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Modelling of Photointermediates Suggests a Mechanism of the Flip of the β-Ionone Moiety of the Retinylidene Chromophore in the Rhodopsin Photocascade

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Light causes an extremely rapid 11-*cis*-to-all-*trans* isomerization of the retinylidene chromophore of rhodopsin.[1] This isomerization leads to bleaching intermediates in the photoactivation cascade. An early photointermediate, bathorhodopsin (Batho), which already contains a photoisomerized all-*trans* retinylidene chromophore, slowly ($\approx$1 μsec) decays by conformational changes to metarhodopsin I (Meta I) through lumirhodopsin (Lumi). The *cis-trans* photoisomerization of the retinylidene chromophore of rhodopsin occurs within the limited space of opsin, which results in a highly strained conformation of the chromophore. In a photoaffinity labeling experiment, Nakanishi et al. showed that a modified β-ionone moiety cross-linked Trp265 on transmembrane segment 6 (TM6) both in the rhodopsin and Batho states, which suggests that the cyclohexenyl moiety remains unchanged in the rhodopsin-to-Batho transition. In the subsequent Batho-to-Lumi transition, the moiety flipped from TM6 towards TM4.[2]

The flip of the modified β-ionone moiety suggests that TM3 and TM4 rearrange to accommodate the modified β-ionone moiety, as schematically shown in Figure 1, while the helix arrangement of Batho remains unchanged. Spectroscopic analyses such as UV/Vis, resonance Raman, and FTIR spectra of Batho and Lumi have revealed that Lumi has an almost relaxed all-*trans* chromophore with the protonated Schiff base (PSB), whereas Batho has a twisted double bond at C11=C12.[3] However, little is known about the structural origins of the twisted structure of the Batho chromophore and the flip of the β-ionone moiety in the Batho-to-Lumi transition.

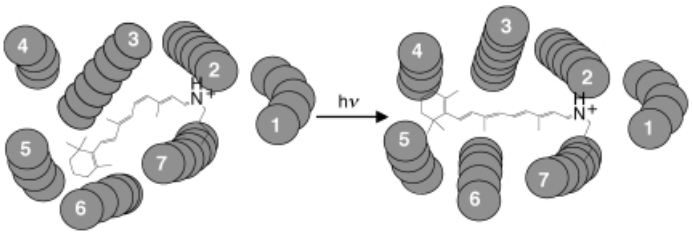

Figure 1. *The flip of the β-ionone moiety and the rearrangement of TM3 and TM4 in the photoactivation. A view from an intradiscal side.*

We previously formulated a structural model of the Batho chromophore[4] by using models of the transmembrane helices constructed from a projection map of rhodopsin.[5] The crystal structure of rhodopsin has recently been solved at a resolution of 2.8 Å[6] and this could greatly aid structural analysis of the process of formation of the photoactivated intermediates in the photocascade.

In the study reported herein, we applied restrained molecular dynamics simulation to the isomerization of the chromophore. Based on the present results, we propose structural models for Batho and Lumi that provide a structural explanation for the flip of the chromophore and the motion of TM3 and TM4.

The extraordinarily rapid photoisomerization ($\approx$200 fs) at a low temperature (77 K) leaves most of the opsin structure unaffected.[7] We generated candidate structures of the Batho chromophore in the crystal structure of opsin by using molecular dynamics and subsequent structure minimization and freezing

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the coordinates of opsin except for the Lys296 residue, which forms the PSB.

All of the Batho chromophore structures we generated showed essentially the same conformation under the conditions described above, which suggests that the binding cleft allows very limited motion of the chromophore. The distance between the PSB and the carboxylate group of Glu113 (3.36 Å) was similar to that in rhodopsin (3.40 Å). The Batho chromophore had a characteristically twisted double bond at C11=C12, with a negative dihedral angle (−148°) for C10−C11−C12−C13, while the cyclohexenyl moiety remained in the original binding cleft with a dihedral angle of −30° for C5−C6−C7−C8 (Figure 2). The steric interaction between the 13-methyl carbon atom and the amide carbonyl oxygen atom of Cys187 (3.11 Å) prevented a complete rotation of the C13−Nζ portion and thus stabilized the Batho chromophore. The instability of the Batho state, as observed in the photoisomerization of the 13-desmethyl retinylidene chromophore,[8] may be due to the removal of this steric interaction, though the interaction may not be of importance for the physiological function.[9] Furthermore, the carbonyl oxygen atom of Cys187 is located close to the Cα atom of Gly114 (3.62 Å).

The twisted double bond presumably makes a major contribution to the bathochromic shift (around 40 nm) in the UV absorption maximum of the Batho chromophore. We used ZINDO, a semiempirical quantum chemical program, to estimate that the bathochromic shift of the Batho chromophore model was about 30 nm. Together with the negatively twisted C11=C12 double bond, the negative dihedral angles of the double bonds for C11=C12−C13=C14 (−172°), and C13=C14−C15=Nζ (−150°) form a left-handed helical conformation in the C10 – Nζ portion of the structure, and are thus associated with the negative Cotton signal at 540 nm observed for the Batho chromophore.[10]

It has been observed that irradiation of rhodopsin generates a highly strained photointermediate, photorhodopsin (Photo),[11] prior to the formation of Batho. Snapshots of molecular dynamics calculations at 60 – 100 fs show a more twisted conformation at the C11=C12 bond (−112°) and the C12−C13 bond (−143°) than in Batho, although the conformation is not stable enough to maintain the highly twisted structure during simulation. The nascent chromophore structure suggests that the ultra-fast *cis*-to-*trans* isomerization (< 200 fs) gives rise to a highly strained all-*trans* chromophore with a highly twisted conformation at the C11=C12 and C12−C13 bonds. This process may reflect the *Hula-twist* isomerization at the C11 – C13 portion of the molecule (**1** →**2** in Scheme 1).[12–14]

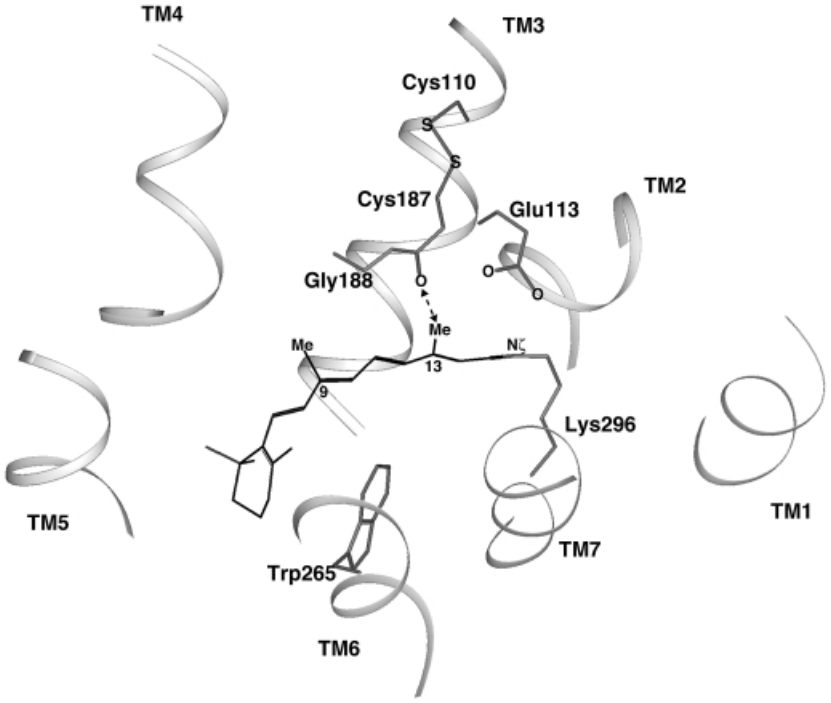
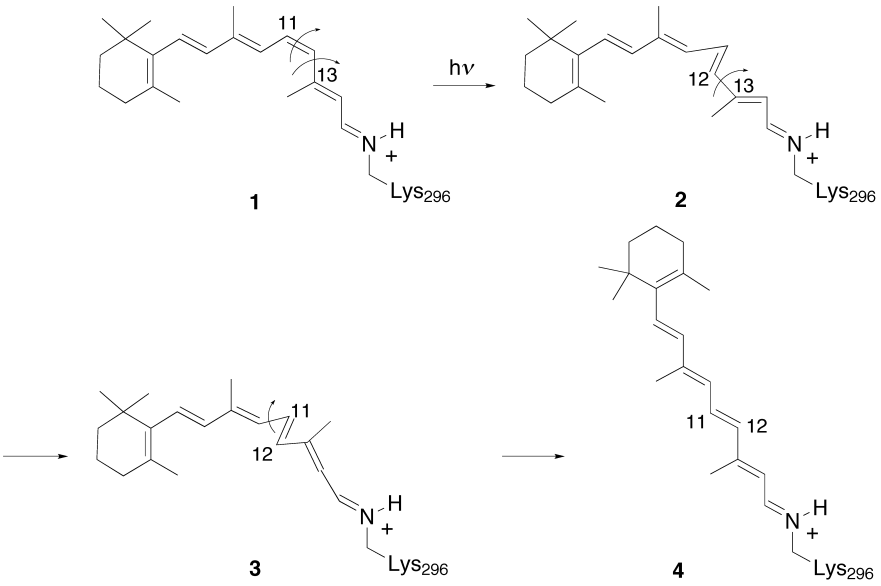


Figure 2. *The structural model of the Batho chromophore with surrounding residues at the binding cleft. A view from an extracellular site. The broken arrow indicates the steric interaction between the C13-Me group and the carbonyl oxygen atom of Cys187. Transmembrane helices (TM) are partially represented by ribbons.*



Scheme 1. *A putative process for the 11-*cis*-to-all-*trans *isomerization of rhodopsin (**1**) to Lumi (**4**). At the first step (**1** →**2**), the photoisomerization only allows a hula-twist of the C11 – C13 portion of the chromophore in the very limited space of opsin, which is followed by rotation of the C11 – Nζ portion (**2** →**3**). The flip of the β-ionone moiety affords the Lumi chromophore. Arrows indicate the rotation of the bonds involved in the formation of each subsequent intermediate of the Batho chromophore.*

The highly twisted C11=C12 double bond of the nascent intermediate requires a rotational motion of the C13 – Nζ portion of the structure to restore the relaxed double bond, while the bulky β-ionone moiety is tightly held at its original position. Then, incomplete rotation of the C13 – Nζ portion described above results in the Batho structure (**2** → **3** in Scheme 1). The structural model described here shows an *s*-trans conformation at the C12–C13 bond, whereas an *s-cis* conformation was seen in our previous model.[4] This difference may be due to the narrower space in the binding cleft in our previous rhodopsin model. The narrow cleft did not allow rotation of the C13 – Nζ portion of the structure.

The twisted conformation at the C11=C12 double bond indicates that restoration of the relaxed C11=C12 double bond leads to a flip of the β-ionone moiety towards TM3, and that outward motion of TM3 is required for formation of the subsequent intermediate, Lumi (Figure 3). We assume that the intracellular portion of TM3 swings outward without significantly affecting the conformation of the extracellular portion, because the conserved disulfide bond between Cys110 and Cys187[15] structurally constrains the extracellular end of TM3. The swinging of the intracellular portion of TM3, which is pivoted on the Cys110 residue, displaces the intracellular end (Val139) by about 1.5 Å. The motion of TM3 also causes displacement of the intracellular end of TM4 by 3.3 Å. Molecular dynamics calculations starting from the Batho chromophore structure and its neighboring residues (4 Å from the chromophore) in the newly generated opsin structure indicated that the cyclohexenyl moiety was flipped toward TM3 and TM4, and that all double bonds of the chromophore were relaxed.

In the Lumi model, the PSB nitrogen atom is located 2.5 Å from the counterion, Glu113. The acyclic polyene portion is twisted at the C12–C13 bond (+160° for the dihedral angle of C11=C12–C13=C14). Dihedral angles of the polyene portion of the chromophore of the photointermediates are summarized in Table 1. The 9-methyl group of the chromophore turned towards TM6 and made tight contact with Trp265. The twist of the C12–C13 bond may be due to steric interaction, which suggests that there is further outward motion of TM3 in the formation of the next intermediate, Meta I. The Lumi model is consistent with results obtained in photoaffinity labeling experiments.[2] In this model, the cyclohexenyl moiety did not significantly rotate at the C6–C7 bond and thus the geminal methyl groups at C1 and the methylene group at C2 were located close to Thr118 and Glu122 (both in TM3), whereas the methylene groups at C3 and C4 faced toward the kinked site between Cys167 and Pro171 (both in TM4; Figure 4).

Table 1. *Dihedral angles for selected bonds in the acyclic portion of the chromophore.*

	C_{10}–C_{11}	C_{12}–C_{13}	C_{14}–C_{15}	C_{11}=C_{12}
Rhodopsin[a]	178	157	180	−2
Batho	180	−172	−150	−148
Lumi	175	160	176	179

[a] Structure B of 2 structures (A and B) of the crystal.[6]

Although experimental results suggest that the cyclohexenyl moiety is located close to Ala169, the cyclohexenyl moiety in the present model was not close to Ala169, which is located at the site opposite the chromophore binding site in the crystal structure. As fairly large extra functional groups were introduced at C3 and C4 in the photoaffinity labeling experiments, the functional groups in the cyclohexenyl moiety of the Lumi model became involved in strong steric interactions with TM4. Molecular dynamics calculations of the Lumi model with the modified chromophore structure predicted deformation of the Cys167 – Pro171 region of TM4, particularly at a site kinked by Pro170 and Pro171 (Figure 5). The model deformed in the Cys167 – Pro171 region, which indicates that Ala169 can be exposed to the reactive portion of the chromophore.

Thus, we conclude that the flip of the β-ionone moiety in the Batho-to-Lumi transition originates from the twisted C11=C12 double bond in the Batho chromophore and that only the modified "bulky" retinylidene chromophore is accessible to the Ala169 residue as a result of the deformation of the Cys167 – Pro171 region of TM4.

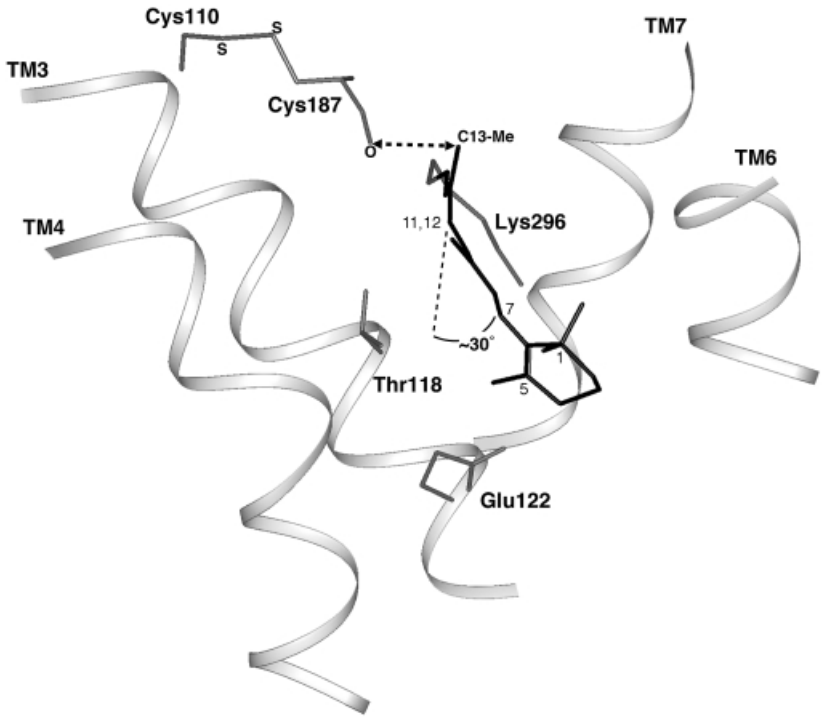


Figure 3. *The conformation of the Batho chromophore twisted at the C11 – C12 double bond in a view along the C11 – C12 axis. For clarity, TM1, TM2, and TM5 are omitted. The broken arrow indicates the steric interaction between the C13-Me group and the carbonyl oxygen atom of Cys187. The twist (≈30°) of the C11 – C12 double bond is indicated by the broken line.*

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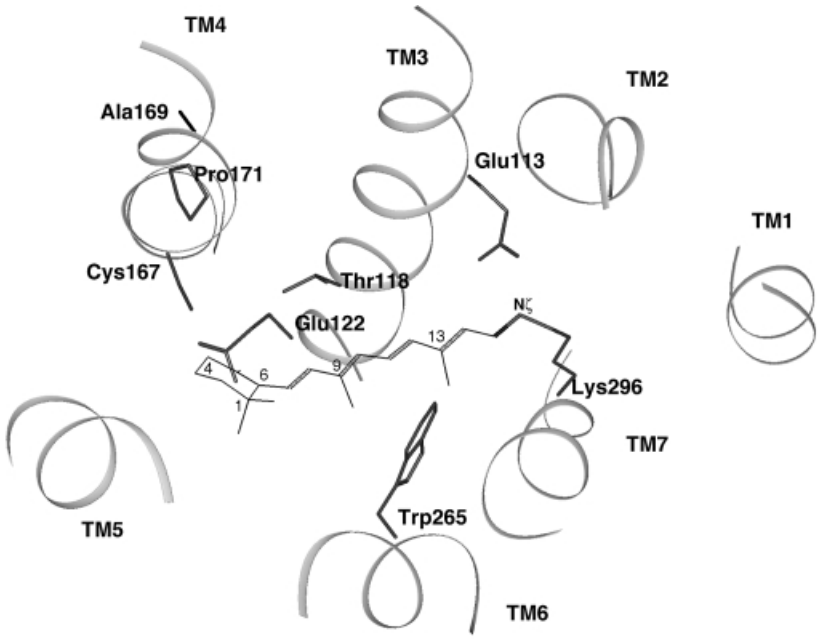

Figure 4. *The structural model of Lumi. A view from an intradiscal side.*

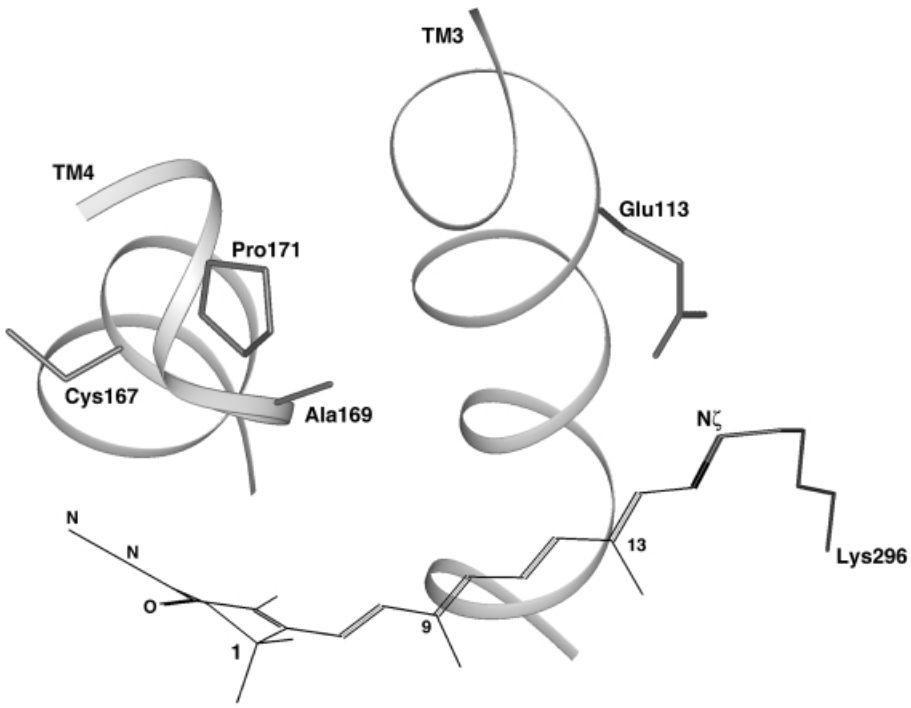

Figure 5. *The modified chromophore and Ala169 on the deformed TM4 in the Lumi model. For clarity, only TM3 and TM4 are shown.*

Methods

Molecular dynamics calculations were performed at 300 K with a cut-off distance of 8.5 Å, a distance-dependent dielectric constant, and a time step of 1 fs, for 50 ps. The conformation was sampled every 1 ps by using CVFF parameters in the Discover 3 program (ver2000, Molecular Simulations Inc., San Diego, CA, USA). At the early stage of isomerization, molecular dynamics calculations were performed at 300 K with a time step of 1 fs, for 1 ps, with sampling of the conformation every 10 fs. Structure minimization of the whole structure was performed until the final root mean square deviation (rmsd) was less than 0.1 kcal mol^{-1} $Å^{-1}$. Cα atoms were fixed to maintain the original helical structure assuming no change occurred in the TM arrangement.

The rigid-body motions of the transmembrane helices were calculated automatically by checking the interhelical distances to the neighbouring helices. TM3 was swung outward pivoted on the Cβ atom of Cys110 every 0.5 Å and a distance of at least 5 Å was maintained between the Cα atoms of TM3 and the adjacent Cα atom of TM2. For each model in which TM3 was moved, TM4 was also automatically swung to avoid steric interactions with TM3. TM4 pivoted on the Cα atom of a C-terminus residue. The details of the algorithm for the motion of TMs will be reported elsewhere.

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