

## Tuning of Retinal Twisting in Bacteriorhodopsin Controls the Directionality of the Early Photocycle Steps

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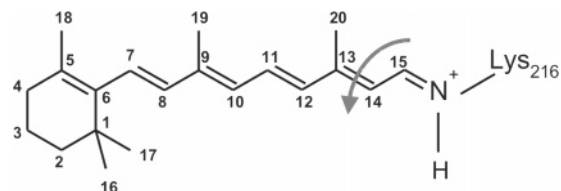
Productive proton pumping by bacteriorhodopsin requires that, after the all-trans to 13-cis photoisomerization of the retinal chromophore, the photocycle proceeds with proton transfer and not with thermal back-isomerization. The question of how the protein controls these events in the active site is addressed here using quantum mechanical/molecular mechanical reaction-path calculations. The results indicate that, while retinal twisting significantly contributes to lowering the barrier for the thermal cis–trans back-isomerization, the rate-limiting barrier for this isomerization is still 5–6 kcal/mol larger than that for the first proton-transfer step. In this way, the retinal twisting is finely tuned so as to store energy to drive the subsequent photocycle while preventing wasteful back-isomerization.

Bacteriorhodopsin (bR) is a light-driven proton pumping protein found in the membrane of *Halobacterium salinarium*.<sup>1</sup> After photoisomerization, the retinal chromophore changes its configuration from all-trans in the ground-state protein (the bR state) to 13-cis, 15-anti (Scheme 1), thus forming the K intermediate. Further relaxation yields the L intermediate (Figure 1) and triggers a sequence of five proton-transfer steps that results in the translocation of one proton from the cytoplasmic to the extracellular side of the membrane. The first of these steps involves the transfer of the retinal Schiff base proton to Asp85 during the transition between the L and M states.

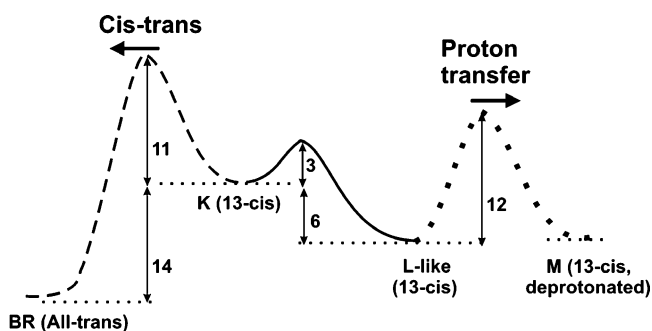
For the photocycle to proceed forward and pump protons, the energy barrier for ground-state thermal back-isomerization of the retinal (i.e., K → bR) must be significantly higher than the highest barrier separating the K state from M (Figure 1). The origin of this directionality is addressed here by calculating and analyzing the energetics of the early steps in the photocycle.

The calculations were performed using a quantum mechanical/molecular mechanical (QM/MM) Hamiltonian and reaction-path finding techniques. Details of the methods are given in refs 2 and 3. The QM region (86 atoms, see Figure 2) was described with the SCC-DFTB method<sup>4</sup> and was embedded in a flexible protein region. The minimum-energy pathways and transition-state barriers were determined with the conjugate peak refinement (CPR) algorithm<sup>5</sup> as implemented in CHARMM.<sup>6</sup> CPR finds a minimum-energy pathway between given reactant and product structures without the requirement of a predefined reaction coordinate. An initial guess of the path which connects the end states, and optionally includes initial intermediates, is refined until a continuous path is found along which the energy

### SCHEME 1: Unphotolyzed Retinal in the All-Trans Configuration<sup>a</sup>



<sup>a</sup> The arrow indicates the bond being isomerized upon photoabsorption.



**Figure 1.** Cis–trans back-isomerization vs proton transfer in early steps of the bacteriorhodopsin photocycle. The schematic energy profile is based on SCC-DFTB/MM-optimized energies (values in kilocalories per mole), as discussed in the present work and in refs 2 and 3. Molecular movies of the K → bR pathway as well as for the first proton-transfer step are available at <http://www.iwr.uni-heidelberg.de/groups/biocomp/fischer/>.

maxima are first-order saddle points that give the transition states of the reaction.

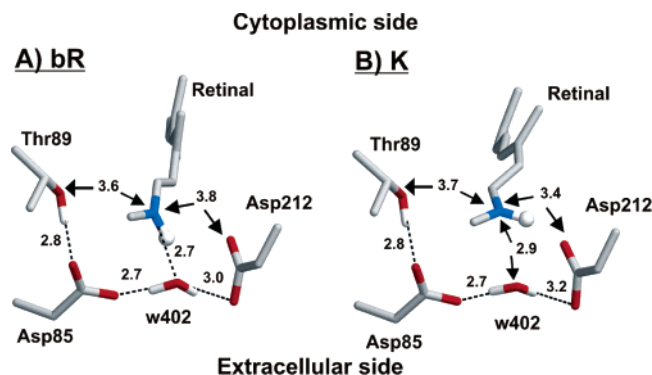
QM/MM-optimized models of the K and bR states were prepared as described previously<sup>2,3</sup> and are schematically depicted in Figure 2. In the all-trans bR state, the Schiff base is

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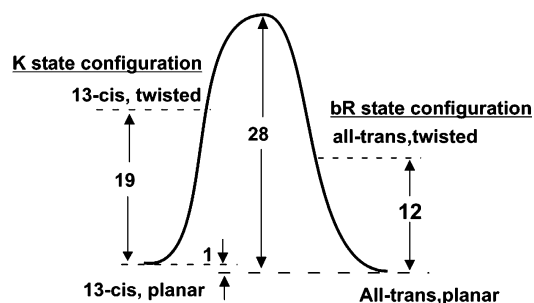
**Figure 2.** Models of the bR all-trans retinal state (A) and the 13-cis K-state (B) structures. In both structures, the Schiff base NH bond points toward the extracellular side. Only the retinal and the protein groups included in the QM region are depicted. The dashed lines indicate hydrogen bonds. Distances between heavy atoms are given in angstroms. Protein structures were taken from refs 7, 9, and 18.<sup>13</sup>

hydrogen bonded to water w402 which is further bridged with Asp85 and Asp212 (Figure 2A). Consistent with experimental<sup>7</sup> and theoretical<sup>8</sup> data, the Schiff base NH segment of the all-trans retinal is twisted toward Asp212. Deviations from planarity are mainly observed for the  $C_{12}-C_{13}=C_{14}-C_{15}$  and  $C_{14}-C_{15}=N-C_e$  dihedral angles, which are  $-153$  and  $-164^\circ$ , respectively (as compared to  $-157$  and  $-163^\circ$ , respectively, in the crystal structure<sup>7</sup>).

Recent crystallographic structures<sup>9,10</sup> and molecular dynamics simulations<sup>11,12</sup> suggest that, in the K state, which is formed in a few picoseconds after photoabsorption, the 13-cis retinal is highly twisted. This is confirmed by the present QM/MM-optimized 13-cis K-state model,<sup>13</sup> which indicates twisting of several retinal bonds. The  $C_{12}-C_{13}=C_{14}-C_{15}$ ,  $C_{13}=C_{14}-C_{15}=N$ , and  $C_{14}-C_{15}=N-C_e$  dihedral angles are  $-37$ ,  $170$ , and  $147^\circ$ , similar to the highest resolution K-state crystal structure from ref 9, which indicates values of  $-2 \pm 39$ ,  $138 \pm 35$ , and  $101 \pm 31^\circ$ . The Schiff base of the highly twisted retinal points toward Asp212 (Figure 2B). The K-state model is 14 kcal/mol higher in energy than the bR state, in agreement with the  $11.6 \pm 3.4$  kcal/mol experimental value.<sup>14</sup>

Distortion of the  $C_{13}$  angle ( $C_{12}-C_{13}=C_{14}$ ) to a value of  $145 \pm 12^\circ$  has been suggested to account for a significant fraction of the energy stored in K.<sup>9</sup> In the present K-state model, upon QM/MM optimization this angle relaxes from  $130^\circ$  in the crystal structure of ref 9 to  $122^\circ$ . The lack of a significant distortion of the  $C_{13}$  angle is consistent with previous observations<sup>15</sup> and with our preliminary molecular dynamics simulations. We performed test calculations that show that increasing the  $C_{13}$  angle to  $130$  or  $139^\circ$  raises the K-state energy by  $\sim 3$  or  $\sim 16$  kcal/mol, respectively. The combined effect of  $C_{13}$  angle distortion and torsional distortions would thus compromise the photocycle directionality by over-destabilizing K, which could facilitate an unproductive  $K \rightarrow$  bR transition. Our results indicate that the  $C_{13}$  angle is unlikely to be significantly distorted in K.

While the highly twisted retinal configuration may be an important element in storing the energy of the absorbed photon,<sup>9,12,15,16</sup> the question arises as to why the twisting does not compromise the productivity of the photocycle by lowering the torsional barrier for thermal back-isomerization to all-trans. Back-isomerization would then compete with the forward step of retinal deprotonation and proton pumping. To address this question, we calculated the QM/MM pathways and barriers for the cis-trans  $K \rightarrow$  bR retinal back-isomerization. The pathway in which the Schiff base NH bond rotates via Thr89 is excluded due to a rate-limiting barrier of  $\sim 23$  kcal/mol. Rotation in the



**Figure 3.** Energies of retinal configurations in a vacuum. The relative energies (in kilocalories per mole) of the retinal end states were obtained with SCC-DFTB. Planar retinals were QM-optimized in vacuo. The twisted retinal coordinates were taken from the QM/MM-optimized protein structure. The 28 kcal/mol vacuum energy barrier for rotation against the  $C_{13}=C_{14}$  bond is taken from ref 19.

opposite direction, via Asp212, has a rate-limiting barrier of  $\sim 11$  kcal/mol (dashed curve in Figure 1). This is approximately twice the barrier calculated for a proton transfer from the Schiff base to Asp85 while in a K-like structure.<sup>2,3</sup> More significantly, this is much higher than the  $\sim 3$  kcal/mol barrier over which the extracellular-oriented K state can escape to a 13-cis L-like configuration through reorientation of the Schiff base toward the cytoplasm<sup>3</sup> (solid curve in Figure 1). From this configuration, proton transfer can occur over a  $\sim 12$  kcal/mol barrier<sup>3</sup> (dotted curve in Figure 1), which agrees with the experimental enthalpy of activation for the L to M transition.<sup>17</sup> These results explain why although the K-state 13-cis retinal is highly twisted, it prefers to proceed with proton transfer rather than with back-isomerization to all-trans.

To understand the origin of the  $\sim 11$  kcal/mol barrier of the  $K \rightarrow$  bR back-isomerization, the following analysis was performed. First, the total energy ( $E$ ) was decomposed according to  $E = E_{QM} + E_{QM/MM} + E_{MM}$ , comprising the interactions between the QM particles ( $E_{QM}$ ), the interactions between the QM particles and the MM atoms ( $E_{QM/MM}$ ), and the interactions between the MM atoms ( $E_{MM}$ ). This decomposition indicates that  $\sim 10$  kcal/mol ( $\sim 90\%$ ) of the 11 kcal/mol barrier arises from  $E_{QM}$ . To determine the origin of  $E_{QM}$ , in a further analysis, the coordinates for the retinal atoms were selected from the QM/MM-optimized pathway in the protein, and the energy profile was recomputed along these coordinates in the absence of the protein environment. This gives an energy barrier of 11.3 kcal/mol at the SCC-DFTB level and 12.1 kcal/mol using B3LYP/6-31G\*, indicating that the rate-limiting barrier is indeed due to the internal energy of the retinal.

The 11–12 kcal/mol isomerization barrier found here is less than half the  $\sim 28$  kcal/mol barrier for the torsion around the  $C_{13}=C_{14}$  bond calculated with SCC-DFTB for a fully relaxed retinal Schiff base model in a vacuum<sup>19</sup> ( $\sim 30$  kcal/mol with B3LYP/6-31G\*<sup>20</sup>). This difference in barriers arises from differences in the twisting of the 13-cis retinal in the respective reactant configurations. In the fully relaxed vacuum model, the retinal polyene chain is planar, whereas, in the protein, the retinal is destabilized by twisting. Indeed, our isolated retinal in the twisted K-state configuration is 19 kcal/mol higher in energy than the planar, vacuum 13-cis retinal (see Figure 3). This energy value, when added to the  $\sim 11$  kcal/mol barrier in the protein, is close to the  $\sim 28$  kcal/mol barrier in a vacuum. This confirms that in the K state there is significant energetic destabilization of the retinal chain due to torsion in the Schiff base segment.

In contrast to the early photocycle steps, in which the retinal twist is tuned so as to keep the cis-trans isomerization barrier high enough to prevent back-isomerization while storing energy,

at the end of the photocycle, the cis–trans energy barrier must be low enough to allow isomerization on the millisecond time scale. It is therefore conceivable that the rate-limiting barrier for the N → O cis–trans retinal isomerization could be lowered by transiently imposing a twisted retinal configuration, in a similar way to the present results on the earlier part of the photocycle.

The QM/MM reaction-path calculations discussed here contribute to our understanding of the directionality in the early steps of the bacteriorhodopsin photocycle. After photoisomerization of the all-trans retinal chromophore to 13-cis, ~14 kcal/mol of energy is stored in the K intermediate. About 7 kcal/mol (=19–12 kcal/mol in Figure 3) of this energy resides in retinal twisting. Consistent with previous observations,<sup>12</sup> the remaining half is due to the perturbation of the interactions between the retinal Schiff base and the active site protein groups. The energy stored through retinal twisting is finely tuned, being large enough to help drive the subsequent proton pumping steps but low enough to maintain a sufficiently high barrier to back-isomerization (5–6 kcal/mol higher than that for forward proton transfer). Thus, unproductive relaxation through thermal cis–trans back-isomerization becomes rare and the photocycle is efficient.

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