Electron Density Deformations Provide New Insights into the Spectral Shift of Rhodopsins

Erix Wiliam Hernández-Rodríguez,[a,b,c] Ana Lilian Montero-Alejo,[c] Rafael López,[b] Elsa Sánchez-García,[d] Luis Alberto Montero-Cabrera,[c] and José Manuel García de la Vega*[b]

Spectral shifts of rhodopsin, which are related to variations of the electron distribution in 11-cis-retinal, are investigated here using the method of deformed atoms in molecules. We found that systems carrying the M207R and S186W mutations display large perturbations of the π-conjugated system with respect to wild-type rhodopsins. These changes agree with the predicted behavior of the bond length alternation (BLA) and the blue shifts of vertical excitation energies of these systems. The effect of the planarity of the central and Schiff-base regions of retinal chain on the electronic structure of the chromophore is also investigated. By establishing nonlinear polynomial relations between BLA, chain distortions, and vertical excitation energies, we are also able to provide a semiquantitative approach for the understanding of the mechanisms regulating spectral shifts in rhodopsin and its mutants. © 2013 Wiley Periodicals, Inc.

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Introduction

Many experimental and theoretical studies of visual pigments focus on the elucidation of their spectral tuning mechanisms.[1] Although the calculation of accurate electronic spectra of these proteins is a challenging task,[2] electronic structures of biological chromophores remain one of the goals of quantum chemical approaches.[14,3] The design of artificial photosensitive proteins[4] and the investigation of abnormal effects related to mutations in human rhodopsin, are also areas of interest.[5]

Several mechanisms have been proposed to explain the spectral tuning in retinal proteins: (i) changes in the interaction of the chromophore with the counterion balancing its positive charge; (ii) changes in the interaction of the chromophore with the remaining amino acid residues and water molecules lining the rhodopsin binding pocket (RBP); (iii) changes of the chromophore geometry due to interactions with the protein binding pocket; and (iv) disturbance of the internal hydrogen-bonding network taking place into the protein binding pocket.[5,6] The general principles governing the absorption spectra of chromophores are basically known[7] and several times reported as clarified at the molecular level.[6a,6c,8] Nevertheless, a graphical and easily understood method able to illustrate electron cloud behaviors that are related to spectral tuning of retinal chromophore in rhodopsins would provide a very useful tool to rationalize both the absorption spectra and other experimental properties of visual pigments. This is especially important in the context of altered protein environments like mutated systems.

In the case of bovine rhodopsin, a vertebrate retinal protein extremely similar to its human counterpart,[5a,9] the retinal binding pocket is stiff[10] and both ends of the chromophores must be fitted into the protein matrix[6b,11] in both bovine and human wild-type (WT) rhodopsins (bWT and hWT), substitutions of residues in key positions with respect to the chromophore creates a scenario suitable to distinguish electronic perturbations of the polyene system. In addition, two mutant human rhodopsins related to the progressive retinal degeneration known as retinitis pigmentosa (RP)[12] M207R, and S186W[13] were strategically selected from the structural point of view in our previous quantum mechanics/molecular mechanics (QM/MM) studies. This kind of investigation allows identifying relevant structural deviations related with spectral shifts.

Electron density perturbations of the retinal system related to changes of the bond length alternation (BLA), spectral...
shifts, electrostatic factors, or excitation in rhodopsins have been indirectly investigated.\textsuperscript{5a,14} A general principle states that any mechanism reducing charge delocalization and increasing BLA in polyenes (even if it has an even number of atoms) should induce hypsochromic shifts in the absorption maxima, although the opposite could also be true.\textsuperscript{7} Consequently, the link between the geometrical distortion and vertical excitation energy (VEE) has been studied through the charge delocalization in retinal. Studies agree that the analysis of the electronic structure of the opsin embedded retinal is a necessary approach to understand the structural basis for the optical properties of rhodopsin.\textsuperscript{11a}

To our knowledge, retinal electron delocalization patterns have not yet been described explicitly up to date, although they would provide a better understanding of the retinal electronic structures for both ground and excited states. Indeed, perturbations of the conjugated \( \pi \) system can influence the VEE, but these perturbations cannot be properly modeled without a method directly describing the conjugated \( \pi \)-clouds.

Previous studies have shown that the chemical behavior of several systems could be rationalized appropriately by means of small nonspherical atomic deformations of the electron density provided by the deformed atoms in molecules (DAM) method.\textsuperscript{16} Electron density deformations, linking the quantum mechanical concept of electron density and empirical chemistry notions,\textsuperscript{17} are able to provide a whole picture of the disturbances of the \( \pi \)-conjugated electron system of retinal due to rhodopsin mutations.

In the present work, we evaluate our previous model of the \( \pi \)-conjugated system of retinal in the dark state of all WT and mutated rhodopsins linked to \( Rp \), using DAM and establish the relationship between perturbations of \( \pi \)-conjugated clouds in mutants with retinal geometry distortions and spectral shifts.

**Computational Details**

**Structural models**

In our previous work,\textsuperscript{5a} molecular dynamics (MD) snapshots were optimized at the QM/MM = (B3LYP/TZVP)\textsuperscript{18}/CHARMM22 level of theory using ChemShell\textsuperscript{18} and Turbomole\textsuperscript{19} in version 5.7.1 with an electrostatic embedding scheme.\textsuperscript{20} These geometries are used for all further calculations in this work. Mutated rhodopsins carrying the M207R and S186W substitutions are named here hM207R and hS186W, respectively. In hM207R, the neutral methionine residue is replaced by the positive charged arginine residue near to the \( \beta \)-ionone ring of retinal and in hS186W, the polar serine is replaced by the aromatic tryptophan near to the Schiff-base linkage (SBL) (Fig. 1). The retinal Schiff-base (SBR) is protonated and Glu113 is deprotonated in bWT, hWT, and the hM207R mutant. SBR is deprotonated and Glu113 protonated in the hS186W mutant. The VMD program\textsuperscript{21} was used for drawing molecular structures and the residue notation is the same as in the Protein Data Bank file 1U19.

** Electron density analysis**

Point single calculations of each \( \text{Rho}_1 \), \( \text{Rho}_2 \), and \( \text{Ret}_{\text{gas}} \) model were performed at the B3LYP/TZVP level of theory using the Gaussian09 package\textsuperscript{22} to compute the electron density that was used for further analysis of the retinal electronic structure. The DAM method\textsuperscript{16} was used to analyze the electron density of 11-cis-retinal. This method is based on atom-centered partition schemes\textsuperscript{17,23} within the linear combination of atomic orbitals framework. In that context, the electron density is expressed as a linear combination of one- and two-center

![Figure 1](image-url) A few residues belonging to the RBP of hWT. The positions to be mutated are given in red. The QM region included the 11-cis-retinal-Lys296-partsystem in green, a part of Glu113 in yellow, and two water molecules (W6 and W8) in blue. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

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The following models are used:

**Rho**\(_1\): protein in explicit water environment, the QM region includes 11-cis-retinal, part of Lys296, part of Glu113, and two water molecules (W6 and W8) (see Fig. 1), for bWT, hWT, hM207R, and hS186W. \( \text{Rho}_1 \) is used to establish the whole retinal electron delocalization pattern (retinal electronic structure) and its perturbations in the protein environments (see Supporting Information Fig. S1 and Tables S1a–S1d).

**Rho**\(_2\): model \( \text{Rho}_1 \) is modified taking into account that the QM region only contains 11-cis-retinal and part of Lys296. All MM point charges are set to zero except those of Glu113, for hWT, hM207R, and hS186W. \( \text{Rho}_2 \) is used to identify the significance of electrostatic influences on the retinal electronic structure, owing to mutated groups (see Supporting Information Fig. S2 and Table S2).

**Ret**\(_{\text{gas}}\): in this model, the QM region only contains 11-cis-retinal and part of Lys296. All MM point charges are set to zero, for hWT, SBR is deprotonated. \( \text{Ret}_{\text{gas}} \) is used to explore the electronic structure of a retinal similar to gas phase (without strict vacuum conformation). This model allows an approach to a \( \pi \)-conjugated electron system of retinal without any electrostatic influences (see Supporting Information Fig. S3 and Table S3).
charge distributions, and each atomic fragment consists of all its one-center distributions plus the part of its two-center distributions closer to it.[23] Besides, the small nonspherical atomic deformations of every atom, the responsible for the chemical behavior,[16] can be separated from the largely dominant spherical term,[24] providing an appropriate insight into the molecular electron density as well as identifying regions of electron accumulation and depletion in agreement with chemical notions.[17,23]

The retinal $\pi$-conjugated system was explored through isosurfaces of electron density deformations. Positive deformations (charge accumulation) of the 11-cis-retinal were explored at 0.001 bohr$^{-3}$, the deformation level corresponding to the characteristic delocalization pattern found in aromatic molecules.[24] The electron delocalization-cloud exploration was used to identify specific perturbation sites throughout the conjugated $\pi$ system. Relevant $\pi$-cloud perturbations involve less contribution to the charge delocalization from any 11-cis-retinal atom in mutated rhodopsins with respect to the reference hWT.

The DAM method also allows quantifying these $\pi$-cloud disturbances at specific retinal atoms by means of atomic electron density contributions. A common axis system for each structure was chosen to quantify the $\pi$-cloud perturbation. In this system, the Z axis is perpendicular to the plane defined by three consecutive retinal atoms including the disturbance-bond atoms. It is easy to prove that the first term in the multipolar expansion of the atomic fragment, which contributes in a likewise manner (accumulating or depleting charge) to both the upper and lower parts of the $\pi$-cloud is one of quadrupole components ($l = 2$). In Figure 2, it can be identified the $d_{322-2}$ component corresponding to $m = 0$.

Therefore, larger values of the $d_{322-2}$ quadrupole moment mean higher contributions to charge delocalization via the $\pi$-cloud. Moment value and moment sign are quantitative descriptors of the contribution of a given atom to the $\pi$-cloud, which can be useful to quantify disturbances in delocalization. The gOpenMol program in version 3.00 has been used throughout this work to visualize the electron density plots.[25]

**Geometrical distortion analysis**

Geometrical distortions linked to density changes are analyzed along the retinal polyene chain. 11-cis-retinal (angles between planes and dihedral angles). The effect of arched twist through angles between planes is defined by two points with coordinates $(x, y, z)$ corresponding to two 11-cis-retinal atoms $(x_1, y_1, z_1$ and $x_2, y_2, z_2)$ and a third point with coordinates $(x_3, y_3, z_3)$ equal to those from one atom of the same plane but with $z_3$ set to zero (i.e., $x_3 = x_1, y_3 = y_1, z_3 = 0$). Both retinal twists are explored at specific sites where relevant electron density changes are found.

**Regression analysis**

Relations between BLA and distortion-angle, and VEE and BLA were explored. The BLA and VEE values employed were taken from our previous QM/MM study on Rho and Ret$_{gas}$ models.[5a] Parameters, regression coefficient ($R^2$), and root-mean-square deviation (rmsd), were evaluated using the Mathematica package.[48] In this way, four new models were proposed for this analysis supported in Rho and Ret$_{gas}$ models:

- **Rho$_4$:** protein in a solvated lipid bilayer of palmitoyl-oleoyl-phosphatidyl-choline, the same QM region as above (Rho$_1$), for bWT, hWT, and hS186W (see Supporting Information Fig. S4 and Tables S4a–S4c).
- **Rho$_5$:** using the Rho$_1$ model but Glu113 (counterion) and water molecules were excluded from the QM region, for hWT, hM207R, and hS186W (see Supporting Information Fig. S5 and Table S5).
- **Rho$_6$:** using the Rho$_1$ model but the QM region only contains 11-cis-retinal and part of Lys296. All MM point charges were retained except those of Glu113, for hWT, hM207R, and hS186W (see Supporting Information Fig. S6 and Table S6).
- **Rho$_7$:** using the Ret$_{gas}$ model but applied to hS186W (see Supporting Information Fig. S7 and Table S7).

**Results and Discussion**

**Electron density deformations**

The electron density deformations for each QM region of the Rho$_1$ models carrying either protonated SBR (bWT, hWT, hM207R) or deprotonated SBR (hS186W) were calculated. Perturbations are found on the retinal $\pi$-conjugated system, which are related to geometrical distortions at specific locations along the retinal chain, due to electrostatic or steric influences.[6d,8d] In Figure 3, positive electron density deformation isosurfaces of the full retinal delocalization pattern in hWT (QM regions) are shown. In order to visualize the density of this system, three different pictures are depicted with its molecular structure. Here, we use a positive deformation (in

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**Figure 2.** Spherical harmonic $m = 0, l = 2$ (component $d_{322-2}$ of the quadrupole) allows to add charge (in red) up and downward of the $xy$ plane (three consecutive retinal atoms, which are perpendicular to $z$ axis). The charge reduction (in blue) is placed between both charge-accumulation regions (in red). The contribution of the component $m = 0 (l = 2)$ quantitatively describes the retinal conjugated $\pi$-clouds. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
red) level of 0.001 bohr$^{-3}$ and the same residue notation as in the pdb file 1U19.$^{[26]}$ We find (see Figs. 3a and 3c), that both the C$_5$A$_{C6}$ bond and the water molecule W6 are implicated with the retinal $\pi$-conjugated system in hWT. In Figure 4, the same kind of isosurfaces using $\text{Rho}_1$ model are shown for both WT rhodopsins. The $\pi$-conjugated electron system of 11-cis-retinal in both WT rhodopsins is continuous. Despite of the known twisted conformations of retinal in the RBP environment, both models show the conjugated $\pi$ system as a continuous ribbon along the retinal polyene chain from the C$_5$A$_{C6}$ bond to the N of the Schiff base. It is important to note that the contour value used for this representation also shows the electron density deformations related to each $\sigma$ bond in the structure. In this case, the electron density accumulations appear with nearly cylindrical symmetry and they are perfectly distinguishable from the $\pi$-clouds of the system.

Interestingly, it is showed an isthmus of the deformed density corresponding to a slight narrowing of the conjugated $\pi$-cloud at the C$_{12}$A$_{1_{1}}$ and C$_{14}$A$_{1_{15}}$ retinal bonds of both bWT and hWT. Isthmus means a reduction of the charge accumulation at the retinal central and Schiff-base regions of 11-cis-retinal when compared with the remaining $\pi$-cloud. The central region is strongly twisted in the 11-cis-retinal of bWT with respect to gas-phase retinal$^{[27]}$ showing red shifted VEE.$^{[28]}$ The geometrical distortion of the retinal central region in bWT has been related with the photoisomerization reaction at the C$_{11}$A$_{C12}$ bond$^{[29]}$ and with the electron density redistribution for the $S_0\rightarrow S_1$ electronic transition.$^{[6c]}$ A previous study on bovine rhodopsin stated that distortions of the C$_{14}$A$_{15}$ single bond could be associated to disturbance of the electron density redistribution by reducing the electron conjugation.$^{[8c]}$ Moreover, the electron density of counterion Glu113 stabilizes the protonated SBR$^{[30]}$ which is significantly implicated with the retinal electronic structure (see Fig. 3b). The C$_5$A$_{C6}$ double bond appears to be involved into the $\pi$-conjugated system at the computed deformation level in 11-cis-retinal adopted a 6-$s$-cis conformation.$^{[5a]}$ Here, we find that a $\pi$-cloud is more extended than the other one on that double bond (Figs. 3b and 3c). Although, a significant BLA change in mutants is found, their C$_5$A$_{C6}$ bond distances are similar to those of WT systems (1.35 Å for hWT, bWT, hS186W and 1.36 Å for hM207R). Therefore, we can conclude that this double bond distance is less sensitive to the electronic structure variations in retinal chromophores.

It is also possible to differentiate the electron density deformations of the 11-cis-retinal related with the oxygen lone pair of the water molecules. Both WT systems show that the water molecule labeled as W6 shares electron density with the chromophore $\pi$-conjugated system around the retinal C$_{14}$ atom (see Figs. 3a and 4). This fact is consistent with studies suggesting a crucial influence of internal water molecules on

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**Figure 3.** Positive electron density deformations isosurfaces (in red) for the QM region in hWT (model $\text{Rho}_1$): (a) picture of the conjugated $\pi$ system of chromophore 11-cis-retinal into the reference rhodopsin environment; (b) isthmus of the electron density deformation at retinal central region; (c) the electron density of counterion Glu113 stabilizing the protonated SBR. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
spectral properties of rhodopsin\textsuperscript{[6d,31]} and with the proximity of the retinal with the water molecule W6 (the average distances between the retinal C\textsubscript{14} atom and W6 oxygen atom (C\textsubscript{14}–O\textsubscript{w6} distance) are 3.39 ± 0.04 and 3.57 ± 0.02 Å for hWT and bWT, respectively). In addition, the electrostatic influence of the counterion on the retinal electronic structure is noticeable from the computed electron density deformations (see Fig. 3c).

In a similar way, positive electron density deformations of 11-cis-retinal in hM207R and hS186W mutants and the comparison with UV-visible spectra calculated previously\textsuperscript{[5a]} are shown in Figure 5. When we compare these pictures with those of WT rhodopsins (Figs. 4 and 5), we found that the π-cloud of one side of the chromophore plane in hM207R is extended as in WT systems and a more pronounced isthmus appears at the retinal C\textsubscript{10}–C\textsubscript{11} bond. The electron density iso-surface shows a break (gap) of the conjugated compound at the C\textsubscript{14}–C\textsubscript{15} bond on the other side of the retinal plane (see Fig. 5a). This disruption around the Schiff-base end reduces the π-cloud extension and obviously the retinal charge delocalization. Hence, electron excitation properties of this chromophore must be changed and the prediction agrees with the obtained spectral blue shift for this mutant with respect to WT systems. In addition, the average C\textsubscript{14}–O\textsubscript{w6} distance is 3.27 ± 0.03 Å, which is similar to those in hWT and bWT systems. W6 is also implicated with the π-cloud of one side of the chromophore plane (see Fig. 5a).

The largest π-cloud gap is observed at the retinal central region on one side of the chromophore plane in hS186W mutant, causing the greatest reduction of the π-cloud (see Fig. 5b) and changes in the retinal electron excitation properties. The shortness of the conjugated π system explains the largest blue shift of this mutant. A less pronounced isthmus persists at the C\textsubscript{14}–C\textsubscript{15} bond with respect to the reference WT (see Fig. 5b). Obviously, the electron density discontinuity at the C\textsubscript{10}–C\textsubscript{11} bond arises as the most relevant disturbance related with spectral properties in the hS186W mutant. In addition, there is no interaction between the water molecules and retinal π-conjugated clouds because of its spatial conformations leading to the largest average C\textsubscript{14}–O\textsubscript{w6} distance (4.10 ± 0.05 Å). Electron density deformations of the Glu113 residue and the Schiff base are not related to each other (see Fig. 5b), which is expected because of their protonation states and the Glu113–Schiff-base distances causing a broken Glu113–SBL salt bridge\textsuperscript{[5a]}

As mentioned above, the disturbance quantification is achieved from the suitable component (m = 0) of the quadrupole, which can describe the atom contributions to the π-conjugated system at the C\textsubscript{10}–C\textsubscript{11} and C\textsubscript{14}–C\textsubscript{15} bonds. In Table 1, average values of the quadrupolar moment m = 0 (l = 2) for Rho\textsubscript{1} model are depicted. Values for the atoms corresponding to the C\textsubscript{10}–C\textsubscript{11} bond in mutants are lower than in both WT systems. This behavior agrees with the charge-accumulation evolution in the retinal central region calculated from the iso-surfaces of positive electron density deformations in WT rhodopsins and mutants (see Figs. 4 and 5). Furthermore, the reduction of the quadrupole-component values is in full agreement with a lower distortion at the dihedral angle φC\textsubscript{9}–C\textsubscript{10}–C\textsubscript{11}–C\textsubscript{12} for hWT, hM207R, and hS186W (see Table 1). The lowest values of the multipolar moment (m = 0, l = 2) were found for 11-cis-retinal of hS186W, which suffers the largest chain twist (ca. 25° with respect to the WT systems) at the C\textsubscript{10}–C\textsubscript{11} bond (see Table 1). Consequently, the geometrical distortion at the C\textsubscript{10}–C\textsubscript{11} bond appears as a relevant factor affecting the atom contributions to the π-conjugated system in mutated human rhodopsins with respect to hWT.

The lowest values of the quadrupolar moment (m = 0, l = 2) is calculated for the C\textsubscript{10} and C\textsubscript{11} atoms of 11-cis-retinal for hS186W.
in agreement with the largest geometrical distortion and the represented \( \pi \)-cloud gap at the retinal central region. The drastic charge reduction at the \( \text{C}_{10} \sim \text{C}_{11} \) bond in hS186W can be enough to achieve the more extensive perturbation of the retinal \( \pi \)-conjugated system. Here, the significant decrease of retinal charge delocalization, in terms of the great shortening of the \( \pi \)-cloud extension, explains the strongest blue shift in this mutant with respect to hM207R and WT rhodopsins (see Fig. 5b).

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**Figure 5.** Electron density deformations isosurfaces of the mutants hM207R and hS186W (model Rho1). At left, the \( \pi \)-conjugated electron system of 11-cis-retinal in both WT rhodopsins is discontinuous (gap). At right, blue shifts of the absorption band calculated previously\(^{17}\) for both mutants. Oscillator strength \((f)\). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Table 1. Multipolar moments \((m = 0, l = 2)\) of implicated atoms with electron density deformations at the retinal \(C_{10}-C_{11}\) and \(C_{14}-C_{15}\) bonds.

<table>
<thead>
<tr>
<th>Structure</th>
<th>(C_{10}) ((m = 0, l = 2))</th>
<th>(C_{11}) ((m = 0, l = 2))</th>
<th>(\varphi_{C_{9}-C_{10}-C_{11}-C_{12}})</th>
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<tbody>
<tr>
<td>bWT</td>
<td>1.273</td>
<td>0.922</td>
<td>170.54</td>
</tr>
<tr>
<td>hWT</td>
<td>1.401</td>
<td>1.077</td>
<td>170.79</td>
</tr>
<tr>
<td>hM207R</td>
<td>1.245</td>
<td>0.920</td>
<td>160.44</td>
</tr>
<tr>
<td>hS186W</td>
<td>0.440</td>
<td>0.762</td>
<td>145.13</td>
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</table>

<table>
<thead>
<tr>
<th>Structure</th>
<th>(C_{14}) ((m = 0, l = 2))</th>
<th>(C_{15}) ((m = 0, l = 2))</th>
<th>(\varphi_{C_{13}-C_{14}-C_{15}-N})</th>
</tr>
</thead>
<tbody>
<tr>
<td>bWT</td>
<td>1.723</td>
<td>0.624</td>
<td>175.57</td>
</tr>
<tr>
<td>hWT</td>
<td>1.617</td>
<td>0.670</td>
<td>173.34</td>
</tr>
<tr>
<td>hM207R</td>
<td>1.342</td>
<td>0.532</td>
<td>165.62</td>
</tr>
<tr>
<td>hS186W</td>
<td>1.786</td>
<td>0.947</td>
<td>170.54</td>
</tr>
</tbody>
</table>

Dihedral angles \((\varphi)\) for the bonds related with the electronic perturbations. These average values correspond to the dark state of retinal optimized using \(\text{RhO}_1\) model at the QM/MM level. Quadrupole components are in atomic units and angles in degrees.

Table 1 shows multipolar moment average values of the \(C_{14}\) and \(C_{15}\) atoms and evidences an unperturbed \(11\text{-cis-retinal}\) at the \(C_{14}-C_{15}\) single bond in the hS186W mutant with respect to both WT, according to the high planarity at this single bond. In contrast, the \(11\text{-cis-retinal}\) region in hM207R is significantly affected near to the Schiff-base end. A notable reduction of atom contributions to the electron charge delocalization is found for \(C_{14}\) and \(C_{15}\) atoms. This finding agrees with the \(\pi\)-cloud representations displaying an electron density gap at the \(C_{14}-C_{15}\) bond, a disturbance that is found only in this mutant (see Figs. 4 and 5). Besides, the dihedral angle \(\varphi_{C_{13}-C_{14}-C_{15}-N}\) shows a change of about \(10^\circ\) in the hM207R mutant, compared with the remaining systems. At the contour values used here, the small extension of the electron density gap at the \(C_{14}-C_{15}\) bond corresponds with the twist degree of retinal at this single bond. Both the electron density discontinuity at the \(C_{14}-C_{15}\) bond and the more pronounced central isthmus explain the reduction of the retinal \(\pi\)-cloud extension in hM207R with respect to WT rhodopsins, according to the spectral properties of this mutant.

Calculations of electron density deformations in \(\text{Retgas}\) and \(\text{RhO}_2\) models provide further insights into the causes of the perturbation of whole charge delocalization pattern of protein embedded retinals. In Figure 6, the pictures using \(\text{Retgas}\) model for DAM calculations show the conjugated \(\pi\) system of the \(11\text{-cis-retinal}\) linked to the Lys296 part via a deprotonated SBR. The \(\pi\)-conjugated system in \(\text{Retgas}\) model is widely continuous and unperturbed along the retinal polyene chain, similar to the picture achieved for hWT (see Figs. 3, 4, and 6). However, retinal-SBR-Lys296 \((\text{Retgas})\) exhibits unnoticeable isthmuses of electron density deformations at the \(C_{10}-C_{11}\) and \(C_{14}-C_{15}\) single bonds of \(11\text{-cis-retinal}\). This system is the most planar structure at the central and Schiff-base regions. Its dihedral angles \(\varphi_{C_{5}-C_{10}-C_{11}-C_{12}}\) and \(\varphi_{C_{13}-C_{14}-C_{15}-N}\) show values of 178 and 180°, respectively. In addition, this system displays the lowest BLA value \(0.22\) Å and a low-energy excited state \((S_1\) energy\) of 1.99 eV \(622\) nm\)\(^{[5a]}\) in agreement with experimental and theoretical studies for retinal in vacuum \((610–635\) nm\)\(^{[1a,8c,28]}\).

In Figure 7, electron density deformation isosurfaces using \(\text{RhO}_2\) model in hWT, hM207R, and hS186W are depicted. Results are very similar to those obtained from model \(\text{RhO}_1\). However, the hM207R mutant using the \(\text{RhO}_2\) model does not show the particular discontinuity of the \(\pi\)-cloud at the characteristic retinal \(C_{14}-C_{15}\) bond of the \(\text{RhO}_1\) model (see Figs. 5a and 7b). This unperturbed electron density around the Schiff-base region is related with a more planar dihedral angle \(\varphi_{C_{13}-C_{14}-C_{15}-N}\) \((178.47^\circ)\) in the hM207R mutant \((\text{RhO}_2)\).  

**Figure 6.** Electron density deformation isosurfaces in the system \(11\text{-cis-retinal-SBR-Lys296}\) from model \(\text{Retgas}\). Two pictures in different planes of the retinal \(\pi\)-conjugated system and an unperturbed electron density at the retinal \(C_{10}-C_{11}\) and \(C_{14}-C_{15}\) bonds. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]
Indeed, the retinal atom contributions of C$_{14}$ (1.435 a.u.) and C$_{15}$ (0.755 a.u.) to the charge delocalization are higher in hM207R of $Rho_2$ than in hM207R of $Rho_1$ model (see Table 1). Although, there are no electron density perturbations at the C$_{14}$–C$_{15}$ bond in the $Rho_2$ model, these disturbances persist at the retinal central regions; here we pay special attention to it.

The central isthmus of the electron density deformation in the $Rho_2$ model remains more pronounced in hM207R mutant than in hWT (see Figs. 4, 5a, and 7b). Again, a great electron density gap corresponds to the hS186W mutant, even when the polarization effects on retinal due to the mutations were not considered in this model ($Rho_2$). In Table 2, multipolar moments $m = 0$ ($l = 2$) of the atoms implicated with electron density deformation for the $Rho_1$ model are shown. The central geometrical distortion is related with the reduction of the charge delocalization in both mutants, being the charge a lot less delocalized in the less planar mutant hS186W at the retinal central region (see Table 2 and Fig. 7).

Both relevant geometrical distortion and electron charge-delocalization reduction increase when going from WT rhodopsins to hM207R and hS186W mutants (see Table 1 and Figs. 4, 5, and 6). These results are in full agreement with significant changes of BLA and the interprotein spectral shift between these rhodopsins.[$^{[5a]}$] Both the BLA and VEE are very sensitive to the electron charge delocalization of chromophores.[$^{[1c,8c,14]}$] Therefore, the spectral differences between these rhodopsins can be explained in terms of the disturbances of electron density deformations.

![Figure 7.](image)

**Figure 7.** Electron density deformation isosurfaces on: (a) hWT, (b) hM207R, and (c) hS186W from model $Rho_2$. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

<table>
<thead>
<tr>
<th>Structure</th>
<th>C$_{10}$ ($m = 0$, $l = 2$)</th>
<th>C$_{11}$ ($m = 0$, $l = 2$)</th>
<th>$\phi$C$<em>{10}$–C$</em>{10}$–C$<em>{11}$–C$</em>{12}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>hWT</td>
<td>1.291</td>
<td>0.830</td>
<td>170.27</td>
</tr>
<tr>
<td>hM207R</td>
<td>1.215</td>
<td>0.732</td>
<td>160.06</td>
</tr>
<tr>
<td>hS186W</td>
<td>0.687</td>
<td>0.872</td>
<td>153.26</td>
</tr>
</tbody>
</table>

Dihedral angle ($\phi$) for the bond related with the electronic perturbation. These values correspond to the dark state of retinal optimized using $Rho_2$ model at the QM/MM level. Quadrupole components are in atomic units and angles are in degrees.
Table 3. Average values of \(\omega_1\), \(\omega_2\), and \(\omega_3\) torsion angles (in degree), which correspond to the dark state of retinal optimized from the Rho model.

<table>
<thead>
<tr>
<th>Structure</th>
<th>(\omega_1)</th>
<th>(\omega_2)</th>
<th>(\omega_3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>bWT</td>
<td>39.32</td>
<td>26.36</td>
<td>7.75</td>
</tr>
<tr>
<td>hWT</td>
<td>40.05</td>
<td>25.63</td>
<td>8.07</td>
</tr>
<tr>
<td>HM207R</td>
<td>63.95</td>
<td>29.43</td>
<td>17.36</td>
</tr>
<tr>
<td>hS186W</td>
<td>49.32</td>
<td>38.56</td>
<td>33.09</td>
</tr>
</tbody>
</table>

See text and Figure 8 for the description of torsion angles.

Geometrical central distortions of retinal

The significant twist of the retinal polyene chain, as a consequence of the rhodopsin environment, is well documented by spectroscopic and molecular modeling studies. Torsion angles for \(\omega_1\), \(\omega_2\), and \(\omega_3\) which correspond to the dark state of retinal optimized from the Rho model (WT and mutants) are depicted in Table 3. The angle \(\omega_1\) is described between the planes formed by atoms \(C_\epsilon-C_\gamma-C_\delta-C_\beta\) \((\rho_1)\) and \(C_\epsilon-C_\beta-N\) \((\rho_2)\). Torsion angles \(\omega_2\) and \(\omega_3\) are described in Figure 8.

Theoretical approaches have described the retinal kink using the \(\omega_1\) angle for bWT, showing values of 40 and 44° from classical and QM/MM MD studies, respectively. These values are in agreement with the average \(\omega_1\) values for the bWT and hWT systems reported in Table 3. These \(\omega_1\) values indicate that the retinal is more twisted in the HM207R mutant than in WT rhodopsins and the hS186W mutant. A similar trend has been documented for the dihedral angle \(H_7-C_7-C_8-C_9\) \((\rho_1)\) and \(C_11-C_12-N\) \((\rho_2)\). The dihedral angle is used as a conventional way to explore the helical molecule torsion.

In Table 3, the \(\omega_2\) angle shows larger values for hM207R and hS186W than for the WT rhodopsins, being again the hS186W mutant the more planar system at the \(C_{10}-C_{11}\) single bond. The \(\omega_2\) angle describes appropriately the arched twist for the systems, displaying a more curved retinal in both mutants with respect to normal rhodopsins. The 11-cis-retinal in the hS186W mutant is about two times more arched than in the other mutant at the retinal central region (see Table 3). These helical and arched torsion of 11-cis-retinal also led to a larger \(C_{10}-C_{11}\) distance in the less planar structures, 1.44 Å (HM207R) and 1.45 Å (hS186W) compared with 1.43 Å in the more planar systems at this bond (bWT and hWT).

The \(\varphi_{C_9-C_{10}-C_{11}-C_{12}}\) angle was also calculated in Ret^gas and all Rho models to identify its relation with the BLA. This dependence was explored because the disturbance of the conjugated \(\pi\) system at the \(C_{10}-C_{11}\) bond becomes larger as \(\varphi_{C_9-C_{10}-C_{11}-C_{12}}\) deviates from 180° (see Table 1 and Figs. 4, 5, and 7). This fact leads to a reduction of the \(\pi\)-cloud extension (charge delocalization) and an increased BLA. In Figure 9, we have studied the regression analysis between BLA (in Å) and the \(\varphi_{C_9-C_{10}-C_{11}-C_{12}}\) (in degree) using both linear and quadratic descriptions. We found that larger BLA values are associated to a larger nonplanarity \(\varphi_{C_9-C_{10}-C_{11}-C_{12}}\) values far from 180 or 0°. As we can observe in Figure 9, linear and quadratic relations display very similar values of \(R^2\) and rmsd. The quadratic function describes the predicted BLA values with more physical meaning that the linear function (see Fig. 9). A more extensive data set including \(\varphi_{C_9-C_{10}-C_{11}-C_{12}}\) values in the range from 0 to 90° would improve the fitting parameters. This geometrical relation (BLA vs. \(\varphi_{C_9-C_{10}-C_{11}-C_{12}}\)) is consistent with the known link between helical twists and changes of the electronic structure of retinal, which can regulate the visual pigment wavelength. Therefore, the retinal kink at the \(C_{10}-C_{11}\) bond can be a relevant factor for the spectral tuning mechanisms.

Recent studies state that changes of the hydrogen-bonding network also regulate the absorption spectra in retinal proteins. Our previous QM/MM study shows a higher...
perturbation of the hydrogen-bonding network in the systems with higher VEE. However, this mechanism can be reduced to changes of the interaction between the chromophore and the amino acid residues around it, being the disturbance of the hydrogen-bonding network a consistent chemical basis for the protein core destabilization. In addition, it is reasonable to state that substitutions of amino acid residues placed far away from the chromophore could also lead to a significant structural reorganization of rhodopsin and retinal distortions due to steric interactions. This has possible implications for the spectral tuning and evidences the importance of other factors in addition to electrostatic effects.

**BLA and VEE**

The tentative relationship between retinal torsion and VEE suggests the need to revisit also the relation VEE versus BLA using values from the same data set (Rho<sub>o</sub> and Ret<sub>gas</sub> models). Several theoretical studies have documented a linear relationship between the S<sub>1</sub> energy and BLA for the wide UV-visible range of some systems and particularly of the 11-cis-retinal. In Figure 10, two relationships between VEE (eV) and BLA (Å) values using all models has been fitted, using both linear and quadratic polynomial functions. The quadratic function shows a higher R<sup>2</sup> than the linear function and a rmsd two times lower (0.03) than the corresponding linear (0.07). The quadratic function describes the dependence between VEE and BLA rather than VEE and 6-s-cis conformation. Perhaps, the 6-s-trans conformation of retinal absorbing at 610 nm would have a BLA value close to the calculated in retinal from Ret<sub>gas</sub> model absorbing at 622 nm. The 6-s-cis conformation is known to influence the VEE, either by affecting the charge delocalization or the BLA. The latter is sensitive not only to changes in the 6-s-cis conformation but also to other factors affecting the conjugated π system.

Conclusions

The ground-state electronic structures of 11-cis-retinal in WT (bovine and human) rhodopsin and two human rhodopsin mutants (hM207R and hS186W) associated to retinitis pigmentosa disease is investigated by means of the analysis of electron density deformations. The DAM computed electron density on these structures provides a detailed picture of conjugated π electron systems along the polyene chain of retinal chromophore and shows differences for the electronic structures of 11-cis-retinal among mutant and WT rhodopsin. Perturbed π-conjugated molecules appear in both mutants, sustaining less charge delocalization when going from WT to hM207R and hS186W rhodopsin. DAM calculations show π-conjugated compound disturbances as linked to relevant geometrical distortions of the retinal chain. Significant nonplanarity at the retinal polyene central
region appears for the hS186W mutant, whereas in the case of the hM207R mutant is also seen at the Schiff-base end. The latter displays large retinal distortions at both regions and a sensitive reduction of charge delocalization when compared to WT rhodopsins, in agreement with the spectral blue shift of this mutant. The largest twist corresponds to the hS186W mutant at the retinal central region, leading to a more evident reduction of the $\pi$-cloud extension. In addition, the calculation of electron density deformations suggests the implication of water in retinal electronic structures.

We also found that helical and arched twists of the retinal chromophore, especially at the central vinyl chain region, influence charge delocalization and spectral tuning of retinal proteins. Additionally, we present second-order polynomial functions that are able to robustly describe relationships between planar distortions and BLA, and those with VEE. Direct nonlinear dependences were found for both relationships.

The analysis of the conjugated $\pi$ electron system of 11-cis-retinal by the electron density deformation together with the geometrical factors associated to the protein environment perturbations provides a new and useful insight into the optical properties of visual pigments carrying the retinal chromophore. The electron density deformations analysis allows rationalizing the link between retinal geometric and electronic changes associated to the absorption maximum and clarifying the spectral tuning mechanisms. Therefore, the DAM method arises as a novel approach able to provide a direct description of retinal delocalization patterns to fully explore the retinal electronic structure.

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**Keywords:** rhodopsin • mutations • retinitis pigmentosa • electronic structure • electron density deformations


Additional Supporting Information may be found in the online version of this article.

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