Communication

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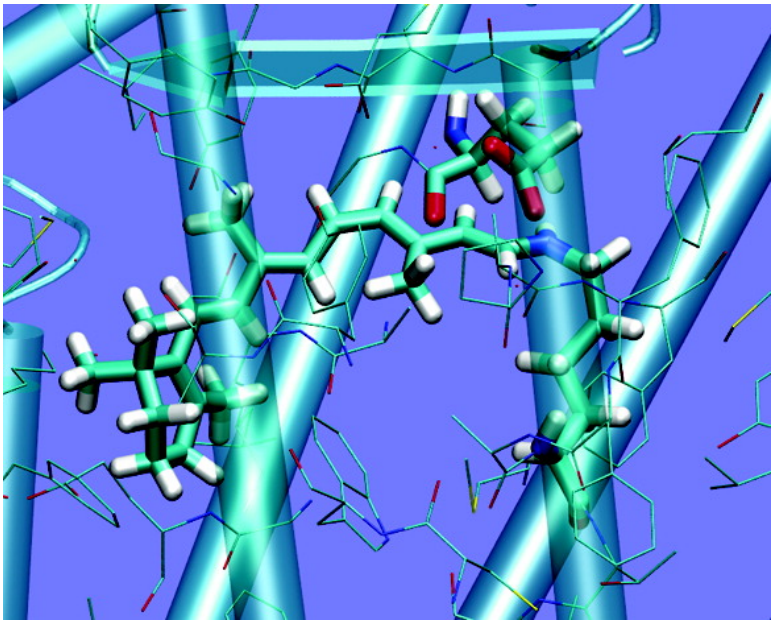

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A Molecular Spring for Vision

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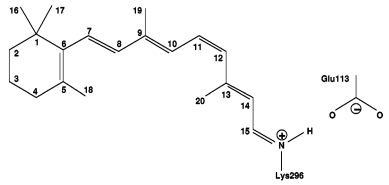
Light absorption by the G-protein-coupled receptor rhodopsin leads to vision via a complex signal transduction pathway that is initiated by the photoisomerization of its chromophore, the retinal protonated Schiff base (RPSB).



Within the protein, the 11-cis to all-trans isomerization of the RPSB is ultrafast (200 fs)[1] and very efficient (quantum yield 0.65),[2] in contrast to the same photoreaction in solution. This fact is puzzling in view of the steric confinement of the RPSB to a small binding pocket that should hamper the large movements required to adopt an all-trans conformation. Much work has been devoted to understanding the molecular mechanism of this reaction.[3,4] Experimental evidence reveals that bathorhodopsin, the first thermally equilibrated intermediate in the signaling cascade, exhibits a strained all-trans RPSB and stores 32 ± 1 kcal/mol of the photon energy.[5] Two different energy storage mechanisms have been discussed: electrostatic energy storage by charge separation between the protonated Schiff base and its counterion Glu113 and mechanical energy storage in the form of strain energy. From the crystal structures of bovine Rh,[6,7] it is known that the RPSB is twisted in the $C_{11}-C_{12}$ region. Recent theoretical studies establish the rotational direction of the twist as uniquely negative and identify the major steric influences.[8−10] Experimental and computational data show that removal of the C_{20} methyl group and thus lowering of the steric strain around the $C_{11}-C_{12}$ bond slows down the photoreaction and decreases the quantum yield.[11,12]

The present work describes this intriguing photoreaction with a hybrid quantum mechanical/molecular mechanical (QM/MM)[13] molecular dynamics (MD) methodology[14] that yields an accurate description (at the level of density functional theory) of the electronic structure of the full chromophore, while taking into account the heterogeneity and complexity of the protein environment by a classical force field. For the description of the first excited singlet state (S_1), a good compromise between accuracy and computational efficiency is provided by the restricted open-shell Kohn−Sham (ROKS) algorithm.[15] Our model system is based on the crystal structure of bovine Rh[7] embedded in a membrane mimetic environment.[8] The QM system is evolved according to the Car−Parrinello algorithm.[16] Additional information about the computational methods can be found in Supporting Information.

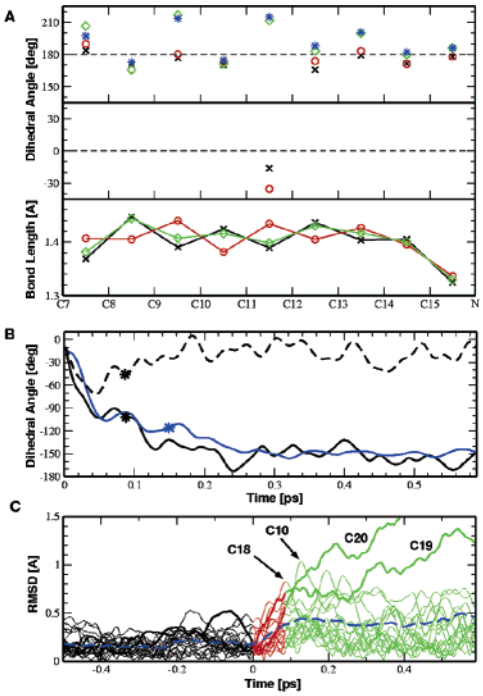


Figure 1. (A) Dihedral angles and bond lengths along the conjugated carbon chain of the RPSB in the dark state (black), in S_1 (red), and in the all-trans ground state (green). The classical dihedral angles of the all-trans ground state are shown in blue. (B) Time evolution of the dihedral angle $\varphi_{C10-C11-C12-C13}$. One ROKS trajectory (dashed black line), one trajectory (same initial conformation) at higher temperature (solid black line, **ROKS_ht**), and the average of the 23 isomerizing classical simulations (blue) are shown. The $S_1 \rightarrow S_0$ transitions are indicated by stars. (C) Displacement of each heavy atom of the RPSB from its average dark state position in the dark state (black), in S_1 (red), and in the all-trans ground-state configuration (green) in **ROKS_ht**. The average value is indicated by a blue dashed line.

Starting from configurations sampled in a dark-state simulation, 23 excited-state QM/MM trajectories of about 100 fs each were calculated. The excited-state configuration of the RPSB is characterized by the well-known inversion of the bond length pattern (Figure 1A). In S_1, especially the bonds C_9-C_{10} (1.44 Å), $C_{11}-C_{12}$ (1.43 Å), and $C_{13}-C_{14}$ (1.43 Å) are elongated, thus lowering the barrier toward isomerization. Whereas the electronic structure would be unselective toward the rotation of any of these double bonds, the protein environment favors $C_{11}-C_{12}$ bond isomerization by steric strain. In fact, the dihedral angles from C_7 to C_{11} and from C_{12} to N deviate in S_1, similar to S_0, only by at most 15° from a perfect trans conformation (Figure 1A). In contrast, the pretwisted dihedral angle $\varphi_{C10-C11-C12-C13}$ rotates toward more negative values, with fluctuations up to −72° and an average of −35° (Figure 1A, middle panel). An angle appropriate for isomerization (≈−90°)[12] is not reached, indicating the presence of a

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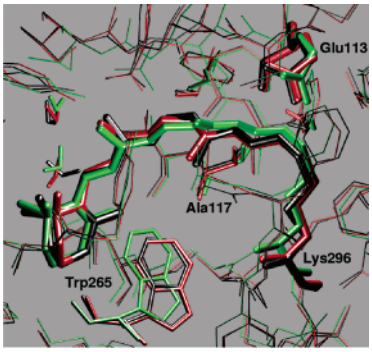

Figure 2. Superposition of the chromophore structure in the protein binding pocket in the dark state (black), at the $S_1 \rightarrow S_0$ transition (red), and after 500 fs of relaxation in the isomerized state (green).

residual barrier probably due to the limited accuracy of our calculations. The barrier is very small, as we can show by performing an excited-state MD simulation in which the initial velocity of the RPSB is increased, so that the local temperature corresponds to 690 K (Figure 1B, solid blue line, **ROKS_ht**).[17]

This enhanced kinetic energy is sufficient to allow the barrier crossing in a very short time: $\varphi_{C10-C11-C12-C13}$ rotates within 50 fs to −103° and fluctuates then around an average value of −100°. No recrossing to the −35° configuration is observed, indicating that the −100° configuration is at least a local minimum on the ROKS S_1 energy surface. When released to the ground state after 90 fs, the RPSB evolves toward a highly twisted all-trans structure. Figure 1C shows the displacement of each RPSB heavy atom from its average position in the dark state (black), in S_1 (red), and in the all-trans S_0 configuration (green). Remarkably, in the excited state, no atom moves more than 0.8 Å, that is, only 0.3 Å more than the maximal thermal displacement in the dark state. In the following 500 fs of ground state relaxation, only the methyl groups C_{19} and C_{20} move further away from their position in the dark state, and the average root-mean-square deviation (RMSD) is 0.4 Å. The strain is propagated through the carbon chain, as can be seen from the deviation of the dihedral angles up to almost 40° (Figure 1A, upper panel). The surprisingly small difference between the primary photoproduct and the dark-state structure is also evident in Figure 2, where the dark-state structure (0 fs, black), the transition structure (90 fs, red), and the all-trans ground-state structure (590 fs, green) are superimposed.[18]

To estimate the energy stored in the system after isomerization, we optimized the chromophore structure both in the 11-cis and in the all-trans configuration in Rh, starting from the initial dark state conformation of **ROKS_ht** and fixing all atoms except for the RPSB. We obtain a total energy difference of 28 kcal/mol in good agreement with the experimentally determined energy storage in bathorhodopsin (32 kcal/mol).[5] In the all-trans conformer, the energy stored in the internal degress of freedom of the RPSB (+18 kcal/mol) and the van der Waals (steric) interaction energy between the RPSB and the protein (+10 kcal/mol) increase substantially, while the electrostatic interaction energy remains unaffected (<0.1 kcal/mol difference). Electrostatic energy storage is negligible, consistent with the finding that the saltbridge between the protonated Schiff base and the counterion Glu113 remains stable.

The QM/MM simulations suggest that the photoreaction is limited to a single pathway that is essentially determined by steric constraints. Therefore classical MD simulations, where the torsional potential around $C_{11}-C_{12}$ is inverted (**CLASS_inv**), can be used in order to gain a statistical picture of the isomerization at a low computational cost. Comparison of the average all-trans structures of the first 500 fs after isomerization shows a remarkable similarity between the structures generated by multiple classical MD trajectories and the DFT results (RMSD = 0.2 Å), once again emphasizing the predominating sterical influence of the protein. Even the strain propagation along the conjugated carbon chain can be described properly by the classical model, as is evident from the average deviation of the torsional angles from a planar conformation (Figure 1A) that parallels the DFT results.

In conclusion, hybrid QM/MM simulations of the photoreaction in rhodopsin confirm that (1) the protein binding pocket selects and accelerates the isomerization exclusively around the $C_{11}-C_{12}$ bond via preformation of a twisted structure, (2) the 11-cis to all-trans isomerization is possible within the binding pocket with a minor atomic rearrangement, producing a highly strained chromophore, and (3) the photon energy in bathorhodopsin is stored in internal strain of the RPSB and in steric interaction energy with the protein. Hence, the initial step of vision can be viewed as the compression of a molecular spring that can then release its strain by altering the protein environment in a highly specific manner.

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Supporting Information Available: Details of the computational methods and of the protein model (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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(17) This approach allows small energy barriers to be crossed without imposing an a priori chosen reaction path and was used, e.g., by: Garavelli, M. et al. *J. Phys. Chem. A* **2001**, *105*, 11496−11504.
(18) We also applied a different approach for crossing the small residual isomerization barrier, namely, by restraining the dihedral angle C10−C11−C12−C13 stepwise from −65 to −100° in the excited state. Here, we obtain the same highly twisted all-trans ground-state structure, suggesting that the isomerization pathway is determined mostly sterically.

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