Russian Chemical Reviews **81** (11) 1071 – 1090 (2012) © 2012 Russian Academy of Sciences and Turpion Ltd

DOI 10.1070/RC2012v081n11ABEH004309

Chemistry and molecular physiology of vision: light-sensitive protein rhodopsin

M A Ostrovsky, T B Feldman

Contents

I. Introduction 1071
II. Rhodopsin structure 1072
III. Molecular physiology of rhodopsin 1076

Abstract. The review concerns literature data on the structure, functions and molecular simulation of rhodopsin. The mechanisms of the spectral tuning of visual pigments, photoisomerization of the rhodopsin chromophore group (11-*cis*-retinal moiety) and the formation of the physiologically active state of rhodopsin as a G-protein-coupled receptor are considered. Particular attention is given to the rhodopsin visual cycle and pathogenetic consequences of its impairment. A knowledge of the structure and photochemistry of rhodopsin was shown to be of importance for the use in nanotechnology of the future. The bibliography includes 219 references.

I. Introduction

During 600 million years of the animal evolution, light, as a source of information, gave rise to the organs of vision, their development and improvement. This culminated in an eye and vision systems in vertebrates and invertebrates. It should be noted that Charles Darwin [1] himself wrote in his classical work 'On the Origin of Species' that the evolution of the eye by natural selection at first glance seemed 'absurd in the highest possible degree'.

In recent years, publications, including excellent reviews by Lamb *et al.*,[2–4] have been reported on the evolution of light-sensitive visual proteins (visual pigments), invertebrate and vertebrate photoreceptor cells, the retina and the eye as the organ of vision. The available data show that Darwin's theory is completely true for an understanding of the evolution of an eye and its photoreceptor apparatus.

The light-sensitive photoinformation protein rhodopsin appeared in eukaryotic cells of simplest multicellular representatives of the animal kingdom about 1 billion years ago. Later on these multicellular organisms were divided into two groups exhibiting radial and bilateral symmetry of the body plans, respectively. In turn, bilateral organisms were separated into two fundamentally important groups about 600 million years ago. One of these groups gave origin to most of the available invertebrates, whereas another group, to vertebrates, whose descendants are highest vertebrates, including primates and humans. In the period dating from 540 to 490 million years ago, at the Cambrian explosion, a large number of new animal species appeared, which were characterized by a unprecedented diversity of organs of vision, including the human eye. Since that period, the organs of vision were divided into two main types: the complex eye in most of invertebrates (for example, in arthropods) and the camera eye in vertebrates.

The complex eye in invertebrates is composed of numerous unit eyes (ommatidia) containing own photoreceptor cells (rhabdoms). It should be noted that cephalopod molluscs (octopus, squids) have the camera eye, like vertebrates, but their photoreceptor cells are the same as those in arthropods (rhabdomers). In the vertebrate camera eye, the retina contains non-rhabdomeric-type photoreceptor cells originating from cilium. In most of vertebrates, including primates and humans, there are two types of photoreceptor cells: rods responsible for highly sensitive twilight vision and evolutionary more ancient cones responsible for photopic and colour vision. However, different types of organs of vision and all photoreceptor cells, from light-sensitive cells in simplest multicellular organisms to photoreceptor cells of the rhamdomeric or ciliary (rods and cones) types in highly organized invertebrates and vertebrates, contain principally the same light-sensitive molecule, the transmembrane protein rhodopsin incorporated into a bilayer photoreceptor membrane.

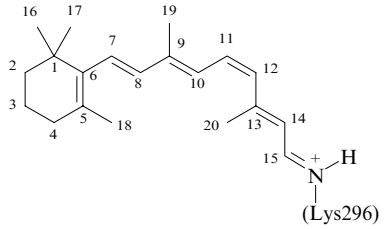
The word 'rhodopsin' comes from two Greek words: 'rhodo' meaning rose and 'opsis' meaning vision. Until recently (late 1950s – early 1960s), this protein was called

M A Ostrovsky, T B Feldman N M Emanuel Institute of Biochemical Physics, Russian Academy of Sciences, ul. Kosygina 4, 119334 Moscow, Russian Federation. Fax (7-499) 137 41 01, Department of Biology, M V Lomonosov Moscow State University, Leninskie Gory 1, 119991 Moscow, Russian Federation. Fax (7-495) 939 43 09, tel. (7-499) 135 70 73, (7-495) 137 41 01,
e-mail: ostrovsky@sky.chph.ras.ru (M A Ostrovsky), feldman@sky.chph.ras.ru (T B Feldman)

Received 12 March 2012
Uspekhi Khimii **81** (11) 1071 – 1090 (2012); translated by T N Safonova

'visual purple', and earlier (after its discovery by Franz Boll in 1876) it was referred to as the 'visual substance' (Sehestoff). As for the evolution of the visual protein, it is evident that rhodopsin is one of the most ancient proteins in the animal kingdom responsible for the photoreception (photoinformation process). The unique photochemical and spectral properties of rhodopsin are determined by one of the most conserved domains of the molecule (the chromophore site), in which the 11-*cis*-isomer of retinal interacts with the nearest protein environment. The main physiological function of the site is to trigger the phototransduction, *i.e.*, to transform the energy of an absorbed quantum of light to the electrical signal in the visual cell (rhabdomeric or ciliary), which is provided by other, also exceptionally conserved, domains of the rhodopsin molecule as well.

Rhodopsin (the molecular weight is 40 kDa) consists of the opsin apoprotein (348 amino acid residues), in which the nitrogen atom of Lys296 is covalently bound to the 11-*cis*-retinal moiety. This chromophore group is a protonated Schiff base (PSB).†



The molar extinction coefficient of rhodopsin is 40 600 litre mol^{-1} cm^{-1} at 500 nm.

The visual pigment rhodopsin is responsible for the photoreception (photoinformation process) and is a typical representative of light-sensitive retinal-containing proteins. This family includes also proteorhodopsin, which is a bacterial rhodopsin, discovered recently in dinoflagellate protists,[5] sensor rhodopsins, halorhodopsin and bacteriorhodopsin from halophilic archaea (see reviews [6, 7]). These retinal-containing proteins are actually among the most ancient proteins found across the biosphere. Bacteriorhodopsin responsible for the anoxygenic photosynthesis (photoenergetic process) appeared in prokaryotic cells about 3.5 billion years ago.[2] Its chromophore-binding domain contains the all-*trans*-retinal moiety covalently bound to opsin. It should be noted that only the 11-*cis*-retinal moiety is involved in virtually all visual pigments. This fact emphasizes that the chromophore-binding domain of retinal-containing proteins is conserved and perfect.

The uniquely arranged chromophore site, which formed in the earliest stages of the evolution of the biosphere, provides the exceptionally high rate and efficiency of the photochemical reaction. Both in vertebrate rhodopsin (for example, bovine rhodopsin)[8] and invertebrates rhodopsin (octopus),[9] the photoisomerization of the retinal chromophore occurs after the absorption of a quantum of light during about 100 fs. The kinetics and efficiency of the photoisomerization of the chromophore in retinal-containing proteins are comparable with the theoretically calculated isomerization rate of retinal in the gas phase.[10] In other words, the isomerization rate of the double bond of retinal in the tight protein environment (the volume of the chromophore site in rhodopsin, into which 11-*cis*-retinal is incorporated, is as small as 660 $Å^3$)[11] is comparable with the rate of isomerization of retinal in the free volume. This surprising phenomenon is indicative of the ideal structure of the chromophore site and the efficient interaction between the chromophore and its nearest protein environment.

Visual rhodopsin is simultaneously the classical class A G-protein-coupled receptor. The typical eukaryotic genome contains genes of more than 800 such receptors structurally similar to rhodopsin. They play a key role in the transduction of intra- and extracellular signals (information), including hormone, nervous and sensor (vision, taste, smell) systems. Investigations of the mechanisms of the transition of rhodopsin from the inactive (dark) state to the physiologically active state provide an insight into the molecular mechanisms of activation of the whole class of G-protein-coupled receptors (see, for example, one of recent reviews [12]).

II. Rhodopsin structure

Rhodopsin is the first transmembrane protein of animal origin whose two-dimensional (Fig. 1)[13, 14] and three-dimensional[15] structures (Fig. 2) were determined. The rhodopsin molecule is composed of several domains: a hydrophobic transmembrane domain consisting of seven α-helices, which form a bundle and traverse the photoreceptor membrane, and two hydrophilic domains located on both sides of the membrane, *viz.*, cytoplasmic and intradiscal (extracellular).

In the dark state of rhodopsin, the 11-*cis*-retinal moiety has a nonplanar (distorted and twisted) conformation.[16, 17] By contrast, the three-dimensional structure of PSB and free 11-*cis*-retinal in solution is virtually planar.[18] The important role of the protein environment in the formation of the energetically unfavourable nonplanar configuration of the chromophore in the visual rhodopsin molecule is beyond doubt (see the review [19]).

The three-dimensional organization of crystalline rhodopsin and its chromophore site was studied in sufficient detail by X-ray crystallography.[15, 20] However, it should be remembered that X-ray crystallography gives a static picture of the three-dimensional organization of the protein molecule. In the crystalline state, some noncovalent bonds (hydrogen, van-der-Waals, electrostatic), which play an important role in the functioning of the visual pigment, can be broken.

First X-ray diffraction studies were followed by a series of theoretical works (quantum mechanical calculations, molecular simulation), where attempts were made to describe the rhodopsin molecule in the dark state and after absorption of a quantum of light.[21–28]

The chromophore site, *i.e.*, the three-dimensional arrangement of the 11-*cis*-retinal moiety and its interaction with the nearest amino acid residues, was described in sufficient detail.[17, 20, 21, 29–31] This information is important for an understanding of the mechanisms of the spectral tuning of visual pigments and for an explanation of their unique photochemical and physiological properties.

In the analysis of the chromophore site, particular attention is given to the following two regions of 11-*cis*-retinal: the β-ionone ring and the covalent bond with

† The notation N–(Lys296) means that the bond is formed by the nitrogen atom involved in the corresponding amino acid.

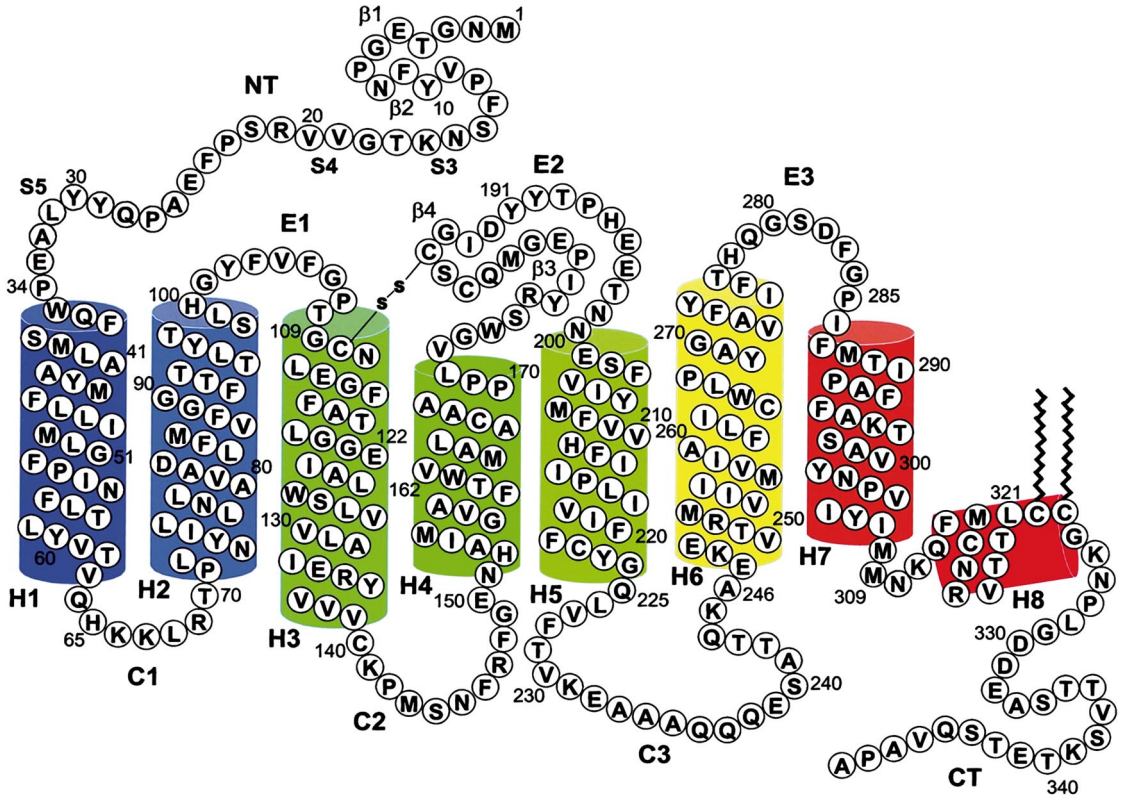


Figure 1. Two-dimensional structure of rhodopsin.[13]

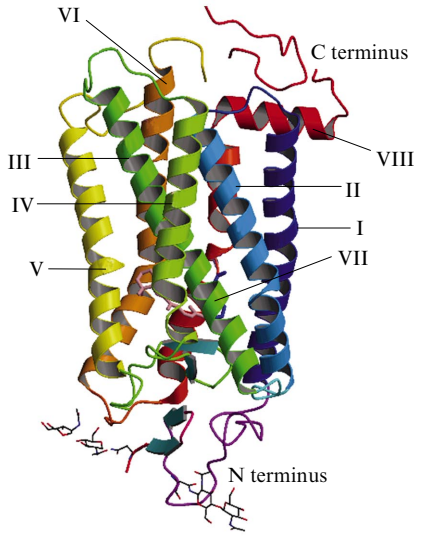


Figure 2. Three-dimensional structure of rhodopsin.[15]

Lys296. Since the photoinduced dynamics of the β-ionone ring is thought to have an important role in the initiation of subsequent conformational rearrangements in the protein part of the molecule,[12, 30, 32–35] investigations of the conformation of this ring with respect to the polyene chain and its interactions with the nearest amino acid residues in the dark and photoactivated states of the visual pigment molecule, are topical problems.

There is currently no consensus on the three-dimensional conformation of the β-ionone ring (6-*s*-*cis* or 6-*s*-*trans*) with respect to the polyene chain in 11-*cis*-retinal.[17, 36, 37] According to the X-ray diffraction data, the β-ionone ring adopts the 6-*s*-*cis* conformation.[20] However, the ^{1}H NMR spectroscopic studies showed that this part of the molecule has different orientations: 6-*s*-*trans*,[37, 38] 6-*s*-*cis*[39–41] and a mixture of 6-*s*-*cis* (74%) and 6-*s*-*trans* (26%).[36] It was hypothesized[42] that the chromophore site can contain both these forms, but the 6-*s*-*cis* conformation is energetically more favourable.

The role of the nonplanar conformation of the chromophore in the unique photochemical properties of the visual pigment remains an open question. It was suggested[16, 43–45] that this conformation of 11-*cis*-retinal in rhodopsin is attributed to the rotation of the β-ionone ring with respect to the plane of the polyene chain by approximately 50–65°.

By using molecular dynamics methods, we showed that the 11-*cis*-retinal moiety and its nearest protein environment are mutually adjusted to each other.[43–45] A change in the three-dimensional conformation of 11-*cis*-retinal, in particular, the rotation of the β-ionone ring around the C(6)–C(7) bond by ~60° with respect to the initial conformation was already observed within 300–400 ps after the beginning of the simulation (Fig. 3). This is in good agreement with both the theoretical calculations performed by other authors[21] and the above-mentioned experimental data.[16] Moreover, the rotation of the β-ionone ring with respect to the plane of the polyene chain can occur, according to the results of the theoretical study,[46] in opposite directions to form two enantiomers of 6-s-*cis*-11-*cis*-retinal.

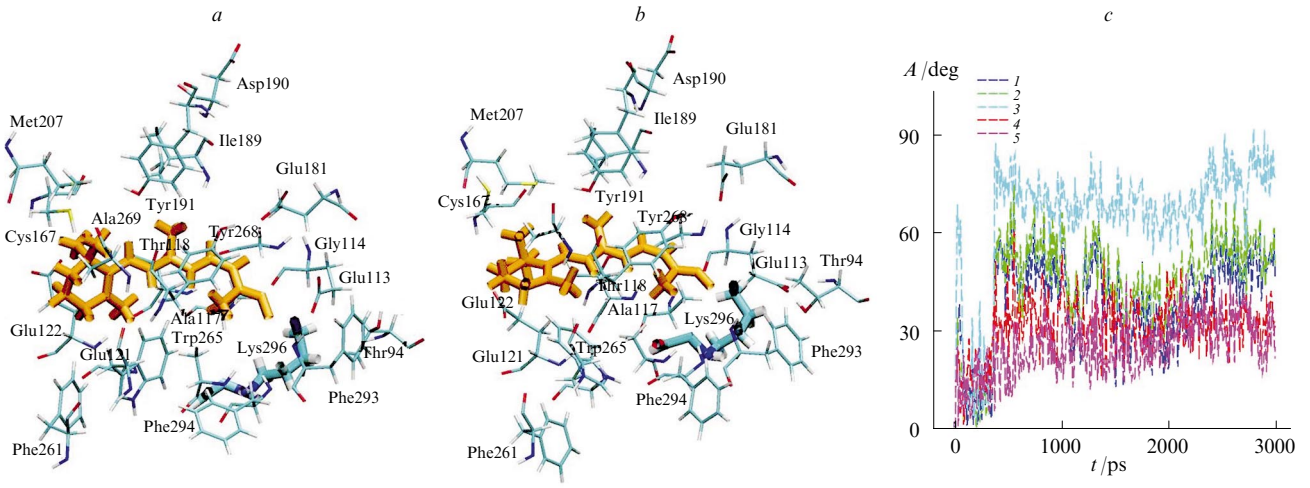


Figure 3. Configuration of the rhodopsin chromophore at the initial moment of the simulation (*a*) and after 3000 ps (*b*) calculated by the molecular dynamics method and the diagrams of changes in the torsion angles (*A*) during rotations for 3000 ps of five methyl groups of 11-*cis*-retinal at positions 16 (*1*), 17 (*2*), 18 (*3*), 19 (*4*) and 20 (*5*) (*c*).[43]

According to *ab initio* quantum chemical calculations,[47] this conformation of the β-ionone ring is characterized by a shift of the absorption band of rhodopsin to longer wavelengths by 10 nm. This shift corresponds to the electronic transition $S_0 \rightarrow S_1$ between the ground and first excited singlet states of the chromophore.[45] To put it differently, the rotation of the β-ionone ring results in the transition of rhodopsin into the 'photochemically prepared' state, because the energy of the transition of this system into the photoexcited singlet S_1 state is lower compared to the structure in which the β-ionone ring is in the plane of the polyene chain.

In addition to the rotation of the β-ionone ring, the middle part of the polyene chain [the C(10)–C(13) atoms] contribute substantially to the formation of the twisted strained conformation of the 11-*cis*-retinal moiety.[43, 48–50]

The questions as to how and in which three-dimensional conformation the 11-*cis*-retinal moiety is incorporated into the opsin chromophore site in the course of regeneration of the visual pigment after its photobleaching remain unclear. Undoubtedly, the conformation of 11-*cis*-retinal delivered to free opsin by the retinal-transferring protein differs from that of the rhodopsin chromophore group.

It should be noted that the chromophore site in the dark state of rhodopsin is not a specific binding site exclusively for 11-*cis*-retinal. This site can incorporate also other its isomers (for example, in the case of 9-*cis*-retinal, the so-called isorhodopsin, which was not found in natural visual pigments, is formed [51]) and its modifications.[52] Apparently, the conformation of 11-*cis*-retinal incorporated into the chromophore site is nearly planar and its subsequent interaction with the surrounding amino acid residues results in the rotation of the β-ionone ring and the twisting of the polyene chain. In this energetically unfavourable conformation, the 11-*cis*-retinal moiety, on the one hand, acts as the chromophore and gets rhodopsin ready for the highly efficient photochemical reaction, and, on the other hand, acts as an inverse agonist and stabilizes rhodopsin as the G-protein-coupled receptor in its physiologically inactive state.

Noncovalent bonds of the β-ionone ring with the nearest aromatic residues (Trp265, Phe261 and Tyr268) play a very important role in the properties of 11-*cis*-retinal as the inverse agonist.[15, 17, 20, 29] The above-mentioned interaction restricts the mobility of the transmembrane α-helix VI, thus preventing the spontaneous activation of rhodopsin in the dark.[29] (See below for the key role of this α-helix in the mechanism of physiological activation of rhodopsin.) At the same time, this aromatic cluster plays an important role in the retention of the twisted strained conformation of 11-*cis*-retinal in the dark inactive state of rhodopsin.[21, 29, 36, 40]

The interactions between the β-ionone ring and the amino acid residues of the aromatic cluster and Leu266 C were described by molecular dynamics simulations.[43, 44] The torsional vibrations relative to the C(6)–C(7) single bond lead to the rotation of the β-ionone ring by $\sim 60^\circ$ with respect to the plane of the polyene chain, resulting in its steric interaction with Leu266 (the shortest distance between these groups is about 3.5 Å) (Fig. 4). This arrangement is fixed by the aromatic rings of Trp265 and Tyr268 in such a way that the β-ionone ring is clamped between these rings, all three rings being located in parallel planes one above another at rather short distances (4–5 Å) (Fig. 5). This may be indicative of the presence of strong electrostatic interactions between the β-ionone ring, Trp265 and Tyr268. This mutual arrangement of Trp265 and the β-ionone ring was also confirmed by the results of the molecular simulation.[53]

Therefore, on the one hand, 11-*cis*-retinal acts as an inverse agonist and stabilizes the α-helix VI and, on the other hand, the amino acid residues of the aromatic cluster fix the chromophore in the twister nonplanar conformation.

Investigations of the dynamics of 11-*cis*-retinal and the surrounding amino acid residues in the vicinity of the iminium bond are also of great importance. Particular attention was given to the residue Glu113 as the main PSB counterion.[54–56] The role of Glu113 in the stabilization of the inactive state of rhodopsin in the dark, the increase in the efficiency of its regeneration, the spectral tuning and the regulation of the lifetime of intermediate photolysis products were the subjects of several studies.[57–59]

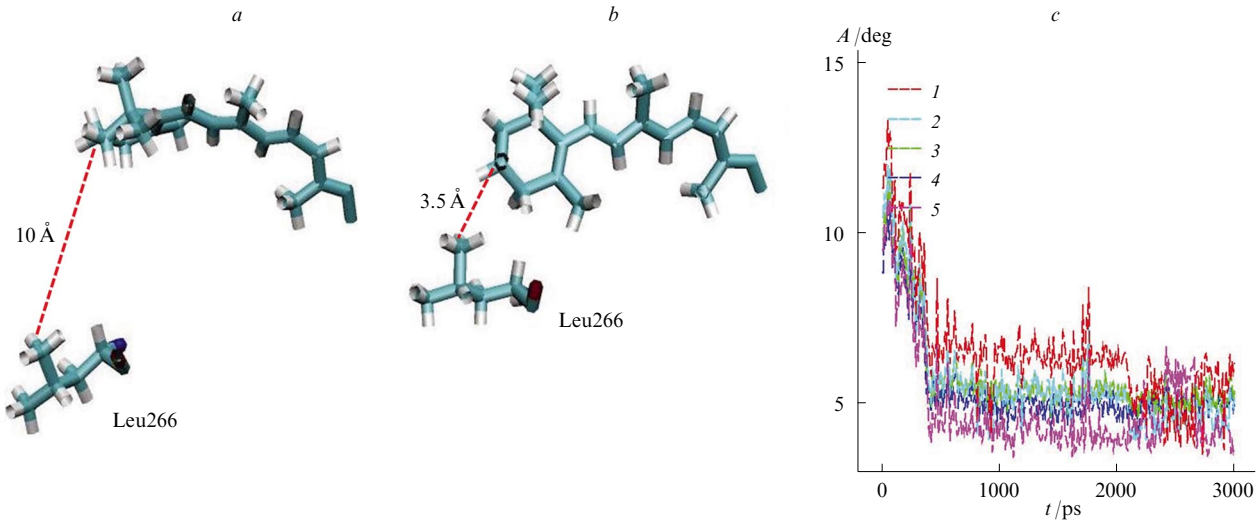


Figure 4. Relative arrangement of 11-*cis*-retinal and Leu266 in the rhodopsin molecule at the initial moment of the simulation (*a*) and after 3000 ps (*b*) calculated by the molecular dynamics method and the diagrams of changes in the interatomic distances (*D*) between the C(3) atom of the β-ionone ring of 11-*cis*-retinal and the atoms of Leu266 in positions δ_1 (*1*), γ (*2*), β (*3*), α (*4*) and δ_2 (*5*).[43]

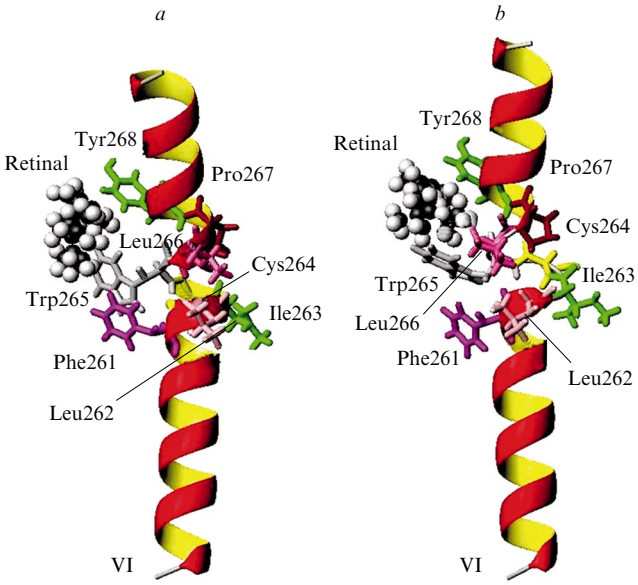


Figure 5. Configuration of the α-helix VI and its amino acid residues located in the vicinity of 11-*cis*-retinal at the initial moment of the simulation (*a*) and after 3000 ps (*b*) calculated by the molecular dynamics method.[43] The atoms of the chromophore are represented as beads and the amino acid residues are shown as a stick model.

Until recently, it was commonly recognized that the negatively charged residue Glu113 in vertebrate rhodopsin is the main and the only PSB counterion (see, for example, Ref. 13). However, according to X-ray crystallographic data, the chromophore site involves the second Glu in position 181 in the direct vicinity of the 11-*cis*-retinal moiety, and this has caused a great interest in the role of this amino acid in the mechanisms of stabilization of PSB and the activation of rhodopsin as the G-protein-coupled receptor.[42,60–62] At present, several models (so-called 'counterion switch' models) describing the contribution of Glu181 to these processes are actively debated.

It was suggested[63] that Glu113 is the main PSB counterion in dark-state rhodopsin, whereas Glu181 becomes a counterion in the step of the formation of metarhodopsin I as a result of the proton transfer from Glu181 to Glu113 through a hydrogen bond network with the participation of Ser186 and water molecules. It was hypothesized[53,64] that Glu113 and Glu181 are simultaneously involved in the stabilization of PSB. Based on theoretical calculations,[65] it was suggested that Glu181 in the dark state of rhodopsin makes an even greater Ser186-mediated contribution to stabilization of PSB than Glu113.

Until recently, the question about the counterion in invertebrate rhodopsins remained open because these proteins do not contain a negatively charged amino acid in position 113 analogous to Glu113. It was hypothesized that it is this difference that is responsible for the different mechanisms of photoconversions of visual pigments in vertebrates (photolysis) and invertebrates (closed cycle).[66]

Recently, a Japanese research group showed[67–69] that Glu181 serves as the PSB counterion in invertebrates. It should be noted that this residue is exceptionally conserved in all opsins, as opposed to Glu113, which is conserved only in the vertebrate visual pigment.[67] Based on these data, it was hypothesized that Glu181 initially served as the counterion but, in the course of the evolution, this role was transferred to Glu113, which appeared in vertebrates as a result of the mutagenesis. Apparently, the residue Glu113 that appeared in vertebrate rhodopsin accepts a proton from the nitrogen atom of PSB in the step of the formation of metarhodopsin II, thus facilitating the hydrolysis of this bond and the release of *trans*-retinal from opsin (see below).

The question as to why retinal in the 11-*cis*-configuration was selected in the earliest stages of the photochemical evolution of light-sensitive visual proteins as the chromophore in the whole family is very interesting and has been long debated. One of the latest attempts to answer this question has been made using a complex method combining quantum mechanical and molecular dynamics calcula-

tions.[70] The structures, stability, energy and absorption spectra of the 7-*cis*-, 9-*cis*-, 11-*cis*- and 13-*cis* isomers of the retinal moiety in vertebrate rhodopsins (bovine, monkey) and invertebrate rhodopsin (squid) were analyzed in detail. From this study it follows that the optimization of the electrostatic interaction between retinal and its nearest protein environment in the dark state of rhodopsin is one of the key factors in the course of natural selection, although the selection of this isomer was, evidently, determined by a number of other factors as well. Among these factors are the efficient enzymatic synthesis of 11-*cis*-retinal catalyzed by the protein RPE65 (retinol isomerase) in retinal pigment epithelium cells, the rates of the binding of 11-*cis*-retinal to opsin and its incorporation into the chromophore site in the course of the regeneration of rhodopsin after its bleaching, stability of dark-state rhodopsin at physiological temperature, the efficiency of 11-*cis*-retinal as an inverse agonist and the quantum yield and stereoselectivity of the superfast photoisomerization with its participation.

III. Molecular physiology of rhodopsin

The visual pigment rhodopsin is involved in several physiological functions in the course of photoreception, where the chromophore – protein interaction plays a key role. Firstly, this is the spectral tuning of visual pigments. The latter can absorb light in a wide spectral range. from ultraviolet to red (from 360 to 620 nm). The spectral tuning provides colour vision.

Secondly, rhodopsin triggers the visual phototransduction event. This process is determined by the following two key intramolecular events: the superfast photoisomerization of the retinal chromophore and, as a consequence, the conformational rearrangement of the protein part of the opsin molecule resulting in the binding and activation of the G-protein transducin.

Thirdly, rhodopsin maintains low thermal dark noise of the visual cell (rod). In the dark, the chromophore locks rhodopsin as the G-protein-coupled receptor in the inactive state, *i.e.*, the 11-*cis*-retinal moiety acts as an efficient inverse agonist. After the absorption of a quantum of light, the chromophore locks the relatively long-lived product metarhodopsin II (see below) in the physiologically active state. In this case, the all-*trans*-retinal moiety acts as a powerful agonist.

Finally, the rhodopsin molecule or the so-called rhodopsin visual cycle has a pathogenic role (more than hundred mutations were described). In particular, the accumulation of (photo)toxic *trans*-retinal, which is released in the last step of the photolysis of rhodopsin, in the photoreceptor membrane is associated with the potentially harmful damaging effect of light on the retina and retinal pigment epithelium (RPE), as well as with the development of retinal degenerative diseases, primarily, the Stargardt disease and age-related macular degeneration.[71, 72]

1. Spectral tuning of visual pigments

The spectral tuning (different positions of the maximum in the absorption spectrum) of visual pigments is possible on two time scales: long-term evolutionary and relatively short-term adaptation (physiological) time scales. The evolutionary tuning is provided by amino acid mutations in the opsin chromophore site around the chromophore group, *viz.*, the 11-*cis*-retinal or 11-*cis*-3,4-dehydroretinal moiety, and its interaction with the counterion complex. The adaptation tuning, either seasonal or light habitat-dependent, is determined by the replacement of the chromophore group, 11-*cis*-retinal (vitamin A_1 aldehyde), by 11-*cis*-3,4-dehydroretinal (vitamin A_2 aldehyde) (see reviews [59, 73, 74]).

The visual pigment of two populations of the opossum shrimp *Mysis relicta* (sea and lake), which became separated at the end of the last glacial period (*ca.* 9000 years ago), is an example of the light habitat-dependent adaptation. These populations live under different illumination conditions and are substantially different in the light and spectral sensitivity. We showed that the absorption maxima of rhodopsin in these shrimps are different: 530 nm for the sea shrimp living under relatively high illumination and 560 nm for the lake shrimp living at great depth.[75 – 78] This difference is most likely associated not with amino acid mutations but with the replacement of 11-*cis*-retinal with 11-*cis*-3,4-dehydroretinal.

The evolutionary tuning is of principal importance. In the course of the evolution, five classes of visual pigments formed in vertebrate retinal photoreceptor cells. These are one type of rod pigments and four types of cone pigments. The absorption maximum of rod pigments (rhodopsin) in almost all ground-dwelling vertebrates is observed at 500 nm. The spectral tuning of cone pigments is much more diverse. There are one long-wavelength-sensitive (with an absorption maximum at 500 – 570 nm), one medium-wavelength-sensitive (480 – 530 nm) and two short-wavelength-sensitive (400 – 470 and 355 – 445 nm) classes. The diversity of cone pigments lost in mammals is restored in the course of the evolution only in primates and humans, thus providing colour vision.

As for the interaction of the chromophore with the protein environment, one amino acid replacement in the chromophore site is sufficient to provide a shift of the absorption spectrum of the short-wavelength-sensitive cone pigment from the ultraviolet to violet-green region of the spectrum (see reviews [73, 74]). In the case of the spectral tuning of long-wavelength-sensitive cone pigments, several amino acid mutations, including anion-binding sites, are required. These are chlorine-binding amino acid residues in red cones in reptiles, birds and mammals.[73, 74, 79 – 81] Our experiments in the isolated goldfish retina showed that the removal of chloride ions not only shifts the absorption maximum of red-sensitive cones to shorter wavelengths (green region) by $\sim$30 nm but also leads to a decrease or even the disappearance of their electrical response (so-called late receptor potential) to red light flash.[81]

Data on the role of water molecules in the spectral tuning of cone visual pigments are gradually accumulated. In this respect, at least two facts should be noted. First, unlike vertebrate and invertebrate rhodopsins, none of cone visual proteins was isolated in the crystalline state and, consequently, their three-dimensional structures are unknown. Second, although the role of the bound water in the maintenance of the structure and the functioning of vertebrate (bovine) and invertebrate (squid) rhodopsins is rather well known, the corresponding data on cone pigments are scarce.

In rhodopsin, bound water molecules were detected and studied in detail by modern methods, such as X-ray crystallography,[15, 82] Fourier-transform infrared spectroscopy [69, 83] and radioactive labelling.[84, 85]

The recent study[86] of red- and green-sensitive cone pigments in monkey by low-temperature Fourier-transform infrared spectroscopy showed that from 3 to 8 bound water molecules are present in the vicinity of the 11-*cis*-retinal chromophore. In rhodopsin, there are 6 bound water molecules in the vicinity of the chromophore group. In other words, the number of bound water molecules in cone pigments is approximately equal to that in the dark state of cone rhodopsin. One of the observed difference is that the bound water in the vicinity of the chromophore was retained in cone pigments after the isomerization of 11-*cis*-retinal, whereas such water in rhodopsin disappeared. This fact was attributed to the presence of the chlorine-binding site in cone pigments in the vicinity of the carbon atoms C(11) and C(9) of the retinal moiety, whereas this site is absent in rhodopsin. Evidently, investigations of the correlation between the amount, localization and the role of the bound water, on the one hand, and the spectral tuning of cone pigments, on the other hand, should be continued. It is interesting that the mode of water binding in rod and cone visual pigments substantially differs from that in the archaeal retinal-containing proton-pumping protein bacteriorhodopsin, in which water molecules are strongly bound.[87]

In discussing intramolecular mechanisms of the spectral tuning of visual pigments, let us mention a series of recent studies[88–91] performed by Japanese researchers, who used a mixed quantum mechanics/molecular dynamics (QM/MM) approach. In these studies, the chromophore of the molecule was considered in terms of quantum mechanics, whereas the protein environment was treated in terms of molecular mechanics. The results of these calculations showed that the spectral tuning is determined by the interaction of the retinal chromophore with the counterion complex and the protein environment. The change in the structure of the chromophore (the presence of 11-*cis*-retinal or 11-*cis*-3,4-dehydroretinal) has no substantial effect.

Therefore, the following three factors affecting the position of the absorption maximum of the visual pigment were considered:

1) the nature of the chromophore and its configuration;

2) the interaction of the chromophore with the counterion complex;

3) the interaction of the chromophore with the nearest protein environment.

The spectral tuning of the pigment is determined by the two last mentioned factors. The nature of the chromophore and its isomeric configuration are the same in all pigments. Thus, they contain retinal in the 11-*cis* configuration. Consequently, the spectral sensitivity tuning of the visual pigment is one of the most important physiological functions of the retinal chromophore in the chromophore site. This intramolecular tuning provides the adaptation of photoreceptor cells in organs of vision in invertebrates and vertebrates to the light habitat and the formation of the mechanism of colour vision.

2. Photochemistry of rhodopsin

The phototransduction starts with the photoisomerization of the rhodopsin chromophore group. The transformation of the 11-*cis*-retinal chromophore into its *trans* form as the photochemical reaction in vision was discovered by Wald and co-workers[92] back in the 1960s (the Nobel Prize in Physiology or Medicine 1967) and was later studied in detail in the 1990s (see, for example, Ref. 93). However, the details of the mechanism of this important photochemical reaction remain the subject of experimental and theoretical investigations, which have been extensively performed in recent years due to the development of femtosecond spectroscopy.

It should be noted that the barrier to thermal isomerization of the retinal chromophore is exceptionally high (45 kcal mol^{-1}).[94,95] The functional sense of such a high thermal barrier, as well as of the high rate and efficiency of photoisomerization, is, on the one hand, to exclude false events of rhodopsin in the dark and, on the other hand, to use the energy of an absorbed quantum for this photochemical reaction with the minimum possibility of its loss as heat or fluorescence. The question about the nature of dark noise in photoreceptor cells is physiologically important.

It is known that the colourless twilight or night vision is provided by the operation of retinal rods. A rod functions as a counter of individual quanta because it can reliably detect one absorbed quantum of visible light. This ability directly depends on the level of thermal dark noise. The dark noise interfering with the physiological mechanism of excitation of a rod with a single quantum originates from the spontaneous activation of rhodopsin. It should be emphasized that the dark noise of rhodopsin, *i.e.*, the spontaneous transformation of the pigment into the physiologically active state, is exceptionally low. Thus, the isomerization in a rod in the primate retina can occur at 36 °C once in 420 years.[95]

It has been shown[96–98] and then recently confirmed[99] that the thermal isomerization of the 11-*cis*-retinal moiety is a source of dark noise. This isomerization initiates the same physiological signal in a rod as the photoisomerization of the chromophore. The molecular mechanism lying behind the appearance of this noise has remained unclear until recently. The quantitative ratio of the energy of photoactivation of rhodopsin to the position of its absorption maximum confirms that it is the isomerization of the chromophore that is a source of dark noise. The larger the shift of the absorption maximum to longer wavelengths, the higher the level of dark noise in the photoreceptor cell. This explains why there are no pigments in a large family of visual pigments that absorb in the infrared region of the spectrum.[99] To put it differently, rhodopsin, as a visual pigment responsible for twilight vision, acquired unique photochemical properties in the course of the evolution, which enabled the photoreceptor cell to optimize the light sensitivity. These properties are the quantum yield of photoisomerization of retinal, which is high for photobiological processes, and the minimization of its dark thermal isomerization, *i.e.*, the suppression of dark noise.

According to the results of the recent study,[100] a network of intramolecular hydrogen bonds formed by 20 water molecules, the iminium bond in PSB and peptide groups in the transmembrane domain play a key role in the decrease in the probability of the thermal isomerization of 11-*cis*-retinal as a source of dark noise. In other words, a network of intramolecular hydrogen bonds and the perfect steric interaction (adaptation) of the chromophore with its nearest protein environment in the opsin chromophore site are two key factors providing the appearance of the molecular mechanism of twilight rod vision in the course of the evolution, which optimizes the ratio of the high quantum yield of photoisomerization to the low probability of the thermal isomerization. The optimization of this ratio is a key evolutionary factor for the appearance of physiologi-

cally more perfect rhodopsin-containing rods as receptors of twilight vision from evolutionary more ancient, less perfect and less light-sensitive cones, which function as receptors of photopic vision containing cone pigments with different spectral tuning.

The exceptionally high rate and efficiency of the photoisomerization of the retinal chromophore are evidenced by the following facts. Firstly, the reaction has a high quantum yield. Initially, the quantum yield was estimated at 0.67,[101] and the refined value obtained later was 0.65.[102] It should be noted that the quantum yield of the *cis–trans* isomerization of the rhodopsin chromophore group in the tight protein environment is almost an order of magnitude higher than that in the case of free retinal in solution.[103–105] Secondly, the first unstable photolysis product, photorhodopsin, is formed in the ground state within 200 fs.[93, 106–111] Thirdly, a considerable energy of the absorbed quantum (35 kcal mol^{-1}) is accumulated in the first intermediate, bathorhodopsin, which is stable at liquid nitrogen temperature and whose stability is temperature-dependent.[112, 113]

Figure 6 shows the general scheme of the photolysis of rhodopsin. Upon absorption of a quantum of light, the rhodopsin chromophore (11-*cis*-retinal moiety) is isomerized into the all-*trans* form to form photorhodopsin as the first product. Then intermediate products showing characteristic absorption spectra are successively formed in the dark in the course of temperature-dependent conformational rearrangements. The resulting relatively long-lived intermediate, metarhodopsin II, can bind and activate the G-protein. The photolysis of rhodopsin culminates in the hydrolysis of the Schiff base covalent bond and the release of free *trans*-retinal (all-*trans*-**1**).

It should be noted that the first photolysis product of rhodopsin was initially called prime-rhodopsin (prime-Rho),[114] and this name is still used by some authors;[9] however, the name photorhodopsin (photo-Rho), which was suggested approximately at the same time,[115] is more commonly accepted. Although bathorhodopsin is generated from photorhodopsin, the former was discovered and investigated much more earlier.[92] The study of photorhodopsin showed that the chromophore in this form also has a distorted *trans* configuration; however, stability of this intermediate is temperature independent as opposed to bathorhodopsin.[116–119]

The superfast dynamics of the transition of visual rhodopsin to photo- and then bathorhodopsin was studied in the 1990s and the early 2000s.[93, 107–111, 120, 121] The results of these studies showed that the time of the formation of photorhodopsin containing *trans*-retinal in a highly distorted conformation is actually ~200 fs. The more recent study[122] using picosecond time-resolved Raman spectroscopy confirmed that photorhodopsin is converted into bathorhodopsin within a few picoseconds.

All these data, along with some other information, provide evidence that the conical intersection of the potential energy surfaces (PES) of the ground and excited electronic states of retinal (Fig. 7) plays a key role in this exceptionally efficient and stereoselective photoisomerization of the chromophore in the rhodopsin molecule.[123–125] However, up to the very recent time no direct experimental evidence was obtained, because changes in the absorption spectra of the molecule accompanying the transition from

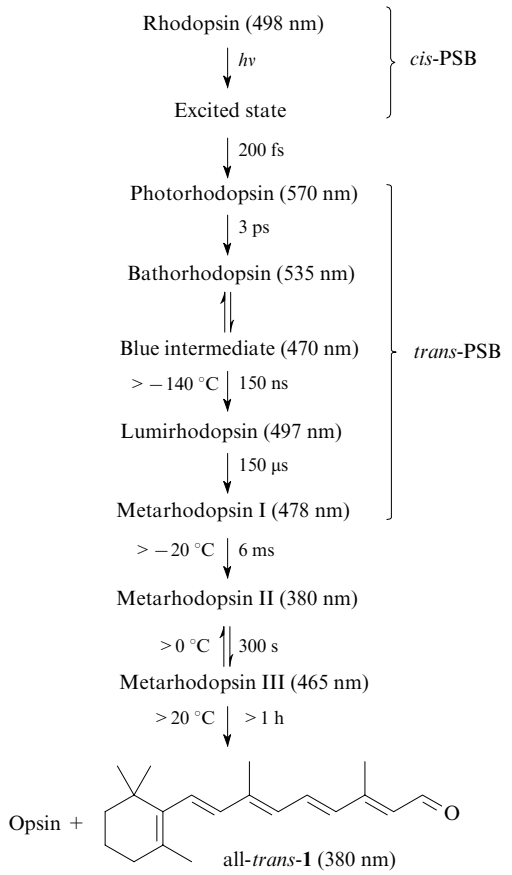


Figure 6. Scheme of the photolysis of vertebrate rhodopsin. The positions of the absorption maxima are given in parentheses; the configuration of the chromophore is given on the right.

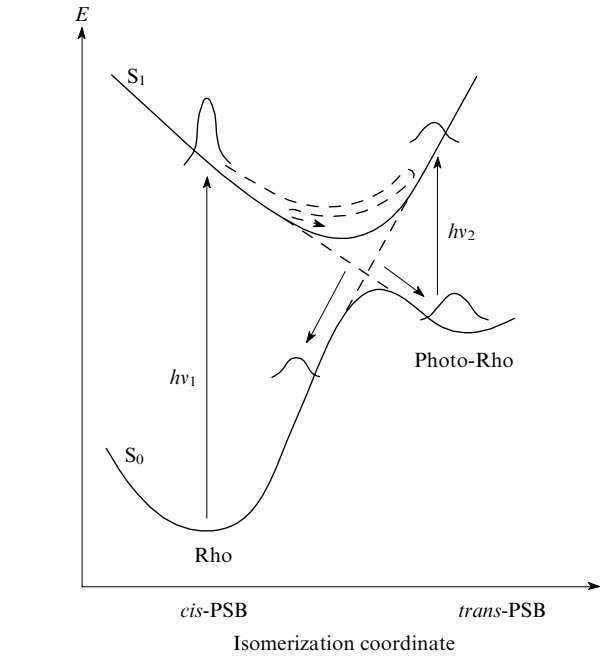


Figure 7. Scheme of the potential energy surfaces of the rhodopsin molecule describing the photoisomerization of 11-*cis*-retinal in the region of the conical intersection.[121]

the excited-state PES to the PES of the products can be observed only by means of superfast high-resolution optical spectroscopy.

The experimental evidence for the existence of the conical intersection point on the PES of the photoisomerization of the 11-*cis*-retinal moiety in the rhodopsin molecule has been recently obtained by the US[8] and Japanese[9] researchers by means of modern femtosecond spectroscopy. It was shown that this point is reached for both vertebrate[8] and invertebrate[9] rhodopsins within $\sim$80 fs, and then, after $\sim$200 fs, ground-state photorhodopsin, in which the chromophore is in a distorted but all-*trans* configuration, is formed. It should be noted that, as opposed to the photoisomerization of retinal in the rhodopsin chromophore site, the conical intersection of the PES for free retinal in solution is reached much more slowly (2.9–3.8 ps).[126]

Recently, we have studied in detail the differential photoinduced absorption spectra of rhodopsin at different delay times, the kinetics of the photoisomerization of 11-*cis*-retinal and the anisotropy of the differential absorption signal using femtosecond laser spectroscopy in polarized light with time resolution of 27 fs.[127] The experimental data allowed us to obtain an alternative estimate of the time during which the conical intersection of PES is reached for the isomerization of the 11-*cis*-retinal moiety in rhodopsin. According to the estimation, this time varies from 60 to 100 fs. To put it differently, the conical intersection of the ground- and excited-state PES of the chromophore in rhodopsin upon photoisomerization is reached within 80 ± 20 fs. Therefore, these data combined with the results of quantum mechanical and molecular simulation calculations indicate that the region of the PES near the conical intersection actually exists and plays an important role in the photochemical reaction.

However, a comparison of the experimental results for vertebrate[8] and invertebrate[9] rhodopsins and their interpretation revealed differences. According to the results obtained by Polli *et al.*,[8] the energy of an absorbed quantum of light is used exclusively for a slight displacement of atoms in a system of the C(9)=C(10)–C(11)=C(12) double bonds. Since the chromophore group is in the tight protein environment,[20] it was suggested that this limits the possibility of the displacement of 11-*cis*-retinal in the region of the iminium bond with the protein and in the region of the β-ionone ring. Once the local displacement of the atoms in the middle of the polyene chain of retinal occurs, the whole highly strained structure of the chromophore rapidly relaxes to form a structure containing the chromophore with an even more pronounced *trans* configuration of the chromophore (photorhodopsin → bathorhodopsin transition). This transition is the main pathway of the photoreaction.[128] It should be noted that there is a side pathway, in which the C(11)=C(12) double bond adopts the starting *cis* configuration after relaxation of the highly strained structure of the chromophore.

It should be noted that the conical intersection of the PES is reached within 80 fs both for octopus visual rhodopsin[9] and bovine rhodopsin,[8] which is almost three times faster than in the case of archaeal rhodopsin, bacteriorhodopsin.[129] This difference can be attributed to both the type of the structural transition of the retinal chromophore in visual (from the 11-*cis*- to all-*trans* configuration) and bacterial (from the all-*trans* to 13-*cis* configuration) rhodopsins and the different structures of the chromophore sites in these two radically different retinal-containing proteins. It is of interest to compare the dynamics of photoisomerization of the retinal chromophore in different retinal-containing proteins of animal and microbial rhodopsins and protozoan rhodopsins.

The time during which the region of the PES near the conical intersection in bovine and octopus rhodopsins is reached is virtually equal to the time predicted by the theoretical simulation of the isomerization of free retinal in the gas phase.[130–132] This fact seems to be surprising because the photoisomerization of the chromophore occurs not in the free, unrestricted space but in the tight protein environment. As mentioned above, the volume of the opsin chromophore site is as small as 660 Å^3, the surface area of the interaction between the 11-*cis*-retinal moiety and the surrounding amino acids is $\sim$230 Å^2 (Ref. 11). Hence, the opsin chromophore site has an ideal arrangement, because the tight protein environment of 11-*cis*-retinal not only does not hinder but even facilitates the superfast and efficient photoinduced change in the geometry of the chromophore. The goal of further investigations is to elucidate how the protein environment of rhodopsin facilitates an increase in the rate and efficiency of the first and the only photochemical reaction in vision.

In this respect, there are at least two promising areas: 1) investigations and comparison of the direct and reverse (photochromic) reactions of rhodopsin; 2) the preparation of rhodopsin genetically modified in the region of the chromophore site and studies of its photoreactions. Investigations in the former area were started in the early 1960s, when Wald and co-workers[92] showed that bathorhodopsin (the *trans* form of the retinal moiety as the chromophore group) can undergo photoconversion back to rhodopsin (11-*cis* isomer) with a small admixture of isorhodopsin (9-*cis* isomer) at liquid nitrogen temperature.

Actually, intermediate photolysis products of rhodopsin are also photoactive. The absorption of a quantum of light by the intermediate can lead to the reverse *trans*–*cis* isomerization of retinal and the regeneration of room-temperature-stable rhodopsin or less stable isorhodopsin. This process is referred to as the visual pigment photoregeneration, the regeneration of the later products (see Fig. 5) to rhodopsin or isorhodopsin being less efficient compared to the early products. Thus, bathorhodopsin is regenerated almost completely,[92] whereas the degree of regeneration of metarhodopsin II is only 45%, as has been shown recently by studying frog retinal rods.[133] It is essential that the reverse reaction does not give intermediates, as evidenced by the presence of an isosbestic point in the absorption spectra recorded at different instants of time.[92, 134]

It should be noted that, as opposed to invertebrates, in which the photoregeneration is a physiological reaction and is necessary for the visual pigment regeneration, the physiological regeneration in vertebrates is a dark process and involves a complex cascade of biochemical reactions. The *in vivo* absorption of the second quantum of light by the visual pigment in vertebrates is virtually reduced to zero.

Nevertheless, the photoregeneration is a good tool for *in vitro* investigations of conformational changes in the visual pigment in different photolysis steps. Studies of the direct and reverse photoreactions at low temperatures allowed Yoshizawa and Wald[92] to propose for the first time the mechanism of the *cis*–*trans* isomerization of the chromo-

phore upon absorption of light by rhodopsin as the primary event of the photoconversion. More recently, this mechanism was confirmed by different spectrophotometric methods [135] and by structural investigations of synthetic analogues of rhodopsin containing the retinal moiety with a fixed structure in the region of the C(11) atom of the polyene chain.[117, 136]

It is investigations of the direct and reverse reactions of the visual pigment, where the isochromism of rhodopsin and its photolysis products was discovered. It was shown that the bleaching of rhodopsin gives two spectrally indistinguishable forms of metarhodopsin I, one of which can undergo photoregeneration, whereas the photoregeneration of another form is impossible.[137] The results of studies of photochromic reactions of rhodopsin at low temperatures showed [134, 138, 139] that, beginning with the step of the formation of metarhodopsin I during the photoregeneration of the visual pigment, two spectrally identical isochromic forms of rhodopsin are generated. One of these forms is stable at room temperature, whereas the other one is not.

In recent years, photoreversible reactions of rhodopsin have attracted increasing interest because these reactions can be considered as a prototype of optical devices for recording, processing and storing information. To put it differently, a retinal-containing protein is a superfast molecular photoswitch having a high quantum yield and spectrally distinguishable dark and photoinduced forms. For example, the natural ability of the product that is formed in the late step of the photolysis in the invertebrate visual pigment (metarhodopsin) to undergo the reverse transition to rhodopsin lies behind both the physiological mechanism of the regeneration of the pigment and the use of photoreversibility in biotechnology.[140, 141] For example, it was shown [140] that the repeated photoconversion of octopus rhodopsin to metarhodopsin and the reverse reaction at room temperature occur even in *in vitro* experiments. Attempts were made (thought not quite successful) to perform this reaction by immobilizing octopus rhodopsin in a polymer matrix.[141] Relatively recently, the reversible photoisomerization of the rhodopsin chromophore was reproduced in the artificial system between two layers of purified natural clay (montmorillonite).[142] A continuation of studies in this area may be promising for the use in modern nanotechnology.

Early steps of photoinduced conversions of rhodopsin, for example, the steps of the formation of photo- and bathorhodopsin, are most attractive from the point of view of achieving the highest performance of such devices. Yan *et al.*[143] made an attempt to perform the reversible superfast photoswitching of intermediates of bovine rhodopsin, in particular, bathorhodopsin, under the action of femtosecond (300 fs) laser pulses. However, the authors did not report convincing evidence for the fact that the photoswitching does occur on a subpicosecond time scale.

We began comparative studies of the direct and reverse photoreactions of rhodopsin on a femtosecond time scale.[111, 121] The dynamics of the coherent photoisomerization of the 11-*cis*-retinal chromophore in bovine rhodopsin was investigated by high-resolution laser absorption spectroscopy. Changes in the absorption spectra of rhodopsin on a picosecond time scale induced by a femtosecond light pulse were recorded with the use of a double-pulse system (Fig. 8).[111]

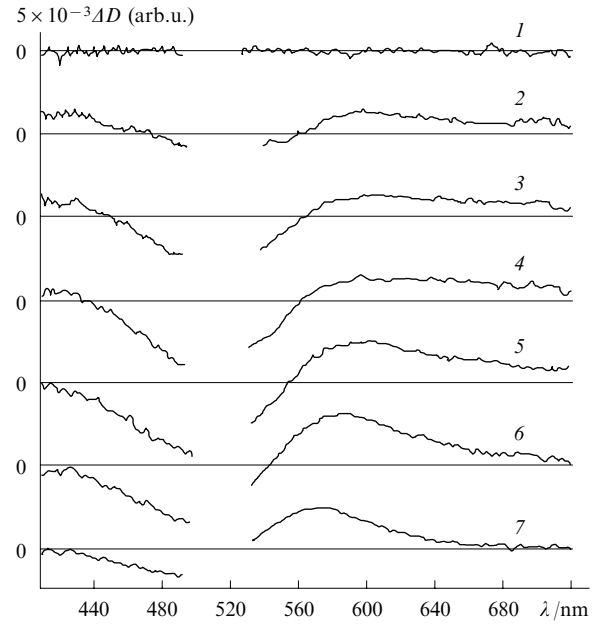


Figure 8. Photoinduced absorption spectra of bovine rhodopsin recorded after pulse excitation at λ = 500 nm in the spectral range of 410–720 nm at delay times, ps: −0.11 (*1*), 0.06 (*2*), 0.08 (*3*), 0.10 (*4*), 0.15 (*5*), 0.20 (*6*) and 3.0 (*7*).[111]
ΔD is the difference between the excited and unexcited sample absorption.

Oscillations of time-resolved absorption signals of photorhodopsin containing the chromophore in the *trans* form as the first photoreaction product and ground-state rhodopsin containing the vibrationally excited 11-*cis*-retinal moiety were studied (Fig. 9). A comparison of the non-

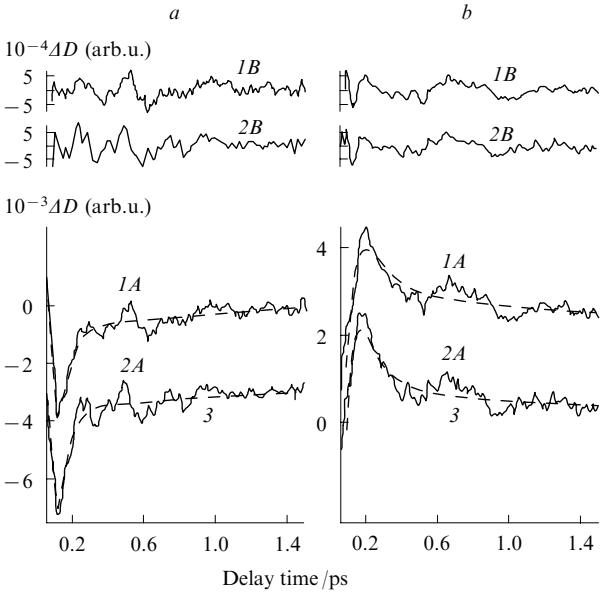


Figure 9. Kinetic curves of photoinduced absorption of rhodopsin measured after pulse excitation at 500 (*1A*) and 560 nm (*2A*) at delay times of 0.06–1.5 ps at probe wavelengths of 480 (*a*) and 610 nm (*b*), model curves (*3*) and oscillation components of the kinetic curves (*1B*, *2B*).[111]
The dashed lines indicate the model curves.

normalized kinetic curves of the photoinduced absorption of rhodopsin was performed at probe wavelengths of 480 (absorption of rhodopsin) and 610 nm (absorption of photorhodopsin). These oscillations were interpreted as the dynamics of coherent vibrational wave packets in reaction products. In other words, there was additional evidence confirming and refining the coherent nature of the photoisomerization of the retinal chromophore in the rhodopsin molecule.

We recorded for the first time the superfast photochromic reversible reaction of the visual pigment rhodopsin on a femtosecond time scale with the use of the specially designed three-pulse femtosecond laser system: the transition of rhodopsin from the dark state (11-*cis* isomer of chromophore) to the primary photoinduced intermediate, ground-state photorhodopsin (*trans* isomer), and then the reverse photoinduced conversion of photorhodopsin to rhodopsin.[121] It was shown that the reverse process can be coherently controlled by means of irradiation of photorhodopsin in the coherent vibrationally excited state at different instants of time within the lifetime of this intermediate.

Therefore, we showed for the first time that the superfast photochromic reaction of rhodopsin can occur at room temperature.[121] This result proves that the visual pigment rhodopsin can be considered as a prototype of a superfast molecular photoswitch.

Investigations of photoreactions of rhodopsin genetically modified in the region of the chromophore site may be considered as a new and very promising area of research with the use of femtosecond spectroscopy. Such studies will aim to understand the structural features of opsin as an ideal protein environment of the chromophore site providing the high rate and efficiency of the photoisomerization of the retinal chromophore.

3. Physiological activation of rhodopsin

The photochemical isomerization of the chromophore in rhodopsin initiates the transion of the visual pigment to the physiologically active state. The steps of this transion are shown in Fig. 5. As mentioned above, photorhodopsin is converted to bathorhodopsin as the next intermediate of the photolysis during 1 ps. The energy of an absorbed quantum of light accumulated in the step of bathorhodopsin ($\sim$35 kcal mol^{-1}, which is about 60% of the energy of the absorbed quantum[112]) is used for conformational rearrangements of the nearest protein environment of the chromophore and then, in subsequent steps of the photolysis, of the whole protein part of the molecule: the transmembrane (a seven α-helix bundle) and cytoplasmic domains. Before the formation of lumirhodopsin and metarhodopsin I, conformational changes take place mainly in the chromophore domain.

The most important intramolecular events occur in the course of the conversion of metarhodopsin I to metarhodopsin II: a) the proton transfer from PSB to the Glu113 counterion; b) the shift of α-helices III, V and, particularly, VI in the transmembrane domain; c) the proton uptake by the ionic lock located between α-helices III and VI. The conversion of metarhodopsin I to metarhodopsin II (millisecond time scale) successively affords the following spectrally identical forms of the latter: Meta IIa, Meta IIb and Meta IIbH$^+$.[144]

As for the proton uptake by the ionic lock, we discovered this phenomenon for the first time in the mid-1980s.[145] It was shown that the formation of metarhodopsin II is accompanied by the proton uptake and binding rather than by the transmembrane proton transfer, as it takes place in bacteriorhodopsin. More recently, it became clear that the intramolecular proton transfer lies behind the two-step switching of rhodopsin from its dark (inactive) to physiologically active state.[146]

Pogozheva *et al.*[147, 148] studied the photolysis of rhodopsin by saturation-transfer ESR spectroscopy with the use of spin labels covalently bound to accessible (hydrophilic) SH groups of cysteine residues (Cys140 and Cys316). We were the first to observe an increase in the conformational mobility of the cytoplasmic loops after the conversion of rhodopsin to metarhodopsin II and a decrease in its mobility after the reverse photoconversion of metarhodopsin II to a mixture of products, including photoregenerated rhodopsin and metarhodopsin III. The results of the study[149] also confirmed the fact that it is these two cysteine residues (Cys140 and Cys316) that are accessible in dark-state rhodopsin for the binding to spin labels.

The dark regeneration of rhodopsin resulting in the return of 11-*cis*-retinal to the chromophore site of opsin leads, as we have recently shown, to the regeneration of the dark conformational state of rhodopsin. Under exposure to light, the photoinduced conformational response of regenerated rhodopsin is again observed.[150]

The covalent binding of spin labels to SH groups was performed for both the native cysteine residues (Cys140/Cys167/Cys185/Cys222/Cys264/Cys316) in the polypeptide chain of rhodopsin and the cysteine residues artificially introduced into this chain using modern site-directed mutagenesis methods. This approach made it possible to determine key conformational changes in the rhodopsin molecule taking place in the course of its physiological activation (see publications[26, 151–153] and reviews[154, 155]).

The shifts of α-helices III, V and VI, as well as of other transmembrane α-helices, after the conversion of rhodopsin into metarhodopsin II are fundamentally important conformational rearrangements. These shifts are directly related to the formation of the sites of binding to the C-terminal polypeptide fragment of the α subunit of the three-subunit G-protein transducin in the cytoplasmic domain.

Recently, 28 cysteine point mutations were performed[151] using modern mutagenesis methods. It was shown that the conversion of rhodopsin into the photoactivated state of metarhodopsin II results in that amino acid residues of transmembrane α-helix III located in the vicinity of the chromophore become accessible to water molecules. In other words, it was clearly shown that the space around the agonist (*trans*-retinal moiety) in the physiologically active state of rhodopsin increases. An increase in the volume of the chromophore site was observed also by X-ray diffraction study (see below).

At least two factors are responsible for the recent progress in investigations of the structure of the activated state of rhodopsin. These factors are of fundamental importance for an understanding of the mechanisms of activation of both rhodopsin and the rhodopsin-like class A family of G-protein-coupled receptors. Firstly, the structures of rhodopsin in the dark and light-activated states can be compared with the available abundant spectroscopic,

biochemical and genetic data (see, for example, Refs 156 and 157). Secondly, rhodopsin can be obtained in sufficient amounts for the crystallization and determination of the three-dimensional structure both from native photoreceptor membranes and as the recombinant protein.

These factors account for the fact that rhodopsin was the first transmembrane protein of animal origin whose structure was reliably determined, whereas the bacterial protein bacteriorhodopsin was the first membrane protein whose primary structure was determined.[158, 159] The first three-dimensional structure of visual rhodopsin was determined for the dark state.[11, 15] Later, the structures of the thermally stable recombinant mutant of rhodopsin [160] and its several metastable intermediates containing the *trans*-retinal moiety in the chromophore site were established,[161, 162] and finally the structure of the physiologically activated opsin apoprotein containing no retinal was determined.[163]

Attempts to crystallize rhodopsin in the physiologically active state (metarhodopsin II) have long been unsuccessful, because the protein is very unstable. However, it was found that metarhodopsin II forms a stable complex with the C-terminal peptide of the α subunit of transducin. The first publication on the crystallization of opsin with the use of this complex appeared in 2008.[164] This gave rise to the hope that metarhodopsin II will be crystallized in the complex as well. Actually, stable structures of activated rhodopsin containing the *trans*-retinal moiety as an agonist, similarly to native metarhodopsin II, were described in 2011 – 2012.[35, 165, 166]

It should be remembered that in the native photoreceptor membrane, metarhodopsin II is formed as a result of the photoinduced conversion of the inverse agonist, *i.e.*, of the 11-*cis*-retinal moiety covalently bound to opsin (to be more precise, to Lys296), into the powerful agonist, the all-*trans*-retinal moiety. It is important that all-*trans*-retinal is also covalently bound to the same amino acid residue of opsin and remains in the tight protein environment of the chromophore site. However, in the step of metarhodopsin II, both the volume of the chromophore site and the surface area of the interaction of the retinal moiety with the amino acid environment are substantially larger (850 Å^3 *versus* 660 Å^3 in the dark state of rhodopsin and ~300 Å^2 *versus* ~230 Å^2, respectively).[11] The results of other studies also indicate that the conversion of the inverse agonist to the agonist is accompanied by an increase in the volume of the chromophore site (see, for example, Ref. 151). In fact, an increase in the volume of this site after the photoisomerization of 11-*cis*-retinal takes place earlier than the formation of metarhodopsin II, that is, in the step of the formation of lumirhodopsin, in which the volume of the chromophore site is ~890 Å^3 (Ref. 162).

Returning to the complex of the α subunit of transducin with metarhodopsin II containing the all-*trans*-retinal moiety, it should be noted that it was prepared using somewhat different strategies in the above-mentioned publications. In the study,[166] a crystal of opsin was initially grown, and then *trans*-retinal (all-*trans*-**1**) was introduced into this crystal. However, the position of the retinal moiety in the chromophore site of opsin remained unclear.

In two other works, the complex contained rhodopsin with particular point mutations.[35, 165] One of these mutations (Glu113Gln) was in the chromophore site.[165] This mutation made it possible to neutralize the Schiff base counterion Glu113, thus preventing the dissociation of the *trans*-retinal from the chromophore-binding site. However, the covalent bond between retinal and opsin is not retained in this mutant form, as opposed to native metarhodopsin II. In the study [35] published in 2012, the mutation (Met257Tyr) was in the vicinity of the G-protein-binding site. In this case, both the chromophore site and the covalent bond between the 11-*cis*-retinal moiety and Lys296 of opsin remained the same as in native metarhodopsin II. Moreover, the introduction of the inverse agonist (11-*cis*-retinal) into this complex results in the complete suppression of the activation of transducin. In the case of the photoisomerization of 11-*cis*-retinal to the *trans* form, *i.e.*, to the agonist, the complex of metarhodopsin II with the C-terminal peptide was activated in a similar way as native metarhodopsin II according to the mechanisms of the phototransduction. This made it possible to prepare a stable complex of metarhodopsin II with the C-terminal peptide of the α subunit of transducin. The structure of this complex was studied in detail.

It should be noted that the constantly activated state of mutant G-protein-coupled receptors was described rather long ago. This state can appear as a result of even a slight distortion of the mutual arrangement of α-helices in the transmembrane domain, which leads to an abnormal increase in their conformational mobility. This state of mutant receptors is responsible for a number of diseases.[167] For example, in the case of rhodopsin, this state causes serious eye disorders, such as congenital stationary night blindness [168] and retinitis pigmentosa .[58] In this respect, the mutation Met257Tyr, which does not cause substantial structural rearrangements but gives rise to the constantly activated state of rhodopsin,[35] can serve as a bright example of a dramatic change in the functional properties of the G-protein-coupled receptor as a result of the only point mutation.

Structural rearrangements in rhodopsin induced by the photoisomerization of the 11-*cis*-retinal moiety and resulting in its interaction with the G-protein transducin can be described based on the results of the three latest studies,[35, 165, 166] which are in good agreement with each other, as well as with a large number of other investigations by NMR, ESR and Fourier-transform infrared spectroscopy and some other methods.

The isomerization and rotation of retinal in the chromophore site lead to the 4 Å shift of the β-ionone ring in the space between transmembrane α-helices V and VI. In the vicinity of the chromophore site, α-helices II, VI and VII move apart from each other and the volume of the chromophore site increases compared to the dark state. Along with the simultaneous shift of Ser298 in α-helix VII and Trp265 in α-helix VI, a free space appears between the chromophore site and the cluster of bound water molecules. The involvement of bound water molecules in the mechanism of activation of rhodopsin is extensively studied and discussed.[15, 69, 82 – 85]

Therefore, there are several key events in the transition of rhodopsin to the physiologically active state. One of these events is the intramolecular proton transfer from PSB to the counterion Glu113 in transmembrane α-helix III.[146, 169] The distance between the nitrogen atom of the Schiff base and the δ-carbon atom of Glu113 increases to 6.6 Å,[35] which is in good agreement with some other X-ray diffraction data.[163 – 166, 169] This is additional evidence that

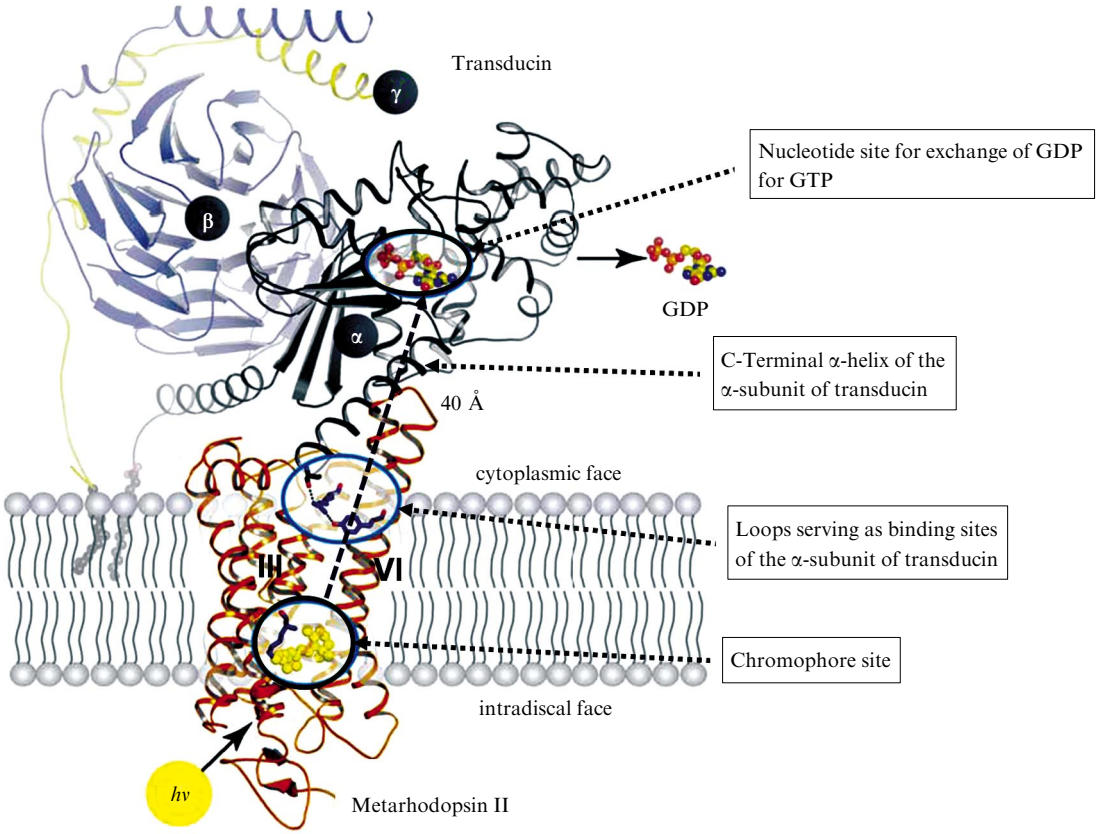


Figure 10. Scheme of the interaction between metarhodopsin II and transducin (the modified figure taken from the study [144]); α, β, γ are subunits of transducin.

the existence and retention of the covalent bond between retinal and opsin in metarhodopsin II is of key importance for the physiological activation of rhodopsin.

The intramolecular displacement and rotation of the β-ionone ring of retinal in the space between the α-helices V and VI is another key event. It is these α-helices of the transmembrane domain that undergo the largest conformational changes in the course of physiological activation of rhodopsin. According to the X-ray diffraction and NMR spectroscopic data, this leads to an increase in the distance between α-helices III and V and the displacement of the second intradiscal loop.[35, 170, 171] The rotation of the β-ionone ring results in the release of the tryptophan Trp265 in α-helix VI, which, in turn, causes the rearrangement of a hydrogen bond network. This rearrangement substantially facilitates a change in the slope of transmembrane α-helix VI and, which is of most importance, it involves the region from the retinal moiety in the chromophore site to the binding site of the G-protein of transducin in the hydrophilic cytoplasmic domain of rhodopsin, *i.e.*, extends to a distance of about 40 Å (Fig. 10). It should be noted that the amino acid residues involved in the rearrangement of the hydrogen bond network (the cluster of hydrogen-bonded water molecules) are highly conserved in the rhodopsin-like class A of G-protein-coupled receptors (see the review [12]).

Therefore, the all-*trans*-retinal moiety, which does not fit into the chromophore pocket after the isomerization of the 11-*cis*-double bond, initiates a cascade of activated conformational states of the protein part of the molecule, resulting finally in the formation of a cleft in the region of the hydrophilic cytoplasmic domain entered by the C-polypeptide terminus of the α subunit of the G-protein transducin.[19, 27, 144, 155] Then the conformational signal is transmitted to the nucleotide-binding site of transducin, resulting in the exchange of guanosine diphosphate (GDP) for guanosine triphosphate (GTP) and the dissociation of transducin to the α and βγ subunits (see Fig. 10). The details of the mechanism of the conformational signal transduction to transducin remain unknown.

The establishment of the molecular mechanisms of the binding and activation of the G-protein using rhodopsin is of fundamental importance for an understanding of the mechanisms of action of a large family of G-protein-coupled receptors and for a search of new drugs. At present, modern pharmaceutical industry invests large efforts and resources to the design of new drugs intended for the regulation of G-protein-coupled receptors and their interaction with the G-protein.

4. Hydrolysis of Schiff-base C=N bond to form opsin and *trans*-retinal

a. Free opsin

The last step of the photolysis of rhodopsin involves the hydrolysis of the iminium bond and the release of free retinal all-*trans*-**1** to the lipid bilayer of the photoreceptor membrane (see Fig. 6). As a result, the opsin apoprotein, which does not contain the chromophore group, remains in the membrane, whereas *trans*-retinal is removed. The bleaching of a considerable amount of rhodopsin is accompanied by the accumulation of a large amount of opsin in

the photoreceptor cell outer segment. It was found that this protein can by itself bind and activate transducin; however, its efficiency as the G-protein-coupled receptor is very low and is 10^{-6} of the activity of metarhodopsin II.[144] Nevertheless, even this very low efficiency is of physiological importance for photoreceptor cells, primarily, for their light adaptation.

Free opsin can initiate the phototransduction by binding and activating transducin, which creates the so-called equivalent background light in visual cells. The existence of this background light was suggested back in the 1930s.[172] Equivalent background light causes a decrease in the light sensitivity of a photoreceptor cell (desensitizes a rod), thus serving as an important event in the molecular mechanism of its light adaptation. The subsequent visual pigment regeneration leads to the disappearance of a nonspecific signal because, as discussed above, the chromophore group in the dark state of rhodopsin is a powerful inverse agonist. In other words, the visual pigment regeneration plays a key role in the mechanism of the dark adaptation of visual cells.

The absence of free opsin and, consequently, equivalent background light, in a dark-adapted rod, as well as very low thermal dark noise (see above) allows the rod to detect a single absorbed quantum of light and transmit information about this event to subsequent retinal nerve cells and then to the brain.[97, 173–175]

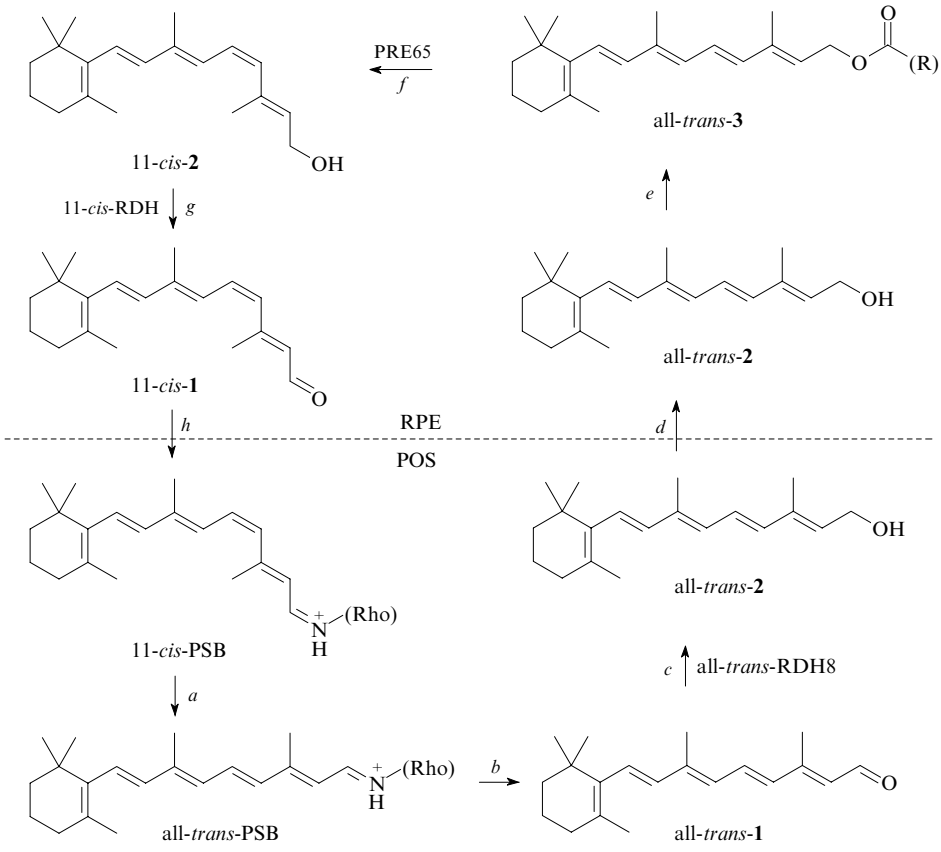
b. Free *trans*-retinal

In the vision, light is not only is a carrier of information but also a risk factor. This is the so-called photobiological paradox of vision.[72] In the course of the evolution, this paradox was resolved. Thus, along with a photoreception system, a multilevel system of protection against the risk of damage was formed in organs of vision.[72, 176, 177] Epidemiological data are indicative of the aggravating effect of light (primarily, in the near-ultraviolet and short-wavelength visible regions of the spectrum) on the development of retinal degenerative diseases, primarily of the severe and widespread age-related macular degeneration.[71, 178]

Photoreceptor cells and retinal pigment epithelium (RPE) cells are highly sensitive to photooxidative stress. These cells contain all three factors responsible for the development of this stress according to the mechanism of free-radical photooxidation: photosensitizers, oxygen and easily oxidizable substrates (polyunsaturated fatty acids, PUFAs). Retinoids, which are products of the conversion of *trans*-retinal (**1**) released from photolyzed rhodopsin, are the main photosensitizers. *Trans*-retinal (**1**) and its conversion products are fairly toxic and phototoxic. Acting as photosensitizers, these compounds can damage artificial membranes,[179–181] proteins (for example, rhodopsin) and photoreceptor membrane lipids[182, 183] an also photoreceptor cells, as was shown by experiments in transgenic mice.[184, 185]

The so-called rhodopsin visual cycle formed in the course of the evolution of organs of vision in vertebrates for the removal of free *trans*-retinal from the photoreceptor membrane, its detoxification, synthesis and delivery of new 11-*cis* isomer to opsin and finally the regeneration of the dark state of the visual pigment (Scheme 1) (see the review[186]). This cycle involves the following two cell structures: rod and cone outer segments and the adjacent RPE

Scheme 1



(Rho) is the opsin moiety

cell monolayer, where a large number of enzymes are responsible for the release of *trans*-retinal and for the synthesis and delivery of the new 11-*cis* isomer. As a rule, the disruption of any step of the visual cycle leads to retinal dystrophy.

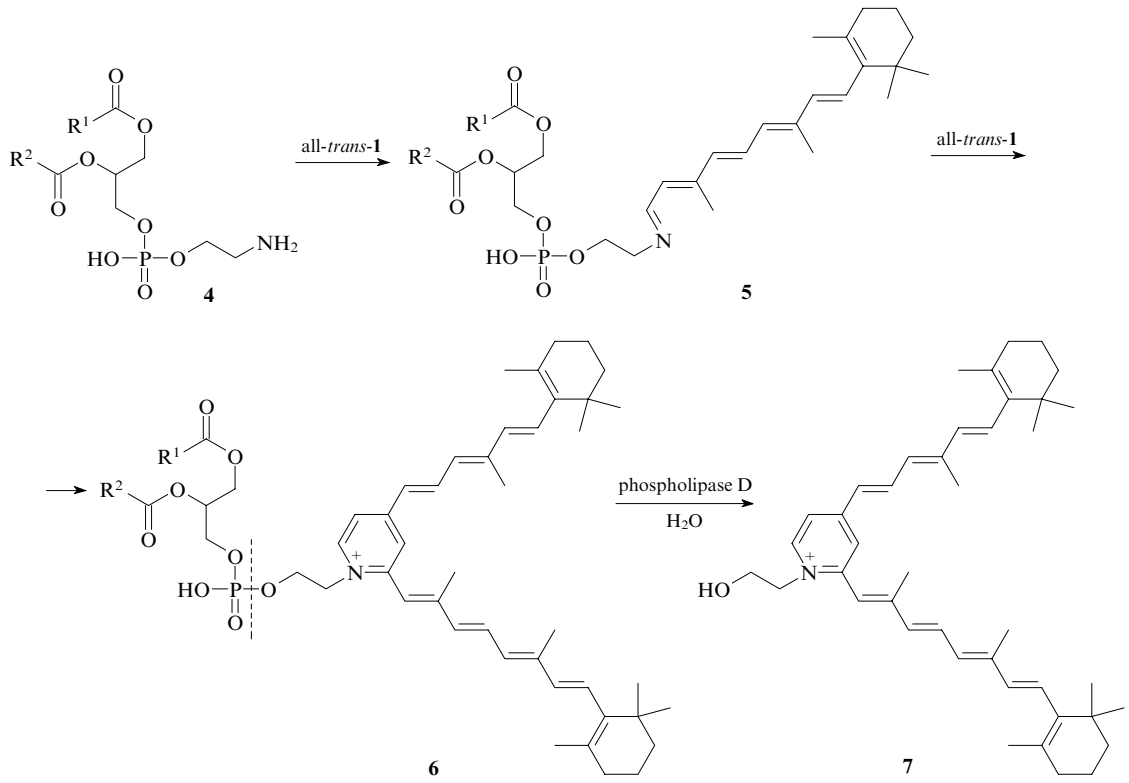
In the step *a* (see Scheme 1), the rhodopsin chromophore group, 11-*cis*-retinal, undergoes photoisomerization to the *trans* isomer. The step *b* involves the release of all-*trans*-retinal from opsin in the last step of the photolysis and its transition to the membrane lipid phase. In the visual cycle, the removal of *trans*-retinal from the photoreceptor membrane is catalyzed by the following two enzymes: all-*trans*-retinol dehydrogenase 8 (all-*trans*-RDH8) and the ATP-binding cassette transporter, the membrane protein ABCR4. In the nicotinamide adenine dinucleotide phosphate (NADPH)-catalyzed reaction, the enzyme RDH8 reduces all-*trans*-**1** to the nontoxic alcohol, *trans*-retinol (all-*trans*-**2**). The step *c* occurs on the outer (cytoplasmic) face of the photoreceptor membrane. Then the retinol-transport protein delivers *trans*-retinol from the outer segment of the photoreceptor cell to the RPE cell. The formation of the *trans* isomer of ester **3** (step *e*) is followed by the dark isomerization and hydrolysis to 11-*cis*-retinol (11-*cis*-**2**) catalyzed by isomerohydrolase RPE65 (step *f*). 11-*cis*-Retinol **2**, whose formation is mediated by isomerohydrolase, is oxidized by the enzyme 11-*cis*-retinol dehydrogenase to form 11-*cis*-retinal (step *g*). The transport of the latter from the RPE cell to the photoreceptor outer segment (POS) is mediated by the retinal-transport protein. The transport of 11-*cis*-retinal to the apoprotein and its incorporation into the chromophore site of opsin complete the visual cycle (see Scheme 1).

Recently, experiments in transgenic mice have shown that the conversion of *trans*-retinal to *trans*-retinol is independent of the presence of the ATP-binding cassette transporter (ABCR4), arestin, rhodopsin kinase or the palmitylation of rhodopsin, *i.e.*, the factors affecting the removal of *trans*-retinal from the photoreceptor membrane.[187] The function of ABCR4 is also to transport all-*trans*-**1** across the membrane; however, the mechanism of the transport remains unclear (see the review[188]). The question of how these two enzymes (RDH8 and ABCR4) located in different parts of the photoreceptor disc membrane perform the coordinated action is also open (see the review[188]).

It should be noted that the product of the interaction of *trans*-retinal with one of the three main photoreceptor membrane phospholipids, phosphatidylethanolamine (**4**), rather than *trans*-retinal by itself is the natural substrate of ABCR4. This product is *N*-retinylidenephosphatidylethanolamine (**5**) (the formula is shown in Scheme 2).[189–191]

The protein ABCR4 attracts great attention because its impairments cause retinal degenerative diseases, primarily the above-mentioned Stargardt disease (see the review[71]). The defects in ABCR4 lead to a considerable accumulation of intracellular inclusions in RPE cells, such as the age-related pigment or lipofuscin granules (LG) containing more than a dozen of retinoid fluorophores, *trans*-retinal conjugates.[192–194] Scheme 2 shows the pathway of the formation of retionoids from *trans*-retinal: *N*-retinylidene-phosphatidylethanolamine (A2PE, **5**), bis(retinylidene)-phosphatidylethanolamine (**6**) and bis(retinylidene)ethanolamine (A2E, **7**). The fluorophore A2E was the first to be identified and characterized.

Scheme 2



R^1, R^2 are hydrocarbon residues PUFAs

Lipofuscin granules, as well as unoxidized, oxidized and/or photooxidized forms of fluorophores in LG, whose formation was shown in *in vitro* [193, 195] and *in vivo* [196, 197] experiments, can exert the toxic and phototoxic effect on RPE cells.

There are the following main risk factors of the disruption of the rhodopsin visual cycle: the generation of active oxygen forms by fluorophores photoinduced with visible light,[196, 198–200] the release of photooxidized products of A2E from LG under illumination, which can initiate the RPE cell apoptosis,[201] the disturbance of the digestive function of lysosomes, resulting in the formation and accumulation of LG in RPE cells,[202] the ability of A2E to specifically inhibit RPE65 (the key enzyme of the visual cycle) [203] and so on. Retinoids absorbing in the short-wavelength region of visible light can serve as photosensitizers for the well-known risk of the damaging effect on the retina and RPE cells caused by this spectral region (blue light hazard).[204, 205] This is evidenced, in particular, by the action spectrum of photodamage observed in numerous biochemical, cytological, electrophysiological and psychophysiological studies (see reviews [72, 206–209]).

Since absorption spectra and, correspondingly fluorescence spectra of unoxidized and oxidized products of LG fluorophores are different, a component analysis of the fundus autofluorescence can be based on the detection of these differences. This novel noninvasive method of diagnosis of age-related changes and retinal degenerative diseases is based on the detection of autofluorescence of fluorophores that are present mainly in LG.

In recent studies,[210, 211] it was shown that cells serving as a source of 11-*cis*-retinal, which is necessary in the final step of the regeneration of rod and cone visual pigments, are different. Retinal pigment epithelium cells play an important role in both types of pigments, but in the case of the regeneration of cone pigments, Müller glial cells in the retina serve as an additional source of 11-*cis*-retinal.[212, 213]

As mentioned above, isomerohydrolase (the enzyme RPE65), which is present in RPE cells and isomerizes *trans*-retinol (all-*trans*-**2**) to 11-*cis*-form, plays a key role in the synthesis of 11-*cis*-retinal in both types of photoreceptor cells (see Scheme 1). It should be emphasized that the mechanism of action of this enzyme remains unclear, although extensive attempts are made to clarify this question.[214] This is of great interest for the chemistry of enzymes catalyzing the stereospecific isomerization. It has been recently shown [215] that isomerohydrolase in Müller glial cells, whose function is to regenerate cone pigments, is somewhat different from isomerohydrolase RPE65 (for example, 22% of amino acid sequence is nonhomologous).

The strategy of the search for agents capable of retarding the development of retinal degenerative diseases, primarily of the Stargardt disease and the so-called dry form of age-related macular retinal degeneration, is based on the inhibition of the RPE65 activity. The goal is to retard the operation of the visual cycle, thus decreasing the accumulation of retinoids, which are (photo)toxic by-products of the photolysis, in photoreceptor cell outer segments. It was shown that the free-radical trapping agent α-phenyl-*N*-*tert*-butylnitrone (PBN), which can protect the retina against photodamage, inhibits the enzymatic activity of RPE65, thus slowing down the regeneration of rhodopsin.[216] The same principle of the suppression of the visual cycle and the regeneration of rhodopsin lies behind the action of the new drug ACU-4429, which has been recently manufactured by the U.S. company Acucela.

The mechanism of incorporation and conversion of 11-*cis*-retinal to the chromophore group (step *h* in Scheme 1) also requires further experimental and theoretical investigations. The channelling hypothesis was proposed as an explanation of this mechanism.[217, 218] According to this hypothesis, the rhodopsin molecule has the entrance (for 11-*cis*-retinal) and exit (for *trans* isomer) chromophore sites, and the exit site becomes free after the incorporation of 11-*cis*-retinal into the chromophore site. However, the published data [219] cast doubt on this hypothesis. Since the formation of the covalent bond between the chromophore and the protein in the chromophore site is irreversible, the flow of 11-*cis*-retinal delivered from RPE and Müller cells to rod and cone outer segments and, correspondingly, the visual cycle are in fact controlled by the amount of free opsin in the photoreceptor membrane of visual cells.

Therefore, the visual cycle shows how *trans*-retinal, which is released in the course of the photolysis, is again converted into 11-*cis*-retinal as a result of the intra- and extracellular transfer events and enzymatic cascade reactions and how the latter returns to the chromophore site of opsin. This completes the regeneration of the dark state of rhodopsin in rods and of the visual pigment in vertebrate retinal cones in animals and humans.

* * *

The visual pigment rhodopsin has several key physiological functions in the mechanism of the visual reception. It provides spectral sensitivity of photoreceptors cells, phototransduction and the light and dark adaptation. Genetically determined defects of both the visual pigment molecule and the proteins involved in the mechanisms of phototransduction and adaptation or the mechanism of the visual cycle, are directly related to the pathogenesis of different forms of retinal degenerative diseases. An understanding of the structure and physicochemical mechanisms of the functioning of light-sensitive visual pigments opens a route to investigations of the pathogenesis of the heavy eye diseases and the design of new modern nanotechnological devices on their basis.

References

1. C Darwin *On the Origin of Species by Means of Natural Selection, or the Preservation of Favoured Races in the Struggle for Life* (London: John Murray, 1859) p. 186
2. T D Lamb, S P Collin, E N Pugh Jr *Nature Rev. Neurosci.* **8** 960 (2007)
3. T D Lamb *Philos. Trans. R. Soc. London, B: Biol. Sci.* **364** 2911 (2009)
4. T D Lamb *Sci. Am.* **305** 64 (2011)
5. J A Fuhrman, M S Schwalbach, U Stingl *Nature Rev. Microbiol.* **6** 488 (2008)
6. M Grote, M A O'Malley *FEMS Microbiol. Rev.* **35** 1082 (2011)
7. C Consani, O Braem, A A Oskouei, R M van der Veen, A Elnahhas, A Cannizzo, G Aublck, F van Mourik, M Chergui *Chimia (Aarau)* **65** 683 (2011)
8. D Polli, P Altoè, O Weingart, K M Spillane, C Manzoni, D Brida, G Tomasello, G Orlandi, P Kukura, R A Mathies, M Garavelli, G Cerullo *Nature (London)* **467** 440 (2010)

9. A Yabushita, T Kobayashi, M Tsuda *J. Phys. Chem. B* **116** 1920 (2012)
10. T J Martinez *Nature (London)* **467** 412 (2010)
11. J Li, P C Edwards, M Burghammer, C Villa, G F Schertler *J. Mol. Biol.* **343** 1409 (2004)
12. X Deupi, J Standfuss *Curr. Opin. Struct. Biol.* **21** 541 (2011)
13. Yu A Ovchinnikov, N G Abdulaev, M Yu Feigina, I D Artamonov, A S Zolotarev, M B Kostina, A S Bogachuk, A I Miroshnikov, V I Martynov, A B Kudelin *Bioorg. Khim.* **8** 1424 (1982) [a]
14. P A Hargrave, J H Mcdowell, D R Curtis, J K Wang, E Juszczak, S L Fong, J K Rao, P Argos *Biophys. Struct. Mech.* **9** 235 (1983)
15. 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 *Science* **289** 739 (2000)
16. G F J Salgado, A V Struts, K Tanaka, N Fujioka, K Nakanishi, M F Brown *Biochemistry* **43** 12819 (2004)
17. D C Teller, T Okada, C A Behnke, K Palczewski, R E Stenkamp *Biochemistry* **40** 7761 (2001)
18. M Sugihara, V Buss, P Entel, M Elstner, T Frauenheim *Biochemistry* **41** 15259 (2002)
19. S O Smith *Annu. Rev. Biophys.* **39** 309 (2010)
20. T Okada, M Sugihara, A-N Bondar, M Elstner, P Entel, V Buss *J. Mol. Biol.* **342** 571 (2004)
21. J Saam, E Tajkhorshid, S Hayashi, K Schulten *Biophys. J.* **83** 3097 (2002)
22. P S Crozier, M J Stevens, L R Forrest, T B Woolf *J. Mol. Biol.* **333** 493 (2003)
23. R S H Liu, L U Colmenares *Proc. Natl. Acad. Sci. USA* **100** 14639 (2003)
24. A Cordomí, J J Perez *J. Biomol. Struct. Dyn.* **27** 127 (2009)
25. S Hayashi, E Tajkhorshid, K Schulten *Biophys J.* **96** 403 (2009)
26. V Hornak, S Ahuja, M Eilers, J A Goncalves, M Sheves, P J Reeves, S O Smith *J. Mol. Biol.* **396** 510 (2010)
27. B Mertz, A V Struts, S E Feller, M F Brown *Biochim. Biophys. Acta* **1818** 241 (2012)
28. I Schapiro, M N Ryazantsev, L M Frutos, N Ferre, R Lindh, M Olivucci *J. Am. Chem. Soc.* **133** 3354 (2011)
29. S T Menon, M Han, T P Sakmar *Physiol. Rev.* **81** 1659 (2001)
30. W L Hubbell, C Altenbach, C M Hubbell, H G Khorana *Adv. Protein Chem.* **63** 243 (2003)
31. Y Liang, D Fotiadis, S Filipek, D A Saperstein, K Palczewski, A Engel *J. Biol. Chem.* **278** 21655 (2003)
32. B Borhan, M L Souto, H Imai, Y Shichida, K Nakanishi *Science* **288** 2209 (2000)
33. T Okada, O P Ernst, K Palczewski, K P Hofmann *Trends Biochem. Sci.* **26** 318 (2001)
34. J E Kim, D Pan, R A Mathies *Biochemistry* **42** 5169 (2003)
35. X Deupi, P Edwards, A Singhal, B Nickle, D Oprian, G Schertler, J Standfuss *Proc. Natl. Acad. Sci. USA* **109** 119 (2012)
36. P J R Spooner, J M Sharples, S C Goodall, P H M Bovee-Geurts, M A Verhoeven, J Lugtenburg, A M A Pistorius, W J De Grip, A Watts *J. Mol. Biol.* **343** 719 (2004)
37. G Gröbner, I J Burnett, C Glaubitz, G Choi, A J Mason, A Watts *Nature (London)* **405** 810 (2000)
38. P L Yeagle, G Choi, A D Albert *Biochemistry* **40** 11932 (2001)
39. S O Smith, J Courtin, H J M de Groot, R Gebhard, J Lugtenburg *Biochemistry* **30** 7409 (1991)
40. A F L Creemers, S Kiihne, P H M Bovee-Geurts, W J De Grip, J Lugtenburg, H J M de Groot *Proc. Natl. Acad. Sci. USA* **99** 9101 (2002)
41. S Ahuja, M Eilers, A Hirshfeld, E C Y Yan, M Ziliox, T P Sakmar, M Sheves, S Smith *J. Am. Chem. Soc.* **131** 15160 (2009)
42. J A Gascon, E M Sproviero, V S Batista *Acc. Chem. Res.* **39** 184 (2006)
43. K T Kholmurodov, T B Feldman, M A Ostrovsky *Mendeleev Commun.* 1 (2006)
44. T B Feldman, Kh T Kholmurodov, M A Ostrovsky *Pis'ma v EChAYa (Elementarnye Chastitsy Atomnogo Yadra)* **5** 226 (2008) [b]
45. T B Feldman, Kh T Kholmurodov, M A Ostrovsky, M G Khrenova, A V Nemukhin *Biofizika* **54** 660 (2009) [c]
46. P W Lau, A Grossfield, S E Feller, M C Pitman, M F Brown *J. Mol. Biol.* **372** 906 (2007)
47. K B Bravaya, A V Bochenkova, A A Granovsky, A V Nemukhin *J. Am. Chem. Soc.* **129** 13035 (2007)
48. P J E Verdegem, P H M Bovee-Geurts, W J de Grip, J Lugtenburg, H J M de Groot *Biochemistry* **38** 11316 (1999)
49. S W Lin, M Groesbeek, I van der Hoef, P Verdegem, J Lugtenburg, R A Mathies *J. Phys. Chem. B* **102** 2787 (1998)
50. G Gröbner, G Choi, I J Burnett, C Glaubitz, P J Verdegem, J Lugtenburg, A Watts *FEBS Lett.* **422** 201 (1998)
51. D Singh, B S Hudson, C Middleton, R R Birge *Biochemistry* **40** 4201 (2001)
52. Y Imamoto, M Sakai, Y Katsuta, A Wada, M Ito, Y Shichida *Biochemistry* **35** 6257 (1996)
53. B Isin, K Schulten, E Tajkhorshid, I Bahar *Biophys. J.* **95** 789 (2008)
54. J Nathans *Biochemistry* **29** 937 (1990)
55. T P Sakmar, R R Franke, H G Khorana *Proc. Natl. Acad. Sci. USA* **86** 8309 (1989)
56. E A Zhukovsky, D D Oprian *Science* **246** 928 (1989)
57. K Tsutsui, Y Shichida *Photochem. Photobiol. Sci.* **9** 1426 (2010)
58. P R Robinson, G B Cohen, E A Zhukovsky, D D Oprian *Neuron* **9** 719 (1992)
59. A Altun, S Yokoyama, K Morokuma *Photochem. Photobiol.* **84** 845 (2008)
60. T Andruniow, N Ferre, M Olivucci *Proc. Natl. Acad. Sci. USA* **101** 17908 (2004)
61. S Sekharan, V Buss *J. Am. Chem. Soc.* **130** 17220 (2008)
62. M N Sandberg, T L Amora, L S Ramos, M H Chen, B E Knox, R R Birge *J. Am. Chem. Soc.* **133** 2808 (2011)
63. E C Y Yan, M A Kazmi, Z Ganim, J-M Hou, D Pan, B S W Chang, T P Sakmar, R A Mathies *Proc. Natl. Acad. Sci. USA* **100** 9262 (2003)
64. S Ludeke, M Beck, E C Yan, T P Sakmar, F Siebert, R Vogel *J. Mol. Biol.* **353** 345 (2005)
65. T B Feldman, K T Kholmurodov, M A Ostrovsky, in *Molecular and Nanoscale Systems for Energy Conversion* (Ed. S Varfolomeev) (New York: Nova Sci. Publ., 2008) p. 157
66. M Nakagava, T Iwasa, S Kikkawa, M Tsuda, T G Ebrey *Proc. Natl. Acad. Sci. USA* **96** 6189 (1999)
67. A Terakita, M Koyanagy, H Tsukamoto, T Yamashita, T Miyata, Y Shichida *Nature Struct. Mol. Biol.* **11** 284 (2004)
68. A Terakita, T Yamashita, Y Shichida *Proc. Natl. Acad. Sci. USA* **97** 14263 (2000)
69. T Ota, Y Furutani, A Terakita, Y Shichida, H Kandori *Biochemistry* **45** 2845 (2006)
70. S Sekharan, K Morokuma *J. Am. Chem. Soc.* **133** 19052 (2011)
71. M A Ostrovsky, T B Feldman, M A Yakovleva, E R Lovyagina, L A Churkina, M P Kirpichnikov, D A Dolgikh, O V Nekrasova, V A Korshun, Kh P Takhchidi, S A Borzenok, M F Shurygina, E K Ginter, O V Khlebnikova, A V Polyakov, in *Postgenomnye Issledovaniya i Tekhnologii* (Post-Genomic Studies and Technologies) (Ed. S D Varfolomeev) (Moscow: Moscow State University, 2011) p. 376
72. M A Ostrovsky *Usp. Biol. Khim.* **45** 173 (2005) [d]
73. D M Hunt, L S Carvalho, J A Cowing, J W Parry, S E Wilkie, W L Davies, J K Bowmaker *Photochem. Photobiol.* **83** 303 (2007)

74. L S Carvalho, W L Davies, P R Robinson, D M Hunt *Proc. Biol. Sci.* **279** 387 (2012)
75. A E Dontsov, I B Fedorovich, M Lindström, M A Ostrovsky *J. Comp. Physiol. B* **169** 157 (1999)
76. M Jokela-Maatta, J Pahlberg, M Lindström, P P Zak, M Porter, M A Ostrovsky, Th W Cronin, K Donner *J. Comp. Physiol. A* **191** 1087 (2005)
77. A E Abu Khamidakh, Yu V Demchuk, P P Zak, M Lindström, M A Ostrovsky *Vestn. Mosk. Univ., Ser. 16, Biol.* (2) 9 (2010) [e]
78. T Feldman, M Yakovleva, M Lindstrlm, K Donner, M Ostrovsky *J. Crustacean Biol.* **30** 636 (2010)
79. E M Slobodyanskaya, E V Abrashin, A M Ostrovsky *Bioorg. Khim.* **6** 223 (1980) [a]
80. I Yu Novitskii, P P Zak, M A Ostrovsky *Spektral'naya Neodnorodnost' Krasnochuvstvitel'nykh Kolbochek Setchatki. Mikrospektrofotometricheskoe i Elektrofiziologicheskoe Issledovanie* (Spectral Heterogeneity of Retinal Cones Sensitive to Red. Microspectrophotometric and Electrophysiology Investigation, Preprint of the Institute of Chemical Physics, Academy of Sciences of the USSR, Chernogolovka, 1986)
81. P P Zak, M A Ostrovsky, J K Bowmaker *Vision Res.* **41** 1755 (2001)
82. M Murakami, T Kouyama *Nature (London)* **453** 363 (2008)
83. Y Furutani, Y Shichida, H Kandori *Biochemistry* **42** 9619 (2003)
84. T E Angel, M R Chance, K Palczewski *Proc. Natl. Acad. Sci. USA* **106** 8555 (2009)
85. T E Angel, S Gupta, B Jastrzebska, K Palczewski, M R Chance *Proc. Natl. Acad. Sci. USA* **106** 14367 (2009)
86. K Katayama, Y Furutani, H Imai, H Kandori *Biochemistry* **51** 1126 (2012)
87. H Kandori *Biochim. Biophys. Acta* **1460** 177 (2000)
88. A Altun, S Yokoyama, K Morokuma *J. Phys. Chem. B* **112** 16883 (2008)
89. A Altun, S Yokoyama, K Morokuma *J. Phys. Chem. B* **112** 6814 (2008)
90. S Sekharan, A Altun, K Morokuma *J. Am. Chem. Soc.* **132** 15856 (2010)
91. S Sekharan, K Morokuma *J. Am. Chem. Soc.* **133** 4734 (2011)
92. T Yoshizawa, G Wald *Nature (London)* **197** 1279 (1963)
93. R W Schoenlein, L A Peteanu, R A Mathies, C V Shank *Science* **4** 412 (1991)
94. D A Baylor *Invest. Ophthalmol. Vis. Sci.* **28** 34 (1987)
95. D A Baylor, B J Nunn, J L Schnapf *J. Physiol.* **357** 575 (1984)
96. L Lagnado, D Baylor *Neuron* **8** 995 (1992)
97. D Baylor *Proc. Natl. Acad. Sci. USA* **93** 560 (1996)
98. T D Lamb *Proc. Natl. Acad. Sci. USA* **93** 566 (1996)
99. D G Luo, W W Yue, P Ala-Laurila, K W Yau *Science* **332** 1307 (2011)
100. J Liu, M Y Liu, J B Nguyen, A Bhagat, V Mooney, E C Y Yan *J. Biol. Chem.* **286** 27622 (2011)
101. H J A Dartnall *Vision Res.* **8** 339 (1967)
102. J E Kim, M J Tauber, R A Mathies *Biochemistry* **40** 13774 (2001)
103. R S Becker, K Freedman *J. Am. Chem. Soc.* **107** 1477 (1985)
104. Y Koyama, K Kubo, M Komori, H Yasuda, Y Mukai *Photochem. Photobiol.* **54** 433 (1991)
105. H Kandori, Y Katsuda, M Ito, H Sasabe *J. Am. Chem. Soc.* **117** 2669 (1995)
106. G Haran, E A Morlino, J Matthes, R H Callender, R M Hochstrasser *J. Phys. Chem. A* **103** 2202 (1999)
107. H Chosrowjan, N Mataga, Y Shibata, S Tachibanaki, H Kandori, Y Shichida, T Okada, T Kouyama *J. Am. Chem. Soc.* **120** 9706 (1998)
108. H Kandori, Y Furutani, S Nishimura, Y Shichida, H Chosrowjan, Y Shibata, N Mataga *Chem. Phys. Lett.* **334** 271 (2001)
109. G G Kochendoerfer, R A Mathies *J. Phys. Chem.* **100** 14526 (1996)
110. O A Smitienko, I V Shelaev, F E Gostev, T B Feldman, V A Nadtochenko, O M Sarkisov, M A Ostrovsky *Dokl. Akad. Nauk* **421** 277 (2008) [f]
111. O A Smitienko, M N Mozgovaya, I V Shelaev, F E Gostev, T B Feldman, V A Nadtochenko, O M Sarkisov, M A Ostrovsky *Biokhimiya* **75** 34 (2010) [g]
112. A Cooper *Nature (London)* **282** 531 (1979)
113. G A Schick, T M Cooper, R A Holloway, L P Murray, R R Birge *Biochemistry* **26** 2556 (1987)
114. H Ohtani, T Kobayashi, M Tsuda, T G Ebrey *Biophys. J.* **53** 17 (1988)
115. Y Shichida, S Matsuoka, T Yoshizawa *Photobiochem. Photobiophys.* **7** 221 (1984)
116. Y Fukada, Y Shichida, T Yoshizawa, M Ito, A Kodama, K Tsukida *Biochemistry* **23** 5826 (1984)
117. B Mao, M Tsuda, T G Ebrey, H Akita, V Balogh-Nair, K Nakanishi *Biophys. J.* **35** 543 (1981)
118. T Kobayashi, M Kim, M Taiji, T Iwasa, M Nakagawa, M Tsuda *J. Phys. Chem. B* **102** 272 (1998)
119. L Zhu, J Kim, R A Mathies *J. Raman Spectrosc.* **30** 777 (1999)
120. H Kandori, H Sasabe, K Nakanishi, T Yoshizawa, T Mizukami, Y Shichida *J. Am. Chem. Soc.* **118** 1002 (1996)
121. M N Mozgovaya, O A Smitienko, I V Shelaev, F E Gostev, T B Feldman, V A Nadtochenko, O M Sarkisov, M A Ostrovsky *Dokl. Akad. Nauk* **435** 262 (2010) [f]
122. J E Kim, R A Mathies *J. Phys. Chem. A* **106** 8508 (2002)
123. B G Levine, T M Martinez *Annu. Rev. Phys. Chem.* **58** 613 (2007)
124. G Tomasello, G Olaso-González, P Altozh, M Stenta, L Serrano-Andrés, M Merchán, G Orlandi, A Bottoni, M Garavelli *J. Am. Chem. Soc.* **131** 5172 (2009)
125. I Schapiro, F Melaccio, E N Laricheva, M Olivucci *Photochem. Photobiol. Sci.* **10** 867 (2011)
126. G Zgrablić, A M Novello, F Parmigiani *J. Am. Chem. Soc.* **134** 955 (2012)
127. V A Nadtochenko, O A Smitienko, T B Feldman, M N Mozgovaya, I V Shelaev, F E Gostev, O M Sarkisov, M A Ostrovsky *Dokl. Akad. Nauk* (2012) [f] (in the press)
128. Q Wang, R W Schoenlein, L A Peteanu, R A Mathies, C V Shank *Science* **266** 422 (1994)
129. T Kobayashi, T Saito, H Ohtani *Nature (London)* **414** 531 (2001)
130. A M Virshup, C Punwong, T V Pogorelov, B A Lindquist, C Ko, T J Marttnez *J. Phys. Chem. B* **113** 3280 (2009)
131. O Weingart *J. Am. Chem. Soc.* **129** 10618 (2007)
132. L M Frutos, T Andruniow, F Santoro, N Ferrí, M Olivucci *Proc. Natl. Acad. Sci. USA* **104** 7764 (2007)
133. A V Kolesnikov, D A Korenyak, S A Shukolyukov, V I Govardovskii *Sens. Sist.* **25** 55 (2011) [h]
134. V A Krongauz, R R Shifrina, I B Fedorovich, M A Ostrovsky *Biofizika* **20** 219 (1975) [c]
135. D S Kliger, J S Horwitz, J W Lewis, C M Einterz *Vision Res.* **24** 1465 (1984)
136. T Mizukami, H Kandori, Y Shichida, A-H Chen, F Derguini, C G Caldwell, C Biffe, K Nakanishi, T Yoshizawa *Proc. Natl. Acad. Sci. USA* **90** 4072 (1993)
137. B N Baker, T P Williams *Vision Res.* **11** 449 (1971)
138. V A Krongauz, R R Shifrina, I B Fedorovich, M A Ostrovsky *Biofizika* **20** 419 (1975) [c]
139. V A Krongauz, R R Shifrina, I B Fedorovich, M A Ostrovsky *Biofizika* **20** 426 (1975) [c]
140. M A Ostrovsky, H H Weetall *Biosens. Bioelectron.* **13** 61 (1998)
141. C Paternolli, M Neebe, E Stura, F Barbieri, P Ghisellini, N Hampp, C Nicolini *J. Biomed. Mater. Res. A* **88** 947 (2009)

142. H Kandori, T Ichioka, M Sasaki *Chem. Phys. Lett.* **354** 251 (2002)
143. M Yan, L Rothberg, R J Callender *Phys. Chem. B* **105** 856 (2001)
144. K P Hofmann, P Scheerer, P W Hildebrand, H W Choe, J H Park, M Heck, O P Ernst *Trends Biochem. Sci.* **34** 540 (2009)
145. T F Shevchenko, G R Kalamkarov, M A Ostrovsky *Sens. Sist.* **1** 117 (1987) [h]
146. M Mahalingam, K Martínez-Mayorga, M F Brown, R Vogel *Proc. Natl. Acad. Sci. USA* **105** 17795 (2008)
147. I D Pogozheva, V A Kuznetsov, V A Livshits, I B Fedorovich, M A Ostrovsky *Biol. Memb.* **2** 880 (1985) [i]
148. I D Pogozheva, V A Kuznetsov, V A Livshits, M A Ostrovsky *Biol. Memb.* **2** 897 (1985) [i]
149. K Cai, R Langen, W L Hubbell, H G Khorana *Proc. Natl. Acad. Sci. USA* **94** 14267 (1997)
150. P V Shelyakin, A L Kovarskii, V V Kasparov, T B Feldman, M A Ostrovsky *Khim. Fiz.* (2012) (in the press) [k]
151. W Ou, T Yi, J-M Kim, H G Khorana *PLoS ONE* **6** e17398 (2011)
152. J Klein-Seetharaman, J Hwa, K W Cai, C Altenbach, W L Hubbel, H G Khorana *Biochemistry* **38** 7938 (1999)
153. K Yang, D L Farrens, W L Hubbell, H G Khorana *Biochemistry* **35** 12464 (1996)
154. D L Farrens *Photochem. Photobiol. Sci.* **9** 1466 (2010)
155. V Jastrzebska, Y Tsybovsky, K Palczewski *Biochem. J.* **428** 1 (2010)
156. S Ye, E Zaitseva, G Caltabiano, G F Schertler, T P Sakmar, X Deupi, R Vogel *Nature (London)* **464** 1386 (2010)
157. X E Zhou, K Melcher, H E Xu *Acta Pharmacol. Sin.* **33** 291 (2012)
158. Y A Ovchinnikov, N G Abdulaev, M Y Feigina, A V Kiselev, N A Lobanov *FEBS Lett.* **84** 1 (1977)
159. H G Khorana, G E Gerber, W C Herlihy, C P Gray, R J Anderegg, K Nihei, K Biemann *Proc. Natl. Acad. Sci. USA* **76** 5046 (1979)
160. J Standfuss, G Xie, P C Edwards, M Burghammer, D D Oprian, G F Schertler *J. Mol. Biol.* **372** 1179 (2007)
161. H Nakamichi, T Okada *Angew. Chem., Int. Ed.* **45** 4270 (2006)
162. H Nakamichi, T Okada *Proc. Natl. Acad. Sci. USA* **103** 12729 (2006)
163. J H Park, P Scheerer, K P Hofmann, H W Choe, O P Ernst *Nature (London)* **454** 183 (2008)
164. P Scheerer, J H Park, P W Hildebrand, Y J Kim, N Krauss, H W Choe, K P Hofmann, O P Ernst *Nature (London)* **455** 497 (2008)
165. J Standfuss, P C Edwards, A D'Antona, M Fransen, G Xie, D D Oprian, G F Schertler *Nature (London)* **471** 656 (2011)
166. H W Choe, Y J Kim, J H Park, T Morizumi, E F Pai, N Krauss, K P Hofmann, P Scheerer, O P Ernst *Nature (London)* **471** 651 (2011)
167. M J Smit, H F Vischer, R A Bakker, A Jongejan, H Timmerman, L Pardo, R Leurs *Annu. Rev. Pharmacol. Toxicol.* **47** 53 (2007)
168. V R Rao, G B Cohen, D D Oprian *Nature (London)* **367** 639 (1994)
169. J M Kim, C Altenbach, M Kono, D D Oprian, W L Hubbell, H G Khorana *Proc. Natl. Acad. Sci. USA* **101** 12508 (2004)
170. S Ahuja, E Crocker, M Eilers, V Hornak, A Hirshfeld, M Ziliox, N Syrett, P J Reeves, H G Khorana, M Sheves, S O Smith *J. Biol. Chem.* **284** 10190 (2009)
171. S Ahuja, V Hornak, E C Yan, N Syrett, J A Goncalves, A Hirshfeld, M Ziliox, T P Sakmar, M Sheves, P J Reeves, S O Smith, M Eilers *Nature Struct. Mol. Biol.* **16** 168 (2009)
172. W S Stiles, B H Crawford, in *Report of a Joint Discussion on Vision* (Cambridge: Physical Society of London, Cambridge University Press, 1932) p. 194
173. J Chariton, C A Lea *Proc. R. Soc. London, Ser. A* **122** 304 (1929)
174. S I Vavilov *Mikrostruktura Sveta* (Microstructure of Light) (Moscow: Academy of Sciences of the USSR, 1950) p. 198
175. S Hecht, S Shlaer, M H Pirenne *J. Gen. Physiol.* **25** 819 (1942)
176. M A Ostrovsky, I B Fedorovich *Sistemy Organov Chuvstv. Morfofunktsional'nye Aspekty Evolyutsii* (Systems of Sense Organs. Morphofunctional Aspects of Evolution) (Leningrad: Nauka, 1987) p. 4
177. M A Ostrovsky, in *Problemy Regulyatsii v Biologicheskikh Sistemakh. Biofizicheskie Aspekty* (The Problems of Regulation in Biological Systems. Biophysical Aspects) (Ed. A B Rubin) (Moscow, Izhevsk: Research Centre 'Regular and Chaotic Dynamics', 2007) p. 133
178. K V Chalam, V Khetpal, R Rusovici, S Balaiya *Eye Contact Lens* **37** 225 (2011)
179. A V Sokolov, V S Sokolov, T B Feldman, M A Ostrovsky *Biol. Membr.* **25** 499 (2008) [j]
180. E A Sokolenko, V S Sokolov, A V Sokolov, O A Finogenova, A E Dontsov, M A Ostrovsky *Eur. Biophys. J.* **34** 772 (2005)
181. A E Dontsov, N L Sakina, M A Ostrovsky *Izv. Akad. Nauk, Ser. Khim.* 438 (2012) [l]
182. M Yu Loginova, E V Rostovtseva, T B Feldman, M A Ostrovsky *Dokl. Akad. Nauk* **419** 838 (2008) [f]
183. M Yu Loginova, E V Rostovtseva, T B Feldman, M A Ostrovsky *Biokhimiya* **73** 162 (2008) [g]
184. A Maeda, M Golczak, T Maeda, K Palczewski *Invest. Ophthalmol. Vis. Sci.* **50** 5435 (2009)
185. A Maeda, T Maeda, M Golczak, S Chou, A Desai, C L Hoppel, S Matsuyama, K Palczewski *J. Biol. Chem.* **284** 15173 (2009)
186. P D Kiser, K Palczewski *Prog. Retin. Eye Res.* **29** 428 (2010)
187. L R Blakeley, C Chen, C K Chen, J Chen, R K Crouch, G H Travis, Y Koutalos *Invest. Ophthalmol. Vis. Sci.* **5** 3483 (2011)
188. Y Tsybovsky, R S Molday, K Palczewski *Adv. Exp. Med. Biol.* **703** 105 (2010)
189. R S Molday, M Zhong, F Quazi *Biochim. Biophys. Acta* **1791** 573 (2009)
190. J M Sullivan *Exp. Eye Res.* **89** 602 (2009)
191. V Vasiliou, K Vasiliou, D W Nebert *Hum. Genomics* **3** 281 (2009)
192. M A Ostrovsky, in *Klinicheskaya Fiziologiya Zreniya* (Clinical Physiology of Vision) (Ed. A M Shamshinova) (Moscow: Scientific-Medical Firm MBN, 2006) p. 109
193. M A Yakovleva, T B Feldman, A S Krupennikova, S A Borzenok, M A Ostrovsky *Izv. Akad. Nauk, Ser. Khim.* 2252 (2010) [l]
194. J R Sparrow, Y Wu, C Y Kim, J Zhou *J. Lipid Res.* **51** 247 (2010)
195. M A Yakovleva, T B Feldman, Z M Polonskaya, A E Dontsov, S A Borzenok, Kh P Takhchidi, M A Ostrovsky *Oftal'mokhirurgiya* (5) 59 (2009) [m]
196. Y P Jang, H Matsuda, Y Itagaki, K Nakanishi, J R Sparrow *J. Biol. Chem.* **280** 39732 (2005)
197. A R Wielgus, R J Collier, E Martin, F B Lih, K B Tomer, C F Chignell, J E Roberts *Photochem. Photobiol. Sci.* **9** 1505 (2010)
198. M A Ostrovsky, A E Dontsov, N L Sakina, M Boulton, J Jarvis-Evans *Sens. Sist.* **6** 51 (1992) [h]
199. M Boulton, A Dontsov, J Jarvis-Evans, M Ostrovsky, D Svistunenko *J. Photochem. Photobiol. B* **19** 201 (1993)
200. R A Radu, N L Mata, A Bagla, G H Travis *Proc. Natl. Acad. Sci. USA* **101** 5928 (2004)
201. A E Dontsov, N L Sakina, A M Golubkov, M A Ostrovsky *Dokl. Akad. Nauk* **425** 683 (2009) [f]
202. F G Holz, F Schutt, J Kopitz, G E Eldred, F E Kruse, H E Volcker, M Cantz *Invest. Ophthalmol. Vis. Sci.* **40** 737 (1999)

203. G Moiseyev, O Nikolaeva, Y Chen, K Farjo, Y Takahashi, J X Ma *Proc. Natl. Acad. Sci. USA* **107** 17551 (2010)
204. W T Ham Jr, H A Mueller, D H Sliney *Nature (London)* **260** 153 (1976)
205. W T Ham Jr, H A Mueller, J J Ruffolo Jr, D Guerry III, R K Guerry *Am. J. Ophthalmol.* **93** 299 (1982)
206. S M Meyers, M A Ostrovsky, R F Bonner *Trans. Am. Ophthalmol. Soc.* **102** 83 (2004)
207. D T Organisciak, D K Vaughan *Prog. Retin. Eye Res.* **29** 113 (2010)
208. P N Youssef, N Sheibani, D M Albert *Eye (London)* **25** 1 (2011)
209. D van Norren, T G Gorgels *Photochem. Photobiol.* **87** 747 (2011)
210. T M Redmond, S Yu, E Lee, D Bok, D Hamasaki, N Chen, P Goletz, J X Ma, R K Crouch, K Pfeifer *Nat. Genet.* **20** 344 (1998)
211. M L Batten, Y Imanishi, D C Tu, T Doan, L Zhu, J Pang, L Glushakova, A R Moise, W Baehr, R N Van Gelder, W W Hauswirth, F Rieke, K Palczewski *PLoS Med.* **2** e333 (2005)
212. N L Mata, R A Radu, R C Clemmons, G H Travis *Neuron* **36** 69 (2002)
213. J S Wang, V J Kefalov *Prog. Retin. Eye Res.* **30** 115 (2011)
214. E Poliakov, T Parikh, M Ayele, S Kuo, P Chander, S Gentleman, T M Redmond *Biochemistry* **50** 6739 (2011)
215. Y Takahashi, G Moiseyev, Y Chen, O Nikolaeva, J X Ma *FEBS J.* **278** 2913 (2011)
216. M N Mandal, G P Moiseyev, M H Elliott, A Kasus-Jacobi, X Li, H Chen, L Zheng, O Nikolaeva, R A Floyd, J X Ma, R E Anderson *J. Biol. Chem.* **286** 32491 (2011)
217. S A Schadel, M Heck, D Maretzki, S Filipek, D C Teller, K Palczewski, K P Hofmann *J. Biol. Chem.* **278** 24896 (2003)
218. M Heck, S A Schadel, D Maretzki, K P Hofmann *Vision Res.* **43** 3003 (2003)
219. D A Korenyak, V I Govardovskii, in *Proceedings of the Visionarium IX, Tvarminne, Finland, 2010* p. 20

[a] — *Russ. J. Bioorg. Chem. (Engl. Transl.)*
[b] — *Part. Nucl. Lett. (Engl. Transl.)*
[c] — *Biophysics (Engl. Transl.)*
[d] — *Biol. Chem. Rev. (Engl. Transl.)*
[e] — *Moscow Univ. Biol. Sci. Bull. (Engl. Transl.)*
[f] — *Dokl. Biochem. Biophys. (Engl. Transl.)*
[g] — *Biochemistry (Moscow) (Engl. Transl.)*
[h] — *Sens. Syst. (Engl. Transl.)*
[i] — *Biol. Membr. (Engl. Transl.)*
[j] — *Biochemistry (Moscow), Ser. A (Engl. Transl.)*
[k] — *Russ. J. Phys. Chem., Ser. B (Engl. Transl.)*
[l] — *Russ. Chem. Bul., Int. Ed. (Engl. Transl.)*
[m] — *Ophthal. Surgery (Engl. Transl.)*