Recent Applications of a QM/MM scheme at the CASPT2//CASSCF/AMBER (or CHARMM) level of theory in Photochemistry and Photobiology

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Abstract. The excited-state properties of chemically different chromophores embedded in diverse protein environments or in solution can be nowadays correctly evaluated by means of a hybrid quantum mechanics/molecular mechanics (QM/MM) computational strategy based on multiconfigurational perturbation theory and complete-active-space-self-consistent-field geometry optimization. In particular, in this article we show how a QM/MM strategy has been recently developed in our laboratory and has been successfully applied to the investigation of the fluorescence of the green fluorescent protein (GFP) and how the same strategy (embedding the chromophores in methanol solution) has been combined with retrosynthetic analysis to design a prototype light-driven Z/E molecular switch featuring a single reactive double bond and the same electronic structure and photoisomerization mechanism of the chromophore of the visual pigment Rhodopsin.

1. Introduction

The study of photochemical problems by means of computer simulations using specialized software tools and strategies enable us to get an understanding at the microscopic level of what happens to a molecule after absorption of a photon. Two molecular events may follow light energy absorption: energy wastage or energy exploitation (figure 1). Technology often requires molecules where this energy is not wasted but exploited to achieve specific chemical, conformational and electronic changes. In contrast, other applications, as those in the field of photoprotection or photostability, need molecules that eliminate the stored photon energy (i.e. be structurally unaffected by light absorption) efficiently via emission and/or internal conversion.

A detailed understanding of the properties of electronically excited state species and the knowledge of the molecular mechanisms, which control the fate of the energy deposited on a molecule after absorption, increase our ability to design efficient photochemical reactions and artificial photosynthetic systems. In this context, the comprehension of the mechanisms that lead to photodegradation processes is essential in order to suggest new methods of photostabilization that one might implement in the attempt of improving the photoresistance of photolabile materials. Furthermore, this represents a fundamental requirement for the rational design of novel materials, molecular devices and molecular level machines. On a more general ground, the ability to simulate,
using complementary computational strategies, photoinduced events often allow exploring areas of chemistry that experiment could touch only indirectly.

In this respect, during the last few years, computational methods have been successful applied to explore photon energy wastage mechanisms (e.g. in fluorescent probes) [1-3] and the mechanism of fast internal conversion in the DNA basis [4-6]. Similarly, as an example of process where light is exploited to drive stereospecific photochemical reactions, we can recall the ultrafast pericyclic reactions [7]. The same types of processes can be found in photobiology. For instance, there are fluorescent proteins, such as the Green Fluorescent Protein (GFP), where the energy of the photon is “wasted” radiatively to produce fluorescence while there are other proteins, such as the visual pigment Rhodopsin, where the energy of the photon is exploited to produce a change in the protein conformation.

![Figure 1. Fate of light Energy at the Molecular Level.](image)

2. Computational Investigation of Reaction Mechanisms in Photochemistry and Photobiology

2.1. Photochemical Reaction Path

A suitable approach to mechanistic problems in photochemistry and photobiology involves the computation of the photochemical reaction path. As illustrated in figure 2, once species A is promoted to the excited state, it starts to evolve on the corresponding potential energy surface. As a consequence of such relaxation, that may involve the overcoming of a transition state (TS), the species reaches a point of conical intersection (CI) and decays. The whole process can be described by computing a Minimum Energy Path (MEP) starting at the Franck Condon (FC) point (i.e., at the ground state equilibrium structure) and ending at the CI.

The CI provides a key mechanistic entity for the description of a photochemical reaction and it can be seen as a very efficient channel for the decay to the ground state. In the past, it has been referred as
the “photochemical funnel” [8-10]. It corresponds to a molecular structure that lives for only few femtoseconds (10^{-15} seconds) and plays, in photochemistry, the role of the transition state of a thermal process. For a complete description of the reaction we need to compute also the MEP on the ground state that describes photoprodut formation. In figure 2 we show that the entire photochemical reaction path is defined and computed in terms of a set of connected MEPs. In particular, the path starting at the FC (structure A*) on the potential energy surface of the spectroscopic excited state and ending at the photoprodut energy minimum B located on the ground state energy surface is constructed by joining two MEPs. The first MEP (grey arrows) connects the FC point to the conical intersection (A→CI). The second MEP (black arrows) connects the conical intersection to the photoprodut (CI→Photoprodut B). One can also compute a third MEP that starts at CI and describes the reactant reconstitution process (CI→A) responsible for partial return of the photoexcited species to the original ground state minimum. This mechanistic scheme is very general.

![Figure 2. Model intersecting the ground (S0) and the excited state (S1) potential energy surfaces. The Franck-Condon point (A*) is geometrically identical to the minimum on the ground state, but located on the S1 surface. The arrows indicate the direction of the minimum energy path connecting the FC point (A*) to the conical intersection (CI) and then to A and the photoprodut B.](image)

The characterization of the molecular structure and relative stability of the “photochemical funnel” in terms of CI (or singlet/triplet crossings) is of central importance in mechanistic photochemistry. Nowadays, computational strategies are available for locating crossing points and for constructing *inter-state* “photochemical” reaction pathways. These tools comprise methodologies for the optimization of low-lying crossings between pair of potential energy surfaces and the computation of relaxation paths from a photoexcited reactant (e.g. from the Franck-Condon (FC) structure) to a deactivation channel. More in general, it is possible to compute the entire pathway connecting an excited state molecule to its ground state product.
2.2. *Ab Initio* Quantum Chemical methods for Excited States

When approaching the study of a photochemical reaction the first problem to be solved is that of computing the excited state energy at a level of accuracy that, hopefully, allows the comparison with spectral data.

Among the possible types of quantum chemical technologies (e.g. semiempirical, DFT and *ab initio*) available to compute the potential energy surface of an excited state molecule, we adopt the *ab initio* CASPT2//CASSCF approach. In this computational method the full reaction coordinate is computed at the CASSCF (Complete Active Space - Self Consistent Field) [11] level of theory while the associated energy profile (determining the reaction energetics) is re-evaluated along a selected series of reaction coordinate points by performing single-point CASPT2 (i.e. an implementation of second-order multireference perturbation theory) [12,13] computations. The CASPT2 correction ensures a proper treatment of the dynamic electron correlation in the wavefunction (note that CASPT2 geometry optimizations are presently not feasible for medium to large size organic and bio-organic chromophores).

In summary, this is considered a “practical” compromise between computational cost and computational accuracy especially when comparison with observed spectroscopic quantities and reaction barriers are needed. Over the last years the CASPT2//CASSCF methodology has been successfully employed to investigate the mechanism of different photophysical and photochemical processes in diverse organic chromophores [9,10,14].

In the following we will show that these methodologies have been also employed to study the molecular mechanism of photochemical processes occurring in photobiological systems.

2.3. The Quantum Mechanics/Molecular Mechanics (QM/MM) strategy in Siena

Very recently a QM/MM computational method based on the use of an *ab initio* CASPT2//CASSCF/6-31G* strategy (i.e. geometry optimization at the CASSCF level and energy evaluation at the CASPT2 level) coupled with a protein force field such as AMBER (or CHARMM) has been implemented in our laboratory. Briefly, the method is based on a hydrogen link-atom scheme [15] where the MM and QM segments interact in the following way: (i) the QM electrons and the full set of MM point charges interact via the one-electron operator, (ii) stretching, bending and torsional potentials involving at least one MM atom are described by the MM potential (iii) QM and MM atom pairs separated by more then two bonds interact via either standard or re-parametrized van der Waals potentials.

The method has been first validated computing the excited state properties of Rhodopsin, including reaction path mapping and conical intersection search [16-19]. The model used for the Rhodopsin computations is shown in figure 3 and special care has been taken in the parametrization of the protonated Schiff base linkage region that describes the delicate border region between the MM (the protein) and QM (the chromophore) subunits. The frontier is placed at the \( \text{C}_\delta\text{C}_\varepsilon \) bond of the Lys296 side chain.
Figure 3. QM/MM model of Rhodopsin. The QM part consists of the chromophore, the retinal, while the MM part is the rest of the protein.

Comparing the CASPT2//CASSCF/AMBER vertical excitation energy with the experimental values for Rhodopsin we found that the absorption maximum is reproduced with only 3 kcal mol$^{-1}$ error (476 nm vs. 498 nm) while for the solution the error is only 1 kcal mol$^{-1}$ (433 nm vs. 442 nm). These results confirm the quality of our approach and allow reproducing the so-called “opsin-shift” (the 445 nm $\lambda_{\text{max}}$ observed for PSB11 in methanol is red-shifted to 498 nm in Rhodopsin) with a 2 kcal mol$^{-1}$ error. Furthermore the method allowed for the semi-quantitative analysis of the factor determining the properties of the protein environment. Thus we demonstrated that the strategy could be correctly applied to the investigation of excited states with a computational error $<5$ kcal mol$^{-1}$.

Following these positive results we have applied the same QM/MM methodology for the investigation of the origin of the fluorescence in green fluorescent proteins [20] that feature an anionic benzylidene-imidazolone chromophore (note that the retinal is a cation) and for the design of a new photochemical biomimetic switch [21].

3. The QM/MM strategy for biological and biomimetic chromophores.

3.1. The Green Fluorescent Protein (GFP)

The first problem we discuss regards the investigation of the excited state properties of the green fluorescent protein (GFP), a protein that can waste light energy with great efficiency through fluorescence and that features an anionic benzylidene-imidazolone chromophore. The importance of GFP is related to its use as marker of gene expression, as biosensors or to the possibility to use it in the photochemical memories.

The GFP has been found in several bioluminescent organisms, like some kind of jellyfish and sea pansy of the North Pacific. The Wild-type GFP consists of a rigid $\beta$-sheet-based structure with the chromophore located at the centre of a barrel-like protein backbone (see figure 4). The prosthetic group that is responsible for the green fluorescence is the anionic structure (of a $p$-hydroxybenzilideneimidazolone (HBDI)), which is coupled to an Arg cation as counterion.
The fluorophore is excited by UV light and fluoresces with an 80% quantum yield. The excited-state lifetime of the GFP chromophore is very long in the protein (ca. 3 ns), but much shorter (less than 0.3 ps) in solution and also in vacuo. The absorption maxima of the fluorophore are also very environment-dependent. In fact, in protein, it is red-shifted compared to the solution but even the gas-phase. In fact, while the absorption of HBDI in solution is 426 nm, GFP has a much red-shifted (495 nm) absorption maximum.

In order to perform an analysis of the factors that cause the different environmental dependent properties and to determine the nature of the wt-GFP emitting domain, we employed our QM/MM computations to model the ground ($S_0$) and first singlet excited ($S_1$) states of the chromophore in the protein, in water and we compared the results with our previous calculations in vacuo and with the experiments (in fact, the GFP chromophore is a particular lucky case study since the gas-phase spectra of its fluorophore are available and one can effort a direct comparison with the experiment in different environments (gas-phase, solution, protein matrix) [20]. These comparisons are reported in Table 1. The computed absorption maxima for all models compare well with the observed quantities, in fact are reproduced within few kcal mol$^{-1}$. Furthermore, the absorption maxima simulation correctly reproduces the closeness of the gas-phase and protein absorption maxima. Since our QM/MM method allows for geometry optimization on the excited state we can also predict the emission maxima. The results indicate that the protein/gas-phase similarity also holds for the emission.

|          | $\lambda_{\text{max}}^a$ (nm) | $\lambda_{\text{max}}^f$ (nm) |
|----------|-------------------------------|-------------------------------|
| **Protein** | Computed: 495  | Experimental: 468 |
|          | Computed: 508  | Experimental: 507    |
| **Solution** | Computed: 426  | Experimental: 434 |
|          | Computed: 480  | Experimental: 469    |
| **Gas-Phase** | Computed: 479  | Experimental: 465 |
|          |                 | Computed: 507          |
|          |                 | Experimental: —        |
The fact that the protein absorption and emission maxima are substantially closer to the gas-phase values rather than to the solution ones suggests the idea that the GFP protein cavity offers an environment more similar to the gas phase than to the solution. The analysis of the optimized geometries indicates that the emitter corresponds to a slightly perturbed H-bonded chromophore---H$_2$O pair. The rest of the protein seems to be designed in such a way to mimic the gas-phase environment. This is confirmed by the computed values of the absorption (470 nm) and emission (502 nm) $\lambda_{\text{max}}$ of the HBDI plus the water molecule taken with its geometry found in the protein that are very similar to the ones obtained with the full protein. Furthermore in a configuration where such interaction has been disrupted, the computed emission maximum becomes less closed to the experimental value. This again demonstrates the importance of the bonding to the water molecule.

To further support this hypothesis we compared the gas-phase structure of HBDI with a structure where the chromophore is hydrogen bonded to a single H$_2$O molecule and we found a change in the equilibrium structure (see figure 5). More specifically, this becomes remarkably similar to that found in the protein. Thus our results demonstrate that, in the protein, the other residues (even the positively charged Arginine 96 counterion) do not perturb much the electronic and molecular structure of the emitter. This can only be explained admitting that the charges distributed in the various parts of the protein cavity counterbalance each other in both the $S_0$ and $S_1$ states.

![Figure 5. CASSCF/MM structures of HBDI in different environments: a) GFP, b) water solution, c) in vacuo, without (left side) and with (right side) a hydrogen bond to a H$_2$O molecule.](image)

Regarding the difference in excited state lifetime of the chromophore in vacuo (or in solution) compared to the protein, the mechanistic idea is that the decay is due to a $Z/E$ isomerization, but while in solution, the fluorophore basically undergoes an ultrafast internal conversion, the protein should act by restraining the isomerization. We have performed very preliminary calculations of two paths on the excited state potential energy surface starting at the minimum found on the excited state and rotating around the two central bond. We have found that there is a minimum on this surface and that the presence of the barrier do not allow a rapid relaxation on $S_0$ of the fluorophore but restrain it on the excited state. This is not the case of the vacuo calculations where no barrier has been found for both paths.
In conclusion, the CASPT2/CASSCF/6-31G*/MM computations can be used to study the structure and spectroscopy of GFPs but can be also used to compute the minimum reaction path inside the protein.

3.2. The design of a new class of bio-mimetic and photochemical molecular switches

Here we show how the combined use of our QM/MM strategy with retrosynthetic analysis and spectroscopic characterization has been very useful and successful for the design of a completely new class of biomimetic and photochemical molecular switches featuring the same photoisomerization mechanism of the chromophore of the visual pigment Rhodopsin [21].

Molecular switches act as mechanical systems that exploit chemical, electrical or radiative energy to achieve a well defined molecular structure deformation. The simplest examples of “molecular motors” are single molecules undergoing a Z->E (or E->Z) double bond isomerization immediately followed by an E->Z (or Z->E) isomerization closing a rotation cycle. In these systems a strict requirement for achieving the status of « motor » is that the Z/E change must ultimately occur only in one torsional direction (i.e. clockwise or counterclockwise).

Among the designed molecules here we present the results of this synthetically accessible molecule, the indanylidene pyrroline switch (IndPyr, top of figure 6), which combines the electronic structure and photoisomerization mechanism of the protonated Schiff base of retinal (PSB11) (see bottom of figure 6), the chromophore of Rhodopsin proteins, which undergoes an ultrafast photoinduced Z/E isomerization with a very high quantum yield (67%) in the protein and the locked skeleton of Diarylidenes (DA) (see bottom of figure 6) that are molecules able to rotate around a single isomerizable bond upon irradiation. They are a class of well-know light-driven molecular rotors. Thus we hope to emulate nature in the design of this new class of potentially very efficient biomimetic molecular rotor.

![Figure 6. The target and synthesized molecular switch compared to the molecular structure of the biomimetic rotors diarylidenes (DA) and the chromophore of Rhodopsin proteins (PSB11).](image)

The target and synthesized molecule (see the synthetic strategy in figure 7) has been characterized by means of spectroscopy and the results have been compared with our calculations of the spectra in methanol solution (neutralizing the solution with a chlorine counterion).
Figure 7. The synthetic strategy used to obtain the molecular switch IndPyr.

With this strategy we have been able to reproduce the absorption maxima of the molecule with a difference compared to the experimental ones of less than 5 kcal mol⁻¹ (see figure 8). In particular we have been able to assign the more intense band to the transition towards the first singlet excited state. The less intense band is assigned to the transition to the third excited state while the transition between ground state and the second excited state is weaker and the band is probably hidden below the shoulder near 300 nm. The values of the oscillator strength (f = 0.55 for the $S_0 \rightarrow S_1$ transition, f = 0.07 for the $S_0 \rightarrow S_3$ transition, and f = 0.06 for the $S_0 \rightarrow S_2$ transition) are qualitatively consistent with the observed absorbance pattern.

Figure 8. Room temperature absorption spectra of IndPyr in methanol. Computed values of absorption maxima are shown in parenthesis.
The similarity between our model and the electronic structure of the retinal molecule is evident if we plot the computed charge distribution. In fact the data suggests that upon the vertical excitation, the switch undergoes a 30% of charge transfer through its reactive central double bond (that is, from the pyrroline to the indanone ring). This is closer in magnitude to the 34% of charge transfer seen for Rhodopsin.

![Figure 9. Excited state charge distribution changes for IndPyr (left side) compared to the ones for retinal (right side).](image)

We have computed, for both the Z→E and E→Z reactions, the photoisomerization path and we have found that, similar to Rhodopsin, the two reaction mechanisms feature a substantially barrierless path. In fact, upon excitation the cis species relaxes to an excited state structure featuring an inverted π-bond order and a twisted central bond. Furthermore, as in the case of Rhodopsin, a conical intersection is accessed, almost at half of the isomerization completed. The barrierless path suggests that the switch has a short excited-state lifetime and, thus, a very weak fluorescence.

In conclusion, by means of calculations we have demonstrated that such molecules act as potentially efficient photochemical switches and that the biomimetic molecule designed can be seen as a prototype of a new photochemical biomimetic switches. As future work we can envision to introduce chiral center in the structure to improve the efficiency of the molecule and move from a molecular switch to a molecular rotor. Furthermore we can introduce this molecules in the peptidic chain of cyclic oligopeptides in order to control their motion thus to photomodulate their conformation.

4. Conclusions

We hope we have provided some evidence that it is, nowadays, possible to investigate, at a semi-quantitative level, photobiological problems using computational tools. In particular the use of the \textit{ab initio} CASPT2/CASSCF computational strategy hybridized with molecular mechanics force fields can be used to investigate both biological photoresponsive molecules and to design new bio-materials. We have been able to perform the modeling of the photochemical reaction path of a completely new molecule, potentially a molecular motor, inside the real environment of the reaction that is in solution. This strategy can be extended to the study of macromolecules and supramolecular assembly and to the investigation of radiation damage in biological materials.
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