DR JOACHIM HEBERLE

Deciphering the mechanisms of membrane proteins

Dr Joachim Heberle applies advanced spectroscopic techniques to analyse the biophysical dynamics of cellular membrane protein workings in a wide variety of contexts

What are your primary research objectives?

My research is focused on membrane proteins; the nanomachines that cells employ to communicate with their environment and catalyse the import and export of foodstuffs, or metabolites.

Could you describe your current investigations into the mechanisms of proteins?

One of my current interests is channelrhodopsin, a retinal protein structurally similar to the rhodopsin that we use for vision. A major difference is that channelrhodopsin is a cation channel which opens upon absorption of visible light.

Ion channels are of central interest to neurophysiology as they are involved in nerve propagation. However, the functional mechanism of this very broad class of membrane proteins is not at all resolved. In particular, the structural changes of the protein and the dynamics that eventually lead to channel opening and closure are not well understood.

The option to trigger and synchronise an ion channel by a short laser pulse is unique and allows us to perform experiments over the range of femtoseconds to minutes and hours (10-15 to 103 seconds). Thus, we can trace the entire functional dynamics of an ion channel by time-resolved molecular spectroscopy. We are able to follow the isomerisation reaction of the retinal chromophore, conformational changes of the protein backbone and, very specifically, proton transfer reactions among amino acid side chains. Proton transfer within a protein leads to changes in electrostatics, which is the strongest non-covalent interaction in proteins. Thus, altered electrostatics drive structural changes of the ion channel.

How is vibrational spectroscopy useful to your work?

Vibrational spectroscopy monitors the motions of atoms. If the atoms form a chemical bond, the vibrational frequency of the atoms is a measure of the strength of the bond. The challenge for vibrational spectroscopy when applied to macromolecules, like proteins, is selectivity. Imagine probing the vibrations of a mid-sized protein that is comprised of 10,000 atoms. It follows from physico-chemical principles that the vibrational spectrum of the protein gives rise to 30,000 vibrational bands. This can be reduced by a simple trick: we record the vibrational spectrum of our protein in one state (eg. the inactive state) and then convert the protein to another state (eg. the active state) and record that spectrum. The difference between the spectra provides a measure for the changes in the protein's structure. These can be as minute as changes in hydrogen bonding between residues.

Could you provide an introduction to surface-enhanced infrared absorption (SEIRA) spectroscopy?

This is where the physics comes in. Surface plasmons are electromagnetic excitations at the interface between a metal and a dielectric material. They have an electric field that is extremely localised to the surface of the metal. This 'compression' of the electric field results in plasmonic field amplitudes that can be two orders of magnitude larger than the electric field that excites them!

Field enhancement is implicated in SEIRA. The intensity of the electric field decays exponentially with the distance from the metallic surface. As the decay length is about 10 nm, surface enhancement applies only to molecules within this distance. Once a biomembrane with a typical thickness of 5 nm is tethered to the metallic surface, the SEIRA spectrum probes only this layer.

Noble metals are employed as electrodes, so membrane proteins that are triggered by electron injection or electric fields can be studied, such as membrane proteins of the respiratory chain or voltage-gated ion channels.

What has been your most exciting discovery to date?

Developing methods is key to my research; the design of a neat experiment is very satisfying once it works. Thus, I am most proud of the methodology which my first student and I described to perform time-resolved Fourier Transform Infrared (FTIR) experiments on membrane proteins under near-native conditions and acute chemical control. Later, we improved the sensitivity of FTIR spectroscopy by introducing SEIRA spectroscopy to address biochemical problems. Quite a few labs from around the globe are now using our methodology for their particular research interests.

How do you see the field evolving over the next few years?

Until now, most vibrational spectroscopic experiments have been performed on purified proteins. The biggest challenge for the future is to apply our methodologies to a more native-like, or even living, system. Needless to say, we have ideas on how we might achieve this!

Exploring Nature's essential **nanomachines**

At the **Free University of Berlin**, studies across a wide range of fields are applying innovative methodologies to observe membrane protein structure and function in both time and space at the nanoscale

AT THE INTERSECTION of biology and nanotechnology, bionanotechnology spans scientific disciplines ranging from engineering and medicine to understand the workings of Nature at the molecular level; this knowledge can then be applied to develop new biological materials or devices. Where defects in biological function become apparent, as in disease, the knowledge gained through bionanotechnology can be harnessed to remedy them.

The processes with which bionanotechnology is concerned are those that define living organisms and manage their fundamental elements, such as DNA information coding and photosynthesis. The key to understanding them is having the correct tools at the nanoscale with which to probe them.

ANALYSING MEMBRANE PROTEINS

Membrane proteins catalyse innumerable functions in living cells and are critical to their health; they are thus core to life. In humans, they are encoded by more than 30 per cent of the genome, and more than 65 per cent of current drugs for disease target them. However, much still remains to be discovered about their functions, structures and mechanisms.

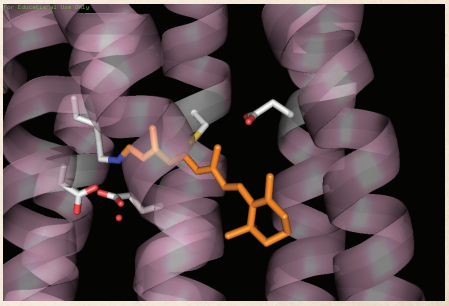
To Dr Joachim Heberle of the Free University of Berlin, the complexity of the structure and dynamics of membrane proteins is intriguing, and he sees fundamental understanding of their sophisticated efficiency as crucial to knowledge-based development of new drugs: "With membrane proteins, there is a hierarchy of how things happen, in both space and time," he muses, "and from a biomedical viewpoint, interest in membrane proteins is enormous and the pharmaceutical market is even bigger".

Heberle is Professor for Experimental Molecular Biophysics at the University, and his research applies biophysical and biochemical methodologies to find answers to how membrane proteins are synthesised, how they act and how changes in their structure affect their functions. In this work, he is aided by his students and instructive interactions with collaborators from many different disciplines and institutions, as well as his wife, who heads the laboratory for Genetic Biophysics at the University, and supplies the group with purified membrane proteins as well as expertise in molecular genetics techniques.

Heberle is particularly interested in discovering new analytical methods and improving existing techniques to explore the thin monolayer that membrane proteins constitute in cellular membranes. In 2006 he and Dr Kenichi Ataka pioneered a surface-enhanced vibrational spectroscopy technique for probing functional changes of membrane proteins at the monolayer level, using biomimetic systems and delivering greater control.

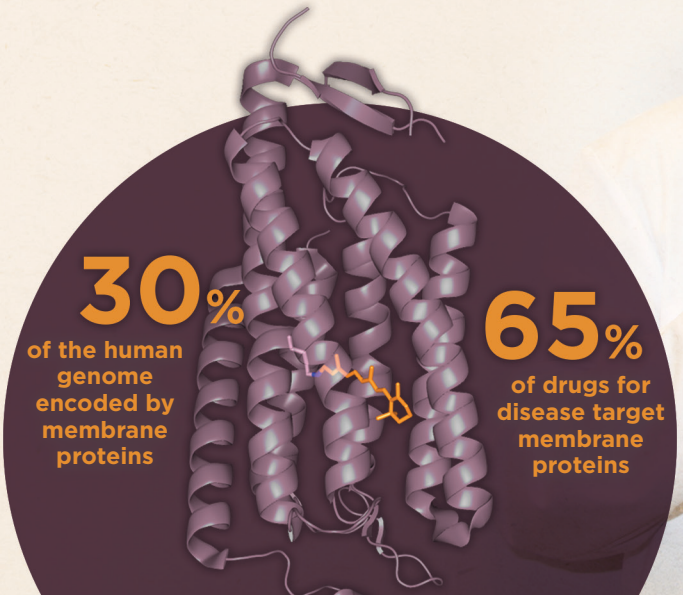
NOVEL METHODS AND APPROACHES

Vibrational spectroscopy allows Heberle and his group to analyse changes in membrane proteins at the molecular level, with minimal interference during observation because of the low level of radiation energy it uses. Step-scanning Fourier Transform Infrared (FTIR) spectroscopy combined with attenuated total reflection is employed to obtain infrared spectra to nanosecond resolutions from samples in aqueous solution with minimal interference from the solution, so in a near-native state. Compared with nuclear magnetic resonance spectroscopy, FTIR is capable of recording spectra with far smaller time resolutions, to the scale of picoseconds.



Retinal-binding pocket of channelrhodopsin. This microbial rhodopsin is a light-gated ion channel which is used as an optogenetic tool in modern neurophysiology.

Heberle's lab has developed and uses a number of other techniques for analysing membrane proteins. Time-resolved ultraviolet/visible spectroscopy is used to determine differences



SURFACE-ENHANCED VIBRATIONAL SPECTROSCOPY PROBES THE MECHANISM OF LIGHT-SWITCHABLE (MEMBRANE) PROTEINS

OBJECTIVES

The research is focused on membrane proteins that are embedded in the biomembrane. These are nanomachines that cells employ to communicate with the environment and to catalyse the import and export of foodstuffs (metabolites).

KEY PARTNERS/ COLLABORATORS

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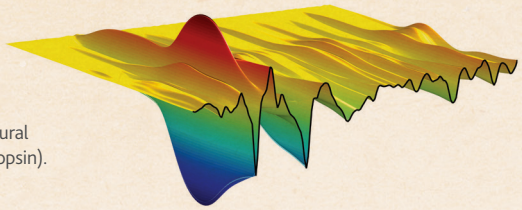
PROF DR JOACHIM HEBERLE defended his PhD in Biophysics at the FU Berlin (1991) and completed his postdoc at the Hahn-Meitner institute Berlin (1991-93). He has served as group leader at the Research Center Jülich (1993-2005) and in 2005 was appointed Full Professor for Biophysical Chemistry at Bielefeld University. Heberle joined the physics department of the Freie Universität Berlin in 2009 where he is a full professor in biophysics.



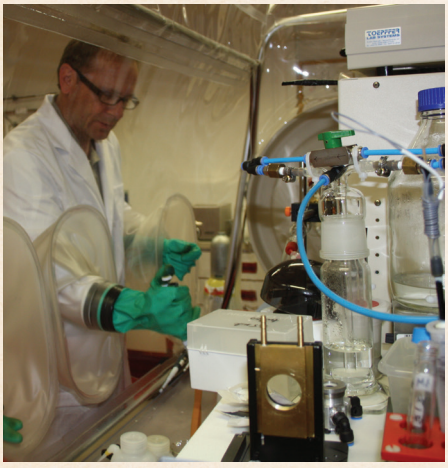
Time-resolved infrared spectroscopy reveals structural changes of membrane proteins (here channelrhodopsin). These are monitored at the atomistic level with a temporal resolution of microseconds.

in absorption of light and scanning near-field infrared microscopy (SNIM) is applied for chemical imaging to nanometre resolutions.

Heberle's method for using surface-enhanced infrared absorption spectroscopy (SEIRAS) enables functional studies of cell membrane characteristics. In probing the cellular membrane, a biological sample is tethered to a thin metal film, typically made of gold or silver; SEIRAS is then applied. Because proximity to metal can degrade or denature proteins, the metal layer may be cushioned with a layer of organic molecules that have a strong affinity for the metal. In any context where the metal film's electrode properties are useful, however, no cushioning is required. Heberle's SEIRAS technique then supports detection of minute changes in peripheral proteins: "A little surface chemistry provides an elegant way to achieve orientated, immobilised membrane proteins, which is crucial in experiments that assess their vectorial functionality," he explains. As well as addressing the functional mechanism of membrane proteins, current experiments performed by the group are exploring the details of how membrane proteins are synthesised, how polypeptides are inserted into the membrane and how they fold.

OPTOGENETICS STUDIES WITH CHANNELRHODOPSIN

The new field of optogenetics is based on the discovery of channelrhodopsin, a membrane protein that acts as a light-gated ion channel: the protein responds to blue light by opening a pore in the membrane that allows positively-charged ions to pass through. Insertion of DNA encoding for channelrhodopsin into the membranes of a type of neurons enables blue light to activate those particular neurons, and other rhodopsins have the ability to silence neural activity. This process selectively holds great promise for the investigation and treatment of brain diseases and disorders, such as Parkinson's and Alzheimer's, epilepsy, schizophrenia, attention deficit hyperactivity disorder and addictions. Tests with animals are in progress and it has been found that neuronal activity in mice can be remotely elicited by light instead of using bulky metal electrodes; a recent study found that obsessive-compulsive grooming behaviour in mice could be eased by these means.



Heberle and his group are exploring various retinal rhodopsins and are now working on resolving the mechanisms of channelrhodopsin so that they can rationally design new variants that improve its optical and channel properties. In this, they are applying time-resolved FTIR spectroscopy to elucidate the functional dynamics of both channelrhodopsin and the new variants.

RENEWABLE PURE ENERGY

In another project, Heberle is seeking to develop an alternative means of generating energy without releasing greenhouse gases such as CO_2: "The grand challenge to be faced is the energy problem when our future society runs out of fossil fuel," he reflects. The path that Heberle is exploring centres on directly converting sunlight into chemical molecules that can be stored for burning afterwards, via photobiological hydrogen production.

The membrane protein photosystem II, which uses sunlight to split water and releases electrons in the process, is the core of his chosen system. The electrons it releases are fed into another enzyme, a nickel-iron hydrogenase, which catalyses the recombination of electrons and protons to form molecular hydrogen, which then can be burned in a fuel cell to generate electricity with fresh water as the sole by-product: "We have shown that such a bio-battery works at a very high efficiency," asserts Heberle. He intends to communicate the results of the experiments with this enzymatic nanomachine to biotechnologists so that the production rate of photobiological hydrogen by green algae and cyanobacteria can be improved.

The team now aims to extend his methods and techniques, with the ultimate goal of investigating living membrane proteins: "Merging spectroscopy and electrophysiology is top of our list to generate a technique that integrates structural sensitivity, spatial resolution and ion specificity of ion channels," Heberle states.