

## CHAPTER 6

# The Photoreceptor Membrane as a Model System in the Study of Biological Signal Transduction

Thomas Huber\* and Thomas P. Sakmar

*Howard Hughes Medical Institute, Laboratory for Molecular Biology and Biochemistry,  
The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA  
E-mail: sakmar@mail.rockefeller.edu*

### Contents

1. Introduction	182
2. Lateral and transverse asymmetry in membranes	184
2.1. Rhodopsin biosynthesis	184
2.2. Membrane lateral organization in model membranes	187
2.3. <i>In vitro</i> folding	192
2.4. Reconstitution in biomembrane models	192
3. Interactions with membranes	195
3.1. Membrane surface potential and water activity	195
3.2. Localization depends on membrane interactions	196
4. Concluding remarks	198
Acknowledgements	198
References	198

### Abstract

Much of biological signal transduction is coupled, either directly or indirectly, to seven-transmembrane helical, G protein-coupled receptors (GPCRs). GPCRs comprise a large family of related membrane proteins that respond to a variety of external stimuli such as small molecules, polypeptides, odorants, and light. The stimulant signals activate specific GPCRs, which couple to G proteins or other molecular switches to facilitate the cellular response. The dim-light photoreceptor rhodopsin, the only GPCR for which a structure is known at atomic resolution, is the prototype of the largest subfamily of GPCR genes in the human genome. Rhodopsin is expressed in rod cells in the vertebrate retina, where it is localized primarily to disk membranes, which are highly specialized intracellular organelles. The disk membranes are assembled from the plasma membrane of the rod outer segment (ROS), and subsequently their composition is remodeled to a unique, highly polyunsaturated phospholipid environment. The dense packing of the photoreceptor membranes with rhodopsin and other components required for visual phototransduction has facilitated the preparation of model systems, which have been studied by a variety of biophysical,

---

\* Corresponding author. E-mail: hubert@mail.rockefeller.edu

biochemical, and molecular biological techniques. Although the disk membrane is highly specialized to detect light, its key molecular components are conserved in essentially all biological membranes in eukaryotic organisms. Yeast mating, chemotaxis, hormone signaling, and synaptic transmission all depend on highly conserved GPCR signaling pathways. Here, we review some recent results regarding the influence of membrane lipid composition on the structural organization and activity of the molecular components in GPCR signaling. We also provide a perspective toward a computational description of the underlying molecular mechanisms in biomembranes.

## 1. INTRODUCTION

*“Getting ready for the decade of the lipids”* [1]. In a recent review, Donald Hilgemann speculated that phospholipids and their metabolites might soon become subject of an information explosion similar to that observed in genomics and proteomics [1]. In fact, a new initiative from the National Institutes of Health supports the LIPID MAPS (LIPID Metabolites and Pathways Strategy; lipidmaps@chem.ucsd.edu) consortium that includes an attempt to establish ‘lipidomics.’ In this chapter, we review several experimental and theoretical techniques to study protein function and lipid/protein interactions in photoreceptor membranes to demonstrate how biophysics, biochemistry, and molecular biology can contribute to this renaissance in phospholipid research.

Let us first introduce the system and make some reference to the vast amount of knowledge accumulated over more than a century of molecular vision research. In his Nobel lecture in 1967, George Wald described the early history of rhodopsin research after its discovery in 1877 by Franz Boll [2] leading to his groundbreaking insight that rhodopsin is a carotenoid-protein that engages in a light-dependent cycle of reactions with retinal and vitamin A [3]. Later it became clear that light reception by rhodopsin triggers a signaling cascade by catalyzing nucleotide exchange in a heterotrimeric guanine nucleotide-binding regulatory protein (G protein), called transducin ( $G_t$ ) [4]. The work by Gilman and Rodbell (Nobel prize 1994) and many others on this universal mechanism of signal transduction involving G proteins emphasized the importance of a large class of homologous receptor proteins, the G protein-coupled receptors (GPCRs), of which rhodopsin is the only member with a known structure at atomic resolution [5]. Members of the GPCR family respond to a variety of different external stimuli ranging from small molecule hormones and neurotransmitters to peptides and polypeptides to odorants and photons. Cell membrane receptors, many of them GPCRs, are very important pharmacologically, comprising an estimated 45% of all molecular drug targets of current therapies [6]. Rhodopsin is a prototypical member of the largest gene family of GPCRs, the rhodopsin-like class A family, with 484 possible candidates identified in the human genome [7,8]. This family

contains the opsins and protein, peptide, and cationic amine receptor subfamilies, constituting about 95% of all documented human GPCRs.

Photoreceptor membranes, containing the GPCR rhodopsin as the major transmembrane protein component and the heterotrimeric  $G_t$  as the major peripheral membrane protein component, are a paradigm for experimental research on GPCRs and heterotrimeric G protein mediated signal transduction [9–12]. Milligram quantities of the purified proteins easily can be obtained from a few bovine retinas – a fact that stimulated the development of biophysical and biochemical methods for membrane proteins (see Methods in Enzymology, Vols. 82, 88, 315/6, Academic Press; San Diego, CA). The amino acid sequence of rhodopsin was among the first to be determined for a membrane protein [13–15]. A heptahelical transmembrane motif was inferred from biochemical methods [16], demonstrated by 2D crystallography [17], and proven by the high-resolution X-ray structure [5,18,19]. Optical spectroscopy revealed a detailed picture of kinetic intermediates during the thermal decay of rhodopsin after light reception [20].

The GPCRs are strictly confined to the eukaryotic domain, with, for example, a single pheromone receptor in baker's yeast and hundreds of receptors in humans. However, other seven transmembrane helical proteins with a retinoid prosthetic group and a fold similar to rhodopsin can be found in archaeal bacteria (bacteriorhodopsin and sensory rhodopsin) and recently in marine bacteria (proteorhodopsin) [21]. Bacteriorhodopsin (bR) has a light-dependent conformational cycle somehow similar to rhodopsin, and advances in crystallography have led to the elucidation of nine intermediates [22]. Until similar detailed information becomes available for rhodopsin, the allosteric mechanism of ligand-dependent activation of the G protein by its receptor will remain unclear.

The photoreceptor cells, which harbor the phototransduction machinery, are specialized neurons of the central nervous system (CNS). The rod cells, specialized for dim-light, or scotopic vision, have a pronounced polarity; the apical part comprises the ROS, which connects *via* a thin cilium to the inner segment of the cell body and an axon with synaptic terminal. The rod cells are aligned in parallel and densely packed in the retina. Adjacent to the outer segment is the retinal pigment epithelium (RPE), followed by the choroid layer. The rod cells are intimately coupled to the RPE cells, *viz.* the apical part of the outer segment sheds several times a day a stack of disks that get phagocytized by the epithelium cells. In addition, the RPE cell isomerizes retinal enzymatically from the all-*trans* form to the 11-*cis* form [23], which is required by the rod cells to form rhodopsin from the apoprotein opsin. On the other hand, light isomerizes the retinyl group in rhodopsin from 11-*cis* to all-*trans*, which is released during thermal decay to opsin. This cycle is called the retinoid visual cycle [24,25]. There is another, not so well known, metabolic cycle between RPE and rod cells: the docosahexaenoic acid (DHA; C22:6 $\omega$ 3) recycling pathway [26,27]. These two cycles minimize the need for essential lipid precursors, the carotenoid vitamin A and the  $\omega$ 3 polyunsaturated fatty acids (PUFAs) such as

$\alpha$ -linolenic acid (C18:3 $\omega$ 3). While the need for the retinoid cycle might be obvious, the DHA recycling pathway needs further clarification.

The major fraction of rhodopsin is localized to the rod cell disk membranes (RDMs) of the ROS. The phospholipids in the RDMs contain about 50% DHA chains [28], the highest known specific enrichment of DHA, followed by brain synaptosomal membranes with about 30%. In comparison, most other cellular membranes contain only trace amounts of DHA, and one can speculate about a specific function of DHA in the CNS [29]. As noted above, DHA biosynthesis in animals is dependent on dietary uptake of  $\omega$ 3 fatty acids, which are generally synthesized in plants and enter the ecological food chain *via* herbivores. For example, certain fish oils contain high amounts of DHA probably as a result of accumulation along the food chain. In contrast to the CNS-specific  $\omega$ 3 series, the role of  $\omega$ 6 series of PUFAs is better understood. The prominent members are the essential linoleic acid (C18:2 $\omega$ 6) and arachidonic acid (C20:4 $\omega$ 6). Arachidonic acid is usually stored in the *sn*-2 chain of phospholipids, released upon action of phospholipase A<sub>2</sub>, and converted to signal transmitting molecules (e.g., prostaglandins, prostacyclin, leukotrienes, and endocannabinoids) that target a large number of GPCRs and possibly other receptors. Apparently, DHA does not generate such a wealth of signaling molecules and its function might be of a structural nature instead.

The following sections are dedicated to biophysical principles of lipid/protein interactions in the cell biology of biomembranes. A special focus is on the GPCR rhodopsin and the question of how polyunsaturated DHA might bear a special function in photoreceptor membranes. The structure of rhodopsin in the membrane bound state [30] might guide the discussion, *cf.* Fig. 1. We adopt a reductionism approach to a multitude of experimental observations and theoretical models, with the hope that the resulting discussion might guide future efforts in lipidomics.

## 2. LATERAL AND TRANSVERSE ASYMMETRY IN MEMBRANES

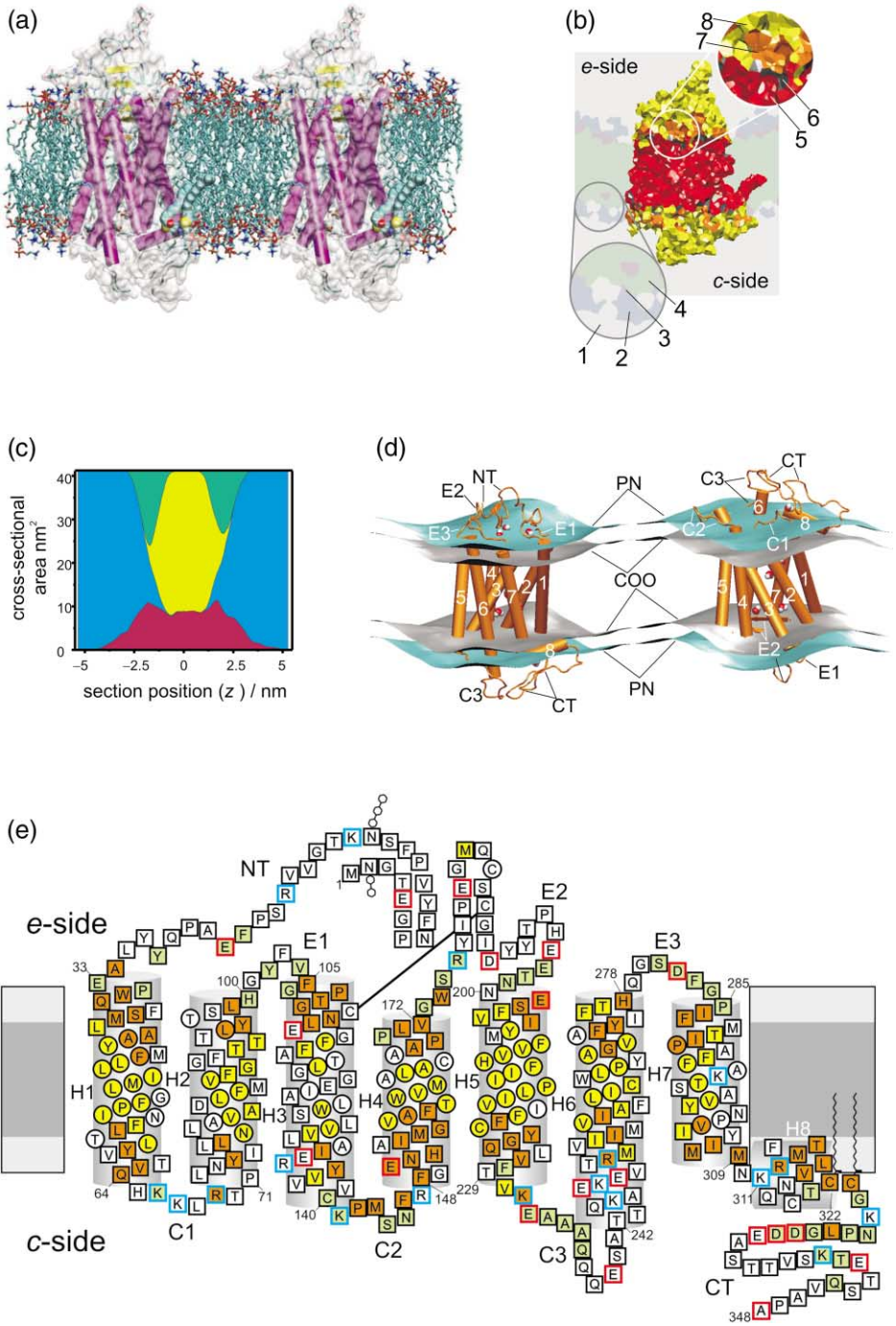
The lateral organization of membranes in photoreceptor cells is a classical example of macroscopic domains in biomembranes [31]. The transverse organization, on the other hand, is determined by the biosynthesis of opsin that is another textbook example [32]. Here, we review some key findings to stimulate further experiments that might reveal the role of the unique lipid composition in the photoreceptor cells with respect to the membrane asymmetry.

### 2.1. Rhodopsin biosynthesis

Briefly, opsin is synthesized by ribosomal translation of a messenger ribonucleic acid (mRNA) and insertion into the endoplasmic reticulum (ER) membrane.

The membrane insertion is believed to be under control of four start-transfer and three stop-transfer amino acid sequences in rhodopsin [33,34]. The sequence topology of rhodopsin relative to the phospholipid/water interface is shown in Fig. 1e. The N-terminus is translocated to the extracellular/luminal side of the membrane under control of a start-transfer sequence with minus end first, located in the first transmembrane helix H-1. The net charge of the N-terminal domain is zero, three negative charges of side chains are neutralized by two basic residues and the N-terminal backbone ammonium group, facilitating translocation until the basic cluster (positive charge +4) in the cytoplasmic loop C-1 acts as a stop-transfer signal. Positive residues interact strongly with the phosphate groups of phospholipids, not only in acidic lipids but also in zwitterionic neutral lipids. This preference of positive residues to the lipid/water interface results in the positive inside rule of transmembrane protein folding in general [35]. The inserted N-terminus gets stabilized by glycosylation at two asparagine residues (N2 and N15), which may prevent the sequence from flipping back to the cytoplasmic side as opsin mutants lacking the N15 glycosylation site tend to be misfolded [36]. The remaining polypeptide chain of opsin is under control of three pairs of start-transfer and stop-transfer sequences in helix pairs H-2/H-3, H-4/H-5, and H-6/H-7, leading to translocation of the corresponding connecting loops E-1, E-2, and E-3 to the extracellular/luminal side of the membrane. The C-terminal sequence remains on the cytoplasmic side and it contains an amphiphilic helix, H-8, which is anchored in the membrane by the transmembrane helix H-7 and a pair of post-translational palmitoylated cysteines [5], cf. Fig. 1a. The folded protein finally gets stabilized by a disulfide bridge between Cys-110 in helix H-3 and Cys-187 in loop E-2, and binding of 11-*cis* retinal ligand. The denaturation temperature in the membrane-bound state increases from about 50 °C for the apoprotein opsin to about 70 °C for the retinal-containing rhodopsin state.

The intracellular trafficking of membrane proteins is thought to be vesicle mediated, originating from the ER *via* the Golgi complex to the plasma membrane. Furthermore, in case of rhodopsin, a densely packed stack of disk-like vesicles is formed in the outer segment by invaginations of the plasma membrane and membrane fission. The stacking of the disks is stabilized by peripherin/rds [37,38]. The vectorial transport of post-Golgi vesicles toward the distal region of the inner segment is under the control of the cytoskeleton. The opsin-containing vesicles are moved along microtubuli by the cytoplasmic dynein motor protein, where a specific light chain (Tctex-1) recognizes the C-terminus of opsin [39]. The specificity of this address tag in the C-terminus can be demonstrated by exchange to homologous sequences that change the transport direction, for example, an opsin chimera with the C-terminus of the  $\alpha_{2A}$  adrenergic receptor gets directed toward the synaptic terminal of the photoreceptor cell, which is the normal destination of the  $\alpha_{2A}$  adrenoreceptor [40].



The open question is now how the rhodopsin-rich post-Golgi vesicles are assembled during the transition from the *cis*- to *trans*-Golgi membrane network. Moreover, DHA-phospholipids are enriched in these rhodopsin-bearing post-Golgi vesicles [41]. The co-localization of DHA-phospholipids and rhodopsin remains after fusion with the plasma membrane, ciliary transport into the outer segment, and disk formation. Hence, one could speculate that rhodopsin has a preference for DHA-phospholipids and drives microdomain formation.

## 2.2. Membrane lateral organization in model membranes

It is difficult to characterize the underlying driving forces of membrane organization from *in vivo* studies due to the context of the cellular protein machinery. If we want to test the hypothesis that thermodynamic properties drive the membrane self-organization, model membranes are necessary tools. Let us, therefore, review several experimental findings involving DHA or rhodopsin. Hubbell and coworkers

---

**Fig. 1.** Lipid/protein interactions in rhodopsin-containing membranes determined from 15 ns all-atom MD simulations of a 45,000 atoms model [30]. (a) A side view of rhodopsin in a POPC bilayer membrane is shown with the extracellular (intradiskal) side up and the cytosolic side down. The secondary structure is shown as a cartoon under a transparent molecular surface. (b) A novel generalized molecular surface (GMS) was developed to quantify the protein interfaces based on generalized Voronoi volume regions (visualized as a color-coded map on a diagonal slice through the cell) associated with aqueous salt solution (light gray; indicated by 1), with the glycerophosphocholine head groups (light blue; 2), the acyl chain ester group atoms (light purple; 3), and the acyl chain hydrocarbon atoms (light green; 4) of the POPC bilayer. The rhodopsin interfaces with the phospholipid acyl chain hydrocarbon atoms (red; 5), ester groups (gray; 6), and glycerophosphocholine head groups (orange; 7), and with the aqueous solvent (yellow; 8) are displayed as a color-coded GMS. (c) Lateral deformation of the bilayer membrane due to the protein inclusion. The GMS method was applied to determine the partial contributions of the different components of the membrane system to the cross-sectional area as a function of position along the bilayer normal: aqueous solvent (blue), hydrophobic (yellow) and polar (green) parts of the phospholipids, and protein (red). Note that the protein has a vase-like shape, with larger cross-sectional area values close to the lipid/water interfaces than in the bilayer center. (d) Approximate lipid/water interfaces corresponding to hydrophobic region (gray) and head groups (cyan) of the bilayer. Note that all helices, except some cytoplasmic parts of helices H-3, H-6, and H-8, are confined by the lipid head group/water interfaces. (e) A sequence topology scheme of rhodopsin illustrates the protein residues associated with the different GMS interfaces: residues exposed to the bilayer hydrocarbon interior (the lipid/protein hydrophobic interface; shown in yellow), head groups (the lipid/protein polar interface; green), and both simultaneously (orange). Squares indicate protein residues exposed to internal or external water. The charge states of the residues in the MD model are designated by red or blue symbol frames.

studied the 2D distribution of rhodopsin in a series of different phospholipid bilayers by freeze-fracture electron microscopy [42–46]. In several cases, their membranes exhibited non-uniform distributions of rhodopsin depending on the lipid composition and the temperature before freezing. The main factors driving rhodopsin aggregation and lateral domain formation were lipid chain length mismatch and temperatures below the main transition temperature of the phospholipids. The drawback of freeze-fracture studies are potential freezing artifacts.

Other methods for direct visualization of lateral microdomain formation under physiological conditions are limited by the resolution of optical microscopy relative to the nanometer scale of microdomains or the need for a solid support in atomic force microscopy (AFM) studies. Recently, evidence for regular organization of rhodopsin in rows of dimers have been presented from AFM studies of solid-supported disk membranes [47,48], but the results led to some controversial discussion about the possibility of artifacts due to the solid support [49,50]. This controversy arose partially due to contradiction with spectroscopic methods that demonstrated free rotational and translational mobility of rhodopsin in disk membranes [51,52], a classical example for the fluid mosaic model of biological membranes [53]. An increasing amount of evidence for a different type of lateral microdomains in biomembranes called lipid rafts, however, challenges the dogma of the fluid mosaic model [54–56].

Despite the elegance of a direct visualization of supramolecular organization, many spectroscopic methods have the strength to address structural as well as dynamical qualities of a system. Especially, in liquid-like patterns of self-organized structures relevant to signal amplification, the lifetime of molecular complexes might impair visualization. However, an inherent weakness of spectroscopic methods is that the interpretation of results is model dependent and oversimplification is often necessary due to insufficient amounts of data. Another problem frequently limiting spectroscopic methods is the requirement for difficult labeling procedures. In the following section, we will review some results from spectroscopic experiments relevant to photoreceptor membranes.

The self-association of rhodopsin in phospholipid bilayers was indirectly studied by measuring the effective rotational correlation time of the spin-labeled protein by electron paramagnetic resonance (EPR) spectroscopy. It was found that the protein rotational correlation time depends on temperature, lipid/protein ratio, lipid acyl chain length, and type. The rotational mobility increases with temperature, especially pronounced during a chain melting phase transition in the phospholipid matrix, with the lipid/protein ratio, and with acyl chain approaching an optimal length [57,58]. As exemplified by these results, protein aggregation can be the result of hydrophobic mismatch of the hydrophobic interface of the protein with the hydrocarbon layer of the surrounding bilayer [59].

Previously, we analyzed the condition of hydrophobic matching by a combination of molecular dynamics (MD) simulations and solid-state deuterium

nuclear magnetic resonance ( $^2\text{H}$  NMR) spectroscopy [30]. The packing of the rhodopsin-containing POPC bilayer membrane is illustrated in Fig. 1. EPR spectroscopy of spin-labeled phospholipids was extensively used to quantify the number of motionally restricted lipids in the boundary of membrane proteins [58,60]. Rhodopsin has about 21–28 motionally restricted lipids [60] with a lifetime in the boundary in the order of 60 ns for the fluid membrane state at ambient temperature [61]. The boundary layer of lipids around a transmembrane protein in a fluid bilayer can be seen as the primary solvation shell of a molecule in a 2D liquid. Since the long-range diffusion cannot occur toward the protein, motional restriction in the boundary layer is expected. In addition to these geometric considerations specific lipid/protein interactions might induce structure in this boundary layer. However, in case of rhodopsin no head group preference was found [60,62], contrasting, for example, the mitochondrial ADP/ATP carrier (AAC) that has a strong affinity for cardiolipin [63]. The positions of cardiolipin molecules, which co-purify with the protein, in the high-affinity binding sites of the AAC were recently revealed by X-ray crystallography [64]. Is there any experimental evidence that suggests specific lipid/protein interactions in rhodopsin-containing membranes?

The alteration of lipid/protein interactions during light activation of rhodopsin was demonstrated by Fourier-transform infrared (FTIR) difference spectroscopy in model membranes [65]. The assignment of the observed spectral effects was possible by using specific acyl chain carbonyl ( $^{13}\text{C}=\text{O}$ ) isotope labeling of the phospholipids (POPC) together with engineered rhodopsin mutants that lack spectral overlap in the carbonyl stretching region. The transition to the metarhodopsin II state affects both acyl chains to the same extent and appears to be restricted to about one lipid in the boundary layer of the protein [66]. A possible molecular mechanism underlying the observations is that rigid-body domain motions in the cytoplasmic region of rhodopsin [10] changes the circumference of the protein and thus the number of lipids in the boundary layer. Other plausible mechanisms influencing the boundary layer during photoactivation involve, for example, changes of the protein self-association, the exposure of amino acyl residues, and the electrostatic potential around the protein. The latter possibility deserves further attention.

The formation of the active metarhodopsin II conformation is accompanied by proton uptake from the cytoplasmic side at residue E134 in the highly conserved D(E)RY sequence motif [67], a mechanism common to GPCRs in general. The protonation changes the net charge of the cytoplasmic domain of rhodopsin by  $+1e$ . The equilibrium distribution of negatively charged phosphatidylserine to the cytoplasmic and extracellular leaflets of the ROS disk membranes [68] suggests that the population difference in favor of the cytoplasmic side exactly cancels the charge difference in rhodopsin over the membrane under physiological conditions. Therefore light-induced protonation of the cytoplasmic domain might reduce the affinity of phosphatidylserine to

the cytoplasmic leaflet, and indeed, light activation of rhodopsin increases selectively the extractability of spin-labeled phosphatidylserine from the cytoplasmic leaflet of disk membranes [69].

The organization in photoreceptor model membranes can be studied by methods based on fluorescence resonance energy transfer (FRET). The FRET effect is a distance-dependent dipolar interaction between the electronic excited states of two fluorophore groups in which excitation is transferred from a donor group to an acceptor group by a zero quantum transition, i.e., without emission of a photon. The FRET transfer efficiency is dependent on the inverse sixth power of the distance between the donor and acceptor groups relative to the so-called Förster distance, a label-specific scaling coefficient. An introduction in theory and experimental realization can be found in the book of Lakowicz [70].

As noted above, the self-association of rhodopsin in membranes can be indirectly measured from the rotational mobility of the protein that changes upon aggregation. An early FRET-based approach to study rhodopsin–rhodopsin interactions in reconstituted vesicles showed that FRET efficiency between rhodopsin molecules is independent of the protein/lipid ratio, suggesting aggregation of rhodopsin independent of the average surface concentration of the protein in the membrane [71]. The reconstitution procedure utilized in those experiments is expected to produce a symmetric or scrambled orientation of inside-out and right-side-out facing proteins [16]. The unphysiological antiparallel alignment of a membrane protein with a pronounced dipole moment is energetically favored (i.e., the parallel orientation is repulsive and the antiparallel orientation is attractive). The 2D crystal forms of rhodopsin prepared from mixed micelles by detergent removal exclusively show symmetric orientation of antiparallel aligned rhodopsin molecules. The exceptions are 2D crystals from lipid depletion of native ROS membranes that preserve the biosynthetically generated asymmetric orientation [72]. The unit cells in the high-resolution 3D crystal structure [5] also contain an antiparallel aligned pair of rhodopsin molecules, but the axes are twisted relative to the orientation in the membrane bound state. A second possible mechanism driving protein aggregation is hydrophobic mismatch [59,73]. The FRET study utilized certain conditions and a mixture of phosphatidylcholine (PC) and phosphatidylethanolamine (PE) lipids [74] that are expected to have a significantly larger hydrophobic thickness from what we estimated for the conditions of hydrophobic matching from the NMR and MD study of rhodopsin in POPC bilayers [30]. Such a hydrophobic mismatch can theoretically drive rhodopsin aggregation and explain the FRET results.

Recently, a different FRET-based method was applied to study cholesterol-dependent recruitment of a DHA-containing phospholipid by rhodopsin. The experiments demonstrated preferential association of rhodopsin with polyunsaturated lipids, while cholesterol associated with saturated lipids [75]. The cholesterol-dependent formation of lateral microdomains, such as lipid rafts and

caveolae [55], is related to a coexistence of cholesterol-rich liquid-ordered ( $l_o$ ) and cholesterol-depleted liquid-disordered ( $l_d$ ) phases [54]. Studying the solubility limit of cholesterol in different lipids, we found from solid-state NMR spectroscopy that cholesterol is up to five times more soluble in lateral domain or lipid raft promoting sphingomyelin (SM) or disaturated PC [76] than in DHA-containing dipolyunsaturated PC, determined by X-ray diffraction [77]. A similar conclusion can be drawn from data of partition coefficients of cholesterol between methyl- $\beta$ -cyclodextrin and liposomes of different lipid composition with head group and acyl chain variations [78].

Despite these recent insights into cholesterol-dependent sorting of DHA lipids and rhodopsin, the molecular mechanism is yet to be resolved. MD simulations of polyunsaturated chains in comparison with monounsaturated chains in phospholipid bilayers demonstrate that the flexible polyallylic motifs in the DHA chains obtain interesting conformations with very long extended, as well as short back-bent hairpin-like structures [79]. This feature might be related to the unusual thermotropic and lyotropic phase transitions with a pronounced hysteresis of the chain melting [80] and a liquid-to-liquid transition during dehydration [81]. An MD model of rhodopsin in a mixed chain phospholipid bilayer suggested that the polyunsaturated DHA chains rapidly adjust to the protein surface [82]. The hydrophobic thickness of the bilayer matching rhodopsin [30], as shown in Fig. 1c and d, is significantly less than the cholesterol-induced equilibrium thickness of lipid raft-promoting phospholipids and sphingolipids [76], and lipid mixtures representative for the plasma or ROS disk membranes [83]. In the theory of bilayer elasticity, related to mechanical stress due to thickness deformation is the aspect of monolayer curvature stress, which we will discuss in the following section.

The lipid compositional effects on light-induced activation of rhodopsin in model membranes suggested an important role of non-lamellar reverse-hexagonal phase promoting lipids in the ROS disk membranes [28] for the stabilization of the active metarhodopsin II conformation [84–86]. On the other hand, increasing cholesterol content [87] and high melting lipids [46] inhibit formation of the active conformation. An extension of the protein conformational free energy to include elastic coupling to the surrounding bilayer, the so-called flexible surface model, allows for a quantitative description of these lipid compositional effects [86]. As we observed for the dark-state (see Fig. 1c), the bilayer adjusts to a vase-like shape of rhodopsin [30]. A modified flexible surface model predicts that flexible lipids with bulky acyl chains and small head groups as found in the ROS disk membranes will have a lower deformation energy than cholesterol-rich stiff raft-like lipids. Together with the hydrophobic thickness, this effect might contribute to the cholesterol-induced formation of microdomains containing rhodopsin and polyunsaturated DHA lipids.

### 2.3. *In vitro* folding

The folding of rhodopsin requires a membrane-like environment. The assembly of the transmembrane fold in opsin has been accomplished *in vitro* by translation of mRNA by wheat germ extract, signal recognition particle (SRP) dependent translocation of the nascent polypeptide into rabbit liver microsomal membranes, followed by luminal core glycosylation [88], and *in vivo* by mRNA injection of *Xenopus oocytes* [89]. However, practical methods for synthesis of opsin for biochemical and biophysical studies involve heterologous expression. The biotechnological optimization of heterologous expression strategies for rhodopsin has led to several systems yielding microgram to milligram amounts of correctly folded protein by expression of synthetic bovine opsin genes [90] in transiently and stably transfected mammalian cell lines [91–95], baculovirus-infected *Sf9* insect cells [96], and yeasts [97,98]. While it would be desirable for biophysical studies requiring labeled amino acids to express the protein in bacteria or yeast, the problem of the yeast systems is the low amount of correctly folded protein. As bacteria are lacking the endoplasmic reticulum and Golgi complex, (re-)folding generally would have to occur *in vitro*. Recently, some first successful bacterial expression and refolding experiments of other GPCRs have been described [99,100]. Hence, there is large interest in a rational and/or universal approach for *in vitro* folding and refolding of GPCRs, as for example opsin.

Let us for a moment deviate to bacteriorhodopsin (bR). The seven transmembrane helix retinal protein bR from the halophilic archaeal bacterium *Halobacterium salinarium* shares many similarities to bovine rhodopsin [12,101], but a direct evolutionary link has still to be established. The common advantage of these retinal proteins is the well-established relation of the UV-vis spectral properties with the functional state of the protein that allows easy assays for the folded protein. It has been shown that bR can be refolded from a completely denatured state in the presence of certain lipid/detergent mixtures [102–105]. Comparing the different lipid/detergent mixtures favoring bR renaturation, it seems that their common feature is to form so-called bicelles. Bicelles are *binary*, *bilayered*, mixed *micelles* [106,107], micellar discoid structures with a central phospholipid-rich bilayer patch stabilized by a detergent-rich rim. It is noteworthy that this *refolding* is conceptually different from *reconstitution* in phospholipid bilayers, which we will discuss next.

### 2.4. Reconstitution in biomembrane models

Rhodopsin can be functionally reconstituted with certain amphiphiles in bilayer and non-bilayer phases, such as micelles, lipidic cubic phases [108], and lamellar bilayer phases. The usage of the term reconstitution is context sensitive, and it has

been suggested to refer to recombination just in case the *in vivo* function is not definitely known [42]. Some characteristic functions of rhodopsin are binding of 11-*cis* retinal to the apoprotein opsin forming rhodopsin, a sequence of photointermediates after illumination of rhodopsin leading again to opsin, activation of nucleotide exchange in the heterotrimeric  $G_t$  by the active photointermediate metarhodopsin II, and termination of the signaling state by recognition of rhodopsin kinase that leads to C-terminal phosphorylation and visual arrestin binding [109].

The case of bR is simpler. Its characteristic function is the light-driven proton pump activity and thermal relaxation of the light-adapted state to the dark-adapted state without retinal dissociation. The size of the pH gradient over a vesicle membrane generated by the photocycle-coupled proton pump activity depends on the distribution of inside-out and rightside-out oriented proteins, and the protein is easily probed by UV-vis absorption spectroscopy. These properties were useful to study details of the incorporation mechanism of bR in proteoliposomes [110]. Rigaud proposed essentially two different mechanisms for the role of detergents in formation of proteoliposomes, *viz.* the detergent-catalyzed direct incorporation of the protein into preformed phospholipid bilayers of liposomes, and the formation of protein-containing phospholipid bilayers from ternary detergent/protein/phospholipid mixed micelles. The mechanism applies also to the reverse process of reconstitution, the detergent solubilization of membranes. Therefore, one can refer to both as the lamellar-to-micellar transition.

A special case of proteoliposome formation with high protein-to-lipid ratio is the 2D crystallization. The route taken in a particular experiment is dependent on factors like composition, nature of the detergent, and the kinetics of detergent removal [111,112]. Representative for different solubilization properties of detergents,  $\beta$ -octyl- $D$ -glucopyranoside (OG) and sodium cholate were studied for their ability to dissolve phospholipids, a mixture of phosphatidylcholine and phosphatidic acid (PC/PA 9:1 mole/mole), from large unilamellar vesicles [113]. The results of the solubilization experiments could be described by a three-stage model [114]. Without lipids, increasing the detergent concentration above the critical micellar concentration (cmc; 17 mM for OG, and 2.8 mM for cholate), the monomeric detergent concentration remains constant and any additional detergent aggregates in the form of micelles. Above the cmc, the effective detergent concentration is the difference of the total detergent concentration and the cmc. In presence of a particular concentration of phospholipid vesicles, the additional detergent binds to the bilayers instead of forming micelles. This proceeds until the bilayers are detergent saturated (1.3 or 0.30 mole effective detergent per mole phospholipid, for OG or cholate, respectively) and start to disintegrate into mixed detergent/phospholipid micelles. At the point of complete solubilization (3.8 or 0.9 mole effective detergent per mole phospholipid, for OG or

cholate, respectively), all bilayers are consumed and additional detergent changes the effective detergent/phospholipid ratio [113].

The incorporation of detergent solubilized bR into phospholipid vesicles at different effective detergent to lipid molar ratios, followed by rapid detergent removal by a polystyrene resin, occurred quite differently for OG and cholate [110]. Below the cmc of OG or cholate, rhodopsin cannot be incorporated into the liposomes and forms protein-rich self-aggregated particles. Apparently, bR is incorporated in OG saturated vesicles with a high preference for inside-out orientation. Cholate favors symmetric incorporation with only a slight preference for inside-out *versus* rightside-out orientation of bR. In addition, for OG concentration below full lipid solubilization, the protein is only partially incorporated into liposomes, and self-aggregated protein-rich particles are coexisting with proteoliposomes and protein-free liposomes. Cholate concentrations above the cmc lead to complete incorporation of bR in proteoliposomes with increasing lipid-to-protein molar ratio coexisting with protein-free liposomes, until at full solubilization only proteoliposomes are formed. Based on these observations, the plausible mechanism for OG-based reconstitution is the direct incorporation of bR in a preferential orientation into preformed bilayers, whereas for cholate-based reconstitution the proteoliposome bilayers assemble from ternary detergent/lipid/protein mixed micelles in random orientation. These results are quite important, as now with this model, we can categorize similar observations for rhodopsin.

The incorporation of rhodopsin in proteoliposomes based on slow detergent removal by dialysis from completely OG-solubilized rhodopsin with different phospholipids led to mixtures of protein-rich aggregates, proteoliposomes, and protein-free liposomes [115]. A modification of the protocol involving a rapid dilution of the mixture to OG concentrations below the cmc favored formation of proteoliposomes [116]. Compared with the bR experiments, the dialysis method from the completely OG-solubilized state apparently proceeds *via* conditions of partial solubilization, whereas the rapid dilution method is comparable to the rapid detergent removal by the polystyrene beads. In a recent experiment, it was demonstrated that detergent removal by dialysis from completely OG-solubilized rhodopsin with asolectin leads to asymmetric incorporation of rhodopsin in proteoliposomes with the cytoplasmic side facing out, in contrast to dialysis of completely cholate-solubilized ROS membranes mixed with egg PC and egg PE that yielded a symmetric distribution of rhodopsin orientations [16,117]. These results are also in agreement with the bR case, where OG favors asymmetric and cholate favors symmetric protein orientations. Unfortunately, it is not possible to conclude anything about the incorporation efficiency of rhodopsin under these conditions, since Niu and coworkers did not analyze the samples for possible formation of protein-rich aggregates or protein-free liposomes in addition to proteoliposomes. Another modification of the OG dialysis protocol is the addition of cholate to ensure homogeneous proteoliposomes with different lipid-to-protein

ratios [57,74]. The possible mechanism is a fast removal of the flexible OG molecule from the mixture, followed by slower removal of the rigid cholate over the dialysis membrane. Therefore, the mechanism might be the same as for the cholate-based reconstitution method from the complete solubilized state in the case of bR. Finally, rhodopsin incorporation into a black lipid membrane of a SPR spectrometer after dilution of OG well below the cmc resulted in only 0.02% incorporation [118], in agreement with the bR results of negligible incorporation in vesicles at low detergent concentration.

### 3. INTERACTIONS WITH MEMBRANES

The membrane interface has a strong effect on the activity and reactivity of molecules. For example, charged species experience long-ranged electrostatic forces altering the diffusion toward the generally negatively charged biomembranes. The phospholipid head groups can form strong hydrogen bonds in the short range. Hydrophobic interactions with the membrane are dependent on the composition of the hydrophobic core of the bilayer. These are some general biophysical principles that make the biomembrane interface a special compartment with selective partitioning of molecules within the cell. In the following sections, we will discuss how these principles affect GPCR-mediated signaling.

#### 3.1. Membrane surface potential and water activity

One of the important factors determining the conformational state of a protein is the local proton activity affecting the equilibria between protonated and deprotonated conformations. In photoactivated rhodopsin, the proton uptake in the cytoplasmic domain leads to the formation of the G protein activating conformation [67,119]. There are some different views on the sequence of intermediates related to the protonation reaction [120–122], but in essence proton uptake favors metarhodopsin II with a deprotonated retinylidene Schiff base. The pH dependency of this reaction is affected by the membrane surface potential, and application of a Poisson-Boltzmann model describes quantitatively the effect of salt, phosphorylated protein side chains, and negatively charged lipids [123,124]. It appears that negatively charged lipids stimulate metarhodopsin II formation by lowering the local pH, while at the same time inhibiting its formation by affecting the bilayer surface elasticity [124].

Protonation reactions are a special case of general acid or base catalyzed reactions. We have shown that hydrogen exchange reactions in the lipid/water interface are acid and base catalyzed, and occur significantly less frequent than in solution [125,126]. Here, the pH dependency suggests that the effects of the

membrane interface reflect the reduced water activity or accessibility rather than the membrane surface potential. Therefore, the slow hydrogen exchange dynamics can be useful to study, for example, the water accessibility of acidic residues in the transmembrane domain during photoactivation of rhodopsin mutants [127,128].

The competition of water with peptide hydrogen bonds is reduced in the membrane interface. Consequently, the reduced water activity is a key factor for membrane protein folding [129]. However, before the hydrogen bond formation stabilizes secondary structure, the peptide has to partition into the membrane interface. Hydrogen bond formation of cationic residues with the phosphate in the lipid head group and hydrophobic interactions of apolar side chains with the hydrocarbon interior of the membrane facilitate partitioning of a peptide to the membrane. In addition, negatively charged lipids favor partitioning of cationic peptides in the membrane interface with resulting stabilization of helical structure, as shown for peptide sequences analogous to the amphipathic helix H-8 of rhodopsin [130]. As noted above, the stop-transfer signal in membrane protein folding is frequently a sequence of cationic residues underlining the biological importance of these interactions.

The partitioning principles are also important for ligand recognition of membrane proteins. For example, the stronger repulsion of the ATP relative to ADP by the net negative charge of the mitochondrial inner membrane contributes to the selectivity of the diphosphate to the positively charged nucleotide binding site of the ADP/ATP carrier (AAC) [131]. In a similar fashion, the increase of the positive charge in the cytoplasmic domain of photoactivated rhodopsin by protonation of the conserved D(E)RY motif [67] might direct the diffusion of the negatively charged transducin ( $G_t$ ) toward the activated receptor. On the other hand, phosphorylation of activated rhodopsin by rhodopsin kinase modulates the binding affinity for the G protein and arrestin [132].

### 3.2. Localization depends on membrane interactions

The association and orientation of peripheral membrane proteins are dependent on the nature of the lipid interface. It is believed, for example, that several positively charged residues in visual arrestin contribute to its specific binding to activated and phosphorylated rhodopsin by purely electrostatic interactions with negatively charged lipids in the membrane [133]. In a complex fashion, the interactions of  $G_t$  with the membrane might be under control of the functional state of the rod cells. The  $G_{\alpha_t}$  subunit is myristoylated at the N-terminal glycine, and the  $G_{\gamma_t}$  subunit is carboxymethylated and farnesylated at the C-terminal cysteine [134]. The complex  $G\beta\gamma_t$  of  $G_{\gamma_t}$  with the  $G\beta_t$  is stable and independent of receptor activation. The inactive heterotrimeric form  $G_t$  in the GDP-bound state exchanges

the nucleotide upon activation to GTP, followed by partial dissociation into GTP-bound  $G\alpha_t$  and  $G\beta\gamma_t$ . The GTP-bound  $G\alpha_t$  activates the phosphodiesterase, and a GTPase activity terminates signaling [109].

Binding studies of the  $G_t$  and the dissociated components  $G\alpha_t$  and  $G\beta\gamma_t$  to model membranes revealed different contributions of the protein charges and post-translational modifications to the interactions with the bilayer. The membrane binding of subunits is not fully synergistic in the heterotrimeric complex. The head group packing density most strongly affects binding of  $G\alpha_t$ , while  $G_t$  and  $G\beta\gamma_t$  are only weakly affected. Negatively charged lipids enhance binding of  $G\beta\gamma_t$ , repel slightly  $G_t$  and more strongly the GTP $\gamma$ S-bound  $G\alpha_t$ , and do not affect the GDP-bound  $G\alpha_t$ . On the other hand, positive charge modification by binding of magnesium ions to the negatively charged membrane strongly enhances the binding of  $G_t$  and  $G\alpha_t$  [135]. Positively charged lipids were shown to have similar effects as magnesium [136], supporting the hypothesis of an electrostatic mechanism of divalent cation action. Light adaptation was shown to drive massive translocation of the G protein from the outer segment to the inner segment in rod cells, and  $G\alpha_t$  leaves the outer segment faster than  $G\beta_t$  (note that the species is most likely the dimeric  $G\beta\gamma_t$  complex). Upon dark adaptation, the kinetics for the reverse process is the same for  $G\alpha_t$  and  $G\beta_t$  [137]. It might be that the differential binding modes drive this translocation, as the dark adapted ROS has a high concentration of intracellular calcium ions that bind stronger than magnesium to the membrane. In the light-adapted state, the calcium concentration drops significantly and the G protein subunits are dissociated. Therefore, the membrane-binding affinity is dramatically reduced and the components might repartition according to the available cytoplasmic volumes in the outer and inner segments. The kinetics of this process most likely reflects the different membrane affinities of the dissociated and associated components [135,137].

The role of the post-translational modifications by prenylation and acylation in the localization of G proteins to membrane microdomains deserves some further comments. The acylation of  $G\alpha_t$  generally comprises a heterogeneous mixture of saturated and unsaturated acyl chains of 12–14 carbons [135]; other  $G\alpha$  subtypes are palmitoylated [138]. The two hydrocarbon chains found in  $G\gamma$  prenylation are farnesyl (15 carbons) and geranylgeranyl (20 carbons) groups. The farnesyl modification in rod cell  $G\gamma_t$  is less hydrophobic than the geranylgeranyl group of other  $G\gamma$  subunits, facilitating dissociation from the membrane as probably involved in the translocation during light adaptation. One might expect that the branched unsaturated prenylation would favor localization into liquid-disordered ( $l_d$ ) domains such as those rhodopsin-containing microdomains with DHA-phospholipids as noted above. The myristoylation would then favor localization into liquid-ordered ( $l_o$ ) domains or lipid rafts or caveolae [54]. This hypothesis was tested for ROS membranes [139,140] with a method involving extraction and fractionation to form detergent resistant membranes (DRM). DRM preparation is

a biochemical tool to study the composition of membrane microdomains by low temperature extraction with the detergent Triton X-100. However, increasing evidence for artifacts in this principal biochemical and operational definition of lipid rafts has challenged the validity of conclusions based on DRM methods [56].

The lipid composition in model membranes affects the coupling of photo-activated rhodopsin to the G protein  $G_t$  [141–143]. It was found that  $G_t$  activation is increased by DHA-phospholipids but reduced by cholesterol and negatively charged PS. These effects are synergistic with the effects of these lipids on metarhodopsin II formation. At this point the molecular mechanism is not clear, but lipid effects on the effective partitioning of  $G_t$  to the membrane surface and thus to the activated receptor appear to be a plausible explanation.

#### 4. CONCLUDING REMARKS

In this chapter, we have discussed several experimental approaches to study the cell biological relevance of lipid/protein interactions in membrane protein biosynthesis, G protein-coupled receptor-dependent signal transduction, and vision. The highly polyunsaturated lipid environment of rhodopsin in photoreceptor membranes appears to be on the opposite side of the spectrum of biomembrane microdomains compared with cholesterol-rich, more saturated lipid rafts or caveolae. Future experimental and computational studies might elucidate the underlying molecular mechanisms to gain insight to the role of different lipid classes in structure and dynamics of biomembranes. The visual signal transduction system in photoreceptor membranes is very attractive for computational studies, especially since the 3D structures for many components have already been determined [109,144].

#### ACKNOWLEDGEMENTS

We thank Klaus Beyer, Ana Vitoria Botelho, Michael F. Brown, Martin Klingenberg, Volker F. Kurze, Santosh T. Menon, Xavier Periole, Horia I. Petrache, Pallavi Sachdev, and Bernhard Steinbauer for many helpful discussions.

#### REFERENCES

- [1] D.W. Hilgemann, Getting ready for the decade of the lipids, *Ann. Rev. Physiol.* 65 (2003) 697–700.
- [2] F. Boll, Zur Anatomie und Physiologie der Retina, *Arch. Anat. Physiol. (Physiol. Abt.)* (1877) 4–36.

- [3] G. Wald, Nobel Lecture, December 12, 1967, The Molecular Basis of Visual Excitation. From Nobel Lectures, Physiology or Medicine 1963–1970, Elsevier, Amsterdam, 1967.
- [4] B.K. Fung, J.B. Hurley, L. Stryer, Flow of information in the light-triggered cyclic nucleotide cascade of vision, *Proc. Natl. Acad. Sci. U.S.A.* 78 (1981) 152–156.
- [5] K. Palczewski, T. Kumasaka, T. Hori, C.A. Behnke, H. Motoshima, B.A. Fox, I. Le Trong, D.C. Teller, T. Okada, R.E. Stenkamp, M. Yamamoto, M. Miyano, Crystal structure of rhodopsin: a G protein-coupled receptor, *Science* 289 (2000) 739–745.
- [6] J. Drews, Drug discovery: a historical perspective, *Science* 287 (2000) 1960–1964.
- [7] E.S. Lander, L.M. Linton, B. Birren, C. Nusbaum, M.C. Zody, J. Baldwin, *et al.*, Initial sequencing and analysis of the human genome, *Nature* 409 (2001) 860–921.
- [8] S. Moro, F. Deflorian, G. Spalluto, G. Pastorin, B. Cacciari, S.K. Kim, K.A. Jacobson, Demystifying the three dimensional structure of G protein-coupled receptors (GPCRs) with the aid of molecular modeling, *Chem. Commun.* (2003) 2949–2956.
- [9] K.P. Hofmann, S. Jäger, O.P. Ernst, Structure and function of activated rhodopsin, *Isr. J. Chem.* 35 (1995) 339–355.
- [10] W.L. Hubbell, C. Altenbach, C.M. Hubbell, H.G. Khorana, Rhodopsin structure, dynamics, and activation: A perspective from crystallography, site-directed spin labeling, sulfhydryl reactivity, and disulfide cross-linking, *Adv. Prot. Chem.* 63 (2003) 243–290.
- [11] T. Ebrey, Y. Koutalos, Vertebrate photoreceptors, *Prog. Retin. Eye Res.* 20 (2001) 49–94.
- [12] T.P. Sakmar, S.T. Menon, E.P. Marin, E.S. Awad, Rhodopsin: insights from recent structural studies, *Annu. Rev. Biophys. Biomol. Struct.* 31 (2002) 443–484.
- [13] Y.A. Ovchinnikov, N.G. Abdulaev, M.Y. Feigina, I.D. Artamonov, A.S. Zolotarev, M.B. Kostina, A.S. Bogachuk, A.I. Miroshnikov, V.I. Martinov, A.B. Kudelin, The complete amino-acid-sequence of visual rhodopsin, *Bioorg. Khim.* 8 (1982) 1011–1014.
- [14] P.A. Hargrave, J.H. McDowell, D.R. Curtis, J.K. Wang, E. Juszczak, S.L. Fong, J.K. Rao, P. Argos, The structure of bovine rhodopsin, *Biophys. Struct. Mech.* 9 (1983) 235–244.
- [15] J. Nathans, D.S. Hogness, Isolation, sequence-analysis, and intron exon arrangement of the gene encoding bovine rhodopsin, *Cell* 34 (1983) 807–814.
- [16] B.K. Fung, W.L. Hubbell, Organization of rhodopsin in photoreceptor membranes. 2. Transmembrane organization of bovine rhodopsin: evidence from proteolysis and lactoperoxidase-catalyzed iodination of native and reconstituted membranes, *Biochemistry* 17 (1978) 4403–4410.
- [17] G.F.X. Schertler, C. Villa, R. Henderson, Projection structure of rhodopsin, *Nature* 362 (1993) 770–772.
- [18] D.C. Teller, T. Okada, C.A. Behnke, K. Palczewski, R.E. Stenkamp, Advances in determination of a high-resolution three-dimensional structure of rhodopsin, a model of G-protein-coupled receptors (GPCRs), *Biochemistry* 40 (2001) 7761–7772.
- [19] T. Okada, Y. Fujiyoshi, M. Silow, J. Navarro, E.M. Landau, Y. Shichida, Functional role of internal water molecules in rhodopsin revealed by x-ray crystallography, *Proc. Natl. Acad. Sci. U.S.A.* 99 (2002) 5982–5987.
- [20] D.S. Kliger, J.W. Lewis, Spectral and kinetic characterization of visual pigment photointermediates, *Isr. J. Chem.* 35 (1995) 289–307.
- [21] O. Beja, L. Aravind, E.V. Koonin, M.T. Suzuki, A. Hadd, L.P. Nguyen, S. Jovanovich, C.M. Gates, R.A. Feldman, J.L. Spudich, E.N. Spudich, E.F. DeLong, Bacterial rhodopsin: evidence for a new type of phototrophy in the sea, *Science* 289 (2000) 1902–1906.
- [22] J.K. Lanyi, B. Schobert, Local-global conformational coupling in a heptahelical membrane protein: Transport mechanism from crystal structures of the nine states in the bacteriorhodopsin photocycle, *Biochemistry* 43 (2004) 3–8.

- [23] R.R. Rando, The biochemistry of the visual cycle, *Chem. Rev.* 101 (2001) 1881–1896.
- [24] D.R. Pepperberg, R.K. Crouch, An illuminating new step in visual-pigment regeneration, *Lancet* 358 (2001) 2098–2099.
- [25] V. Kuksa, Y. Imanishi, M. Batten, K. Palczewski, A.R. Moise, Retinoid cycle in the vertebrate retina: experimental approaches and mechanisms of isomerization, *Vision Res.* 43 (2003) 2959–2981.
- [26] N.M. Giusto, S.J. Pasquare, G.A. Salvador, P.I. Castagnet, M.E. Roque, M.G.I. de Boschero, Lipid metabolism in vertebrate retinal rod outer segments, *Prog. Lipid Res.* 39 (2000) 315–391.
- [27] M.A. Crawford, I. Golfetto, K. Ghebremeskel, Y. Min, T. Moodley, J. Poston, A. Phylactos, S. Cunnane, W. Schmidt, The potential role for arachidonic and docosahexaenoic acids in protection against some central nervous system injuries in preterm infants, *Lipids* 38 (2003) 303–315.
- [28] G.P. Miljanich, L.A. Sklar, D.L. White, E.A. Dratz, Disaturated and dipolyunsaturated phospholipids in the bovine retinal rod outer segment disk membrane, *Biochim. Biophys. Acta* 552 (1979) 294–306.
- [29] M.A. Crawford, M. Bloom, C.L. Broadhurst, W.F. Schmidt, S.C. Cunnane, C. Galli, K. Ghebremeskel, F. Linseisen, J. Lloyd-Smith, J. Parkington, Evidence for the unique function of docosahexaenoic acid during the evolution of the modern hominid brain, *Lipids* 34 (1999) S39–S47.
- [30] T. Huber, A.V. Botelho, K. Beyer, M.F. Brown, Membrane model for the GPCR rhodopsin: hydrophobic interface and dynamical structure, *Biophys. J.* 84 (2004) 2078–2100.
- [31] R.B. Gennis, *Biomembranes*, Springer, New York, 1993.
- [32] B. Alberts, A. Johnson, J. Lewis, M. Raff, K. Roberts, P. Walter, *Molecular Biology of the Cell*, Garland, New York, 2002.
- [33] M. Friedlander, G. Blobel, Bovine opsin has more than one signal sequence, *Nature* 318 (1985) 338–343.
- [34] Y. Audigier, M. Friedlander, G. Blobel, Multiple topogenic sequences in bovine opsin, *Proc. Natl. Acad. Sci. U.S.A.* 84 (1987) 5783–5787.
- [35] G. von Heijne, Membrane-protein structure prediction – hydrophobicity analysis and the positive-inside rule, *J. Mol. Biol.* 225 (1992) 487–494.
- [36] S. Kaushal, K.D. Ridge, H.G. Khorana, Structure and function in rhodopsin – the role of asparagine-linked glycosylation, *Proc. Natl. Acad. Sci. U.S.A.* 91 (1994) 4024–4028.
- [37] C.J.R. Loewen, R.S. Molday, Disulfide-mediated oligomerization of peripherin/Rds and Rom-1 in photoreceptor disk membranes – implications for photoreceptor outer segment morphogenesis and degeneration, *J. Biol. Chem.* 275 (2000) 5370–5378.
- [38] T.P. Sakmar, Restoration of compact discs, *Nature Genet.* 25 (2000) 245–246.
- [39] A.W. Tai, J.Z. Chuang, C. Bode, U. Wolfrum, C.H. Sung, Rhodopsin's carboxy-terminal cytoplasmic tail acts as a membrane receptor for cytoplasmic dynein by binding to the dynein light chain Tctex-1, *Cell* 97 (1999) 877–887.
- [40] B.M. Tam, O.L. Moritz, L.B. Hurd, D.S. Papermaster, Identification of an outer segment targeting signal in the COOH terminus of rhodopsin using transgenic *Xenopus laevis*, *J. Cell Biol.* 151 (2000) 1369–1380.
- [41] E.B.R. de Turco, D. Deretic, N.G. Bazan, D.S. Papermaster, Post-Golgi vesicles cotransport docosahexaenoyl-phospholipids and rhodopsin during frog photoreceptor membrane biogenesis, *J. Biol. Chem.* 272 (1997) 10491–10497.
- [42] K. Hong, W.L. Hubbell, Preparation and properties of phospholipid bilayers containing rhodopsin, *Proc. Natl. Acad. Sci. U.S.A.* 69 (1972) 2617–2621.
- [43] Y.S. Chen, W.L. Hubbell, Temperature-dependent and light-dependent structural-changes in rhodopsin-lipid membranes, *Exp. Eye Res.* 17 (1973) 517–532.

- [44] K. Hong, W.L. Hubbell, Lipid requirements for rhodopsin regenerability, *Biochemistry* 12 (1973) 4517–4523.
- [45] P.A. Baldwin, W.L. Hubbell, Effects of lipid environment on the light-induced conformational-changes of rhodopsin .1. Absence of metarhodopsin-II production in dimyristoylphosphatidylcholine recombinant membranes, *Biochemistry* 24 (1985) 2624–2632.
- [46] P.A. Baldwin, W.L. Hubbell, Effects of lipid environment on the light-induced conformational changes of rhodopsin. 2. Roles of lipid chain length, unsaturation, and phase state, *Biochemistry* 24 (1985) 2633–2639.
- [47] D. Fotiadis, Y. Liang, S. Filipek, D.A. Saperstein, A. Engel, K. Palczewski, Atomic-force microscopy: Rhodopsin dimers in native disc membranes, *Nature* 421 (2003) 127–128.
- [48] Y. Liang, D. Fotiadis, S. Filipek, D.A. Saperstein, K. Palczewski, A. Engel, Organization of the G protein-coupled receptors rhodopsin and opsin in native membranes, *J. Biol. Chem.* 278 (2003) 21655–21662.
- [49] M. Chabre, R. Cone, H. Saibil, Biophysics – is rhodopsin dimeric in native rods?, *Nature* 426 (2003) 30–31.
- [50] D. Fotiadis, Y. Liang, S. Filipek, D.A. Saperstein, A. Engel, K. Palczewski, Biophysics – is rhodopsin dimeric in native rods? Reply, *Nature* 426 (2003) 30–31.
- [51] R.A. Cone, Rotational diffusion of rhodopsin in the visual receptor membrane, *Nat. New Biol.* 236 (1972) 39–43.
- [52] P.A. Liebman, G. Entine, Lateral diffusion of visual pigment in photoreceptor disk membranes, *Science* 185 (1974) 457–459.
- [53] S.J. Singer, G.L. Nicolson, Fluid mosaic model of structure of cell-membranes, *Science* 175 (1972) 720–731.
- [54] D.A. Brown, E. London, Structure and function of sphingolipid- and cholesterol-rich membrane rafts, *J. Biol. Chem.* 275 (2000) 17221–17224.
- [55] K. Simons, E. Ikonen, How cells handle cholesterol, *Science* 290 (2000) 1721–1726.
- [56] M. Edidin, The state of lipid rafts: from model membranes to cells, *Annu. Rev. Biophys. Biomol. Struct.* 32 (2003) 257–283.
- [57] A. Kusumi, J.S. Hyde, Spin-label saturation-transfer electron-spin resonance detection of transient association of rhodopsin in reconstituted membranes, *Biochemistry* 21 (1982) 5978–5983.
- [58] N.J.P. Ryba, D. Marsh, Protein rotational diffusion and lipid protein interactions in recombinants of bovine rhodopsin with saturated diacylphosphatidylcholines of different chain lengths studied by conventional and saturation-transfer electron-spin-resonance, *Biochemistry* 31 (1992) 7511–7518.
- [59] J.A. Killian, Hydrophobic mismatch between proteins and lipids in membranes, *Biochim. Biophys. Acta* 1376 (1998) 401–416.
- [60] D. Marsh, L.I. Horvath, Structure, dynamics and composition of the lipid–protein interface. Perspectives from spin-labelling, *Biochim. Biophys. Acta* 1376 (1998) 267–296.
- [61] N.J.P. Ryba, L.I. Horvath, A. Watts, D. Marsh, Molecular-exchange at the lipid rhodopsin interface – spin-label electron-spin-resonance studies of rhodopsin dimyristoylphosphatidylcholine recombinants, *Biochemistry* 26 (1987) 3234–3240.
- [62] A. Watts, I.D. Volotovskii, D. Marsh, Rhodopsin–lipid associations in bovine rod outer segment membranes – identification of immobilized lipid by spin-labels, *Biochemistry* 18 (1979) 5006–5013.
- [63] L.I. Horvath, M. Drees, K. Beyer, M. Klingenberg, D. Marsh, Lipid–protein interactions in ADP–ATP carrier egg phosphatidylcholine recombinants studied by spin-label ESR spectroscopy, *Biochemistry* 29 (1990) 10664–10669.
- [64] E. Pebay-Peyroula, C. Dahout-Gonzalez, R. Kahn, V. Trezeguet, G.J.M. Lauquin, R. Brandolin, Structure of mitochondrial ADP/ATP carrier in complex with carboxyatractyloside, *Nature* 426 (2003) 39–44.

- [65] M. Beck, F. Siebert, T.P. Sakmar, Evidence for the specific interaction of a lipid molecule with rhodopsin which is altered in the transition to the active state metarhodopsin II, *FEBS Lett.* 436 (1998) 304–308.
- [66] J. Isele, T.P. Sakmar, F. Siebert, Rhodopsin activation affects the environment of specific neighboring phospholipids: An FTIR spectroscopic study, *Biophys. J.* 79 (2000) 3063–3071.
- [67] S. Arnis, K. Fahmy, K.P. Hofmann, T.P. Sakmar, A conserved carboxylic-acid group mediates light-dependent proton uptake and signaling by rhodopsin, *J. Biol. Chem.* 269 (1994) 23879–23881.
- [68] G.P. Miljanich, P.P. Nemes, D.L. White, E.A. Dratz, The asymmetric transmembrane distribution of phosphatidylethanolamine, phosphatidylserine, and fatty acids of the bovine retinal rod outer segment disk membrane, *J. Membr. Biol.* 60 (1981) 249–255.
- [69] E. Hessel, P. Müller, A. Herrmann, K.P. Hofmann, Light-induced reorganization of phospholipids in rod disc membranes, *J. Biol. Chem.* 276 (2001) 2538–2543.
- [70] J.R. Lakowicz, *Principles of Fluorescence Spectroscopy*, Kluwer, New York, 1999.
- [71] H. Borochoy-Neori, M. Montal, Rhodopsin in reconstituted phospholipid vesicles. 1. Structural parameters and light-induced conformational changes detected by resonance energy transfer and quenching, *Biochemistry* 22 (1983) 197–205.
- [72] G.F.X. Schertler, P.A. Hargrave, Preparation and analysis of two-dimensional crystals of rhodopsin, *Meth. Enzym.* 315 (2000) 91–107.
- [73] P.A. Kralchevsky, V.N. Paunov, N.D. Denkov, K. Nagayama, Stresses in lipid-membranes and interactions between inclusions, *J. Chem. Soc.-Faraday Trans.* 91 (1995) 3415–3432.
- [74] H. Borochoy-Neori, P.A.G. Fortes, M. Montal, Rhodopsin in reconstituted phospholipid vesicles. 2. Rhodopsin-rhodopsin interactions detected by resonance energy transfer, *Biochemistry* 22 (1983) 206–213.
- [75] A. Polozova, B.J. Litman, Cholesterol dependent recruitment of di22,6-PC by a G protein-coupled receptor into lateral domains, *Biophys. J.* 79 (2000) 2632–2643.
- [76] W. Guo, V. Kurze, T. Huber, N.H. Afdhal, K. Beyer, J.A. Hamilton, A solid-state NMR study of phospholipid-cholesterol interactions: sphingomyelin-cholesterol binary systems, *Biophys. J.* 83 (2002) 1465–1478.
- [77] M.R. Brzustowicz, V. Cherezov, M. Caffrey, W. Stillwell, S.R. Wassall, Molecular organization of cholesterol, in polyunsaturated membranes: microdomain formation, *Biophys. J.* 82 (2002) 285–298.
- [78] S.L. Niu, D.C. Mitchell, B.J. Litman, Manipulation of cholesterol levels in rod disk membranes by methyl-beta-cyclodextrin. Effects on receptor activation, *J. Biol. Chem.* 277 (2002) 20139–20145.
- [79] T. Huber, K. Rajamoorthi, V.F. Kurze, K. Beyer, M.F. Brown, Structure of docosahexaenoic acid-containing phospholipid bilayers as studied by  $^2\text{H}$  NMR and molecular dynamics simulations, *J. Am. Chem. Soc.* 124 (2002) 298–309.
- [80] J.A. Barry, T.P. Trouard, A. Salmon, M.F. Brown, Low-temperature  $^2\text{H}$  NMR-spectroscopy of phospholipid-bilayers containing docosahexaenoyl (22:6 $\omega$ 3) chains, *Biochemistry* 30 (1991) 8386–8394.
- [81] H. Binder, K. Gawrisch, Effect of unsaturated lipid chains on dimensions, molecular order and hydration of membranes, *J. Phys. Chem. B* 105 (2001) 12378–12390.
- [82] S.E. Feller, K. Gawrisch, T.B. Woolf, Rhodopsin exhibits a preference for solvation by polyunsaturated docosohexaenoic acid, *J. Am. Chem. Soc.* 125 (2003) 4434–4435.
- [83] D. Huster, K. Arnold, K. Gawrisch, Influence of docosahexaenoic acid and cholesterol on lateral lipid organization in phospholipid mixtures, *Biochemistry* 37 (1998) 17299–17308.
- [84] T.S. Wiedmann, R.D. Pates, J.M. Beach, A. Salmon, M.F. Brown, Lipid protein interactions mediate the photochemical function of rhodopsin, *Biochemistry* 27 (1988) 6469–6474.

- [85] N.J. Gibson, M.F. Brown, Lipid headgroup and acyl chain composition modulate the M<sub>I</sub>–M<sub>II</sub> equilibrium of rhodopsin in recombinant membranes, *Biochemistry* 32 (1993) 2438–2454.
- [86] A.V. Botelho, N.J. Gibson, R.L. Thurmond, Y. Wang, M.F. Brown, Conformational energetics of rhodopsin modulated by nonlamellar-forming lipids, *Biochemistry* 41 (2002) 6354–6368.
- [87] D.C. Mitchell, M. Straume, J.L. Miller, B.J. Litman, Modulation of metarhodopsin formation by cholesterol-induced ordering of bilayer lipids, *Biochemistry* 29 (1990) 9143–9149.
- [88] B.M. Goldman, G. Blobel, In vitro biosynthesis, core glycosylation, and membrane integration of opsin, *J. Cell Biol.* 90 (1981) 236–242.
- [89] H.G. Khorana, B.E. Knox, E. Nasi, R. Swanson, D.A. Thompson, Expression of a bovine rhodopsin gene in *Xenopus oocytes* – demonstration of light-dependent ionic currents, *Proc. Natl. Acad. Sci. U.S.A.* 85 (1988) 7917–7921.
- [90] L. Ferretti, S.S. Karnik, H.G. Khorana, M. Nassal, D.D. Oprian, Total synthesis of a gene for bovine rhodopsin, *Proc. Natl. Acad. Sci. U.S.A.* 83 (1986) 599–603.
- [91] D.D. Oprian, R.S. Molday, R.J. Kaufman, H.G. Khorana, Expression of a synthetic bovine rhodopsin gene in monkey kidney-cells, *Proc. Natl. Acad. Sci. U.S.A.* 84 (1987) 8874–8878.
- [92] J. Nathans, C.J. Weitz, N. Agarwal, I. Nir, D.S. Papermaster, Production of bovine rhodopsin by mammalian-cell lines expressing cloned cDNA – spectrophotometry and subcellular-localization, *Vision Res.* 29 (1989) 907–914.
- [93] P.J. Reeves, R.L. Thurmond, H.G. Khorana, Structure and function in rhodopsin: high level expression of a synthetic bovine opsin gene and its mutants in stable mammalian cell lines, *Proc. Natl. Acad. Sci. U.S.A.* 93 (1996) 11487–11492.
- [94] P.J. Reeves, J.M. Kim, H.G. Khorana, Structure and function in rhodopsin: a tetracycline-inducible system in stable mammalian cell lines for high-level expression of opsin mutants, *Proc. Natl. Acad. Sci. U.S.A.* 99 (2002) 13413–13418.
- [95] P.J. Reeves, N. Callewaert, R. Contreras, H.G. Khorana, Structure and function in rhodopsin: High-level expression of rhodopsin with restricted and homogeneous N-glycosylation by a tetracycline-inducible N-acetylglucosaminyltransferase I-negative HEK293S stable mammalian cell line, *Proc. Natl. Acad. Sci. U.S.A.* 99 (2002) 13419–13424.
- [96] J.J.M. Janssen, W.J.M. Vandeven, W. Vangroningenluyben, J. Roosien, J.M. Vlak, W.J. Degrip, Synthesis of functional bovine opsin in insect cells under control of the Baculovirus polyhedrin promoter, *Mol. Biol. Rep.* 13 (1988) 65–71.
- [97] R. Mollaaghababa, F.F. Davidson, C. Kaiser, H.G. Khorana, Structure and function in rhodopsin: expression of functional mammalian opsin in *Saccharomyces cerevisiae*, *Proc. Natl. Acad. Sci. U.S.A.* 93 (1996) 11482–11486.
- [98] N.G. Abdulaev, M.P. Popp, W.C. Smith, K.D. Ridge, Functional expression of bovine opsin in the methylotrophic yeast *Pichia pastoris*, *Protein Expr. Purif.* 10 (1997) 61–69.
- [99] S. Luca, J.F. White, A.K. Sohal, D.V. Filippov, J.H. van Boom, R. Grisshammer, M. Baldus, The conformation of neurotensin bound to its G protein-coupled receptor, *Proc. Natl. Acad. Sci. U.S.A.* 100 (2003) 10706–10711.
- [100] J.L. Baneres, A. Martin, P. Hullot, J.P. Girard, J.C. Rossi, J. Parello, Structure-based analysis of GPCR function: Conformational adaptation of both agonist and receptor upon leukotriene B-4 binding to recombinant BLT1, *J. Mol. Biol.* 329 (2003) 801–814.
- [101] T.P. Sakmar, Structure of rhodopsin and the superfamily of seven-helical receptors: the same and not the same, *Curr. Opin. Cell Biol.* 14 (2002) 189–195.
- [102] K.S. Huang, H. Bayley, M.J. Liao, E. London, H.G. Khorana, Refolding of an integral membrane-protein – denaturation, renaturation, and reconstitution of intact bacteriorhodopsin and 2 proteolytic fragments, *J. Biol. Chem.* 256 (1981) 3802–3809.

- [103] E. London, H.G. Khorana, Denaturation and renaturation of bacteriorhodopsin in detergents and lipid-detergent mixtures, *J. Biol. Chem.* 257 (1982) 7003–7011.
- [104] P.J. Booth, M.L. Riley, S.L. Flitsch, R.H. Templer, A. Farooq, A.R. Curran, N. Chadborn, P. Wright, Evidence that bilayer bending rigidity affects membrane protein folding, *Biochemistry* 36 (1997) 197–203.
- [105] S.J. Allen, J.M. Kim, H.G. Khorana, H. Lu, P.J. Booth, Structure and function in bacteriorhodopsin: the effect of the interhelical loops on the protein folding kinetics, *J. Mol. Biol.* 308 (2001) 423–435.
- [106] C.R. Sanders, J.H. Prestegard, Magnetically orientable phospholipid-bilayers containing small amounts of a bile-salt analog, *Chapso*. *Biophys. J.* 58 (1990) 447–460.
- [107] C.R. Sanders, G.C. Landis, Reconstitution of membrane-proteins into lipid-rich bilayered mixed micelles for NMR-studies, *Biochemistry* 34 (1995) 4030–4040.
- [108] J. Navarro, E.M. Landau, K. Fahmy, Receptor-dependent G-protein activation in lipidic cubic phase, *Biopolymers* 67 (2002) 167–177.
- [109] S.T. Menon, M. Han, T.P. Sakmar, Rhodopsin: structural basis of molecular physiology, *Physiol. Rev.* 81 (2001) 1659–1688.
- [110] J.L. Rigaud, M.T. Paternostre, A. Bluzat, Mechanisms of membrane-protein insertion into liposomes during reconstitution procedures involving the use of detergents. 2. Incorporation of the light-driven proton pump bacteriorhodopsin, *Biochemistry* 27 (1988) 2677–2688.
- [111] J.L. Rigaud, B. Pitard, D. Levy, Reconstitution of membrane-proteins into liposomes – application to energy-transducing membrane-proteins, *Biochim. Biophys. Acta* 1231 (1995) 223–246.
- [112] J.L. Rigaud, M. Chami, O. Lambert, D. Levy, J.L. Ranck, Use of detergents in two-dimensional crystallization of membrane proteins, *Biochim. Biophys. Acta* 1508 (2000) 112–128.
- [113] M.T. Paternostre, M. Roux, J.L. Rigaud, Mechanisms of membrane-protein insertion into liposomes during reconstitution procedures involving the use of detergents. 1. Solubilization of large unilamellar liposomes (prepared by reverse-phase evaporation) by Triton X-100, octyl glucoside, and sodium cholate, *Biochemistry* 27 (1988) 2668–2677.
- [114] D. Lichtenberg, R.J. Robson, E.A. Dennis, Solubilization of phospholipids by detergents – structural and kinetic aspects, *Biochim. Biophys. Acta* 737 (1983) 285–304.
- [115] M.L. Jackson, B.J. Litman, Rhodopsin phospholipid reconstitution by dialysis removal of octyl glucoside, *Biochemistry* 21 (1982) 5601–5608.
- [116] M.L. Jackson, B.J. Litman, Rhodopsin-egg phosphatidylcholine reconstitution by an octyl glucoside dilution procedure, *Biochim. Biophys. Acta* 812 (1985) 369–376.
- [117] L. Niu, J.M. Kim, H.G. Khorana, Structure and function in rhodopsin: asymmetric reconstitution of rhodopsin in liposomes, *Proc. Natl. Acad. Sci. U.S.A.* 99 (2002) 13409–13412.
- [118] Z. Salamon, Y. Wang, M.F. Brown, H.A. Macleod, G. Tollin, Conformational-changes in rhodopsin probed by surface-plasmon resonance spectroscopy, *Biochemistry* 33 (1994) 13706–13711.
- [119] K. Fahmy, T.P. Sakmar, F. Siebert, Transducin-dependent protonation of glutamic acid 134 in rhodopsin, *Biochemistry* 39 (2000) 10607–10612.
- [120] I. Szundi, T.L. Mah, J.W. Lewis, S. Jäger, O.P. Ernst, K.P. Hofmann, D.S. Kliger, Proton transfer reactions linked to rhodopsin activation, *Biochemistry* 37 (1998) 14237–14244.
- [121] S. Dickopf, T. Mielke, M.P. Heyn, Kinetics of the light-induced proton translocation associated with the pH-dependent formation of the metarhodopsin I/II equilibrium of bovine rhodopsin, *Biochemistry* 37 (1998) 16888–16897.

- [122] S.K. Gibson, J.H. Parkes, P.A. Liebman, Phosphorylation alters the pH-dependent active state equilibrium of rhodopsin by modulating the membrane surface potential, *Biochemistry* 38 (1999) 11103–11114.
- [123] J.H. Parkes, S.K. Gibson, P.A. Liebman, Temperature and pH dependence of the metarhodopsin I-metarhodopsin II equilibrium and the binding of metarhodopsin II to G protein in rod disk membranes, *Biochemistry* 38 (1999) 6862–6878.
- [124] Y. Wang, A.V. Botelho, G.V. Martinez, M.F. Brown, Electrostatic properties of membrane lipids coupled to metarhodopsin II formation in visual transduction, *J. Am. Chem. Soc.* 124 (2002) 7690–7701.
- [125] K. Beyer, T. Huber, Mixed micelle formation between gramicidin-S and a nonionic detergent: a nuclear magnetic resonance model study of peptide/detergent aggregation, *Eur. Biophys. J. Biophys. Lett.* 28 (1999) 166–173.
- [126] V. Kurze, B. Steinbauer, T. Huber, K. Beyer, A <sup>2</sup>H NMR study of macroscopically aligned bilayer membranes containing interfacial hydroxyl residues, *Biophys. J.* 78 (2000) 2441–2451.
- [127] K. Fahmy, F. Jäger, M. Beck, T.A. Zvyaga, T.P. Sakmar, F. Siebert, Protonation states of membrane-embedded carboxylic-acid groups in rhodopsin and metarhodopsin-II – a Fourier-transform infrared-spectroscopy study of site-directed mutants, *Proc. Natl. Acad. Sci. U.S.A.* 90 (1993) 10206–10210.
- [128] P. Rath, L.L.J. Decaluwe, P.H.M. Boveegeurts, W.J. Degrip, K.J. Rothschild, Fourier-transform infrared difference spectroscopy of rhodopsin mutants – light activation of rhodopsin causes hydrogen-bonding change in residue aspartic acid-83 during Meta-II formation, *Biochemistry* 32 (1993) 10277–10282.
- [129] S.H. White, W.C. Wimley, Membrane protein folding and stability: physical principles, *Annu. Rev. Biophys. Biomolec. Struct.* 28 (1999) 319–365.
- [130] A.G. Krishna, S.T. Menon, T.J. Terry, T.P. Sakmar, Evidence that helix 8 of rhodopsin acts as a membrane-dependent conformational switch, *Biochemistry* 41 (2002) 8298–8309.
- [131] T. Huber, M. Klingenberg, K. Beyer, Binding of nucleotides by the mitochondrial ADP/ATP carrier as studied by <sup>1</sup>H nuclear magnetic resonance spectroscopy, *Biochemistry* 38 (1999) 762–769.
- [132] S.K. Gibson, J.H. Parkes, P.A. Liebman, Phosphorylation modulates the affinity of light-activated rhodopsin for G protein and arrestin, *Biochemistry* 39 (2000) 5738–5749.
- [133] J.A. Hirsch, C. Schubert, V.V. Gurevich, P.B. Sigler, The 2.8 angstrom crystal structure of visual arrestin: A model for arrestin's regulation, *Cell* 97 (1999) 257–269.
- [134] D.E. Clapham, E.J. Neer, G protein beta gamma subunits, *Annu. Rev. Pharmacol. Toxicol.* 37 (1997) 167–203.
- [135] H.R. Seitz, M. Heck, K.P. Hofmann, T. Alt, J. Pellaud, A. Seelig, Molecular determinants of the reversible membrane anchorage of the G-protein transducin, *Biochemistry* 38 (1999) 7950–7960.
- [136] T.J. Melia, J.A. Malinski, F. He, T.G. Wensel, Enhancement of phototransduction protein interactions by lipid surfaces, *J. Biol. Chem.* 275 (2000) 3535–3542.
- [137] M. Sokolov, A.L. Lyubarsky, K.J. Strissel, A.B. Savchenko, V.I. Govardovskii, E.N. Pugh, V.Y. Arshavsky, Massive light-driven translocation of transducin between the two major compartments of rod cells: A novel mechanism of light adaptation, *Neuron* 34 (2002) 95–106.
- [138] P.B. Wedegaertner, P.T. Wilson, H.R. Bourne, Lipid modifications of trimeric G-proteins, *J. Biol. Chem.* 270 (1995) 503–506.
- [139] K. Seno, M. Kishimoto, M. Abe, Y. Higuchi, M. Mieda, Y. Owada, W. Yoshiyama, H. Liu, F. Hayashi, Light- and guanosine 5'-3-O-(thio)triphosphate-sensitive localization of a G protein and its effector on detergent-resistant membrane rafts in rod photoreceptor outer segments, *J. Biol. Chem.* 276 (2001) 20813–20816.

- [140] K.S. Nair, N. Balasubramanian, V.Z. Slepak, Signal-dependent translocation of transducin, RGS9-1-G beta 5L complex, and arrestin to detergent-resistant membrane rafts in photoreceptors, *Curr. Biol.* 12 (2002) 421–425.
- [141] S.L. Niu, D.C. Mitchell, B.J. Litman, Optimization of receptor-G protein coupling by bilayer lipid composition II – Formation of metarhodopsin II-transducin complex, *J. Biol. Chem.* 276 (2001) 42807–42811.
- [142] D.C. Mitchell, S.L. Niu, B.J. Litman, Optimization of receptor-G protein coupling by bilayer lipid composition I – kinetics of rhodopsin–transducin binding, *J. Biol. Chem.* 276 (2001) 42801–42806.
- [143] P.A. Liebman, A.V. Botelho, M.F. Brown, J.H. Parkes, Membrane surface charge control of transducin activity on rhodopsin reconstituted membranes, *Biophys. J.* 80 (2001) 244.
- [144] K.D. Ridge, N.G. Abdulaev, M. Sousa, K. Palczewski, Phototransduction: crystal clear, *Trends Biochem. Sci.* 28 (2003) 479–487.