QM/MM simulation of the first step of vision

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1 Introduction

The absorption of light in the visual range by the retina protein rhodopsin initiates the process of vision [1]. The first step, i.e. the transformation of light into chemical energy, is known to consist of a photochemical reaction, namely the cis-trans isomerization of the C_{11}-C_{12} double bond of the rhodopsin chromophore (Fig. 1). About the details of this reaction, however, there is a long-standing debate in the literature [2, 3, 4] and it has been the subject of many theoretical studies [5, 6, 7, 8, 9, 10]. Since the chromophore is covalently linked to the protein at the Schiff base end, the isomerization would imply a large movement of the ionone ring end (Fig. 1). In the tight protein binding pocket, the movement of this bulky group would inevitably be slowed down by collisions with the environment. However, it is known that the photoisomerization is an extremely fast and efficient process, which is completed in less than 200 femtoseconds after excitation [11] with a quantum yield of 65 percent [12]. To resolve this antagonism, two models have been proposed in the literature, involving the rotation of a second bond in order to compensate the motion resulting from the C_{11}-C_{12} isomerization. This compensation can be achieved by the simultaneous rotation of either a single bond (hula twist mechanism [13]) or a double bond (bicycle pedal mechanism [14]). By combining a quantum mechanical (QM) first principles approach with a molecular mechanics (MM) methodology we have developed a tool to check these hypotheses and to investigate the reaction mechanism of this highly optimized biological nanomachine.

2 Methodology

The methodology has been described in detail previously [15]. We use first-principles molecular dynamics (MD) for the chromophore in combination
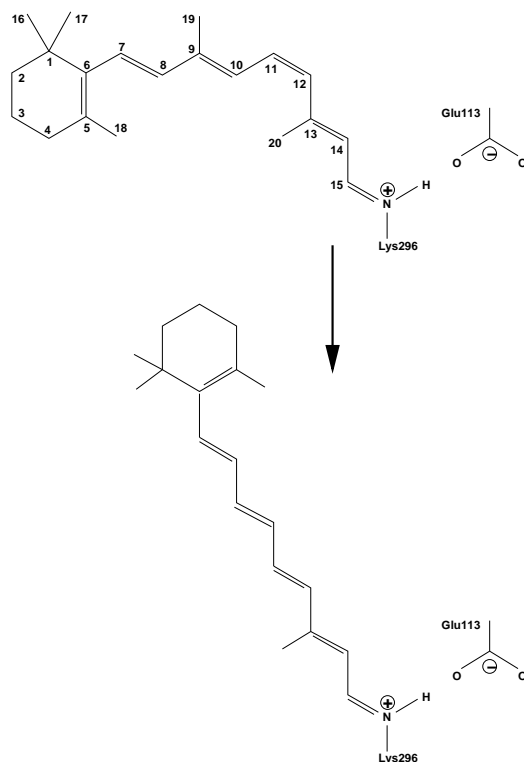
Fig. 1. The first step of vision: *cis-trans* isomerization of the rhodopsin chromophore

with a classical MD scheme for the protein. The protein is modeled in a membrane mimetic environment [16]. This model, consisting of about 24000 atoms, (see Figure 2) is based on a recent X-ray structure [17] and has been demonstrated to be stable both in purely classical and in QM/MM simulations. We consider all nuclear degrees of freedom in the simulation and do not prescribe the reaction path. Breaking and formation of bonds is possible in the QM part only.

We describe the electronic structure of the excited state with the restricted open-shell Kohn-Sham (ROKS) method [18] that has been applied to the description of the isolated chromophore previously [19, 20]. First-principles molecular dynamics according to Car and Parrinello (CPMD) [21, 22] is used to describe the dynamics of molecular systems in the ground state [23]. We use the Kohn-Sham Hamiltonian [24, 25, 26, 27] with the standard density functionals LDA and BLYP [28, 29, 30]. The chemically unreactive part is described by the AMBER force field [31]. The hybrid quantum mechanics/molecular mechanics (QM/MM) code developed at the ETH Zurich [32, 33] has been combined with the ROKS approach [34].
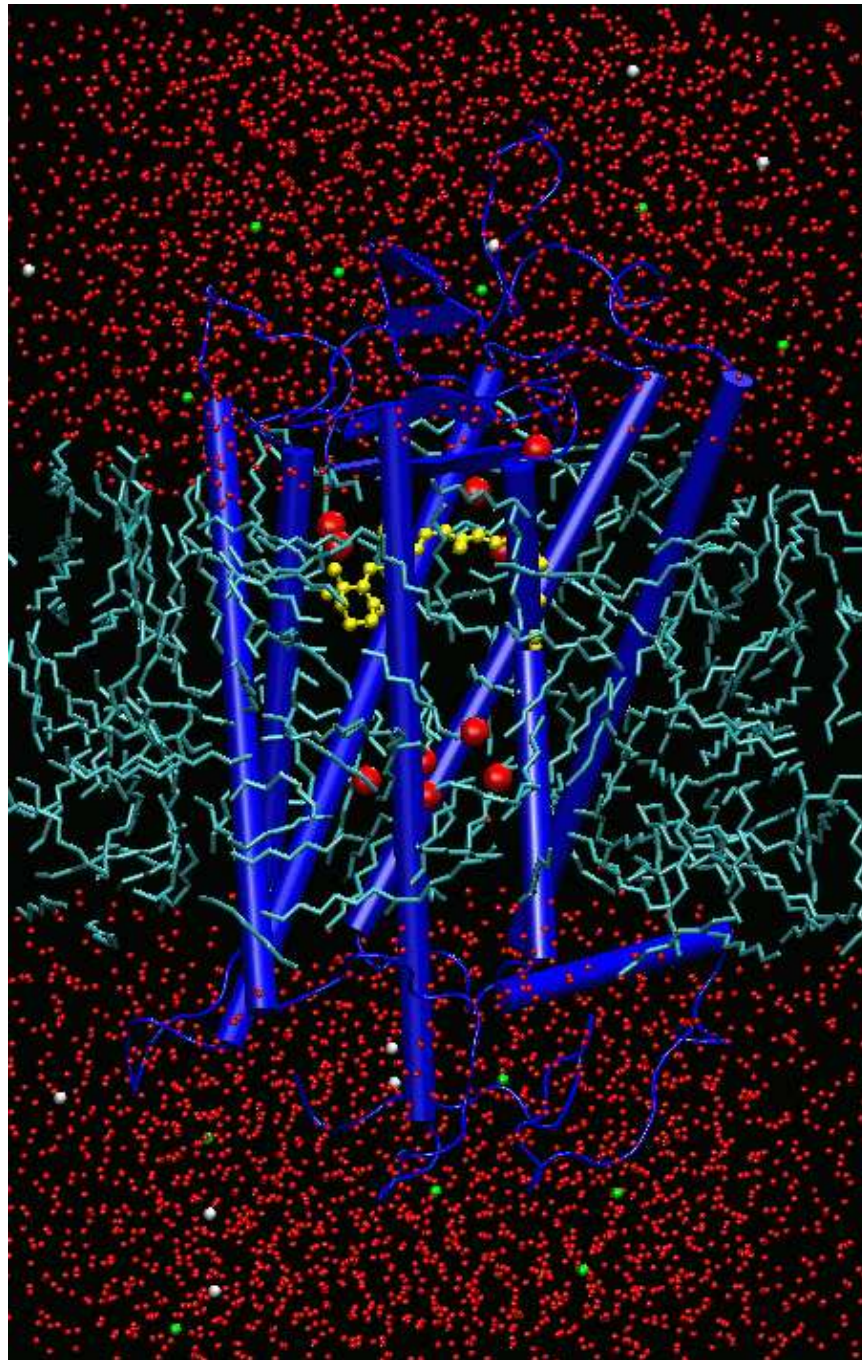
Fig. 2. Model of rhodopsin plus environment

	70 Ry	200 Ry
Hitachi SR8000, 16 nodes	1.6	8.3
IBM Power 4, 16 processors	4.6	19.5
HP/Compaq SC45, 16 processors	3.1	20.4
AMD Opteron, 1.4 GHz, 16 processors	3.8	24.4

Table 1. Benchmarks for CPMD simulations. Data are given for single SCF steps (in seconds) of the wavefunction optimization for a system consisting of 32 water molecules using two different plane wave cutoffs (70 and 200 Ry, respectively). In the rhodopsin simulation a single MD step, corresponding to 0.1 femtoseconds, takes about 30 s on 12 nodes of the Hitachi SR8000.

3 Results

3.1 The protonated Schiff base

For the investigation of $\pi - \pi^*$ excitations, we have developed a special self-consistent field (SCF) algorithm that represents a modification of the commonly used Goedecker-Umrigar algorithm [35] for open-shell SCF calculations [36]. The modified algorithm guarantees, in the case of non-degenerate orbitals, that the self-consistently computed orbitals have the correct molecular symmetry, which is non-trivial for open-shell systems. To test the methodology, we have systematically applied the modified Goedecker-Umrigar algorithm to the computation of the first excited states of several polyenes, cyanines, and protonated Schiff bases [36] (Figure 3).
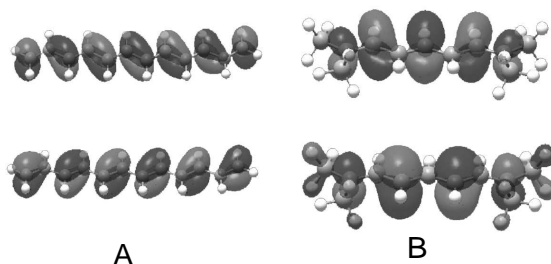

Fig. 3. Singly occupied orbitals of dodecahexaene (A) and of a cyanine molecule (B) as obtained with ROKS using the modified Goedecker-Umrigar algorithm.

We concentrate the discussion on the protonated Schiff bases. Compared to the experimental 0-0 transitions in solution, the vertical singlet excitation energies are redshifted by roughly 1.1 eV (Figure 4).

By comparison with the results for polyenes and cyanines it is concluded that this shift is largest for the vertical singlet excitation, while triplet excitations are very well reproduced. We attribute the shift to an insufficient
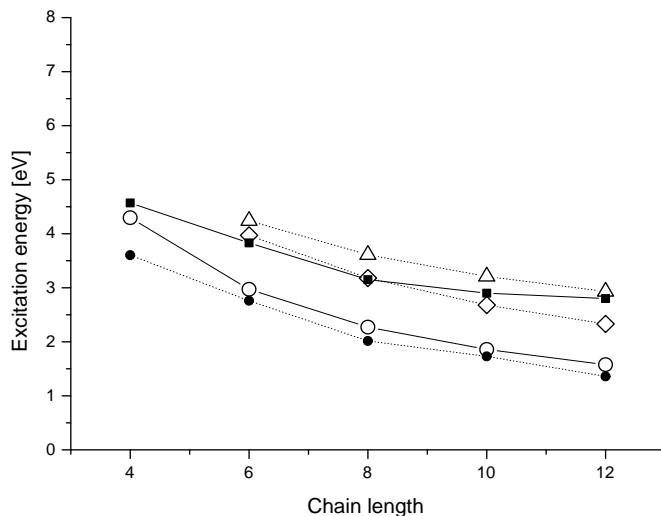
Fig. 4. The vertical singlet excitation energies for the protonated imines. Filled symbols: substituted Schiff bases, open symbols: unsubstituted Schiff bases. Squares: experiment, circles: ROKS(BLYP), triangles: CNDO(SCI), diamonds: CNDO(DCI) [36]. The substituted Schiff base with chain length 12 corresponds to the isolated rhodopsin chromophore.

description of the singlet-triplet splitting by the exchange term of the homogeneous electron gas in the Kohn-Sham approximation. For $\pi - \pi^*$ singlet excitations, it leads to an energetic lowering of the Franck-Condon region of the excited state potential energy surface (PES).

The optimized geometries for both ground and excited states exhibit a significant bond alternation near the ionone ring only. Upon excitation, the short bonds become longer and the long bonds become shorter, but none of the original double bonds fully adopts single bond character [36]. This makes it plausible that — in contrast to e.g. ethylene or butadiene, where the double bond alternation is very pronounced in the ground and excited states — a certain barrier to double bond rotation persists also in the excited state. This finding is in line with the notion that e.g. large cyanines, that do not exhibit significant double bond alternation in the ground state, represent excellent fluorescence dyes while fluorescence would be quenched by cis-trans isomerizations.

3.2 Rhodopsin simulations

The ambitious aim of our study is to simulate the rhodopsin photoreaction on the basis of the quantum chemical equations without prescribing the reaction path. Our first attempts to simulate the reaction by putting the system to the excited state after equilibration in the ground state at 300 K did not lead to a successful isomerization. The dihedral angle spontaneously rotates to -65 degrees, but then goes back to -40 degrees instead of continuing the rotation to -90 degrees or further. Obviously there is still a barrier that prevents free rotation. Such a barrier is to be expected for the free chromophore in solution, since experimentally the chromophore without the protein environment isomerizes significantly slower and less specific. In previous ROKS calculations for the free chromophore, we found a barrier on the excited state potential energy surface and were able to show that it is reduced to about 6 kcal/mol by the presence of the counter ion [19]. Due to the complexity of the system, only an approximate estimate can be given for the barrier height in case of the chromophore in the protein environment. The protein environment leads to a further elongation of the C_{11}-C_{12} bond and thus to an additional reduction of the barrier [20]. The remaining small barrier in the protein simulation can be due to several of the approximations in our calculations; we attribute it to shortcomings of the ROKS/BLYP functional. There are several approaches to overcome a small free energy barrier, the simplest of which is to increase the kinetic energy. On the femtosecond timescale, this allows to cross barriers in the order of kT only, i.e., barriers with a height of not more than a few kcal/mol. By raising the kinetic energy of the chromophore, we in fact succeed to observe an isomerization. As expected, the dihedral angle of the C_{11}-C_{12} bond changes fast and selectively to values beyond -90 degrees. It stays at about -100 degrees in the excited state, indicating that there is a minimum on the excited state energy surface. The rotation continues when the chromophore is put back to the ground state and the all-trans structure is formed. Analysing the motion during the rotation in detail, two points are important to note: Firstly, no other bond isomerizes simultaneously with the C_{11}-C_{12} bond. Instead, the rotation of the C_{11}-C_{12} bond is compensated by smaller rotations of several dihedral angles in the chain. Secondly, the atoms do not experience large displacements during the isomerization. The specific distortion of the chromophore from a planar structure by the protein environment, as it was found by X-ray analysis [38], allows an isomerization of the C_{11}-C_{12} bond without large-scale motions.

That means, the protein environment influences the reaction both sterically and electronically (the latter mainly by the counter ion). Both effects lead to the ultrafast and highly specific photoreaction.
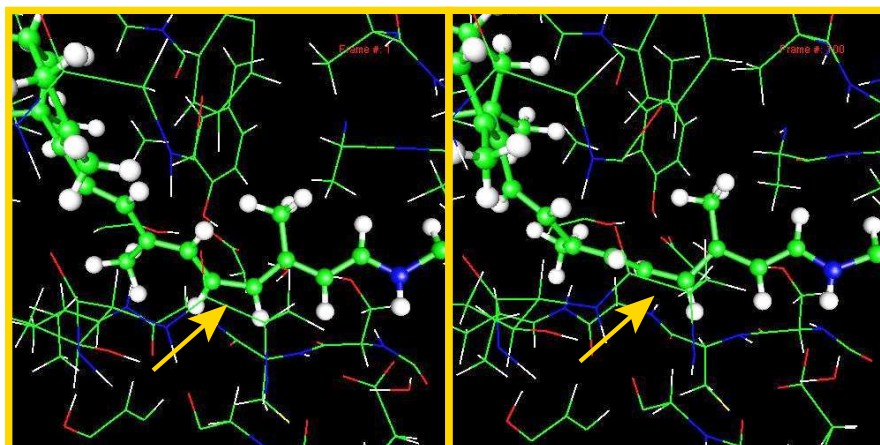
Fig. 5. The rhodopsin chromophore at the beginning and at the end of the excited state simulation. While the single atoms have hardly moved, the C_{11}-C_{12} bond is rotated beyond -90 degrees at the end of the simulation.

4 Summary and Outlook

We have combined the restricted open-shell Kohn-Sham method with a QM/MM scheme and have applied it to the photoisomerization of rhodopsin. We were able to observe the cis-trans isomerization only at elevated kinetic energy of the chromophore. This is attributed to shortcomings of the ROKS potential surface. By treating the complete chromophore quantum mechanically and simulating the full system at finite temperature without imposing any restraints, our simulations set new standards in the modeling of rhodopsin. We obtain a reaction path that convincingly explains the high efficiency of the system by the interplay between chromophore and apoprotein. The present study demonstrates that the aim of modeling photoreactions in complex biological systems without *a priori* knowledge of the reaction path is coming into reach.

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