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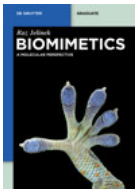

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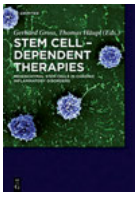

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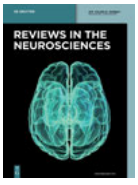

Reviews in the Neurosciences
Editor-in-Chief: Joseph P. Huston
ISSN 0334-1763

Optogenetics

Edited by Peter Hegemann and Stephan Sigrist

DE GRUYTER

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ISBN 978-3-11-027071-6
e-ISBN 978-3-11-027072-3
ISSN 2196-4769
e-ISSN 2196-4777

Photographs (Appendix) have been kindly provided by Denise Stewin.

Library of Congress Cataloging-in-Publication Data
A CIP catalog record for this book has been applied for at the Library of Congress.

Bibliographic information published by the Deutsche Nationalbibliothek
The Deutsche Nationalbibliothek lists this publication in the Deutsche Nationalbibliografie; detailed bibliographic data are available in the Internet at http://dnb.dnb.de.

Typesetting: PTP-Berlin Protago-TEX-Production GmbH, Berlin
Printing and binding: Hubert & Co. GmbH & Co. KG, Göttingen
♾ Printed on acid-free paper
Printed in Germany

www.degruyter.com

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Content

Introduction

When I was asked by my colleague and member of the Dahlem Conferences Scientific Advisory Board, Robert Bittl, in 2010 to organize a Dahlem conference about optogenetics, I was extremely reluctant and skeptical about the purpose of such a conference. There are already too many conferences, and we are presenting similar data about our research on many occasions and locations around the world. Moreover, the Dahlem conferences are unstructured in the sense that there is no fixed program or schedule, the number of attendants is limited to 40 and there is no big audience listening to what the participants have to say. Even worse, there are no talks and very little chance to present any of the latest research. What should encourage the best researchers in a certain field to come to such a conference or workshop and to be locked up for a few of days despite the extremely tight schedules they already have in most cases?

The idea of the Dahlem Conferences is to discuss challenges and potential risks of a novel technology, traditionally during five days in a closed venue, and not to present data. It is anticipated that the participants know the state of the art prior to the meeting. Why optogenetics? Optogenetics is a new technology that combines genetics with the latest optical technology to study neuronal networks on different scales of space and time. This technology developed very rapidly, from zero at the year 2002 to a widely accepted research field 10 years later. It has now reached a level where it is even considered for clinical applications. This rapid development convinced the Scientific Advisory Board members of the Dahlem Conferences to bring researchers of the optogenetics field together to discuss future perspectives of the technology.

Prior to the conference, Karl Deisseroth, Stephan Sigrist, Uwe Heinemann, Thomas Oertner, Zhuohu Pan, and Sabine Schleiermacher identified candidate subtopics that were later used in the initial discussion groups, before the participants mixed and reassembled during the following days. A number of participants had sent discussion manuscripts with provocative questions and considerations, and all participants were asked to send in "seed questions" that they wanted to be discussed during the workshop. The idea originally brought up by Stefan Sigrist was extremely useful, and we collected some 140 "seeds" as starting material for the conference. The topics that we selected during the pre-conference stage are the following:

1. *Optogenetic tools*, chaired by Karl Deisseroth (Stanford), Roger Tsien (San Diego), and myself. As optogenetics is a comparatively young discipline, many of its tools are currently under investigation and active development. Light-gated protein switches (*i.e.*, photoreceptors), with improved or entirely new molecular function, enable enhanced light control over cellular processes, and expand the scope of optogenetics. Several lines of research are pursued to address the need for additional and streamlined optogenetic tools. Firstly, multiple research groups try to obtain a description at the molecular level of the structure, function, and signaling mechanism of photoreceptors. Insights into these properties

allow rational improvement of proteinaceous light switches. For example, several channelrhodopsin variants, with differing spectral sensitivities and photocurrent kinetics, have been produced. Secondly, genome databases are sifted for previously unknown light-regulated proteins and enzymes. These new light switches permit optogenetic control in ways complementary to existing approaches, if they possess molecular functions different from other optogenetic tools. For example, recently several light-activated adenyl cyclases have been discovered that perform their enzymatic activity in a light-regulated manner. Thirdly, the repertoire of natural photoreceptors has recently been expanded by the design of synthetic photoreceptors. Inspired by natural systems, custom-made light switches allow light control over yet other cellular processes. In the most striking demonstration to date of synthetic photoreceptors, the motility of fibroblasts has been controlled by blue or red light, via a small light-activated GTPase, the Rac1 protein. In an ideal scenario, any arbitrary protein activity could be subjected under light control; if this can be accomplished, metabolism, signaling networks, and the behavior of cells and organisms could be manipulated in precise ways with only minimal perturbation of other processes.

2. *Application in cellular systems and lower model organisms*, chaired by Stephan Sigrist (Berlin), Alexander Gottschalk (Frankfurt), and Erik Jorgensen (Utah). Two kinds of devices address complementary needs for the research with lower model organisms: light-driven actuators control electrochemical signals, while light-emitting sensors report them. When actuators are expressed in genetically defined neurons in the intact animal, previously unattainable insight into the organization of neural circuits, the regulation of their collective dynamics, and the causal relationships between cellular activity patterns and behavior can be achieved. Animal model systems, which combine high optical transparency with easy and efficient genetics, are particularly effective in further progressing these aspects of optogenetics. The nematode *Caenorhabditis elegans*, with a comparatively simple nervous system, is clearly suitable for optogenetics, *e.g.*, neurotransmission has been analyzed with high temporal precision in a neurotransmitter-selective manner. The fruitfly *Drosophila* affords similar advantages, although it resembles a significantly higher level of complexity. Quite a few tools for remotely activating neural circuits by light in *Drosophila* have become available as well. As for vertebrate systems, the translucent brain of zebrafish (*Danio rerio*) offers superior experimental conditions for optogenetic approaches *in vivo*. Enhancer and gene-trapping approaches have generated many Gal4 driver lines in which the expression of UAS-linked effectors can be targeted to subpopulations of neurons. Local photoactivation of genetically targeted light-activated channels or pumps, such as channelrhodopsin and halorhodopsin, or channels chemically modified with photoswitchable agents, such as LiGluR, have uncovered novel functions for specific areas and cell types in zebrafish behavior. Despite widespread and growing use, very little work has been done to characterize exactly how optoge-

netic tools affect activity in model system neurons. We discussed these aspects in addition to new exciting examples of optogenetic tools for circuit analysis of model systems.

3. *Mapping neuronal networks*, chaired by Thomas Oertner (Hamburg). Recent progress in optics, genetics, and chemistry has provided new tools for the morphological dissection and functional analysis of neuronal networks, both *in vitro* and *in vivo*. Not only can light-controlled actuators of neuronal activity, *e.g.*, channelrhodopsin, be activated with millisecond precision, but this activation can also be performed in a targeted, cell-specific manner. Alternatively, the activity of distinct neurons can be blocked by ion pumps, *e.g.*, halorhodopsins, or by the use of recently designed K-selective ionotropic glutamate receptors. The specificity in the optical control of the activity of neuronal networks can be enhanced by various ways of targeting the light specifically to individual neurons by new scanning devices. Of particular promise is two-photon microscopy for neuron-specific activation, which grants access to deeper tissue layers. With these approaches, the control of activity can be exerted at various levels of neuronal circuits, ranging from neuronal subcompartments, such as axons and dendritic spines, up to entire classes of neurons within a circuit; for example, all or specific GABAergic inhibitory interneurons. The range of conceivable applications is enormous and includes the identification of synapses within the networks that control synaptic plasticity, the study of how neurons are connected to each other to control defined behaviours *in vivo*, or the determination of basic mechanisms of default circuitries in the brain, such as those underlying the central pattern generators (CPGs) which generate periodic motor commands for rhythmic movements.
4. *Clinical application*, chaired by Uwe Heinemann (Berlin), and Luis de Lecea (Stanford). Optogenetic methods have already been applied to study circuits and symptoms relevant to narcolepsy, blindness, depression, fear, anxiety, addiction, schizophrenia, autism, Parkinson's disease, and epilepsy. Moreover, the potential of the technology to fundamentally advance our understanding of neural circuit dysfunction is enormous. This session covered clinical applications of optogenetics, including efforts dedicated to understanding disease circuitry in animal models, and efforts focused on direct clinical translation. Topics in the latter category included applications to deep brain stimulation, peripheral nerve stimulation, and motor prosthetics. Topics in the former category were motivated by the fact that a most fundamental impact of optogenetics need not arise from direct introduction of opsins into human tissue, but rather from use as a research tool to obtain insights into complex tissue function, as has already been the case for Parkinson's disease. Many opportunities exist in both categories. Due to technological limitations in probing intact neural circuits with cellular precision, our current understanding of brain disorders does not do full justice to the brain as a high-speed cellular circuit. Rather than conceptualizing the brain as a mix of neurotransmitters, ideally we would be able to move toward a circuit-engineering

approach, in which devastating symptoms of disease are understood to causally result from specific spatiotemporal patterns of aberrant circuit activity relating to specific neuronal populations. But technology has been lacking for the requisite high-speed, targeted, causal control of intact neural circuit function, and this challenge extends to basic neuroscience and other biological systems as well. Optogenetics now provides a means to address this challenge.

5. *Restoration of vision and hearing*, chaired by Zhuohua Pan (Detroit) and Botond Roska (Basel). Retinitis pigmentosa (RP) refers to a diverse group of progressive, hereditary diseases, leading to incurable blindness, and affecting two million people worldwide. There is no general cure for RP, but several approaches that offer some degree of treatment in some forms of RP are in clinical trials and others are on the horizon. Gene replacement shows great promise if the disease is caused by the lack of function of the mutated gene, which mostly occurs in recessive forms of RP. Progress in replacing mutated RPE65 in the retinal pigment epithelium in Leber congenital amaurosis not only offers hope for patients of this disease, but also shows promise for other gene-replacement strategies by demonstrating the safety and efficacy of adeno-associated viral vectors for gene therapy in the human eye. Gene replacement can only be envisioned if the cell type expressing the gene is still alive and therefore, in the case of the most common rod-specific genes, early diagnosis and gene therapy in childhood might be necessary. When the mutation creates a "toxic" protein or the gene is too large to fit the viral vectors authorized in clinical trials, this approach is limited. Nevertheless, in the cases when it is feasible and unlike other approaches documented below, gene replacement may provide a real cure for a group of patients. Secondly, approaches to decrease the speed of degeneration of photoreceptors attempt to slow down the progression of the disease. This approach is feasible until visual function is preserved. Thirdly, a number of approaches attempt to restore photosensitivity without interfering with the intrinsic progress of the disease by creating new photosensors and couple them into the remaining retinal circuitry. Patients who are legally blind are the key target population of these approaches. Three different approaches in this group are the implantation of differentiated or undifferentiated photoreceptors, electronic retinal implants, and optogenetic approaches. The symposium introduced, contrasted, and debated the different approaches to restore photosensitivity to animal models of Retinitis pigmentosa and to human patients. Current clinical and preclinical trials were discussed in terms of safety, efficacy, and impact on society.

Conclusions and final considerations: the key issue of the optogenetic technology is its cell specificity, but at the same time, this is also its major limitation. Neuroscientists might apply optogenetic approaches to cure, or at least alleviate, diseases in the near future, and the first trials will probably be carried out within the next two years for retinal prosthesis or Parkinson's disease. But optogenetics is limited

to those brain diseases that localize to a clearly defined area of the brain. These diseases are extremely rare, whereas most brain disorders are of a much higher level of complexity, involving many cells distributed over a large area of the brain. Not only is the causality of these malfunctions unknown, but they are also out of reach for any optogenetic applications. Optogenetics is certainly an innovative technology and of great analytical value in the context of many diseases, but at present we should be humble about the potential as a therapeutic technology to cure brain malfunction by any means. This will only become true for a very small number of diseases, based on defects of single genes with very local activity of the gene products. Last, but not least, ethical questions should be constantly discussed – from early experiments on mammals, to non-human primates, and to eventual applications in humans.

I personally was extremely amazed about how the discussion developed during the progress of the conference, about the precision with which key issues crystallized during these days, the cross-border discussions that developed between *tool makers* and *appliers*, and the careful consideration of potential application, including ethical perspectives. Finally, I was enlightened that most of the organization and bureaucracy that we experience in our usual conferences is not necessarily needed; thus, fruitful discussions were not unduly hampered and went into the depth required to address questions that really matter for the promotion of a new field.

Finally, I thank my colleagues who worked with me on the planning of the conference, especially Karl Deisseroth, Uwe Heinemann, Andreas Möglich, Zuohuo Pan, Sabine Schleiermacher, Stephan Sigrist, and several others that sent in suggestions and discussion manuscripts. I am also indebted to the three graduate students, Elena Knoche, Franziska Schneider, and Stephanie Wegener, who meticulously recorded the main ideas and outcomes of the sessions and provided these to the authors that you, as the reader, will find in this book. Last, but not least, I thank Michael Brückner, a person quite invisible during the conference, but who ran the organization smoothly, did all the logistics, the financing, and everything that made the conference enjoyable.

I hope that this conference helped to develop the field of optogenetics in a direction where it brings insight into the organization of neuronal networks, where it uncovers origins of brain diseases, and where it might even help to develop curative strategies which make the life of patients more enjoyable.

Berlin, April 2013 *Peter Hegemann*

Keith Moffat, Feng Zhang, Klaus Hahn, Andreas Möglich

1 The biophysics and engineering of signaling photoreceptors

1.1 Photoreceptors

Image formation, vision, and certain developmental and behavioral processes in diverse organisms are naturally sensitive to light. The primary event is absorption of a photon by a photoreceptor protein comprised of at least two units: a photosensor which absorbs light and an effector whose light-dependent activity ultimately elicits a physiological response. (Other units may be present, *e.g.*, those that confer specific intermolecular interactions, but these two units are essential). Since the common constituents of organisms (amino acids and proteins, ribonucleic acids, lipids, carbohydrates, small metabolites) do not absorb the wavelengths significantly present in sunlight, absorption by the photosensor typically occurs in a covalently or non-covalently bound, small organic moiety known as a chromophore. Retinal, flavin nucleotides, and bilin are common examples of chromophores (Figure 1.1). A quite different example is offered by UV-sensitive photoreceptors exemplified by UVR8 [1] where the "chromophore" is believed to be a cluster of tryptophan side chains which naturally absorb in the near-UV region of the spectrum.

When photoreceptors are classified by the chemical nature of their chromophore and the photochemistry that follows photon absorption, they fall into seven distinct classes [2]: UV receptors; photoactive yellow protein and relatives [PYP]; light-oxygen-voltage [LOV]; sensors of blue light utilizing FAD [BLUF]; cryptochromes; rhodopsins; and phytochromes (Figure 1.2). To these may be added cyanobacteriochromes [3, 4]. The term "distinct classes" is loosely defined. Quite different chromophores and photochemistry are found in LOV domains (flavins) and PYP-like molecules (*p*-coumaric acid), yet the photosensor proteins that contain these two distinct chromophores are structurally related. Each forms a subclass of Per-ARNT-Sim domains, which are widely distributed in signaling proteins more generally [5, 6].

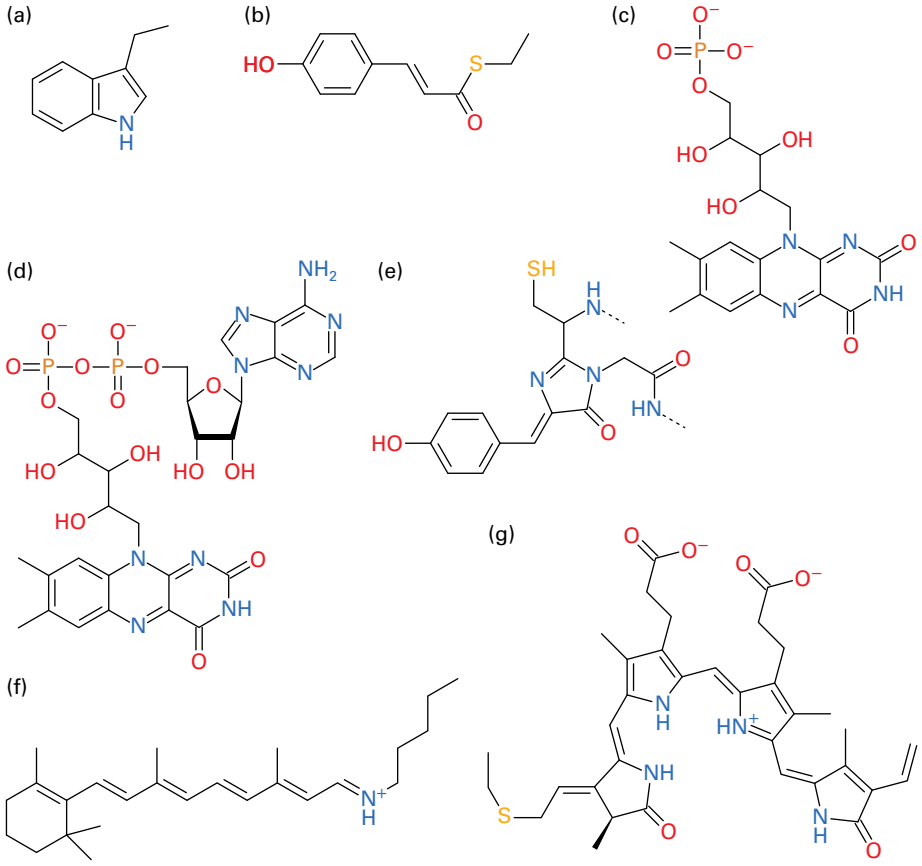

Figure 1.1: Chromophores of photoreceptors. Each photoreceptor harbors an aromatic chromophore molecule that absorbs electromagnetic radiation in the near-UV, visible or near-infrared ranges. As chromophores, plant UV photoreceptors use tryptophan side chains (a); photoactive yellow protein uses *p*-coumaric acid (b); LOV photoreceptors use flavin mononucleotide (c) or dinucleotide (d); cryptochromes and BLUF domains use flavin adenine dinucleotide (d); rhodopsins use retinal (f); and phytochromes use linear tetrapyrroles such as biliverdin (g). In jellyfish fluorescent proteins, the heterocyclic chromophore is formed autocatalytically from three amino acid side chains (e).

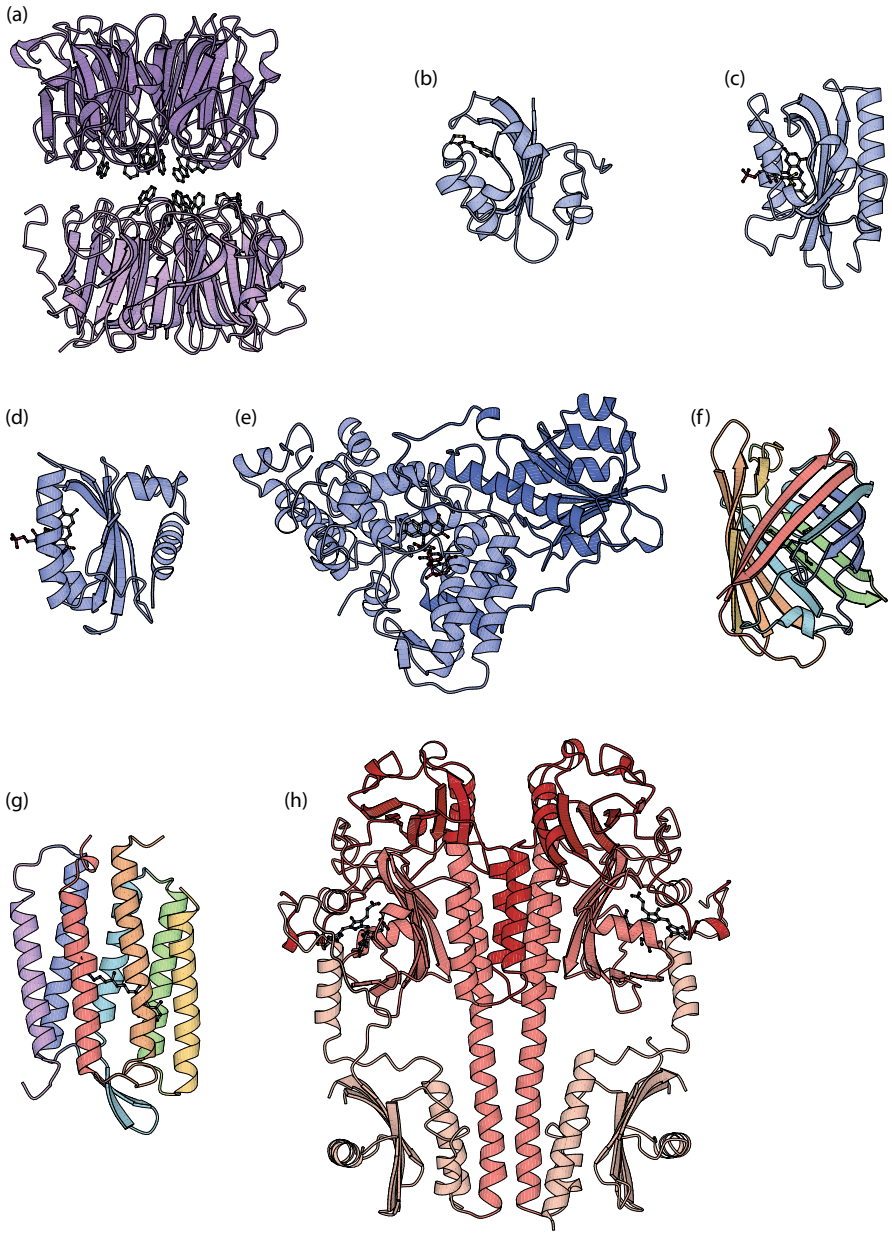

Figure 1.2: Architecture of photoreceptors. Three-dimensional folds of representative members of the different photoreceptor families where the color is meant to indicate which colors of light can be absorbed by a given photoreceptor. (a) *Arabidopsis thaliana* UVR8 (PDB code 4D9S; [7]). (b) *Halorhodospira halophila* photoactive yellow protein (1MWZ; [8]). (c) *Avena sativa* phototropin 1 LOV2 domain (2V0U; [9]). (d) *Rhodobacter sphaeroides* AppA BLUF (2BYC; [10]). (e) *Drosophila melanogaster* cryptochrome (4GU5; [11]). (f) *Echinophyllia sp.* Dronpa (2IE2; [12]). (g) *Halobacterium salinarum* bacteriorhodopsin (1M0L; [13]). (h) *Pseudomonas aeruginosa* bacteriophytochrome (3C2W; [14]).

1.1.1 Novel photoreceptors

It is likely that other classes of photoreceptors remain to be discovered in addition to those noted above. The process for photoreceptor discovery typically originates in identifying a novel, light-dependent process in one, often little studied, organism. Its photochemical action spectrum is obtained, the cell type housing the putative photoreceptor is located, the candidate photoreceptor is purified (often challenging, since its abundance may be very low) and chemically characterized, and its photochemistry *in vitro* matched with that of the biological process *in vivo*. From its protein and gene sequences, related examples in other organisms are quickly found. A recent example is the discovery and characterization of a light-modulated adenylyl cyclase in a marine bacterium [15, 16]. To qualify as an authentic signaling photoreceptor, direct evidence that a particular biological process in that organism is modulated by light absorbed by the candidate photoreceptor must be sought. Sequence similarity is powerful in initial identification but does not substitute for direct demonstration!

Since photons readily traverse membranes, most photoreceptors such as LOV proteins or phytochromes are cytoplasmic, soluble proteins, which allows light to directly regulate an intracellular process. In contrast, rhodopsin-based photoreceptors, *e.g.*, visual rhodopsins, channelrhodopsins or sensory rhodopsins, are integral membrane proteins in which light alters an activity of the protein intrinsic to its location in the membrane, such as its ability to act as a channel or ion pump. Many of the more widely studied chemoreceptors are also integral membrane proteins that respond to extracellular chemical signals which cannot traverse the cell membrane. An interesting question is the extent to which there are parallels in general mechanisms of signal transduction between chemoreceptors and photoreceptors [6].

The key feature of signaling photoreceptors is that absorption of a photon produces a change in a specific biological activity, either directly in the photoreceptor molecule itself, or more usually, in a spatially distant downstream component such as a metabolic enzyme, kinase or transcription factor; light serves as a *specific source of information*. In contrast, light-driven electron transfer processes in photosynthesis generate a change in membrane potential that ultimately drives many biological processes; light serves as a *general source of energy*. Optogenetics is based on genetically encoded, light-dependent control of a biological activity [17]. Thus, we concentrate here on the features of those natural and engineered signaling photoreceptors that exhibit this control.

1.1.2 Biophysics of photoreceptors and signal transduction

Absorption of a photon excites the chromophore to higher electronic and vibrational energy levels; internal conversion on the picosecond time scale rapidly dissipates energy and thus returns the chromophore to the lowest vibrational level of the first