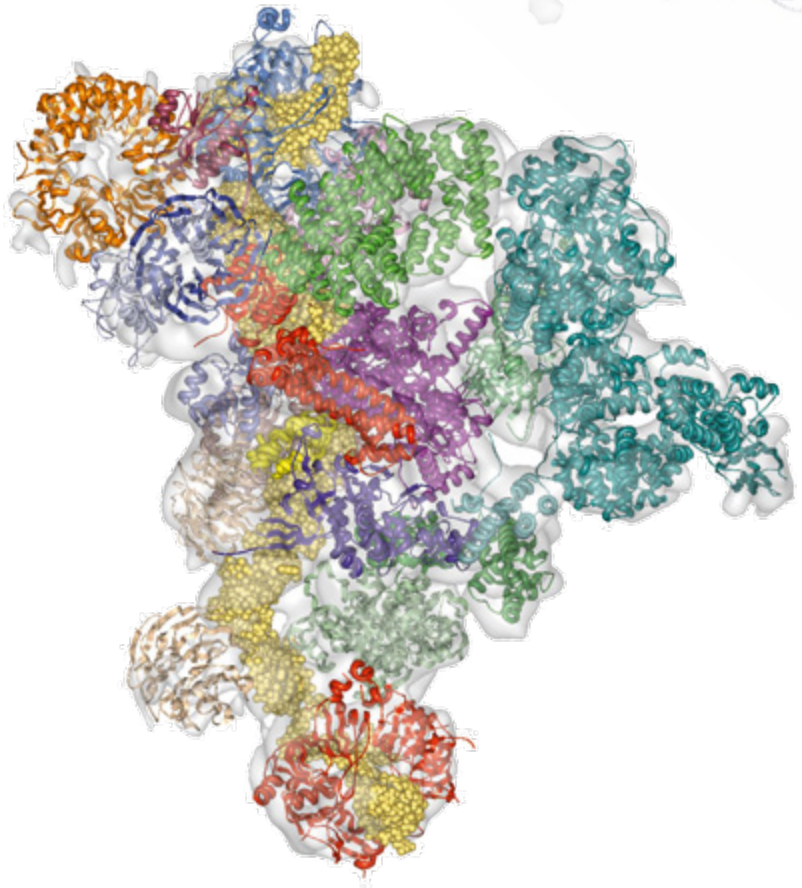
For example, we investigate how the cell's protein factory, the ribosome, produces proteins by reading the genetic information. At present, we are also working with spliceosomes. They are triggered into action after the genetic blueprints for proteins have been copied into the draft version of a messenger 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 cryo-electron microscopy, we cannot only determine the spatial structure at very high resolution. We can also observe the movements of such different molecular machines directly at work – and thus learn to understand their function in detail.



▲ The ribosome schematically as a nanomachine «at work».





▲ Tri-snRNP. Left: Electron microscopic images of the U4/U6.U5 tri-snRNP complex. Right: The three-dimensional structure of the biggest building block of the spliceosome, calculated from these images.

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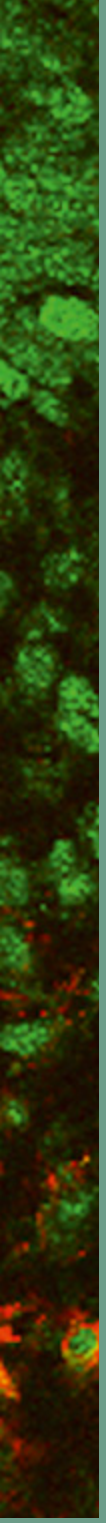
Communication and logistics



How cells transmit signals and distribute cargo

Catch a ball, recognize danger in time, remember something, or solve a mathematics problem – all seemingly effortless, our nervous system stores experiences from earliest childhood, controls complex movements, and creates our consciousness. The human nervous system comprises approximately 100 billion nerve cells. And each individual cell can make contact with thousands of neighbors. Tiny membrane vesicles release special messenger substances that affect the behavior of adjacent cells. How precisely this works on the molecular level is investigated by a number of labs at the institute. The researchers aim to shed light on the molecular processes which enable nerve cells to collect and process information – including complex brain functions such as learning and memory.

Also within every single cell the communication has to function flawlessly. Moreover, cellular logistics need to be well-organized. Scientists at the institute investigate how cells structure their interior, how molecules get into and out of the nucleus, and how cells transport proteins over membranes.

Neurobiology

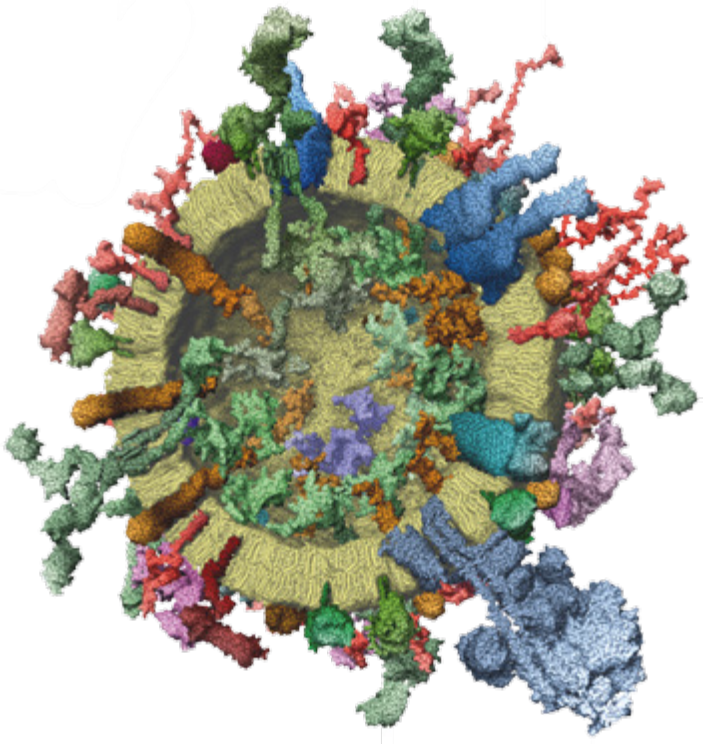
Reinhard Jahn
studied biology and chemistry and obtained his PhD at the University of Göttingen in 1981. Between 1983 and 1986, he worked at Rockefeller University (United States), and then at the Max Planck Institute for Psychiatry (now: Neurobiology) in Munich. In 1991, he became a professor at Yale University in New Haven (United States). In 1997, he was appointed head of the Department of *Neurobiology* at the Max Planck Institute for Biophysical Chemistry. He works as an adjunct professor of biology at the University of Göttingen. Reinhard Jahn has received numerous awards such as the Leibniz Prize (2000), the Ernst Jung Prize for Medicine (2006), the Sir Bernhard Katz Award (2008), the Heinrich Wieland Prize (2014), and the Balzan Prize (2016). He is a member of several academies, including the National Academy of Sciences of the United States.

Contact
rjahn@mpibpc.mpg.de
www.mpibpc.mpg.de/jahn

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 signaling molecules, called 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.

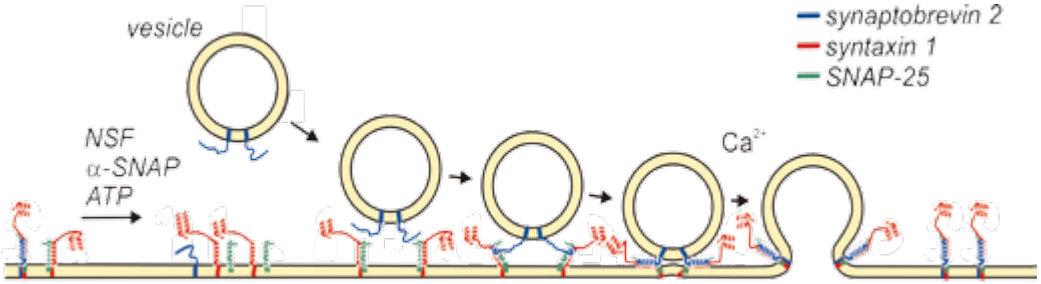
Synaptic vesicles contain a fascinating array of proteins, which execute the main jobs the vesicles must perform. Some of them work as transporters and pump neurotransmitters into the vesicle. These transporters are driven by an ion gradient, or to be more precise, a proton gradient, and we want to understand how exactly they manage to fill a vesicle with high amounts of transmitter in a few seconds.

The second main job of synaptic vesicles is to rapidly fuse with the plasma membrane once an electrical signal arrives in the synapse. Fusion is mediated by a group of specialized proteins, termed SNAREs. Together with other research groups, we want to find out how exactly SNAREs bring about membrane fusion and how they are activated by signaling. 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 a 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.

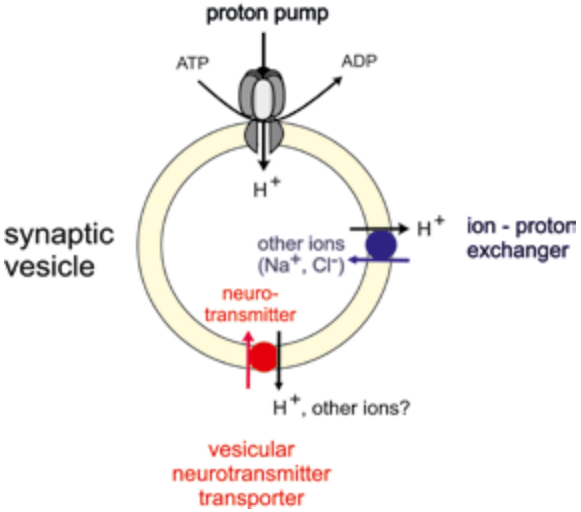


▲ Model of a synaptic vesicle, sliced open, depicting the circular vesicle membrane in which proteins are embedded. Neurotransmitters are omitted.

But 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 thereby generate 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,



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



▲ Neurotransmitter transport by synaptic vesicles is energized by a proton gradient. Neurotransmitter transporters use this gradient to load the vesicle with neurotransmitters.

and only if they are told to do so. Synaptic vesicles, for instance, only fuse if they need to transmit a signal. Intriguingly, some SNAREs are hijacked by pathogenic bacteria such as those responsible for Legionnaires' disease. These bacteria create special vesicles in which they multiply, requiring fusion with intracellular vesicles.

One of our objectives is to find out what all these membrane fusions have in common and how they differ from each other.

Project Group of Hans Dieter Schmitt

Hans Dieter Schmitt and colleagues also work on SNAREs. As a model organism, they use baker's yeast because it is easy to genetically modify. Their main interest is focused on intracellular transport vesicles. These vesicles have to be covered by a protein coat in order to form and detach from the precursor membrane. The group found that the coat is still present when vesicles coming from the Golgi apparatus arrive at their target membrane, the endoplasmic reticulum (ER). There, a large SNARE-associated tethering complex performs the first contact between the vesicle and the ER.

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Nanoscale Cell Biology

Manfred Lindau

studied physics at the Technical University of Berlin and the University of Hamburg, and received his doctoral degree in physical chemistry from the Technical University of Berlin in 1983. This was followed by postdoctoral training at the Max Planck Institute for Biophysical Chemistry and at the Free University of Berlin, where he has led his own research group since 1988 and habilitated in 1991 in experimental physics. After positions as a senior research scientist and lecturer in Heidelberg, he was an associate professor at Cornell University in Ithaca (United States), where he has been a full professor since 2003. In 2013, he rejoined the Max Planck Institute for Biophysical Chemistry as head of the Research Group *Nanoscale Cell Biology*.

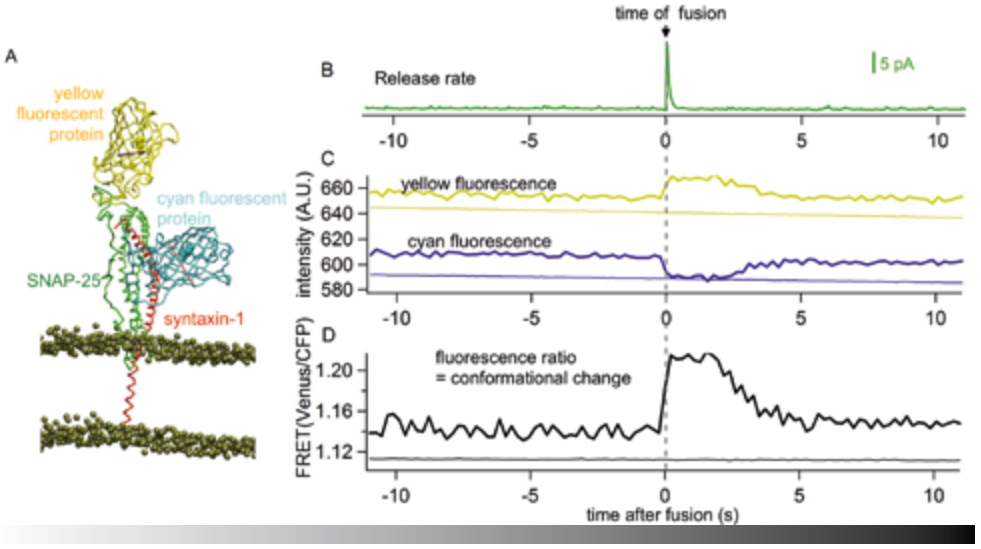
Contact

manfred.lindau@mpibpc.mpg.de
www.mpibpc.mpg.de/lindau

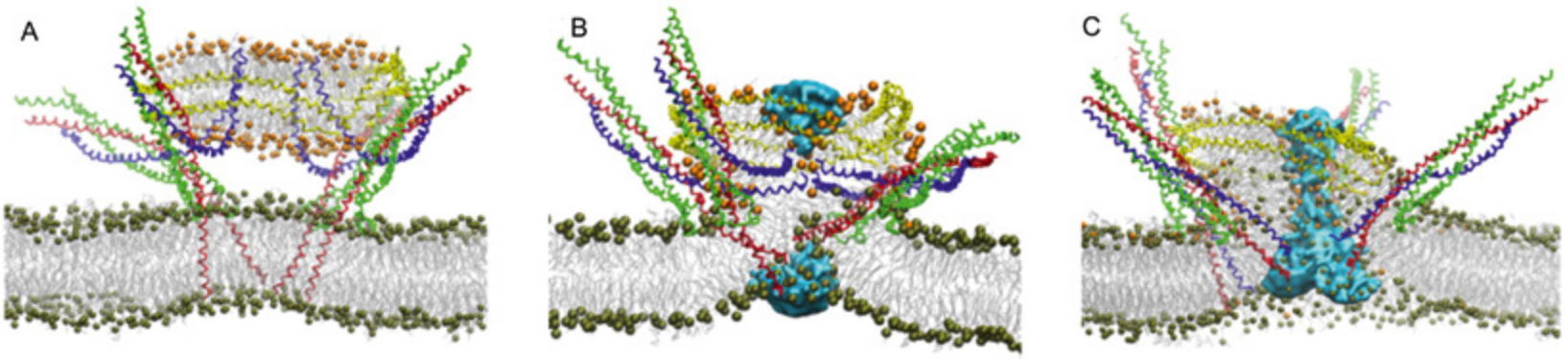
Cells perform their essential roles in the body using sophisticated molecular machines – uniquely assembled protein complexes with a dimension of a few nanometers (millionths of a millimeter). Elucidating the structure and function of these machines can help us understand how they fulfill the tasks that are the basis of all life. The central project in our lab is to investigate the SNARE (soluble NSF attachment receptor) complex. Like a motorized door opener, this nanomachine opens a gate in the cell membrane that has a diameter of about one nanometer – the fusion pore. This allows the release of neuronal messenger molecules (neurotransmitters) and hormones from tiny storage compartments, the so-called secretory vesicles. These vesicles need to fuse with the cell membrane. For this, SNARE proteins on the vesicle membrane interact with SNARE proteins on the cell membrane, pulling the two so close that they eventually fuse, opening a gate between them.

To determine how exactly SNARE complexes accomplish their task, we combine two strategies: We perform experiments in which we characterize the opening of these gates using specially developed versions of the SNARE complex, and we utilize computer simulations that visualize the molecular mechanics.

SNARE complexes are of high medical interest: Tetanus toxin found in soil bacteria destroys essential components of this machine and can lead to death if untreated. Furthermore, the widely known Botox treatment modifies the SNARE complex and thereby reduces transmitter release, which locally paralyzes



◀ Figure 1: **A)** A SNAP-25 construct incorporating cyan and yellow fluorescent proteins reports conformational changes. **B-D)** At the time of membrane fusion, yellow fluorescence increases and cyan fluorescence decreases, producing a transient change in the fluorescence ratio that indicates a temporary conformational change.



▲ Figure 2: **A)** Computer simulation of a possible *trans* (between two opposing membranes) SNARE complex conformation. Four SNARE complexes bridge a small portion of a membrane, a so-called nanodisc (top) and a larger, planar membrane (bottom). Synaptobrevin-2 (blue), Syntaxin-1 (red), and SNAP-25 (green) are shown, as well as water surfaces in cyan. **B)** Movement of the transmembrane domain of Synaptobrevin-2 after 0.3 microseconds of simulating the MD. **C)** Fusion pore structure at 1.7 microseconds simulation time. The proteins of the SNARE complexes have zippered up and waters from both sides of the membranes are now connected.

muscles. The precise understanding of the molecular mechanics of SNARE-induced vesicle fusion may lead to new treatment strategies. It will also advance our understanding of the mechanisms by which viruses enter cells, since they use closely related mechanisms.

A transient change in SNARE complex structure opens the fusion pore

In mammalian neurons and neuroendocrine cells, the SNARE complex is composed of the proteins Synaptobrevin-2, Syntaxin-1, and SNAP-25. The discovery of the key components of vesicle fusion and their regulation was honored with the Nobel Prize for Physiology or Medicine in 2013. However, although the components are known, the question remains: How exactly do the nanomechanics of fusion pore opening work?

When the SNARE complexes of two opposing membranes pull these membranes together, the proteins in the SNARE complex undergo so-called conformational changes – they slightly change their shape. We want to find out how such conformational changes are related to fusion pore formation.

For our experiments, we use a SNARE complex reporter (SCORE), where SNAP-25 is marked with two different fluorescent molecules (Figure 1 A). If the conformation of SNAP-25 changes, the two fluorescent molecules move relative to each other. This is especially useful in a microscopy technique called fluorescence resonance energy transfer (FRET). The FRET technique takes advantage of the fact that the color of the fluorescence shifts when distance or orientation between two fluorescent molecules change. We relate such changes in space and time to the opening of individual fusion pores. In this way, we could show that just before release of the neurotransmitters through the fusion pore, the conformation of SNAP-25 changes – and this induces membrane fusion (Figure 1 B-D). Within a few seconds after fusion, SNAP-25 reverts back to its original state.

Computer simulations reveal fusion pore opening

There is currently no experiment available that allows us to view the detailed molecular conformations and opening of a fusion pore in real time. We therefore use computer simulations of the molecular dynamics (MD). In such simulations we saw that the SNARE complexes zipper up, driven by the molecular interactions between the proteins in the complexes, and thereby open a fusion pore (Figure 2).

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Erwin Neher

studied physics in Munich and at the University of Wisconsin (United States). He redirected his interests to biophysics and carried out a PhD project at the Max Planck Institute for Psychiatry (now: Neurobiology) in Munich. He joined the Max Planck Institute for Biophysical Chemistry in 1972 and spent an interim year working at Yale University in New Haven (United States). From 1983 to 2011, he headed the Department of *Membrane Biophysics* at the Max Planck Institute for Biophysical Chemistry, where he continues his studies in the framework of an emeritus group. Erwin Neher is an honorary professor at the University of Göttingen. For his research on ionic currents flowing through individual membrane pores, he received the Nobel Prize for Physiology or Medicine jointly with Bert Sakmann in 1991.

Contact

eneher@mpibpc.mpg.de
www.mpibpc.mpg.de/neher

Membrane Biophysics

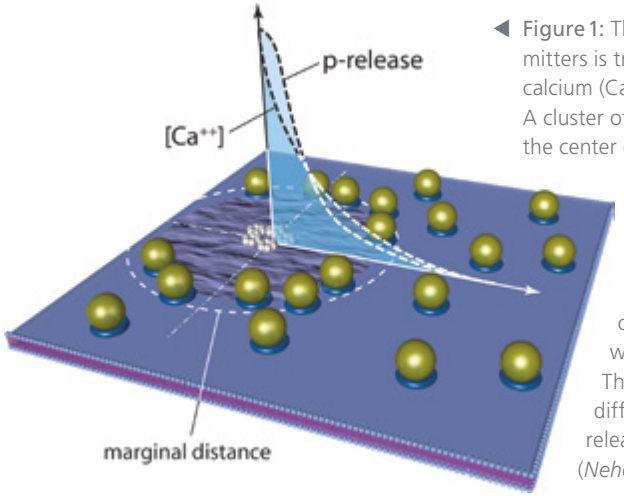
Many vital processes rely on the exchange of signals between subcellular structures, but also between our body's cells. In this context, biological membranes occupy a key position, because they not only compartmentalize tissues and organs, but also provide communication between the compartments. We use biophysical and molecular approaches to study the relevant signaling mechanisms.

Flexible circuits in the brain

Synapses are the contact points for the information flow in our brain, where signals are exchanged between individual neurons. Contrary to the circuit elements in electronic computers, where connections are hardwired, synaptic strength – the electrical response of the receiving neuron to a nerve impulse in the transmitting one – is not constant, but is use-dependent. Each type of synapse has its own «personality» with regard to this so-called synaptic plasticity. In some types of synapses, synaptic strength increases upon repeated stimulation, whereas it decreases in other synapses. Changes, which are caused by short, intensive bursts of activity, may be only transient, or they might persist for hours or days. Neuroscientists consider long-term changes as the basis of learning and memory. Likewise, the short-term changes in synaptic strength play an important role in second-by-second information processing, for instance, when we perceive and process sensory signals.

The research projects of the emeritus group concentrate on short-term plasticity and its molecular and physiological mechanisms. A nerve impulse causes the release of a signal substance from the nerve ending of the transmitting neuron. The immediate trigger for the release of this so-called neurotransmitter is an increase in the presynaptic concentration of calcium ions, caused by the opening of calcium-specific ion channels. This leads to the fusion of storage vesicles containing the neurotransmitter with the cell membrane. Thereby, the 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 containing neurotransmitters ready for release. Synapse strength then depends on how many synaptic vesicles the transmitting cell uses per nerve impulse, and how rapidly such vesicles can be regenerated. In addition to calcium ions, other signal substance such as cyclic AMP, for example, are involved in the regulation of replenishment. Short-term synaptic plasticity is thus the result of numerous intertwined processes.

How is it possible that one and the same signaling substance, calcium, can control several processes in different ways? The answer lies in quantitative detail, as we could show through biophysical investigations: The triggering of neurotransmitter release



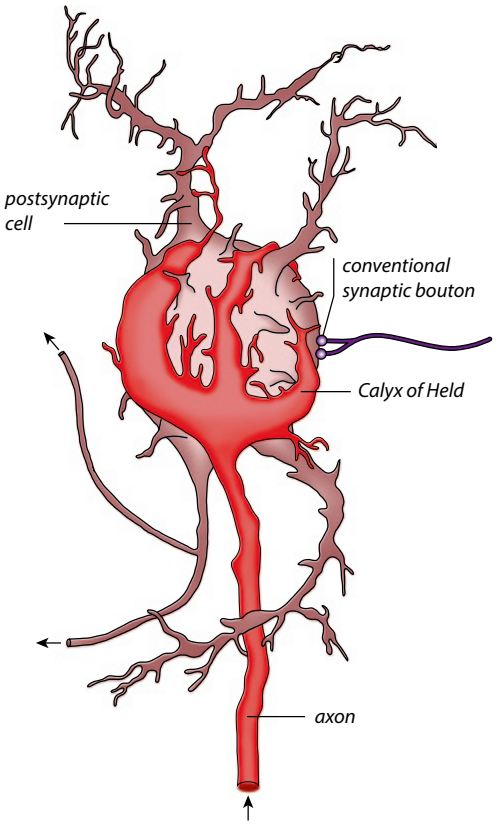
◀ **Figure 1:** The release of neurotransmitters is triggered by the opening of calcium (Ca^{++})-specific ion channels. A cluster of such channels is shown at the center of the dashed white circle. Release-ready vesicles are located at various distances from the channels. Therefore, they are exposed to different concentrations of calcium when channels open up. This, in turn, leads to strongly different probabilities of release (p-release). (*Neher,* 2015)

by calcium is a highly cooperative process, which only sets in at rather high calcium concentrations, but then accelerates greatly. The re-supply of vesicles accelerates linearly with increased calcium and saturates at calcium levels below the release threshold. Depending on the calcium level, either one or the other of these processes will be preferentially activated and that, in turn, depends on the distance between a given site of release and the nearby calcium channels (Figure 1).

Short-term plasticity and brain states

Our brain is able to change its functional state within seconds, such as from sleep to wakefulness or from absence-minded contemplation to vigilance. It is known that certain neuronal populations project to large brain areas, transmitting so-called modulatory signals. On a given target synapse, these signals can induce rapid changes in synaptic strength and short-term plasticity, causing profound changes in functional connectivity between the neurons in their target areas. We hypothesize that such processes are at the basis of the switching between brain states.

Our research in recent years has focused on the Calyx of Held synapse, an important relay station in the auditory pathway. The cup-like structure of its presynaptic nerve terminal is large enough that it can be easily manipulated, which is not possible with very small bouton-like nerve terminals of other synapses (Figure 2). In particular, it allows us to record electrical and fluorescence signals simultaneously from pre- and postsynaptic compartments. We were struck by the finding that there is remarkable heterogeneity in short-term plasticity between individual Calyx of Held synapses, in spite of the fact that they are all very similar in morphology and with respect to their physiological role. Therefore, we tested whether modulatory signals that act in the background could cause such differences. Using a chemical that mimics one of these modulators, we could indeed switch the synapse between short-term plasticity states. We were able to describe the alterations, induced by the chemical, by means of a kinetic model that assumes two functionally distinct states of synaptic vesicles: «normally primed» vesicles and «superprimed» vesicles. Superprimed vesicles are characterized by a release probability about five times as high as that of a normally primed vesicle upon arrival of a nerve impulse. However, the regeneration of superprimed vesicles takes much longer than that of normally primed ones. This combination of slow regeneration and efficient consumption causes rapid depletion of superprimed vesicles during repetitive activation of synapses. Therefore, superprimed vesicles are most effective in boosting synaptic strength at the onset of burst-like activity after pauses. This may make them particularly relevant for the switching-on of high-activity brain states.



◀ **Figure 2:** The Calyx of Held is an exceptionally large nerve ending that makes contact with a compact postsynaptic nerve cell body. It is an important connection in the auditory pathway. Due to its size, it is particularly well suited for biophysical investigations because it is accessible for electrophysiological measurements using the patch-clamp technique.

Internal Research Groups

Dr. Andrew Woehler,
now Berlin Institute for Medical Systems Biology, MDC Berlin
(supported by the DFG Research Center CNMPB).

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Cellular Logistics

Dirk Görlich
studied biochemistry in Halle (Saale) and received his PhD at the Humboldt University of Berlin in 1993. After a two-year research period at the Wellcome/CRC Institute in Cambridge (Great Britain), he was first appointed head of a research group in 1996 and then, in 2001, professor for molecular biology at the ZMBH of the University of Heidelberg. 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 Heinz Maier-Leibnitz Prize (1997), the EMBO Gold Medal (1997), and the Alfried Krupp Promotion Prize (2001). He is an elected member of the European Molecular Biology Organization (EMBO) and the Leopoldina – National Academy of Sciences.

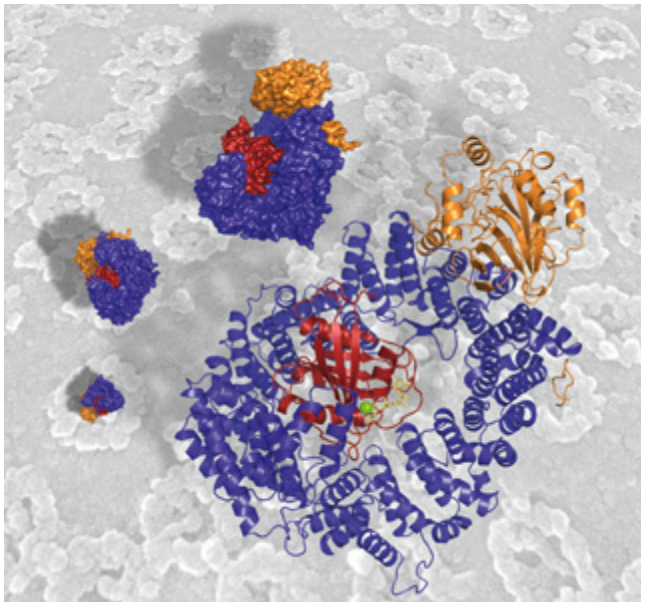
Contact
goerlich@mpibpc.mpg.de
www.mpibpc.mpg.de/goerlich

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 therefore must 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 the 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.

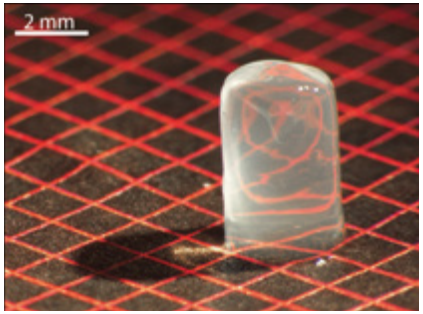
The mobile part of this transport machinery is comprised of nuclear import receptors (importins) and exportins. Although the nuclear pores appear tightly closed to the majority of macromolecules above a certain size limit, importins and exportins have the privilege of being able to pass nearly unimpeded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



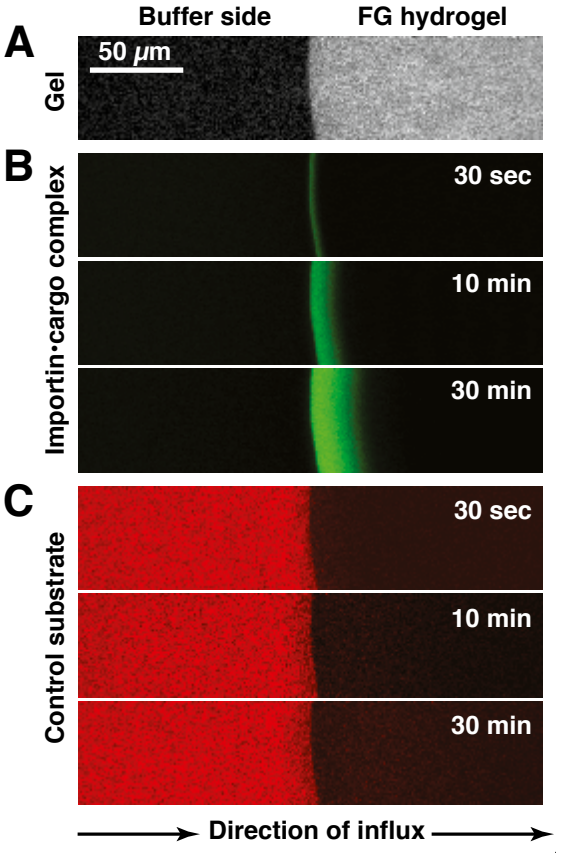
▲ An export complex at atomic resolution. The exportin CRM1 (blue) with its cargo molecule snurportin (orange) and Ran (red) bound. Ran is a molecular switch, which determines the direction into which the cargo is carried (in this case from the cell nucleus to the cytosol). CRM1 exports, for example, 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 is shown – the giant transport channels in the nuclear envelope.

How does the sorting unit of nuclear pores work?

Nuclear pores are extremely effective sorting machines and each of them can transport up to 1,000 cargo complexes per second. Nuclear pores have an extremely complex structure, and each pore 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 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.



◀ The permeability barrier of the nuclear pore is a hydrogel, that is, 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 1,000-fold and moves so rapidly into the gel that it could traverse a nuclear pore within ten milliseconds. **C)** A red fluorescent control substrate in comparison. It does not bind the importin and therefore cannot penetrate into the gel.

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Membrane Protein Biochemistry

Alexander Stein
studied biochemistry at the Free University of Berlin. He conducted his doctoral research at the Max Planck Institute for Biophysical Chemistry and received his PhD in 2010 from the Free University of Berlin. From 2010 to 2014, he was a postdoctoral fellow at Harvard Medical School in Boston (United States). Since 2014, he has been running the Research Group of *Membrane Protein Biochemistry* at the Max Planck Institute for Biophysical Chemistry within the Otto Hahn program of the Max Planck Society.

Contact
alexander.stein@mpibpc.mpg.de
www.mpibpc.mpg.de/stein

Not only cities rely on a properly functioning waste disposal system. Even living cells must eliminate «waste». In particular, they tightly regulate the degradation of proteins.

Proteins that are located in the cytoplasm are degraded by the so-called ubiquitin-proteasome system (UPS) in all highly developed cells. To accomplish this, enzymes called ubiquitin ligases first tag the proteins as waste by attaching chains of a small protein called ubiquitin to the proteins. These ubiquitin chains are recognized by a large cytoplasmic garbage collector, the proteasome, which breaks down the proteins into smaller blocks.

Degradation of non-cytoplasmic proteins

It has been known for some time that the UPS is also involved in the degradation of non-cytoplasmic proteins. After their production in the cytoplasm, such proteins are either incorporated into the membrane of a particular organelle, the endoplasmic reticulum (ER), or transported through the ER membrane. The degradation of these proteins by the UPS is called ER-associated protein degradation (ERAD).

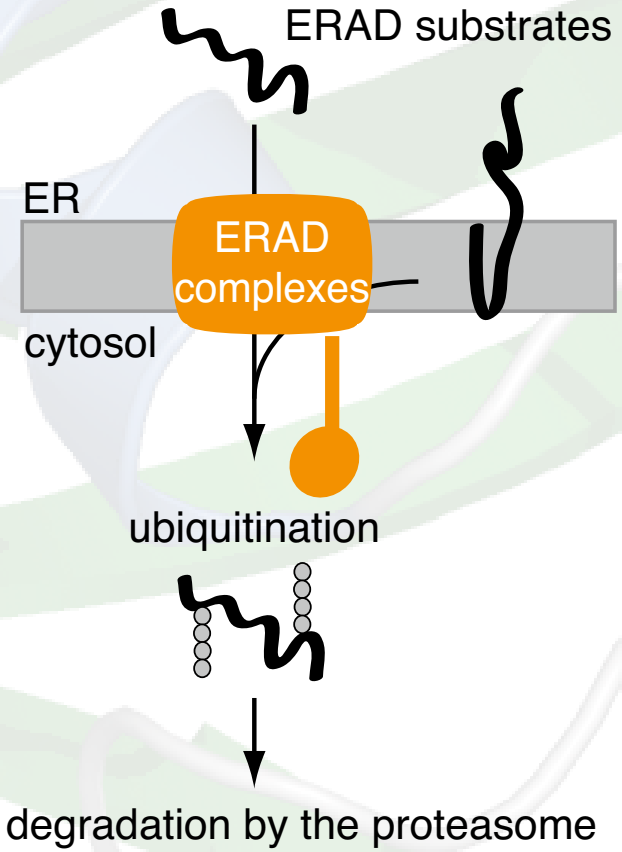
A protein can be degraded by ERAD for several reasons: Some proteins cannot fold stably and are therefore discarded. This prevents misfolded proteins from clumping together and causing disease due to their toxic effects on the cell. Other proteins are degraded when they are simply no longer required.

ERAD differs from the conventional UPS in that the protein is recognized in a different compartment of the cell than where it is degraded. Since the two participating compartments – ER and cytoplasm – are separated by a membrane, the protein must first be transported through the ER membrane before it can be degraded. While scientists have been able to identify many proteins involved in ERAD, it remains largely unanswered how a protein that needs to be degraded is detected and then transported through the ER membrane.

Our work focuses on ubiquitin ligases in the ER membrane. We suspect that they not only attach ubiquitin to proteins which are intended to be degraded, but also form channels, through which ERAD substrates pass through the ER membrane. On the one hand, we investigate ERAD in living cells and try to find out which functions individual proteins have by specifically interfering with the process. For this, we use baker's yeast as a model organism because we can easily manipulate it genetically. Furthermore, it allows conclusions about how this process, which is conserved in all higher developed creatures, generally works. On the other hand, we try to gain a better understanding of the ERAD mechanism by replicating the process in the test tube from purified components.

Transport of proteins into the apicoplast

In a second project, we examine how the ERAD process has been transformed in a completely different context. Some parasites such as the pathogens of malaria or toxoplasmosis have a plastid-like organelle surrounded by four membranes. Since this so-called apicoplast hardly has any genetic information of its own and thus lacks instructions for building proteins, it has to import a large part of its proteins over the surrounding membranes. Evidently an ERAD-like apparatus is involved. We want to find out which proteins are part of this apparatus and transport other proteins into the apicoplast and define these proteins in molecular terms. By comparison with the ERAD process, we hope to understand how ERAD has been transformed into a pure protein import machine in these organisms in the course of evolution.



◀ Schematic representation of the ERAD process: ERAD complexes transport misfolded or no longer required proteins of the endoplasmic reticulum (ER) through the ER membrane. On the cytoplasmic side (cytosol) of the membrane, these proteins are marked as waste with ubiquitin chains (ubiquitination) and are ultimately degraded by the proteasome.

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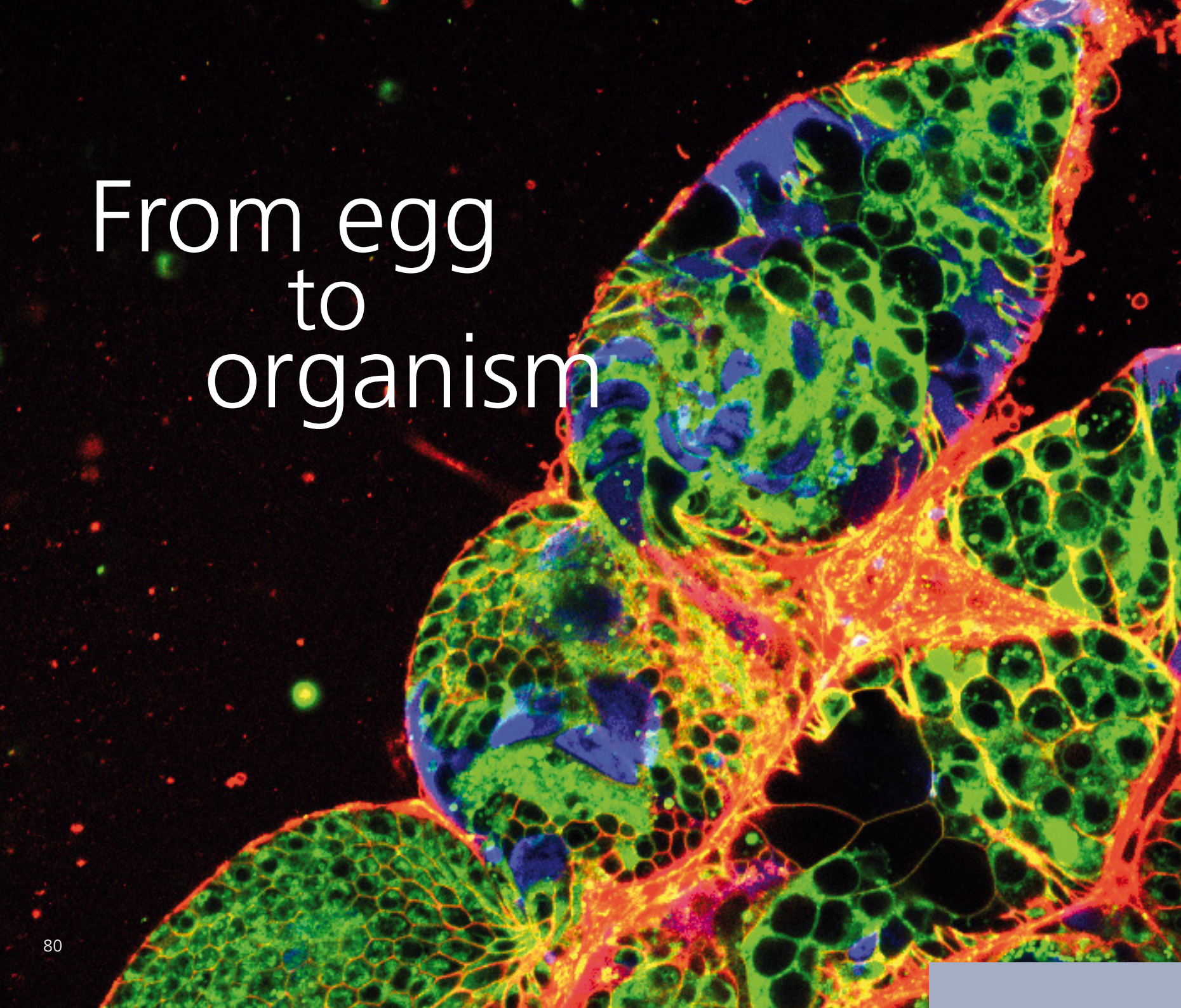
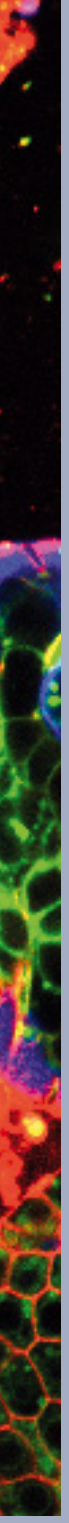


From egg to organism



How living beings are formed and controlled

Heart and brain, liver and lung – all parts of our body originate from a single fertilized egg cell. But how is this egg cell formed? And how does it develop into a complex organism? How do the cells in the embryo form complex organs that interact reliably? Researchers at the institute unravel these fundamental processes on the molecular level using such different organisms as fly and mouse. Even though these animals do not appear to have much in common at first glance, their embryonic development as well as that of human beings follows similar genetic programs. Among other things, scientists use these organisms to investigate in how far diabetes and obesity are influenced by genes. This knowledge helps us to better understand and treat such diseases.

Furthermore, researchers at the institute are interested in the fascinating phenomenon of sleep: A large portion of our life is spent asleep – but why is this so? How is our «biological clock» controlled so that it stays in step? And how does sleep work in principle?

Last but not least, the scientists improve imaging methods like magnetic resonance tomography to obtain «live» images from inside our body and to visualize vital processes such as breathing or the beating heart in real-time.

Meiosis

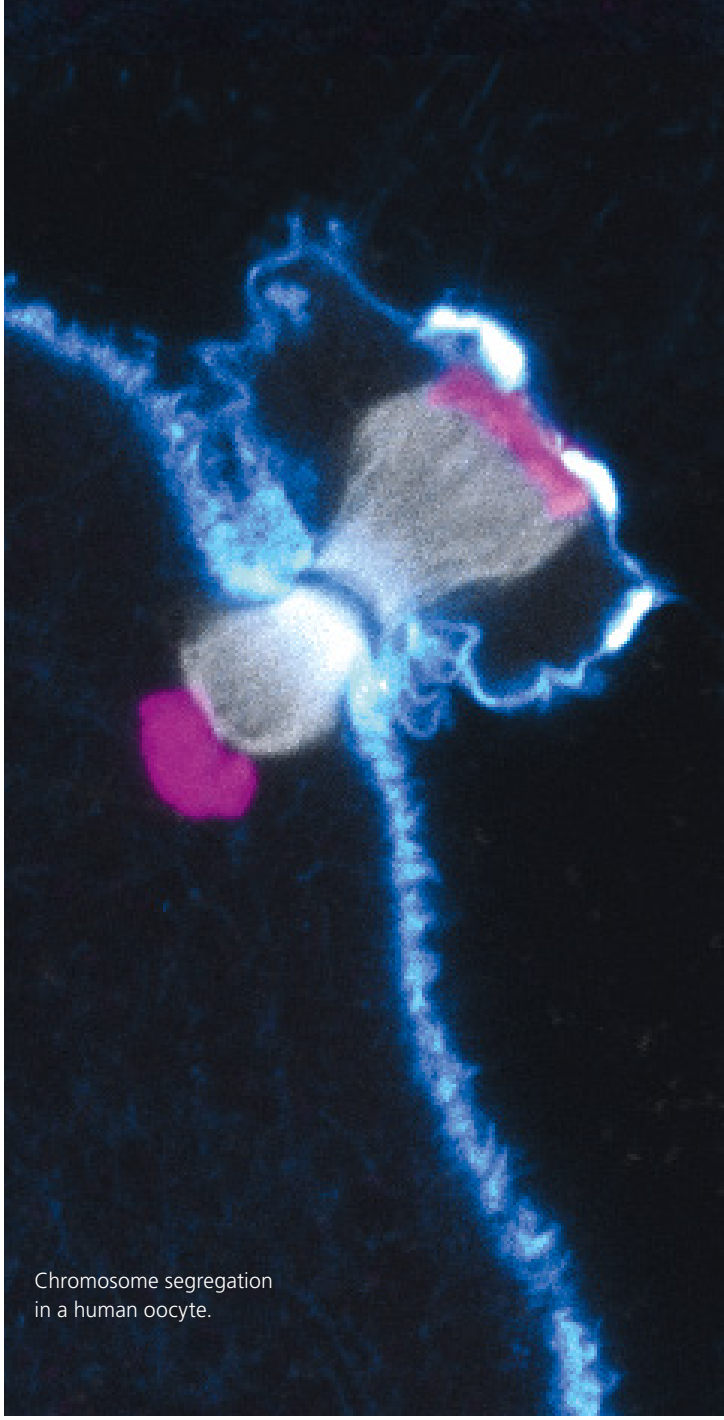
Melina Schuh
studied biochemistry at the University of Bayreuth. For her PhD, she worked at the European Molecular Biology Laboratory (EMBL), and received her degree from the University of Heidelberg in 2008. She then moved to Cambridge (Great Britain), where she was a group leader at the *MRC Laboratory of Molecular Biology* from 2009 until the end of 2015. Since 2016, she is a Director at the Max Planck Institute for Biophysical Chemistry and head of the Department of *Meiosis*. She received several awards for her work, including the John Kendrew Young Scientist Award, the Biochemical Society Early Career Award, the Lister Research Prize, an EMBO Young Investigator Award, and the BINDER Innovation Prize.

Contact
melina.schuh@mpibpc.mpg.de
www.mpibpc.mpg.de/mschuh

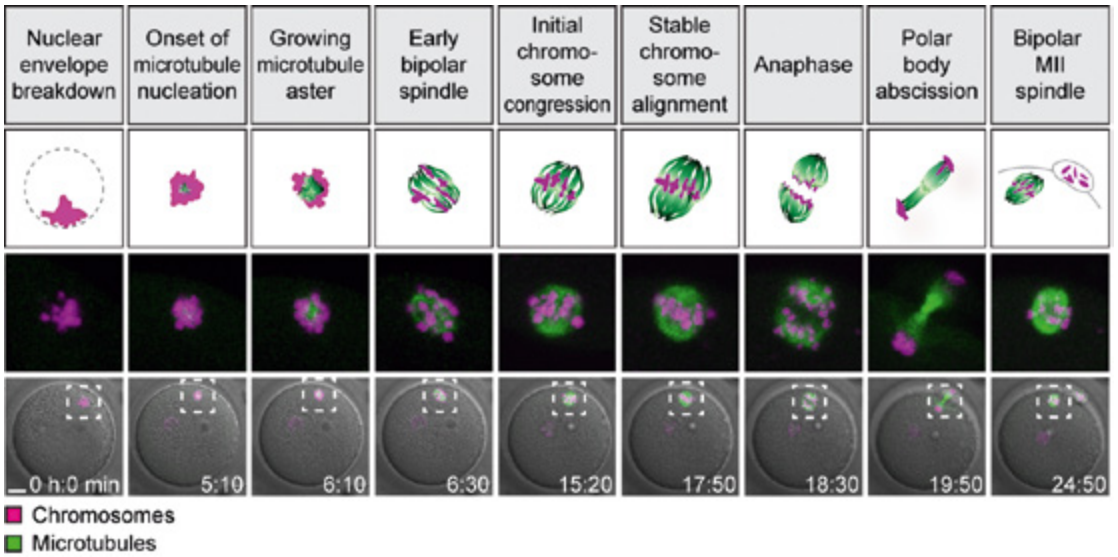
Human life starts with the fertilization of an egg cell by sperm. During fertilization, the genetic material of the mother and the father – which is stored in the form of chromosomes – is united. Egg and sperm differ from all other cells in our body in one central aspect: They contain only half the number of chromosomes. Normally, each chromosome is present in two copies. In contrast, the egg has only one of the two copies of each of the mother's chromosomes, and the sperm contains only one of the two copies of each of the father's chromosomes.

An egg develops out of a progenitor cell, the oocyte, which still possesses two copies of each chromosome. To become a fertilizable egg, the oocyte has to eliminate one of the two copies. This happens once every menstrual cycle through a specialized cell division called meiosis. During meiosis, one of the two copies of each chromosome is eliminated from the oocyte and discarded in a small cell called polar body. Often, this does not work reliably, resulting in an egg with the wrong number of chromosomes. If such an egg is fertilized, the embryo will also have the wrong number of chromosomes. In most cases, an embryo with the wrong number of chromosomes will die. In other cases, the embryo may be viable, but it will suffer from congenital disorders such as Down syndrome, which is caused by an extra copy of chromosome 21. Eggs with the wrong number of chromosomes become more frequent as women get older. This is called the maternal age effect.

Despite decades of work, we still know relatively little about meiosis in mammalian oocytes. Especially human oocytes have hardly been studied. At the same time, fertility problems become more and more prominent in our society, also because childbearing is more frequently postponed until later in life. To improve fertility treatments it is essential that we learn more about the



Chromosome segregation in a human oocyte.



◀ Stages of meiosis observed in live human oocytes.

mechanisms that govern accurate progression through meiosis and that we analyze the causes of chromosome segregation errors in mammalian oocytes. Thus, research into mammalian oocytes, with its many open questions and medical implications, has enormous potential to grow and will remain an attractive field for a long time to come.

Chromosome segregation is error-prone

To find out why eggs frequently have the wrong number of chromosomes, we need to investigate how eggs develop. Our aim is to understand how the chromosomes become prepared for elimination into the polar body, and how the machinery distributing the chromosomes between polar body and egg works. This machinery is called the microtubule spindle and consists of protein fibers, which separate the chromosomes. If the spindle is abnormal, chromosomes cannot be separated accurately. Indeed, our work has revealed that chromosomes are frequently abnormally attached to the spindle in human oocytes, which may contribute to the high error rates in meiosis.

We are also investigating the causes of declining female fertility as women get older. We have found that chromosomes in human oocytes are disintegrating as women age. This leads to more and more errors during the segregation of chromosomes as women get older. This decline in oocyte quality may be due to the fact that eggs are as old as the woman: A 40-year-old woman has 40-year-old eggs.

New tools to study meiosis

To have a solid foundation for future research, we are developing new tools to study meiosis in mammalian oocytes. For instance, we have been able to carry out the first so-called high content screen for genes controlling meiosis in mammals. We have also been able to establish methods that now allow us, for the first time, to study the causes of chromosome distribution errors directly in live human oocytes. This opened an exciting new area of research in our lab that we plan to expand significantly in the future.

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Molecular Developmental Biology

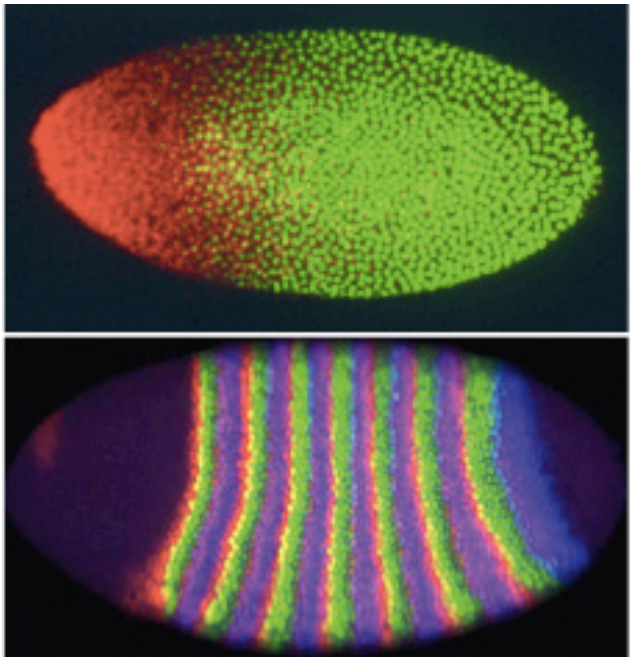
▲ The fruit fly *Drosophila melanogaster.*

Herbert Jäckle
received his PhD from the University of Freiburg in 1977. He subsequently worked at the University of Texas at Austin (United States), the European Molecular Biology Laboratory (EMBL) in Heidelberg, and the Max Planck Institute of Developmental Biology in Tübingen. In 1987, he became professor for genetics at the Ludwig Maximilian University in Munich and was subsequently appointed a Director at the Max Planck Institute for Biophysical Chemistry in 1991. He was head of the Department of *Molecular Developmental Biology* there until 2017. Since then, he has headed an emeritus group at the institute. Herbert Jäckle received numerous awards including the Leibniz Prize, the Otto Bayer Award, the Louis-Jeantet Prize, and the State Prize of the People´s Republic of China. He served as Vice President of the Max Planck Society from 2002 to 2014.

Contact
hjaeckl@mpibpc.mpg.de
www.mpibpc.mpg.de/jaeckle

The fruit fly *Drosophila* is a very popular scientific research object for good reasons. Undemanding and immensely prolific, despite its small 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 single cell? And, once the body is established, how does the animal regulate its energy requirement to maintain its organismal functions?

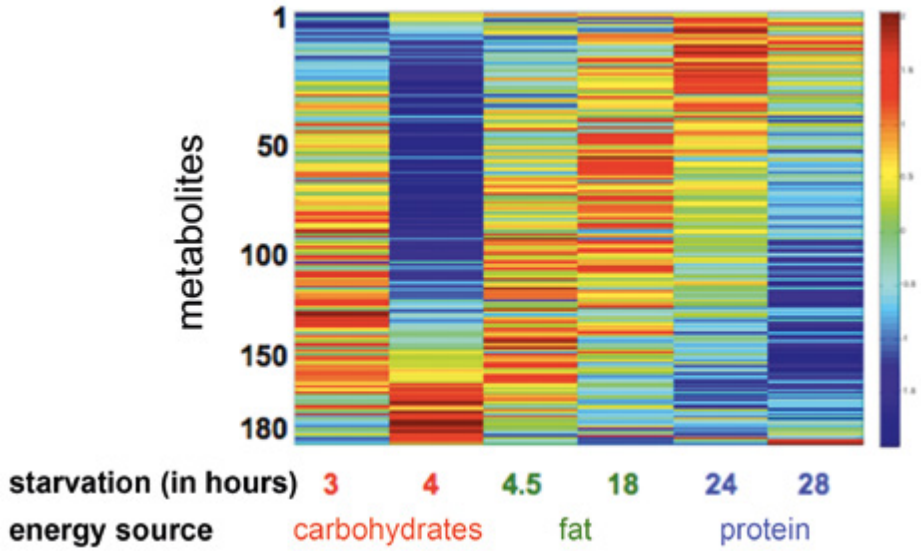
To answer these great biological riddles, we delve deeply into the molecular control mechanisms that regulate developmental processes from egg to fly and to the control of the body´s energy requirement, so that flies become neither obese nor underweight. The astonishing outcome of our analyses is that 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, therefore, is also informative in medical questions: When developmental or metabolic processes derail in humans, in many instances the genes and entire control systems which we know from flies are disturbed.



▲ A fruit fly embryo shortly after fertilization (top). The embryo was stained with antibodies directed against two maternally provided key factors called Bicoid (red) and Caudal (green), forming opposing concentration gradients along the anterior-posterior axis (red is anterior). About 90 minutes later (bottom), these factors have initiated the control of a hierarchy of about 20 interacting segmentation gene activities (three patterns are shown; red, green, blue; yellow are overlap regions), which blueprint the not yet morphologically visible segmented body pattern (head region is to the left).

Setting up the body structure

The body structure of the fly has already been determined before the egg cell is fertilized. Female flies not only supply their eggs with nutrients, they also provide components which intervene as control factors in development. They are asymmetrically distributed in the egg and subsequently 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



◀ Metabolic changes during starvation of adult flies, showing which energy storage molecules of the organism are consumed over time. Small molecules (180) are used as diagnostic markers to indicate that flies first use their stored carbohydrates (up to 4 hours after food deprivation), then fat (4 to 24 hours after food deprivation), and finally the proteins before they die after about 50 hours of starvation.

with its segments and organs are predetermined molecularly. They are constructed accordingly but become visible much later during embryogenesis. In this context, communication processes between neighboring cells and cell areas also play a role. They define the specific developmental fate of a cell at the proper position in the body via the interaction of signal substances with corresponding receptor molecules. While the molecular key players of the body design are identified, we aim at understanding their molecular interplay and the molecular mechanisms to solve the big puzzle that illustrates the path of a single cell, the fertilized egg, to a complex three-dimensional organism that interacts with the environment.

Maintenance of body functions

All aspects of body function require energy, such as movements or metabolic actions of the organism as a whole, individual cells, or the internal cellular processes. Thus, we ask the question of how the fly controls its energy budget since in nature, like all other animals, it is exposed to periods of sufficient food supply as well as famines. The big question is how an organism knows how much energy it must store or deliver to keep the genetically controlled body weight. Fat deposits are the main form of energy storage in the body. They cover the organism's energy requirements in times of famine. We address the question of how organismal energy homeostasis is maintained by showing how the metabolism adapts in response to food deprivation and resumption of food-intake.

Further, we analyze the genetic adaptations that allow flies to survive in environments with varying periods of starvation. These projects are also designed to help us better understand human obesity. Due to our life style, obesity – with its consequences such as cardiovascular diseases, diabetes, and certain types of cancer – is spreading pandemically around the globe. In our biomedical studies we use the fly as a model to contribute to improvement of diagnostic methods and development of new drugs that cure obesity in flies and later, hopefully, in humans.

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Gene Expression and Signaling

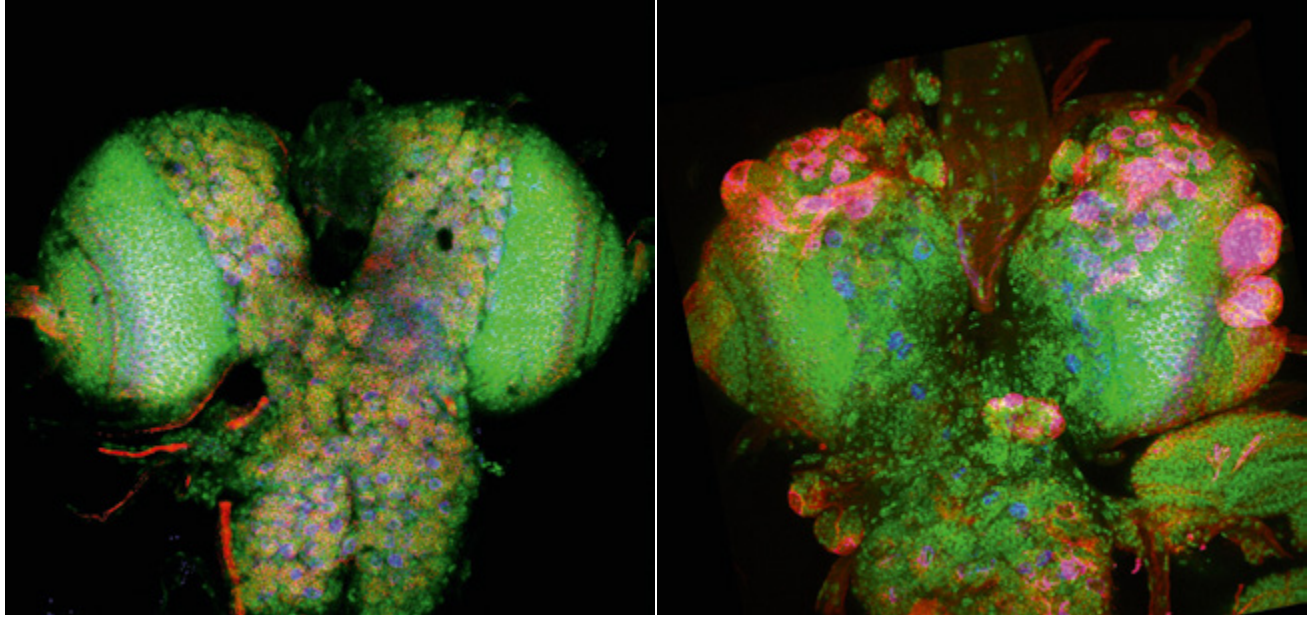
Halyna R. Shcherbata
studied biology and chemistry at the Ivan Franko National University of Lviv (Ukraine), where she also received her PhD and worked as an assistant professor at the Department of *Genetics and Biotechnology*. In 2003, she became a postdoctoral fellow and later research professor at the Department of Biochemistry, University of Washington in Seattle (United States). Since 2008, Halyna R. Shcherbata has been head of the Max Planck Research Group *Gene Expression and Signaling* at the Max Planck Institute for Biophysical Chemistry. In 2012, she habilitated at the University of Göttingen.

Biological processes in all organisms, including humans, are controlled by genes. But what controls genes in turn? A number of mechanisms that regulate gene activity have been discovered. Among these regulators are tiny RNAs, called microRNAs (miRNAs), which are capable of silencing genes.

Since their discovery only two decades ago, it has become clear that miRNAs are implicated in virtually all biological processes. Remarkably, each miRNA may target up to 200 genes, and multiple miRNAs can coordinately target one gene. This illustrates how complex miRNA-mediated gene regulation can be. Even though we still do not know the complete repertoire of genes each miRNA regulates or how exactly miRNAs work, miRNAs have already been developed as new therapeutic targets for many diseases. To better understand the biological roles of miRNAs, we combine experimental and computational approaches and use the fruit fly *Drosophila melanogaster* as a model organism.

◀ Dystrophic *Drosophila* develops an age-dependent muscular dystrophy.

Contact
halyna.shcherbata@mpibpc.mpg.de
www.mpibpc.mpg.de/shcherbata



◀ Deregulation of *Dystroglycan* expression in the brain causes the cobblestone brain or lissencephaly type II.

Not just X and Y

Each cell has a sexual identity, meaning that male and female cells differ in sets of expressed proteins. Unexpectedly, we discovered that cellular sexual identity, which is established during embryogenesis, does not only depend on X and Y chromosomes – it has to be actively maintained during its entire lifetime. This is controlled by a sexually biased steroid hormone and its dependent miRNA. We identified sets of miRNAs that are differentially expressed in multiple male and female tissues across various stages of development.

We also found that miRNAs regulate other very important processes. With the help of miRNAs, cells adjust their metabolism to fluctuations in nutrient composition and availability. Furthermore, miRNAs guide how cells develop from stem cells into specialized cells, a process called differentiation, and more importantly, how stem cells *per se* are maintained. As these two processes are affected in aging and cancer in humans, miRNAs have emerged as potential therapeutics in regenerative medicine and cancer therapy.

Muscular dystrophy in the fruit fly

Furthermore, we study the role of miRNAs under stress conditions and in disease. In particular, we focus on muscular dystrophies. In Western Europe, more than 70,000 people suffer from forms of muscular dystrophy. Unfortunately, no cure exists for these deadly neuromuscular disorders. The patients face neuronal defects as well as progressive muscle weakening and loss. *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 developed a *Drosophila* model for studying different types of muscular dystrophy. We have shown that the phenotypes caused by *Drosophila Dystroglycan* and *Dystrophin* mutations are remarkably similar to phenotypes observed in human muscular dystrophy patients. Mutant flies exhibit a shortened lifespan, decreased mobility, age-dependent muscle degeneration, and brain defects. Importantly, we have shown that several miRNAs regulate *Dystroglycan* expression. In the future, we plan to analyze in depth how the Dystrophin-Dystroglycan complex interacts with miRNAs. This will help us understand the mechanisms contributing to brain defects and dystrophic muscle degeneration and facilitate the development of miRNA-based therapeutics.

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Molecular Organogenesis

Reinhard Schuh
received his PhD in biology from the University of Tübingen in 1986. From 1986 to 1988, he worked as 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 Maximilian University in Munich. In 1992, he joined the Department of *Molecular Developmental Biology* at the Max Planck Institute for Biophysical Chemistry. There, he established his own Research Group *Molecular Organogenesis* in 2005. Reinhard Schuh also teaches as an adjunct professor on the Biological Faculty of the University of Göttingen.

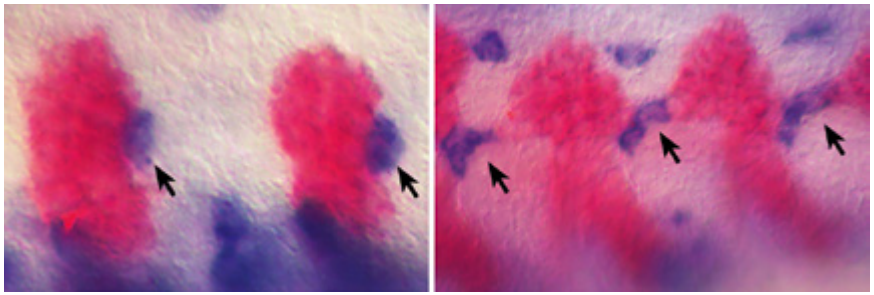
We breathe in and out approximately 20,000 times a day without thinking about it. Each time, the respiratory air flows through the delicate tube system of our lungs, which contain five to six liters of air and exchange approximately half a liter of air with every breath. Like the crown of a tree, the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

Our research group wants to understand how these highly branched pathways for the respiratory air develop. However, examining the underlying molecular mechanisms is very difficult and time-consuming in mammals. As a consequence, we investigate our complex questions on one of biology's most popular model organisms: the fruit fly *Drosophila melanogaster*.

Drying out the respiratory system

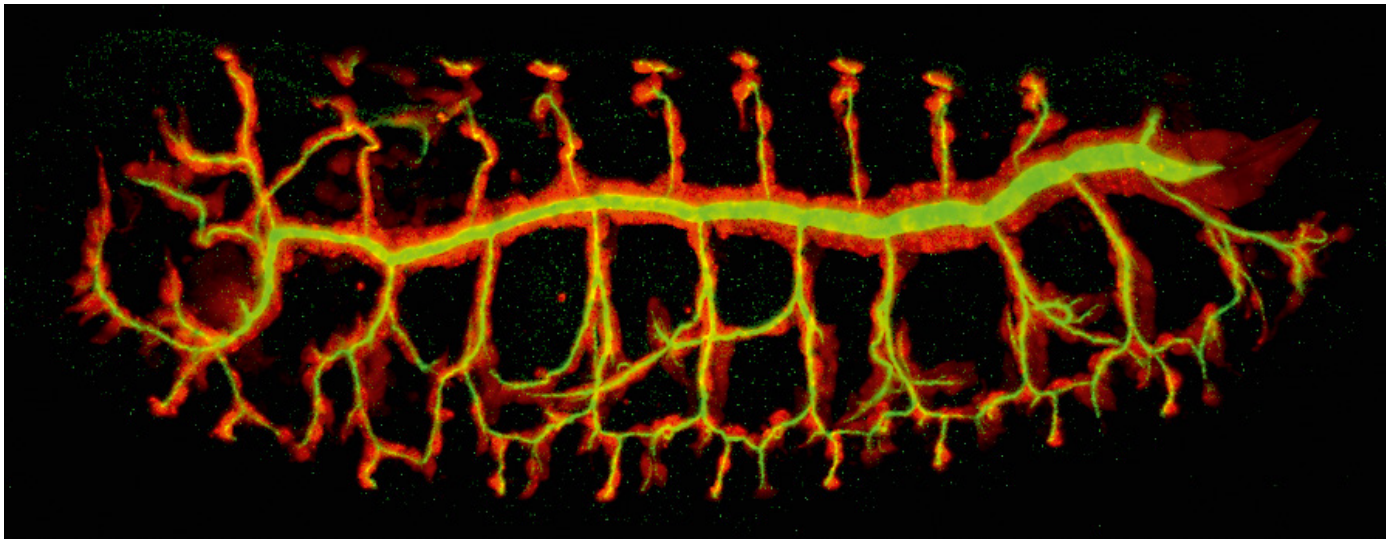
The fly and the human being are more similar in many aspects than one might think. From a total of 13,600 *Drosophila* genes approximately 7,000 genes are also present in similar form in the human genome. The fruit fly indeed has no lungs, but instead a system of arboreally branched tubular pathways for the respiratory air, the trachea. In the meantime, we know that the development of this system of tubes is organized very similarly 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 do not end up too narrow or too wide.

Common to both organisms is also that, during the developmental phase, the respiratory tubes are initially filled with liquid.



◀ Left: The cross-linkage of tracheal cell groups (red) is enabled by closely associated bridge cells (blue and designated by arrows). Right: During development, the bridge cells stretch and connect the tracheal cell groups (red). The tracheal cells migrate along the bridge cell and thus form a connecting network.

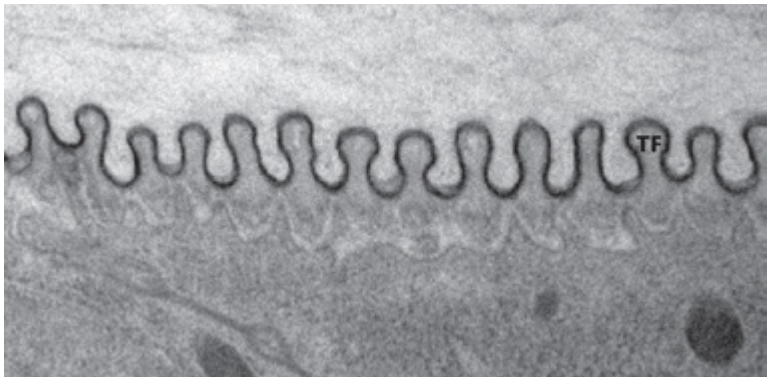
Contact
rschuh@mpibpc.mpg.de
www.mpibpc.mpg.de/schuh



◀ The lung of the insects, the tracheal system, permeates the entire embryo of the fly. Tracheal tubes are marked in green, tracheal cells in red.

Therefore, before birth or hatching of the larva, they must be dried out at the proper time, otherwise severe respiratory problems will arise. Babies born prematurely, for example, are in danger of developing 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 species boundaries.



▲ High resolution electron microscopy of the tracheal cuticle. Teanidial folds (TF) are cuticle ridges that compose a helical structure running perpendicular to the tube length along the lumen.

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Molecular Cell Differentiation

Ahmed Mansouri
received his PhD in chemistry from the Technical University of Braunschweig in 1978. Subsequently, he performed research as a postdoctoral fellow at 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 scientist at the Max Planck Institute for Biophysical Chemistry in the Department of *Molecular Cell Biology*. In 1999, he habilitated at the Medical Faculty of the University of Göttingen. Since 2002, he has been head of the Research Group *Molecular Cell Differentiation* at the Max Planck Institute for Biophysical Chemistry. Ahmed Mansouri has held the professorship of the Dr. Helmut Storz Foundation at the University Medical Center Göttingen since 2005.

Whether it be heart or kidney, pancreas or brain, the organs in our bodies are equivalent to 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 produce digestive juices, the smaller cell group produces hormones such as insulin, which regulates the blood sugar level. The mid-brain also comprises highly specialized cells such as nerve cells, for example, which produce the messenger molecule dopamine.

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

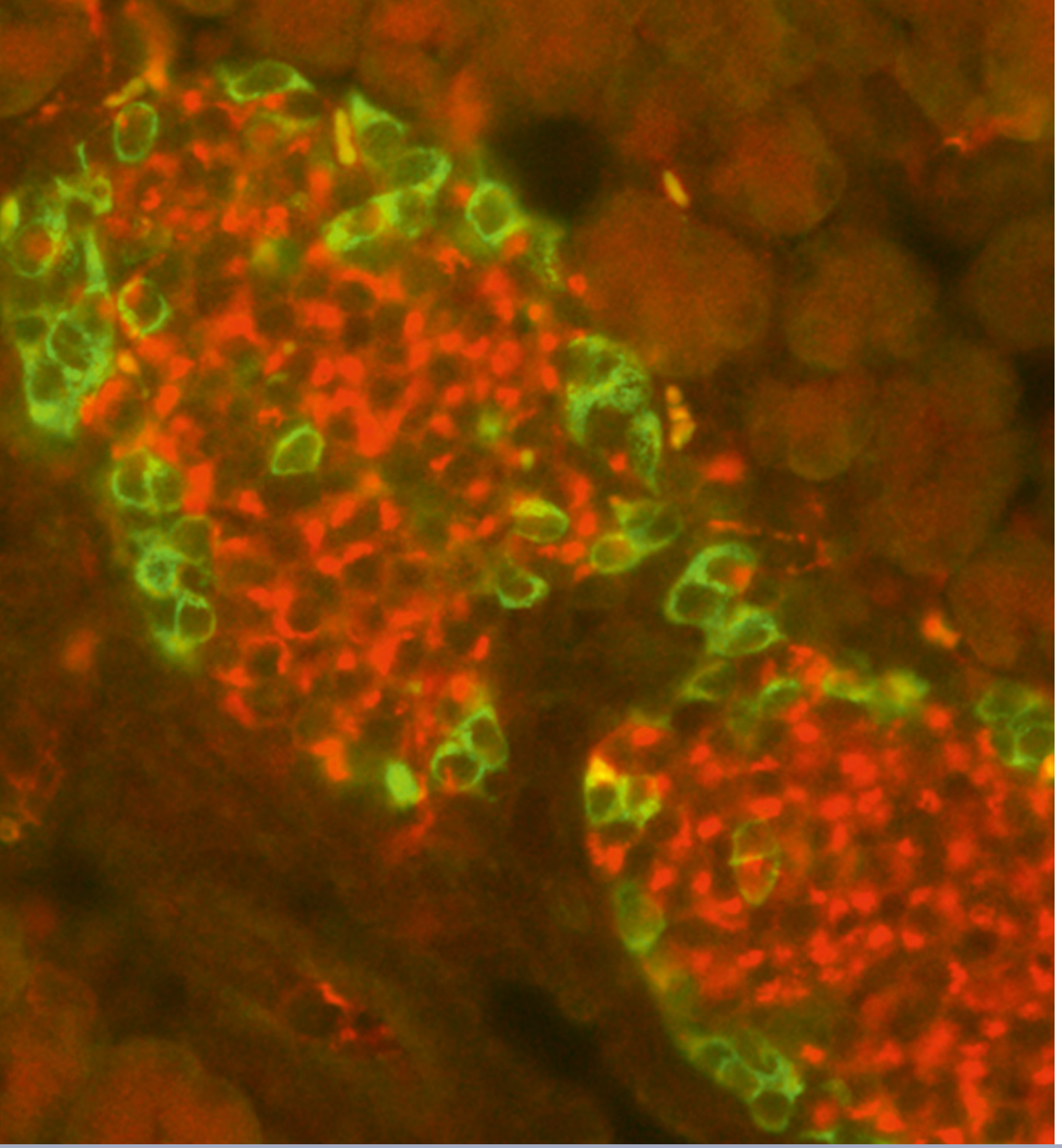
We already know that certain genes control the maturation of an organ and thus determine the subsequent fate of the cells. These control genes provide the blueprint for specific proteins, so-called transcription factors. These factors selectively switch on genetic programs or suppress them and thus transform progenitor cells into cells with specific characteristics. This has been shown by tests in which the control genes were inactivated. For example, without the control gene *Pax4*, no insulin-producing cells develop in the pancreas. Other factors cause cells to produce glucagon, insulin's antagonist. Similar control mechanisms are at work in the mid-brain. There, for example, the factor *Lmx1a* activates a genetic program in a specific group of nerve cells, which enables them to produce dopamine. In order for cells with different tasks to be formed in the correct ratio in an organ, the respective transcription factors interact with each other and thus create the required balance.

Mouse research for human beings

We investigate the maturation of an organ in mice, because we can very easily modify this 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. For example, it can be used to find new strategies for the selective treatment of certain diseases. Such a therapy could be the conversion of glucagon-producing cells into insulin-producing ones to treat patients suffering from type I diabetes.

Contact
amansou@mpibpc.mpg.de
www.mpibpc.mpg.de/mansouri

▶ Two islets of Langerhans in the pancreas, with insulin- (red) and glucagon-producing cells (green). The two hormones regulate blood sugar.



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Genes and Behavior

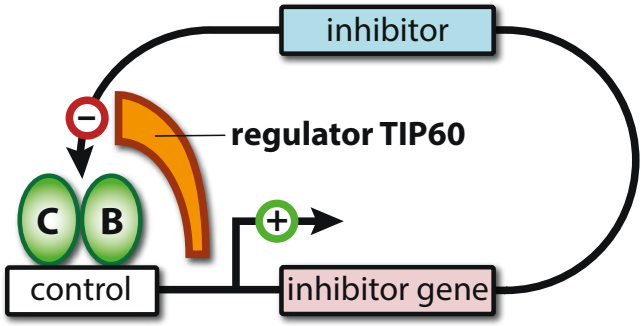
Gregor Eichele

studied chemistry and structural biology and completed his doctorate in 1980 at the University of Basel (Switzerland). He then worked as a postdoctoral fellow at the University of California, San Francisco (United States) from 1981 to 1984. Thereafter, he was a member of the faculty of the Harvard University School of Medicine in Boston (United States) until 1990, and, from 1991 to 1998, he conducted research as *Alvin Romansky Professor* at Baylor College of Medicine in Houston (United States). From 1997 to 2006, he was Director at the Max Planck Institute for Experimental Endocrinology. In 2006, he was appointed as Director at the Max Planck Institute for Biophysical Chemistry, where he has been heading the Department of *Genes and Behavior* ever since. Gregor Eichele received numerous distinctions for his research, including the Friedrich Miescher Prize, the McKnight Neuroscience Development Award, and the Innovation Award in Functional Genomics.

Contact

gregor.eichele@mpibpc.mpg.de
www.mpibpc.mpg.de/eichele
genesandbehavior.mpibpc.mpg.de

Time moves in one direction, and this sequence of events is irreversible. But this does not apply to biological systems! Many life processes show characteristic recurring patterns. A striking example is the strong influence of 24-hour rhythms on the physiology and behavior of living things. These internal clocks, also called circadian clocks, are the research topic of our department, where we examine this phenomenon using genetically modified mice. Much like in humans, a circadian clock is present in all cells of the mouse. One of the questions that interests us is how these clockworks are coordinated: Is there an overriding control center that regulates all the clocks of every cell and aligns them with each other, or are the individual clockworks controlled jointly?

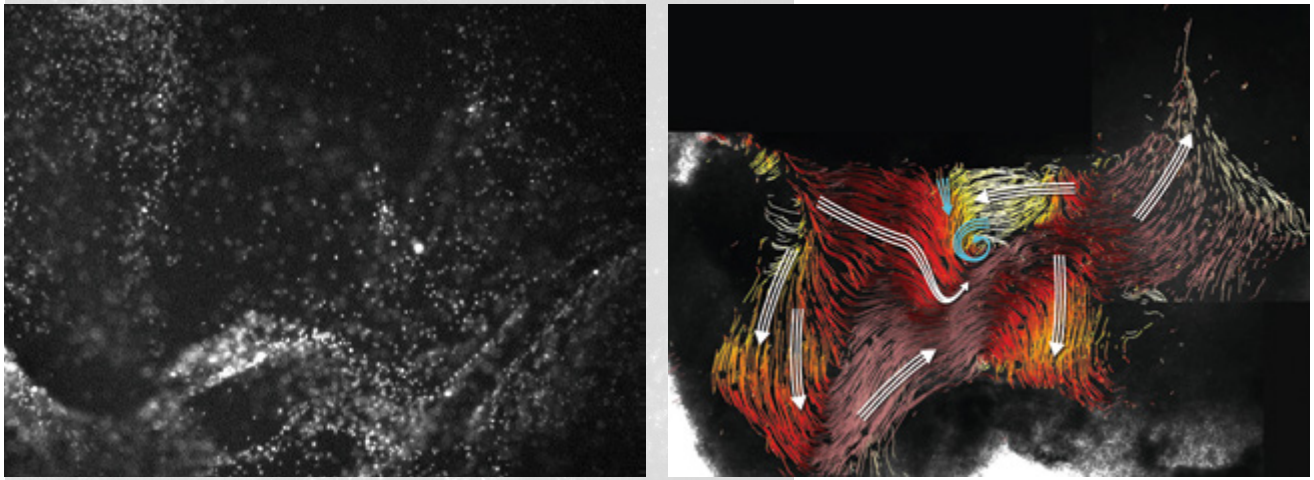


▲ **Figure 1:** Schematic circuit of the circadian clock. In the morning, the activating transcription factors Clock (C) and Bmal1 (B) bind to the control region of an inhibitor encoding gene and turn on this gene (plus sign) by means of the RNA polymerase. The so-called TIP60 protein changes Bmal1 in such a way that it subsequently activates the RNA polymerase. The polymerase then produces numerous inhibitor gene transcripts that are then used to produce the inhibitor proteins. At night, the inhibitor blocks the activators (minus sign) by preventing TIP60 from altering Bmal1. The RNA polymerase no longer produces inhibitor transcripts, the inhibitor is degraded, and a new cycle can begin.

We know that circadian clocks are controlled by positive and negative feedback (Figure 1). At the beginning of the day, activator proteins stimulate the production of inhibitor proteins, which then later in the evening block the activators. We are mainly focused on the nature of these circuits: Which proteins are responsible for the activation? As is already known, certain proteins (so-called transcription factors) play an important role here. But there are only vague ideas about what ultimately turns on the clock genes in the morning and how the inhibitors accomplish their negative feedback later in the day. By means of a gene-based screen, we discovered that a protein called TIP60 acetyl transferase is involved in the turning on of the clock genes, but is also a target of the negative feedback.

Molecular conveyor belts in the brain

We extend these molecular genetic studies to tissues. For example, we investigate the mode of action of circadian clocks in epithelial tissues lining the brain ventricles. Inside the brain of vertebrates, there are four interconnected ventricles which are filled with cerebrospinal fluid. Tiny cilia that extend into the ventricles are important for the transport of cerebrospinal fluid which is rich in signaling molecules. Through their synchronized beating pattern, the cilia create a complex network of dynamic flows that act like conveyor belts and transport molecular «cargo». We were able to observe complex motion profiles in these flows: There are



◀ **Figure 2:** Cilia-powered flows in the third ventricle of the mouse: Thousands of fluorescent beads move along the wall of the ventricle (left). This snapshot is part of a movie from which the depicted flow map is calculated by means of particle tracking software (right). Colored lines represent near-wall flows, and main flow directions in the individual areas are highlighted with white arrows. Dividing line and a whirl are highlighted in blue.

dividing lines and zones that change with the daily rhythm. The whirl in Figure 2, for example, can only be seen at the end of the night. Furthermore, vertical or horizontal barriers form depending on the time of day. For neurophysiologists the cilia-based flows indicate complex logistics through which the signaling molecules seem to be transported accurately, quickly, and under expenditure of energy inside the brain to where they are needed. We are investigating whether a circadian clock controls these rhythms and how this clock could influence such complex processes as the orientation of cilia. To get answers to our questions, we are combining mouse genetics with cellular and structural biology.

Circadian rhythms in ecosystems

Unlike the recently discovered cilia-based complex flows in the brain, the daily recurring vertical migration of an entire ecosystem consisting of plankton has been known for a long time. Both in inland freshwaters and in the ocean this migration is controlled by a circadian clock, and very likely many of the plankton components have such a clock. But there is still no clear answer to the question whether merely the change of light and dark or also additional factors, such as nutrients or the avoidance of plankton-eating sea creatures, play a critical role in migration. It is also unknown to what extent the circadian clocks in this ecosystem differ at the molecular level. Since the plankton population is complex, an individual spatio-temporal analysis of vertical movement is not feasible with classical approaches. Therefore, we use so-called metagenomic methods to capture the totality of the genome of an ecotope in order to explore the diversity of the circadian clock and the rhythm coordination of these clocks between organisms in the plankton ecosystem.

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Biomedical NMR

Jens Frahm

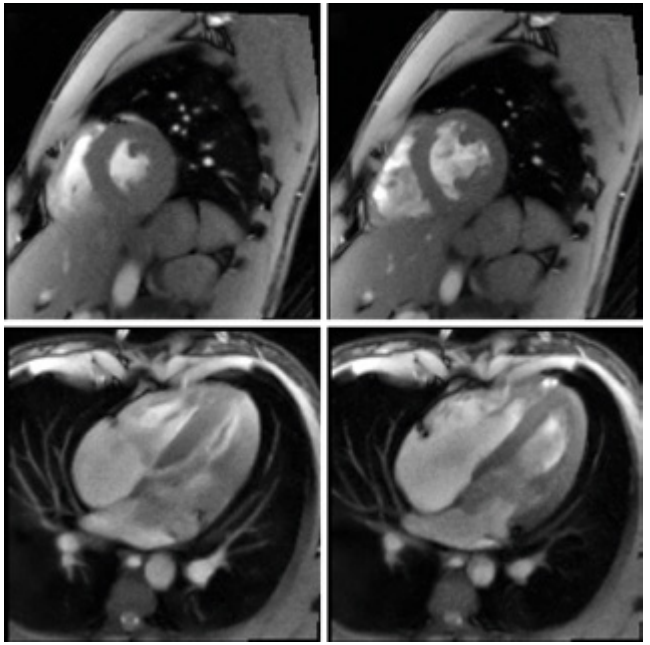
received a PhD in physical chemistry from the University of Göttingen in 1977. After leading an independent research group at the Max Planck Institute for Biophysical Chemistry until 1992, he became Director of the *Biomedizinische NMR Forschungs GmbH*, a non-profit company financed by royalties of the group's patents. Jens Frahm received multiple honors for his achievements in magnetic resonance imaging, including the Gold Medal of the Society for Magnetic Resonance in Medicine (1991), the Karl Heinz Beckurts Award (1993), the State Award of Lower Saxony (1996), the Sobek Research Award (2005), and the *Wissenschaftspreis* of the *Stifterverband für die Deutsche Wissenschaft* (2013). In 2005, he was elected as Ordinary Member of the Academy of Sciences at Göttingen. He is member of the *Hall of Fame der deutschen Forschung* since 2016.

Contact

jfrahm@mpibpc.mpg.de
www.mpibpc.mpg.de/frahm
www.biomednmr.mpg.de

The visualization of structures and functions by imaging plays an increasingly important role in biological and medical research. This also applies to magnetic resonance imaging (MRI), which offers noninvasive, detailed, and quantitative insights into the bodies of animals and humans.

Research in our group is devoted to the development of advanced MRI techniques for novel applications in science and medicine. Applying MRI allows us to link findings in molecular biology and genetics to anatomic, biochemical, and physiologic properties in the intact organism. Our studies of individual animals primarily focus on mouse models of human brain disorders, which are monitored during disease progression and treatment. In humans, for example, MRI methods measuring the directional mobility of water molecules helped us redefine the topography of nerve fiber bundles in the corpus callosum connecting the brain hemispheres. These experiments are based on a diffusion-weighted MRI technique that is insensitive to tissue susceptibility differences and that may, in the future, markedly improve clinical applications in the diagnosis of stroke and cancer.



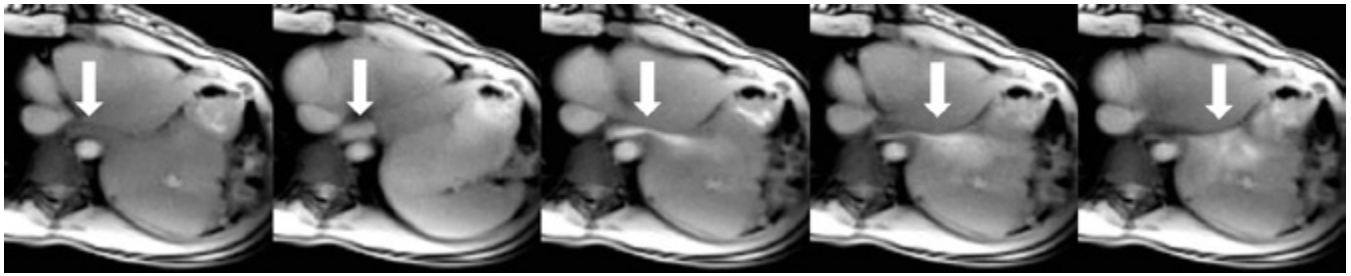
▲ Selected systolic (left) and diastolic images (right) with a temporal resolution of 33 milliseconds of real-time MRI movies of the human heart at 30 frames per second in a short-axis view (top) and a four-chamber-view (bottom).

In light of ground-breaking progress in real-time MRI, our recent work aims at the further development of optimized data acquisition techniques and extended iterative image reconstruction methods. This project is complemented by a wide range of hitherto impossible dynamic MRI applications successfully performed by our group for the first time as well as translation of the technology into broader clinical use.

Real-time MRI

As early as 1985, we invented a new principle for the acquisition of rapid images (FLASH = fast low-angle shot) that revolutionized the scientific potential and clinical impact of MRI. Based on this technique, we recently succeeded in another fundamental acceleration towards dynamic MRI at a temporal resolution of up to 100 frames per second. The technology can be fully integrated into commercial MRI systems, where it may be used for clinical trials without expert knowledge or user interference.

Current real-time MRI studies of body motions deal with movements of the wrist, knee, foot, or temporomandibular joint. We further expanded real-time MRI to completely new applications, for example in the oropharyngeal area, ranging from monitoring articulatory processes such as speaking, stuttering, and brass playing to swallowing dynamics including reflux diagnostics. One specific real-time MRI result of utmost scientific and clinical interest is our discovery that inspiration rather than cardiac pulsation is the major regulator of human cerebrospinal fluid flow.

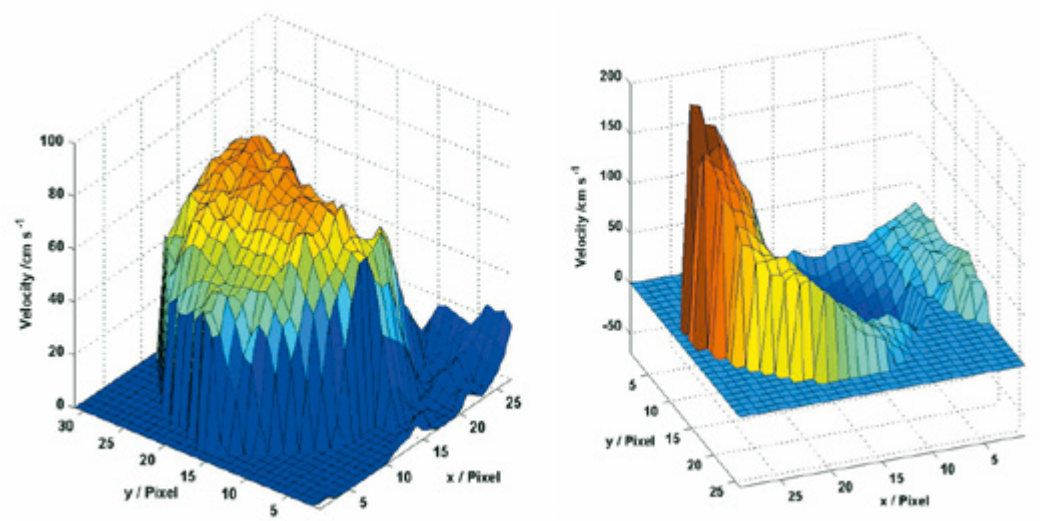


Selected images with a temporal resolution of 40 milliseconds of a real-time MRI movie at 25 frames per second of a single swallow of pineapple juice (light grey). From left to right: before arrival in the lower esophagus, with closed esophageal sphincter, and during entrance into the stomach.

Cardiovascular applications are attractive for several reasons. First of all, real-time MRI – without the need for synchronization to the electrocardiogram and during free breathing – for the first time enables reliable studies of patients with cardiac arrhythmias. Moreover, a comprehensive real-time MRI protocol, combining access to cardiac function, blood flow, and myocardial tissue characterization, will lead to much shorter and more tolerable examinations. Finally, the possibility to monitor immediate physiologic responses of the cardiovascular system to stress or exercise offers new diagnostic opportunities, for example during early phases of heart insufficiency.

Future developments

The increasing use of quantitative MRI techniques will benefit from so-called model-based reconstructions, which take advantage of a known signal model (for example, a relaxation process) to map the underlying physical or physiologic model parameters. Rather than calculating serial images, which are then fitted to a model function, our proposed methods directly determine parameter maps from raw data and thereby optimally exploit redundancies inherent to the model, that is, *a priori* knowledge. First developments deal with highly accurate maps of the T1 relaxation time as well as velocity maps of human blood flow at much improved spatiotemporal acuity. Another class of foreseeable clinical applications will be the use of real-time MRI for guiding minimally invasive interventions.



Real-time MRI of blood flow in the human aorta of a healthy person (left) and a patient with partial valve insufficiency and stenosis (right). The diagrams represent selected systolic frames with a temporal resolution of 35 milliseconds of the velocity distribution inside the ascending aorta.

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Sleep and Waking

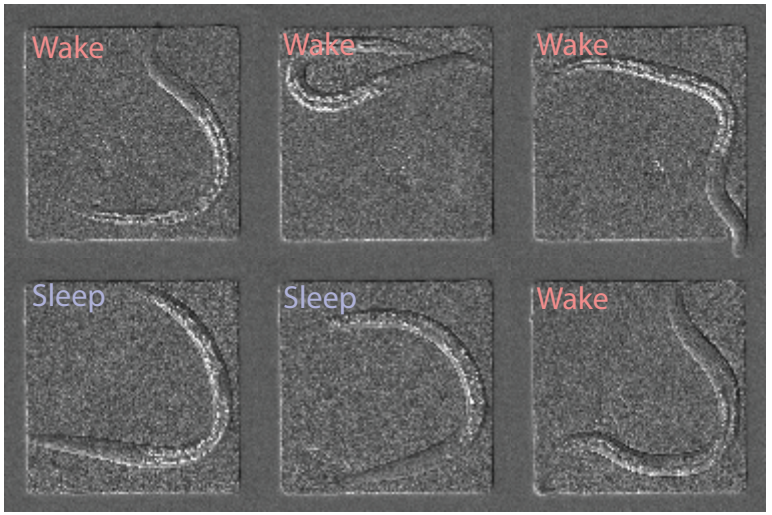
Henrik Bringmann
studied biology in Göttingen and Heidelberg and completed his PhD at the Max Planck Institute for Cell Biology and Genetics in Dresden in 2007. Subsequently, he worked as a postdoctoral fellow at the MRC Laboratory of Molecular Biology in Cambridge (Great Britain). Since 2009, he has been heading the Max Planck Research Group *Sleep and Waking* at the Max Planck Institute for Biophysical Chemistry. Henrik Bringmann was awarded the Otto Hahn Medal of the Max Planck Society in 2008 and an ERC Grant in 2015.

Sleep and waking are part of life for every animal and every human. It appears obvious why we are awake. 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 humans feel tired and are inefficient, and experiments show that animals can die from sleep deprivation. Today, researchers believe that sleep is not only important for energy conservation but also for learning and memory, fundamental cell biological processes such as macromolecule synthesis as well as for nervous system regeneration.

In our research group, we are studying the regulation and functions of sleep. We want to find out how a nervous system falls asleep and wakes up, how it knows when it is tired and has to sleep. We also hope to understand how sleep carries out essential functions, thereby making it indispensable for animals and humans.

The sleeping worm

We are studying sleep and waking in the simplest organism accessible by modern molecular biology tools: the roundworm (nematode) *Caenorhabditis elegans*. Although roundworms and humans are apparently quite different from each other, sleep in *C. elegans* appears to be highly similar to that of humans: Worms move less, exhibit reduced responsiveness, yet can be woken up, and sleep deprivation increases sleep pressure and thus a return to sleep. As sleep is an evolutionary ancient process, it is expected that many



◀ Worm sleep can be followed over long time periods by culturing the worms in microfluidic chambers. During sleep, worms stop moving and eating, and have an elevated arousal threshold.
Sleep deprivation leads to increased sleep pressure and rebound sleep.

Contact
henrik.bringmann@mpibpc.mpg.de
www.mpibpc.mpg.de/bringmann

aspects of worm and human sleep are regulated by similar molecular mechanisms.

Another advantage of our model organism is its simple nervous system: It consists of 302 neurons only, and the connectivity between these neurons is known. Because the animals are transparent, we can observe and manipulate the nervous system in intact animals both during sleep and waking.

Our main objective is to find out how sleep is regulated on the molecular level. This would then enable us to eliminate sleep in a controlled manner, allowing us to study the consequences of sleep loss on development, regeneration, aging, and general well-being.

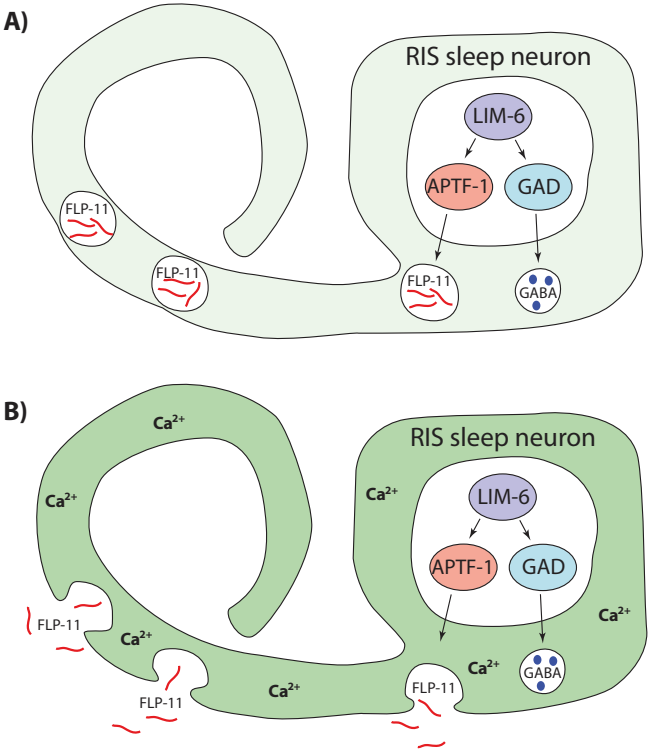
Sleep-active neurons

Scientists already know that sleep is controlled by sleep-active, sleep-promoting neurons that specifically activate at the onset of sleep and release inhibitory signaling molecules (so-called neurotransmitters) such as GABA and neuropeptides. Such neurons are found in the human and mammalian brain alike. Little is known, however, about how sleep neurons are regulated and how they regulate sleep. Understanding the control of these sleep neurons will allow an understanding of sleep regulation and functions.

Yet, the complexity of the mammalian brain makes the molecular mechanistic dissection of sleep difficult. Studying sleep in *C. elegans* is much easier compared with studying mammals. The worm's sleep patterns can be dissected on the molecular level by means of genetic screening and generation of genetically modified worm lines.

In the recent past, our lab has identified a single neuron called RIS, which is indispensable for sleep induction. Like its mammalian counterparts, it is active at sleep onset, actively induces sleep, and releases inhibitory neuropeptides as well as GABA. We found that sleep induction requires a network of so-called transcription factors that leads to the synthesis and release of a neuropeptide called FLP-11. Intriguingly, these factors are also linked to human sleep disorders. This highly simplified, yet evolutionarily conserved system should make it possible to solve the molecular biology of sleep: It is just one single sleep-active neuron in a defined circuit within a genetically amenable system. We are currently figuring out what controls the activation of the sleep neuron RIS and how it induces a systemic sleep state. Ablation of this sleep system is easily possible by genetic or surgical removal of RIS, and by studying the consequences of sleep loss we can elucidate how sleep exerts its restorative functions.

The genes, which we found to control sleep in worms, appear to be conserved from worm to human. We are currently testing the results from our worm sleep studies in more complex animal models as well as in human beings to learn more about the increased complexity of sleep and to study the bases of human sleep disorders.



◀ A molecular machinery drives sleep in the roundworm: Sleep is induced by a single neuron called RIS. At the onset of sleep, this neuron depolarizes and releases inhibitory neurotransmitters including GABA and FLP-11 neuropeptides to induce sleep. **A)** Wake: The transcription factors LIM-6 and APTF-1 specify sleep-promoting RIS function by causing FLP-11 expression. **B)** Sleep: Calcium (Ca^{2+}) transients trigger FLP-11 release at sleep onset.

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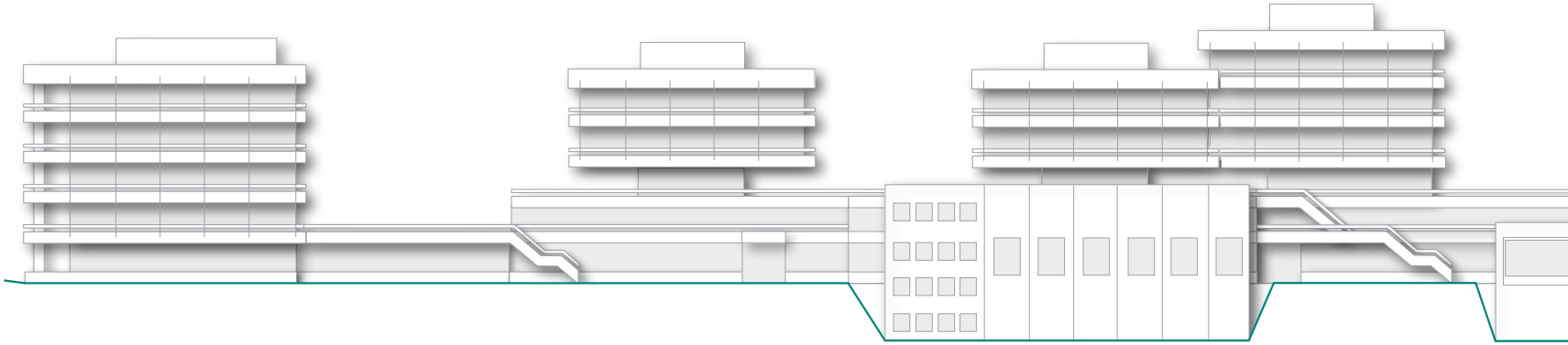


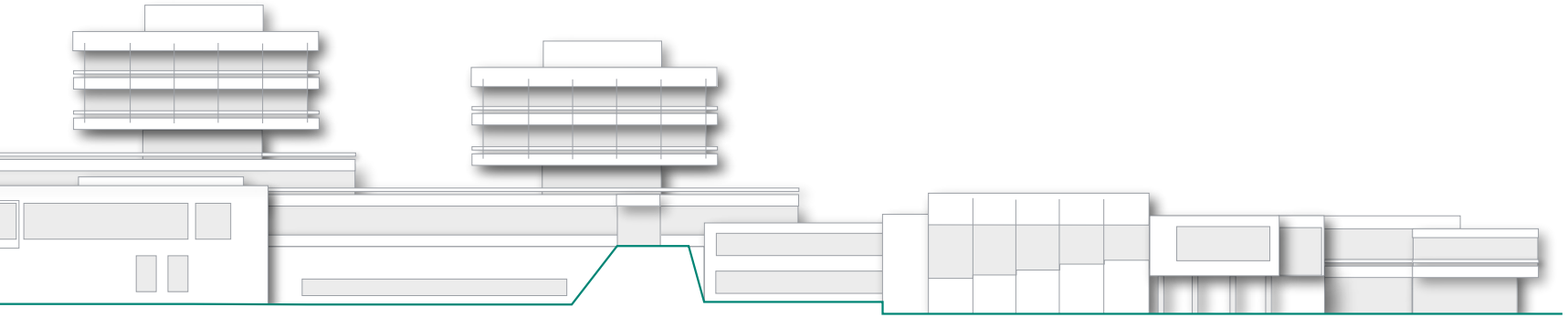
Excellent service for cutting-edge research

What to do when an important element is missing, be it in a complicated experimental set-up or in one's stock of knowledge? Outstanding research at the institute would not be possible without the corresponding service infrastructure. This is provided by top-trained colleagues in the central scientific facilities, workshops, administration, and additional service groups.

Administration

Canteen and Espresso Bar

Carpentry



Child Care Facility

Facility Management

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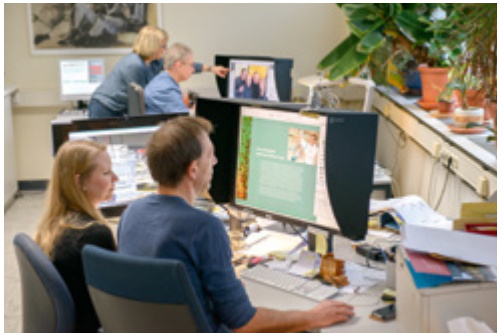
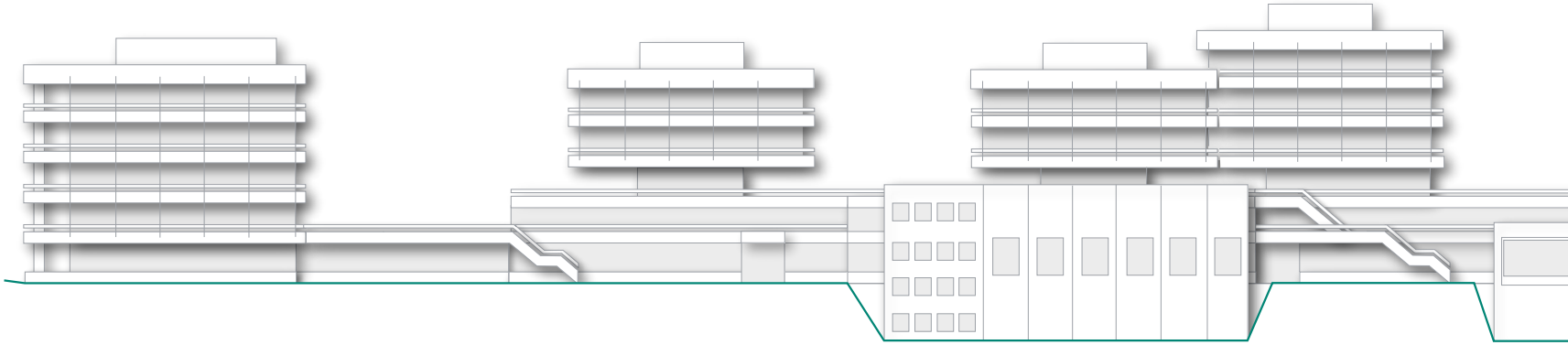
Otto Hahn Library



Public Relations Office & MediaService

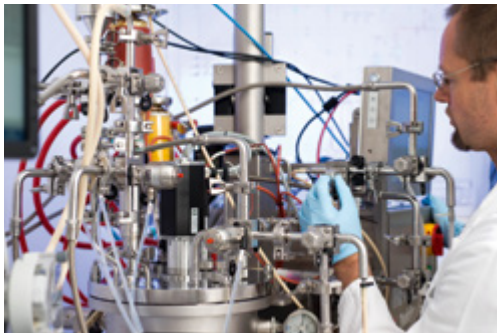
Workshop for Precision Mechanics



Service for scientists

Advanced Light Microscopy

Animal Facility

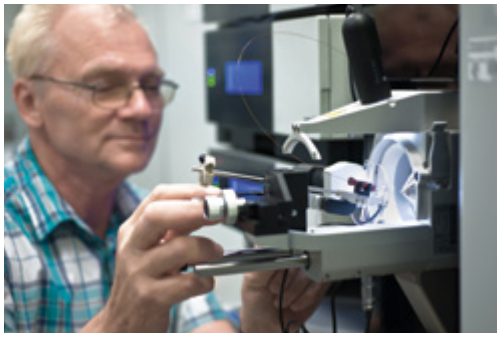


Bioreactor

Career Service for Junior Researchers

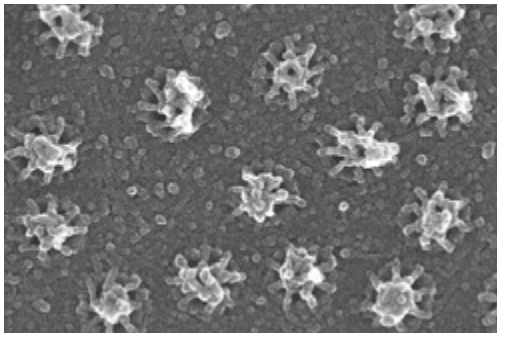
Crystallization

EU Liaison Office



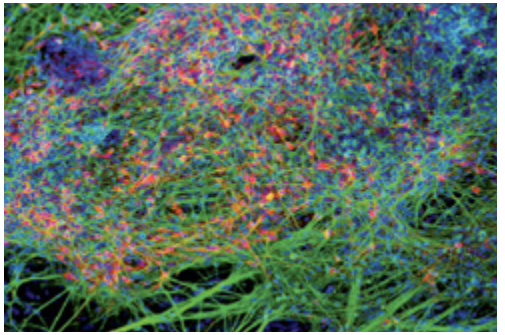
Proteomics

Research Support



Scanning Electron Microscopy

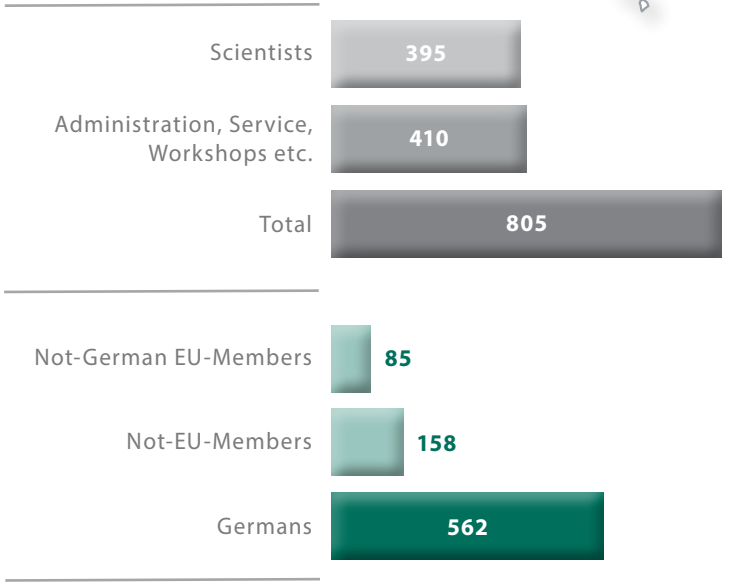
Synthetic Chemistry

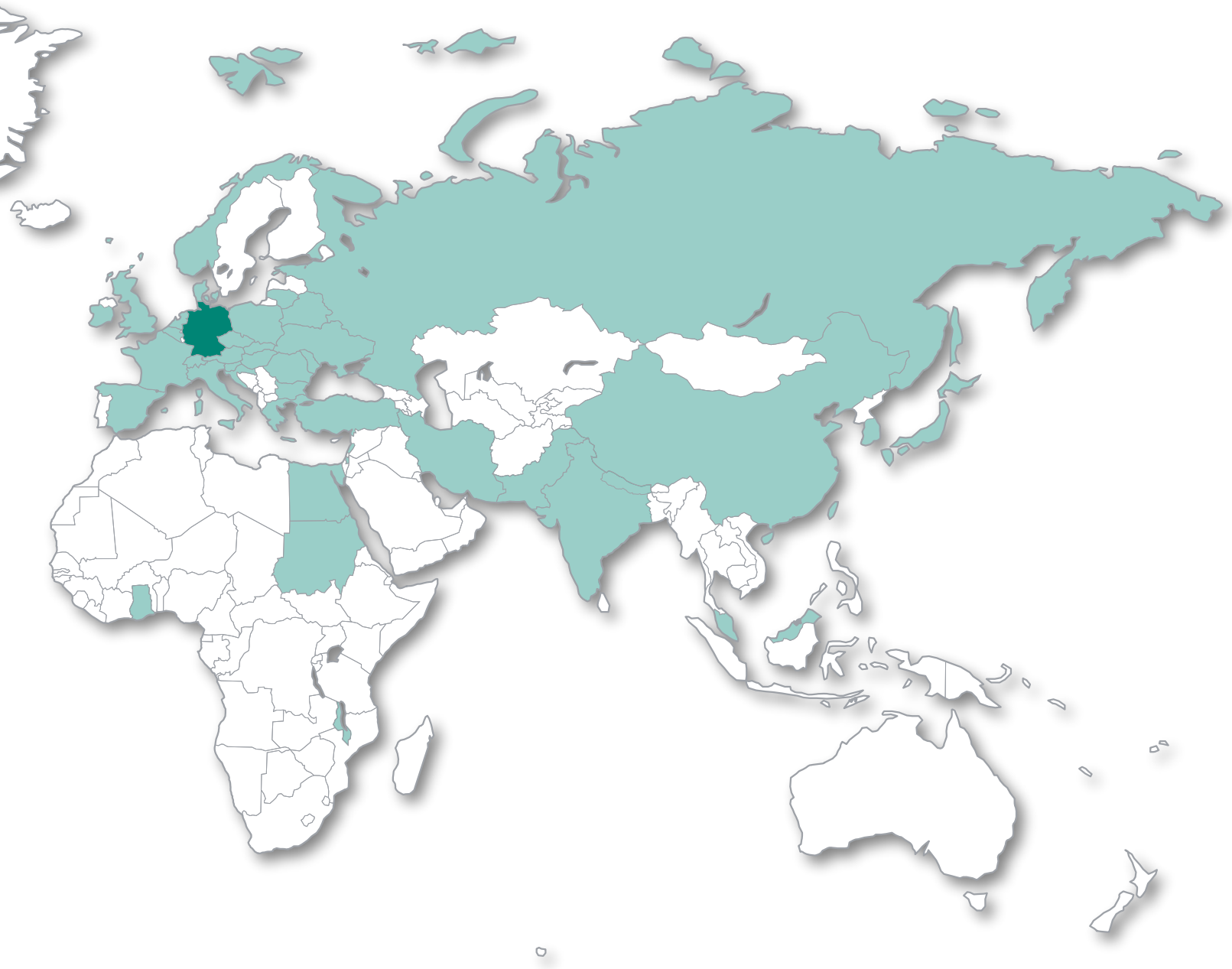


Transgenic Cell Culture

Transmission Electron Microscopy

Employees from 51 countries at the institute



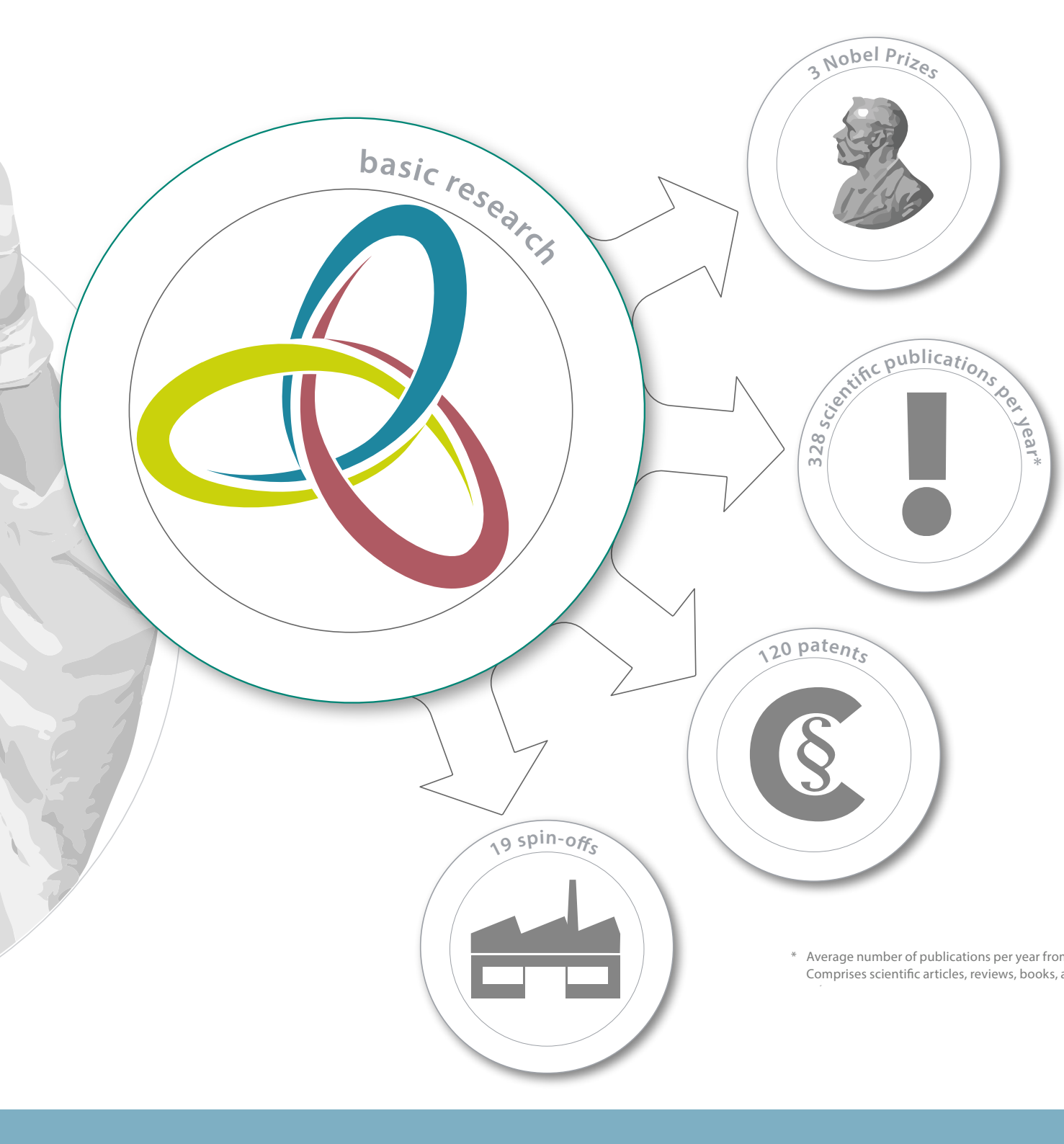


Knowledge transfer

How can one measure the success of basic research?

The most common criterion are scientific publications. How many articles are published in scientific journals, and how many of these have appeared in the renowned journals such as *Nature, Science*, and *Cell*?

However, outstanding research is never only an end in itself. Again and again, it promotes great discoveries and inventions. At the Max Planck Institute for Biophysical Chemistry this is not only documented by three Nobel Prizes – ground-breaking research findings from the institute are also of economical relevance: The list of successful patents granted to scientists at the institute is long. Furthermore, researchers transferred their insights from the lab to practical applications by founding numerous spin-offs, thus creating more than 1,000 jobs.



* Average number of publications per year from 2011 to 2016.
Comprises scientific articles, reviews, books, and book chapters.

The Max Planck Society

The Max Planck Institute for Biophysical Chemistry is one of presently 83 institutes of the Max Planck Society for the Advancement of Science. As an independent, non-profit research organization, the Max Planck Society carries out basic research as service to the general public in the sections Biology & Medicine, Chemistry, Physics & Technology, and Humanities & Social Sciences.

The Max Planck Society is mainly financed by public funds of the Federation and the States. It recruits scientists who are leaders in their field world-wide as Directors, and allows them all freedom for their research. New and innovative ideas are especially promoted.

With this strategy, today, the Max Planck Society is one of the leading research institutions in the world. Since its foundation in 1948, many of its scientists have received renowned awards. 18 times the Nobel Prize has been awarded to Max Planck researchers.

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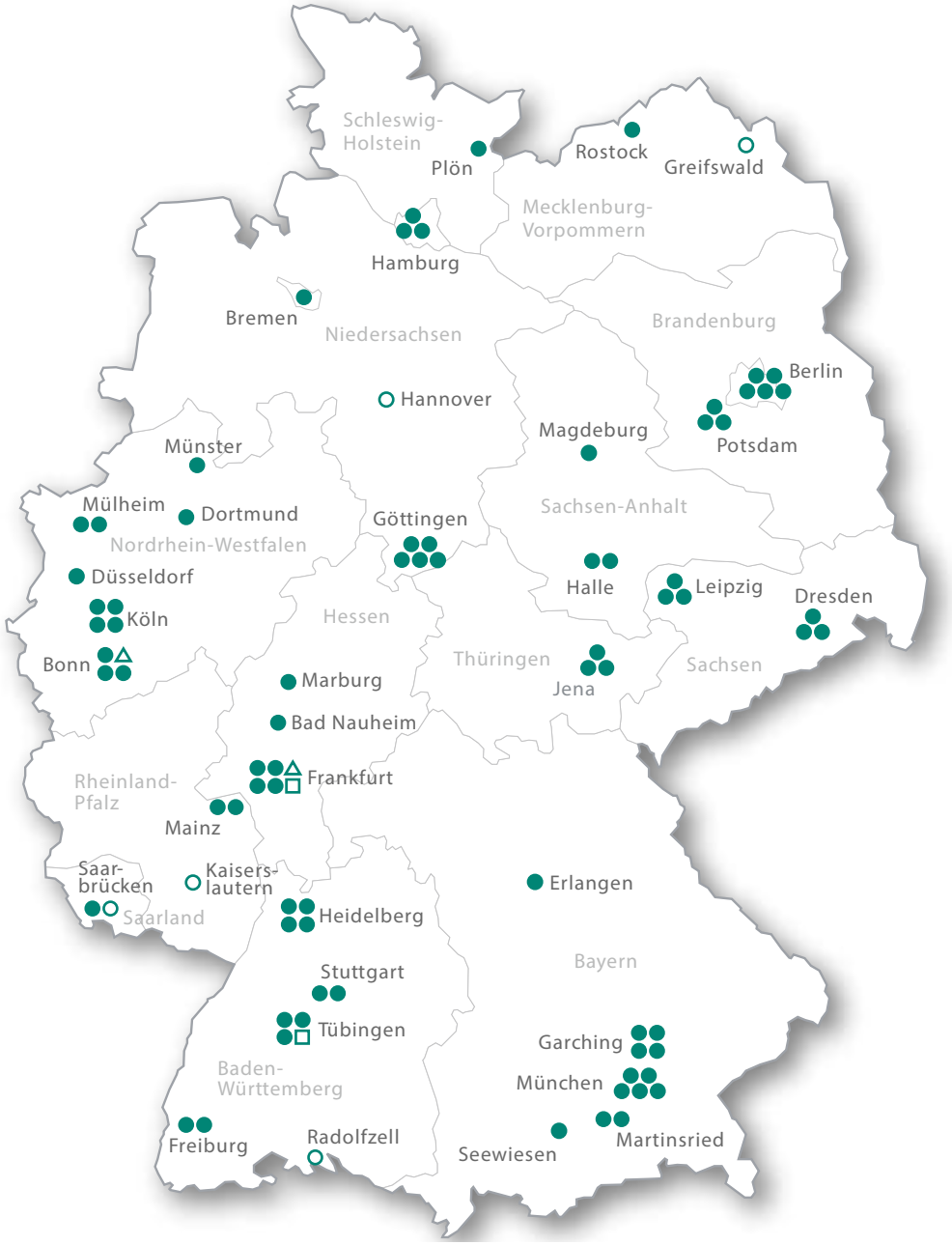

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Linguistics
Frankfurt a.M., Jena, Leipzig, München, Nijmegen (Netherlands).

Emeritus Directors of the institute

Manfred Eigen
Biochemical Kinetics
(1971-1995)

On Manfred Eigen's initiative the Max Planck Institute for Biophysical Chemistry was founded in 1971. It was his vision to investigate complex processes of life with biological, chemical, and physical methods at this new institute. After his pioneering studies on ultra-fast reactions, for which he was honored with the Nobel Prize in Chemistry in 1967, he turned to biochemistry and worked on questions concerning evolution. His theories on self-organization of complex molecules and his development of «evolution machines», with which he translated his theories into practice, established a new branch of the German biotechnology industry – the evolutionary biotechnology.

Hans Kuhn †
Molecular Systems
(1971-1984)

Hans Kuhn worked on the chemistry of interfaces. He furthermore investigated the self-organization of molecular systems. In this context, he designed supra-molecular machines and analyzed the physicochemical conditions for the origin of life. His research contributed to the understanding of the mechanisms of photosynthesis, of proton pumps, and of ATP synthases. One of his associate researchers was the later Nobel laureate Erwin Neher.

Hans Strehlow †
Electrochemistry and Reaction Kinetics (1971-1984)

Hans Strehlow became Director at the Max Planck Institute of Physical Chemistry in 1966 and joined the Max Planck Institute for Biophysical Chemistry after its foundation in 1971. After having dealt with aspects of electrochemistry early in his career, Hans Strehlow was later interested in questions concerning reaction kinetics of elemental processes in solution. Important insights into the kinetics of ion reactions in liquid solution and detergent mixtures were born of his research. One of his doctoral students was Jens Frahm, head of the Biomedical NMR.

Albert Weller †
Spectroscopy (1971-1990)

Before the foundation of the Max Planck Institute for Biophysical Chemistry, Albert Weller was Director at the Max Planck Institute for Spectroscopy. His research dealt with the physicochemical basis of photochemistry. He worked on the molecular absorption and emission spectroscopy of crystals, solutions, and fumes as well as on electron-spin resonance spectroscopy. He additionally performed pioneering kinetic, thermodynamic, and spectroscopic experiments with electronically stimulated molecules.

Otto D. Creutzfeldt †
Neurobiology (1971-1992)

Otto D. Creutzfeldt made fundamental contributions to the understanding of epilepsy and the role of the cerebral cortex in seeing and speaking. Moreover, using intracellular derivation of cortical neurons he provided substantial insights into the neurophysiological basis of EEGs and the functioning of the visual system. He trained a number of important neurobiologists, among them Nobel laureate Bert Sakmann and Max Planck Directors Wolf Singer and Heinz Wässle.

Fritz Peter Schäfer †
Laser Physics (1971-1994)

Fritz Peter Schäfer applied spectroscopic techniques in order to investigate stimulated emission and quantum processes of chemical compounds. He furthermore worked with lasers and thus developed the dye laser (at the same time as Peter Sorokin) which was applicable on a broad spectral band. Later, he was also interested in X-ray lasers. He founded the *Laser-Laboratorium Göttingen e.V.* together with Jürgen Troe and Dirk Basting.

Emeritus Directors of the institute

Leo C.M. De Maeyer †
Experimental Methods
(1971-1995)

Initally, as associate researcher with Manfred Eigen, Leo C.M. De Maeyer substantially contributed to further development of the so-called relaxation techniques. Besides these, he later investigated, among others, the kinetics of chemical processes and worked on electronic data processing and process control as Director at the Max Planck Institute for Biophysical Chemistry. His experimental methods have found broad application in many areas of biology, chemistry, and physics. He was deeply involved in the consolidation of the Max Planck Institute for Spectroscopy and the foundation of the new Max Planck Institute for Biophysical Chemistry.

Manfred Kahlweit †
Kinetics of Phase Transitions
(1971-1996)

Manfred Kahlweit's research on crystal growth, phase diagrams of complex systems, and micro-emulsions provided important insights for the application of physicochemical methods and contributed to the elucidation of fundamental mechanisms of biological processes. As a member of the University of Bremen's founding senate and as temporary head during the foundation of the Max Planck Institute of Colloids and Interfaces in Potsdam, he made important contributions to the development of the scientific landscape in Germany.

Thomas M. Jovin
Molecular Biology
(1971-2007)

Two research areas are at the focus of interest of Thomas M. Jovin's emeritus group at the institute: the activation of normal or cancerous cells with growth factors or other external factors as well as the molecular mechanisms of Parkinson's disease. Thomas M. Jovin's Emeritus Group *Laboratory of Cellular Dynamics* applies a number of methods in biophysics, molecular, and cell biology to find the cause and possible preventive measures of the toxicity of so-called amyloid aggregates in the brain's nerve cells.

Victor P. Whittaker †
Neurochemistry (1973-1987)

Victor P. Whittaker profoundly contributed to the understanding of neuronal synapses. He was the first to isolate so-called synaptosomes that made it possible to investigate synaptic signal transduction. He also succeeded in proving that synaptic vesicles contain neurotransmitters. Victor P. Whittaker trained a number of successful neurobiologists, among them later Nobel laureate Thomas C. Südhof, who performed his PhD research at the institute.

Klaus Weber †
Biochemistry and Cell Biology (1975-2004)

Klaus Weber played an important role in the change in the scientific direction at the institute so that today it includes also key areas in molecular, cell, and developmental biology. His research focused on the cytoskeleton and biochemical anatomy of actin-containing structures such as stress fibers and microvilli, on microtubules, and intermediate filaments. He pioneered the use of immunofluorescence microscopy to visualize the arrangements of these and other structures in cells and tissues. Using protein chemistry, six different actins as well as five different intermediate filament types were defined. The development of monoclonal antibodies specific for each intermediate filament type provided reagents to classify human tumors.

Erwin Neher
Membrane Biophysics (1983-2011)

Together with Bert Sakmann, Erwin Neher received the Nobel Prize in Medicine or Physiology in 1991 for his ground-breaking discoveries concerning the function of single ion channels in the cell membrane. With his Emeritus Group *Membrane Biophysics*, Erwin Neher investigates the mechanisms of neurotransmitter release and synaptic short-term plasticity. Furthermore, the role of calcium ions in signal transduction is a focus of his interest.

Bert Sakmann
Cell Physiology (1985-1988)

Six years after his appointment as Director at the institute, Bert Sakmann received the Nobel Prize in Physiology or Medicine together with Erwin Neher for his ground-breaking discoveries concerning the function of single ion channels in the cell membrane. In 1988, he moved to the Max Planck Institute for Medical Research in Heidelberg. With his emeritus group at the Max Planck Institute of Neurobiology he studies the functional anatomy of circuits in the cerebral cortex and works on the *in silico* reconstruction of a cortical column.

Emeritus Directors of the institute

Dieter Gallwitz
Molecular Genetics
(1986-2004)

Dieter Gallwitz' research provided important insights in the fields of histone biosynthesis and -modifications, RNA splicing, and protein transport between different cellular compartments. A special focus of his work were so-called Ypt/Rab-GTPases discovered by him, which play a central role in vesicle trafficking during exo- and endocytosis.

Peter Gruss
Molecular Cell Physiology
(1986-2014)

Peter Gruss put his research focus on gene regulation and analyzed processes that control genetic programs of tumor viruses and during embryonic development. In mice he identified the so-called pax genes that regulate the development of various organs. Moreover, he significantly contributed to understanding the development of the pancreas' insulin-producing islets of Langerhans. From 2002 until 2014, he was President of the Max Planck Society.

Jürgen Troe
Spectroscopy and Photochemical Kinetics (1990-2008)

With his Emeritus Group *Spectroscopy und Photochemical Kinetics* Jürgen Troe investigates reaction kinetics and reactions of molecule ions in plasma. Based on their findings Jürgen Troe and his co-workers develop theoretical models that can be applied in various fields: in astro- and atmosphere chemistry as well as in plasma- and photochemistry and in combustion chemistry. Jürgen Troe is one of the founders of the *Laser-Laboratorium Göttingen e.V.*

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Cover picture (background): Sperm cells of the fruit fly *Drosophila melanogaster* expressing the fluorescent protein rsEGFP2 bound to α-Tubulin (Sebastian Schnorrenberg, Stefan Jakobs; Research Group *Structure and Dynamics of Mitochondria*)

Front cover (circles from left to right): artistic representation of atoms reacting with a surface (Alec M. Wodtke, Department of *Dynamics at Surfaces*); structure of a mitochondrial membrane channel (Rodolfo Briones, Research Group *Computational Biomolecular Dynamics*); structure of the ribosome (gray and white) with the helper protein SelB (red) (Niels Fischer, Department of *Structural Dynamics*); embryo of the fruit fly *Drosophila melanogaster* (Halyna R. Shcherbata, Max Planck Research Group *Gene Expression and Signaling*)

Page 8: Manfred Eigen (Peter Blachian)

Page 9 (left): Erwin Neher and Bert Sakmann (Ulla Lüthje, Max Planck Institute for Biophysical Chemistry)

Page 10: Karl Friedrich Bonhoeffer (Max Planck Institute for Biophysical Chemistry, archive)

Page 14: STED microscopy (inner circle) provides about ten times more detailed images of a nerve cell's filament structure compared to a conventional microscope (outer image). (Gerald Donnert, Stefan W. Hell; Department of *NanoBiophotonics*)

Page 32/33: fotolia, Marc Rossmann

Page 40: Mass spectrum of a peptide with the structure of an RNA-bound protein in the background (Romina Hofele, Christoph Lenz, Henning Urlaub; Research Group *Bioanalytical Mass Spectrometry*)

Page 52: Structure of the ribosome (gray and white) with the helper protein SelB (red) (Niels Fischer, Department of *Structural Dynamics*)

Page 64/65 (background): Structure of a ribosome (Marina Rodnina, Department of *Physical Biochemistry*)

Page 68: Expression of the transcription factor Uncx4.1 (green) and the tyrosin hydroxylase (orange) in dopamin producing nerve cells, which play a role in Parkinson's disease (Tamara Rabe, Ahmed Mansouri; Research Group *Molecular Cell Differentiation*)

Page 79 (background): Structure of ubiquitin (Rogerdodd, Wikimedia Commons)

Page 80: Fruit flies, which due to a mutation cannot produce the micro-RNA mir-310s, develop ovaries whose follicles have epithelial defects and deposit fat. Cell membranes are colored in red, fat in green, cell nuclei in blue. (Halyna R. Shcherbata, Ömer Çiçek; Max Planck Research Group *Gene Expression and Signaling*)

Page 100/101: Scanning electron microscopy picture of nuclear pores (Volker Cordes, Facility for *Scanning Electron Microscopy*), transgenic cell culture (Ahmed Mansouri, Facility for *Transgenic Cell Culture*), Career Service for Junior Researchers (Peter Heller)

Page 102: fotolia, macrovector

Page 107: Map of Germany (Max Planck Society)

Page 108: Manfred Eigen (Peter Goldmann, Max Planck Institute for Biophysical Chemistry), Hans Kuhn (Max Planck Institute for Biophysical Chemistry, archive), Hans Strehlow (Max Planck Institute for Biophysical Chemistry, archive)

Page 109: Albert Weller (Max Planck Institute for Biophysical Chemistry, archive), Fritz Peter Schäfer (Peter Goldmann, Max Planck Institute for Biophysical Chemistry), Otto D. Creutzfeldt (Max Planck Institute for Biophysical Chemistry, archive)

Page 110: Leo C.M. De Maeyer (Max Planck Institute for Biophysical Chemistry, archive), Manfred Kahlweit (Max Planck Institute for Biophysical Chemistry, archive)

Page 111: Victor P. Whittaker (Max Planck Institute for Biophysical Chemistry, archive), Bert Sakmann (Max Planck Institute for Neurobiology)

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Pages 20-39, 42-51, 54-67, 70-79, 82-97: Images on pages of departments and research groups by the scientists of the respective labs, if not stated otherwise

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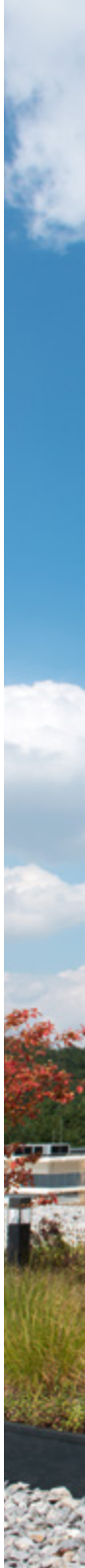

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Frederik Köpper, Carmen Rotte

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