

# A FIRST-PRINCIPLES STUDY OF 11-*CIS*-RETINAL: MODELLING THE CHROMOPHORE-PROTEIN INTERACTION IN RHODOPSIN

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## Abstract

The 11-*cis*-retinal protonated Schiff base is the chromophore of rhodopsin, the photoreceptor in the vertebrate eye. The photochemical isomerization from 11-*cis* to the all-*trans* form triggers a series of enzymatic reactions known as the visual cascade which eventually leads to a neural signal. Experiments such as resonance Raman, NMR *etc.*, have shown that 11-*cis*-retinal is probably highly twisted in the protein pocket. Because detailed knowledge about the kind of interaction with the protein is missing, a theoretical description of the chromophore conformation is difficult. In the simulations the results of which will be presented here, we assume that the retinal chromophore, as a consequence of the steric fit into the protein binding pocket, undergoes a specific kind of conformational change. The structure we obtain is in good agreement with the experimentally observed highly twisted conformation of the chromophore backbone.

*Keywords:* Rhodopsin, 11-*cis*-retinal, *Ab initio* molecular-dynamics simulations

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\*Dedicated to Professor M. A. Ikeda on the occasion of his 70th birthday.

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# 1. INTRODUCTION

Light is essential for living organisms because it provides the energy through photosynthesis as well as the means to effectively interact with the environment through visual sensations. Rhodopsin is the protein responsible for generating an optic nerve impulse in the visual receptors of the three phyla which possess eyes: Mollusks, arthropods, and vertebrates. Classical studies by Wald and coworkers first revealed the photochemical process that initiates visual excitation (Yoshizawa and Wald, 1963). In particular Wald showed that the primary action of light is the photo-isomerization of 11-*cis* retinal to all-*trans* (Fig. 1), which eventually leads to the chemical signal of a nerve impulse (Wald, 1968). Of all the double bonds in the molecule which can theoretically undergo *cis-trans*-isomerization, only the C11–C12 double bond is affected by the action of light. When the 11-*cis* isomer of native rhodopsin absorbs green light of  $\sim 500$  nm (Mathies and Lugtenburg, 2000), the chromophore is excited to the Frank-Condon region of the first excited state, from which it isomerizes to the all-*trans*-isomer bathorhodopsin within only 200 fs. The efficiency and selectivity of this extremely fast reaction can only be understood if proper account is taken of the effects of the environment on the structure and dynamics of the chromophore.

In rhodopsin the retinal chromophore is embedded in a pocket formed by seven  $\alpha$ -helices, which contain about 305 amino acids (Palczewski, Kumasaka, Hori, Behnke *et al.*, 2000). The chromophore of rhodopsin is bound to Lys-296 as a protonated Schiff base (PSB). Recent investigations by resonance Raman (Lin, Groesbeek, van der Hoef, P. Verdegem *et al.*, 1998) and NMR experiments (Verdegem, Bovee-Geurts, de Grip, Lugtenburg *et al.*, 1999) have shown that the 11-*cis*-retinal is highly twisted in the protein pocket, presumably because of intramolecular steric interaction between the methyl group at C13 and the hydrogen atom at C10 in the chromophore (the numbering of the atoms is shown in Fig. 1). On the other hand, 13-demethyl-11-*cis*-retinal PSB, which has no methyl group at C13, is closer to planar in the protein pocket (Wang, Kochendoerfer, Schoenlein, Verdegem *et al.*, 1996). The isomerization reaction rate of this rhodopsin analogue is  $\sim 400$  fs, which is about two times slower than the corresponding rate in rhodopsin. This suggests that the steric interaction between the 13-methyl group and the 10-hydrogen atom facilitates the C11–C12 double bond rotation during the photoisomerization.

In this work we have investigated the effect of the protein pocket on the retinal. Because detailed knowledge about the kind of interaction in the protein binding pocket is missing, a theoretical description of the chromophore conformation is difficult. However, it appears safe to assume that the retinal chromophore undergoes some kind of conformational change by the steric fit into the protein binding pocket. So we add external strain to the 11-*cis*-retinal and perform a molecular dynamics (MD) relaxation, after which the molecule has a highly twisted structure in good agreement with experimental

data.

For the MD calculations the Vienna *ab initio* simulation package (VASP) was used which is a program designed for MD simulation based on density functional theory (DFT). It uses a plane wave basis set and Vanderbilt ultrasoft pseudopotentials. Details about the program can be found in the literature (Kresse and Furthmüller, 1996). All simulations are done for a sufficiently large cell and periodic boundary condition.

## 2. SIMULATION OF THE 11-*CIS*-RETINAL CHROMOPHORE

The chromophore of rhodopsin, 11-*cis*-retinal PSB, is connected via amino acid Lys-296 to the protein. The nitrogen atom is protonated and the chromophore has a net positive charge (Fig. 2, top). Two-photon spectroscopy indicates that the binding site of rhodopsin is neutral (Birge, Murray, Zidovetzki and Knapp, 1987), and consequently there is a negatively charged counter ion in the vicinity of the chromophore. There is evidence that the primary counter ion of the Schiff base is Glu-113 (Zhukovski and Oprian, 1989) Also, one or more water molecules are present within the rhodopsin binding site, and the high C = N stretching frequency and large ND isotope shift indicate that the Schiff base is strongly hydrogen bonded (Rodman-Gilson, and Honig, 1988), possibly to water in the binding site (Cossette and Vocelle, 1987; Tallent, Hyde, Findsen, Fox *et al.*, 1992). A possible model of the rhodopsin binding site that is consistent with available experimental data, and which we call the minimal 11-*cis*-retinal PSB complex, is shown in Fig. 2 (bottom). The counter ion HCOO<sup>-</sup> models part of the negatively charged amino acid Glu-113. The whole structure is electrically neutral, and this is the simplest structure, where the proton can move from the nitrogen via the water molecule to a negatively charged counter ion HCOO<sup>-</sup> (Terstegen and Buß, 1998).

Minimization of the total energy of these models has been performed by simulated annealing. The supercell has been chosen large enough to avoid interaction with images:  $24 \times 18 \times 13 \text{ \AA}^3$ . The calculation has been done with a time step for the MD simulation of 0.5 fs and for one *k*-point ( $\Gamma$  point only). The cut-off energy was 261.1 eV and we have used the generalized gradient approximation (GGA). In the simulation of the isolated 11-*cis*-retinal PSB (Fig. 2, top) we assume a uniform negative back ground charge  $-e$  to achieve charge neutrality. The minimum energy conformations of these models are both planar except for the C6–C7 bond which is strongly twisted because of non-bonded interaction between the methyl group at C5, and the hydrogen atom at C8. The steric hindrance between the methyl group at C13 and a hydrogen atom at C10 seems to induces a twist around the region from C10 to C13. The planarization effect, however, is so strong that it causes an in-plane bending of the C13 methyl group instead. Complexation at this

level does not induce non-planarity into the chromophore. It does influence the bond lengths, however: all double bonds become longer, all single bonds become shorter, which stabilizes the resonance structure and enhances the bond alternation. It has been suggested that the counter ion  $\text{HCOO}^-$  group near the nitrogen atom of the PSB in bacteriorhodopsin will stabilize the resonance structure (Song, El-Sayed and Lanyi, 1993). These minimum energy structure of 11-*cis*-retinal is in good agreement with previous results obtained by using the Gaussian simulation package (Terstegen and Buß, 1998). Bifone *et al.* have optimized the 11-*cis*-retinal with Car-Parrinello *ab initio* MD based on DFT within the local density approximation (LDA) and found a non-planar structure with a dihedral angle of  $38^\circ$  around the C12-C13 single bond (Bifone, de Groot and Buda, 1996). Although this structure seems to be in good agreement with the experimentally observed twisted structure, it appears not be a minimum energy structure, because the boundary box which has been used ( $15.9 \times 10.6 \times 10.6 \text{ \AA}^3$ ) is too small to exclude the interaction with its images. The isolated chromophore and the minimal complex have essentially planar structures, and it is important to consider the influence of the protein environment on the chromophore adequately to explain the twisted structure observed by experiments.

To treat the effect of the environment around the chromophore, we assume that the retinal chromophore, as a consequence of the steric fit into the protein pocket, undergoes some kind of conformational change. As a first step we have introduced a nonspecific strain in the molecule by widening all external bond angles (C7-C8-C9, C9-C10-C11, *etc.*) and contracting all internal bond angles (C6-C7-C8, C8-C9-C10, *etc.*) by the same amount,  $2.5^\circ$ , to fit the chromophore into the protein pocket. The C6-N<sup>+</sup> distance, as a consequence, is reduced from 11.53 Å to 10.97 Å for the isolated molecule and from 11.49 Å to 10.98 Å for the minimal complex, respectively. These molecules with the reduced C6-N<sup>+</sup> distance are still approximately planar. Then we performed MD relaxation with fixed C6 and nitrogen atoms to keep the C6-N<sup>+</sup> distance constant until all bond lengths and angles, including dihedral angles, had equilibrated. The equilibrium bond lengths calculated with and without constraint differ by less than 0.008 Å. The behavior of the dihedral angles of the C11-C12 and the C12-C13 bonds is shown on Fig. 3. Dashed lines refer to the isolated 11-*cis*-retinal PSB and dotted lines to the minimal complex. The molecule is mainly twisted in the region from C11 to C13. The regions from C7 to C12 and from C13 to N<sup>+</sup> are essentially planar. The average of the dihedral angles of the final 200 fs for both MD runs are shown in Table I (Buß, Weingart and Sugihara, 2000). The counter ion affects only the C11-C12 bond, resulting in a twist angle of this bond which is about 5 degree smaller. Our previous investigations have shown that the isomerization around the C11-C12 bond in the ground state of the retinal chromophore is more difficult in the presence of the counter ion than without it. The counter ion returns charge to the chromophore and the C11-C12 bond becomes more double bond like. (Sugihara, Entel, Meyer,

Table 1: The dihedral angles of the 11-*cis*-retinal PSB calculated with constraint. These values are the average of the final 200 fs of MD run for the isolated 11-*cis*-retinal PSB (a) and the minimal 11-*cis*-retinal PSB complex (b). (c) is the equilibrium structure of the isolated 11-*cis*-retinal PSB calculated with another constraint: Each bond length between C6 and N<sup>+</sup> atoms is shortened by 0.07 Å. The numbering of the atoms is shown in Fig. 1.

	C6–C7	C7–C8	C8–C9	C9–C10	C10–C11
(a)	-29.04	180.60	178.69	183.23	182.55
(b)	-43.86	181.97	176.77	183.81	179.84
(c)	-31.41	181.38	176.92	185.66	177.56

	C11–C12	C12–C13	C13–C14	C14–C15	C15–N
(a)	14.08	192.19	174.02	185.31	177.30
(b)	9.42	193.79	177.02	179.98	179.45
(c)	14.26	193.00	174.77	186.17	179.93

Buřet *et al.*, 2000). The quantitative difference of the C11–C12 bond twist angle is understandable from our previous investigation.

There are different experimental methods to study the structure of the retinal chromophore. Among the spectroscopic methods for obtaining structural information about the chromophore in rhodopsin are resonance Raman (Lin, Groesbeek, van der Hoef, P. Verdegem *et al.*, 1998) and NMR (Verdegem, Bovee-Geurts, de Grip, Lugtenburg *et al.*, 1999). They indicate that the molecule is twisted in the central region but it is difficult to get more quantitative information.

One of the most powerful methods is solid-state NMR techniques in combination with selective isotope enrichment, which is capable of extracting direct molecular structural information. Here we discuss the experimental results obtained by the NMR methods. Verdegem *et al.* have determined the nuclear distances C10–C20 ( $r_{10,20}$ ) and C11–C20 ( $r_{11,20}$ ) in the chromophore by using rotational resonance MAS NMR, which allows the measurement of internuclear distances between spin 1/2 nuclei (Creuzet, McDermott, Gebhard, van der Hoef *et al.*, 1987; Verdegem, Helmle, Lugtenburg and de Groot, 1997) They have obtained  $r_{10,20} = 0.304 \pm 0.15$  Å and  $r_{11,20} = 2.93 \pm 0.15$  Å, respectively. The  $r_{10,20}$  and  $r_{11,20}$  distance measurements provide direct evidence that a considerable out-of-plane distortion is present in the central region of the rhodopsin chromophore. Modelling retinal geometry with MD using these intramolecular distances provides an estimate of the angle between the C6–C10 and the C13–C15 plane of 44°, with an error margin of  $\sim 10^\circ$  due to the uncertainties in the distance measurements (Verdegem, Bovee-Geurts, de Grip, Lugtenburg *et al.*, 1999). Another information about the chromophore of rhodopsin was obtained by Feng *et al.* who have measured the relative orientation of pairs of molecular spins (Feng, Lee, Sandström, Edén

Table 2: Bond distance, plane angle and dihedral angle of 11-*cis*-retinal PSB: comparison between experimental and different theoretical models: (a) isolated 11-*cis*-retinal PSB: Each bond angle between C6 and N<sup>+</sup> atoms is changed alternatively by  $\pm 2.5$  degree. (b) the same as (a), but with added HCOO<sup>-</sup> and a water molecule (minimal complex).

	Experiment	(a)	(b)
C10–C20 distance	$3.04 \pm 0.15 \text{ \AA}^a$	3.08 Å	3.11 Å
C11–C20 distance	$2.93 \pm 0.15 \text{ \AA}^a$	3.12 Å	3.14 Å
C6–C10 & C13–C14 plane angle	$44^\circ \pm 10^\circ^a$	31.62°	30.12°
H–C10–C12–H torsional angle	$160^\circ \pm 10^\circ^b$	153.50°	160.63°

<sup>a</sup>(Verdegem, Bovee-Geurts, de Grip, Lugtenburg *et al.*, 1999)

<sup>b</sup>(Feng, Verdegem, Lee, Sandström *et al.*, 1997)

*et al.*, 1996) with solid-state techniques and determined the H–C10–C12–H torsional angle of rhodopsin chromophore to be  $160^\circ \pm 10^\circ$ , which indicates a significant deviation from the planar *cis*-configuration (Feng, Verdegem, Lee, Sandström *et al.*, 1997). The angles estimated by these experiments and theoretical results are shown in Table II. The chromophore is not completely planar in the regions C6–C10 and C13–C15 and we show the dihedral angle between C6–C10 and C13–C14 planes in the third row of Table II. The structures we obtained are in good agreement with the experimentally obtained highly twisted conformation of the chromophore backbone.

There is another possibility to add external strain to the chromophore. When each bond length between C6 and N<sup>+</sup> atoms is shortened by 0.07 Å, the C6–N<sup>+</sup> distance is reduced to 10.98 Å. During the MD relaxation process, the C10–C11 bond at first twists in the opposite direction, but after 500 fs it goes back to the same direction as in the previous MD simulation. The equilibrium configuration of this molecule is shown in Table 1 (c), which is very similar to the previous results, (a) and (b).

Finally we discuss the 13-demethyl-11-*cis*-retinal PSB, which has no methyl group at the C13 and therefore a closer to planar structure, (Wang, Kochendoerfer, Schoenlein, Verdegem *et al.*, 1996). We have investigated the influence of the environment on the 13-demethyl-11-*cis*-retinal PSB in a manner similar to the one used before. This molecule does not relax into a non-planar conformation under the external strain, which agrees with experimentally observed results.

### 3. CONCLUSION

In this work we have investigated the effect of the environment on the 11-*cis*-retinal PSB. Free optimized structures of the 11-*cis*-retinal PSB are planar and this is not changed in the minimal complex with HCOO<sup>-</sup> and H<sub>2</sub>O. We

have investigated the effect of the protein pocket on the chromophore by reducing the distance between C6 and the N atom. The re-optimized structures with the external strain are highly twisted structures in the isomerization region, and the twist is shared between the C11–C12 and C12–C13 bonds, which is in good agreement with the experimentally observed results. The counter ion affects the C11–C12 bond which acquires more double-bond in character. Because of the effect of the counter ion, the dihedral angle of the C11–C12 bond of the 11-*cis*-retinal PSB complex is about 5 degree smaller than in the isolated 11-*cis*-retinal PSB. 13-demethyl-11-*cis*-retinal PSB stays planar and does not relax into a non-planar form under the external strain, in agreement with experimental evidence.

### ***Acknowledgements***

We thank K. Kolster and Y. Sakamoto for discussions. Funding is acknowledged by the Graduate School on *Structure and Dynamics of Heterogeneous Systems* at the University of Duisburg.

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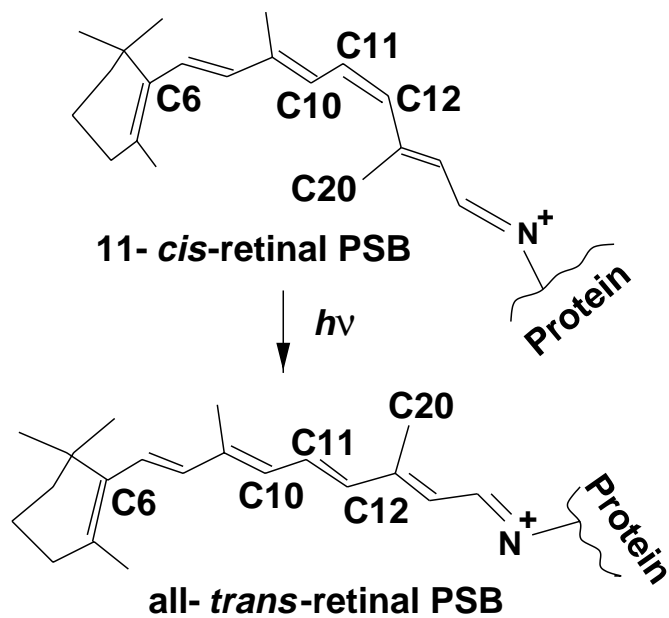


Figure 1: Photoisomerization of the chromophore of rhodopsin from 11-*cis*-retinal PSB (top) to all-*trans*-retinal PSB (bottom).

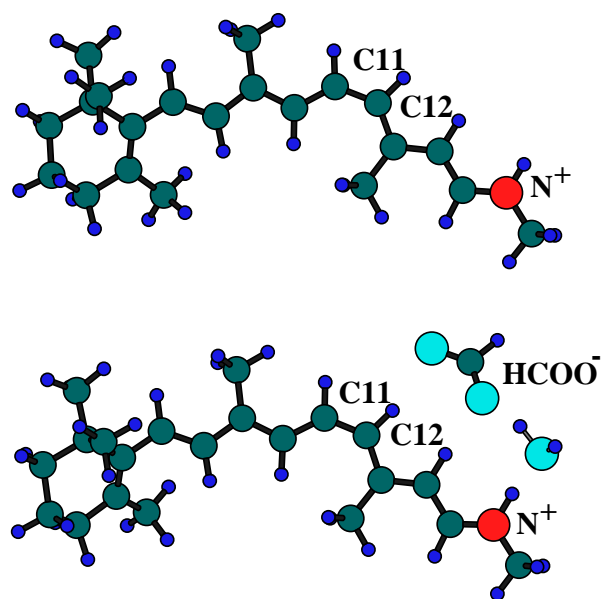


Figure 2: Models of the rhodopsin chromophore of increasing complexity used for calculations. 11-*cis*-retinal PSB (top) and 11-*cis*-retinal with a counter ion HCOO<sup>-</sup> and a water molecule, the minimal complex (bottom).

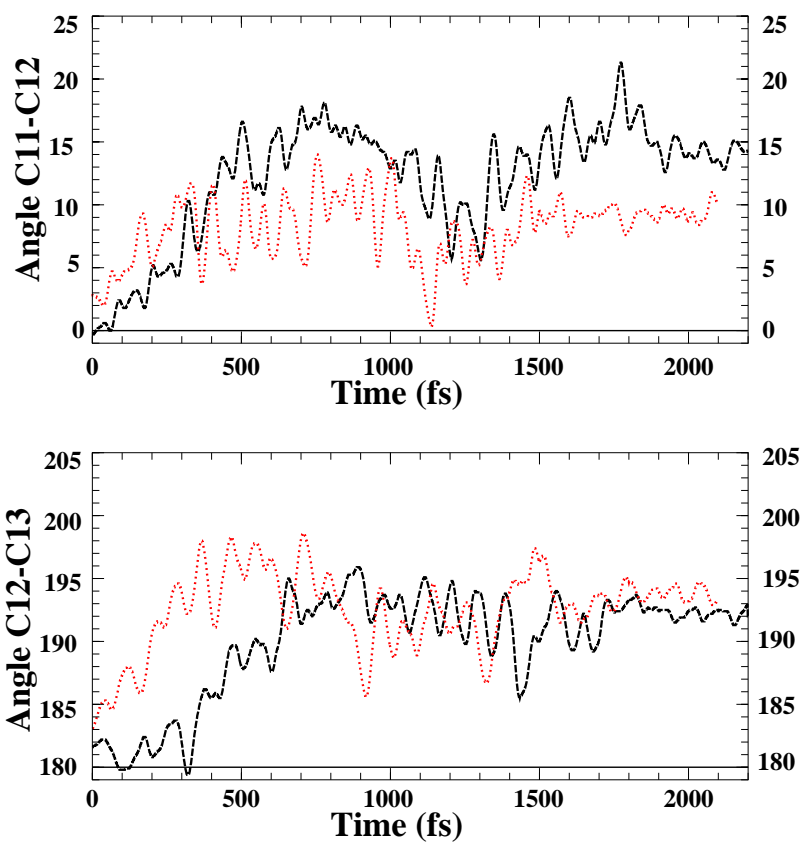


Figure 3: The dihedral angle of the C11-C12 and C12-C13 bonds. Dashed lines are the results for the isolated 11-*cis*-retinal PSB and dotted lines are the results for the 11-*cis*-retinal PSB with counter ion and a water molecule.