HHS Public Access

Author manuscript

Methods Mol Biol. Author manuscript; available in PMC 2015 October 05.

Published in final edited form as:

Methods Mol Biol. 2015 ; 1271: 3–18. doi:10.1007/978-1-4939-2330-4_1.

The G Protein-Coupled Receptor Rhodopsin: A Historical Perspective

Lukas Hofmann and **Krzysztof Palczewski**

Abstract

Rhodopsin is a key light-sensitive protein expressed exclusively in rod photoreceptor cells of the retina. Failure to express this transmembrane protein causes a lack of rod outer segment formation and progressive retinal degeneration, including the loss of cone photoreceptor cells. Molecular studies of rhodopsin have paved the way to understanding a large family of cell-surface membrane proteins called G protein-coupled receptors (GPCRs). Work started on rhodopsin over 100 years ago still continues today with substantial progress made every year. These activities underscore the importance of rhodopsin as a prototypical GPCR and receptor required for visual perception—the fundamental process of translating light energy into a biochemical cascade of events culminating in vision.

Keywords

Rhodopsin; Rod cell(s); Phototransduction; G protein-coupled receptor(s); Receptor phosphorylation; Structure of membrane proteins; Signal transduction

1 Introduction

Molecular studies of rhodopsin began with the work of German physiologist Friedrich Wilhelm Kühne (1837–1900) who extracted rhodopsin from bovine retina with a precursor of modern detergent bile salts [1]. This scientist made the critical observation that rhodopsin's red color faded after exposure to light in the visible range. Denatured by organic solvents but not by salt, rhodopsin could be precipitated out of aqueous solutions with ammonium sulfate, a strategy used later for crystallization of this transmembrane protein [2, 3].

From early work it was clear that rhodopsin's red color could be restored when an illuminated retina was placed on the retinal pigmented epithelium (RPE), a monolayer of cells located in the back of the eye [1]. This regenerative process, known as the visual or retinoid cycle, is achieved by a series of enzymatic reactions that regenerate the light-sensitive chromophore [4]. The identity of the chromophore, the light-sensitive 11-*cis*-retinal ligand of rhodopsin, was not discovered until the work of George Wald [5].

Since ancient times it was known that absence of carotenoids in a diet lacking retinoids could lead to progressive retinal degeneration and blindness. But it was Wald who provided chemical evidence that rhodopsin is composed of two elements: an apoprotein opsin and a prosthetic, covalently linked 11-*cis*-retinal [6 – 8]. First, bleaching of rhodopsin caused

isomerization of the chromophore to the all- *trans*-isomer that eventually was released from the binding pocket of rhodopsin [9]. Then the spent chromophore was recycled back through the retinoid cycle to regenerate the photoactive chromophore which recombined with opsin.

The color of rhodopsin is derived from the chromophore 11-*cis*-retinal, but surprisingly this chromophore absorbs light at 360 nm rather than at 500 nm like rhodopsin. This shift is caused by interaction of the chromophore with the protein and is termed the “opsin shift.” Interactions of this universal chromophore of vision with other visual pigment apoproteins also lead to significantly shorter (hypsochromic) and longer wavelength (bathochromic) light absorption shifts producing the “spectral tuning” of cone pigments. The protonated Schiff base linkage of 11-*cis*-retinal with opsin [7, 10, 11] is critical for specifically tuning its spectral absorbance.

Exposure of rhodopsin to light leads to the highly unstable intermediates metarhodopsin I (Meta I) and metarhodopsin II (Meta II) that achieve an equilibrium between these two states within milliseconds [7]. Meta II is the signaling form of rhodopsin that subsequently interacts with the G protein transducin, rhodopsin kinase (GRK1), and arrestin (reviewed in ref. 12, 13).

Though rhodopsin has been studied by almost all molecular techniques, there is still more to discover. Our level of understanding increases as novel approaches are developed. With its exquisite sensitivity to detect a single photon of light in a highly reproducible way, rhodopsin provides our scotopic window to the world. As such, rhodopsin comprises the center of our interest, and hopefully this series of articles will provide inspiration for pursuing all remaining unanswered questions about this molecular complex.

2 Expression Systems

The rhodopsin transcript is among the most highly expressed in the eye and retina, accounting for 9,114 and 11,745 normalized fragments per kilobase of exon per million mapped reads (FPKM), respectively [14]. The retina is a neuronal tissue composed of several cell types but rods constitute about 80 % (or about 10^8 photoreceptor cells) of the cells in the human, mouse, and bovine retina [15, 16]. Once expressed, rhodopsin is transported to and inserted in elongated cilia called rod outer segments (ROS), which consist of stacks of 600–1,600 independent disk membranes surrounded by a plasma membrane. Rhodopsin is the major protein in rod outer segment membranes (>90 % with a 5 mM concentration within ROS) [17]. This high abundance in membranes of a native source was initially one of the main attractions of this GPCR. The amount of material isolated from just one bovine retina was about 0.5–1 mg of protein [18]. The native protein also lacked any artifacts generated by heterologous expression systems (such as changes in posttranslational modifications), making the study of native rhodopsin highly relevant to mammalian/human physiology. Expression of this protein in other model systems was also needed to probe its structure using mutagenesis, but the key to these approaches was rhodopsin’s reliable expression and purification. Toward this goal the most critical work was pioneered by Oprian and colleagues [19]. A number of mutagenesis studies followed, including spin labeling of Cys residues throughout the rhodopsin structure [20] and employment of

unnatural amino acids to obtain structural information by the Sakmar group [21, 22]. Today, rhodopsin can be expressed in heterologous systems ranging from transformed cells to whole organisms such as *Caenorhabditis elegans* [23]. Because in heterologous systems rhodopsin can couple to Go/I, illumination causes a sudden and transient loss of worm motility dependent on cyclic adenosine monophosphate [24].

3 Three-Dimensional Structure of Rhodopsin

The high expression level and newly developed purification methods for rhodopsin led to the first crystallization of any GPCR [25]. For the first time, a single study revealed the internal organization of rhodopsin at amino acid resolution. Much has been written about the structure of rhodopsin as an archetypical membrane-bound GPCR [12, 18, 20, 26 – 28], and there is no need to repeat it here. As predicted, rhodopsin is composed of seven-transmembrane α-helical segments embedded in the plasma membrane with an almost equally distributed mass between the extracellular (intradiscal) and intracellular domains. The chromophore is embedded in the hydrophobic region, about 2/3 of the way from the cytoplasmic surface (Fig. 1). Many other GPCR structures followed that of rhodopsin crystallized under different conditions or as photoactivated intermediate states [29 – 42] (recently reviewed in ref. 43).

4 Posttranslational Modifications of Rhodopsin

The amino acid sequence of opsin was determined by the laboratories of Ovchinnikov [44] and Hargrave [45]. It was noted that rhodopsin's predicted topology resembles that of bacteriorhodopsin [44]. Once the sequence was obtained, it became possible to assemble the seven-transmembrane helix topology and posttranslational modifications of this protein required for its function (Figs. 2, 3, 4, 5, and 6).

4.1 Disulfide Bridge

The primary sequences of GPCRs are highly diverse [46] but structurally very similar [43], with frequently conserved specific features. One of these is the extracellular disulfide bridge that connects loop II to helix III (Fig. 2) [47]. This bridge between Cys-110 and Cys-187 is essential for the correct tertiary structure of the protein [48, 49]. In rhodopsin, this part also forms a "plug" underneath the chromophore. When this disulfide bridge is formed remains to be determined, so it could be a co-translational rather than a posttranslational modification.

4.2 Palmitoylation and Acylation

Among class A GPCRs, most contain single- and double-Cys residues at the end of cytoplasmic helix 8 that are frequently, if not always, palmitoylated. Rhodopsin is double palmitoylated (Fig. 3) [50, 51]. The palmitoylated Cys residues are close to the NPxxY region, which suggests they are important for activation. Separate in vivo studies indicate they are also important for the structural integrity of the protein [52]. It is unclear if S-palmitoylation is an enzymatic or nonenzymatic reaction in vivo. In addition to S-acylation at these Cys residues, the N-terminus is acetylated as well (Fig. 4).

4.3 Glycosylation

Glycosylation of family A GPCRs usually occurs at the N-terminal end and extracellular side of these receptors. As in other GPCRs, rhodopsin is glycosylated at the (N-X-S/T) site or, more precisely, at the two Asn2 and Asn15 residues located within the N-terminal region [53 – 55] (Fig. 4). N-terminal glycosylation, especially at Asn15, is crucial for proper folding and function of rhodopsin [53]. Furthermore, it has been reported that the N15S mutation causes autosomal dominant retinitis pigmentosa in humans due to the lack of glycosylation [56]. Thus, glycosylation of rhodopsin and members of family A GPCRs in general is essential for the transportation and function of these receptors. It was believed that the core structure of $(Man)_3(GlcNAc)_2$ is fairly uniform [57], but recently more sensitive methods have revealed some heterogeneity of the glycosylation modifications at both sites [58].

5 Regeneration with *Cis*-Chromophores

Rhodopsin forms a permanent Schiff base linkage with only some *cis*-retinals. Though the native chromophore is 11-*cis*-retinal (Fig. 5), visual pigment in biochemical assays can be formed with 9-*cis*-retinal (isorhodopsin), 7-*cis*-retinal, and some of the double *cis*-retinals, but not with 13-*cis*-retinal. Many retinal analogs have been successfully used to probe rhodopsin photoactivation (e.g., the desmethyl series) [59, 60]. All-*trans*-retinal only increased the basal activity of opsin, but the mechanism is unknown [61]. Regeneration with 9-*cis*-retinal (or derivatives) could have clinical applications when the visual cycle is non-functional as in Leber congenital amaurosis (LCA) [62].

6 Phosphorylation

Rhodopsin phosphorylation was accidently discovered in 1972–1973 when rod outer membranes were incubated with radioactive γ-^{32} PATP (reviewed in ref. 63). Today, we know that this is one of the major desensitizing mechanisms of GPCRs. One of the first applications of mass spectrometry in vision research [64], in combination with a specific cleavage of rhodopsin at the C-terminal region by Asp endopeptidase, provided information as to the major site of phosphorylation [65]. Hurley and colleagues showed that photoactivated rhodopsin is repeatedly phosphorylated and dephosphorylated in an ordered fashion [66, 67]. All phosphorylation sites are located in the C-terminal region of rhodopsin. The phosphorylated molecules include Ser334, Ser338, and Ser343 (Fig. 6). Phosphorylation is strictly dependent on photoactivation of rhodopsin and multiple sites can be phosphorylated in photoactivated rhodopsin, contributing to subsequent recognition by arrestin.

7 Photoactivation Mechanism of Rhodopsin

Conformational changes in the opsin moiety occur after rhodopsin is activated by light and the chromophore is isomerized from 11-*cis*-retinylidene to all-*trans*-retinylidene. Generally, these changes were much smaller than anticipated from biophysical studies prior to X-ray crystallography and found mostly in the area of the cytoplasmic end of helix VI (reviewed in ref. 29). Based on solid state NMR data, Brown and colleagues proposed a multiple step

activation mechanism and reported helix fluctuations in the Meta I-Meta II equilibrium on a microsecond-to-millisecond timescale [68]. This proposal would simply suggest that rhodopsin becomes more flexible during the activation process, allowing formation of new productive complexes with partner proteins. Perhaps small conformational changes, changes in protonation of the transmembrane and cytoplasmic residues, and an increase in overall dynamics is how rhodopsin achieves a conformation that can induce a specific fit with prebound transducin. Subsequent nucleotide exchange on the α-subunit of the G protein would then activate the visual cascade.

7.1 Water Molecules

Water molecules, perhaps as many as 30, are integral components of rhodopsin. Identified by various methods, these are located within the transmembrane segment of rhodopsin and some are exchangeable with bulk water. However, many are not and likely were incorporated during biogenesis and inserted into the membrane of rhodopsin [69, 70]. Internal waters are located within a cavity that extends from the chromophore to the cytoplasmic surface (Fig. 7). Water is also required for chromophore hydrolysis from all-*trans*-retinylidene [71, 72]. Water is a critical element for the activation process [73] and is involved in multiple steps, including the protonation and deprotonation of key intermolecular sites within the core and cytoplasmic surface of rhodopsin [74]. Importantly, internal water is conserved among all GPCRs, suggesting a universal role for these prosthetic-like groups in receptor activation [75].

8 Conformationally Sensitive Regions

Three regions in rhodopsin were Identified that are critical for photoactivation (Fig. 8). All protein and water molecule changes are initiated by chromophore isomerization. This signal is propagated to two independent surface regions, namely, the DRY and NPxxY regions [76]. The latter are also conserved regions among GPCRs, suggesting some commonality in the activation mechanism among these receptors.

9 Human Diseases Associated with Mutations in the Opsin Gene

Mutations in the opsin gene can cause a hereditary retinal degenerative disease called retinitis pigmentosa (RP) (RetNet, https://sph.uth.edu/RetNet/) [77]. RP is manifested by progressively decreased vision under low light and loss of peripheral visual fields [78, 79]. To date, more than 100 mutations were Identified to be associated with autosomal dominant RP (30–40 % of all cases) [79]. In contrast, the c.448G > A (p.E150K) mutation and severe truncation of the opsin gene are inherited in an autosomal recessive pattern [80, 81]. These inherited diseases remain without a cure, and active research is ongoing to retain the vision and stop the progression of retinal degeneration of those affected [82, 83].

10 Future Directions

In the opinion of these authors, there are five crucial areas for research that have yet to be fully pursued. Judging from the great interest in this receptor, it is only a question of time when a fuller picture of how rhodopsin works will become available.

There is a need to understand how rhodopsin specifically interacts with the G protein transducin, rhodopsin kinase, and arrestin. Although some low resolution studies have been accomplished [84], the most informative would be X-ray structures of these complexes followed by their biophysical probing. No single structure will be fully informative for any of these complexes, as it would represent only one stable conformation trapped in extremely high concentrations of a precipitating agent. But such structures will set the boundaries for possible conformational changes of this receptor. Several of these structures would provide an even fuller picture and possibly the mechanism of activation of these partner proteins. NMR methods could also add much more information about the dynamics of these complexes.

Rhodopsin is a highly dynamic, chromophore-bound protein with intrinsic water molecules. How this receptor and these waters reorganize during activation needs to be solved. Perhaps a combination of computational [85, 86] and NMR studies [68, 87] will dominate in this area of investigation.

Like almost all other GPCRs, rhodopsin forms oligomers in native membranes [88 – 90]. Here, two questions remain as top priorities. One is how are these rhodopsin molecules specifically arranged in rod outer segment membranes? It is unclear which helices of rhodopsin are involved and form complexes. Perhaps recently developed methodologies [91] will provide tools to answer these questions and provide thermodynamic parameters for these interactions along with their specificities. The measured Kd between two opsin molecules was about 10^{-5} M [91]. Second, what are functional consequences of rhodopsin oligomerization? Improved tools combined with knowledge derived from previous reports [92, 93] are needed to answer this question.

Comparative studies between rhodopsin and cone visual pigments are needed to understand the spectral tuning of these pigments, which use a common chromophore. Again, the first step could involve X-ray crystallography to obtain and analyze the structure of these pigments.

And finally, pharmacological and genetic rescue of mutant rhodopsin molecules should be anticipated. Toward this goal, proper animal models must be generated, as has already been achieved recently with two informative mutations of this receptor [94 – 96]. Taken together, innovative approaches could bring an end to blinding diseases caused by mutations in the opsin genes.

Thus, there remain many challenges, and much needs to be accomplished!

Acknowledgments

We thank Dr. Leslie T. Webster, Jr., and the members of Palczewski's laboratory for their comments on the manuscript. The work was supported by funding from the National Eye Institute, National Institutes of Health Grants R01EY008061 (to K.P.). K.P. is the John H. Hord Professor of Pharmacology.

References

1. Kuhne W. Chemical processes in the retina. Vision Res. 1977; 17:1269–1316. [PubMed: 345610]

2. Salom D, Le Trong I, Pohl E, et al. Improvements in G protein-coupled receptor purification yield light stable rhodopsin crystals. J Struct Biol. 2006; 156:497–504. [PubMed: 16837211]
3. Salom D, Lodowski DT, Stenkamp RE, et al. Crystal structure of a photoactivated deprotonated intermediate of rhodopsin. Proc Natl Acad Sci U S A. 2006; 103:16123–16128. [PubMed: 17060607]
4. Kiser PD, Golczak M, Palczewski K. Chemistry of the retinoid (visual) cycle. Chem Rev. 2014; 114:194–232. [PubMed: 23905688]
5. Wald G. Molecular basis of visual excitation. Science. 1968; 162:230–239. [PubMed: 4877437]
6. Wald G. Carotenoids and the visual cycle. J Gen Physiol. 1935; 19:351–371. [PubMed: 19872932]
7. Hubbard R, Wald G. Cis-trans isomers of vitamin A and retinene in the rhodopsin system. J Gen Physiol. 1952; 36:269–315. [PubMed: 13011282]
8. Wald G, Brown PK. The molar extinction of rhodopsin. J Gen Physiol. 1953; 37:189–200. [PubMed: 13109155]
9. Matthews RG, Hubbard R, Brown PK, et al. Tautomeric forms of metarhodopsin. J Gen Physiol. 1963; 47:215–240. [PubMed: 14080814]
10. Zhukovsky EA, Robinson PR, Oprian DD. Changing the location of the Schiff base counterion in rhodopsin. Biochemistry. 1992; 31:10400–10405. [PubMed: 1329948]
11. Zvyaga TA, Min KC, Beck M, et al. Movement of the retinylidene Schiff base counterion in rhodopsin by one helix turn reverses the pH dependence of the metarhodopsin I to metarhodopsin II transition. J Biol Chem. 1993; 268:4661–4667. [PubMed: 8444840]
12. Palczewski K. G protein-coupled receptor rhodopsin. Annu Rev Biochem. 2006; 75:743–767. [PubMed: 16756510]
13. Palczewski K. Chemistry and biology of vision. J Biol Chem. 2012; 287:1612–1619. [PubMed: 22074921]
14. Mustafi D, Maeda T, Kohno H, et al. Inflammatory priming predisposes mice to age-related retinal degeneration. J Clin Invest. 2012; 122:2989–3001. [PubMed: 22797304]
15. Jeon CJ, Strettoi E, Masland RH. The major cell populations of the mouse retina. J Neurosci. 1998; 18:8936–8946. [PubMed: 9786999]
16. Curcio CA, Sloan KR Jr, Packer O, et al. Distribution of cones in human and monkey retina: individual variability and radial asymmetry. Science. 1987; 236:579–582. [PubMed: 3576186]
17. Nickell S, Park PS, Baumeister W, et al. Three-dimensional architecture of murine rod outer segments determined by cryoelectron tomography. J Cell Biol. 2007; 177:917–925. [PubMed: 17535966]
18. Filipek S, Stenkamp RE, Teller DC, et al. G protein-coupled receptor rhodopsin: a prospectus. Annu Rev Physiol. 2003; 65:851–879. [PubMed: 12471166]
19. Oprian DD, Molday RS, Kaufman RJ, et al. Expression of a synthetic bovine rhodopsin gene in monkey kidney cells. Proc Natl Acad Sci U S A. 1987; 84:8874–8878. [PubMed: 2962193]
20. Hubbell WL, Altenbach C, Hubbell CM, et al. Rhodopsin structure, dynamics, and activation: a perspective from crystallography, site-directed spin labeling, sulfhydryl reactivity, and disulfide cross-linking. Adv Protein Chem. 2003; 63:243–290. [PubMed: 12629973]
21. Grunbeck A, Huber T, Abrol R, et al. Genetically encoded photo-cross-linkers map the binding site of an allosteric drug on a G protein-coupled receptor. ACS Chem Biol. 2012; 7:967–972. [PubMed: 22455376]
22. Daggett KA, Sakmar TP. Site-specific in vitro and in vivo incorporation of molecular probes to study G-protein-coupled receptors. Curr Opin Chem Biol. 2011; 15:392–398. [PubMed: 21571577]
23. Salom D, Cao P, Sun W, et al. Heterologous expression of functional G-protein-coupled receptors in Caenorhabditis elegans. FASEB J. 2012; 26:492–502. [PubMed: 22090314]
24. Cao P, Sun W, Kramp K, et al. Light-sensitive coupling of rhodopsin and melanopsin to G(i/o) and G(q) signal transduction in Caenorhabditis elegans. FASEB J. 2012; 26:480–491. [PubMed: 22090313]
25. Palczewski K, Kumasaka T, Hori T, et al. Crystal structure of rhodopsin: a G protein-coupled receptor. Science. 2000; 289:739–745. [PubMed: 10926528]

26. Teller DC, Okada T, Behnke CA, et al. Advances in determination of a high-resolution three-dimensional structure of rhodopsin, a model of G-protein-coupled receptors (GPCRs). Biochemistry. 2001; 40:7761–7772. [PubMed: 11425302]
27. Menon ST, Han M, Sakmar TP. Rhodopsin: structural basis of molecular physiology. Physiol Rev. 2001; 81:1659–1688. [PubMed: 11581499]
28. Smith SO. Structure and activation of the visual pigment rhodopsin. Annu Rev Biophys. 2010; 39:309–328. [PubMed: 20192770]
29. Park JH, Morizumi T, Li Y, et al. Opsin, a structural model for olfactory receptors? Angew Chem Int Ed Engl. 2013; 52:11021–11024. [PubMed: 24038729]
30. Choe HW, Kim YJ, Park JH, et al. Crystal structure of metarhodopsin II. Nature. 2011; 471:651–655. [PubMed: 21389988]
31. Scheerer P, Park JH, Hildebrand PW, et al. Crystal structure of opsin in its G-protein-interacting conformation. Nature. 2008; 455:497–502. [PubMed: 18818650]
32. Park JH, Scheerer P, Hofmann KP, et al. Crystal structure of the ligand-free G-protein-coupled receptor opsin. Nature. 2008; 454:183–187. [PubMed: 18563085]
33. Park JH, Krauss N, Pulvermuller A, et al. Crystallization and preliminary X-ray studies of mouse centrin1. Acta Crystallogr Sect F Struct Biol Cryst Commun. 2005; 61:510–513.
34. Singhal A, Ostermaier MK, Vishnivetskiy SA, et al. Insights into congenital stationary night blindness based on the structure of G90D rhodopsin. EMBO Rep. 2013; 14:520–526. [PubMed: 23579341]
35. Deupi X, Edwards P, Singhal A, et al. Stabilized G protein binding site in the structure of constitutively active metarhodopsin-II. Proc Natl Acad Sci U S A. 2012; 109:119–124. [PubMed: 22198838]
36. Standfuss J, Xie G, Edwards PC, et al. Crystal structure of a thermally stable rhodopsin mutant. J Mol Biol. 2007; 372:1179–1188. [PubMed: 17825322]
37. Li J, Edwards PC, Burghammer M, et al. Structure of bovine rhodopsin in a trigonal crystal form. J Mol Biol. 2004; 343:1409–1438. [PubMed: 15491621]
38. Nakamichi H, Okada T. X-ray crystallographic analysis of 9-cis-rhodopsin, a model analogue visual pigment. Photochem Photobiol. 2007; 83:232–235. [PubMed: 17576343]
39. Schreiber M, Sugihara M, Okada T, et al. Quantum mechanical studies on the crystallographic model of bathorhodopsin. Angew Chem Int Ed Engl. 2006; 45:4274–4277. [PubMed: 16729349]
40. Nakamichi H, Okada T. Local peptide movement in the photoreaction intermediate of rhodopsin. Proc Natl Acad Sci U S A. 2006; 103:12729–12734. [PubMed: 16908857]
41. Okada T, Sugihara M, Bondar AN, et al. The retinal conformation and its environment in rhodopsin in light of a new 2.2 A crystal structure. J Mol Biol. 2004; 342:571–583. [PubMed: 15327956]
42. Nakamichi H, Buss V, Okada T. Photoisomerization mechanism of rhodopsin and 9-cis-rhodopsin revealed by x-ray crystallography. Biophys J. 2007; 92:L106–L108. [PubMed: 17449675]
43. Palczewski K, Orban T. From atomic structures to neuronal functions of g protein-coupled receptors. Annu Rev Neurosci. 2013; 36:139–164. [PubMed: 23682660]
44. Ovchinnikov YA. Structure of rhodopsin and bacteriorhodopsin. Photochem Photobiol. 1987; 45:909–914. [PubMed: 3306728]
45. Hargrave PA. Rhodopsin structure, function, and topography the Friedenwald lecture. Invest Ophthalmol Vis Sci. 2001; 42:3–9. [PubMed: 11133841]
46. Mirzadegan T, Benko G, Filipek S, et al. Sequence analyses of G-protein-coupled receptors: similarities to rhodopsin. Biochemistry. 2003; 42:2759–2767. [PubMed: 12627940]
47. Applebury ML, Hargrave PA. Molecular biology of the visual pigments. Vision Res. 1986; 26:1881–1895. [PubMed: 3303660]
48. Karnik SS, Sakmar TP, Chen HB, et al. Cysteine residues 110 and 187 are essential for the formation of correct structure in bovine rhodopsin. Proc Natl Acad Sci U S A. 1988; 85:8459–8463. [PubMed: 3186735]
49. Karnik SS, Khorana HG. Assembly of functional rhodopsin requires a disulfide bond between cysteine residues 110 and 187. J Biol Chem. 1990; 265:17520–17524. [PubMed: 2145276]

50. Papac DI, Thornburg KR, Bullesbach EE, et al. Palmitylation of a G-protein coupled receptor. Direct analysis by tandem mass spectrometry. J Biol Chem. 1992; 267:16889–16894. [PubMed: 1512231]
51. Ovchinnikov YA, Abdulaev NG, Bogachuk AS. Two adjacent cysteine residues in the C-terminal cytoplasmic fragment of bovine rhodopsin are palmitylated. FEBS Lett. 1988; 230:1–5. [PubMed: 3350146]
52. Maeda A, Okano K, Park PS, et al. Palmitoylation stabilizes unliganded rod opsin. Proc Natl Acad Sci U S A. 2010; 107:8428–8433. [PubMed: 20404157]
53. Kaushal S, Ridge KD, Khorana HG. Structure and function in rhodopsin: the role of asparagine-linked glycosylation. Proc Natl Acad Sci U S A. 1994; 91:4024–4028. [PubMed: 8171029]
54. Wheatley M, Wootten D, Conner MT, et al. Lifting the lid on GPCRs: the role of extracellular loops. Br J Pharmacol. 2012; 165:1688–1703. [PubMed: 21864311]
55. Wheatley M, Hawtin SR. Glycosylation of G-protein-coupled receptors for hormones central to normal reproductive functioning: its occurrence and role. Hum Reprod Update. 1999; 5:356–364. [PubMed: 10465525]
56. Kranich H, Bartkowski S, Denton MJ, et al. Autosomal dominant ‘sector’ retinitis pigmentosa due to a point mutation predicting an Asn-15-Ser substitution of rhodopsin. Hum Mol Genet. 1993; 2:813–814. [PubMed: 8353500]
57. Fukuda MN, Papermaster DS, Hargrave PA. Rhodopsin carbohydrate. Structure of small oligosaccharides attached at two sites near the NH2 terminus. J Biol Chem. 1979; 254:8201–8207. [PubMed: 468821]
58. Salom D, Wang B, Dong Z, et al. Posttranslational modifications of the serotonin type 4 receptor heterologously expressed in mouse rod cells. Biochemistry. 2012; 51:214–224. [PubMed: 22145929]
59. Crouch RK. Studies of rhodopsin and bacteriorhodopsin using modified retinals. Photochem Photobiol. 1986; 44:803–807. [PubMed: 3550831]
60. Estevez ME, Kolesnikov AV, Ala-Laurila P, et al. The 9-methyl group of retinal is essential for rapid Meta II decay and phototransduction quenching in red cones. J Gen Physiol. 2009; 134:137–150. [PubMed: 19635855]
61. Buczylko J, Saari JC, Crouch RK, et al. Mechanisms of opsin activation. J Biol Chem. 1996; 271:20621–20630. [PubMed: 8702809]
62. Palczewski K. Retinoids for treatment of retinal diseases. Trends Pharmacol Sci. 2010; 31:284–295. [PubMed: 20435355]
63. Maeda T, Imanishi Y, Palczewski K. Rhodopsin phosphorylation, 30 years later. Prog Retin Eye Res. 2003; 22:417–434. [PubMed: 12742390]
64. Ohguro H, Palczewski K, Ericsson LH, et al. Sequential phosphorylation of rhodopsin at multiple sites. Biochemistry. 1993; 32:5718–5724. [PubMed: 8504090]
65. Palczewski K, Buczylko J, Kaplan MW, et al. Mechanism of rhodopsin kinase activation. J Biol Chem. 1991; 266:12949–12955. [PubMed: 2071581]
66. Kennedy MJ, Lee KA, Niemi GA, et al. Multiple phosphorylation of rhodopsin and the in vivo chemistry underlying rod photoreceptor dark adaptation. Neuron. 2001; 31:87–101. [PubMed: 11498053]
67. Lee KA, Nawrot M, Garwin GG, et al. Relationships among visual cycle retinoids, rhodopsin phosphorylation, and phototransduction in mouse eyes during light and dark adaptation. Biochemistry. 2010; 49:2454–2463. [PubMed: 20155952]
68. Struts AV, Salgado GF, Martinez-Mayorga K, et al. Retinal dynamics underlie its switch from inverse agonist to agonist during rhodopsin activation. Nat Struct Mol Biol. 2011; 18:392–394. [PubMed: 21278756]
69. Renthal R. Buried water molecules in helical transmembrane proteins. Protein Sci. 2008; 17:293–298. [PubMed: 18096637]
70. Buss V, Sugihara M, Entel P, et al. Thr94 and Wat2b effect protonation of the retinal chromophore in rhodopsin. Angew Chem Int Ed Engl. 2003; 42:3245–3247. [PubMed: 12876733]
71. Jastrzebska B, Palczewski K, Golczak M. Role of bulk water in hydrolysis of the rhodopsin chromophore. J Biol Chem. 2011; 286:18930–18937. [PubMed: 21460218]

72. Padayatti PS, Wang L, Gupta S, et al. A hybrid structural approach to analyze ligand binding by the serotonin type 4 receptor (5-HT4). Mol Cell Proteomics. 2013; 12:1259–1271. [PubMed: 23378516]
73. Angel TE, Gupta S, Jastrzebska B, et al. Structural waters define a functional channel mediating activation of the GPCR, rhodopsin. Proc Natl Acad Sci U S A. 2009; 106:14367–14372. [PubMed: 19706523]
74. Hofmann KP, Scheerer P, Hildebrand PW, et al. A G protein-coupled receptor at work: the rhodopsin model. Trends Biochem Sci. 2009; 34:540–552. [PubMed: 19836958]
75. Angel TE, Chance MR, Palczewski K. Conserved waters mediate structural and functional activation of family A (rhodopsin-like) G protein-coupled receptors. Proc Natl Acad Sci U S A. 2009; 106:8555–8560. [PubMed: 19433801]
76. Fritze O, Filipek S, Kuksa V, et al. Role of the conserved NPxxY(x)5,6F motif in the rhodopsin ground state and during activation. Proc Natl Acad Sci U S A. 2003; 100:2290–2295. [PubMed: 12601165]
77. Dryja TP, McGee TL, Reichel E, et al. A point mutation of the rhodopsin gene in one form of retinitis pigmentosa. Nature. 1990; 343:364–366. [PubMed: 2137202]
78. Nathans J. Rhodopsin: structure, function, and genetics. Biochemistry. 1992; 31:4923–4931. [PubMed: 1599916]
79. Malanson KM, Lem J. Rhodopsin-mediated retinitis pigmentosa. Prog Mol Biol Transl Sci. 2009; 88:1–31. [PubMed: 20374723]
80. Rosenfeld PJ, Cowley GS, McGee TL, et al. A null mutation in the rhodopsin gene causes rod photoreceptor dysfunction and autosomal recessive retinitis pigmentosa. Nat Genet. 1992; 1:209–213. [PubMed: 1303237]
81. Kumaramanickavel G, Maw M, Denton MJ, et al. Missense rhodopsin mutation in a family with recessive RP. Nat Genet. 1994; 8:10–11. [PubMed: 7987385]
82. Hamel C. Retinitis pigmentosa. Orphanet J Rare Dis. 2006; 1:40. [PubMed: 17032466]
83. Hartong DT, Berson EL, Dryja TP. Retinitis pigmentosa. Lancet. 2006; 368:1795–1809. [PubMed: 17113430]
84. Jastrzebska B, Ringler P, Lodowski DT, et al. Rhodopsin-transducin heteropentamer: three-dimensional structure and biochemical characterization. J Struct Biol. 2011; 176:387–394. [PubMed: 21925606]
85. Sekharan S, Morokuma K. QM/MM study of the structure, energy storage, and origin of the bathochromic shift in vertebrate and invertebrate bathorhodopsins. J Am Chem Soc. 2011; 133:4734–4737. [PubMed: 21391708]
86. Gonzalez-Luque R, Garavelli M, Bernardi F, et al. Computational evidence in favor of a two-state, two-mode model of the retinal chromophore photoisomerization. Proc Natl Acad Sci U S A. 2000; 97:9379–9384. [PubMed: 10944211]
87. Mertz B, Struts AV, Feller SE, et al. Molecular simulations and solid-state NMR investigate dynamical structure in rhodopsin activation. Biochim Biophys Acta. 2012; 1818:241–251. [PubMed: 21851809]
88. Liang Y, Fotiadis D, Maeda T, et al. Rhodopsin signaling and organization in heterozygote rhodopsin knockout mice. J Biol Chem. 2004; 279:48189–48196. [PubMed: 15337746]
89. Fotiadis D, Liang Y, Filipek S, et al. Atomic-force microscopy: rhodopsin dimers in native disc membranes. Nature. 2003; 421:127–128. [PubMed: 12520290]
90. Park PS, Filipek S, Wells JW, et al. Oligomerization of G protein-coupled receptors: past, present, and future. Biochemistry. 2004; 43:15643–15656. [PubMed: 15595821]
91. Comar WD, Schubert SM, Jastrzebska B, et al. Time-resolved fluorescence spectroscopy measures clustering and mobility of a G protein-coupled receptor opsin in live cell membranes. J Am Chem Soc. 2014; 136:8342–8349. [PubMed: 24831851]
92. Fotiadis D, Jastrzebska B, Philippsen A, et al. Structure of the rhodopsin dimer: a working model for G-protein-coupled receptors. Curr Opin Struct Biol. 2006; 16:252–259. [PubMed: 16567090]
93. Jastrzebska B, Orban T, Golczak M, et al. Asymmetry of the rhodopsin dimer in complex with transducin. FASEB J. 2013; 27:1572–1584. [PubMed: 23303210]

94. Sakami S, Maeda T, Bereta G, et al. Probing mechanisms of photoreceptor degeneration in a new mouse model of the common form of autosomal dominant retinitis pigmentosa due to P23H opsin mutations. J Biol Chem. 2011; 286:10551–10567. [PubMed: 21224384]

95. Sakami S, Kolesnikov AV, Kefalov VJ, et al. P23H opsin knock-in mice reveal a novel step in retinal rod disc morphogenesis. Hum Mol Genet. 2013; 23:1723–1741. [PubMed: 24214395]

96. Zhang N, Kolesnikov AV, Jastrzebska B, et al. Autosomal recessive retinitis pigmentosa E150K opsin mice exhibit photoreceptor disorganization. J Clin Invest. 2013; 123:121–137. [PubMed: 23221340]

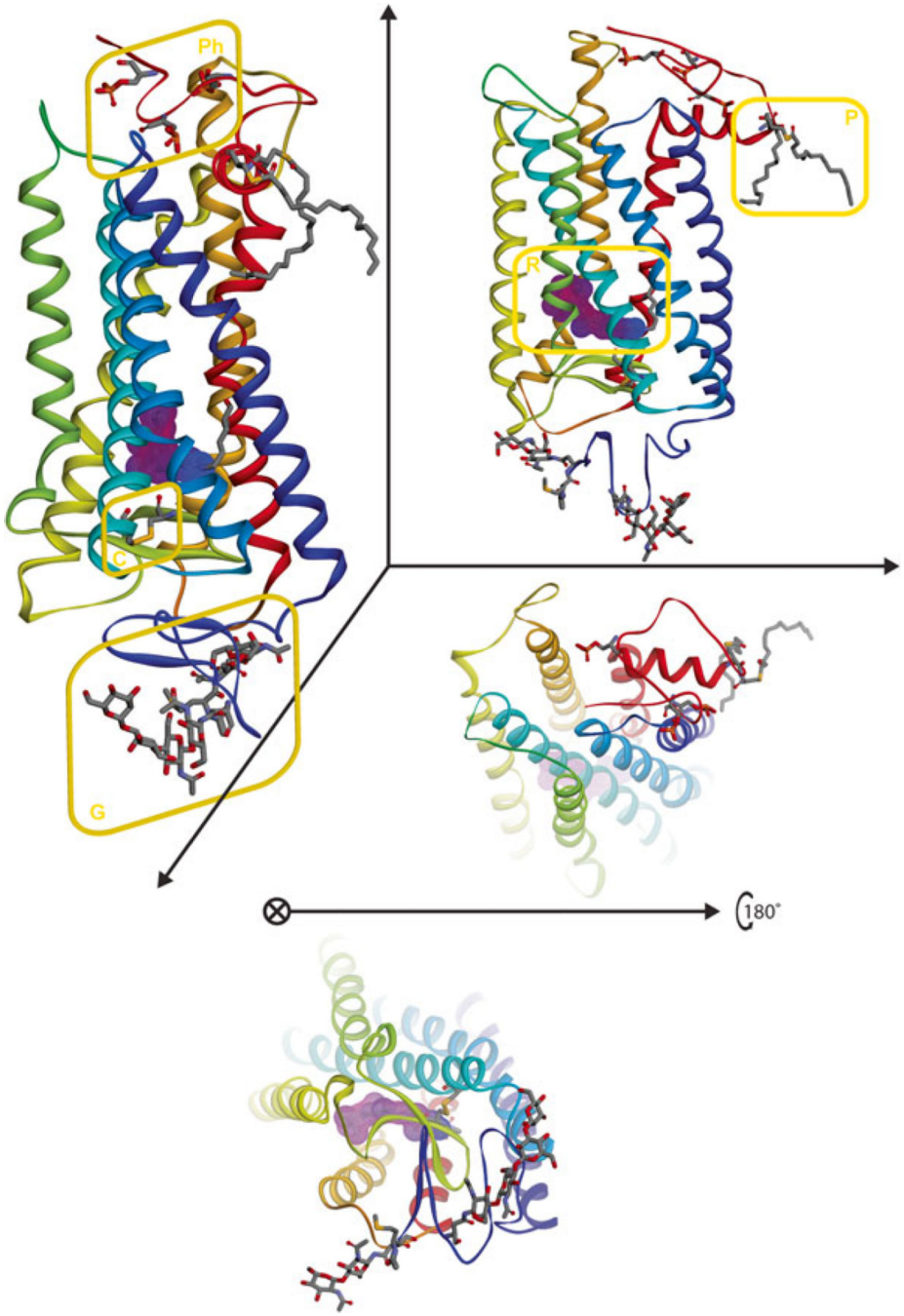

Fig. 1.

Three-dimensional structure of rhodopsin. Rhodopsin is depicted in a perspective with *x*, *y*, and *z* axes with structures colored in *blue* to *red* from the N- to C-termini in a ribbon representation. Posttranslational modifications are highlighted with *yellow panels. P* palmitoylation, *R* 11-*cis*-N-retinylidene-Lys, *Ph* phosphorylation, *C* disulfide bond, and *G* glycosylation

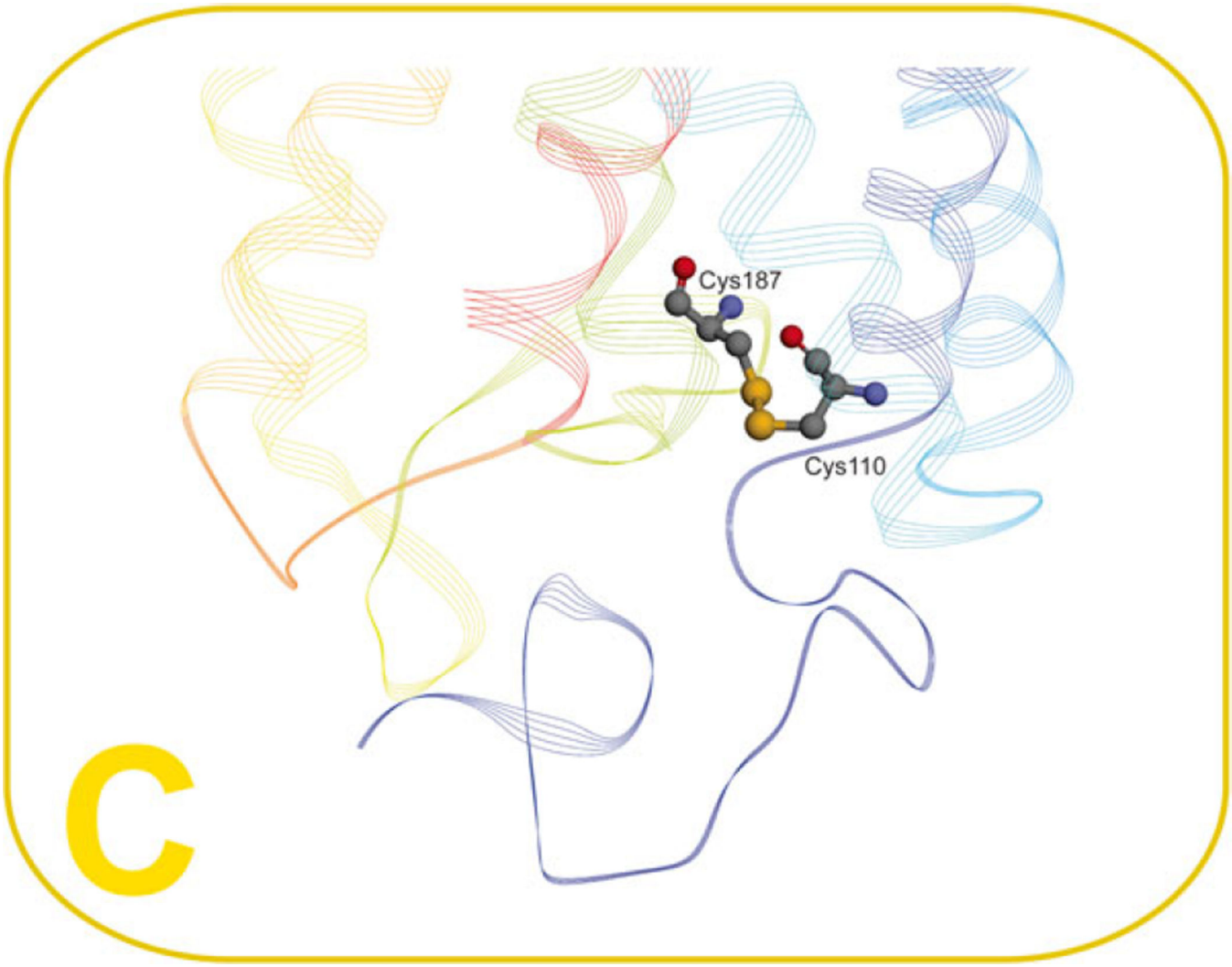

Fig. 2.

Conserved disulfide bonds in rhodopsin. Conserved disulfide bonds are found in many family A GPCRs between Cys187 and Cys110. Rhodopsin is colored in *blue* to *red* from N- to the C-terminus in a wire representation. Cys residues are shown in a *scaled ball* and *stick* representation according to element color

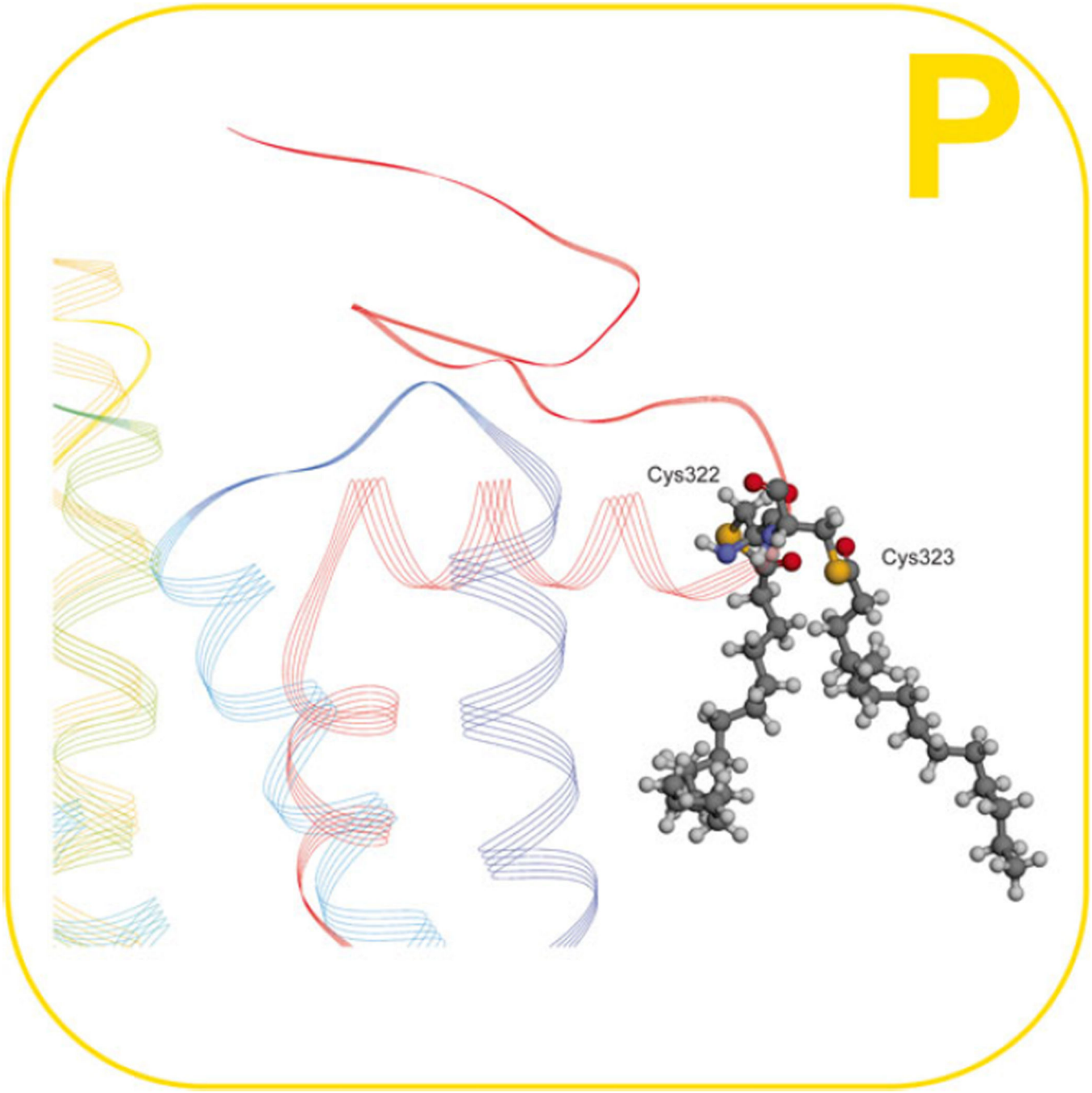

Fig. 3.

Palmitoylation sites on rhodopsin. Palmitoylation of rhodopsin takes place at the C-terminus on Cys322 and Cys323 portrayed in a s *caled ball stick* representation according to element colors. Rhodopsin is colored in *blue* to *red* from N- to the C-terminus in a *wire* representation

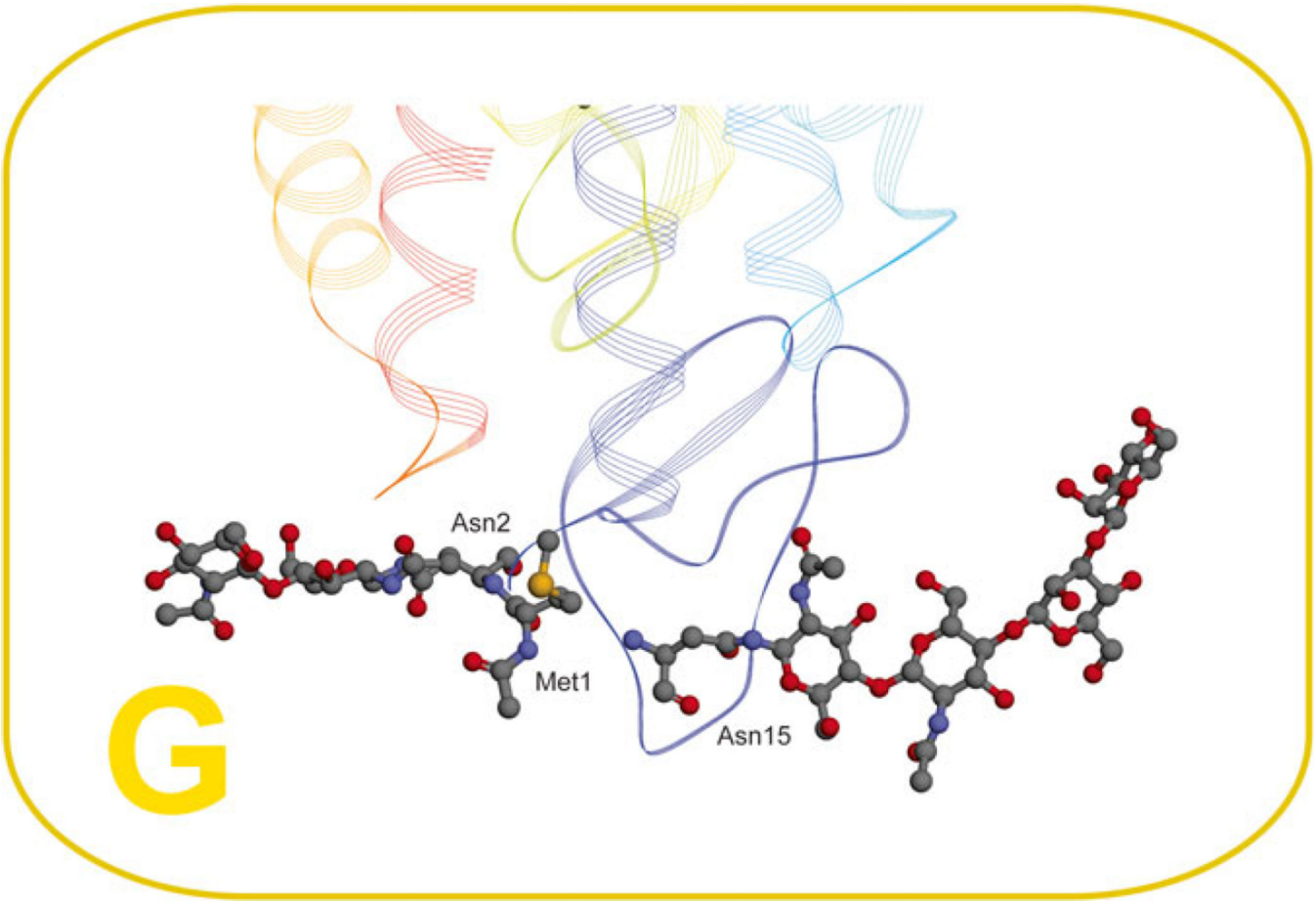

Fig. 4.

Glycosylation sites on rhodopsin. Glycosylation sites on rhodopsin are located at Asn2 and Asn 15 of the N-terminus. The N-terminal Met1 is acetylated and depicted in a *scaled ball stick* representation according to element colors. Rhodopsin is colored in *blue* to *red* from the N- to C-terminus in a *wire* representation

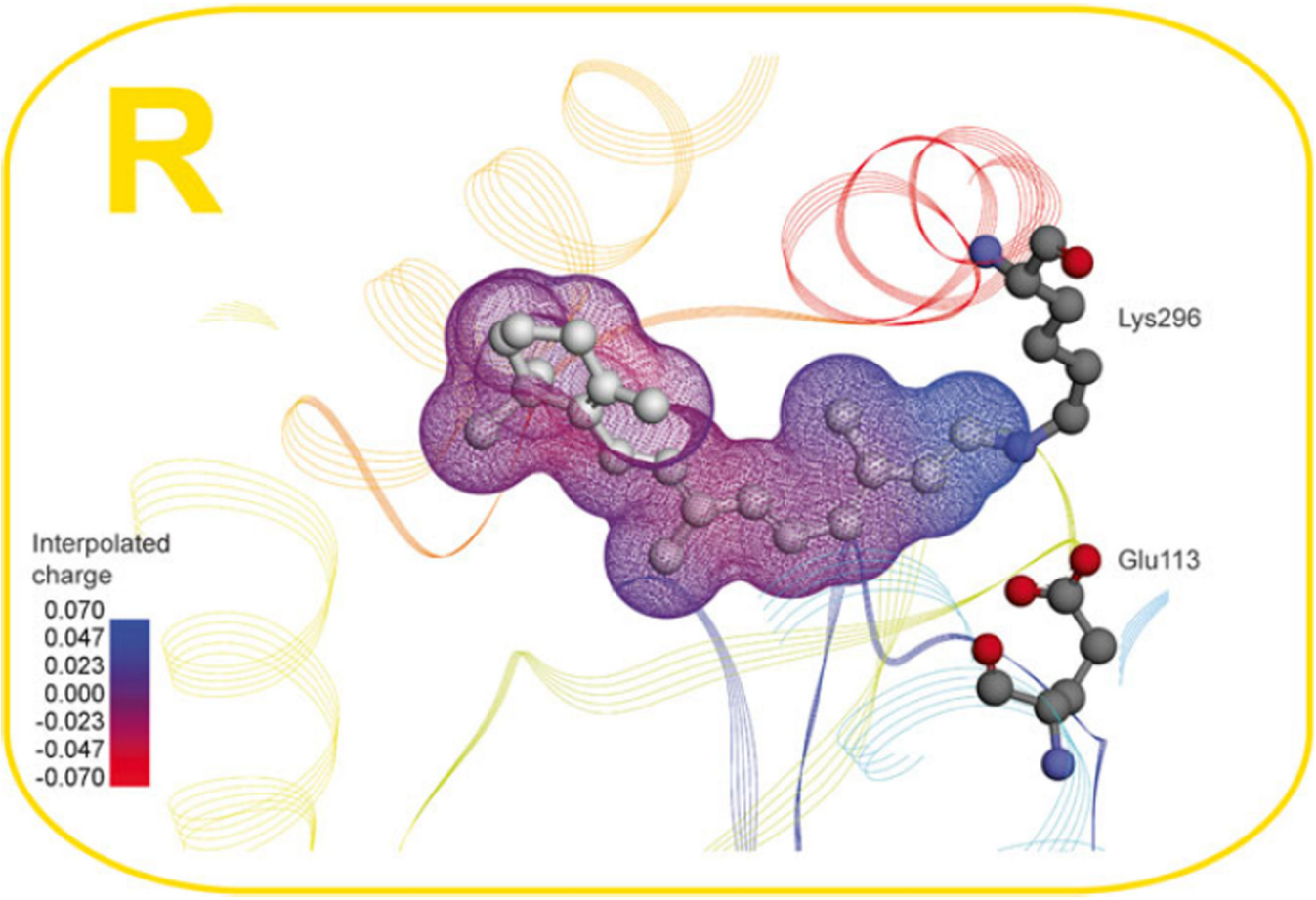

Fig. 5.

The chromophore-binding site of rhodopsin. The 11-*cis*-retinal chromophore is covalently attached to rhodopsin via a Schiff base at Lys296. The counter ion, Glu113, causes protonation of the Schiff base. 11-*cis*-N-Retinylidene-Lys is depicted in a *scaled ball stick* representation; coloring is according to elements except for the chromophore, which is shown in *white*. The surface of 11-*cis*-N-retinylidene-Lys is portrayed in mesh and stained according to interpolated charges determined with Accelrys Discovery Studio software. Rhodopsin is colored in *blue* to *red* from N- to the C-terminus in a *wire* representation

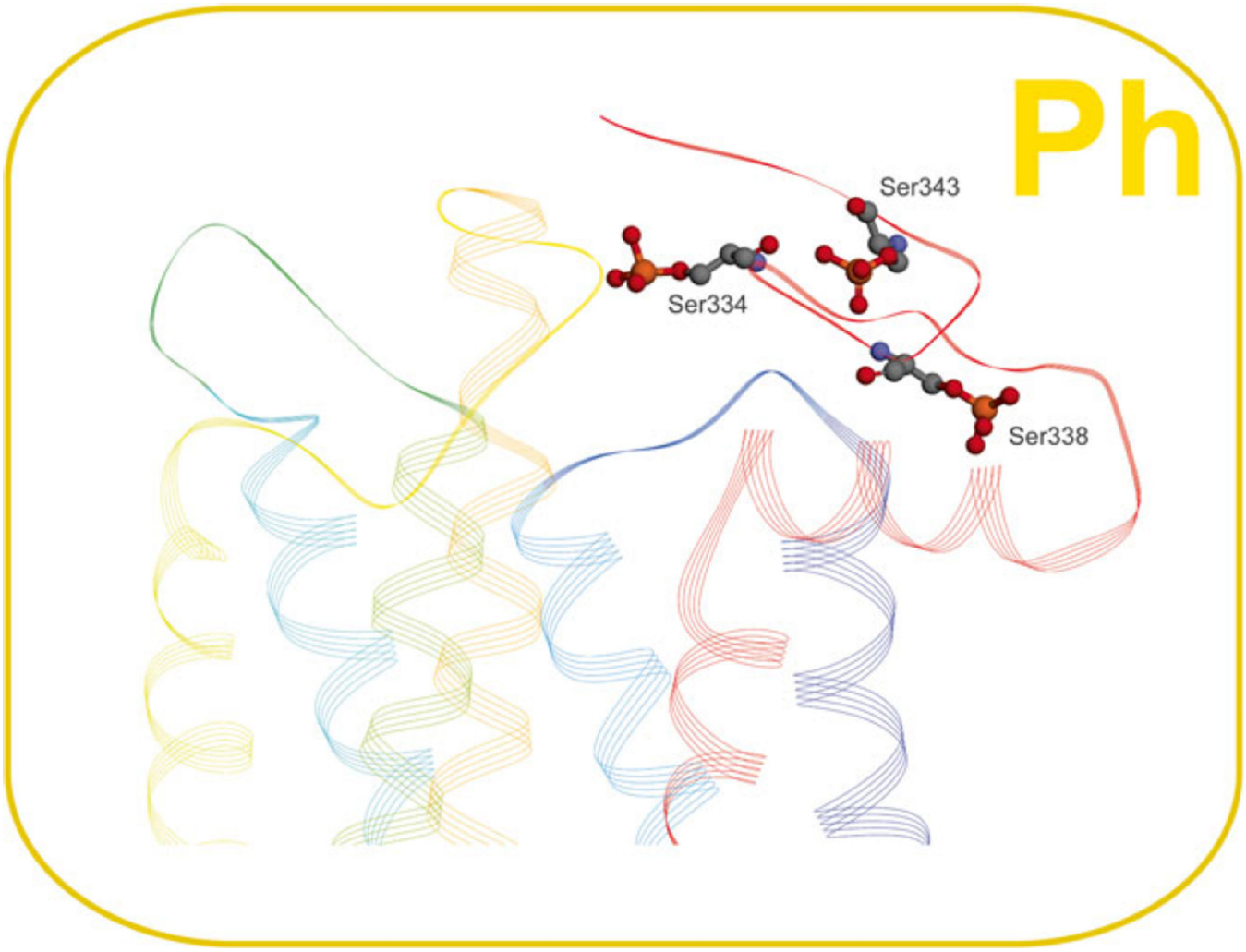

Fig. 6.

Phosphorylation sites on rhodopsin. Phosphorylation sites on rhodopsin are localized at the C-terminus on the three Ser334, Ser338, and Ser343 residues shown in a *scaled ball stick* representation according to element colors. Rhodopsin is colored in *blue* to *red* from N- to the C-terminus in a *wire* representation

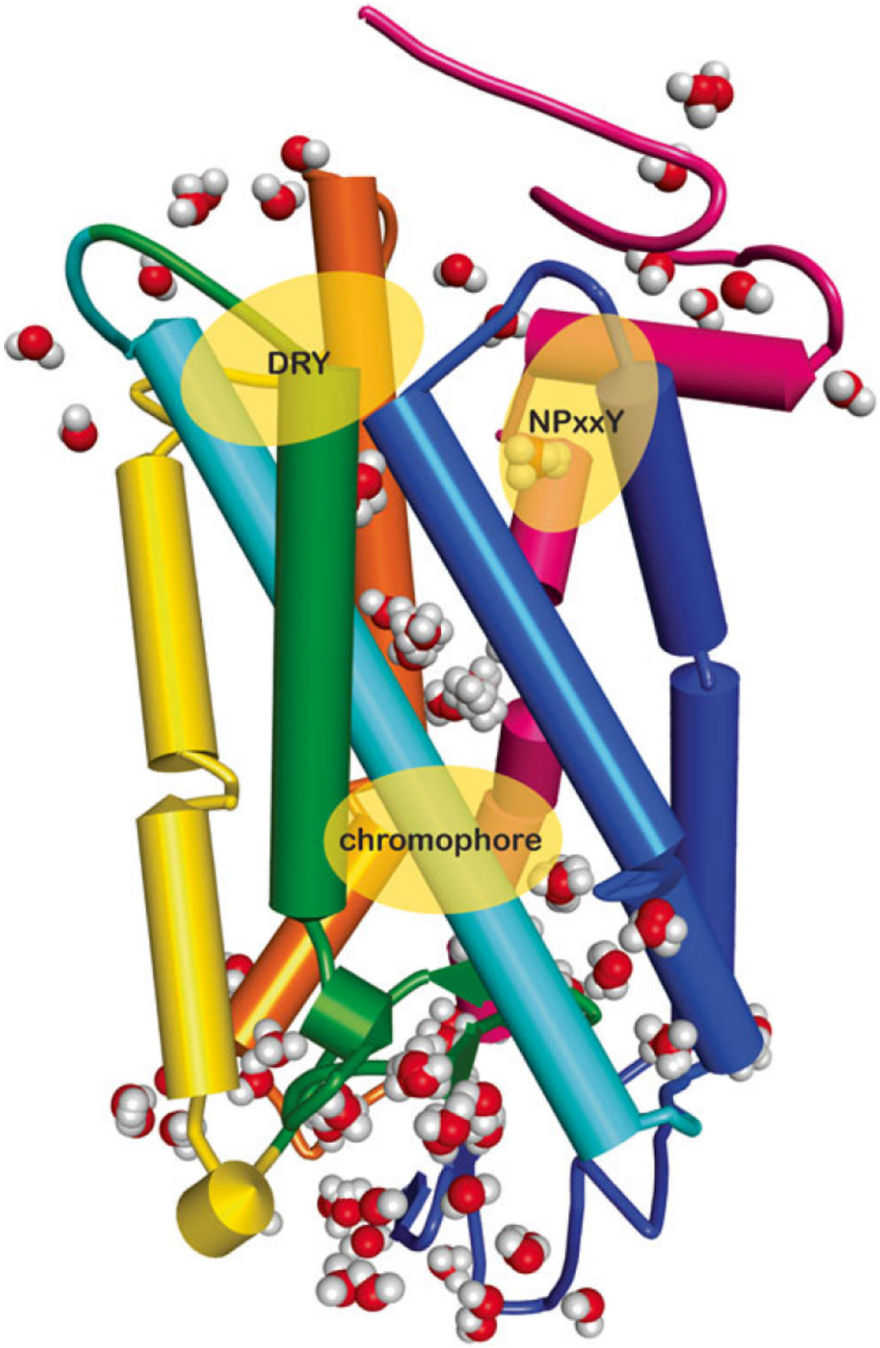

Fig. 7.

Water molecules in rhodopsin. Internal water molecules are shown as *spheres* represented with element colors (H, *white*; O, *red*). Water molecules were combined and aligned according to the protein structure derived from ten published rhodopsin coordinates. This picture shows that the waters are distributed throughout rhodopsin in a channel-like alignment. Furthermore, the number of water molecules is greater at the N-terminal cytoplasmic site than at the C-terminal luminal site. The regions DRY, NPxxY, and chromophore, believed to be involved in the activation and transformation of photoactivated

rhodopsin, are highlighted with *yellow ovals*. Rhodopsin is colored in *blue* to *red* from N- to the C-terminus in a schematic representation

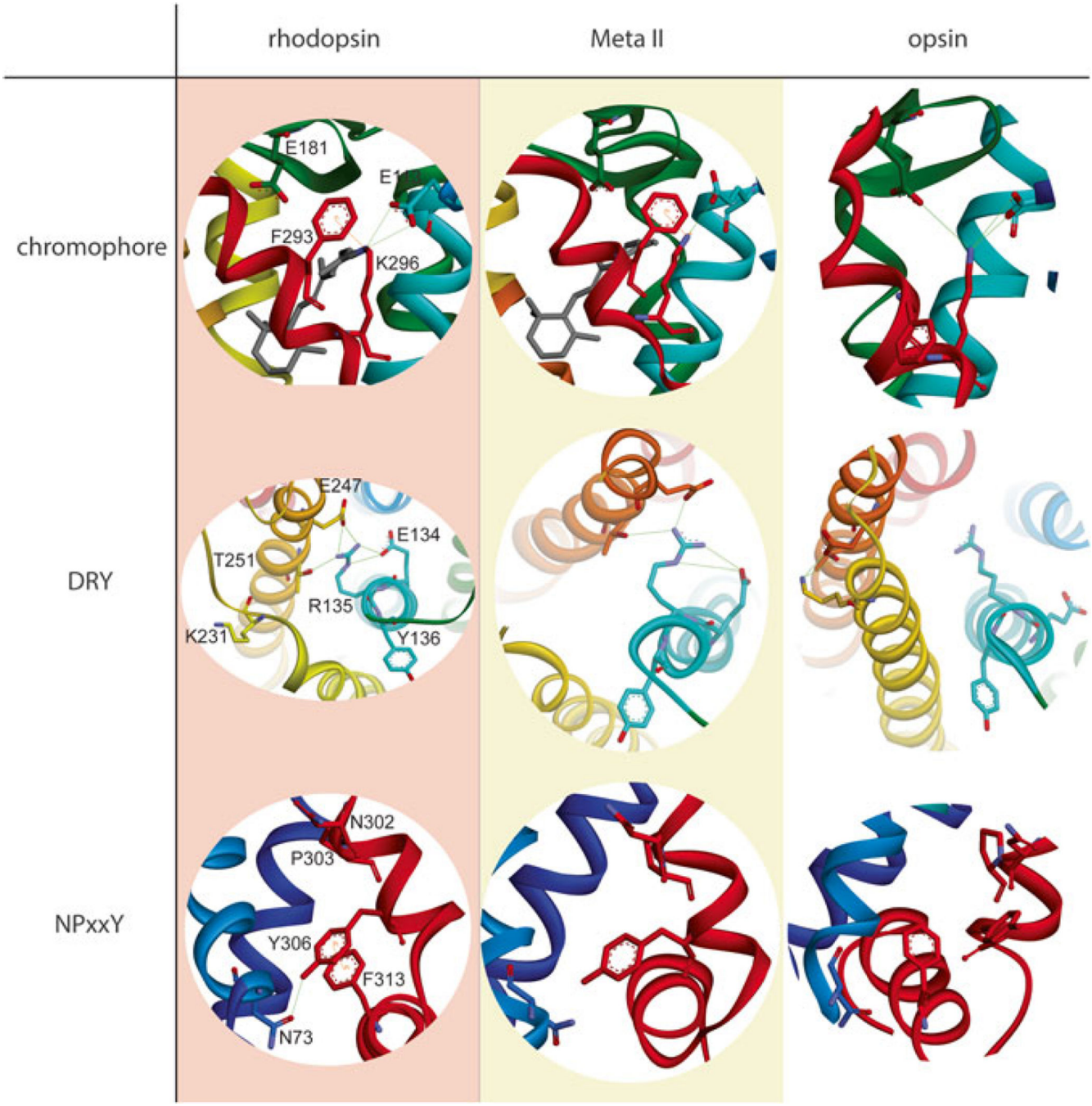

Fig. 8.

Key regions within rhodopsin that undergo conformational changes upon photoactivation. The three regions, chromophore, DRY, and NPxxY, believed to be involved in activation and transformation of photoactivated rhodopsin, are shown at three horizontal levels. The different states of rhodopsin, Meta II, and opsin are distributed in the three rows. The amino acids which undergo a significant change in their conformation and/or an interaction between these states are depicted in a *stick* representation. Rhodopsin is colored *blue* to *red* from N- to the C-terminus in a ribbon representation. Changes at the chromophore site are

dominated by interactions with Lys296. In the rhodopsin state, Phe293 residue coordinates Lys296 residue via π-interactions, whereas the Asp113 residue stabilizes the positive charge located at the Schiff base. These interactions undergo changes in the Meta II state that finally produce a different rotamer for the Phe293 residue and a coordination of the Lys296 residues through Asp181 and Asp113 residues. Changes found in the DRY motif are dominated by the interactions of Arg135 residues. In the rhodopsin state Arg135 residues are coordinated by Asp247, Asp134, and Thr251 residues. During photoactivation, interactions with Arg135 residues are weakened and finally abolished. Asp247 and Thr251 are found as different rotamers which interact with Lys231 through electrostatic interactions in the opsin state. Changes in the NPxxY motif are mainly found in the hydrogen bond interactions between the Tyr306 and Asp73 residues, whereas this conformation is further stabilized by the π–π interaction between the Tyr306 and Phe313 residues. These interactions are weakened during photoactivation and found abolished in the opsin state