Activation of rhodopsin: new insights from structural and biochemical studies

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G-protein-coupled receptors (GPCRs) are involved in a vast variety of cellular signal transduction processes from visual, taste and odor perceptions to sensing the levels of many hormones and neurotransmitters. As a result of agonist-induced conformation changes, GPCRs become activated and catalyze nucleotide exchange within the G proteins, thus detecting and amplifying the signal. GPCRs share a common heptahelical transmembrane structure as well as many conserved key residues and regions. Rhodopsins are prototypical GPCRs that detect photons in retinal photoreceptor cells and trigger a phototransduction cascade that culminates in neuronal signaling. Biophysical and biochemical studies of rhodopsin activation, and the recent crystal structure determination of bovine rhodopsin, have provided new information that enables a more complete mechanism of vertebrate rhodopsin activation to be proposed. In many aspects, rhodopsin might provide a structural and functional template for other members of the GPCR family.

Significant progress in understanding the structure and function of rhodopsin has been made in recent years. Electron cryomicroscopy on two-dimensional crystals of bovine rhodopsin provided the first direct visualization of the seven transmembrane helices of a G-protein-coupled receptor (GPCR)\(^1,2\), and led to structural information about other members of the superfamily through homology-modeling studies\(^3\). In parallel to these advances, biochemical and biophysical studies, often combined with site-directed mutagenesis, have provided valuable information on the nature of rhodopsin structure and the mechanism of rhodopsin activation. In addition, the recent structural determination of bovine rhodopsin by X-ray crystallography\(^4\) offers a new opportunity to assemble these related studies, providing further insight into the mechanism of activation of rhodopsin and GPCRs in general.

### G-protein-coupled receptors

Signal detection and transmission across biological membranes is initiated by the interaction of a chemical or physical stimulus with a specific membrane receptor which, in turn, becomes activated and initiates a chain of intracellular reactions that result in modulation of target protein activity. GPCRs are a superfamily of such membrane proteins that transmit a signal by coupling to heterotrimeric guanine nucleotide-binding proteins (known as large G proteins), which consist of three subunits (α, β and γ). In the activated form, the cytoplasmic domain of a GPCR is competent for binding a G protein, leading to subsequent catalytic nucleotide exchange on the α subunit and G-protein activation. As can be predicted by their amino acid sequences, GPCRs share the structural motif of seven transmembrane α-helical (H) segments, and thus belong to an even larger family of proteins that serve a broad variety of functional properties, from ion translocation (e.g. bacteriorhodopsin) to signal transduction. In the absence of an activating ligand, the GPCR apoprotein (opsin in the case of rhodopsin) has low basal activity; this activity is greatly enhanced upon binding of an agonist and reduced by inverse agonists. An enhanced ability of GPCRs to activate G proteins in the absence of ligand is characteristic of constitutive activity and can be caused by, for example, specific mutations. The G protein in retinal rod cells is called transducin (or Gt), according to its tissue-specific expression of the α subunit (reviewed in Refs 5,6).

In spite of the structural similarities between the receptor apoproteins, the ligands can be exceedingly diverse. The majority of ligands reach GPCRs by diffusion and bind to a site near the extracellular surface of the receptor\(^7\). In the case of rhodopsin, the chromophoric ligand 11-cis-retinal is permanently bound to the receptor as a prosthetic group that efficiently inactivates the receptor. This dormant visual pigment is activated by light-induced cis-trans isomerization of the bound retinal. During the activation process retinal plays a dual role, both as a chromophore in the initial rapid photochemistry and, after relaxation of the photoexcited state, as an agonist in producing the active state of the receptor (metarhodopsin II). It is this latter function for which we anticipate similarities between rhodopsin and other GPCRs, in particular for members of the so-called class I or rhodopsin subfamily of GPCRs. Class I GPCRs share sequence homology\(^8\) and also key structural features (Fig. 1), such as a disulfide bond between H-III and the extracellular region, and a tripeptide Asp(Glu)-Arg-Tyr motif located at the intracellular end of H-III. There are several other highly conserved residues, such as an Asn–Asp pair located in H-I and H-II, respectively, Pro residues in H-V and H-VI, aromatic residues in H-IV and H-VI, and a common Asn-Pro-X-X-Tyr motif in H-VII.

### Topology of rhodopsin

Vertebrate rhodopsin is located in the membranes of rods – flat vesicles that fill the outer segment of rod cells. The extracellular (intradiscal) and

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intracellular regions of rhodopsin each consist of three interhelical loops (given the prefix E or I, for extracellular and intracellular, respectively) and a terminal (COOH- or NH₂-) tail region. A fourth cytoplasmic loop (H-VIII) is formed by anchoring the C-terminal tail to the membrane via two Cys residues (Fig. 1), which carry palmitates in the native structure. Although the mass distribution to the intra- and extracellular regions is comparable, the three-dimensional structure demonstrates a clear contrast; whereas the four extracellular domains associate significantly with each other, only a few interactions are observed among the cytoplasmic domains. In addition, the E-II loop is connected to H-III via a disulfide bridge and fits tightly into a limited space inside the bundle of helices as a result of both interactions with other extracellular loops and the arrangement of its side chains.

The seven transmembrane helices of rhodopsin vary in length from 20 to 33 residues. There are also differences in the degree of bending around Gly–Pro residues, and in the tilt angles between the helices and the predicted membrane surface. A significant feature is a strong distortion imposed by Pro267 in H-VI, one of the most conserved residues among G-protein-coupled receptor are shown, including the tripeptide Asp(Glu)-Arg–Tyr motif (E134, R135, Y136), the Asn-Pro-X-X-Tyr motif in H-VII (N302, P303, Y306), and the disulfide bridge between Cys323 and Cys322 are palmitoylated and anchor the C-tail to the membrane. (b) The most conserved residues among G-protein-coupled receptor are shown, including the tripeptide Asp(Glu)-Arg–Tyr motif (E134, R135, Y136), the Asn-Pro-X-X-Tyr motif in H-VII (N302, P303, Y306), and the disulfide bridge between Cys323 and Cys322. This figure was prepared with Raster3D (Ref. 52).

contrast, a pronounced kink in H-II is caused by flexibility in the Gly–Gly sequence in the middle of this helix. H-VII shows a considerable elongation in the region around the retinal attachment site Lys296, and contains Pro291 and Pro303 (Pro303 is part of the highly conserved Asn-Pro-X-X-Tyr motif). The short helix H-VIII, adjacent to H-VII, runs parallel to the cytoplasmic surface. This helix constitutes part of the binding site for the C-terminus of the Gtα subunit and plays a role in Gtγ binding.

Phosphorylation of an activated receptor is a common mechanism for desensitizing signaling to a G protein. For rhodopsin, the major phosphorylation sites are Ser338, Ser343 and Ser334 (Ref. 9). In the crystal structure, these residues form a cluster in a turn region that does not appear to be exposed to the solvent. In addition, it is probable that the middle part of the C-terminal tail makes contact with a part of the C-III loop, presumably via hydrogen bonds between hydroxyl groups. These arrangements suggest that photoactivation of rhodopsin results in the breaking of these interactions, thus enabling the hydroxyl groups to be phosphorylated by rhodopsin kinase. The terminal penta-peptide region, QVAPA, is essential for translocation of newly synthesized rhodopsin molecules from the inner to the outer segment of the rod photoreceptor cell.

Recent studies emphasized the importance of dimerization and oligomerization for the functions of many GPCRs (Ref. 11). For rhodopsin, within intact functioning rods, the light-induced change in the diffusional speed of the activated receptor expected when it dimerizes with another (active or inactive) rhodopsin molecule was not observed. Elucidation of the crystal structure has now demonstrated that parts of the cytoplasmic loops do not fold over the helical bundle (C-III and H-VIII) but instead extend over the lipid bilayer, forming a large cytoplasmic surface. Thus, a 1:1 complex of the receptor and the trimeric Gt seems possible and could explain the apparent mismatch between the size of the helix bundle and the size of the receptor-binding domain on the heterotrimeric Gt (Ref. 13).

Rhodopsin structure and retinal diseases

Visual pigments contain the 11-cis-retinal chromophore, bound via a protonated Schiff base linkage to a Lys side chain in the middle of H-VII. Because the retinal protonated Schiff base model compounds absorb ~440 nm light, additional interactions between the chromophore and amino acids of the protein moiety of visual pigments cause the desirable shift of the optimal absorption (opsin shift), for instance ~500 nm for rhodopsin and ~400–600 nm for color pigments. Several studies on visual pigments (reviewed in Ref. 14) correctly predicted the localization of the chromophore-binding site visualized in the crystal structure of bovine rhodopsin (Fig. 2). However, a major

Fig. 1. Crystal structure of bovine rhodopsin (a). Seven transmembrane helices are labeled I to VII, cytoplasmic and extracellular domains as C-I, -II, -III and C-tail, and E-I, -II, -III and N-tail, respectively. A short helix (VIII), corresponding to Lys311–Cys322, is found between H-VII and the C-tail. Cys322 and Cys323 are palmitoylated and anchor the C-tail to the membrane.
unexpected finding is the proximity of an anti-
parallel β sheet to the chromophoric ligand. A highly
conserved disulfide bridge (Cys110–Cys187) holds
this β sheet, a part of loop E-II, close to retinal,
bringing the side chain of Glu181, another residue in
the β sheet, into the vicinity of the polyene chain of
retinal. Intriguingly, in red and green color
pigments15. His residues in this position are probably
involved in chloride binding, resulting in an
additional mechanism of spectral tuning.

More than a hundred rhodopsin mutants have
been identified in patients afflicted with eye diseases
(reviewed in Ref. 16; Fig. 3). Three mutations,
Gly90Asp, Ala292Glu and Thr94Ile, cause congenital
stationary night blindness. These mutations appear
to directly affect the salt bridge between the Schiff
base and the counterion Glu113 in the crystal
structure, thus leading to greatly enhanced
constitutive activity of the apoprotein in the absence
of the retinal ligand. Thr94, and the peptide amide of
Cys187, are in the close vicinity of Glu113. Cys187
forms a disulfide bridge with Cys110, and mutation of
both residues was found to be associated with retinitis
pigmentosa (RP), most probably by causing
destabilization of the opsin structure. Many of the
mutations causing RP were found in the E-II loop,
which would lead to either disarrangement of the
extracellular domains or disruption of the retinal-
binding pocket. Understanding the influence of these
mutations on the ground and signaling states of
rhodopsin is important for further pharmacological
approaches aimed at rescuing the phenotype in eye
diseases, and could also impact on the treatment of
other GPCR-linked diseases.

**Ground state**
The activity of ligand-free opsin is equal to 10–6 of
the activity of the all-trans-retinal-bound active
metarhodopsin II state17. However, the 11-cis-retinal-
bound rhodopsin ground state exhibits an even lower
level of activity against Gt, which shows that the
11-cis-retinal acts as an inverse agonist and imposes
further structural constraints. The interactions can
now be seen in the ground-state structure and explain
why the binding of the chromophore is concomitant
with a decrease in enthalpy [ΔH = −11 kcal mol−1
(−46 KJ mol−1)]18. The region around the Schiff
base, which has the lowest crystallographic temperature
factors of the structure, is stabilized by many
interactions. This stabilization is crucial for the
exceedingly low activity of rhodopsin; the resulting
low noise, in the presence of a huge amount of
activatable rhodopsin that forms a dense target for
the light, enables the rod cell to operate as a single
photon detector19.

The salt-bridge between the protonated Schiff
base in H-VII and its counterion, Glu113, in H-III
(Fig. 2), is neutralized in active forms of rhodopsin20.
This interaction is possible because of the extended
conformation of the side chain of Lys296, to which
retinal is covalently linked6. Corresponding salt
bridges between H-III and H-VII were shown to be
crucial for other GPCRs (Ref. 21).

Besides interacting with the chromophore,
numerous structural modules are found in the
crystal structure that stabilize the ground state, and

### Fig. 2. Chromophore-binding site of bovine rhodopsin, viewed (a) in perpendicular and (b) in parallel to the retinal polyene chain. The colors of the side chains are: red for acidic, blue for basic, light blue for polar, brown for hydrophobic and purple for Trp residues. A disulfide bond between Cys110 and Cys187 is shown in yellow. The colors for the polypeptide backbones are the same as in Fig. 1. One of the β sheets (shown as arrows) in E-II loop runs almost in parallel to part of the polyene chain (C11–C15) of 11-cis-retinal. The counterion Glu113 for the protonated Schiff base (between the retinal and Lys296) can also interact with the OH group of Thr94 and peptide amide of Cys187. One of the C-O of Glu181 in E-II loop points towards C12 of the retinal. Three OH groups from Thr118, Tyr268 and Ser186 come close to C9, C11 and C14 of the retinal, respectively. This figure was prepared with Raster3D (Ref. 52).

### Fig. 3. Two-dimensional model of bovine rhodopsin14,15. Residues shown in black circles indicate the position of mutations associated with retinitis pigmentosa. Positions of truncation mutants (Δ68–71, 252–termination, 343–termination) are indicated by gray circles. In rhodopsin, extracellular corresponds to the intradiscal space. The colors for H-I to H-VIII are the same as in Fig. 1.
most of these are mediated by highly conserved residues in GPCRs (Ref. 4). These modules include several distinct hydrogen-bonded networks, each between several helices, as well as hydrophobic interactions. The tripeptide Glu134-Arg135-Tyr136, which is a highly conserved Asp(Glu)-Arg-Tyr motif found in GPCRs (Fig. 4b), is important for maintaining the inactive conformation22. These residues participate in several hydrogen bonds with surrounding residues.

Mutations of Met257 result in strong constitutive activity of the opsin apoprotein23. In H-VI, this Met residue is surrounded by a Leu cluster (Fig. 4a), including Leu76 and Leu79 in H-II, and Leu128 and Leu131 in H-III. Among GPCRs, Leu79 is a highly conserved residue, whereas the other three residues can be variable hydrophobic residues. Mutation of the Leu residue corresponding to Leu128 of bovine rhodopsin makes several GPCRs constitutively active24,25, supporting the general importance of this interaction in maintaining the ground state of GPCRs. The distribution of the side chains in the short helix (H-VIII) from Lys311 to Cys322 exhibits an amphiphilic pattern. The hydrophobic side chains appear to interact with H-I (Leu57) and H-VII (Ile307). There is probably also a hydrophobic interaction between Tyr306 and Phe313. All of these residues are found frequently in GPCRs.

**Photochemistry and early intermediates**

Absorption of a photon provides rhodopsin with the energy to form the active state. As shown in Fig. 5, three phases of the activation process can be distinguished: (1) light induced cis–trans isomerization of the retinal; (2) thermal relaxation of the retinal–protein complex; and (3) the late equilibria that are affected by the interaction of rhodopsin with the G protein.

Two-thirds of the energy of 57 kcal mol⁻¹ (238 kJ mol⁻¹) taken up by light absorption are stored in the photoisomerized chromophore–(all-trans-retinylidene)–opsin complex26, lifting the receptor from the dormant 11-cis-retinal–opsin conformation, via photorhodopsin, to bathorhodopsin (Fig. 5). Isomerization might proceed by rotation around the Schiff-base side of the double bond between C11 and C12 of the retinal, which would bring the polyene chain closer to the side chain of Ser186 (Fig. 2). The steric restriction should limit the degree of rotation, thus leading to a distorted all-trans-configuration, in agreement with the spectroscopic data27.

In the relaxation phase, and during the transition from batho- to lumirhodopsin, such distortion can be released by relocation of the β-ionone ring of retinal, as suggested recently by a low temperature photolabeling study28. The relaxation phase is more complex than is shown in Fig. 5a, and additional photoproducts have been identified29. However, there is good spectroscopic evidence that the β-ionone ring is a crucial element in triggering the formation of the signaling state, metarhodopsin II (MII)30.

**The meta states**

The intermediate MII is the signaling state capable of interacting with the G protein3. Formation of MII from its predecessor, metarhodopsin I (MI), accompanies a large shift in the absorption maximum (first ‘bleached’ product of rhodopsin2), the breakage of the stabilizing salt bridge between the negatively charged side chain of Glu113 and the protonated Schiff base between Lys296 and retinal31,32, and the motion of transmembrane helices33,34. All of these events occur within the millisecond time interval of the net translocation of the Schiff base proton to the Glu113 counterion35. Generally, proton-transfer reactions occur when factors such as the relative orientation and distance of proton donor and acceptor are adjusted36, and can thus be determinants and indicators of conformational changes. From these considerations, it is apparent that the proton-transfer reaction destroys one of the crucial stabilizing elements of the ground state.

Formation of MII is also linked to proton uptake from the cytoplasm; the reaction depends on, and probably involves the protonation of, the highly conserved residue Glu134 (Refs 31,37,38). This residue, a part of the highly conserved Asp(Glu)-Arg-Tyr motif in GPCRs (Fig. 4), forms a salt bridge with the adjacent Arg135. Although Arg135 appears to interact also with Glu247 and Thr251 in H-VI, the rest of the environment surrounding the charge pair is mostly hydrophobic. Thus, protonation of Glu134 would directly destabilize the constraint on Arg135. A mutation

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**Fig. 4.** Hydrophobic interactions around Met257 and the Glu-Arg-Tyr region. (a) The Met257 region. Met257 is surrounded by a cluster of Leu residues from H-II (Leu76 and Leu79) and H-III (Leu128 and Leu131). (b) The Glu-Arg-Tyr region. Glu134-Arg135 is part of a tripeptide Asp(Glu)-Arg-Tyr motif found in most G-protein-coupled-receptors, and also known to be crucial for the function of G-protein activation. The Glu-Arg side chains point to the interior of the helical bundle and interact directly with each other. Two hydrogen-bonding side chains, Glu247 and Thr251, are close to Arg135, whereas the rest of the region consists of a series of hydrophobic side chains from the three cytoplasmic loops, C-I (Pro71, Leu72), C-II (F348) and C-III (Val230), and from H-III (Val137–139), H-IV (Ala153), H-V (Leu226) and H-VI (Val250, Met253). The colors for H-I to H-VIII are the same as in Fig. 1. This figure was prepared with Raster3D (Ref. 52).
eliminating the negative charge at Glu134 evokes constitutive activity of opsin and abolishes light-induced proton uptake, but leaves the proton transfer to Glu113 unchanged. Thus, at least in the solubilized receptor, the two proton translocations can be decoupled, consistent with the MIIa–MIIb two-step activation scheme shown in Fig. 5 (Refs 6,37).

We note here an analogous two-step mechanism found in the retinal-binding proteins of Archaea. In archaeal rhodopsins, structural information on the active M1a and M2 states (the possible analogs of MIIa and MIId in rhodopsin) is available. Of special interest are the sensory rhodopsins from Halobacterium salinarum, which combine with transducer proteins to form a receptor that can bind and activate intracellular phosphoregulatory proteins. Both sensory rhodopsins are proton pumps in their free state, but the bound transducer causes part of the free energy that is used for the transport mode in free sensory rhodopsin to be channeled into a signaling state. 

To explain the sequential flow of events, we propose that first, the N-terminal part of H-III moves into a position to induce proton transfer near the Schiff base and H+ uptake with helix movement are also seen in these rhodopsins. It might thus be useful to consider this part of signal transmission in rhodopsin as a sensory rhodopsin–transducer-like partial proton pump. The mechanism of signal transmission; that is, of forming MIId from MIIa, might well include an extended H-bonded network as is arising for the archaeal rhodopsins. Proton uptake with formation of MIId would be accompanied by the relocation of protons and water in the half-channel from the Schiff base–counterion to the cytoplasmic surface.

**Formation of the signaling state**

The positive enthalpy (ΔH, Fig. 5a) of MII formation indicates that molecular interactions built up in MI are lost upon transition to MII. To drive the conversion, the entropy, and thus the overall disorder in the protein, must increase. This observation would be consistent with the idea that formation of the active state is merely a release of constraints in the helix bundle, thus exposing cytoplasmic binding sites. Experimentally, it was indeed found that the insertion of successively concatenated Gt-binding sites into a surface loop of thioredoxin yields a fusion protein that binds Gt and shows limited nucleotide exchange capacity. The full activation capacity requires both the whole opsin apoprotein and the retinal ligand, which controls even the last steps of activation in the native receptor. A crucial element in the activation process is the 9-methyl group of retinal, suggesting a model in which the retinal provides a scaffold for the proton transfers required for forming the active state.

To explain the sequential flow of events, we propose that first, the N-terminal part of H-III moves into a position to induce proton transfer from the Schiff base to Glu113 (MIIIA). The Gly120–Gly121 tandem would then allow amplified...
movement of the C-terminal part of this helix, thus inducing a larger structural change at the cytoplasmic surface, which is linked to the repyrotonation and rearrangement events around the Glu134-Arg135-Tyr136 tripeptide. This mechanism would require a separate conformational change, following neutralization of Glu113, in agreement with a separate MI Ib state.

Although this property explains the activating effect of Glu113 or Glu134 mutations, it cannot account for several other observations; for example, mutant opsins, in which Glu113 or Glu134 are replaced by a neutral residue, show some constitutive activity but can also be normally activated by light after regeneration with 11-cis-retinal. Consistently, electron paramagnetic resonance (EPR) analyses of the cytoplasmic surface of rhodopsin with a Glu134Gln substitution have shown a local change of conformation around helices H-III and H-VII in the ground state; however, only a small change was seen in helix H-VI, which undergoes a dominant motion upon photoactivation. Moreover, Fourier transform infrared (FTIR) spectroscopic analysis of native rhodopsin has shown that interacting C-terminal peptides from both the α and γ subunits of Gt do not only produce the infrared absorption bands of fully protonated MII but also a pattern assigned to an additional change of the protein structure. Thus, formation of the signaling state might also depend on the (temporary) release of constraints—hydrogen-bonded networks and hydrophobic interactions—as a late consequence of retinal isomerization and because of changes in steric interaction between opsin and all-trans-retinal. In addition, the all-trans-retinylidene group might act not only as scaffold for proton transfers to H-III (Ref. 46) but also as an element that couples H-III with H-VI. The key question, not answered by the kinetic studies, is whether these events can only occur as a consequence of the protonation changes of Glu113 and Glu134.

### Rhodopsin as a model for other GPCRs

In the rhodopsin family of GPCRs, biogenic amine ligands are analogs of the retinal structure, with cationic ammonium groups at one end and a ring-like structure at the other. The inverse agonist 11-cis-retinal is covalently linked to the protein via a flexible Lys side chain. The ground state structure of rhodopsin provides clues as to how this ‘safety cord’ ensures a permanent occupancy of the ligand-binding site and stabilizing interactions, including a salt bridge-like stable apposition between the Schiff base hydrogen and its counterion, Glu113. After isomerization to the strained all-trans chromophore and relaxation of the ‘loaded spring’, the gate to the metarhodopsin conformation is open. There is an analogy of the MI and MI Ib states to the low- and high-affinity binding conformations (R and R*, respectively) of ligand (L) -activated GPCRs (Eqns 1, 2):

For rhodopsin

\[
\text{MI} + G \rightleftharpoons \text{MI a} + G \rightleftharpoons \text{MI b} \cdot G
\]

(light-induced pathway)

For GPCRs

\[
L + R + G \rightleftharpoons LR' + G \rightleftharpoons LR^* \cdot G
\]

(in general)

In rhodopsin, the high-affinity conformation is either stabilized by direct proton uptake, and probably by protonation of the acidic Glu side chain in the Asp(Glu)-Arg-Tyr motif from the aqueous phase, or by binding of the G protein, which might induce protonation of this Glu residue. Analogous proton translocation reactions have been proposed for the α1β-adrenergic and thrombin receptors. We have seen that disrupting the inactivating cluster of interactions in the Glu-Arg-Tyr structure by the Glu134Gln mutation leads to constitutive activity of the apoprotein. The activity of this mutant can be greatly enhanced by reconstitution with retinal, followed by illumination. This observation could explain why GPCRs of the rhodopsin family in which the acidic residue is naturally substituted are functional, and why mutational replacement of the neighboring Arg residue does not generally result in a loss of coupling to G proteins upon activation.

### Concluding remarks

Photoreceptors are designed for the extreme conditions of very low and high activities in dark and light, respectively. The strong constraints seen in the ground-state structure and the early energy-rich intermediates, which can only be reached by uptake of photonic energy, reveal the necessary barriers against spontaneous activation. However, many of the structural details are similar to those in other GPCRs, and the late meta intermediates show close analogies to the low- and high-affinity states in chemically activated receptors. On this basis, the results obtained with rhodopsin will have their impact on GPCRs in general, and unifying principles for GPCR structure and function will emerge. Even a low-resolution structure of MI or its complex with the G protein would be exceedingly valuable because some differences to the ground state, expected from EPR spectroscopy, appear to be substantial. However, the example of the archaeal rhodopsins shows that even a high-resolution structure of the active state does not imply an immediate understanding of its mechanism of formation. Rhodopsin, with its redundant constraints and light trigger, is ideally suited for NMR, EPR and FTIR studies, and we expect solutions from combined structural and spectroscopic studies on native and mutant rhodopsin.
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