QM/MM Study of Rhodopsin

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1 Introduction

The investigation of the first step of vision is a long-standing problem in theoretical chemistry [1]. It has been text-book knowledge for a long time [2, 3, 4], that irradiation of the retina protein rhodopsin with light in the visible range induces a *cis-trans* isomerization at the C_{11}-C_{12} double bond of the chromophore, i.e. of the light-absorbing part of the protein (Fig. 1). However, it is extremely difficult to study the reaction mechanism in full detail on experimental basis. The consecutive signal transduction cascade that finally leads to a neuron signal also still poses many questions.

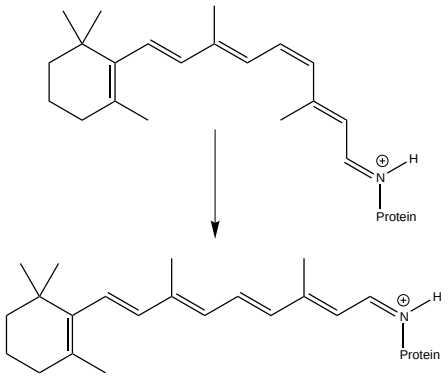

Fig. 1. The first step of vision: *cis-trans* isomerization of the rhodopsin chromophore

Experimental investigations are challenging for several reasons. First, sufficient amounts of bovine rhodopsin have to be isolated. Then, it is difficult to obtain good crystal structures of a membrane protein since the three-dimensional protein structure is determined by the specific membrane environment. Finally, a sample of rhodopsin undergoes the *cis-trans* isomerization just one single time, i.e. outside a living organism it never returns to the original state. For these reasons, most experimental and also many theoretical

studies have been performed on bacteriorhodopsin, one of the light-absorbing proteins of the *halobacterium salinarium* [5, 6, 7]. In this case, accumulation and isolation of the protein are relatively simple, and the protein undergoes an optical cycle which renders it of high interest for technical applications [8].
Nevertheless, the uniquely fast and efficient reaction in the eye (quantum yield $\approx$ 67%, reaction time $<$ 200 fs [9]) is of high interest, and many issues raised by experiments suggest a theoretical approach to the problem. However, theoretical investigations of such a complicated system must rely on a good experimental basis. The first high resolution crystal structure of rhodopsin published in August 2000 [10] provided the impetus to start a collaboration with the aim to model the first step of vision on a first-principles basis.

2 Methodology

The theoretical investigation of rhodopsin bears three major challenges:

- The electronic structure of the chromophore: The chromophore (the protonated Schiff base of retinal) is quite large for an *ab-initio* investigation. CASSCF calculations have been performed but yield up to now no consistent view [11, 12, 13, 14, 15].
- The environment: The *cis-trans* isomerization depends strongly on the environment. This is for example indicated by the fact that in bacteriorhodopsin a different isomerization takes place (C_{13}-C_{14} *trans-cis* instead of C_{11}-C_{12} *cis-trans*), although the chromophore is similar and only the protein environment differs.
- The dynamics: A condensed phase isomerization differs significantly from a gas phase isomerization. While in gas phase the isomerization can be described relatively easily on the basis of the potential curve that is obtained when varying the isomerization angle, in condensed phase the interactions with the environment during the isomerization play an important role. The dynamics of this environment and the response of the chromophore to it cannot be neglected. The sterically demanding *cis-trans* isomerization expected in gas phase cannot occur in the protein, since it would result in collisions with the surrounding.

Our approach consists in using first-principles molecular dynamics (MD) for the chromophore in combination with a classical MD scheme for the protein. The protein is modeled in a membrane-mimetic environment. We plan to describe the electronic structure of the excited state with the restricted open-shell Kohn-Sham (ROKS) method [16, 17, 18].

First-principles molecular dynamics according to Car and Parrinello (CPMD) [19, 20] is nowadays a widely used tool to describe the dynamics of molecular systems in the ground state [21]. Using the Kohn-Sham Hamiltonian [22, 23, 24, 25] with standard density functionals [26, 27, 28], the methodology is applicable to a great variety of systems. The use of a plane-wave basis set for the electronic wavefunction allows the description of gas phase and condensed phase on an equal footing. In contrast to classical MD, the quantum-chemical description of chemical bonds renders the simulation of chemical reactions feasible. CPMD uses the on-the-fly approach, i.e. only the points that are reached during the dynamics are computed instead of the complete potential surface. In this manner, it is possible to simulate chemical reactions in complex systems without any initial knowledge about the reaction mechanism [29, 30].
CPMD is computationally expensive compared to classical simulations. This puts limitations on the size of the reactive system and the time scale of an unconstrained simulation, *i.e.*, in a simulation in which no *a priori* knowledge of the reaction pathway is used. The rhodopsin photoreaction is fast enough to be observable on the accessible (picosecond) timescale. However, the environment is too large for a complete quantum chemical description. For this chemically unreactive part of the system, a classical description is sufficient. The hybrid quantum mechanics/molecular mechanics (QM/MM) code developed at the ETH Zurich is able to describe a quantum chemical system embedded in a classical environment [31, 32] and allows thus MD simulations of the complete system.

In order to describe the photochemical reaction in the protein, it is necessary to combine the QM/MM code with an approach that is able to cope with excited states 'on the fly'. The treatment of excited states is more difficult than that of ground states; electron correlation is very important. For the use with molecular dynamics, an excited-state method has to be simple to use, numerically stable and computationally affordable. In an MD simulation, some 10000 points on a potential energy surface are calculated, and it is not practicable to readjust the input parameters for every point. Furthermore it is desirable that a wavefunction (and not just the energy) for the excited state is computed, and that this wavefunction has a simple structure. Finally, the method should be self-consistent.

A method that essentially fulfills these conditions has been developed recently for the computation of first excited singlet states [16]. The restricted open-shell Kohn-Sham (ROKS) method uses a single spin-adapted function instead of a single determinant to represent the wavefunction [17, 18]. A self-consistent scheme was derived using the Kohn-Sham approximation for the exchange-correlation part of the energy. Application to aldehydes, ketones and imines yield very good results [16]. First excited-state MD simulations

have been performed for photoisomerization reactions [16, 33, 34].

3 Results

3.1 Protein modeling

The experimentally determined structure of rhodopsin (Fig. 2) is not stable outside a membrane, but modeling a lipid membrane is difficult due to the many slow degrees of freedom that have to be relaxed. Since the simulation of the photoreaction and of the consecutive motion within the protein it is not necessary to have a very accurate description of the membrane, we use a membrane-mimetic environment consisting of n-octane surrounded by water containing a physiological concentration of sodium chloride. While on this basis it is not possible to describe the full dynamics of the membrane, the model is able to maintain the structure of the protein on the time scale of several nanoseconds. This has been demonstrated for other membrane proteins [35, 36], and we observed the same behaviour in both classical [37] and QM/MM simulations. With both approaches only vibrational motion around the equilibrium position was observed. The complete model contains $\approx$ 24000 atoms (see Figure 3); we consider all 72000 atomic degrees of freedom in our dynamics. Breaking and formation of bonds is possible in the QM part only. That is, the chromophore is chemically reactive, while the apoprotein, the membrane and the water molecules are inactive.

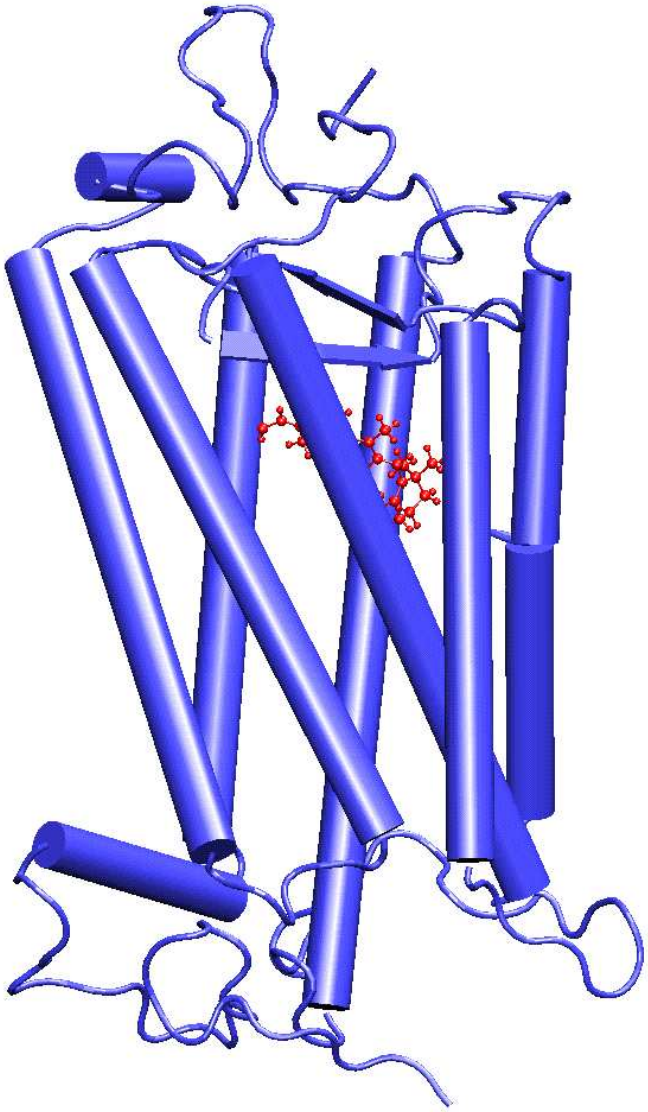

Fig. 2. The experimental protein structure

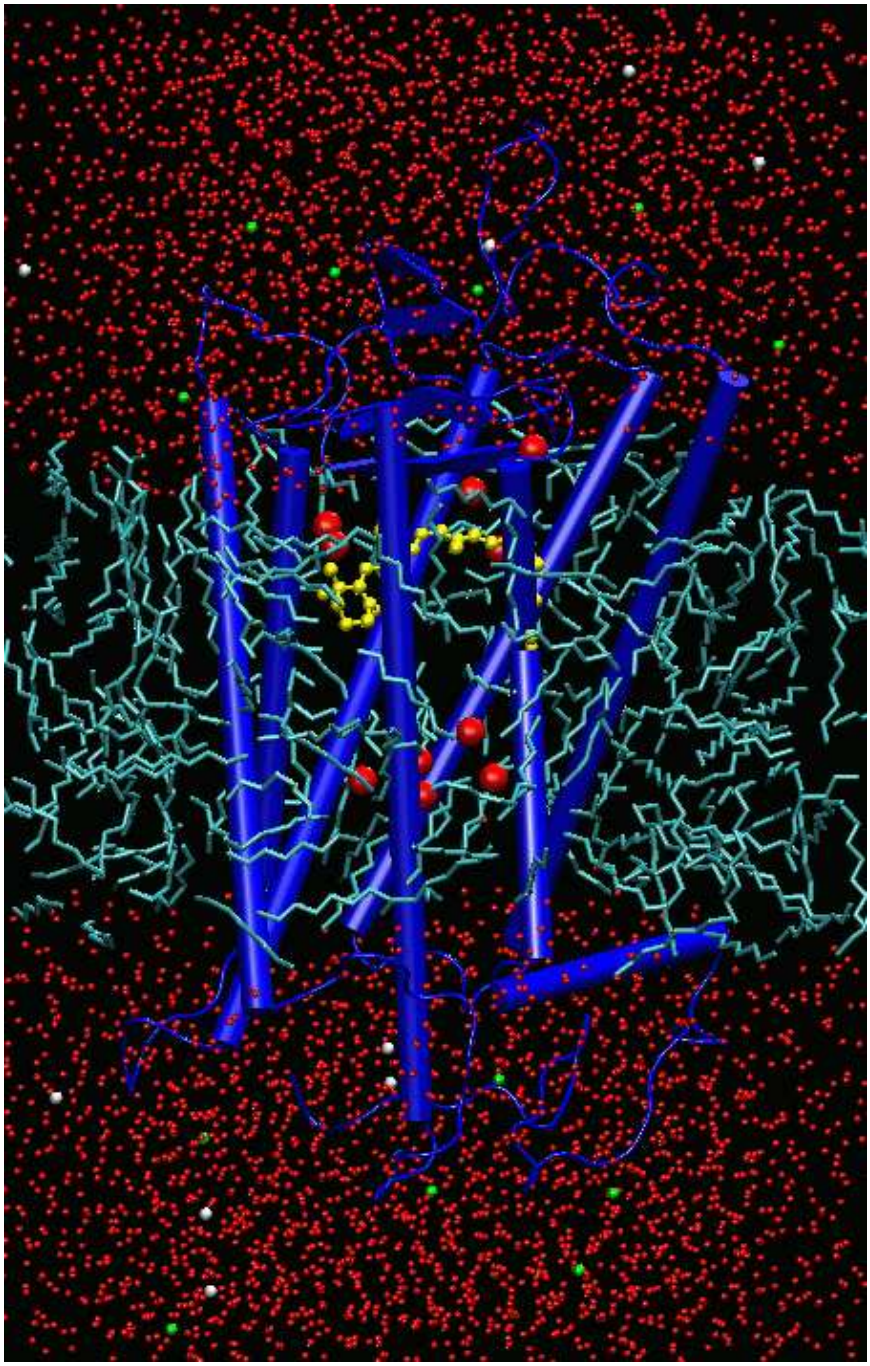

Fig. 3. Model of rhodopsin plus environment

3.2 Electronic structure

In previous gas phase calculations using ROKS [33, 34], it was found that the photoisomerization of the isolated chromophore of rhodopsin exhibits a high barrier in the excited state. This barrier is significantly lowered by adding a single negatively charged amino acid from the protein binding pocket. A linear correlation between the C_{11}-C_{12} bond length and the isomerization barrier was found and indicates that in the protein the barrier is small (< 3 kcal/mol) [34].
In parallel to the calculations on the large chromophore, we are presently performing calculations on smaller conjugated molecules. These systems exhibit a $\pi \rightarrow \pi^*$ electronic transition, in contrast to the molecules investigated earlier [16]. Here, additional difficulties emerge from the fact that the two orbitals involved in the transition belong to the same spatial symmetry. This can eventually cause an unphysical rotation of the two orbitals with the minimization algorithm used up to now [38]. The effect is shown in Fig. 4.

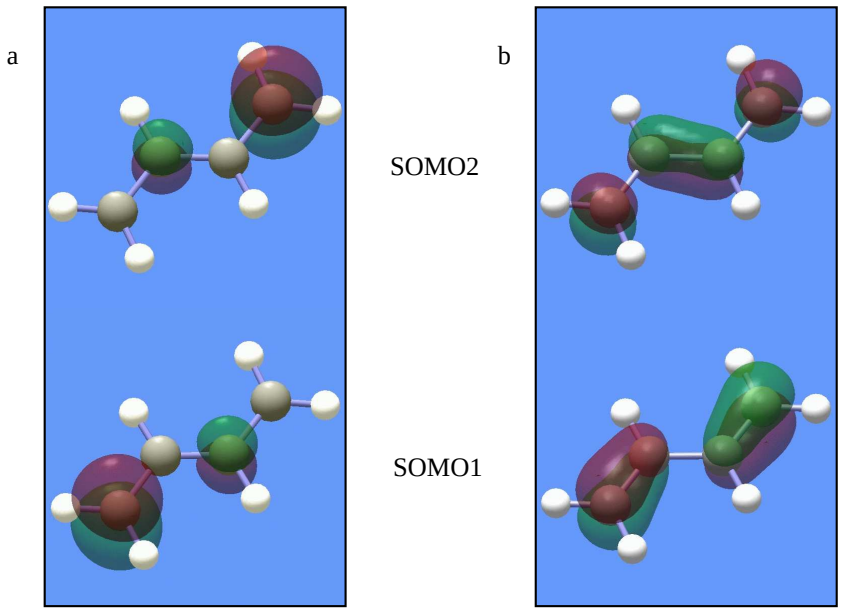

Fig. 4. Frontier orbitals of butadiene. a) unphysical localization. b) correct molecular orbitals.

The rotation leads to a collapse of the singlet energy to the triplet energy, i.e. the singlet-triplet gap is minimized by unphysical localization of the orbitals. Using a modification of the optimization algorithm we are meanwhile able to avoid this problem in a fully self-consistent calculation for small molecules like butadiene. In very recent calculations we were able to perform

a Born-Oppenheimer MD simulation of the *cis-trans* isomerization of butadiene with the correct orbitals.
In future work we plan to achieve this also for the more complicated electronic structure of the rhodopsin chromophore. From first calculations it is evident that in this case the effect of the rotation is much smaller than in the case of butadiene (butadiene: $\approx$ 35 kcal/mol; rhodopsin: $\approx$ 1 kcal/mol). Nevertheless this difference might influence the reaction rate substantially. Before starting the excited-state MD simulation, it must be assured that during the simulation the proper orbital rotation is maintained. Furthermore, we want to combine the modification of the optimization algorithm with the more efficient Car-Parrinello MD scheme in order to reduce the computational cost.

4 Summary and Outlook

We have combined the restricted open-shell Kohn-Sham method with a QM/MM scheme, which brings very attractive applications into reach. One of the most interesting problems in this field is the photoisomerization of rhodopsin. We have developed a model system for rhodopsin in a membrane mimetic environment and have shown that it is stable without constraints in a classical MD simulation on a nanosecond timescale, and in a QM/MM simulation on a picosecond timescale. Our next aim is to simulate the photoreaction that initiates the process of vision.

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