Published in final edited form as:

J Am Chem Soc. 2011 November 30; 133(47): 19052–19055. doi:10.1021/ja208789h.

Why 11-*cis*-Retinal? Why Not 7-*cis*, 9-*cis* or 13-*cis*-Retinal in the Eye?

Sivakumar Sekharan[†] and Keiji Morokuma^{†,‡,*}

[†]Cherry L. Emerson Center for Scientific Computation and Department of Chemistry, Emory University, Atlanta, Georgia 30322

[‡]Fukui Institute for Fundamental Chemistry, Kyoto University, 34-4 Takano Nishihiraki-cho, Kyoto 606-8103, Japan

Abstract

One of the basic and unresolved puzzles in the chemistry of vision concerns the natural selection of 11-*cis*-retinal as the light-sensing chromophore in visual pigments. A detailed computational examination of the structure, stability, energetics and spectroscopy of 7-*cis*, 9-*cis*, 11-*cis* and 13-*cis*-retinal isomers in vertebrate (bovine, monkey) and invertebrate (squid) visual pigments is carried out using hybrid quantum mechanics/molecular mechanics (QM/MM) method. It is shown that the electrostatic interaction between retinal and opsin dominates the natural selection of 11-*cis*-retinal over other *cis*-isomers in the dark-state. In all the pigments, 9-*cis*-retinal is found to be only slightly higher in energy than 11-*cis*-retinal, which provides strong evidence for the presence of 9-*cis*-rhodopsin in nature. 7-*cis*-retinal is suggested to be an “upside-down” version of 11-*trans*-isomer because structural rearrangements observed for 7-*cis*-squid rhodopsin is found to be very similar to that of squid bathorhodopsin. The progressive red shift in the calculated absorption wavelength (λ_{max}) of 7-*cis*- (431 nm), 9-*cis*- (456 nm), 11-*cis*- (490 nm) and 13-*cis*-retinal isomers (508 nm) are attributed to the decrease in bond length alternation of the retinal.

Rhodopsin, the visual pigment found in the rod outer segments of the vertebrate and invertebrate photoreceptor mediates the transformation of light into vision.¹ Bovine and squid rhodopsin belong to the class of vertebrate and invertebrate photoreceptors,² respectively, and are also the only members of the G_t and G_q signaling type of G-protein coupled receptors (GPCRs) with a known X-ray structure.³ The hepta-helical membrane protein is composed of a light absorbing 11-*cis*-retinal chromophore covalently bound to the ϵ -amino group of a lysine residue of an apoprotein (opsin) via a protonated Schiff base (PSB11) linkage. The positive charge of the chromophore is balanced with a negative charge of the glutamate counterion. Glu113 serves as a H-bonded counterion in bovine rhodopsin,⁴ while Glu180 serves as a non-H-bonded counterion in squid rhodopsin.⁵ Remarkably, irrespective of the difference in their H-bonding schemes, both counterions exert a strong blue shift of ~120 nm that steers the first ($S_0 \rightarrow S_1$) vertical excitation energy (λ_{max}) of the PSB11 going from gas phase (experimental=610; calculation=616/604; in nm which is omitted hereafter) to the protein environments (in bovine: exp=498, calc=495; in squid exp=489; calc=490).⁵

7-*cis*-rhodopsin is an artificial analog of rhodopsin that contains protonated Schiff base of 7-*cis*-retinal (PSB7) as its chromophore. It is characterized by its low reaction rate for pigment

*morokuma@emory.edu .

SUPPORTING INFORMATION Cartesian coordinates of all the retinal models discussed in this study. This material is available free of charge via the Internet at <http://pubs.acs.org>

formation, low thermal stability and strongly blue-shifted λ_{max} (exp=450) from 11-*cis* (exp=498) in bovine.⁶ 9-*cis*-rhodopsin or isorhodopsin is the most studied analog of rhodopsin that contains protonated Schiff base of 9-*cis*-retinal (PSB9) as its chromophore. It undergoes an identical bleaching sequence to that of rhodopsin and is characterized by a weakly blue-shifted λ_{max} in both the vertebrate (exp=485 in bovine)⁷ and invertebrate (exp=465 in squid) pigments.⁸ Because both the PSB9 and PSB11 isomers can bind to opsin and form pigments with distinct spectral properties, PSB9 is often used as an artificial analog to probe the structure and function of native rhodopsin.⁹

13-*cis*-rhodopsin, albeit a less studied isomer of the visual pigments is also an artificial analog of rhodopsin that contains protonated Schiff base of 13-*cis*-retinal (PSB13) bound to the opsin. Due to the difficulties involved in synthesizing the pure form of 13-*cis*-rhodopsin, earlier studies could only incorporate 9,13-*dicis* (because it already contains 9-*cis*-configuration) or a locked form of 13-*cis* (because it is not the pure form of 13-*cis*) into bovine opsin.¹⁰ In fact archeal and proteorhodopsins were found to contain mixtures of all-*trans* and 13-*cis* isomers in the dark, because they are completely different protein families, where the binding site has been optimized for the 13-*cis*- and all-*trans*-isomer.¹¹ Apparently, in the visual pigments only PSB11 was found to be present in the dark.¹² Given the fact that primary event in vision involves no breaking of chemical bonds but change in the shape of the molecule from bent 11-*cis* to distorted all-*trans* (PSBT), one wonders why the reactant is always 11-*cis* and not 7-*cis*, 9-*cis* or 13-*cis*? Theoretical and experimental studies have argued that the non-bonded interaction between C10-H and C13-Me group can facilitate an efficient, ultrafast and stereoselective isomerization of 11-*cis* to all-*trans* isomer.¹³ However, in the absence of direct evidence from pigments containing pure form of PSB7 and PSB13 isomers, resolving the fundamental question concerning the natural selection of PSB11 remains still an open question.

In light of the exponential development of theoretical methods and computational resources, we are now in a position to prepare models of visual pigments across different animal kingdom. Especially, comparative analysis of structure, stability, energetics and spectroscopy of retinal isomers in vertebrate and invertebrate visual pigments can be performed using hybrid quantum mechanics/molecular mechanics (QM/MM) methods.¹⁴ In such methods, a relatively small region of the system in which chemical reactions and spectroscopy occur is modeled at QM level and the remaining part is treated with MM force fields. By employing one such method, namely the ONIOM (Our own N-layered Integrated Molecular Orbital) QM/MM protocol,¹⁵ we aim to provide insights into the dark side of vertebrate and invertebrate rhodopsins.

The QM/MM optimized structure of wild-type bovine and squid rhodopsin in which the retinal (PSB11) is treated via quantum mechanics and the opsin containing 348 (in bovine) or 448 (in squid) amino acids is treated via molecular mechanics are taken from references.^{5,16} To probe the impact of evolutionary displacement of amino acid positions in the natural selection of PSB11, we have also modeled monkey rhodopsin, which contains 22 different amino acids (22/348) compared to bovine rhodopsin.¹⁷ Details of the optimization method and optimized structures are given in the Supporting Information (SI).

Although all the retinal isomers are incorporated into an *identical binding site* (see Figure 2A), geometry optimization (see below) allows relaxation of the immediate environment that may stabilize or destabilize PSB7/9/13 relative to PSB11, as originally proposed by Birge *et al.*¹⁸ Especially as the *cis*-conformation is present at different positions; at the beginning (for PSB7), inbetween (for PSB9), in the middle (for PSB11) and at the very end (for PSB13) of polyene side chain for the different retinal isomers, binding to the opsin induces significant non-planar distortions into the retinal. Also, the retinal backbone appears

to be perpendicular to the plane of the β -ionone ring for PSB7 and PSB13. As a result, length of the retinal conjugation (from C5 to SBN⁺) between PSB7 and PSB13 is found to be similar (~ 10.85 Å) to each other, while it shortens by 0.24 Å for PSB11 (10.61 Å) and by 0.40 Å for PSB9 (~ 10.45 Å). Due to the steric interaction between C5-Me and C9-Me groups the C6-C7-C8 angle widens and creates the space required for fitting PSB7 into the binding pocket. Apparently, conformational distortions induced into the PSB7 are analogous to the findings from NMR measurements on 10Me-PSB11 analogs.¹⁹ In 10Me-PSB11, C10-Me and C13-Me groups interact with each other to induce the out-of-plane distortions, which induces increase in the distance between C10-Me and C20-Me positions (*i.e.*) from 3.04 Å in PSB11 to 3.47 Å in 10Me-PSB11. This property can be compared to the increase in distance between C6-Me and C19-Me position (*i.e.*) from 2.66 Å in PSB11 to 3.00 Å in PSB7.

The peaks in Figure 2B correspond to the single bond lengths that range as high as 1.48 Å for C6-C7 bond, and the troughs indicate sharp reduction in double bond lengths that range as low as 1.32 Å for C15=N bond. The average BLA of C5-N moiety (which is defined as the average of the bond lengths of single bonds minus that of double bonds) shows a gradual decrease going from PSB7 to PSB13 isomer. This decrease is attributed to the increase in localization of positive charge by the counterion at the SBN terminal.²⁰ The smallest bond angles found at both C9 and C13 are due to the presence of spacious methyl groups, which serves as the signature motif of PSB11. Negative pre-twist of the isomerizing double bond is also a characteristic feature of all the protein bound retinal isomers.²¹ Orientation of the β -ionone ring (C6-C7 dihedral angle) relative to polyene chain varies from -60° in PSB7 to -40° in PSB13 and is calculated to be different for all isomers.²² Significant non-planar distortions (in the range of $10 \pm 10^\circ$) induced into the chromophore are also a common feature of the retinal chromophore across different visual pigments.

All QM/MM calculations in this study were performed using the two-layer ONIOM (QM:MM) scheme, in which the QM part contains the full retinal and the MM part contains the full opsin depicted as point charges plus van der Waals interaction with the interface between QM and MM region being treated by the hydrogen link atom (see Figure 3). The total energy of the system is obtained as ^{EE}E, the energy in the electronic embedding (EE) scheme, in which the QM:MM interaction is included in the QM calculation and therefore the QM wavefunction is polarized by the MM point charges. Throughout this study we employed the QM/MM-EE scheme for geometry optimization, in which the retinal and all the amino acid residues and water molecules within of 4 Å radius from any chromophore atom are optimized, while the other atoms are fixed at the wild-type structure. An alternative QM:MM energy is ^{ME}E, the energy in the mechanical embedding (ME), in which the QM:MM interaction is calculated in the classical Hamiltonian and the wavefunction is not polarized.²³

Mathies and Lugtenburg have shown that, control of the photochemical reaction by the ground-state conformation is achieved through the induced fit of the retinal in rhodopsin.²⁴ In order to disentangle the factors that contribute to the stabilization of 11-*cis*-retinal over the induced misfit of other retinal isomers into rhodopsin, we have performed the following analysis of the relative energies of each isomer form of rhodopsin. At the above-mentioned EE-optimized geometries of each rhodopsin, the single-point ME energy (^{ME}E), as well as the EE energy (^{EE}E), was calculated. The difference between ^{EE}E and ^{ME}E comes mainly from the polarization of the retinal chromophore by the MM charges of the opsin. ^{ME}E can be divided into the QM energy (^{ME}E_{QM}) of retinal and the MM contributions (^{ME}E_{MM}) from the interaction with opsin and the opsin itself:

$${}^{\text{ME}}E = {}^{\text{ME}}E_{\text{QM}} + {}^{\text{ME}}E_{\text{MM}}$$

$${}^{\text{ME}}E = [{}^{\text{ME}}E_{\text{MM}}(\text{retinal+opsin}) - {}^{\text{ME}}E_{\text{MM}}(\text{retinal})]$$

where, ${}^{\text{ME}}E_{\text{QM}}$ is the QM energy of retinal alone at the rhodopsin-optimized geometry. This is higher than its energy at the gas-phase optimized geometry and the difference represents the deformation energy (E_{DEF}) of retinal in the protein:

$$E_{\text{DEF}} = E_{\text{QM}}(\text{retinal}/(\text{retinal+opsin})) - E_{\text{QM}}(\text{retinal}/\text{gas})$$

In Table 1, we list the difference Δ of ${}^{\text{ME}}E_{\text{QM}}$, (E_{DEF}), ${}^{\text{ME}}E_{\text{MM}}$, ${}^{\text{ME}}E$ and ${}^{\text{EE}}E$ for different isomer rhodopsins relative to those of the reference 11-*cis* rhodopsin in the same protein.

In all the animal pigments in terms of the ultimate EE energy (${}^{\text{EE}}E$) the rhodopsin with PSB11 is the most stable, followed closely by PSB9 by 0.8-2.1 kcal/mol and that PSB7 and PSB13 are very unstable and the overall order of stability is given as PSB11>PSB9>PSB7>PSB13. The fact that PSB9 is only slightly higher in energy than PSB11 across all pigments provides evidence for the presence of 9-*cis*-rhodopsin in nature. The analysis using Table 1 provides further insight into the origin of this stability order. One notices that this order is well maintained in ${}^{\text{ME}}E$. The difference between ${}^{\text{EE}}E$ and ${}^{\text{ME}}E$, the polarization energy due to the polarization of the chromophore wavefunction by the protein electrostatic potential, is at most ~2 kcal/mol and is not important. In contrast, the order of stability is significantly altered if we compare only the energy of PSBR in protein (${}^{\text{ME}}E_{\text{QM}}$). In this case, the order of stability is PSB9>PSB11>PSB7>PSB13, with the exception of squid where the order is PSB9>PSB11>PSB13>PSB7. The stability of PSB13 over PSB7 in squid may be related to the position of the counterion, which when H-bonded to the retinal (as in bovine/monkey) may destabilize PSB13 more than PSB7. However, it is important to recognize that PSB9 is more stable than PSB11 in ${}^{\text{ME}}E_{\text{QM}}$. Interestingly, molecular dynamics studies of deep red cone pigments have found PSB9 to be more stable than PSB11.²⁵ Recently, experimental studies have also shown 9-*cis*-13-isopropylretinal to act as a superagonist after illumination and that the opsin binding site shows preference for 9-*cis*-analogues over 11-*cis*-analogues.²⁶ Further, while some analogs of 9-*cis*-isomer performs better than their 11-*cis*- counterparts there is abundant evidence in the literature that this is usually not the case.

Energies of the isomeric forms of retinal optimized in gas phase (without the presence of protein) has the following order of stability: PSB9 (−3.2 kcal/mol) > PSB13 (−3.1) > PSB11 (0.0) > PSB7 (5.3). This means that in the gas phase PSB9 and PSB13 are the more stable isomers than the naturally occurring (in protein) PSB11; PSB7 is intrinsically unstable due to the steric interaction between C5-Me and C9-Me groups as mentioned above. Of course this argument is a little biased, as the naturally occurring protein is optimized in evolution to stabilize PSB11. To this protein, PSB13 fits poorly, making the energy of protein plus chromophore higher than that of PSB11. PSB9 fits well to a certain extent, although the energetic advantage in gas phase over PSB11 is lost.

To calculate the λ_{max} of PSBR geometries in both the gas phase (QM-none) and in protein environments (QM/MM), spectroscopic oriented configuration interaction method²⁷ with +Q Davidson correction (SORCI+Q) and 6-31G* basis set was used (see Table 2). For the planar *cis*-PSBR isomers in *vacuo* where the retinal is cut off from any external environmental perturbation, the calculations yielded an average value ($\Delta\lambda$) around 610 nm²⁸ [7-*cis*- (591 nm), 9-*cis*- (615 nm), 11-*cis*- (625 nm) and 13-*cis*- (623 nm)]. However, as the

retinal enters into the protein environment, the calculated $\lambda_{\max}$ is considerably blue-shifted in both the gas phase [7-*cis*- (553 nm), 9-*cis*- (566 nm), 11-*cis*- (611 nm) and 13-*cis*- (623 nm)] and in the protein environments [7-*cis*- (431 nm), 9-*cis*- (456 nm), 11-*cis*- (490 nm) and 13-*cis*-rhodopsin (508 nm)] due to geometric distortions induced by the binding pocket. Origin of the progressive red shift going from 7-*cis*- to 13-*cis*- is traced back to the decrease in the BLA of the retinal.

Compared to the gas phase, interaction of the retinal with counterion (Glu113 in vertebrates, Glu180 in invertebrates) induces a strong blue shift of ~120 nm and moves the calculated $\lambda_{\max}$ very close to the experimental value of 500 nm for 11-*cis*-rhodopsin.²⁸ The shift is conceivable as the excited state charge density is shifted against the charge of the counterion leading to change in the dipole moment $\Delta\mu_{(S1-S0)}$ calculated to be ~12.0 D for all the retinal isomers in pigments in excellent agreement with the experimental measurements of Mathies et al.³⁰ Similarly, difference in the dipole moment going from gas phase to protein ($\Delta\mu_{(S0)}^{P-g}$) environments is calculated to be ~6.5 D. This value is almost equal to that (6.8 D) calculated for the all-*trans*-retinal in gas phase and in bacteriorhodopsin.²⁹

In the absence of electrostatic interaction with the counterion, the calculated $\lambda_{\max}$ is found to be ~600 nm, which is almost equal to that found in the gas phase.³¹ Apparently, effect of the neutral residues lining the binding pocket is negligible compared to the strong effect of counterion as originally predicted by mutagenesis studies more than 20 years ago.⁴ Negative and positive twist about the C11=C12 and C12-C13 bonds impart a positive helicity³² on all the *cis*-PSBRs. Spectral manifestation of the twist is evident in the calculated rotatory strengths (R), which is positive in sign and undergoes a slight increase in magnitude as the retinal enters the protein from gas-phase. Note that, of all the retinal isomers, the least stable isomer, PSB7, has the largest oscillator ($\Delta f=1.59$) and rotatory strengths ($\Delta R=0.41$), while the most stable isomer, PSB11 has the smallest values ($\Delta f=1.32$, $\Delta R=0.19$) in protein.

Of all the retinal isomers, we find an interesting correlation between PSB7 and PSBT isomers. In the case of squid, the Schiff base NH bond that was originally oriented towards Y111 in rhodopsin is found to be reoriented towards N87 in 7-*cis*-rhodopsin. Apparently, a similar structural rearrangement was also found in the QM/MM³³ and X-ray³⁴ structures of squid bathorhodopsin. Although the Schiff base environment of PSB7 and PSBT is identical in squid rhodopsin, the geometric and spectroscopic properties of these two isomers are found to be opposite to each other. In PSBT, the C9 and C13 methyl groups are pointing upward, whereas in PSB7 they are pointing downward. Further, a red shift of ~50 nm separates bathorhodopsin (~540 nm) from rhodopsin (~490 nm), whereas a blue shift of ~50 nm separates 7-*cis*-rhodopsin (~440 nm) from rhodopsin (~490 nm). While the red shift is attributed to twisting of the double bonds in PSBT, the blue shift is attributed to twisting of the single bonds in PSB7. Therefore, we suggest that PSB7 is an “upside-down” version of PSBT (Figure 4).

In conclusion, the present study not only enables us to answer why nature selects 11-*cis*-retinal? But also helps us to understand why not 7-*cis*, 9-*cis*- or 13-*cis*-retinal in the eye? Although factors such as, accessibility of the retinal to enzymatic biosynthesis, physiological stability, rate of binding, quantum yield and stereoselectivity of the photoisomerization are critical, it remains to be seen how far the evidence presented in this communication can aid in optimizing the parameters required for the natural selection of the light-sensing chromophore. As the strength of rhodopsin appears to lie in the selection of 11-*cis*-retinal, reducing this strength in order to enhance the weakness of accommodating other retinal isomers is not the goal pursued by evolution.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The authors thank Prof. H. Kandori and Kota Katayama for valuable discussions during the preparation of monkey rhodopsin and A. Altun, H. Hirao and L. W. Chung for their technical assistance. This work at Emory is supported in part by a grant from the National Institutes of Health (R01EY016400) and at Kyoto by a Core Research for Evolutional Science and Technology (CREST) grant in the Area of High Performance Computing from JST.

REFERENCES

1. Birge RR. *Ann. Rev. Biophys. Bioeng.* 1981; 10:315–354. [PubMed: 7020578]
2. Terakita A, Yamashita T, Tachibanaki S, Shichida Y. *FEBS Lett.* 1998; 439:110–114. [PubMed: 9849889]
- 3 a). Palczewski K, et al. *Science.* 2000; 289:739–745. [PubMed: 10926528] b) Murakami M, Kouyama T. *Nature.* 2008; 453:363–367. [PubMed: 18480818]
- 4 a). Sakmar TP, Franke RR, Khorana HG. *Proc. Natl. Acad. Sci. USA.* 1989; 86:8309–8313. [PubMed: 2573063] b) Zhukovsky EA, Oprian DD. *Science.* 1989; 246:928–930. [PubMed: 2573154] c) Nathans J. *Biochemistry.* 1990; 29:9746–9752. [PubMed: 1980212]
- 5 a). Sekharan S, Altun A, Morokuma K. *Chem. Eur. J.* 2010; 16:1744–1749. [PubMed: 20066712] b) Sekharan S, Altun A, Morokuma K. *J. Am. Chem. Soc.* 2010; 132:15856–15859. [PubMed: 20964383]
- 6 a). Degrip WJ, Liu RSH, Ramamurthy V, Asato A. *Nature.* 1976; 262:416–418. [PubMed: 958397] b) Maeda A, Shichida Y, Yoshizawa T. *J. Biochem.* 1978; 83:661–663. [PubMed: 565355]
- 7 a). Schick GA, Cooper TM, Holloway RA, Murray LP, Birge RR. *Biochemistry.* 1987; 5:2556–2562. [PubMed: 3607033] b) DeGrip WJ, Bovee-Geurts PHM, van der Hoef I, Lugtenburg J. *J. Am. Chem. Soc.* 2007; 129:13265–13269. [PubMed: 17918843]
- 8 a). Kliger DS, Horwitz JS, Lewis JW, Einterz CM. *Vis. Res.* 1978; 24:1465–1470. [PubMed: 6533980] b) Sulkes M, Lewis A, Marcus MA. *Biochemistry.* 1978; 17:4712–4722. [PubMed: 728380] c) Milder SJ, Kliger DS. *Biophys. J.* 1986; 49:567–570. [PubMed: 3955186]
9. Sekharan S, Sugihara M, Weingart O, Okada T, Buss V. *J Am Chem Soc.* 2007; 129:1052–1054. [PubMed: 17263385]
- 10 a). Crouch R, Purvin V, Nakanishi K, Ebrey T. *Proc Natl Acad Sci USA.* 1975; 72:1538–1542. [PubMed: 1055424] b) Shichida Y, Nakamura K, Yoshizawa T, Trehan A, Denny M, Liu RSH. *Biochemistry.* 1988; 27:6495–6499. [PubMed: 2975508]
- 11 a). Harbison GS, et al. *Proc. Natl. Acad. Sci. USA.* 1984; 81:1706–1709. [PubMed: 6584904] b) Sasaki J, et al. *Science.* 1995; 269:73–75. [PubMed: 7604281]
- 12 a). Birge RR, Sullivan MJ, Kohler BE. *J. Am. Chem. Soc.* 1976; 98:358–367. [PubMed: 1245683] b) Rando RR. *Angew. Chem. Int. Ed. Engl.* 1990; 29:461–480. c) Nakanishi K. *Amer. Zool.* 1991; 31:479–489.
- 13 a). Warshel A. *Nature.* 1976; 260:679–683. [PubMed: 1264239] b) Schoenlein RW, Peteanu LA, Mathies RA, Shank CV. *Science.* 1991; 254:412–415. [PubMed: 1925597]
14. Warshel A, Levitt M. *J. Mol. Biol.* 1976; 103:227–49. [PubMed: 985660]
15. Vreven T, Morokuma K, Farkas O, Schlegel HB, Frisch MJ. *J. Comp. Chem.* 2003; 24:760–769. [PubMed: 12666168]
16. Altun A, Yokoyama S, Morokuma K. *J. Phys. Chem. B.* 2008; 112:6814–6827. [PubMed: 18473437]
17. Oprian DD, Asenjo AB, Lee N, Pelletier SL. *Biochemistry.* 1991; 30:11367–11372. [PubMed: 1742276]
18. Birge RR, Einterz CM, Knapp HM, Murray LP. *Biophys. J.* 1988; 53:367–385. [PubMed: 2964878]

- 19 a). Delange F, et al. *Biochemistry*. 1998; 37:1411–1420. [PubMed: 9477970] b) Verdegem PJE, Bovee-Geurts PHM, DeGrip WJ, Lugtenburg J, de Groot HJM. *Biochemistry*. 1999; 38:11316–11324. [PubMed: 10471281]
20. Okada T, Sugihara M, Bondar AN, Elstner M, Entel P, Buss V. *J. Mol. Biol.* 2004; 342:571–583. [PubMed: 15327956]
21. Sugihara M, Hufen J, Buss V. *Biochemistry*. 2006; 45:801–810. [PubMed: 16411756]
22. Honig B, Hudson B, Sykes BD, Karplus M. *Proc. Natl. Acad. Sci. USA*. 1971; 68:1289–1293. [PubMed: 5288377]
23. Vreven, T.; Morokuma, K. Hybrid Methods: ONIOM (QM:MM) and QM/MM. In: Spellmeyer, D., editor. *Ann. Rep. Comp. Chem.* Vol. 2. Elsevier; 2006. p. 35-52.
24. Kochendoerfer GG, Verdegem PJE, van der Hoef I, Lugtenburg J, Mathies RA. *Biochemistry*. 1996; 35:16230–16240. [PubMed: 8973196]
25. Amora TL, Ramos LS, Galan JF, Birge RR. *Biochemistry*. 2008; 47:4614–4620. [PubMed: 18370404]
26. deGrip WJ, Bovee-Geurts PH, Wang Y, Verhoeven MA, Lugtenburg J. *J. Nat. Prod.* 2011; 74:383–390. [PubMed: 21309593]
27. Neese FA. *J. Chem. Phys.* 2003; 119:9428–9443.
- 28 a). Gascon JA, Batista VS. *Biophys. J.* 2004; 87:2931–2941. [PubMed: 15339806] b) Sekharan S, Sugihara M, Buss V. *Angew. Chem. Int. Ed.* 2007; 46:269–271.
29. Hoffmann M, et al. *J. Am. Chem. Soc.* 2006; 128:10808–10818. [PubMed: 16910676]
30. Mathies R, Stryer L. *Proc. Natl. Acad. Sci. USA*. 1976; 73:2169. [PubMed: 1065867]
31. Andersen LH, et al. *J. Am. Chem. Soc.* 2005; 127:12347–12350. [PubMed: 16131214]
32. Shichida S, Tokunaga F, Yoshizawa T. *Biochim. Biophys. Acta*. 1978; 504:413–430. [PubMed: 718881]
33. Sekharan S, Morokuma K. *J. Am. Chem. Soc.* 2011; 133:4734–4737. [PubMed: 21391708]
34. Murakami M, Kouyama T. *J. Mol. Biol.* 2011; 453:363–367.

Figure 1.

Schematic representation of 7-*cis* (PSB7) in green, 9-*cis* (PSB9) in blue, 11-*cis* (PSB11) in black, 13-*cis* (PSB13) in purple and all-*trans* (PSBT) retinal in red. R refers to Lys-305 in squid and Lys-296 in bovine and monkey rhodopsins.

Figure 2.

A) QM/MM-EE optimized geometries of the retinal binding pocket containing PSB7 (green), PSB9 (blue), PSB11 (black) and PSB13 (purple) models. B) Comparison of average bond lengths along the carbon atoms of the retinal conjugation in vertebrate (bovine, monkey) and invertebrate (squid) visual pigments.

Figure 3. Schematic representation of the two-layer ONIOM (QM:MM) scheme employed in this study. The retinal treated in the QM part (red circle) is connected to the opsin (yellow) via a hydrogen link atom (H_{LA}).

Figure 4.
Overlay of PSB7 (in green) and PSBT (in red) isomer.

Table 1

Role of the opsin in the natural selection of PSB11 in visual pigments is evaluated using the difference Δ of ${}^{\text{ME}}E_{\text{QM}}$, (E_{DEF}) , ${}^{\text{ME}}E_{\text{MM}}$, ${}^{\text{ME}}E$ and ${}^{\text{EE}}E$ for different isomer rhodopsins in vertebrate (bovine, monkey) and invertebrate (squid) pigments. Values from PSB11 serve as the reference point. See the Supporting Information for details.

Model	Δ of [${}^{\text{ME}}E_{\text{QM}}(E_{\text{DEF}})/{}^{\text{ME}}E_{\text{MM}}/{}^{\text{ME}}E/{}^{\text{EE}}E$] in kcal/mol		
	Bovine	Monkey	Squid
PSB7	5.3(4.2)/8.1/13.4/10.8	5.4(4.3)/7.8/13.2/11.4	9.0(7.9)/3.1/12.1/12.2
PSB9	-3.1(0.1)/5.9/2.8/0.9	-2.9(0.3)/4.9/1.9/0.8	-2.4(0.8)/2.9/0.5/2.1
PSB11	0.0(0.0)/0.0/0.0/0.0	0.0(0.0)/0.0/0.0/0.0	0.0(0.0)/0.0/0.0/0.0
PSB13	3.1(6.2)/27.9/31.0/28.4	8.9(12.0)/28.9/37.8/35.2	-1.2(1.9)/16.8/15.5/15.8

Table 2

Average values of the SORCI+Q calculated $S_0 \rightarrow S_1$ absorption wavelength ($\Delta\lambda$), oscillator (Δf), rotatory strengths (ΔR) in a.u and difference in the ground- (S_0) and excited state (S_1) dipole moments in protein ($\Delta\mu_{(S_1-S_0)}$) and between the gas phase and protein environments ($\Delta\mu_{(S_0)}^{P-g}$) are given in Debye for all the PSBR isomers in vertebrate (bovine, monkey) and invertebrate (squid) pigments.

PSBR	SORCI+Q Ground (S_0) and Excited (S_1) State Properties									
	Gas phase (QM-none)					Protein (QM/MM)				
	$\Delta\lambda$	Δf	$\Delta\lambda$	Δf	ΔR	$\Delta\mu_{(S_1-S_0)}$	$\Delta\mu_{(S_0)}^{P-g}$			
PSB7	553	1.20	431	1.59	0.41	12.1	6.00			
PSB9	566	1.20	456	1.42	0.23	11.7	6.32			
PSB11	611	1.10	490	1.32	0.19	11.7	6.95			
PSB13	623	1.27	508	1.45	0.17	12.5	6.43			