

## First-principles simulation of photoreactions in biological systems

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### 1. ABSTRACT

First-principles simulations start to be applicable to the photochemistry and photophysics in biological systems. In this review the prerequisites for investigating such excited state phenomena in large systems are outlined. Generally, a quantum mechanical description of the electronic structure is combined with molecular dynamics simulations, which allows to describe the motion of the atoms in the field produced by the quantum-mechanical potential. Like this, bonds can be formed and broken, that is, chemical reactions can be simulated. The review focuses on applications of first-principles molecular dynamics to photoactive proteins.

### 2. INTRODUCTION

The enormous advances in the field of computational chemistry, allied to the advent of high speed and parallel computing technologies, have encouraged the development and application of computational simulations for the comprehension of structure, dynamics and chemical reactions of complex molecular systems. Most of these studies are based on molecular dynamics simulations where the systems are described by classical, quantum mechanics or a combined approach, depending on the phenomena investigated.

The theoretical approach most broadly applied to biological systems is classical molecular dynamics. This

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method allows the investigation of structure, dynamics and thermodynamics of inorganic, organic, and biological systems, including proteins, membranes and nucleic acids. It solves the classical Newtonian equations of motion, whereby the forces are calculated from an empirical force field. Molecules are described as atoms being connected by 'springs' that oscillate at finite temperature, typically 300 K. Since many biological systems are composed of but a few different moieties, in particular of amino acids and nucleic acids, this approach is most successful in the application to biosystems, where very good parametrizations exist. The approximations which render the method so extremely efficient, result however in an insufficient description of systems where charge transfer, electronic excitation, bond-breaking or bond-formation take place. For describing such phenomena where details of the electronic structure are essential, it is necessary to include quantum mechanical (QM) calculations in the simulation schemes. Therefore the introduction of density functional theory (DFT) based simulations in biology was motivated by the need of a first-principles description for the interatomic interactions whenever a classical force-field description is not sufficient. An advantage of the first-principles molecular dynamics approach is the fact that it does not make use of atom-type dependent parameters. That is, there is only one type of carbon atoms instead of  $sp^2$  carbons and  $sp^3$  carbons etc. The atoms may change their connectivity and hybridization at any time. This fact in combination with the use of quantum mechanically computed potentials renders the study of quantum phenomena such as bond formation and bond breaking processes possible.

In a first-principles MD simulation, which is capable of combining dynamics and quantum mechanics, the forces acting on the nuclei are iteratively obtained from an electronic structure calculation in every time step, corresponding to an "on the fly" treatment. Pioneering work in this field was done by Car and Parrinello in 1985 (1,2,3). Car-Parrinello molecular dynamics (CPMD) has seen widespread use in materials science, chemistry, and condensed matter physics, but also offers an ideal method which combines the accuracy of DFT and the power of MD necessary for biological systems which are intrinsically dynamical (4). The basic concept is simple: instead of trying to compute the potential energy surface of the system in as many dimensions as possible and performing dynamics on this surface in a second step, the *on-the-fly* methods consider only those points that are reached during a molecular dynamics run. Only for these structures, the energy and the energy gradient are determined by a quantum-chemical method during the simulation. It is apparent that this approach has the advantage to allow very complex systems to be treated without prior knowledge of the possible reaction pathways. A disadvantage is that relatively high demands have to be made concerning the quantum-chemical method used. The development of modern density functionals (5) represented an important step in the development of practical *on-the-fly* schemes. The most successful *on-the-fly* concept is still the one originally developed by Car and Parrinello (see for example (3)):

Description of the valence electrons by density functional theory (6, 7) using a plane-wave basis set and periodic boundary conditions;

Description of the inner electrons by previously computed, fixed pseudopotentials;

Simultaneous motion of electrons and nuclei according to the Car-Parrinello equations (1).

Density functional theory in such simulations - like in most practical calculations - is always used within the Kohn-Sham formalism and may hence also - more accurately - be called Kohn-Sham theory. The Kohn-Sham formalism involves the decomposition of the total density into molecular orbitals (approximate single electron functions). This decomposition leads to approximate functionals but also to a very simple and illustrative picture of the electronic structure in terms of orbitals.

A serious limitation of first-principles MD techniques is the CPU cost which essentially limits the size of tractable systems to some hundred atoms. An extension of the method to a mixed quantum-classical (QM/MM) hybrid scheme is particularly attractive for the investigation of biochemical reactions. QM/MM methods divide the system into a chemically active part, treated by first-principles QM methods, whereas the effect of the protein environment is modeled by a classical force field (8-10). Such an approach has the advantage that the computational effort can be focused on the part of the system where it is most needed, whereas the effects of the surroundings are taken into account with a more expedient model. In this way, large biomolecular systems can be treated in a computationally efficient manner. In addition, long range effects which are important for a proper modeling of protein structures, are better described by a classical parametrization than by present-day density functionals. Several mixed QM/MM Car-Parrinello approaches have been implemented (11,12), in which the steric and electrostatic effects of the surroundings are explicitly taken into account. For recent reviews of applications of QM/MM simulations using the Car-Parrinello scheme, see (4, 13,14).

Very attractive applications of first principles molecular dynamics get into reach by extending the scheme to the treatment of electronically excited states. Particularly the description of photochemical reactions which occur upon electronic excitation by photon absorption, is very appealing because there the full power of the approach can be exploited. Photoreactions usually occur on the timescales that are accessible to free (unconstrained) first principles molecular dynamics simulations. The complex dynamics of photoreactions is an actual field of intense experimental studies (15-20).

For the theoretical investigation of the electronically excited states of molecular systems a large variety of quantum chemical approaches is available. These methods comprise *ab initio* methods such as configuration interaction singles (CIS) (21,22), complete active space self-consistent field theory (CASSCF) (23,24), complete active space perturbation theory in second order (CASPT2) (25-27), and multi-reference configuration interaction (MRD-CI) (28,29), as well as density functional theory (DFT) approaches such as time-dependent density functional theory (TDDFT) (30-33). For a recent overview of the quantum chemical methods which are most

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frequently used for the investigation of excited states in biological systems see (34). Most of these methods, however, are not suitable for *on-the-fly* simulations. This is due to the fact that they are either not fast enough or cannot be used as black-box methods for the automated calculation of thousands of points on a potential energy surface during a simulation. In addition, particularly for the more accurate methods like multi-reference configuration interaction, it is very difficult to obtain analytic energy gradients to compute the forces on the nuclei. Furthermore the quantum chemical approach should be applicable with moderate effort in connection with plane wave basis sets. This latter requirement excludes all methods except DFT methods. The use of delocalized plane waves which have no origin in space, is advantageous for the computation of energies and forces during dynamics simulations. In addition, plane wave basis sets allow the treatment of individual molecules and condensed phases such as solids, surfaces, and solutions, on an equal footing.

To the aim of treating photoreactions restricted open-shell Kohn-Sham (ROKS) was developed in 1998 (35,36). Later the TDDFT gradients were programmed and it became possible to do geometry optimizations and molecular dynamics with this method (37,38). TDDFT is the superior method for describing electronic spectra. However, severe problems occur when moving on excited-state potential energy surfaces, which renders ROKS the better alternative for simulating photoreactions.

In the next section, we briefly introduce the most important concepts of excited state DFT methods and describe the advancements that are relevant for the application of the methodologies to photo-biological processes. Thereafter, we report a few applications and illustrate what can be achieved with first principles molecular dynamics simulations.

### 3. DENSITY FUNCTIONAL THEORY FOR EXCITED STATES

According to Kohn and Sham (7) the exact ground state density, and with it all observable quantities, of the many-body problem can be obtained in principle from a single set of molecular orbitals. In the simplest case of non-degenerate orbitals and an even number of electrons, this means that each orbital is filled with one electron with alpha spin and one electron with beta spin. To satisfy the Pauli principle, these orbitals are not just multiplied to yield the total wavefunction, but are composed to an antisymmetric Slater determinant. In practical applications, always approximate solutions are obtained due to the fact that the exact density functional is unknown.

If one wants to extend the theory to the simulation of photoreactions, the question arises: What are excited states? And: Which excited states are relevant for the description of photoreactions? The first question is not as trivial as it might seem and different communities tend to answer this question in a different way. Particularly in molecular physics the description of the electronically excited states via a perturbation from the ground state is very common. In quantum chemistry, methods starting from a reoccupation of orbitals have been more broadly

used for many years. For vertical excitations this usually leads to equivalent results, meaning that the results from the different approaches can directly be compared. In both descriptions the excited states are orthogonal to the corresponding ground state. Let us, however, consider the situation of two states adopting the same energy which usually occurs during a photoreaction. Now it is a matter of definition what is the ground state and how the excited states are numbered. One might speak of a degenerate ground state at this point and renumber the excited states. In a simulation of a photoreaction, however, one has to decide how to handle this situation in an automated way. Present implementations of perturbation theory based methods tend to fail in such situations.

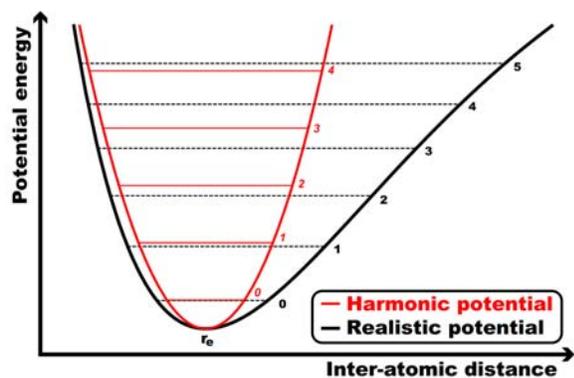
A second problem arises at this point, namely the way how the electronic and nuclear degrees of freedom are separated. The approximation usually employed in molecular calculations is the adiabatic approximation in which the nuclei are fixed in space and the electrons are fully relaxed. This approximation which is based on the paper of Born and Oppenheimer (39) leads to the picture of Born-Oppenheimer potential energy surfaces (Figure 1). In this adiabatic approximation (a-diabatic: 'non-intersecting'), electronic states 'hardly ever cross' (more precisely: states of different spin symmetry or different spatial symmetry do not cross). In the early days of quantum chemistry, this led to the discussion, how molecular systems may return to the ground state as efficiently as it is observed in photoreactions. In the past decades it has been noted on the basis of ab-initio calculations that also in the adiabatic approximation there are so-called conical intersections (40) between ground and excited states present in most molecular systems (41). These conical intersections have been discussed as the points through which the systems proceed during photoreactions on their way back to the ground state. Today it is commonly known that the conical intersections are in general multidimensional objects (conical seams, conical intersection spaces).

An alternative description represents the diabatic approximation for which no unique definition exists. The simplest way to arrive at diabatic surfaces is by making an additional approximation, namely the single-configuration approximation. This approximation forces a system to remain in a certain electronic configuration (for example in a ionic configuration), while during the motion on an adiabatic surface the character of a state may change (for example from ionic to radicalic). In the diabatic approximation crossings between states of the same symmetry are no longer forbidden and a one-dimensional conical intersection is replaced by a two-dimensional crossing.

A complete solution of the problem is possible by using adiabatic approximations (i.e. not diabatic approximations (42)) and computing the non-adiabatic coupling elements. Such powerful calculations which yield the complete picture, are possible for small molecules (see for example 43,44). For larger systems simplifications are desirable.

We consider the simplest situation of a molecular system that has all electrons paired in energetically non-degenerate molecular orbitals in its ground state and has

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**Figure 1.** Schematic representation of typical potential energy surfaces (PES) that are relevant for photoreactions. Plotted is the potential energy  $E$  vs. some reaction coordinate  $R$ . Vibrational levels are neglected, that is, nuclear motion is considered to be classical. Depending on the energy of the photon, the electronic excitation of the singlet ground state ( $S_0$ ) leads vertically from the educt minimum to one of the excited singlet states. In the scheme, fast deactivation to the first excited singlet state  $S_1$  may occur diabatically through the diabatic crossing D1. The system may continue its motion in the  $S_1$  state till it reaches the diabatic crossing D2. From there it may return diabatically to the ground state  $S_0$  and relax in the product state minimum (photoreaction). A back reaction to the ground-state minimum of the educt may occur thermally across the ground-state barrier on longer time scales. If the velocity along the relevant coordinate is too high for a diabatic return to the ground state, the system may continue its motion in the excited state till it reaches the depicted conical intersection through which return to the ground state can occur. Note that this two-dimensional sketch can just give a first idea of the different possibilities in a  $3N-6$  dimensional PES space. (In particular, it does not show that moving along a diabatic crossing space usually leads to a conical intersection space.) The green line marks the process which is described by the diabatic ROKS approximation.

singlet spin symmetry ( $S_0$ ). If it is irradiated with light, it is transferred to some energetically higher-lying singlet state, depending on the energy of the absorbed photon. The transition to triplet states is symmetry forbidden since the photon does not transfer spin. According to Kasha's rule (45), deactivation to the lowest excited singlet state ( $S_1$ ) is very fast, so that usually no photochemistry takes place in the higher-lying excited states.

The  $S_1$  state may be long-lived enough for photoreactions to occur – if there is no close-lying conical intersection through which the system is rapidly transferred back to its ground state. Also, there is a finite probability for conversion to the close-lying triplet state  $T_1$  (intersystem crossing). Since the transition from the triplet state back to the singlet ground state is forbidden,  $T_1$  is usually long-lived and photoreactions may occur in this state. So the states that are most relevant for describing photoreactions in organic and biological systems are  $S_1$  and  $T_1$ .

Which quantum chemical methods may be used to describe these states? As mentioned before, one possibility is to obtain an electronic spectrum as response to a perturbation of the ground state. This concept is most successfully realized with TDDFT in the approximation developed by Casida (32), which has become the standard method to compute spectra of medium-sized to large molecules. Electronic spectra are usually reliably obtained with TDDFT with shortcomings particularly in the description of charge transfer states. Generally, for excited states, one cannot expect the same accuracy as for ground states and comparison to other theoretical and/or experimental results is necessary. Nevertheless, in combination with modern density functionals like B3LYP, a remarkably high accuracy is reached with TDDFT. When dealing with photoreactions, the question arises if TDDFT should be considered as a diabatic or adiabatic approach and how conical intersections should be treated. In a recent publication the TDDFT surfaces were compared to adiabatic surfaces (33).

The second approach mentioned above is used by the restricted open-shell methods, restricted open-shell Hartree-Fock (ROHF) and restricted open-shell Kohn-Sham (ROKS). Here, excited states are constructed by reoccupying orbitals. This allows to propagate the system in the excited state without keeping the knowledge of the corresponding ground state. In the example of a ground-state singlet with all orbitals doubly occupied, the excited state  $S_1$  is generated by removing one electron from the highest occupied molecular orbital (HOMO) and putting it in the lowest unoccupied molecular orbital (LUMO). To describe the spin symmetry correctly, such a situation must in general be described not only by a single Slater determinant but by a linear combination of Slater determinants, namely a spin-adapted configuration. Due to the single-configuration approximation, the restricted open-shell methods are diabatic. With such a restricted open-shell approach, only the few lowest open-shell states can be described, typically  $S_1$  and  $T_1$ . To obtain more states of the spectrum, the concept can be extended in the case of Hartree-Fock theory to a linear combination of many configurations, leading to the adiabatic multi-configuration self-consistent field (MCSCF) theories like CASSCF. At present, such an extension is not possible in Kohn-Sham theory in a satisfying way. However, Kohn-Sham theory offers the advantage of having electron correlation included already in a single configuration description. The different treatments of electron correlation in CASSCF and ROKS lead to different restrictions. In CASSCF the correlation caused by the few orbitals in the active space ('static correlation') is very well considered, while the contributions of all the other virtual orbitals ('dynamic correlation') is completely neglected. This may lead to an unbalanced treatment of correlation unless a large part of the electronic system is included in the active space. Hence CASSCF calculations can be done in a very accurate way for small molecules, but for larger systems a reasonable choice of the active space becomes difficult. In ROKS the correlation of all orbitals is considered in an averaged way through the exchange-correlation term of Kohn-Sham theory. This may lead to qualitatively wrong results if static correlation is of high relevance for the description of  $S_1$ . In practical applications it turned out, however, that a precise description of static correlation is not critical for

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surprisingly many cases (46,47), quite in contrast to what has been concluded from Hartree-Fock based calculations. Empirically, the “Kohn-Sham hope” that a simpler description of the many-body problem is possible on the basis of a single set of orbitals with integer (or rational) occupation numbers holds for low-lying excited states, too. However, a red-shift of typically 0.5-0.7 eV of the ROKS results was observed which is due to an insufficient description of the exchange interaction in connection with the functionals employed (GGA functionals). This usually does not influence the motion on the excited state potential energy surface, but prevents the determination of absolute excitation energies.

One of the nice features of ROKS is the possibility of a simple return to an open-shell ground state (Figure 1): If there is a crossing to an open-shell ground state, the simulation will continue diabatically from the excited to the ground state. This turned out to be a good approximation already in medium-size systems (47). In larger systems the kinetic energy is even easier distributed among the vibrational degrees of freedom and the behaviour is even more diabatically. Note that, when passing such a crossing, the ROKS singlet state becomes the ground state while the restricted closed shell state becomes the excited state. In other words, if talking about restricted and restricted open-shell methods, instead of discriminating between ground and excited states, one might just as well discriminate between restricted closed-shell states, restricted open-shell states, and 'real' excited states (the latter being not easily accessible to a single-configuration method). In this framework, Kasha's rule reads: Photoreactions hardly ever take place in real excited states.

As discussed before, the description of the nuclear dynamics is approximated by the classical equations of motion. Thus quantum effects on nuclear motion are ignored (but could also be described in a CPMD manner (48)). Particularly for photoreactions which usually involve high kinetic energies and negligible barriers, these effects are not relevant. Classical Newton dynamics is simple, but affords the knowledge of the energy gradients. The computation of the gradients for ROKS is straightforward using the Hellmann-Feynman theorem as it is usually done in CPMD calculations; TDDFT gradients have been implemented in several program packages some years ago (37, 38). As is shown in the Applications section, many studies still do not attempt to compute excited-state gradients and excited-state structures, but use ground state structures as obtained for example from DFT-QM/MM calculations. This is significantly simpler to do than QM/MM calculations in the excited states, but gives an information only about the first instances after excitation, i. e. before significant relaxation can take place in the excited state. In a real excited-state dynamics not only the initial relaxation of the system but eventually also photoreactions can be observed. As ultrafast photoreactions typically occur far from equilibrium at high kinetic energies, dynamical effects are most relevant and unconstrained excited-state molecular dynamics represents an irreplaceable tool for the description of such phenomena. It will be seen in the next section, however, that it is not an easy task to simulate spontaneous photoreactions in biological systems due to limitations in accuracy and in CPU time, and but a few successful cases are reported in the literature.

## 4. APPLICATIONS

Several applications of first principles molecular dynamics in a variety of systems of chemical importance and under a broad range of thermodynamic conditions were reviewed quite recently in (49). The comprehensive review illustrates the broad applicability of first-principles molecular dynamics calculations in the study of structures, reaction mechanisms, and electronic properties for both isolated molecules and condensed phases.

Examples for biological systems are reviewed in (4,14). Most studies of such large systems use the QM/MM implementations in CPMD (11,12). These applications include for example the investigation of the catalytic mechanisms and structural features of a variety of enzymes (50-59) and the selectivity mechanism of ion channels (60). Also interactions between a ligand and its target in a *cis*-platin-DNA adduct (61), and DNA-binding organo-ruthenium compounds (62) were investigated, as well as the DNA damage caused by radicals (63).

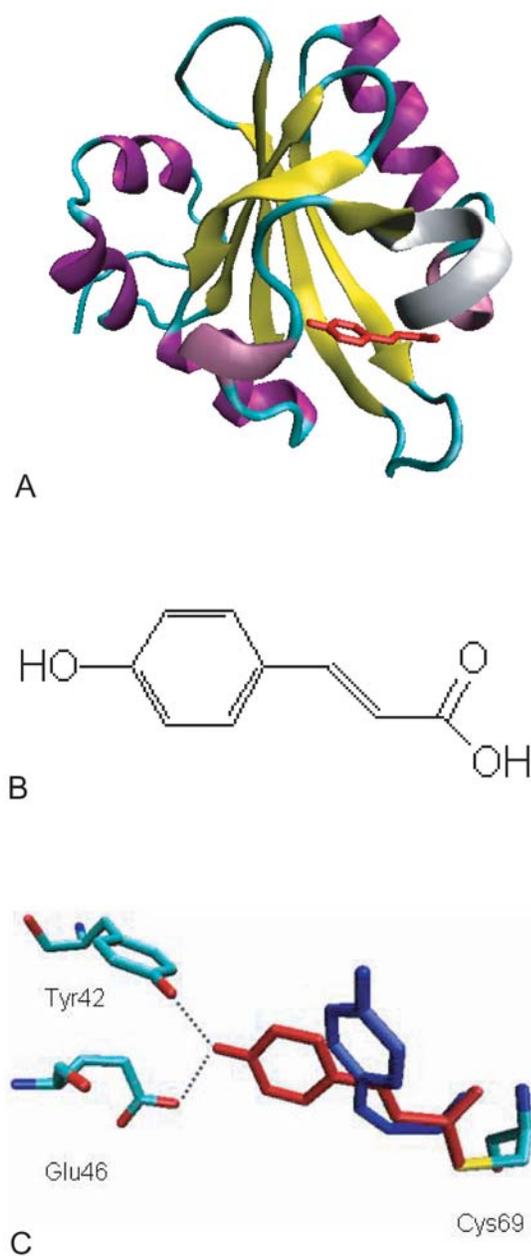
In the present review we concentrate on the application of first-principles molecular dynamics to photoreactions in biological systems. Photoactive proteins fulfill important biological functions. They represent a specific class of proteins which give to different organisms the capability to absorb and convert light. Small organic molecules, known as chromophores, are bound to these proteins to do the first task in the light transduction pathway. Upon absorption of a photon, these chromophores may undergo photochemical reactions which alter their conformation or chemical composition.

Here, we present a short overview of recent applications of DFT-QM/MM approaches to the most important photoactive proteins with chromophores absorbing in the visible region, namely photoactive yellow protein, green fluorescent protein, rhodopsin, and bacteriorhodopsin. In all these systems the chromophores contain extended  $\pi$  systems with double bonds which eventually rotate in the excited state from their *cis* to their *trans* forms or vice versa (photoisomerization). To understand the specific behavior of these photoactive proteins, it is necessary to understand the photoisomerizations in the particular  $\pi$  systems and how they are influenced by the particular protein environments. We report the present state of first-principles simulations on these systems which will show that there are still quite a few open questions. Particularly it turns out that observing a spontaneous (photo)reaction in a biological system and thus determining every detail – the mechanism, the reaction time, etc. - still remains a formidable task for modern quantum chemical simulations.

### 4.1. Photoactive yellow protein

Photoactive yellow protein (PYP) is a small and structurally well characterized protein (Figure 2A), which has a relatively simple light-initiated photocycle with features in common with the visual pigments and other photoactive proteins. For this reason, PYP has extensively been studied, using numerous experimental techniques (64-72). PYP is a water-soluble photoreceptor protein, first isolated from *Ectothiorhodospira halophila* (64,65). The protein is believed to serve as the photosensor initiating the

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**Figure 2.** A) Ribbon representation of PYP. The protein consists of a single polypeptide chain of 125 residues. The chromophore consists of a para-hydroxy cinnamic acid molecule represented by balls and sticks. B) Chemical structure of the para-coumaric acid. C) PYP's chromophore binding pocket. The para-coumaric acid is attached via a thioester linkage to the only cysteine residue (Cys69) in the protein. In the pG state of the photocycle, the chromophore (red) is in the *trans* conformation and is deprotonated. The structure of the intermediate is shown in blue. The buried charge on the chromophore is stabilized by hydrogen bonds from Tyr42 and Glu46.

negative phototactic response to blue light (66). It contains the chromophore para-coumaric acid which gives the protein its characteristic yellow color. This

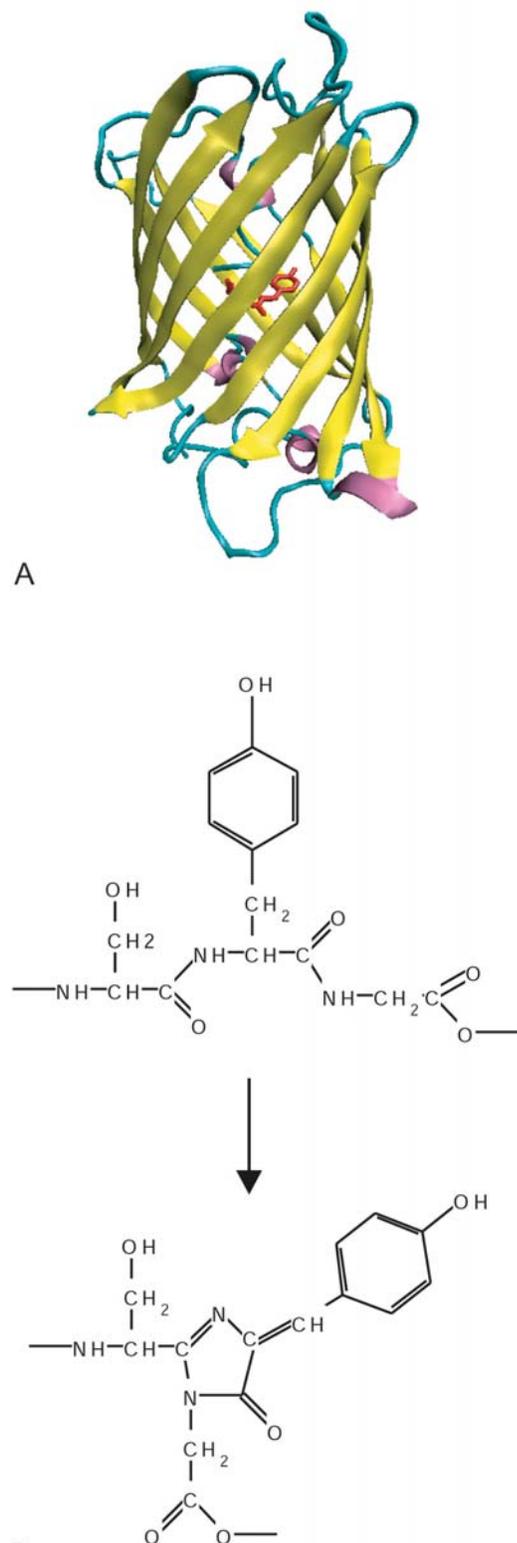
chromophore is bound via a thiol ester linkage to the only cysteine residue in the protein (67,68). Upon excitation, it undergoes an isomerization reaction from its *trans* to its *cis* form (Figure 2B, 2C), leading to a photocycle consisting of several transient intermediates, and recovers the initial state on a subsecond time scale (69). It is sometimes claimed that PYP has properties which make it a candidate for application in photobioelectronics and as a consequence is a potential biomaterial for the next generation of electronic devices.

Several computational studies describe the isomerization of the chromophore (73-79). The isomerization of the isolated chromophore as well as the isomerization of the chromophore in its binding pocket have been studied using static DFT calculations (73). For the isolated chromophore, the computations show that the *trans* configuration is lowest in energy, both for the neutral and for the deprotonated system. The negative charge is found to induce modifications in the structure of the molecule and, specifically, a significant elongation of the isomerizable double bond. Such an elongation of a chemical bond usually correlates to a lowering of the rotational barrier which was also found for the PYP ground state. It was also confirmed that the protein environment induces an out-of-plane torsion in the ground state *trans* pCA chromophore. By means of TDDFT calculations, the electronic excitation relevant for the photochemistry of PYP was determined to be dominated by the HOMO-LUMO transition. Computation of the excited-state potential energy curves using ground state structures with TDDFT (76) yielded a small barrier in the excited state of a few kcal/mol. Note however, that for a more precise determination the structures would have to be relaxed in the excited state. This latter paper is also an example how difficult it can be to make clear statements about protonation states of biological moieties on the basis of DFT calculations which may render an assignment to the spectroscopically observed intermediates difficult.

The protonation of pCA after excitation and isomerization of this chromophore was studied using classical molecular dynamics and DFT-QM/MM (79). It was shown that the stabilization of the charge is a very important factor, indicating that the direct environment of the chromophore is highly important for this reaction. In pure DFT simulations of the chromophore binding pocket in vacuum, very frequent proton transfer was observed in contrast to the QM/MM simulations. This difference was attributed to rearrangements in the environment after isomerization which could not be modelled on the timescale accessible to QM/MM simulations.

Even if we want to restrict our review to DFT calculations, a CASSCF-QM/MM study must be mentioned at this point (78). To our knowledge this remarkable study represents the first simulation of a spontaneous isomerization of PYP. A rather small CASSCF space was chosen (6 electrons in 6 orbitals, 3-21G basis set) which however seems to be sufficient to get the essential features of the photoreaction (reaction time, quantum yield) with very good accuracy. Switching to the ground state was allowed if a conical intersection was reached, thus conserving the total energy during the dynamics. The change of the internal degrees of freedom, that is, the full

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**Figure 3.** A) Cartoon representation of GFP containing the chromophore (in red) in its center. B) Chemical structure of the chromophore *p*-hydroxybenzylidene-imidazolinone that originates from an internal *Ser-Tyr-Gly* sequence which is post-translationally modified.

motion of the chromophore as it occurs spontaneously upon excitation, was monitored at this high theoretical level. The simulations show how the isomerizing double bond is rotated from 180 degrees (*trans* form) to about 90 degrees in the excited state and continues the rotation to a value of about 0 degrees (*cis* form) after return to the ground state.

### 4.2. Green fluorescent protein

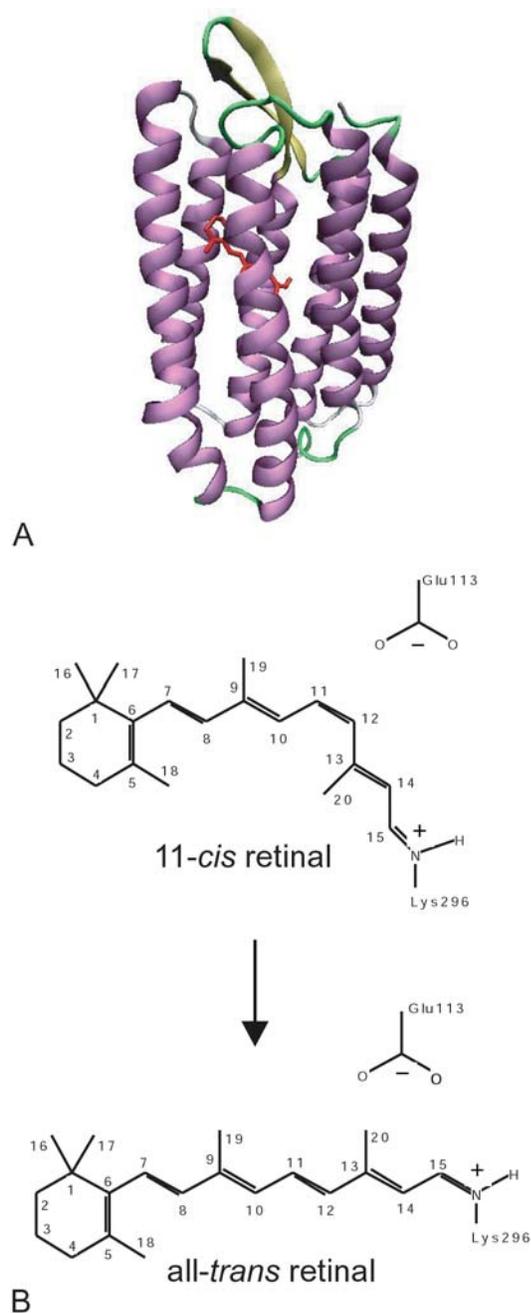
Green fluorescent protein (GFP), an intrinsically fluorescent protein (IFP) isolated from coelenterates, such as the Pacific jellyfish *Aequorea Victoria* (80), is one of the most widely studied and exploited proteins in biochemistry and cell biology. It is used as a marker of gene expression and protein targeting in intact cells and organisms (81-83). Efforts to improve and vary the properties of GFP were made for its application as a pH sensor or in microscopy with the generation of GFP mutants. Many different mutants of GFP were engineered with new spectroscopic properties by mutating amino acids in either the chromophore or in the surrounding part of the protein (84-86).

The cylindrical beta-barrel structure (Figure 3A) formed by 11 beta-strands constitutes a perfect cylinder structure containing in its center the fluorescent chromophore *p*-hydroxybenzylidene-imidazolinone. This fluorophore is derived from post-translational cyclization of a serine-tyrosine-glycine tripeptide, followed by dehydrogenation of the tyrosine (Figure 3B). The chromophore shows two thermodynamically stable protonation states, corresponding to two absorption peaks of the protein spectrum. The wild-type exhibits two absorption maxima at 395 and 475 nm which relax to an emission maximum around 508 nm, emitting a greenish yellow light. The 395 nm peak is associated with the protonated (neutral or A) form, whereas the 475 nm peak is associated with the deprotonated (anionic or B) form. The equilibrium between the two states can be controlled by external factors such as pH and mutations which affect the chromophore environment (84,87).

TDDFT calculations of neutral and anionic chromophore structures determined from ground state QM/MM calculations have been performed and good agreement with experiment was found (88). Another study presents DFT calculations (using TDDFT for the description of the excited states) of the structural and electronic properties of the chromophore in presence of close residues (89). A relationship between optical and structural properties of the chromophore was found. The same approach was also extended to the whole series of the IFP chromophores (90). TDDFT proves to be appropriate to accurately analyze the absorption properties of a large family of molecules.

The excited-state dynamics of a fluorescing protein cannot be expected to be very exciting, as fluorescence usually means that there is no major excited-state motion in the system. Otherwise in most systems a conical intersections would be reached which would allow a radiation-less return to the ground state. More interesting than GFP for performing excited-state MD simulations is asFP595. This protein can repeatedly be switched from a non-fluorescent state to a fluorescent one. The chromophore in the protein environment and its isomerized

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**Figure 4.** A) Ribbon representation of rhodopsin containing the protonated Schiff base of retinal as chromophore (in red). The chromophore is a derivative of vitamin A1 (retinol) with a total of 20 carbon atoms. C) Upon absorption of a photon in the visible range, 11- $\chi\sigma$  retinal can isomerize to all- $\tau\rho\alpha\nu\sigma$  retinal. Retinal is covalently bound to the nitrogen atom of a lysine residue (Lys296) and Glu113 stabilizes the protonated state of retinal.

state were investigated using QM/MM and TDDFT (91). The authors discuss the difficulty of assigning the protonation states for different intermediates, a problem which is ubiquitous in DFT-QM/MM. The shallow

potentials of hydrogen bonds in biological systems and the delicate equilibria are difficult to describe in an accurate way with present-day density functionals. Further method development is needed.

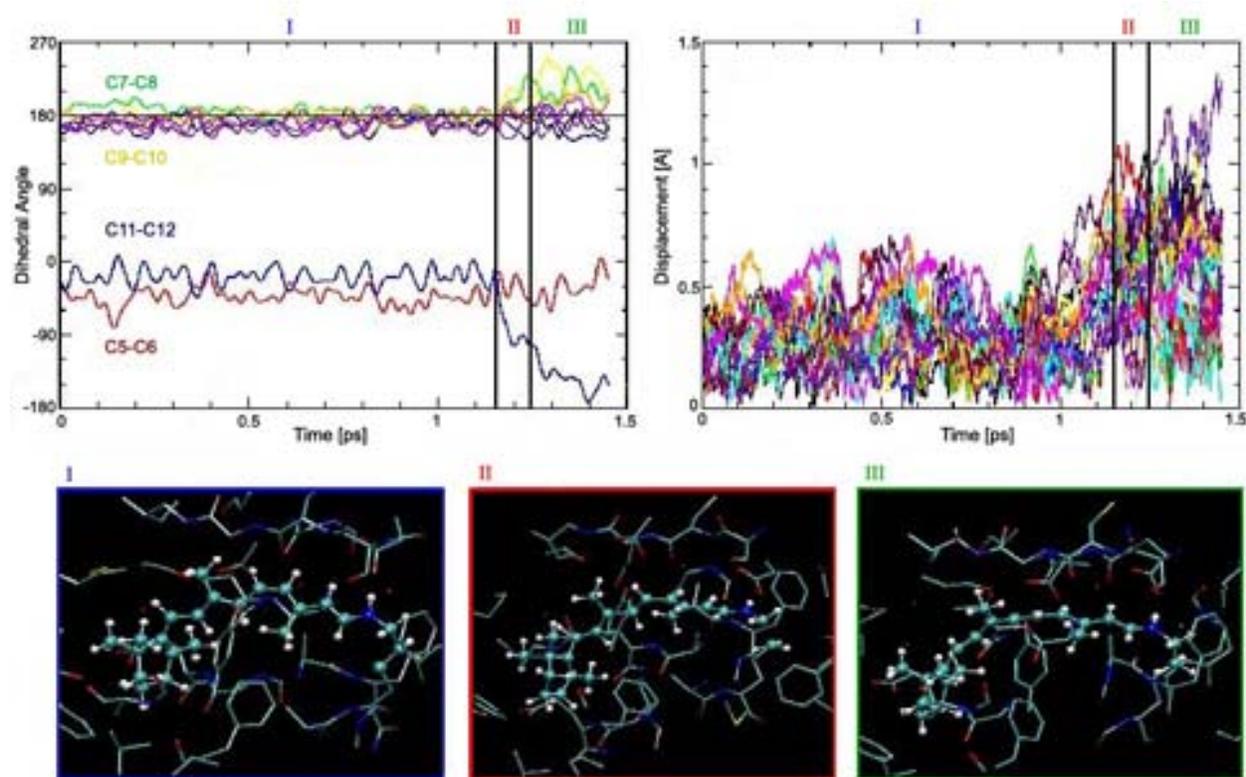
### 4.3. Rhodopsin

Rhodopsin is a highly specialized G protein-coupled receptor (GPCR) which detects photons in the rod photoreceptor cell. Studies on rhodopsin have unveiled many structural and functional features that are common to the large and pharmacologically important group of the GPCR superfamily, of which rhodopsin is the best-studied member (92,93). Rhodopsin is composed of the protein opsin linked to 11-*cis* retinal, a polyene chromophore bound through a protonated Schiff base linkage to Lys-296 (92) (Figure 4). Rhodopsin is involved in the transduction of light into visual signals, i.e. nerve impulses, in the visual system of the central nervous system (94).

The first step of vision consists in absorption of a photon by the rhodopsin chromophore which causes its isomerization to the all-*trans* form in an extremely fast and efficient process (Figure 4B). This photoisomerization initiates a photocycle during which a series of interconversions between different conformational states happens. These structural changes are driven by the release of the photon energy which is in part stored in the system as chemical energy (95-99). Many experimental and theoretical studies have been devoted to the understanding of the underlying mechanism which is of fundamental importance for the process of human vision (100-112).

The isolated chromophore and the chromophore plus counterion were investigated using static ROKS calculations in (102), showing the strong dependence of the reaction on the environment. While for the isolated molecule a significant barrier was observed, the presence of the counterion reduces this barrier significantly. This is in agreement with the observation that in solution the isomerization is slower and less specific, meaning that also other double bonds rotate. In a subsequent study (111), the combination of the QM/MM approach with the ROKS method was used for describing the dynamics in the first excited-singlet state of rhodopsin. Such a QM/MM study became possible after the first X-ray structure of rhodopsin was published (104). A model system (106) consisting of rhodopsin, the chromophore, a hydrophobic membrane, and a saline solution was employed. No photoisomerization could be observed in the ROKS-QM/MM simulations at ambient temperatures. Obviously a small free energy barrier remained also in the protein environment. Exactly the same behavior was observed in TDDFT-QM/MM simulations of the system. Hence shortcomings in the description of the interaction with the environment are a very probable reason. Nevertheless, spontaneous ultrafast isomerization was obtained with ROKS-QM/MM when the local temperature of the chromophore was artificially increased. Then, it could be shown (Figure 5) how the environment induces the selective isomerization from the 11-*cis* to the all-*trans* form of retinal which is possible with surprisingly small atom displacements (111). With this minimum motion, the photoisomerization leads to formation of a strongly distorted *trans* form of the chromophore ('molecular spring'). The release of the strain then induces consecutive reactions. The resulting

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**Figure 5.** ROKS-QM/MM simulation of rhodopsin. Upper row: change of dihedral angles and displacements during the isomerization. Lower row: the chromophore in the binding pocket during the three stages of the simulation. I: Ground state, *cis* form, II: excited state, III: ground state, formation of *trans* form. In the excited state, the C<sub>11</sub>-C<sub>12</sub> bond rotates to 90 degrees and continues this rotation in the ground state, while all other bonds remain essentially unchanged. This rapid rotation is possible with very minor atom displacements in the specific environment of the binding pocket.

intermediates have been investigated with ground state dynamics in (112).

### 4.4. Bacteriorhodopsin

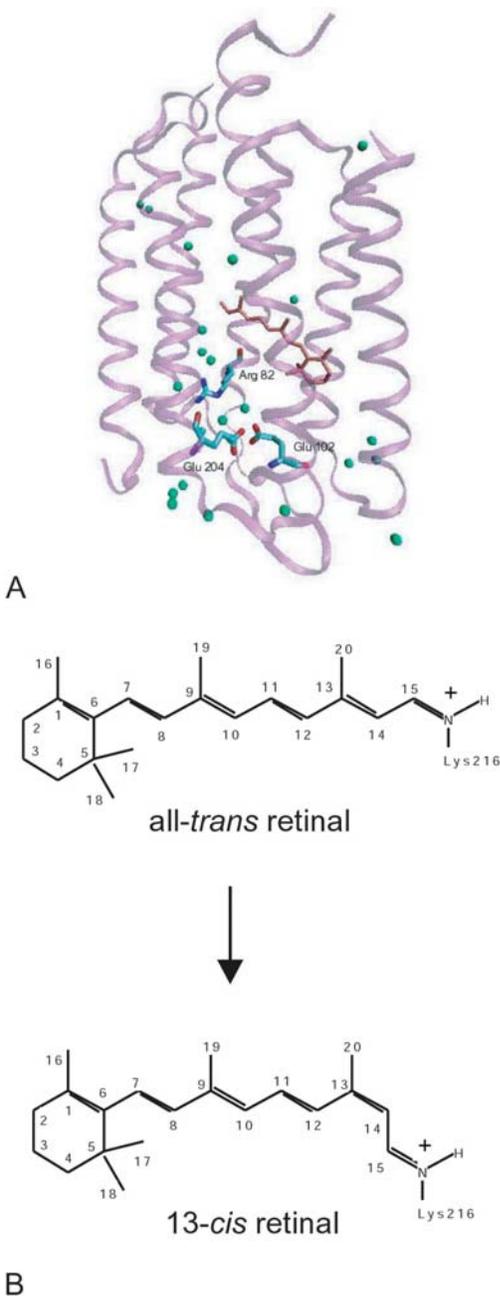
Bacteriorhodopsin (bR), a photoactive trans-membrane protein, is a light driven pump which transports protons across the cell membrane of the halophilic organism *Halobacterium salinarum* (113,114). bR has some features which render an investigation (113-121) more attractive than that of rhodopsin: The extraction from bacteria is much simpler and it seems more suitable for technical applications. It is the best understood ion transport protein and has become a paradigm for membrane proteins in general and transporters in particular (116). bR contains seven trans-membrane helices that enclose a cavity in which a retinal is contained (Figure 6A). Much like in rhodopsin, the retinal moiety which acts as the chromophore, is covalently bound via a Schiff base linkage to the Lys-216 residue. However, in dark state of bacteriorhodopsin the chromophore is not in the 11-*cis*, but in the all-*trans* form.

Absorption of light energy leads to an isomerization of the retinal chromophore from the all-*trans* to the 13-*cis* configuration (Figure 6B), which induces a series of further conformational changes in bR. The

resulting photocycle intermediates are characterized as the sequence of K, L, M, N and O. All steps are well characterized by spectroscopic methods (113,114). From the last intermediate, bR returns to the original conformation, completing the photocycle (115). In the transition from the L to M intermediate, bR releases a proton into the extracellular space, and in the subsequent transition from the M to the N state, a proton is taken up from the cytoplasm. The uptake and release of protons increase the trans-membrane electrochemical H<sup>+</sup> gradient (proton-motive force), converting light energy into biological processes, like ATP production and the maintenance of iso-osmolarity of the cytoplasm during cell growth. Most of the structures involved have been determined with crystallographic methods (117,119).

It was proposed that water networks are involved in the vectorial proton translocation and release (120), contrary to the model that proposes that amino acid side chains are mainly responsible for the proton transport mechanism. Ground-state DFT-QM/MM dynamics simulations were applied to a model of bR in a solvated lipid bilayer membrane where the protonated water network within a hydrophilic pocket on the extracellular side of bR was treated as the QM part (120,121). Simulations of the photoreaction which induces the proton

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**Figure 6.** A) Structural representation of bacteriorhodopsin showing the protonated network consisting of water molecules (represented by green spheres), stabilized by Glu 194, Glu 204, and Arg 82. The structure of bacteriorhodopsin consists for the most part of seven trans-membrane helices. There is a polar channel through the center of the structure that contains a retinal prosthetic group (in red). B) *trans-cis* isomerization of the chromophore. The retinal is covalently attached to Lys216 via a Schiff base linkage.

translocation are not yet available. Simulating the photoreaction of bR and comparison to the rhodopsin simulations would be particularly interesting, as the chromophores isomerize at different positions within the conjugated chains which must be due to the protein environment. Comparison of the X-ray structures shows that in both proteins the counterions are close to the respective isomerizing bond, which suggests that this

interaction with the protein environment determines which bonds are isomerized in a longer conjugated chain.

## 5. PERSPECTIVE

The simulations of chemical reactions in biological systems represent a big challenge to theoretical approaches. The need for an accurate quantum mechanical

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description of the electronic structure combined with the description of the nuclear dynamics at finite temperature leads to enormous requirements concerning computer time. Nevertheless it is nowadays feasible to do Car-Parrinello simulations for systems of about 1000 atoms (122).

Particularly fascinating but even more difficult than ground state dynamics is the investigation of excited state motion of large molecular systems. The first successful unconstrained simulations of ultrafast photoreactions in biological systems have been published in recent years and there is more to come. Proceeding in this direction, however, will afford further developments not only concerning efficiency but also concerning accuracy.

## 6. ACKNOWLEDGMENT

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**Abbreviations:** QM: quantum mechanics; DFT: density functional theory; CPMD: Car Parrinello molecular dynamics; QM/MM: quantum mechanics/molecular modeling; CIS: configuration interaction singles; CASSCF: complete active space self-consistent field theory; CASPT2: complete active space perturbation theory, second order; MRD-CI: multi-reference (doubles) configuration interaction; TDDFT: time-dependent density functional theory; ROKS: restricted open-shell Kohn-Sham; ROHF: restricted open-shell Hartree-Fock; HOMO: highest occupied molecular orbital; LUMO: lowest occupied molecular orbital; MCSCF: multi-configuration self-consistent field; PYP: photoactive yellow protein; GFP: green fluorescent protein; GPCR: G protein-coupled receptor; IFP: intrinsically fluorescent protein; bR: bacteriorhodopsin

**Key Words:** Density Functional Calculations, CPMD, QM/MM, Photoactive Proteins, Fluorescent Proteins, Reaction mechanisms, Review

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