UNDERSTANDING THE RELATIONSHIP BETWEEN THERMAL AND PHOTOCHEMICAL ISOMERIZATION IN VISUAL RECEPTORS

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ABSTRACT

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Visual sensitivity in vertebrates depends on the photochemical and thermal processes occurring in visual pigments of the retina. Indeed, the efficiency of the primary event in vision, the cis-trans photoinduced isomerization of the chromophore embedded inside visual pigments, must be high to achieve such sensitivity. Meanwhile, thermal processes are responsible for generating spontaneous electrical signals even in the absence of light, thus imposing a limit on visual sensitivity. While the photochemical process in visual pigments has been extensively studied both experimentally and computationally, there are fewer studies targeting the thermal process, and such studies are often conflicting. However, the observation of a relationship between the maximum absorption wavelength ($\lambda_{\text{max}}$), a photochemical property, and the activation kinetic constant ($k$), a thermal property, of visual pigments suggests that the thermal and photochemical processes are related.

The understanding of these processes has been largely aided by computational studies. Indeed, computer simulations can be used to investigate certain features of the potential energy surfaces (PESs) driving these processes. However, a quantitative (or even qualitative) description of these PESs requires computational methods capable of correctly describing the ground ($S_0$) and excited ($S_1$) state regions of concern. Therefore, in the following, we first employ a reduced model of the chromophore of visual pigments
(the penta-2,4-dieniminium cation) to design a stringent test of the performance of quantum mechanical methods along regions of interest on the $S_0$ and $S_1$ PESs. We then select a suitable method and employ it in a full hybrid quantum mechanical / molecular mechanical model of the prototypal bovine rod pigment, rhodopsin, to provide a molecular-level understanding of the thermal process in such pigments and its relation to the photochemical isomerization mechanism. We find that the transition state mediating thermal activation has the same electronic structure as the photoreceptor excited state, thus creating a direct link between $\lambda_{\text{max}}$ and $k$. Such a link appears to be the manifestation of intrinsic chromophore features associated with the existence of a conical intersection between its ground and excited states.
To my parents, Rania and Samir

I will never forget all that you have done for me
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CHAPTER I: INTRODUCTION

Vision in vertebrates begins with light interacting with visual pigments found in photoreceptor cells lining the retina of the eye. These visual pigments, rhodopsins, are characterized by a protein structure comprising seven transmembrane helices and that typically host a positively charged and conjugated organic chromophore, the retinal protonated Schiff base (rPSB), which is covalently linked to a lysine side chain (Figure 1.1).¹

**Figure 1.1.** The protein structure of a prototypal rhodopsin (in green) with the retinal protonated Schiff base (rPSB) chromophore embedded within (in red). rPSB in the dark state typically exists in the 11-*cis* conformation (in visual rhodopsins) or the all-*trans* conformation (in microbial rhodopsins). The structure of both conformers is shown on the right. The numbers typically used to refer to some of the backbone atoms of rPSB are displayed on the 11-*cis*-rPSB structure.
Rhodopsins in nature are by no means exclusive to the retina of vertebrates, nor their functions exclusive to vision. For instance, bacterial (or microbial) rhodopsins serve a wider variety of functions, including phototaxis, energy storage, development, and retinal biosynthesis. Among those microbial rhodopsins (Figure 1.2) are bacteriorhodopsin (bR, a proton pump), halorhodopsin (NpHR, a chloride ion pump\(^3\)), channelrhodopsin (ChR, a light-gated ion channel\(^4\)), and sensory rhodopsins (SRs, typically transducin activators).\(^5\) The properties of such microbial rhodopsins (i.e. their ability to act as light-gated ion pumps or proton pumps) have made them very valuable to the growing field of optogenetics.\(^6\) In this field, a light-gated ion channel (most commonly, ChR or NpHR) is delivered into neurons, allowing the activation or silencing of the neuron by light with high spatio-temporal resolution.\(^7\) Optogenetics techniques have allowed scientists to understand neural activity and its relationship with certain behaviors in living creatures, or even control it.\(^8,9\)

Visual and microbial rhodopsins are part of an even bigger family of proteins called retinylidene proteins, or opsins, which comprise several subfamilies.\(^10\) Remarkably, this family of proteins have functions ranging from regulating circadian rhythms\(^11\) to controlling anxiety.\(^12,13\) Even members of the visual rhodopsin subfamily serve a wide range of functions. The Mantis Shrimp has more than 16 different types of photoreceptors\(^14,15\) which allow it to perceive polarized light and multispectral images (Figure 1.3).\(^16\) Moreover, recent studies have found that rhodopsins may be involved in temperature sensing in the fruit fly Drosophila,\(^17\) and even in the auditory function (i.e. hearing).\(^18,19\)
Figure 1.2. Some examples of microbial rhodopsins and their various functions. This figure is adapted from ref. 5

![Diagram of microbial rhodopsins](image)

Figure 1.3. A photo of the fruit fly *Drosophila* (left) and the mantis shrimp *Odontodactylus scyllarus* (right). Rhodopsins in Drosophila have been found to be involved in functions such as temperature sensing and hearing. The mantis shrimp may have up to 16 different types of rhodopsins. The two photos are adapted from the following sources, respectively:

http://www.npr.org/2012/02/21/147207785/cheers-fruit-flies-drink-to-their-health-literally

http://planetanimalzone.blogspot.com/2012/05/mantis-shrimp-amazing-beautiful.html
In microbial rhodopsins, the rPSB chromophore is typically in the all-\textit{trans} conformation and isomerizes to the 13-\textit{cis} conformer upon photoexcitation. The back-isomerization could then occur thermally (as in bR)\textsuperscript{20} or photochemically in photochromic systems (as in Anabaena Sensory Rhodopsin, ASR).\textsuperscript{21} Visual pigments, on the other hand, typically have the 11-\textit{cis} form of rPSB which photochemically isomerizes to all-\textit{trans}. Arguably, one of the most studied members of the opsin family is the prototypal bovine Rhodopsin (Rh), a G-protein coupled receptor found in the rod photoreceptors of \textit{Bos taurus}. Indeed, there are many experimental and theoretical studies on this particular pigment. This is partly due to the fact that Rh is one of the few opsins for which x-ray crystal structures exist.\textsuperscript{10,22,23} Such studies (experimental studies started since the discovery of rhodopsins in the latter half of the 19th century,\textsuperscript{24} while theoretical studies were made possible in the 1970’s\textsuperscript{25,26}) have gradually revealed an intriguing story about one of the most efficient photoactive proteins in nature.

When light shines on Rh, it causes an electronic transition in the opsin-embedded rPSB from the ground state (S\textsubscript{0}) to the first electronic excited state (S\textsubscript{1}). This transition then triggers the isomerization of 11-\textit{cis}-rPSB to all-\textit{trans}, eventually leading to the formation of the metastable intermediate bathorhodopsin (bathoRh).\textsuperscript{27} This photochemical isomerization is sometimes referred to as the primary process in vision. BathoRh can then thermally decay through a series of intermediates (Figure 1.4) in a process that ultimately causes an electrical signal to be generated and sent to the brain, signaling that light has been detected thus allowing vision.
Ultrafast spectroscopic measurements have shown that the cis-trans isomerization of rPSB occurs on a femtosecond timescale.\(^{27,29,30}\) An observation of \(S_0\) vibrational coherence,\(^ {31}\) an ultrafast infrared spectroscopy study,\(^ {32}\) as well as hybrid quantum mechanical / molecular mechanical (QM/MM) studies\(^ {32-34}\) all support a mechanism which involves direct transfer of the excited-state (\(S_1\)) population to the photoproduct along a downhill path passing through a conical intersection (CI), an entity that will be introduced and discussed in Chapter II. Computational QM/MM studies have found that isomerization of the rPSB chromophore inside the opsin cavity occurs via an aborted bicycle-pedal space saving mechanism.\(^ {26,35}\)

The primary photochemical event in vision has been extensively studied not only due to its biological and medical importance (i.e. its role in vision), but also due to its high efficiency, which is important for understanding the physical limits of light perception and visual sensitivity. On the other hand, another important factor that limits visual sensitivity is so-called “thermal
noise” evolving from signals triggered even in the absence of light. While the origin of such signals is unknown, evidence points to the culprit being spontaneous thermal isomerizations of rPSB in rhodopsins. Signals generated by such thermal processes are indistinguishable from those caused by light, and while such events are rare in a single rhodopsin (occurring once every ca. 400 years), the fact that there are many rhodopsins in the retina of the eye (e.g. in humans, there are approximately 125 million rod cells in the retina, each containing around 2 billion Rh pigments) means that these signals are continuously being generated in the retina. This creates a background noise that is caused by body heat rather than light against which photoinduced signals must compete to be recognized by the brain. Thermal noise is one of the major factors reducing the ability to see in dim light. It is also one of the reasons why cone cells are generally much less sensitive than rod cells.

Neurobiological studies have found that a relationship exists between some features of the thermal isomerization and photochemical properties of rPSB. In particular, these studies indicate that there is a correlation between the maximum absorption wavelength of rPSB ($\lambda_{\text{max}}$) and the thermal activation kinetic constant ($k$) of different visual pigments. Such a relationship was predicted by the British neuroscientist Horace G. Barlow in 1957, and we therefore refer to it as the Barlow correlation throughout this work. Luo et al. have attempted to explain the Barlow correlation by suggesting that both the thermal and photochemical processes must go through the same ground-state isomerization barrier. However, this explanation is unlikely in the light of the well-established CI-driven photochemical mechanism which occurs on a femtosecond timescale.
In the following, we employ computer models of the visual pigment Rh to study its mechanism. These models, if accurate, would in principle allow us to map the ground and first excited state potential energy surfaces (PESs) driving both the thermal and photochemical reactions, respectively. In the following chapters, we ultimately attempt to provide a molecular-level understanding of the thermal isomerization mechanism in Rh, and to provide a link with the maximum absorption wavelength. First, in Chapter II, we introduce and explain some of the terminology and concepts important for the understanding of this work. In Chapter III, we investigate the performance of different quantum mechanical methods on certain ground state paths of a reduced model of rPSB. In Chapter IV, we use the same reduced model to map regions of the excited state PES. The purpose of Chapters III and IV is to determine which methods are able to quantitatively describe both the thermal and photochemical properties of a Rh model. After than, in Chapter V, we present an investigation of the thermal noise in Rh.
References:


2.1. Introduction

In this chapter, we revise some of the terms and concepts important for the understanding of this work. A brief description of some of the computational methods used in this work is also presented.

2.2. Potential energy surfaces

A potential energy surface (PES) describes the way the potential energy of a system varies with changes in its nuclear coordinates. The simplest PES would be one-dimensional with the energy plotted against a change on single coordinate (See Figure 2.1A). Diatomic systems are an example of systems where a one-dimensional PES is sufficient to describe the chemistry of the system, since such systems only have one vibrational mode (bond stretching). However, the complexity of these surfaces grows with the number of atoms involved. In fact, the dimensionality of an entire PES is determined by the vibrational number of degrees of freedom, which is 3N-5 for linear systems and 3N-6 for non-linear systems and where N is the number of atoms. Therefore, polyatomic systems have a multidimensional PES which cannot be represented in a single figure. A PES of a polyatomic system can only be represented along select
coordinates, either in one dimension (Figure 2.1A) or in two dimensions (Figure 2.1B). The selection of these coordinates may lead to completely different surface shapes which impart different narratives.

Figure 2.1. A schematic representation of a PES in one dimension (A) and two-dimensions (B) along certain nuclear coordinates ($q_1, q_2$)

Each electronic state in a system has an associated PES. Ground state chemistry (e.g. thermal chemistry) normally occurs entirely on a single PES (but the ground state PES may be strongly influenced by the presence of higher states; an example of such a case will be presented in later chapters). In single-PES processes, reactivity can typically be understood by locating certain stationary points, namely minima and saddle points, on the PES (see Section 2.3). However, an understanding of processes involving more than one state (e.g. photochemical and photophysical processes) may also require an understanding of avoided crossings and conical intersections (see Section 2.4)
2.3. Stationary points: Minima and saddle points

Stationary points are rigorously defined as points where the first derivative is zero. With regards to a PES, a stationary point is a point where the gradient of the energy with respect to a nuclear coordinate at such a point is zero. A **minimum** (Figure 2.2A) is a stationary point where no further decrease in energy is possible upon infinitesimal changes in any nuclear coordinate (i.e. it is a minimum in all dimensions of the PES). A **saddle point**, on the other hand, is a minimum in some dimensions and a maximum in others (Figure 2.2B). A **first order saddle point** is a maximum in only one dimension but a minimum in all others. To assign chemical meaning to these terms, we note that minima on the PES represent equilibrium structures (reactants, intermediates, or products), whereas first order saddle points represent transition states.

![Figure 2.2. Schematic representation of a minimum (A) and a saddle point (B).](image)
2.4. Conical intersections and avoided crossings

Here we look at how different PESs (i.e. PESs corresponding to different states) may interact. In a diatomic system, PESs of electronic states of the same symmetry cannot cross. This non-crossing rule was formulated in 1929 by von Neumann and Wigner, although at the time was thought to be of more general validity for all systems.\(^1\) The reasoning behind the non-crossing rule follows. An electronic state can be described by an adiabatic wavefunction, which consists of diabatic components. Here, for the sake of simplicity, we will consider only two states with two diabatic components:

\[
\Psi = c_1 \phi_2 + c_2 \phi_1
\]

The diabatic components (\(\phi_1\) and \(\phi_2\)) only describe the energy of a particular spin-coupling, whereas the adiabatic functions (\(\Psi\)) are what ultimately determine the energy for each state. We can represent the equation above as a secular equation:

\[
\begin{bmatrix}
H_{11} - E & H_{12} \\
H_{21} & H_{22} - E
\end{bmatrix}
\begin{bmatrix}
c_1 \\
c_2
\end{bmatrix}
= 0
\]

In order to obtain a degenerate solution (\(E_1=E_2\)), it is necessary that both of these conditions are satisfied:

\[
H_{11} = H_{22} \\
H_{12} = H_{21} = 0
\]
However, simultaneously satisfying the two conditions above requires at least two independently variable nuclear coordinates, as explained in ref. 2. Therefore, it is impossible to satisfy these conditions in a diatomic system, where there is only one vibrational mode. In these cases (see Figure 2.3A), it is possible for the diabatic states to cross, but the adiabatic states would be repulsive, leading to an avoided crossing (the point of nearest approach of the two surfaces).

Figure 2.3. A. Schematic representation of an avoided crossing along a one-dimensional PES. $\Psi_1$ and $\Psi_2$ represent the wavefunction of the two adiabatic states (which represent the real surfaces of the two electronic states), while $\phi_1$ and $\phi_2$ represent the diabatic components of these states. B. Schematic representation of a conical intersection in a two dimensional representation of the PES corresponding to the branching plane. The gradient difference vector (GDV) and derivative coupling vector (DCV) correspond to the branching plane vectors and are the degeneracy-lifting modes. Green regions on the surfaces correspond to regions dominated by the $\phi_1$ electronic configuration while brown regions are dominated by $\phi_2$. 
In the case of polyatomic systems, it was found that crossing of potential energy surfaces is possible even if they have the same symmetry and spin. This is because the two conditions above could be satisfied by two independent coordinates, which allowing $E_1$ and $E_2$ to be equal. A point of degeneracy of two or more states is termed a conical intersection (CI). These two coordinates that allow the degeneracy have an important significance, since changes in these coordinates would lead to a splitting in the energy degeneracy (See Figure 2.3B). They are are termed the gradient difference vector (GDV) and derivative coupling vector (DCV), respectively, and are collectively referred to as the branching plane vectors (and the plane which they lie on is the branching plane). At a crossing of two states, all other nuclear coordinates that are orthogonal to the branching plane lie in the intersection subspace, and should have no effect on the energy degeneracy of these two states. Therefore, the intersection subspace is a subspace where every point is a CI. If one starts at a CI structure and follows a single coordinate orthogonal to the branching plane, he or she would be tracing a series of CIs along an intersection seam.

At an avoided crossing, the diabatic states from which the adiabatic states are constructed intersect. Therefore, at such a point, the adiabatic states change character (e.g. $\Psi_1$ changes character from mostly being described by $\phi_1$ to mostly being described by $\phi_2$ along the reaction coordinate of Figure 2.3A). Similarly, two electronic states exchange electronic character at a CI, as shown in Figure 2.3. 

At a CI, the degeneracy of the electronic states, in addition to the large changes in electronic character with respect to small changes in nuclear coordinates (i.e. the breakdown of
the Born-Oppenheimer approximation), allows for a high probability of non-adiabatic transitions between the degenerate states.\textsuperscript{6,7} Therefore, CI points, if accessible, could be very effective at funneling excited state populations from higher states to lower ones.

2.5. Minimum energy path calculations

In Chapters III and IV we run minimum energy path (MEP) calculations. Such calculations, as the name may imply, follow the path of steepest decent (or path of least resistance) along a PES, therefore effectively mapping a path where the energy is minimized at each step along the path. In a computer, this is done by taking a certain structure as an input and searching for a nearby structure with the lowest energy but with a constraint that the change in mass-weighted nuclear coordinates must equal a certain value (the step size). For instance, if a step size of 0.1 Å.amu\(^{1/2}\) is used (the units of amu are there to represent mass-weighted coordinates), the calculation searches for a minimum on a hypersphere (multidimensional sphere) on the PES with a radius of 0.1 Å.amu\(^{1/2}\). Once such a minimum is located, it is considered a minimum energy point and is used as a starting point for another constrained optimization. The calculations terminates once a further decrease in energy cannot be obtained. The series of minimum energy points map a path on the PES that is the MEP.
2.6. Computational methods: Post-SCF methods

The **self-consistent field** (SCF), or the **Hartree-Fock** (HF) method, is a computational method that treats the interaction of electrons of a system in a mean-field way. With this method, the ground state wavefunction is described with a single slater determinant:

\[
\Phi_0 = \frac{1}{(N!)^{\frac{1}{2}}} \left| \begin{array}{cccc}
\phi_1(1) & \phi_2(1) & \ldots & \phi_N(1) \\
\phi_1(2) & \phi_2(2) & \ldots & \phi_N(2) \\
\vdots & \vdots & \ddots & \vdots \\
\phi_1(N) & \phi_2(N) & \ldots & \phi_N(N)
\end{array} \right|
\]

where N is the total number of electrons in the system and \( \phi \) is a spin orbital. While the HF method may be accurate in describing certain regions of the ground state potential energy of a system, it lacks electron correlation and so cannot properly describe a PES were the extent of electron correlation changes along the surface. Electron correlation can be divided into **static electron correlation**, which describes long range effects arising from mixing of degenerate or near-degenerate states, and **dynamic electron correlation**, which describes short-range effects due to the instantaneous repulsion of electrons when they are close together.

In order to account for electron correlation, more advanced methods than HF (e.g. post-SCF methods) are required. **Configuration interaction** methods are an example of such post-SCF methods. Such methods allow for a linear combination of **configuration state functions** (CSFs), which themselves are symmetry-adapted linear combinations of Slater determinants that
are eigenfunctions of the squares of the angular momentum and spin operators, to describe the wavefunction of each state. For example, the ground state wavefunction may be described by:

\[ \Psi_0 = c_0 \Phi_0 + \sum_{i=1}^{\infty} c_i \Phi_i \]

where \( \Phi_0 \) is the HF configuration, \( \Phi_i \) are CSFs made up of configurations where one or more electrons are moved to virtual orbitals, and \( c_i \) is the coefficient for each CSF. The final wavefunction is obtained by varying the \( c_i \) variables. If all possible excitations are performed, accounting for all configurations within a certain basis set, this would be a full configuration interaction (FCI) calculation within that basis set. In theory, using FCI with an infinite basis set would allow us to achieve an exact solution of the wavefunction and energy. However, the computational cost of such calculations rises factorially with the size of the system and basis set due to the number of possible configurations. Therefore, FCI may typically only be achieved for very small systems, and more approximate methods are usually necessary. CIS (single excitations configuration interaction) and CISD (single and double excitation configuration interaction) are examples of methods where the number of configurations are limited to single or double excitations in order to reduce computational cost.

**Multiconfigurational SCF (MCSCF)** methods overcome part of the limitations of the methods above.\(^{11,12}\) With MCSCF, a linear combination of CSFs is also used to describe different electronic configurations (hence the name, multiconfigurational), but in addition to varying the coefficient of each configuration the molecular orbitals are also varied. MCSCF methods generally account well for static electron correlation, but usually not for dynamic electron
correlation. One of the most commonly used MCSCF methods is the complete-active-space SCF (CASSCF) method\textsuperscript{13} (indeed MCSCF is often used as a synonym for CASSCF). CASSCF is intermediate between HF (where there is only one Slater determinant and the molecular orbitals are varied) and FCI (where all possible configurations are included). It requires the user to choose an active space, a certain subset of orbitals and electrons for which all electronic configurations will be included in the description of the wavefunction (see Figure 2.4). All orbitals and electrons outside of the active space (inactive orbitals) are treated simply at the HF level of theory (i.e. no configurations except the ground state are considered), although these inactive orbitals are optimized together with the active space. This allows a certain level of flexibility since the user may choose an active space that includes all orbitals important for describing the chemical problem of interest (this means choosing the size of the active space and the nature of the orbitals to use). However, one main criticism of CASSCF is that it is not a black box method, and requires that the user have a good understanding of the chemical problem under investigation before even starting. Another main criticism is that it usually does not account for the dynamic electron correlation of electrons outside the active space. The CASSCF wavefunction may therefore be used as a zero-order wavefunction for subsequent multireference calculations discussed in the following section.
Figure 2.4. Schematic representation of the CASSCF wavefunction with an active space of six electrons and six orbitals. The CASSCF wavefunction in this case would include 175 Slater determinants corresponding to 175 different electronic configurations. The active space is highlighted in yellow, while inactive orbitals are grayed out.

2.7. Computational methods: Multireference methods

Multireference methods use a zero-order wavefunction that is multiconfigurational (e.g. CASSCF). One main advantage of such methods is that the reference wavefunction is already optimized for a certain state, and therefore the multireference method does not need to be too elaborate to obtain a quantitatively accurate description of a PES. One example of such a method is the multireference configuration interaction (MRCI) method.\textsuperscript{14} MRCI is a variational method that uses a certain subset of configurations (e.g. those obtained by CASSCF) and performs single excitations (MRCIS), double excitations (MRCISD), or higher excitations (e.g. as in MRCISDT, MRCISDTQ) out of and into the reference configurations (see Figure 2.5).
Figure 2.5. Schematic representation of the MRCI wavefunction employing the CASSCF wavefunction of Figure 2.4 as a reference. In classical (uncontracted) MRCI, excitations are performed out of and into the active space from all inactive orbitals for each of the reference CASSCF configuration state functions (here we use the second configuration of Figure 2.4 as an example of one such reference configurations). MRCISD would include both single and double excitations, as shown here.

Other well known multireference methods that use CASSCF as a zero-order wavefunction are the complete-active-space second order perturbation (MRPT2) methods. These methods account for correlation between active space and inactive electrons perturbatively using Møller–Plesset perturbation theory. While they are not as accurate as MRCI, these methods are much faster and therefore may be used for larger systems. Examples of such methods are CASPT2, NEVPT2, and XMCQDPT2, all of which are tested in Chapters III and IV.
2.8. Hybrid quantum mechanical / molecular mechanics models

In Chapter V, we use a hybrid quantum mechanical / molecular mechanical (QM/MM) model to study the chromophore of bovine rhodopsin embedded in its native environment, and to study the effect of changes in this environment.\textsuperscript{19} With the QM/MM protocol it is possible to separate a system into one part that is treated with an electronic structure method (the QM subsystem), while the other part is treated using parameterized classical mechanics (the MM subsystem). MM calculations are much cheaper than QM and allow for the simulation of thousands or up to millions of atoms.

The energy of such a QM/MM system can be described using the following equation:

\[ E_{\text{Total}} = E_{\text{QM}} + E_{\text{MM}} + E_{\text{QM/MM}} \]

The first two terms account for the contribution to the energy from the non-interacting QM and MM subsystems, while the third describes their interaction. The classification of QM/MM methods is based on the formulation of the \( E_{\text{QM/MM}} \) term. QM/MM schemes can be divided into two classes: mechanical embedding and electrostatic embedding. With mechanical embedding, the MM subsystem cannot polarize the QM subsystem. Therefore, to capture the electronic effect of the MM atoms on the QM subsystem, electrostatic embedding is required, where the electrostatic interactions between the two subsystems are included in the one-electron operators of the QM Hamiltonian. The remaining nonbonding interactions (van der Waals) and bonding (stretching, bending, and torsional) interactions are treated at the MM level.
When there is direct bonding between the QM and MM subsystems, the $E_{QM/MM}$ term must also include bond, angle, and torsional parameters to treat bond at the QM/MM frontier. One simple approach to treat the frontier bond is to use a link-atom (LA) approach, where the QM subsystem is capped by a hydrogen atom that is included in the quantum chemical treatment.

For a while now it has been possible to use Multiconfigurational or even multireference methods to describe the QM subsystem in a QM/MM protocol. Since these methods allow for the description of excited states as well as ground states, this has helped advance the field of computational photobiology. Indeed, it is now possible to study large photoactive proteins where the chromophore is largely responsible for the photochemical properties (e.g. rhodopsins and the green fluorescent protein). For such proteins, the chromophore could be treated with QM while the effect of the protein is accounted for with MM. A common approach has been to treat such systems with a CASPT2//CASSCF/6-31G*/MM QM/MM protocol, where geometries are optimized at the CASSCF level, energies are evaluated at the CASPT2 level, all while employing the 6-31G* basis set and accounting for the effect of the environment using MM electrostatic embedding. This approach has been shown to be quite successful (see Figure 2.6), and is used in the last chapter of this study.
Figure 2.6. Accuracy of the CASPT2//CASSCF/6-31G*/MM protocol for modeling photoreceptors. Comparison between observed and computed absorption ($S_0 \rightarrow S_1$, $S_0 \rightarrow S_2$) and emission ($S_1 \rightarrow S_0$) $\lambda_{\text{max}}$ values of a set of protein pigments (rhombuses indicate data from the labs of Prof. Massimo Olivucci. Triangles indicate data from other groups). These include bovine rhodopsin (Rh),$^{21}$ bathorhodopsin (bathoRh),$^{22}$ isorhodopsin (iso-Rh),$^{23}$ the Rh mutants E113D and G121L,$^{24,25}$ bacteriorhodopsin (bR),$^{26}$ Anabaena sensory rhodopsin (ASR),$^{27}$ the green fluorescent protein (GFP),$^{28}$ the photoactive yellow protein (PYP),$^{29}$ the tryptophan containing proteins parvalbumin (Parv) and Monellin (Mone),$^{30}$ luciferase,$^{31}$ the GFP-like mutant Dronpa$^{32}$ and phytochrome.$^{33}$ This figure is adapted from ref. 34.
2.9. References

(1) Von Neumann, J.; Wigner, E. Phys. Z. 1929, 30, 467-70.


(9) Fock, Z. Physik. 1930, 61, 126.


3.1. Abstract

The ground state potential energy surface of the retinal chromophore of visual pigments (e.g., bovine rhodopsin) features a low-lying conical intersection surrounded by regions with variable charge-transfer and diradical electronic structures. This implies that dynamic electron correlation may have a large effect on the shape of the force fields driving its reactivity. To investigate this effect, we focus on mapping the potential energy for three paths located along the ground state CASSCF potential energy surface of the penta-2,4-dieniminium cation taken as a minimal model of the retinal chromophore. The first path spans the bond length alternation coordinate and intercepts a conical intersection point. The other two are minimum energy paths along two distinct but kinetically competitive thermal isomerization coordinates. We show that the effect of introducing the missing dynamic electron correlation variationally (with MRCISD), perturbatively (with MRPT2 methods), and using equation-of-motion coupled-cluster theory (EOM-CC) leads, invariably, to a stabilization of the regions with charge transfer character and to a significant reshaping of the reference CASSCF potential energy surface and suggesting a change in the dominating isomerization mechanism. The possible impact of such a correction on the thermal and photochemical properties of the retinal chromophore is discussed.

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\[\text{i} \text{This Chapter is based on the following articles. Adapted with permission from:}

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3.2. Introduction

The penta-2,4-dieniminium cation (PSB3) is a conjugated and protonated imine which has been used extensively as a low-cost computational model of the retinal protonated Schiff base chromophore (rPSB) of visual pigments\(^1\)-\(^7\) (see Figure 3.1). Indeed, PSB3 reproduces several features of 4-cis-nona-2,4,6,8-tetraeniminium cation (PSB5)\(^8\),\(^9\), a model system that incorporates the five conjugating double bonds of native rPSB (since the sixth double bond residing on the β-ionone ring has a reduced conjugation\(^10\)).

As illustrated in Figure 3.1, at the complete-active-space-self-consistent-field (CASSCF) level of theory, the first singlet excited state (S\(_1\)) surface of cis-PSB3 is characterized by a barrierless relaxation path connecting the Franck-Condon (FC) region to a peaked conical intersection (CI)\(^1\),\(^11\) with the ground state (S\(_0\)) energy surface. The CI structure is highly twisted about the central C2=C3 double bond and mediates the photochemical isomerization of cis-PSB3 to trans-PSB3\(^11\). The lowest energy CI\(^12\) displays a geometry with a ca. 90° twisted central bond and a decreased bond length alternation (BLA) with respect to the S\(_0\) equilibrium structures (energy minima) cis-PSB3 and trans-PSB3\(^3\),\(^13\). The same figure shows that, after decay in the CI region, the system must relax along the S\(_0\) potential energy surface. In spite of the fact that an exact knowledge of the properties of this surface is mandatory for the understanding of the relaxation process, little work has been devoted to its study.
Figure 3.1. Top. The structures of the cis-penta-2,4-dieniminium cation (PSB3), the 4-cis-nona-2,4,6,8-tetraeniminium cation (PSB5), and the 11-cis-retinal protonated Schiff base (rPSB) which is connected to the Lys296 residue in the prototypal pigment bovine rhodopsin. Bottom. Schematic, one-dimensional representation of the potential energy surfaces of PSB3.
As will be discussed in more detail in Chapter V for rPSB, the CASSCF $S_0$ potential energy surface of PSB3 is similarly characterized by two transition states (see $T_{S\text{CT}}$ and $T_{S\text{DIR}}$ in Figure 3.1) featuring a ca. 90° twisted central bond but opposite BLA patterns and both connecting \textit{cis}-PSB3 to \textit{trans}-PSB3. Therefore, both $T_{S\text{CT}}$ and $T_{S\text{DIR}}$ mediate the thermal isomerization of \textit{cis}-PSB3 to \textit{trans}-PSB3. These transition states lie on opposite sides of a CI point. Comparison of the optimized CI, \textit{cis}-PSB3, \textit{trans}-PSB3, $T_{S\text{CT}}$ and $T_{S\text{DIR}}$ structures and relative CASSCF stabilities (see Figure 3.2) supports the landscape depicted in Figure 3.3.A. This represents a low-lying two-dimensional cross-section of the PSB3 $S_0$ CASSCF/6-31G* potential energy surface spanning a reaction coordinate (RC) dominated by the twisting deformation about the C2=C3 double bond and a BLA coordinate dominated by an inversion between double and single bonds. As we will explain below, in the CI region the coordinate space along RC and BLA is roughly parallel to the branching plane$^{14}$ associated with the CI.
Figure 3.2. Main CASSCF/6-31G* geometrical parameters (bond lengths in Angstrom and C1-C2-C3-C4 dihedrals in degrees) and relative energies (value in parenthesis in kcal/mol) for the S₀ stationary points and S₁/S₀ conical intersection of PSB3. The resonance formula also provides a qualitative representation of the singlet electron pairing and charge distribution. The resonance formula used for the CI represents a mixture of the intersecting TSₜₐₜ and TSₜ₃ₜ electronic configurations.
Figure 3.3. Low-lying ground state potential energy surface of PSB3. A. Schematic two-dimensional cross-section of the $S_0$ potential energy surface of PSB3. The two geometrical coordinates are dominated by bond length alternation (BLA) and the C2-C3 twisting reaction coordinate (RC), respectively. The surface region dominated by a charge-transfer wavefunction ($\psi_{CT}$) is displayed in brown, while that dominated by a covalent/diradical wavefunction ($\psi_{DIR}$) is displayed in green. The electronic structures of the two transition states are described with a bubble diagram representing the total Mulliken charge along each atom along backbone of PSB3 (charges summed onto heavy atoms). The values of these charges are displayed. Finally, the three paths used in the present study (BLA coordinate, MEP$_{CT}$, and MEP$_{DIR}$) are schematically represented as dashed lines on the surface. B. $S_0$ and $S_1$ 2-root SA-CASSCF/6-31G* energy profiles along the three paths represented in part A. Brown regions correspond to regions dominated by a charge transfer wavefunction while green regions are predominantly covalent (although the contribution of charge transfer character in these regions also varies).
We show in Figures 3.2 and 3.3A that one of the transition states (TS\textsubscript{DIR}) displays a diradical character consistent with an S\textsubscript{0} isomerization mechanism driven by the homolytic breaking of the central C2=C3 double-bond. Therefore TS\textsubscript{DIR} has two unpaired electrons located on the \(\pi\)-systems of the nearly orthogonal allyl (C5H\textsubscript{2}-C4H-C3H-) and protonated imine heteroallyl (-C2H-C1H-NH\textsubscript{2}) moieties, respectively. In contrast, the other transition state (TS\textsubscript{CT}) displays a charge-transfer (closed-shell) character with an augmented electron density (and therefore a reduced positive charge) in the N=C1 region with respect to that of the \textit{cis} and \textit{trans} equilibrium geometries. The contrasting charge distributions of TS\textsubscript{DIR} and TS\textsubscript{CT} indicate the existence of two low-lying regions on the S\textsubscript{0} potential energy surface of protonated Schiff bases where qualitatively different \(\pi\)-electron densities are observed. This can be understood due to the varying nature of a electronic character of an adiabatic wavefunction around a CI (see Sections 2.4 and 5.3) where each region is predominantly described by a different diabatic function. One region encompasses most of the S\textsubscript{0} potential energy surface (including TS\textsubscript{DIR} and the \textit{cis}-PSB3 and \textit{trans}-PSB3 equilibrium structures) and is characterized by a wavefunction with a predominantly correlating covalent/diradical configuration (\(\psi\textsubscript{DIR}\)), while the other region is dominated by a charge-transfer (singly excited) configuration (\(\psi\textsubscript{CT}\)). Note that since we generally describe regions in the proximity of a CI, we chose to use the “DIR” label to describe the covalent wavefunction although the corresponding electronic structure varies from closed shell at the reactant and product to diradical for twisted geometries due to homolytic cleavage of the central \(\pi\) bond.

While the regions of the S\textsubscript{0} potential energy surface describing the transition between the charge-transfer and covalent states of PSB3 are presently unknown, the difference in the \(\pi\)-
electron density associated with $\psi_{\text{DIR}}$ and $\psi_{\text{CT}}$ suggests that dynamic electron correlation would play a key role in determining their relative extension and stability.\textsuperscript{15,16} Therefore dynamic electron correlation may significantly alter the shape of the reference CASSCF landscape of Figure 3.3.A with potentially important chemical consequences. See the conclusion for a discussion of such chemical consequences.

In a recent study, Mori et al.\textsuperscript{7} investigated the effect of dynamic electron correlation on the geometry of the minimum energy CI in PSB3. Moreover, Valsson et al.\textsuperscript{6} studied the effect of dynamic electron correlation on the $S_1$ isomerization of PSB3, with special attention to the FC region and the minimum energy path connecting the FC to the CI. In the present work, we build on these two studies by investigating the effect of dynamic electron correlation on the shape and stability of the $S_0$ potential energy surface surrounding the CI. Accordingly, we characterize the CASSCF/6-31G* surface of the PSB3 along three potential energy paths (see Figure 3.3.B). The first corresponds to a coordinate obtained via a linear interpolation (and extrapolation) of the optimized $T_{\text{CT}}$ and $T_{\text{DIR}}$ structures (BLA). This interpolation intercepts a single CI point that is located between the two transition structures (the structure of this CI intercepted by the BLA path is shown in Figure 3.2). The second (MEP\textsubscript{DIR}) corresponds to the minimum energy path connecting $T_{\text{DIR}}$ to the \textit{cis} reactant and the \textit{trans} product where the wavefunction remains predominantly covalent ($\psi_{\text{DIR}}$). The third (MEP\textsubscript{CT}) corresponds, again, to a minimum energy path describing the isomerization coordinate passing through $T_{\text{CT}}$ and connecting reactant and product. However, this path intercepts regions dominated by different electronic configurations ($\psi_{\text{CT}}$ or $\psi_{\text{DIR}}$).
The results reported below show that incorporation of dynamic electron correlation on top of the CASSCF wavefunction using either variational (MRCISD), perturbative (MRPT2), or equation-of-motion coupled-cluster (EOM-CC) theories leads to a stabilization of the region dominated by the charge-transfer configuration ($\psi_{CT}$) with respect to the rest of the surface. Accordingly, the TS$_{CT}$ structure becomes the lowest energy saddle point on the PSB3 S$_0$ potential energy surface. Consistently with the results of Mori et al.\textsuperscript{7}, the same effect leads to a shift in the position and shape of the CI along the BLA coordinate and towards TS$_{DIR}$.

3.3. Methods

The dynamic electron correlation is introduced on top of the reference CASSCF wavefunction in a variational scheme using the MRCISD and MRCISD+Q methods and also using different implementations of multireference second order perturbation theory (MRPT2) such as single state CASPT2\textsuperscript{17} (referred to hereafter as simply CASPT2), multistate-CASPT2 (MS-CASPT2)\textsuperscript{18}, XMCQDPT2\textsuperscript{19} and QD-NEVPT2\textsuperscript{20-22}. We also test the effect of using single-reference EOM-CC methods\textsuperscript{23-26} along the CASSCF generated path. In this section we revise the methods used for generating structures along the paths of Figure 3.3 and present details about the multireference methods used.
3.3.1 Reference potential energy surface

CASSCF structures and energies were computed using the MOLCAS 7.6 quantum chemistry software package.\textsuperscript{27} TS\textsubscript{CT} and TS\textsubscript{DIR} were both optimized at the equal-weight 2-root state averaged (SA)-CASSCF/6-31G* level. The basis set employs Cartesian d polarization functions. The active space comprises the 6 $\pi$-orbitals of PSB3 occupied with 6 electrons (6-in-6). The CASSCF wavefunctions were then used as zero-order approximations for the higher level methods described below. From the two transition state structures, the BLA path was produced by using a linear interpolation/extrapolation of their coordinates to give a set of 14 structures (including the two transition states) spanning the corresponding BLA coordinate. Throughout this work we define the value along the BLA coordinate as the difference between the average bond length (in Angstroms) of formal double bonds (N=C1, C2=C3, and C4=C5) and formal single bonds (C1-C2 and C3-C4). The other two paths (MEP\textsubscript{CT} and MEP\textsubscript{DIR}) were produced by running two minimum energy path (MEP) calculations for each path at the CASSCF level of theory with a step size of 0.01 Angstrom•(amu)\textsuperscript{\textfrac{1}{2}} and starting from the corresponding transition state. The resulting MEP\textsubscript{CT} and MEP\textsubscript{DIR} paths represent thermal isomerizations from cis-PSB3 to trans-PSB3. Since, in this study we are mainly concerned with the region surrounding the CI, we do not consider the whole path, which comprises over 100 geometries, but rather only the 8 structures closest to the transition states from each side (thus each path includes 17 geometries including the transition state). The CASSCF/6-31G* energy profiles along the BLA, MEP\textsubscript{CT}, and MEP\textsubscript{DIR} paths are shown in Figure 3.3.B. Unless otherwise specified, the CASSCF reference wavefunction and basis set are the same as the ones used for the geometry optimization.
In order to determine the relaxed structure and branching plane vectors characterizing the CI, the Gaussian '03 software package was used. The CI structure shown in Figure 3.2 provided a guess initial structure for the CI optimization. A conical intersection optimization was performed with the 2-root SA-CASSCF method and the reduced maximum step size of 0.05 Bohr, which converged to a solution in 5 iterations and provided the branching plane vectors corresponding to the CI geometry.

### 3.3.2 Multireference calculations

Single point CASPT2 and MS-CASPT2 energies were computed using the MOLCAS 7.6 quantum chemistry software package. For these calculations, an imaginary shift of 0.2 was used to exclude intruder states, consistent with previous studies. The effect of the ionization potential-electronic affinity (IPEA) shift on CASPT2 energies is considered. The IPEA parameter reduces a systematic error through a modification of the zeroth order Hamiltonian and its 0.25 value has been obtained by fitting a specific set of experimental data (See also the discussion in ref. 30 for systems that require different IPEA values). CASPT2 energies reported with the default IPEA shift are labeled with “IPEA=0.25,” otherwise the reported CASPT2 values and geometries are obtained with IPEA=0. The reported QD-NEVPT2 energies are based on the partially contracted variant of NEVPT2 using state-specific sets of canonical inactive and virtual orbitals as opposed to the same set of state average orbitals. XMCQDPT2 energies were computed using Firefly version 7.1.G, build number 5620. For the XMCQDPT2 calculations, the intruder state avoidance (ISA) shift was set to 0.02 for avoiding the intruder states. MRCISD energies were computed using the internally contracted variant with the Molpro package. The
full CASSCF active space (6-in-6) was included in the reference space for MRCISD calculations. The original Davidson correction \( (Q) \) with a relaxed reference was used for MRCISD+Q calculations. The 1s core orbitals in carbon and nitrogen atoms were kept frozen for all multireference calculations, and in all cases the 6-31G* basis set with Cartesian d polarization functions was consistently used.

All charges reported in this study are computed with the Mulliken population analysis. The charge-transfer and covalent/diradical characters (i.e. the weights of the \( \psi_{\text{DIR}} \) and \( \psi_{\text{CT}} \) configurations, respectively) is measured indirectly by computing the total Mulliken charge of the allyl moiety (C5H2-C4H-C3H-) which is separated from the Schiff base moiety (-C2H-C1H-NH2) by the isomerizing C2=C3 bond. The XMCQDPT2 charges (computed in Firefly) and QD-NEVPT2 and MS-CASPT2 charges (computed externally) were computed from the wavefunctions obtained by the diagonalization of the effective Hamiltonian evaluated at the 0+1+2 order. These wavefunctions are linear combinations of the two SA-CASSCF wavefunctions and thus they belong to the CAS space. The charges therefore include the most important effects caused by state mixing while omitting other contributions from the first-order interacting space.

In this study we have invariably employed a 2-root state averaging for the reference CASSCF wavefunctions. However, we test the effect of additional roots in the state-averaging scheme in Section 3.4.5.

In Section 3.4.4 we present the effect of optimizing the BLA, MEP\(_{\text{CT}}\), and MEP\(_{\text{DIR}}\) paths with two representative MRPT2 methods, CASPT2 and XMCQDPT2. Numerical gradients were used (these methods are available in Molcas 7.6 for CASPT2 and in the developer’s version of
Firefly for XMCQDPT2). The *cis*-PSB3, *trans*-PSB3, TS\textsubscript{CT}, and TS\textsubscript{DIR} structures were reoptimized with the MRPT2 methods in a manner consistent with the CASSCF optimizations (also employing the same 6-31G* basis set and number of roots). The BLA path was then generated by interpolation/extrapolation of the MRPT2 optimized TS\textsubscript{CT} and TS\textsubscript{DIR} structures, and the MEP\textsubscript{CT} and MEP\textsubscript{DIR} paths were generated using MEP calculations employing numerical gradients, and started from the MRPT2 optimized transition states. CASPT2 MEP calculations used identical parameters to those performed with CASSCF since the same software package was employed for both calculations. XMCQDPT2 MEP calculations employed the Gonzalez-Schlegel second order method\textsuperscript{36} and a stride of 0.0053 Angstrom•(amu)\textsuperscript{½}.

### 3.3.3 EOM-CC calculations

EOM-CC calculations were performed with the 6-31G* basis set using Cartesian d-functions, consistent with the CASSCF and MRCISD calculations. All electrons were correlated. In this work, we consider two different EOM-CC models which employ different excitation operators.\textsuperscript{24} Namely, we use EOM-EE (EOM for excitation energies), which employs a particle-conserving operator, and EOM-SF (spin-flip EOM), which employs a spin-flipping operator.\textsuperscript{37-40} In both cases, we use an excitation level limited to single and double substitutions (EOM-CCSD). However, in the case of EOM-SF, we also consider triple excitations perturbatively added to give rise to EOM-CCSD(dT) or EOM-CCSD(fT) models.\textsuperscript{41-43}

In EOM-EE, the lowest closed-shell Hartree-Fock solution was used as the reference. In EOM-SF, the lowest high-spin triplet was employed as the reference. The quality of the EOM-SF
wave functions can be affected by spin-contamination; this can be mitigated by employing a ROHF triplet reference. Thus, for highest-accuracy SF results using ROHF is recommended; however, if the UHF solution is not strongly spin-contaminated, it can also be used. We present both UHF and ROHF based EOM-SF-CCSD results. The $<S^2>$ values of the target EOM-SF wave functions present a reliable diagnostic of spin-contamination. For PSB3, the spin-contamination of the UHF triplet reference is too large, spoiling the description of the target states. For example, at the $T_{SCt}$ geometry, the $<S^2>$ values of the three lowest EOM-SF/UHF states are: 0.87 (open-shell singlet corresponding to $\Psi_{\text{dir}}$), 1.24 ($M_s=0$ triplet), and 0.07 (closed-shell singlet, ionic state $\Psi_{\text{CT}}$), whereas using the ROHF reference, the $<S^2>$ values become much closer to the spin-pure values (0.012, 2.026, and 0.063).

All EOM-CC calculations were performed using the Q-Chem electronic structure package.\textsuperscript{44}

3.4. Results and discussion Part 1: Multireference methods

Here we systematically describe the changes in the shape of the PSB3 potential energy surface along the BLA, MEP\textsubscript{CT}, and MEP\textsubscript{DIR} coordinates following MRCISD and MRPT2 computations using the common 6-31G* basis. Then we investigate the effect of the geometrical relaxation of the surface stationary points at the MRPT2 level, the effect of the number of roots used in the state-averaging scheme, and the effect of the active space orbitals. A discussion of the quality of CASPT2//CASSCF/6-31G* calculations widely used in photochemical and photobiological applications is given in the last subsection.
3.4.1. BLA path and branching Plane

The energy profiles along the BLA coordinate computed with CASSCF, MRCISD, MRCISD+Q, and various MRPT2 methods are shown in Figures 3.4.A and 3.4.B. We assume here that MRCISD+Q provides the quantitatively correct description of the different electronic structures of PSB3. It is apparent that the selected reference CASSCF method not only overestimates energy barriers of the two transition states with respect to MRCISD+Q but displays a reverse stability order of $\text{TS}_{\text{CT}}$ and $\text{TS}_{\text{DIR}}$. A discussion on the effect of dynamic electron correlation on the topology of the ground state is presented at the end of this section.
Figure 3.4. Energy profiles along the BLA coordinate compared with 2-root SA-CASSCF (red) and MRCISD+Q (black) which are present in both parts A and B. The energy values are relative to the reactant (cis-PSB3). The position of the CI for each method is indicated with a filled circle. The curves are labeled at the left margin to distinguish between diabatic curves with predominantly charge transfer ($\psi_{CT}$) and covalent-diradical ($\psi_{DIR}$) character for each method. A. The S0 and S1 energies for MRCISD (grey), CASPT2 (dark blue), MS-CASPT2 (magenta), QD-NEVPT2 (orange), and XMCQDPT2 (green). The QD-NEVPT2 CI does not lie within the selected BLA coordinate values, and so its position is estimated by extrapolating the QD-
NEVPT2 curve using a polynomial fit (dashed line). B. The S₀ and S₁ energies for CASPT2 (IPEA=0.25) (blue), MS-CASPT2(IPEA=0.25) (violet), QD-NEVPT2 with 6-in-8 active space (brown), XMCQDPT2 with a diagonal fit (pink), and XMCQDPT2/F(Γns) (dark green). C and D. schematic Valence Bond-like state mixing diagrams for the S₀ and S₁ energy profiles along the BLA coordinate at the CASSCF and MRCISD+Q levels of theory, respectively. The diabatic states are represented with dashed lines. Mixing of the diabatic states produces the adiabatic states represented with solid lines. Brown curves are dominated by a charge transfer wavefunction while green curves are dominated by a covalent one.

Consistent with the MRCISD+Q result, all MRPT2 methods seem to yield the same change characterized by the TS_{CT} stabilization and the CI shifting towards TS_{DIR}. On the other hand, the magnitude of the charge-transfer stabilization depends on the method. CASPT2 and MS-CASPT2 yield the same energy profile for the charge-transfer state as MRCISD+Q but, as expected, an overstabilized covalent curve due to the lack of IPEA shift. In Figure 3.4.B, we show that the incorporation of the IPEA shift yields CASPT2(IPEA=0.25) and MS-CASPT2 (IPEA=0.25) profiles substantially identical to MRCISD+Q. Our findings are consistent with a study on diatomic molecules where it was also found that CASPT2 with a modified zeroth-order Hamiltonian performs better than unmodified CASPT2 when compared to FCI. XMCQDPT2 (Figure 3.4.A) yields curves similar to MRCISD but both shifted to lower energies, and with a slightly overstabilized covalent curve with respect to MRCISD+Q. In Figure 3.4.B we report XMCQDPT2 results obtained using slightly different definitions of the model Fock-like operator used to define the zero-order Hamiltonian within the XMCQDPT2 formalism. Those modifications of the “classical” XMCQDPT2, termed “XMCQDPT2/diagonal fit” and “XMCQDPT2/F(Γns)”, provide a more balanced description of states of very different nature and therefore produce energy profiles which are in better agreement with MRCISD+Q. Finally, the
QD-NEVPT2 method leads to an over-stabilization of the $\psi_{\text{CT}}$ configuration with respect to MRCISD+Q. In contrast, the covalent curve is substantially on top of MRCISD+Q. For this reason, when using the reference CASSCF wavefunction defined above, QD-NEVPT2 yields a considerably shifted position of the CI. The QD-NEVPT2 CI is shown in an extrapolation of the path in Figure 3.4.A. This over-stabilization of the charge transfer configuration is due to the limit in the zero-order CASSCF wavefunction and may be resolved by using a larger active space. In fact, we find that the expansion of the active space to 6-in-8 allows the QD-NEVPT2 energy profile to virtually overlap with that of MRCISD+Q(6-in-6) (Figure 3.4.B). We discuss the effect of the active space on the CASSCF, CASPT2, CASPT2(IPEA=0.25), and XMCQDPT2 energy profiles later in this chapter as well (see Section 3.4.6).

In Figure 3.4.A, we find an artifact along the MS-CASPT2 profile. This artifact is located in the vicinity of 0.00 Angstrom along the BLA coordinate and therefore in correspondence with the CASSCF CI. Such a behavior has been shown to arise in both CASPT2 and MS-CASPT2 if the corresponding CASSCF $S_0$ and $S_1$ wavefunctions (which in this case correspond to the $\psi_{\text{DIR}}$ and $\psi_{\text{CT}}$ configurations) are highly mixed, e.g. near a CI. Solutions to this problem have already been proposed and XMCQDPT2 does not suffer from this problem while QD-NEVPT2 reduces it. The XMCQDPT2 extension has recently also been used for the development of the XMS-CASPT2 method.
Figure 3.5. Results for a path centered around the 3-root CASSCF CI. The energies are relative to the point $-5 \, S_0$ energy. Only the $S_0$ and $S_1$ energies are shown. A. The 3-root SA-CASSCF energies. B. 3-root CASPT2 and MS-CASPT2 energies. C. The energies displayed in the diagonal elements of the XMCQDPT2 effective Hamiltonian in the basis of CASCI eigenvectors, and the 3-root XMCQDPT2 energies. D. The 3-root SS-NEVPT2 and QD-NEVPT2 energies.
Although such artifacts in CASPT2 and MS-CASPT2 energies have been investigated before and solutions have been proposed, here we again demonstrate the occurrence of such artifacts in PSB3. To that effect, we have produced a fourth set of geometries that are centered around a 3-root state averaged CASSCF CI point between \( S_0 \) and \( S_1 \) where there is strong mixing of the wavefunctions of the two states. The 3-root CASSCF energies and charge transfer character for \( S_0 \) and \( S_1 \) are shown in Figure 3.5.A. At point 0, there is a near degeneracy, and the wavefunction has mixed \( \psi_{\text{CT}} \) and covalent \( \psi_{\text{DIR}} \) character (while at the other points, the each state has mostly pure \( \psi_{\text{CT}} \) or \( \psi_{\text{DIR}} \) character). As expected, the CASPT2 correction stabilizes \( \psi_{\text{CT}} \) more than \( \psi_{\text{DIR}} \). This stabilization of one of the states relative to the other should lead to the splitting of the degeneracy at point 0. However, as shown in Figure 3.5.B, point 0 remains a degeneracy at the CASPT2 level due to the mixed character of the reference wavefunction, while at all the other points the CASPT2 energies are described correctly. This causes a visible artifact in the CASPT2 energy profile. Meanwhile, the opposite effect is observed with MS-CASPT2, which overcompensates in its correction of this artifact, in turn causing a different artifact in the same region. As already pointed out,\(^9\), this behavior of MS-CASPT2 is due to a large electron coupling in the second order effective Hamiltonian matrix. As a result of this, the CASPT2 energy degeneracy at point 0 is split by ca. 32 kcal/mol at the MS-CASPT2. This may explain large differences between reported results between single state CASPT2 and MS-CASPT2 in literature such as in ref. 49. In Figures 3.5.C and 3.5.D we also show the XMCQDPT2 and NEVPT2 energies along the same scan. While the irregularity persists with single state (SS)-NEVPT2 and in the diagonal elements of the XMCDPT2 hamiltonian, both QD-NEVPT2 and
XMCQDPT2 actively correct for these artifacts. However, a small artifact still persists with QD-NEVPT2.

It is of interest to explore the changes imposed by the dynamic electron correlation on the location, topology and electronic structure of the CI originally located at the CASSCF level. The changes in energy profile along the BLA path can be interpreted in terms of a valence-bond diagram reporting the energies of the $\psi_{CT}$ and $\psi_{DIR}$ configurations/diabatic states along the path. The transition states are located, roughly, at the minima of these two diabatic states. At the CASSCF level the energies of the $\psi_{CT}$ at TS$_{CT}$ and $\psi_{DIR}$ at TS$_{DIR}$ (see dashed curves in Figure 3.4.C) are relatively close (see double arrow in Figure 3.4.C). As a consequence the CI point has a “peaked” shape and both transition states reside on $S_0$. However, at the MRCISD+Q level the $\psi_{CT}$ state is largely stabilized with respect to $\psi_{DIR}$ (see double arrow in Figure 3.4.D). Consequently, the CI moves to larger BLA values and changes shape becoming a sloped or intermediate CI.$^{14}$ In this situation TS$_{DIR}$ disappears from $S_0$ and becomes an $S_1$ minimum along the BLA coordinate.

At the CI, the molecular modes labeled $X_1$ and $X_2$ correspond to the gradient difference and derivative coupling and define the branching plane.$^{14}$ As shown in Figure 3.6.A, it is apparent that the $X_1$ and $X_2$ modes are primarily related to the RC and BLA coordinates, respectively.$^{50}$ With this information we compute a set of branching plane structures located along a small circle centered at the CI. In Figure 3.6.B we show the CASSCF/6-31G* $S_0$ energy profile along the circle and the corresponding charge distribution (to monitor the wavefunction character). The plot shows two energy maxima (MAX$_{DIR}$ and MAX$_{CT}$) at ca. 80° and 270°. These correspond to ridges developing towards TS$_{DIR}$ and TS$_{CT}$. MAX$_{DIR}$ and MAX$_{CT}$ are roughly
oriented in the direction of $X_2$ and feature a covalent and charge-transfer character, respectively, and consistent with the results of Figure 3.4. The two minima along the profile of Figure 3.6.B (ca. 10° and 180°) are associated to the cis-PSB3 and trans-PSB3 energy valleys (i.e. equilibrium structures) and are located along $X_1$. In Figure 3.6.C and 3.6.D we show that the changes imposed by the dynamic electron correlation on the relative stability of $S_0$ and $S_1$ energy are consistent with the results of Figure 3.4. For instance, at the XMCQDPT2 level it is clear that the CI point has shifted so much that it is not inside the loop anymore and the wavefunction character is exclusively dominated by $\psi_{CT}$. Clearly the CASPT2 represents an intermediate case where the CI point is now located on the loop itself at ca. 110°.
Figure 3.6. The CASSCF/6-31G* branching plane of PSB3. A. Modes corresponding to the gradient difference vector (GDV) and derivative coupling vector (DCV). GDV corresponds to the RC (isomerization/pyramidalization) mode, while DCV represents a BLA mode. B, C, and D. the CASSCF, CASPT2 and XMCQDPT2 $S_0$ and $S_1$ energies (solid lines) and charge transfer character (blue area) along a circle (radius=0.002 Angstrom, 1 step every 10º) centered on the CI structure intercepted along the BLA coordinate. The energies are relative to cis-PSB3. The charge transfer character is described by the value of the sum of the Mulliken charges on the allyl fragment (C5H2-C4H-C3H-). Brown regions correspond to regions dominated by a charge transfer wavefunction while green regions are predominantly covalent.
3.4.2. MEP\(_{\text{CT}}\) path

The energy profiles along MEP\(_{\text{CT}}\) are shown in Figure 3.7.A. Of course, the order of the energies at TS\(_{\text{CT}}\) reflects the order of the energies seen in Figure 3.4. Consistently, the barrier at the TS\(_{\text{CT}}\) is lowest at the QD-NEVPT2 level, followed by XMCQDPT2, CASPT2, CASPT2 (IPEA=0.25), and MS-CASPT2(IPEA=0.25) all giving barrier heights similar to the one computed at the MRCISD+Q level. In all cases, the dynamic electron correlation decreases the activation energy along MEP\(_{\text{CT}}\). The MEP shows substantially equivalent energy changes when moving towards cis-PSB3 and trans-PSB3. Thus, starting at TS\(_{\text{CT}}\) one sees that the CASSCF/6-31G* electronic structure changes from a pure charge-transfer to one where the covalent character eventually becomes predominant. This evolution can be monitored by plotting the charge-distribution along the MEP\(_{\text{CT}}\) profile (Figure 3.7.B). TS\(_{\text{CT}}\) has nearly 90\% of its positive charge on the allyl moiety. However, as the molecule moves away from TS\(_{\text{CT}}\) the system quickly loses its charge-transfer character.
Figure 3.7. Energy profiles along the MEP\textsubscript{CT} coordinate. A. The $S_0$ energies computed using 2-root SA-CASSCF (red), MRCISD (grey), MRCISD+Q (black), CASPT2 (dark blue), CASPT2 (IPEA=0.25) (blue), MS-CASPT2 (IPEA=0.25) (violet), QD-NEVPT2 (orange), XMCQDPT2 (green), XMCQDPT2 with a diagonal fit (pink), and XMCQDPT2/F($\Gamma_n$) (dark green). The energies are relative to the reactant (\textit{cis}-PSB3, point -0.54). B. The charge transfer character along...
MEP\textsubscript{CT} for CASSCF, MRCISD, XMCQDPT2, CASPT2, MS-CASPT2 and QD-NEVPT2. \textbf{C and D.} Schematic Valence Bond-like state mixing diagrams for the \textit{S}_0 and \textit{S}_1 energy profiles along MEP\textsubscript{CT} at the CASSCF and MRCISD+Q levels of theory, respectively. The diabatic states are represented with dashed lines. Mixing of the diabatic states produces the adiabatic states represented with solid lines. Brown regions correspond to regions dominated by a charge transfer wavefunction while green regions are predominantly covalent.

The charge transfer profiles for multireference methods are also plotted in Figure 3.7.B. We find that, in general, methods that stabilize the $\psi\textsubscript{CT}$ wavefunction with respect to $\psi\textsubscript{DIR}$ have a more extended charge transfer region along MEP\textsubscript{CT}. CASPT2 has a very limited effect on the charge transfer profile because (as shown in Figure 3.4.A) it stabilizes the $\psi\textsubscript{CT}$ and $\psi\textsubscript{DIR}$ energy profiles to an almost equal extent. The fact that the CASPT2 charge transfer profile is slightly reduced compared to CASSCF may be due to the missing contribution from the relaxation of the orbital and CI-coefficients in CASPT2, which is expected to slightly increase ionic character.

At the MS-CASPT2 level, on the other hand, there is a large extension of the MS-CASPT2 charge transfer region as seen in Figure 3.7.B. This is due to an overestimation of off-diagonal matrix elements of the effective Hamiltonian and thus to overestimation of zero-order state mixing.\textsuperscript{19} This is also reflected in the MS-CASPT2 energy profile (Figure 3.7.A) where there is a large stabilization of \textit{S}_0 (and destabilization of \textit{S}_1 as shown in the SI) as the molecule moves away from TS\textsubscript{CT}, resulting in an irregular energy profile.

The rest of the methods (XMCQDPT2, QD-NEVPT2, and MRCISD) all lead to an extension of the charge transfer region compared to CASSCF. XMCQDPT2 and MRCISD have similar charge transfer profiles because they both have a similar relative stability of the $\psi\textsubscript{CT}$ and $\psi\textsubscript{DIR}$ wavefunctions (as indicated by the fact that they display a similar position of the CI along
the BLA coordinate; see Figure 3.4.A). QD-NEVPT2 has a more extended charge transfer region due to its overstabilization of $\psi_{CT}$ with respect to $\psi_{DIR}$. MRCISD+Q would be expected to have a charge transfer profile in between that of MRCISD and QD-NEVPT2 since it stabilizes $\psi_{CT}$ with respect to $\psi_{DIR}$ more than MRCISD does, but not to the same extent as QD-NEVPT2.

Again, these results can be interpreted in terms of a valence-bond state mixing diagram and focusing on the MEP$_{CT}$ path. In Figure 3.7.C we show schematic energy profiles reporting the change in energy of the $\psi_{CT}$ and $\psi_{DIR}$ configurations/diabatic states along the path. The adiabatic $S_0$ and $S_1$ energy profiles originate from the mixing of the two configurations that also determine the variation in electronic structure (charge-transfer vs. covalent-diradical) in different regions of the energy profile. At the CASSCF level the two states are rather distant in energy and effectively cross only in a limited region corresponding to the vicinity of the TS$_{CT}$ structure. As a consequence, upon relaxation towards cis-PSB3 and trans-PSB3, the $S_0$ electronic structure rapidly changes character and becomes dominated by a covalent-diradical electron distribution. This explains why the CASSCF charge-transfer region of the $S_0$ energy potential energy surface has a limited extension as shown in Figure 3.7.B and schematically illustrated in Figure 3.7.C. The inclusion of dynamic electron correlation energy leads to the changes shown in Figure 3.7.D as it stabilizes the $\psi_{CT}$ state with respect to the $\psi_{DIR}$ state. As a consequence the $\psi_{CT}$ energy profile is shifted to lower energies and $\psi_{CT}$ and $\psi_{DIR}$ intersect along a larger region. Consequently, the energy of the TS$_{CT}$ is lowered with respect to cis-PSB3 and trans-PSB3 (the MEP$_{CT}$ path becomes the energetically dominating $S_0$ path) and the charge-transfer region dominates a larger and flatter region of the $S_0$ potential energy surface around the TS$_{CT}$ point (see Figure 3.7.D).
3.4.3. MEP_{DIR} path

The energy and charge transfer profiles along MEP_{DIR} are shown in Figures 3.8.A and 3.8.B, respectively. The effect of the electron dynamic correlation on the CASSCF/6-31G* energy profile is similar to the one found for MEP_{CT}. The barriers (with respect to cis-PSB3) are always decreased with respect to the CASSCF level but the magnitude of this change is substantially smaller than that observed for MEP_{CT}. In the case of MS-CASPT2(IPEA=0.25), we see the same artifact as in MEP_{CT}, displaying an extremely rapid decrease in energy when moving away from the transition state. The other methods all give a much smoother profile. There is a strong agreement between the XMCQDPT2, CASPT2, and QD-NEVPT2 in their energy profile along MEP_{DIR}.

The charge transfer profile along MEP_{DIR} shows that CASSCF has the widest diradical region, while the MRPT2 methods and MRCISD reduce the size of the diradical region In general, we find that methods that extend the charge transfer profile along MEP_{CT} (Figure 3.7.B) tend to reduce the diradical region along MEP_{DIR} (Figure 3.8.B) because \( \psi_{DIR} \) gets destabilized relative to \( \psi_{CT} \). Since the MEP_{DIR} path intersects with the BLA path at a BLA coordinate of around 0.025 Å, methods that result in a CI with a higher BLA than 0.025 Å (i.e. a sloped or intermediate CI) would still have an \( S_0 \) state with charge transfer rather than diradical character at the TSDIR geometry. Effectively, this moves the corresponding TS_{DIR} minimum along the BLA coordinate from the \( S_0 \) state to the \( S_1 \) state. Such methods would display an inverted charge transfer profile along MEP_{DIR} with respect to the other methods (see, for example, the QD-NEVPT2 charge transfer profile in Figure 3.8.B). This is expected to be the case for all the multireference methods shown in Figure 3.4.B.
Figure 3.8. Energy profiles along the MEP\textsubscript{DIR} coordinate. A. The $S_0$ energies computed using 2-root SA-CASSCF (red), MRCISD (grey), MRCISD+Q (black), CASPT2 (dark blue), CASPT2 (IPEA=0.25) (blue), MS-CASPT2(IPEA=0.25) (violet), QD-NEVPT2 (orange), XMCQDPT2 (green), XMCQDPT2 with a diagonal fit (pink), and XMCQDPT2/F($\Gamma_{\text{mp}}$) (dark green). The
energies are shown relative to the reactant (cis-PSB3, point -0.52). B. The charge transfer character along MEP\textsubscript{DIR} for CASSCF, MRCISD, XMCQDPT2, CASPT2, MS-CASPT2 and QD-NEVPT2. C and D. schematic Valence Bond-like state mixing diagrams for the S\textsubscript{0} and S\textsubscript{1} energy profiles along MEP\textsubscript{DIR} at the CASSCF and MRCISD+Q levels of theory, respectively. The diabatic states are represented with dashed lines. Mixing of the diabatic states produces the adiabatic states represented with solid lines. Brown regions correspond to regions dominated by a charge transfer wavefunction while green regions are predominantly covalent.

Again, the charge transfer profiles along MEP\textsubscript{DIR} can be explained when comparing the valence bond diagrams in Figures 3.8.C and 3.8.D. At the CASSCF level the diabatic states do not cross and the diabatic and adiabatic states have similar profiles. Stabilization of the $\psi\textsubscript{CT}$ diabatic state with MRCISD+Q causes the $\psi\textsubscript{CT}$ state to become lower in energy than the $\psi\textsubscript{DIR}$ state along part of the MEP\textsubscript{DIR} path (with the two states being nearly degenerate at TS\textsubscript{DIR}).

3.4.4. Effect of the geometry optimization

The analysis above has consistently been based on structures located using CASSCF/6-31G* analytical gradients. In this section we will investigate the effect of re-optimizing the geometries of the four stationary points (cis-PSB3, trans-PSB3, TS\textsubscript{CT} and TS\textsubscript{DIR}) at the CASPT2/6-31G* and XMCQDPT2/6-31G* levels using a 2-root SA-CASSCF wavefunction as the zeroth-order wavefunction and numerical gradients. The high computational costs prevents a study at the MRCISD+Q level due to the size of our reference space used. While the CASSCF and MRPT2 structures display very similar dihedral and bending angles, there are differences between bond lengths, consistent with earlier studies.\textsuperscript{2,6} The structures optimized at the MRPT2 level of theory are shown in Figure 3.9. While CASSCF tends to give a larger BLA value\textsuperscript{2,6} this
change is limited with the exception of C1-N bond and (to a smaller extent) the C5-C4 bond (compare Figure 3.2 and 3.9).

Figure 3.9. The structures of cis-PSB3, trans-PSB3, TS\textsubscript{CT} and TS\textsubscript{DIR} all optimized at the MRPT2 level of theory, as well as the structure of the CI intercepted by the BLA scans in Figure 3.10.A. Bond lengths, as well as the central isomerization angle, are labeled for both the CASPT2 and XMCQDPT2 levels of theory (XMCQDPT2 in parentheses).

We plot the CASSCF, CASPT2, and XMCQDPT2 energies (optimized at the respective method) along the BLA coordinate in Figure 3.10.A (CASPT2 displays an artifact near the center of the curve due to its proximity to the CASSCF CI\textsuperscript{19,47}). We find that while the position of TS\textsubscript{CT} along the BLA coordinate is not largely affected, TS\textsubscript{DIR} displays the largest variation and moves to lower BLA values with both CASPT2 and XMCQDPT2. However, the position of the CI is relatively unaffected by the MRPT2 optimization. We find, consistently with the results of Figure 3.4.A, that the CASPT2 CI is close to the CASSCF CI but has slightly higher BLA, while the XMCQDPT2 CI is over 0.01 Angstrom higher along the BLA coordinate. In spite of the
geometrical differences observed for TS\textsubscript{DIR} there is a limited change in the relative energies with respect to the cis-PSB3 geometry (see Table 3.1, where we compare MRPT2 relative energies for CASSCF and MRPT2 geometries). TS\textsubscript{DIR} is stabilized with respect to TS\textsubscript{CT} after CASPT2 and XMCQDPT2 optimizations, but in both cases the effect is such that TS\textsubscript{CT} remains the lowest in energy (with the XMCQDPT2 TS\textsubscript{CT} lower in energy than the CASPT2 TS\textsubscript{CT}, consistently with the results in Figure 3.4.A). In Figures 3.10.B and 3.10.C we display the energy profiles along MEP\textsubscript{CT} and MEP\textsubscript{DIR} coordinates optimized starting from the TS\textsubscript{CT} and TS\textsubscript{DIR} structures at the corresponding MRPT2 levels (i.e. starting from the MRPT2-optimized transition states, the path points were generated at the respective MRPT2 level of theory as well). The results are compared with the MRPT2//CASSCF MEP\textsubscript{CT} and MEP\textsubscript{DIR} in the same figure. We find that the minimum energy path geometries generated with the MRPT2//MRPT2 methods are similar to the ones generated at the MRPT2//CASSCF level. Overall, the results of Figure 3.10 and Table 3.1 indicate that the MRPT2//MRPT2 S\textsubscript{0} potential energy surface near the CI is very similar to the MRPT2//CASSCF one, except that the valleys corresponding to the MEP\textsubscript{CT} and MEP\textsubscript{DIR} paths must be geometrically closer to each other since the distance between TS\textsubscript{CT} and TS\textsubscript{DIR} along the BLA coordinate is closer at the MRPT2 levels with respect to the CASSCF level.
Figure 3.10. The CASSCF, CASPT2, and XMCQDPT2 energies plotted along a BLA coordinate. The interpolated structures were generated from TS\textsubscript{CT} and TS\textsubscript{DIR} optimized at the respective level of theory. The energies are relative to \textit{cis}-PSB3 optimized at the same level of theory. The positions of the optimized transition states along the BLA coordinate are marked with grey circles. CASPT2 displays an artifact near the center of the curve due to its proximity to the CASSCF CI. (See also text) \textbf{B} and \textbf{C}. The MRPT2 energies along the MEP\textsubscript{CT} and MEP\textsubscript{DIR} paths computed using MRPT2 numerical gradients (solid lines) and their energies along the same path computed at the CASSCF level of theory (dashed lines).
Table 3.1. The relative MRPT2 energies of $\text{trans}$-PSB3, $\text{TS}_{\text{CT}}$, and $\text{TS}_{\text{DIR}}$ computed at the MRPT2 and CASSCF optimized geometries. The single point energy calculations and optimizations were performed with the 6-31G* basis set. The energies are relative to $\text{cis}$-PSB3.

<table>
<thead>
<tr>
<th>Energy</th>
<th>CASPT2</th>
<th>CASPT2</th>
<th>XMCQDPT2</th>
<th>XMCQDPT2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geometry</td>
<td>CASSCF</td>
<td>CASPT2</td>
<td>CASSCF</td>
<td>XMCQDPT2</td>
</tr>
<tr>
<td>$\text{trans}$-PSB3 (kcal/mol)</td>
<td>-3.1</td>
<td>-3.1</td>
<td>-2.8</td>
<td>-2.8</td>
</tr>
<tr>
<td>$\text{TS}_{\text{CT}}$ (kcal/mol)</td>
<td>49.3</td>
<td>49.4</td>
<td>46.9</td>
<td>46.9</td>
</tr>
<tr>
<td>$\text{TS}_{\text{DIR}}$ (kcal/mol)</td>
<td>50.6</td>
<td>50.3</td>
<td>50.5</td>
<td>50.0</td>
</tr>
</tbody>
</table>

3.4.5. Effect of the number of roots in the state-averaging scheme

In this section we explore the effect of averaging additional roots in the CASSCF reference wavefunction. This allows a better understanding of whether the chosen reference wavefunction could provide a balanced description of the potential energy of the $S_0$ and $S_1$ states (the intersecting states) and if this remains true after performing multireference calculations. To do so we have performed a series of $n$ ($n = 2, 3, 4 \ldots 10$) root state average computations (the roots are always taken with equal weights) for one selected structure. This corresponds to the transition state $\text{TS}_{\text{CT}}$. 
Figure 3.11. Absolute energies of the electronic states at $\text{TS}_{\text{CT}}$ as a function of the number of roots averaged in the CASSCF/6-31G* reference wavefunction. The number of roots goes from 2
to 10 except for MRCISD and MRCISD+Q where it goes from 2 to 7 due to computational cost. All roots have equal weights. States (levels) with covalent/diradical character are in green, while states with charge transfer character are in red. States with correlating characters are connected by straight lines.

**Figure 3.12.** Energy Profiles along the BLA coordinate computed with $n$-roots averaged with equal weights in the CASSCF reference wavefunction with $n=2, 3, 4,$ and 5. **A.** The $n$-root state averaged CASSCF energy profiles. For $n=3,4,$ and 5 the CIs do not appear within the selected BLA geometries presented in the manuscript and therefore their positions are determined by extrapolating the graphs using a polynomial fit. **B and C.** The $n$-root CASPT2(IPEA=0.25) and MS-CASPT2(IPEA=0.25) energy profiles respectively. Energies are relative to cis-PSB3.
Figure 3.13. Energy Profiles along the MEP\textsubscript{CT} and MEP\textsubscript{DIR} coordinates computed with \(n\) roots averaged with equal weights in the CASSCF reference wavefunction with \(n=2, 3, 4,\) and 5. \textbf{A.} \(n\)-root state averaged CASSCF MEP\textsubscript{CT} energy profile. \textbf{B.} \(n\)-root state averaged CASSCF MEP\textsubscript{DIR} energy profile.
energy profile. C. n-root CASPT2(IPEA=0.25) MEP\textsubscript{CT} energy profile. D. n-root CASPT2 (IPEA=0.25) MEP\textsubscript{DIR} energy profile. E. n-root MS-CASPT2(IPEA=0.25) MEP\textsubscript{CT} energy profile. F. n-root MS-CASPT2(IPEA=0.25) MEP\textsubscript{DIR} energy profile.

**Figure 3.11** shows the results for CASSCF, CASPT2, CASPT2(IPEA=0.25) MS-CASPT2(IPEA=0.25), XMCQDPT2, QD-NEVPT2, MRCISD, and MRCISD+Q. We found that the computed energy levels are, in general, quite sensitive to the number of roots used. However, the computed changes follow a regular trend. In fact, at the CASSCF level the addition of roots with covalent character (i.e. with a wavefunction producing a positive charge located on the Schiff base fragment -C2H-C1H-NH\textsubscript{2}) stabilizes all covalent states displaying a dominant \(\psi\textsubscript{DIR}\) configuration. Similarly, addition of states with charge transfer character stabilizes states displaying a dominant \(\psi\textsubscript{CT}\) configuration. This trend is easily rationalized by considering that when a covalent state is added to the state average set, the weight of these states and, therefore, of the corresponding charge distribution, is increased in the averaged molecular orbital description. The molecular orbitals have then more contribution from the roots which are dominating the state-average CASSCF wavefunction. This leads to a stabilization of all states sharing the same electronic character and to a destabilization of the states with different character. To better quantify the effect of the number of roots used while state averaging, we present the CASSCF energy profile along the BLA coordinate with a different number of roots averaged in **Figure 3.12A**. The position of the CASSCF CI along the BLA coordinate varies dramatically with different state averaging schemes. This behavior explains the difference between our 2-root results and the 3-root results by Mori et al.\textsuperscript{7} showing a displaced CI in PSB3
at the CASSCF level of theory. Also, as expected, we see a large difference in the energy gap between \( S_0 \) and \( S_1 \) along the entire CASSCF MEP\(_{CT} \) and MEP\(_{DIR} \) paths shown in Figure 3.13.A and 3.13.B.

In Figures 3.11.B - 3.11.D we find that the CASPT2 both with and without the IPEA and MS-CASPT2(IPEA=0.25) energy levels are relatively unaffected by the number of roots in the state average set. MS-CASPT2(IPEA=0.25) only slightly differs from CASPT2(IPEA=0.25) when 7 or more states are averaged. In CASPT2, there is a degeneracy between two states in the case of 9- and 10-root averaging, but MS-CASPT2 splits this degeneracy. This is consistent with the purpose of MS-CASPT2.\(^{18} \) It is also clear from Figures 3.12.B and 3.12.C that the CASPT2(IPEA=0.25) and MS-CASPT2(IPEA=0.25) energy profiles along the BLA coordinate are also unaffected by the number of states averaged. However, in Figures 3.13.C - 3.13.F we find that the situation is a little different along the MEP\(_{CT} \) and MEP\(_{DIR} \) paths. This is especially clear in Figure 3.13.C which shows the CASPT2(IPEA=0.25) energy profiles along MEP\(_{CT} \), where once the geometry is displaced from TS\(_{CT} \) along the MEP coordinate, the energy profiles computed with a different number of states behave differently. With 2-root CASPT2(IPEA=0.25), the ground state energy profile appears regular, while the excited state seems to have a slight irregularity due to the \( S_1 \) energy at TS\(_{CT} \) being too high in energy, causing the points at -0.01 and 0.01 \( \text{Åamu}^{\frac{1}{2}} \) to appear as shallow \( S_1 \) minima. However, with 3, 4, and 5 root CASPT2 (IPEA=0.25), TS\(_{CT} \) is no longer a transition state but instead appears as an \( S_0 \) minimum, with two nearby \( S_0-S_1 \) crossings or avoided crossings near -0.02 and 0.02 \( \text{Åamu}^{\frac{1}{2}} \). MS-CASPT2 (IPEA=0.25) corrects these artifacts, but suffers from artifacts of its own since it causes a large splitting in the energy gap between \( S_0 \) and \( S_1 \).
Uncorrected MRCISD (Figure 3.11.E) displays a root dependence that is similar in trend to that observed in CASSCF, but the extent of the behavior is not as large. However, the Davidson correction (i.e. the MRCISD+Q level, Figure 3.11.F) reduces this root dependence and the energy levels become less sensitive to the number of roots used in the zeroth order wavefunction. Finally, we find that both XMCQDPT2 and QD-NEVPT2 (Figures 3.11.G and 3.11.H respectively) display a reverse dependence on the number of roots than that found for CASSCF. While, for instance, at the CASSCF level additional covalent roots induce a relative stabilization of all covalent states, at the QD-NEVPT2 and XMCQDPT2 levels these roots are destabilized. This is due to an overcompensation for the CASSCF wavefunction deterioration upon averaging of additional roots. It is shown that minimal state-averaging may be the best compromise in order to get more accurate CASSCF and MRPT2 energies. Better MRPT2 accuracies may be achieved by increasing the dimension (i.e. adding more CASCI states) in the construction of the effective Hamiltonian (e.g. in XMCQDPT2) while keeping the underlying state-averaging scheme for CASSCF the same, as shown in the case of butadiene in ref. 19.
Figure 3.14. A - E. Energy profiles along the BLA coordinate with different active space sizes for CASSCF, CASPT2, CASPT2(IPEA=0.25), XMCQDPT2, and QD-NEVPT2 respectively. Results are shown for 6-in-6 in red, 6-in-8 in blue, and 6-in-10 in violet in each case. The energies are relative to cis-PSB3 computed with the respective active space, and are compared with MRCISD +Q energies computed with a 6-in-6 reference space shown as a black dotted line in each graph (a larger active space for MRCISD+Q is currently not affordable).
3.4.6. Effect of the active space

With the aim to clarify the origin of the difficulties found for a balanced description of the charge transfer and diradical/covalent states, we have investigated the effect of increasing the active space size on the energy profiles computed along the BLA coordinate. Starting from the reference 6-in-6 active space, the space is gradually enlarged by adding more $\pi^*$-orbitals, obtaining the 6-in-8 and 6-in-10 active spaces. The 2-root state average CASSCF results with the different active spaces are reported in Figure 3.14.A together with the MRCISD+Q 6-in-6 data. Comparison of the energy profiles shows that the MRCISD+Q behavior is gradually approached by CASSCF when the active space is enlarged (the CASSCF wavefunction is improved), with the CI moving to the right of the figure. This is due to a marked stabilization of the charge transfer state when more $\pi$-orbitals are active. The application of MRPT2 approaches on these CASSCF wavefunctions leads to the results reported in Figures 3.14.B - F. While expansion of the active space stabilizes the charge transfer state at the CASSCF level, the opposite effect is observed with the MRPT2 method where there is generally a destabilization of the charge transfer state with respect to the covalent state (most prominently with the 6-in-8 active space, with a partial recovery when the basis set is further expanded to 6-in-10). For QD-NEVPT2, the expansion of the active space allows the energy profile to markedly approach that of MRCISD+Q with a 6-in-6 reference space. However, CASPT2, CASPT2(IPEA=0.25), and XMCQDPT2 show a more modest variation where their agreement with 6-in-6 MRCISD+Q decreases.
3.4.7. Remarks on the CASPT2//CASSCF/6-31G* level

The CASPT2//CASSCF/6-31G* protocol has been widely used for evaluating vertical and 0-0 excitation energies as well as ground and excited state reaction barriers. For instance, this methodology has been used in quantum-mechanics/molecular-mechanics computation to investigate the spectroscopy of different proteins yielding absorption maxima with errors of a few kcal/mol with respect to experimentally determined values. Here we use the PSB3 results to assess the validity of this protocol by comparing CASPT2//CASSCF/6-31G* relative energies with the corresponding quantities computed at the CASPT2(IPEA=0.25)/ANO-VDZP, CASPT2(IPEA=0.25)/ANO-VTZP using the CASPT2/6-31G* geometry.

<table>
<thead>
<tr>
<th>Energy</th>
<th>CASPT2(IPEA=0) (6-31G*)</th>
<th>CASPT2(IPEA=0.25) (6-31G*)</th>
<th>CASPT2(IPEA=0.25) (ANO-L-VDZP)</th>
<th>CASPT2(IPEA=0.25) (ANO-L-VTZP)</th>
<th>CASPT2(IPEA=0.25) (ANO-L-VDZP)</th>
<th>CASPT2(IPEA=0.25) (ANO-L-VTZP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-PSB3 S0-S1 excitation energy (kcal/mol)</td>
<td>92.0</td>
<td>98.2</td>
<td>95.1</td>
<td>94.1</td>
<td>93.2</td>
<td>92.3 (92.2)*</td>
</tr>
<tr>
<td>trans-PSB3 S0-S1 excitation energy (kcal/mol)</td>
<td>94.4</td>
<td>100.9</td>
<td>98.6</td>
<td>97.6</td>
<td>97.1</td>
<td>96.2</td>
</tr>
<tr>
<td>TSCT S0 energy relative to cis-PSB3 (kcal/mol)</td>
<td>-3.1</td>
<td>-3.1</td>
<td>-2.9</td>
<td>-2.8</td>
<td>-2.9</td>
<td>-2.8</td>
</tr>
<tr>
<td>TSDIR S0 energy relative to cis-PSB3 (kcal/mol)</td>
<td>49.3</td>
<td>48.9</td>
<td>47.6</td>
<td>48.0</td>
<td>47.6</td>
<td>48.0</td>
</tr>
</tbody>
</table>

Table 3.2. A table investigating the effect of using CASPT2(IPEA=0.25), expanding the basis set, and optimizing the structures at the CASPT2 level on the vertical excitation energies at the cis-PSB3 and trans-PSB3 geometries, the activation barriers via TSCT and TSDIR, and on the relative energies of S0 cis-PSB3 and trans-PSB3. Highlighted cells correspond to the methods that best reproduce the energies obtained at the superior CASPT2(IPEA=0.25)/ANO-L-VTZP//CASPT2/6-31G* level of theory (right column) but at a much lower computational cost. * The value in parentheses is an experimentally determined excitation energy of a PSB3 derivative from ref. 53. Note however that this value was measured in methanol and dichloromethane, and corresponds to the absorption maximum, and therefore only gives us an idea of the 0-0 absorption of gas-phase PSB3.
The results in Table 3.2 indicate that the success of the CASPT2/CASSCF/6-31G* protocol in predicting vertical excitation energies can be attributed to a cancellation of errors. Larger basis sets would red-shift the computed excitation energy. We also find that optimizing the ground state reactant at the CASPT2 level rather than the CASSCF level before evaluating the excitation energy also red-shifts the absorption, consistent with a recent study.\textsuperscript{56} On the other hand, the more accurate CASPT2(IPEA=0.25) level (i.e. the use of the IPEA shift), in all cases leads to blue-shifting. The data in Table 3.2 show that, for PSB3, the red-shifting and blue-shifting effects counterbalance each other when using CASPT2(IPEA=0.25) and large basis sets such as ANO-VTZP and more so when also using the CASPT2 optimized geometry. This is consistent with findings in a previous study on adenine,\textsuperscript{57} and with data presented in ref. 6. As a result, we find that the computed CASPT2/CASSCF/6-31G* value is close to experimentally determined absorption maxima available for a PSB3 derivative\textsuperscript{53} which, however, have been recorded in methanol and dichloromethane.

The cancellation effects do not appear to operate when evaluating the reaction barriers or the relative stability of TS\textsubscript{CT} and TS\textsubscript{DIR}. In this case it appears to be more accurate to use the CASPT2(IPEA=0.25)/CASSCF/6-31G* level. In fact this level produces barriers and stabilities close to the ones computed at the CASPT2(IPEA=0.25)/ANO-VTZP level using the CASPT2/6-31G* optimized geometry. The failure of the cancellation effect in this case can be understood by considering the nature of the wavefunction characterizing S\textsubscript{0} and S\textsubscript{1} at cis- and trans- PSB3 and of the wavefunctions characterizing TS\textsubscript{DIR} and TS\textsubscript{CT}. A simple analysis reveals that the S\textsubscript{1} wavefunction of cis-PSB3 and trans-PSB3, which is dominated by a charge-transfer configuration, has a certain percentage of diradical character with respect to the closed-shell
character of the ground state. Since the IPEA shift is known to increase the energy of open-shell states\textsuperscript{29} it is evident that the CASPT2(IPEA=0.25) level blue-shifts the absorption (opposite to the basis set effect that stabilizes charge-transfer states with respect to the ground state). In contrast, when computing the relative transition state stabilities the situation is inverted. In fact, in this case, TS\textsubscript{DIR} has an almost purely diradical character state while TS\textsubscript{CT} is closed-shell such that the IPEA shift and basis set effect go in the same direction. Therefore we find that the IPEA shift increases the TS\textsubscript{DIR} barrier while the improvement of the basis set primarily stabilizes the TS\textsubscript{CT} barrier.

3.5. Results and Discussion Part 2: EOM-CC methods and comparison with MRCISD+Q

Here we systematically describe and compare the shapes of the potential energy profiles along the BLA, MEP\textsubscript{CT}, and MEP\textsubscript{DIR} coordinates obtained with EOM-CC methods, and compare them to those of CASSCF, MRCISD, and MRCISD+Q

3.5.1. BLA path

The S\textsubscript{0} and S\textsubscript{1} energies computed with various EOM-CC methods are compared to those of CASSCF, MRCISD and MRCISD+Q in Figure 3.15. We again assume that MRCISD+Q provides the highest-quality potential energy surfaces.
Figure 3.15. The $S_0$ and $S_1$ energy profiles along the BLA coordinate. The methods displayed are EOM-EEMCCSD (green), EOM-SF-CCSD/ UHF (blue), EOM-SF-CCSD/ROHF (violet), EOM-SF-CCSD(dT)/ ROHF (orange), EOM-SF-CCSD(fT)/ROHF (brown), uncorrected MRCISD (gray), and MRCISD+Q (black), along with CASSCF (red). The energy values are relative to the reactant (cis-PSB3). The position of the CI for each method is indicated with a filled circle. The curves are labeled on the left to distinguish between the diabatic curves with ionic/charge transfer ($\psi_{CT}$) character and the covalent/diradical ($\psi_{DIR}$) character for each method.

Below, we will discuss the effect of the various methods on the relative stabilities of the $\psi_{CT}$ and $\psi_{DIR}$ curves along the BLA coordinate. To better quantify this effect, we report (Table 3.3) the $S_0$-$S_1$ energy gap at the $T_{SDIR}$ and $T_{SDIR}$ geometries, to complement the information shown in Figure 3.15. To provide additional measure of the discrepancies between different methods, here we also report non-parallelity errors (NPEs). NPEs are useful for quantitatively comparing different methods along a potential energy path. They are computed by finding the energy deviation of each point along the path from the reference method (in our case taken to be MRCISD+Q), and then subtracting the minimum deviation from the maximum deviation. A large
NPE generally indicates that a method differentially treats different regions of the PES, whereas a zero NPE corresponds to the identical-shape surfaces (note that NPE does not say anything about the relative positions of the two surfaces).

<table>
<thead>
<tr>
<th>Method</th>
<th>$\Delta E$ at TSCT</th>
<th>$\Delta E$ at TSDIR</th>
<th>$\Delta E$ at cis-PSB3</th>
<th>NPE along $\Psi_{CT}$ curve</th>
<th>NPE along $\Psi_{DIR}$ curve</th>
<th>S0 NPE</th>
<th>S1 NPE</th>
<th>S0 NPE</th>
<th>S1 NPE</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRCISD+Q</td>
<td>10.2</td>
<td>0.6</td>
<td>101.4</td>
<td>-</td>
<td>-</td>
<td>0.0</td>
<td>0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MRCISD</td>
<td>8.8</td>
<td>1.6</td>
<td>104.8</td>
<td>0.6</td>
<td>0.6</td>
<td>1.7</td>
<td>1.3</td>
<td>0.6</td>
<td>0.4</td>
</tr>
<tr>
<td>CASSCF</td>
<td>4.5</td>
<td>7.4</td>
<td>110.3</td>
<td>0.8</td>
<td>2.4</td>
<td>5.6</td>
<td>4.3</td>
<td>2.3</td>
<td>2.3</td>
</tr>
<tr>
<td>EE-CCSD/UHF</td>
<td>16.6</td>
<td>6.9</td>
<td>-</td>
<td>0.4</td>
<td>0.3</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SF-CCSD/UHF</td>
<td>2.4</td>
<td>7.5</td>
<td>-</td>
<td>0.5</td>
<td>0.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>SF-CCSD(dT)/ROHF</td>
<td>5.8</td>
<td>6.0</td>
<td>105.5</td>
<td>2.2 (2.2)</td>
<td>0.2 (0.1)</td>
<td>3.3</td>
<td>2.9</td>
<td>1.8</td>
<td>3.2</td>
</tr>
<tr>
<td>SF-CCSD(T)/ROHF</td>
<td>11.1</td>
<td>0.6</td>
<td>102.1</td>
<td>1.4 (1.0)</td>
<td>0.9 (0.2)</td>
<td>0.4</td>
<td>0.4</td>
<td>1.3</td>
<td>0.8</td>
</tr>
</tbody>
</table>
| S0-S1 energy gaps: ΔE, kcal/mol at TSCT, TSDIR, and cis-PSB3, as well as the non-parallelity errors (NPEs, kcal/mol) along the BLA, MEPCT, and MEPDIR paths for various methods examined in this study. MRCISD+Q is our reference method against which all NPEs are computed. Energy gaps reported in bold have a different order of states with respect to MRCISD+Q at the TS_DIR geometry. NPEs were computed along the entire BLA path of Figure 3.15, and along a section (ranging from -0.08 to 0.08 Angstrom•amu$^{1/2}$) of the MEP_CT and MEP_DIR paths. Values reported in parentheses are NPEs calculated when ignoring the kinks along the BLA energy profile which are discussed in the Results and Discussion section on the BLA path.

As Figure 3.15 illustrates, EOM-EE-CCSD yields potential energy curves that have correct shapes. The NPEs relative to MRCISD+Q are 0.4 and 0.3 kcal/mol for the $\Psi_{CT}$ and $\Psi_{DIR}$, respectively. This is not surprising, as the analysis of the EOM-SF wave function amplitudes (below) confirms essentially single-configurational character of $\Psi_{CT}$, which is dominated by the closed-shell determinant at all BLA values (the leading EOM-SF amplitude corresponding to this configuration is 0.86-0.94 along the scan). Thus, the CCSD wave function is qualitatively correct.
for $\Psi_{\text{CT}}$ and the open-shell diradical state, $\Psi_{\text{DIR}}$, can be correctly described by EOM. However, the two curves are considerably shifted with respect to each other, i.e., the $\Psi_{\text{CT}}$ curve is overstabilized and the $\Psi_{\text{DIR}}$ curve is destabilized (the gap between the two states at $T_{\text{ST}}$ is 16.6 kcal/mol, to be compared to the MRCISD+Q value of 10.2 kcal/mol). As a result, the EOM-EE-CCSD CI is shifted to a much larger BLA value, and does not even appear within the selected BLA coordinate values shown in Figure 3.15. This is expected, as EOM-EE treats the two states on a different footing, i.e., $\Psi_{\text{CT}}$ as the reference and $\Psi_{\text{DIR}}$ as an EOM state. Consequently, EOM-EE fails to describe the degeneracy between the two states correctly.

The description of the relative position of the two states is improved when using the spin flip (SF) approach. EOM-SF-CCSD/UHF yields the $\Psi_{\text{DIR}}$ curve that is only slightly overstabilized with respect to MRCISD+Q, and the $\Psi_{\text{CT}}$ curve that is not stable enough, thus shifting the CI geometry to the BLA value of ca. -0.005 Å, which is too low compared to that of the MRCISD+Q CI (the BLA value of ca. 0.030 Angstroms).

Next, we investigate the effect of reducing spin-contamination in EOM-SF-CCSD by employing a ROHF reference. Consistently with large EOM-SF-CCSD/UHF spin-contamination (see the Methods section above), we observe a noticeable change in the curves. Whereas the effect on the $\Psi_{\text{DIR}}$ curve is small, reducing spin-contamination does stabilize the $\Psi_{\text{CT}}$ curve and, therefore, brings it closer to the MRCISD+Q $\Psi_{\text{CT}}$ one. Consequently, this also moves the CI geometry towards that of MRCISD+Q.
Interestingly, we observe a kink on the EOM-SF-CCSD/ROHF BLA energy profile near 0.00 Angstroms of the BLA coordinate, that is, near the CI. The analysis of the SF wave function of the $\Psi_{CT}$ state reveals that the discontinuity is correlated with the changes in the wave function composition. As we show in Figure 3.16, the leading coefficient in the $\Psi_{CT}$ wave function, which corresponds to the closed-shell determinant, changes from 0.86 to 0.95 along the BLA scan except for the point at 0.00 Å where it drops to 0.84 (thus, the share of the diradical configurations in the wave function increases). This change in the wave function is also responsible for the large NPE for $\Psi_{CT}$ (see Table 3.3). Note that the character of the triplet reference state varies smoothly, and the respective curves (ROHF, CCSD, and EOM-SF-CCSD, see Figure 3.17) have no kinks. We note that the computed energies correspond to adiabatic states, and the diabatic (i.e., $\Psi_{CT}$ and $\Psi_{DIR}$) curves are drawn by simply connecting the dots following the leading character of the adiabatic wave functions. Thus, the discontinuity of the so-
drawn diabatic curves should disappear if proper diabatization is performed. A similar behavior is sometimes observed in the MRCISD wave functions giving rise to kinks in MRCISD(+Q) curves near a MRCISD CI. This problem is rectified by “rotating” the MR-CISD states, which is exploited in the modified Davidson correction; a similar procedure could be used within the EOM-SF-CCSD formalism.

Figure 3.17. A, B, and C. A plot of the ROHF, CCSD, and EOM-SF-CCSD reference triplet state energies along the BLA path, respectively. Notice that there are no kinks along these reference states.
Finally, we investigate the effect of including the correction due to triple excitations in EOM-SF-CCSD. The two corrections, denoted as (dT) and (fT), are derived by using second-order Rayleigh-Schroedinger perturbation theory starting from the EOM-SF-CCSD solutions as zero-order states. The difference between the two corrections is in the definition of the triples-triples block of $H_0$. In (dT), the diagonal of the full similarity transformed Hamiltonian is employed, whereas in (fT) the Fock matrix (i.e., orbital energy differences) is used. We consider (dT) to be superior. In previous benchmark studies, the performance of both corrections was found to be similar.

The inclusion of triples correction leads, as expected, to a further stabilization of the $\Psi_{CT}$ curve and, therefore, to a better agreement with the MRCISD+Q energy profile. Both the EOM-SF-CCSD(dT)/ROHF and EOM-SF-CCSD(fT)/ROHF BLA energy profiles of Figure 3.15 are effectively reproducing the MRCISD+Q result, with both the $\Psi_{CT}$ and $\Psi_{DIR}$ curves being only slightly lower in energy. However, we note that the artifact from the EOM-SF-CCSD/ROHF calculations persists. There is a clear discontinuity in the BLA energy profile occurring near the BLA coordinate of 0.00 Å for all three EOM-SF/ROHF methods. This is expected as the discontinuity originates in the mixing of the zero-order wave functions of the two diabatic states near the CI. In such situations, it would be more appropriate to apply perturbation theory for the degenerate states, rather than follow non-degenerate formalism as was done in deriving (dT) and (fT) triples corrections.

Since this kink also corresponds to the change in the wave function composition, we note that the triple excitation correction curves behave slightly differently before and after the kink. At negative BLA values (before the kink) the EOM-SF-CCSD(fT)/ROHF profile is in better
agreement with MRCISD+Q, as indicated by the agreement of the $S_0$-$S_1$ energy gap at the $TS_{CT}$
geometry, while EOM-SF-CCSD(dT)/ROHF slightly overstabilizes the $\Psi_{CT}$ state yielding a
larger gap. However, at positive BLA values (after the kink), we find that EOM-SF-CCSD(dT)/
ROHF is in better agreement with MRCISD+Q, as indicated by the energy gap at $TS_{DIR}$ and the
similar position of the CI in Figure 3.15. On the other hand, EOM-SF-CCSD(fT)/ROHF
reverses the state ordering at the $TS_{DIR}$ geometry, shifts the CI towards lower BLA values with
respect to MRCISD+Q. These differences appear to fall within 1-2 kcal/mol and yield a
difference in the position of the crossing of ca. 0.005 Å.

3.5.2. MEP$_{CT}$ path

Figure 3.18.A presents the $S_0$ and $S_1$ EOM-SF-CCSD/ROHF and EOM-SF-CCSD(dT)/
ROHF energies along the MEP$_{CT}$ path and compares them to CASSCF, MRCISD, and MRCISD
+Q. The energies at 0 Å of the MEP$_{CT}$ path are identical to the point at -0.016 Å along the BLA
coordinate since they correspond to the same geometry ($TS_{CT}$, where the two paths cross). At the
$TS_{CT}$ geometry, the $S_0$ state is of the $\Psi_{CT}$ character, whereas $S_1$ is $\Psi_{DIR}$. As we move away from
$TS_{CT}$ along the MEP$_{CT}$ path either towards the reactant or the product, we find that the $S_0$ wave
function gradually loses its charge-transfer character and becomes more mixed with the diradical
configurations (see Figure 3.18.B). In light of this, the shape of the EOM-SF-CCSD/ROHF
energy profile along MEP$_{CT}$ can be rationalized by the tendency of this method to overstabilize
the $\Psi_{DIR}$ wave functions with respect to $\Psi_{CT}$. At the cis-PSB3 and trans-PSB3 geometries, where
the $S_0$ state is $\Psi_{DIR}$ and the $S_1$ state is $\Psi_{CT}$, we find that EOM-SF-CCSD/ROHF overestimates the
$S_0$-$S_1$ energy gap, as compared to MRCISD+Q (see the $S_0$-$S_1$ gaps reported in Table 3.3).
Meanwhile, at the TSCT geometry, where the S₀ state is Ψₐ and S₁ is ΨDIR, EOM-SF-CCSD/ROHF underestimates the S₀-S₁ energy gap (also see Table 3.3). This change in wave function along the MEPₐ path is responsible for the large NPE for EOM-SF-CCSD/ROHF (NPEs of 3.3 and 2.9 kcal/mol for S₀ and S₁, respectively).
Figure 3.18. Energy profiles along the MEP\textsubscript{CT} coordinate. A. The S\textsubscript{0} and S\textsubscript{1} energies computed using EOM-SF-CCSD/ROHF (violet), EOM-SF-CCSD(dT)/ROHF (orange), uncorrected MRCISD (grey), MRCISD+Q (black), and CASSCF (red). The energies are relative to the reactant (\textit{cis}-PSB3, -0.54 Å). The region defined by -0.02 to 0.02 Å is magnified in the inset. B. The charge-transfer character along MEP\textsubscript{CT} for CASSCF (red), uncorrected MRCISD (grey), and EOM-SF-CCSD/ROHF (violet). The inset shows that at the MRCISD level the charge-transfer character of S\textsubscript{0} extends from points -20 to 22.
On the other hand, the EOM-SF-CCSD(dT)/ROHF method yields the MEP\(_{\text{CT}}\) profile that is much more similar to MRCISD+Q (NPEs of 0.4 kcal/mol for both S\(_0\) and S\(_1\)); however, it has consistently lower S\(_0\) and S\(_1\) energies for the non-planar structures (i.e. structures along the -0.08 to +0.08 Angstroms range of the MEP\(_{\text{CT}}\) path). In the case of planar structures (\textit{cis} and \textit{trans}-PSB3) the EOM-SF-CCSD(dT)/ROHF S\(_0\) and S\(_1\) relative energies agree quantitatively with MRCISD+Q (for \textit{cis}-PSB3: 102.1 kcal/mol compared to 101.4 kcal/mol, respectively).

**Figure 3.18.B** illustrates the EOM-SF-CCSD/ROHF S\(_0\) charge-transfer character (as quantified by partial Mulliken charges) along the MEP\(_{\text{CT}}\) path. We find that at the TS\(_{\text{CT}}\) geometry, S\(_0\) has slightly less charge-transfer character than it does at the CASSCF and MRCISD levels. Moreover, we find that the EOM-SF-CCSD/ROHF charge transfer profile is intermediate between that of CASSCF and MRCISD. This is consistent with the observation that EOM-SF-CCSD/ROHF stabilizes \(\Psi_{\text{CT}}\) more than \(\Psi_{\text{DIR}}\) compared to CASSCF, but not as much as MRCISD, and therefore has an intermediate extension of the charge transfer region.

### 3.5.3. MEP\(_{\text{DIR}}\) path

The S\(_0\) and S\(_1\) EOM-SF-CCSD/ROHF and EOM-SF-CCSD(dT)/ROHF energies along the MEP\(_{\text{DIR}}\) path are presented in **Figure 3.19.A** along with the CASSCF, MRCISD and MRCISD+Q energies. Along this scan, the point at 0.00 Å of the MEP\(_{\text{DIR}}\) path corresponds to TS\(_{\text{DIR}}\) and is identical to the point at around 0.025 Å of the BLA coordinate. At the MRCISD and EOM-SF-CCSD/ROHF levels of theory, the entire S\(_0\) state along this path is described by a \(\Psi_{\text{DIR}}\) wave function, whereas the S\(_1\) state is \(\Psi_{\text{CT}}\). As a result, we find that EOM-SF-CCSD/ROHF uniformly overstabilizes the S\(_0\) state and destabilizes the S\(_1\) state at the \textit{cis}-PSB3, TS\(_{\text{DIR}}\), and
trans-PSB3 geometries with respect to MRCISD+Q, leading consistently to an overestimated $S_0$-$S_1$ energy gap (by ca. 5 kcal/mol at both the equilibrium structures and at TS$_{\text{DIR}}$). However, along the path connecting the TS to the reactant and product there is an agreement between the EOM-SF-CCSD/ROHF and MRCISD+Q $S_1$ energy profiles along both the MEP$_{\text{CT}}$ (Figure 3.18.A) and MEP$_{\text{DIR}}$ (Figure 3.19.A) paths. The NPE for EOM-SF-CCSD/ROHF along the MEP$_{\text{DIR}}$ path is 1.8 kcal/mol and 3.2 kcal/mol for $S_0$ and $S_1$, respectively.
Figure 3.19. Energy profiles along the MEP\textsubscript{DIR} coordinate. \textbf{A.} The S\textsubscript{0} and S\textsubscript{1} energies computed using EOM-SF-CCSD/ROHF (violet), EOM-SF-CCSD(dT)/ROHF (orange), uncorrected MRCISD (grey), MRCISD+Q (black), and CASSCF (red). The energies are relative to the reactant (\textit{cis}-PSB\textsubscript{3}, -0.52 Å). The inset magnifies the region from -0.02 to 0.02 Å. \textbf{B.} The charge-transfer character along MEP\textsubscript{DIR} for CASSCF (red), uncorrected MRCISD (grey), and EOM-SF-CCSD/ROHF (violet).
As for the EOM-SF-CCSD(dT)/ROHF energy profile along the MEP\textsubscript{DIR} path (Figure 3.19.A), we find, again, that it is in good agreement with MRCISD+Q, but (consistently with the results shown in Figures 3.15 and 3.18.A) is lower in energy along the entire path (NPE error is 1.3 kcal/mol and 0.8 kcal/mol for S\textsubscript{0} and S\textsubscript{1} respectively). As shown in the inset of Figure 3.19.A, the TS\textsubscript{DIR} geometry is very close to the CI (i.e., the two states are almost degenerate), and, in fact, the S\textsubscript{0} state is of \(\Psi\textsubscript{CT}\) character at this level of theory (this is clear in Figure 3.15).

Finally, in Figure 3.19.B we show the EOM-SF-CCSD/ROHF S\textsubscript{0} charge-transfer character along the MEP\textsubscript{DIR} path where we find that at the TS\textsubscript{DIR} geometry, the wave function has slightly less diradical character (i.e. more charge-transfer character) than it does at the CASSCF and MRCISD levels. Here the extension of the diradical region shrinks compared to CASSCF as a result of the stabilization/increase in extension of the charge transfer region, but again not to the same extent as MRCISD.

3.6. Conclusions

Our computations demonstrate that the low-lying cross-sections of the S\textsubscript{0} CASSCF potential energy surface of PSB3 investigated in this work are qualitatively different when computed with methodologies accounting for dynamic electron correlation. In general, MRCISD +Q, all MRPT2, and higher level EOM-CC implementations point to a large stabilization of the region dominated by the charge-transfer configuration (\(\psi\textsubscript{CT}\)) with respect to the region dominated by a covalent-diradical structure (\(\psi\textsubscript{DIR}\)). The stabilization of the charge-transfer region is also reflected by its extension along the RC coordinate.
Figure 3.20. A. Schematic shape of the S₀ CASSCF potential energy surface consistent with the results of our computations. B. The same schematic representations based on the results of MRCISD+Q computations.

The rather dramatic change in shape of the CASSCF energy surface (Figure 3.20.A) with respect to electron correlated surfaces (Figure 3.20.B) has important implications. While we find that the optimization of the TS\textsubscript{CT} and TS\textsubscript{DIR} transition states yields acceptable geometries, and the shape of the \(\psi\textsubscript{CT}\) and \(\psi\textsubscript{DIR}\) curves is similar at all levels of theory presented in this work, the relative energies of these two regions is different. This implies that optimizations may be performed at the CASSCF level of theory, but dynamic electron correlation is important to obtain quantitative energies, especially regarding the relative height of the two transition state barriers. This can be achieved with MRPT2 methods or EOM-CCSD with triples correction.
The results presented in this chapter, while focusing on the $S_0$ PES, also have important implications for the photochemistry of PSB3. A recent study presented semi-classical trajectory computations for a CASSCF/6-31G*/AMBER model of bovine rhodopsin (Rh), a dim-light rod visual pigment$^{58}$. Such trajectories provided a simulation of the 11-$cis$ to all-$trans$ photochemical isomerization of rPSB in Rh. The analysis of the charge distribution along different trajectories indicated that upon $S_1$ to $S_0$ decay in the region of a CI (where the reactive C11=C12 double-bond is fully twisted) the rPSB chromophore may evolve along a charge-transfer region of the $S_0$ potential energy surface. A direct chemical consequence of such an event is that the double-bond reconstitution is delayed as $\psi_{CT}$ imposes a single bond character along C11=C12. This has been demonstrated for PSB3 by a quantitative valence-bond analysis of the CASSCF wavefunction$^{11}$ showing that in $\psi_{DIR}$ singlet spin-paired $\pi$-electrons are ready to re-couple along the reactive bond while in $\psi_{CT}$ the $\pi$-electrons are spin-paired along adjacent bonds. In other words, even if the chromophore starts twisting along the reaction (isomerization) coordinate it will have to find its way to a region dominated by the $\psi_{DIR}$ configuration before the $\pi$-bond reconstitution can begin. As detailed in ref. 58, such a delay has an impact on the fate of the trajectory that may result in either a reactive (i.e. leading to the bathorhodopsin photoproduct) or non-reactive (i.e. leading back to the Rh reactant) event and, in principle, on the reaction quantum yield. The results presented here suggest that the relaxation dynamics occurring immediately after the decay at the CI could be qualitatively different in these two situations. The peaked CI of the CASSCF energy surface would probably favor a “ballistic” decay where the majority of the population would hop during the first approach to the CI region. The same surface suggests that after decay the population will mainly be collected in a covalent/diradical region favoring reconstitution of
the central double bond. In contrast, the MRCISD+Q energy surface featuring a sloped-intermediate CI would probably drive a less straightforward decay process. As a consequence, while our study indicates that the use of CASSCF is still possible to study the mechanism of the photochemical process in question (i.e. the minima, transition states, and a CI are successfully located in PSB3), but caution must be exercised when drawing conclusions from CASSCF semiclassical trajectory calculations especially in terms of dynamics and statistical branching of an excited state population after the decay. One remarkable feature is the flattening and widening of the charge transfer region which suggests that a larger number of trajectories will be collected in this region after decaying to \( S_0 \), thus producing longer-lived transient species with the features of an intramolecular twisted charge-transfer state. In principle, similar changes may also have an impact on our understanding of the dynamics associated to the photoisomerization of visual pigments.

The PSB5 model incorporating five conjugating double bonds (and therefore closer to rPSB) has the \( T_{\text{DIR}} \) and \( T_{\text{CT}} \) transition states separated by 0.7 kcal/mol on the CASSCF energy surface. In this situation, it is likely that the charge-transfer state becomes, as indicated by our shorter model, stabilized by the dynamic electron correlation yielding similar dramatic effects on the ground state relaxation dynamics even when embedded in a protein cavity. Of course, as previously documented, and as we will see in Chapter V, the environment (be it solvent or protein) around rPSB may have important additional effects on the relative stability of the charge transfer and diradical/covalent regions of the \( S_0 \) potential energy surface.
3.7. References


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4.1. Abstract

The photoisomerization of the retinal chromophore of visual pigments proceeds along a complex reaction coordinate on a multidimensional surface that comprises a hydrogen-out-of-plane (HOOP) coordinate, a bond length alternation (BLA) coordinate, a single bond torsion and, finally, the reactive double bond torsion. These degrees of freedom are coupled with changes in the electronic structure of the chromophore, and therefore the computational investigation of the photochemistry of such systems requires the use of a methodology capable of describing electronic structure changes along all those coordinates. Here, we employ the penta-2,4-dieniminium (PSB3) cation as a minimal model of the retinal chromophore of visual pigments and compare its excited state isomerization paths at the CASSCF and CASPT2 levels of theory. These paths connect the cis isomer and the trans isomer of PSB3 to two structurally and energetically distinct conical intersections (CIs) which belong to the same seam of CIs. MRCISD +Q energy profiles along these paths provide benchmark values against which other ab initio methods are validated. Accordingly, we compare the energy profiles of MRPT2 methods (CASPT2, QD-NEVPT2, and XMCQDPT2) and EOM-SF-CC methods (EOM-SF-CCSD and EOM-SF-CCSD(dT)) to the MRCISD+Q reference profiles. We find that the paths produced

\[\text{i This Chapter is based on the following article:}\]

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with CASSCF and CASPT2 are topologically and energetically different, partially due to the existence of a “locally excited” region on the CASPT2 excited state near the Franck-Condon point that is absent in CASSCF and that involves single bond, rather than double bond, torsion. We also find that MRPT2 methods as well as EOM-SF-CCSD(dT) are capable of quantitatively describing the processes involved in the photoisomerization of systems like PSB3.

4.2. Introduction

The penta-2,4-dieniminium cation (PSB3, see Figure 4.1) is a conjugated and protonated imine which has been used extensively as a computational model of the retinal protonated Schiff base chromophore (rPSB) of visual pigments.\textsuperscript{1-4} In the previous chapter, we benchmarked the effect of introducing dynamic electron correlation along three paths spanning the ground state ($S_0$) potential energy surface (PES) of PSB3 using several electronic structure methods. These included multireference configuration interaction with single and double excitations\textsuperscript{5,6} (MRCISD) with and without the Davidson correction Q\textsuperscript{7}, multireference second order perturbation theory (MRPT2) methods, and equation-of-motion coupled-cluster (EOM-CC) methods\textsuperscript{8,9}. 

In the present chapter, we map a different region of the PES, this time focusing on three paths generated on the first singlet excited state \((S_1)\) state rather than the \(S_0\) state. Two paths (named MEP\(_{\text{CIS}}\) and MEP\(_{\text{TRANS}}\)) lead from the Franck-Condon (FC) point of one isomer of PSB3 (cis- or trans-PSB3, shown in Figure 4.1) to a conical intersection (CI). MEP\(_{\text{CIS}}\) is similar to the path already computed in ref. 10. The third path is along a seam of CIs that connects the two above paths; this path is named the CI seam path. Note that while the first two paths are characteristic paths on the PES, the third is artificially constructed by an interpolation of coordinates (see methods section).

Upon photoexcitation, PSB3 undergoes a partial charge transfer of its positive charge from the \(=\text{C}2\text{H}−\text{C}1\text{H}=\text{NH}_2\) side (the Schiff base moiety) to the \(\text{C}5\text{H}_2=\text{C}4\text{H}−\text{C}3\text{H}=\) side of the molecule (the allyl moiety).\(^1\) Studies of PSB3 conducted at the CASSCF level of theory showed a barrierless path leading from the FC point to a CI.\(^2\) In fact, Migani et al. mapped a part of the \(S_1\) PES of PSB3 at the CASSCF level of theory by following a minimum energy path (MEP) coordinate along the \(S_1\) surface starting from the FC point of cis-PSB3.\(^{11}\) However, a number of
studies have emerged showing inconsistencies between CASSCF, which is missing dynamic electron correlation, and higher-level electronic structure methods. Fantacci et al.,\textsuperscript{12} used the CASSCF paths from Migani et al.\textsuperscript{11} to test the effect of running single point CASPT2 and TD-DFT energy calculations along these paths. They found that the barrierless CASSCF path is no longer barrierless when dynamic electron correlation is properly accounted for. Similar findings have subsequently emerged at various levels of theory and on a variety of rPSB models.\textsuperscript{13-19} As a result of those studies, it became clear that dynamic electron correlation may be important for a proper description the $S_1$ PES of PSB3.

In order to quantify these effects, we map the $S_1$ PES at both the CASSCF and CASPT2 levels of theory. Therefore, the MEP\textsubscript{CIS}, MEP\textsubscript{TRANS}, and CI seam paths were generated independently at both levels of theory, as described in the methods section below, and then single point MRCISD, MRCISD+Q, MRPT2, and EOM-CC energy calculations were performed along these paths. We found that dynamic electron correlation does indeed have an important effect on the topology of the $S_1$ PES in PSB3. The results of this work, together with the results of the previous chapter, point to the fact that dynamic electron correlation is important for a proper quantitative and qualitative description of the mechanism and dynamics of the PSB3 isomerization and, possibly, also for higher homologues.
4.3. Methods

This section outlines how the three $S_1$ paths were generated at each of the CASSCF and CASPT2 levels of theory, and how the dynamic electron correlation was introduced by single point energy calculations.

4.3.1. Reference potential energy surfaces

To prepare the reference PES, three paths have been generated at the CASSCF and CASPT2 levels of theory. All optimizations and MEP calculations employed the 6-31G* basis set with Cartesian $d$ polarization functions and with an active space of 6 electrons in 6 $\pi$ orbitals (6-in-6). In the case of CASPT2 optimizations and MEPs, the IPEA parameter was kept at the default value of 0.25. To avoid confusion, from here on all references to CASPT2 geometries and paths assume that the IPEA is for these calculations is the default 0.25 value. However, when we discuss single point calculations along the reference paths, a tag will be used to indicate whether the calculation is performed without an IPEA shift (IPEA=0) or with (IPEA=0.25). CASPT2 optimizations and MEP calculations were performed with numerical gradients.

The first path, MEP$_{\text{CIS}}$, is generated for each of CASSCF and CASPT2 by an MEP calculation started from the cis-PSB3 Franck-Condon point optimized at the respective level of theory and with a radius of $0.01 \, \text{Å} \cdot (\text{amu})^{1/2}$ until a CI is reached (the CI intercepted by the MEP$_{\text{CIS}}$ path will be called CI$_{\text{CIS}}$). In the case of CASPT2, however, the MEP started from the FC point does not lead to a CI but rather to a minimum (MIN$_{\text{CIS}}$) reached by a twisting of the C1-C2
single bond and a pyrimidilation of the Schiff base nitrogen and Cl carbon. Therefore, a transition state (TSCIS) separating MINCIS from the CI had to be located by an S1 transition state optimization, and an MEP was launched in both the forward and backward directions from this transition state. The backward MEP eventually lead to the MINCIS structure, while the forward MEP lead to a CI_CIS. The three MEP calculations were combined into one continuous path (i.e. the CASPT2 MEP_CIS path) which starts from the cis-PSB3 FC point, passes first through MINCIS, then increases in energy to reach TSCIS, and finally ends at CI_CIS.

The CASSCF and CASPT2 MEP_TRANS paths were generated in exactly the same way as the CASSCF and CASPT2 MEP_CIS paths, respectively, but starting from a trans-PSB3 structure optimized at the respective level of theory. The CASSCF MEP_TRANS path is barrierless and connects the trans-PSB3 FC point to CI_TRANS directly, while the CASPT2 MEP_TRANS path encounters a MIN_TRANS structure and a TS_TRANS structure on the way. The structures of all S1 stationary points encountered by the CASPT2 paths are shown in Figure 4.2.
Figure 4.2. Structure of MIN\textsubscript{CIS}, MIN\textsubscript{TRANS}, TSCIS, and TS\textsubscript{TRANS} optimized at the CASPT2 level of theory. The bond-line formulas are a schematic representation of the dominant electronic configurations of the corresponding states. The geometry parameters are shown in parenthesis. Bond length values are in Ångstroms and are displayed in black while torsions are displayed in a red font.

In the case of the CASSCF MEP\textsubscript{CIS} and MEP\textsubscript{TRANS} paths, the first eleven structures were chosen to be 0.01 Å·(amu)$^{1/2}$ apart, but then a structure was selected every 0.02 Å·(amu)$^{1/2}$. However, in the case of the CASPT2 MEP\textsubscript{CIS} and MEP\textsubscript{TRANS} paths, the first nine structures were chosen to be 0.01 Å·(amu)$^{1/2}$ apart, but then a structure was selected every 0.08 Å·(amu)$^{1/2}$ from after the FC region up to the TS\textsubscript{CIS} (in MEP\textsubscript{CIS}) or TS\textsubscript{TRANS} (in MEP\textsubscript{TRANS}) structure. After the transition state, a point was selected every 0.02 Å·(amu)$^{1/2}$ up to the point where the MEP intercepts a CI point.

Finally, the CI seam path was generated at each level of theory by a parabolic interpolation of internal coordinates using three structures. In the case of the CASSCF CI seam path, the three structures are the CI\textsubscript{CIS} structure intercepted by the CASSCF MEP\textsubscript{CIS} path, the
\( \text{Cl}_{\text{BLA}} \) structure which is the CI intercepted by the CASSCF BLA path of Chapter III (Figure 3.2), and the \( \text{Cl}_{\text{TRANS}} \) structure intercepted by the CASSCF ME\( \text{P}_{\text{TRANS}} \) path. As for the CASPT2 CI seam path, it is a parabolic interpolation of the \( \text{Cl}_{\text{CIS}} \) structure intercepted by the CASPT2 ME\( \text{P}_{\text{CIS}} \) path, the \( \text{Cl}_{\text{BLA}} \) structure which is the CI intercepted by the CASPT2(IPEA=0.25) BLA path of Chapter III, and the \( \text{Cl}_{\text{TRANS}} \) structure intercepted by the CASPT2 ME\( \text{P}_{\text{TRANS}} \) path. Remember that the “BLA path” is a path that follows a bond length alternation coordinate (which is the origin of the acronym BLA). The parabolic interpolation was done by fitting each internal coordinate with a polynomial fit to the second order, and using the resulting equation to generate the interpolated internal coordinate values. The geometries of \( \text{Cl}_{\text{CIS}}, \text{Cl}_{\text{BLA}}, \) and \( \text{Cl}_{\text{TRANS}} \) which were used to generate the CI seam paths are shown in Figure 4.3. While the bond lengths are not very different in the three CIs, their structures mainly differ along the hydrogen-out-of-plane (HOOP) coordinate. The HOOP (rigorously defined as the C1-C2-C3-C4 minus the H-C2-C3-H dihedral) is positive at \( \text{Cl}_{\text{CIS}} \), almost zero at \( \text{Cl}_{\text{BLA}} \), and negative at \( \text{Cl}_{\text{TRANS}} \). However, the \( \pi \) orbital overlap (\( \tau \), rigorously defined as the average of the two dihedrals involved in the HOOP) is almost 90° in all three cases, meaning that the \( \pi \) orbitals are orthogonal as would be expected at a CI.
4.3.2. Multireference configuration interaction calculations

MRCISD and MRCISD+Q calculations were performed with Molpro\textsuperscript{20} and are of the internally contracted variant\textsuperscript{21}. The reference space employed the same electrons and orbitals as in the 6-in-6 active space used in CASSCF. The 1s core orbitals in carbon and nitrogen atoms were kept frozen. For MRCISD and MRCISD+Q calculations, as well as all other calculations throughout this work, the 6-31G* basis set with Cartesian d polarization functions was consistently used. In MRCISD+Q, the Davidson correction\textsuperscript{7} with a relaxed reference\textsuperscript{22} was used.

Figure 4.3. Structures of CI\textsubscript{CIS}, CI\textsubscript{BLA}, and CI\textsubscript{TRANS} used to generate the CI seam path at both the CASSCF and CASPT2 levels of theory. The CASSCF and CASPT2 geometry parameters are shown (CASPT2 in parenthesis). Top: Bond-line structures showing bond length values in Ångstroms. Bottom: Newman projection along the C2=C3 isomerizing bond. The the H-C2=C3-H and C1-C2=C3-C4 dihedrals are labeled.
4.3.3. Multireference perturbation theory calculations

The MRPT2 methods investigated in this chapter are CASPT2\textsuperscript{23}, XMCQDPT2\textsuperscript{24} and NEVPT2\textsuperscript{25,26}.

In the case of CASPT2, we investigate both single-state CASPT2 (hereon referred to as simply CASPT2) and multistate CASPT2 (MS-CASPT2)\textsuperscript{27}, both with and without the default IPEA parameter. CASSCF and CASPT2 energy calculations, optimizations, and MEP calculations were all performed with the Molcas 7.8 quantum chemistry software package.\textsuperscript{28} An imaginary shift of 0.2 was used in CASPT2 calculations to exclude intruder states.

The quasi-degenerate (QD-) formulation of NEVPT2 is used throughout this work. The reported QD-NEVPT2 energies are based on the partially contracted variant\textsuperscript{29}

XMCQDPT2 energies were computed using Firefly version 8.0.0.\textsuperscript{30} In these calculations, the intruder state avoidance (ISA) shift was set to 0.02 to avoid intruder states. In addition to the classical version of XMCQDPT2, two more recently developed methods were also tested in this chapter (as in the previous chapter). The first, XMCQDPT2/diagonal fit, makes use of semi-canonical orbitals obtained by block-diagonalization of a standard closed-shell-like canonical Fock operator built from the two-electron integrals and the first order spin-free state-averaged CASSCF density matrix. Upon obtaining orbitals of different types, the energies of the orbitals belonging to the active space are redefined to provide the best possible one-particle least-square approximation to the diagonal of the CASSCF Hamiltonian. The second, termed XMCQDPT2/\textsuperscript{f}\textsuperscript{(}\Gamma_{ns}) applies a modified Fock-like operator which incorporates some terms arising due to the non-separable part $\Gamma_{ns}$ of the CASSCF state-averaged second-order density matrix $\Gamma$. 
4.3.4. Equation-of-motion coupled-cluster calculations

In CCSD and EOM calculations, all electrons were correlated. Only the spin-flip (SF) variant of EOM\textsuperscript{31-34} was tested due to its success in the ground state benchmark in Chapter III. The lowest high-spin triplet was employed as the reference, which was computed at the ROHF level of theory to reduce spin-contamination. In this work, we employ EOM-SF-CCSD (or, for brevity, SF-CCSD) and SF-CCSD(dT). The former includes only single and double substitutions, while the latter is augmented by a perturbative triples correction obtained by using second-order Rayleigh-Schrödinger perturbation theory.\textsuperscript{35} In SF-CCSD(dT) the diagonal of the full similarity transformed Hamiltonian is employed. All EOM-SF calculations were performed using the Q-Chem electronic structure package.\textsuperscript{36}

4.3.5. Charge transfer character

In the following, we probe the electronic character of PSB3 by looking at the charge transfer character across a certain bond. The charge transfer character across the C2=C3 bond for a certain geometry is computed as the sum of the charges of all the atoms in the allyl moiety (C5H=:\textsuperscript{2}C4H-C3H=) of PSB3. The charge transfer character across the C1-C2 bond is computed by taking the sum of the charges on the -C2H=C3H=C4H=C5\textsuperscript{2} moiety of PSB3. These charge transfer characters are computed at both the CASSCF and CASPT2 levels of theory using Mulliken population analysis computed in MOLCAS.
4.3.6. Non-parallelity errors

In the Results and Discussion section, we present and discuss non-parallelity errors (NPEs). NPEs are useful for quantitatively comparing different methods along a potential energy path. They are computed by finding the energy deviation of each point along the path from the reference method (in our case taken to be MRCISD+Q), and then subtracting the minimum deviation from the maximum deviation. A large NPE generally indicates that a method differentially treats different regions of the PES, whereas a small NPE indicates that the potential energy computed at that level of theory is parallel to that of the reference method along the path of interest.

4.4. Results and Discussion

In this section we analyze the CASSCF and CASPT2 reference paths, and then present the results for MRCISD, MRCISD+Q, MRPT2, and EOM-CC energy corrections on the two surfaces.
Figure 4.4. $S_0$ (blue) and $S_1$ (green) energy profiles along the composite CASSCF (A) and CASPT2 (B) paths comprising the MEP$_{CIS}$, CI seam, and MEP$_{TRANS}$ paths. The points corresponding to the structures shown in Figures 4.2 and 4.3 are labeled in the graph. Energies are relative to the $trans$-PSB3 $S_0$ energy, and are reported at the respective level of theory at which the geometries were generated.
4.4.1. Analysis of reference potential energy surfaces

The energy profile along the composite reaction path, comprising the MEP\textsubscript{CIS}, CI seam, and MEP\textsubscript{TRANS} paths, is shown in Figure 4.4 for each of the CASSCF and CASPT2 levels of theory. There are several differences between the CASSCF and CASPT2 paths, as highlighted above. Most notably, the molecule at the CASSCF level isomerizes immediately around the C2=C3 double bond, whereas with at the CASPT2 level it follows a barrierless but very shallow path along a C1-C2 single bond isomerization coordinate, with a barrier along the C2=C3 double bond which needs to be overcome to lead to \textit{cis-trans} isomerization. Notice that the MIN\textsubscript{CIS} and MIN\textsubscript{TRANS} structures occupy a very flat region of the S\textsubscript{1} PES.

At the FC point of \textit{cis}-PSB3 and \textit{trans}-PSB3, there is steep decrease in energy in the S\textsubscript{1} state (and a corresponding rise in energy in the S\textsubscript{0} state). This sudden change in energy in the FC regions is associated with a large change of bond length alternation (BLA) associated with an increase in double-bond lengths and decrease in single bond lengths (see Figure 4.5.A). This phenomenon has been well documented in rPSB and its models.\textsuperscript{11,37-39} We find that this initial relaxation along the BLA coordinate is predicted by both CASSCF and CASPT2, but is much more pronounced in CASSCF where the decrease in BLA is on the order of 0.15 Å and the associated change in energy is ca. 10 kcal/mol (compared to CASPT2 that has a BLA decrease of 0.04 Å and an associated energy change of ~ 5 kcal/mol). In both cases, the large change in BLA is accompanied by a very limited increase in charge transfer character with respect to the reactive C2=C3 bond (see Figure 4.5.B).
Figure 4.5. A. The BLA value along the CASSCF (top) and CASPT2 (bottom) composite paths. The dotted vertical lines are used to distinguish the three paths (from left to right: MEP\textsubscript{CIS}, CI seam, and MEP\textsubscript{TRANS}). The BLA value is computed as the difference between the average of the single bond and double bond lengths. B. The charge transfer character across the C2=C3 bond along the CASSCF and CASPT2 composite paths.
This difference in BLA behavior of CASSCF and CASPT2 near the FC point arises due to the existence of a so-called “locally excited” (LE) region on the CASPT2 \( S_1 \) PES. Such a region has been recently documented in a hybrid quantum mechanics / molecular mechanics (QM/MM) model of bovine rhodopsin incorporating a rPSB chromophore with the isomerizing double bond locked in a 5-membered ring.\(^{40}\) In this work, it has been proposed that the LE region hosts an \( S_1 \) intermediate from which fluorescence may occur, or which may lead to an alternate decay pathway for the \( S_1 \) population (i.e. other than the double-bond isomerization mechanism).

At the CI seam, we see a marked difference in the BLA profile of Figure 4.5.A between CASSCF and CASPT2. Indeed, the BLA along the interpolation coordinate in that region has positive curvature at the CASSCF level and negative at the CASPT2 level. This is due to the fact that while CI\(_{\text{CIS}}\) and CI\(_{\text{TRANS}}\) have similar BLA values at the CASSCF and CASPT2 levels of theory, CI\(_{\text{BLA}}\) is different for each method (with a BLA value of ca. 0 Å and 0.3 Å for CASSCF and CASPT2, respectively) causing the CI seam profile to be different. As expected, along the CASSCF path, the two states appear to have mixed character along most of the CI seam. As shown in Figure 4.5.B, the charge transfer character remains at almost 0.5 e- for both \( S_0 \) and \( S_1 \). This indicates that both states have a mixed electronic character that is in between that of a full charge-transfer and diradical configuration. There are sudden changes in the charge transfer character along the CASSCF CI seam due to the near degeneracy situation.\(^{41}\)

Along the CASPT2 CI seam, the geometries have a complete charge transfer character on \( S_1 \) (accompanied by a completely diradical \( S_0 \) character). Technically this can be explained by the increase in BLA with respect to the CASSCF CI seam, which brings the molecule into charge
transfer region on S1 in the underlying CASSCF level of theory where the states are no longer degenerate. This charge transfer region is characterized by large twisting about the C2=C3 double bond, and an electronic structure consistent with that of a twisted intramolecular charge transfer (TICT)42,43 state reaching top charge separation at CI_{BLA} with a ca. 90° C1-C2-C3-C4 dihedral angle and a ca. 0° HOOP. As reported in previous work, such TICT character may also develop along paths and trajectories approaching the seam.44 In such cases a sudden polarization effect45 is documented when along the path the charge transfer suddenly raises from a ca. 0.5 to ca. 1.0 value.

Figure 4.6.A displays the dihedral angles related to the double bond isomerization along the CASSCF and CASPT2 paths. We find that near the FC regions of cis-PSB3 and trans-PSB3, there is little torsional change due to the initial relaxation from the FC being dominated by a BLA mode. As discussed in the methods section, in the case of CASSCF, isomerization of the double bond begins immediately and leads to a CI, whereas for CASPT2 the double bond isomerization does not occur until later in the MEP_{CIS} and MEP_{TRANS} coordinates. A comparison of Figures 4.5.A and 4.6.A reveals a relation between the BLA coordinate and the isomerization. At (or just before) the start of the isomerization, the BLA value decreases rapidly, only to reach a minimum and increase again as the twisting occurs, until a CI is reached. This trend in BLA is observed in both CASSCF and CASPT2 (although in CASSCF the onset is earlier than in CASPT2)
Figure 4.6. A. The C1-C2-C3-C4, H-C2-C3-H, and π-orbital overlap (τ, calculated as the average of the C1-C2-C3-C4 and H-C2-C3-H dihedrals) along the CASSCF (top) and CASPT2 (bottom) composite paths. The dotted vertical lines are used to distinguish the three paths (from left to right: MEP_{CIS}, CI seam, and MEP_{TRANS}). B. The hydrogen-out-of-plane (HOOP, defined as the difference between the C1-C2-C3-C4 and H-C2-C3-H dihedrals) along the CASSCF (top) and CASPT2 (bottom) composite paths.
The role of HOOP in the isomerization of rPSB in visual pigments has been documented in several QM/MM trajectory calculations. Here, we see that the HOOP mode is largely involved in the isomerization even in a MEP calculation of a gas-phase PSB3 model (Figures 4.6.A and 4.6.B). Its involvement is thus not due to dynamics reason exclusively but, mainly, to the actual $S_1$ force field. The C1-C2-C3-C4 dihedral is coupled with an even larger change in H-C2-C3-H dihedral in both MEP$_{CIS}$ and MEP$_{TRANS}$, and therefore the absolute value of the HOOP increases from 0° at the FC points to 30°-60° at the CI. Notice that isomerization starting from cis-PSB3 leads to a positive value of HOOP, favoring isomerization to trans-PSB3, while starting from trans-PSB3 yields a negative HOOP, favoring isomerization to cis-PSB3. Therefore, the CI$_{CIS}$ and CI$_{TRANS}$ have completely opposite HOOP values, and the path along CI seam is dominated by a change in the HOOP coordinate (Figure 4.6.B). Meanwhile, the $\pi$-orbital overlap (roughly represented by the geometrical variable $\tau$, defined in the Figure 4.6 caption) remains almost constant along the CI seam (Figure 4.6.A). Interestingly, we find that the CI$_{TRANS}$ has a larger absolute HOOP value than CI$_{CIS}$ at both the CASSCF (CI$_{TRANS}$ is -60.8° and CI$_{CIS}$ is 34.6°) and CASPT2 (CI$_{TRANS}$ is -56.4° and CI$_{CIS}$ is 47.1°) levels of theory.

In Figure 4.7.A we find that while CASSCF displays virtually no change in the N-C1-C2-C3 dihedral along MEP$_{CIS}$ and MEP$_{TRANS}$, at the CASPT2 level of theory isomerization around the C1-C2 single bond starts to occur almost immediately from the FC point (after just a small relaxation in the BLA shown in Figure 4.5.A bottom). This isomerization leads to an $S_1$ minimum that is $\sim$ 70° twisted with respect to the FC point (MIN$_{CIS}$ or MIN$_{TRANS}$). This single bond isomerization is associated with a very limited change in the BLA (Figure 4.5.A bottom), but with a significant charge transfer across the C1-C2 bond such that the molecule has the
positive charge almost fully localized on the -C2H=C3H-C4H=C5H₂ moiety (Figure 4.7.B bottom). Therefore the MINCIS or MINTRANS structures may be seen as single bond TICT states corresponding to real and, in principle, fluorescent S₁ intermediates. Finally, we find from a comparison of Figures 4.6.A and 4.7.A that along MEP_CIS the single bond and double bond isomerizations are almost independent (the dihedral around the C2=C3 double bond remains planar while single bond isomerization occurs), but the same is not true for MEP_TRANS. This last observation is also related to the fact that TS_TRANS has a larger torsional deformation in both the C1-C2 single and C2=C3 double bond than TS_CIS.
Figure 4.7. A. The N-C1-C2-C3 dihedral along the CASSCF (top) and CASPT2 (bottom) composite paths. The dotted vertical lines are used to distinguish the three paths (from left to right: MEP_{CIS}, CI seam, and MEP_{TRANS}). B. The charge transfer character across the C1-C2 bond along the CASSCF and CASPT2 composite paths.
In summary, we found several differences between the CASSCF and CASPT2 paths. The main difference along the MEP\textsubscript{CIS} and MEP\textsubscript{TRANS} paths is the existence of a LE region on the S\textsubscript{1} CASPT2 PES of PSB3 near the FC point which is absent in CASSCF. This corresponds to a difference in topology of the potential energy surface and it is, therefore, of mechanistic significance. Such a region must have a significant effect on the initial relaxation dynamics along the BLA coordinate from the FC point, as observed in several studies\textsuperscript{16,18,47} However, it is noteworthy to mention that even along the CASPT2 path the BLA does decrease significantly, as in CASSCF, once the molecule leaves the LE region. The LE region in CASPT2 also introduces an S\textsubscript{1} barrier for isomerization along the C2=C3 double bond, instead favoring a torsional deformation along the C1-C2 single bond. This is consistent with TD-DFT and CC2 studies of rPSB models\textsuperscript{17} However, it is important to stress that such single bond deformation does not lead to a degeneracy of the S1 and S0 states and may be considered photochemically (but not photophysically) of a limited importance. Indeed, note that a removal of the path segments dominated by single bond deformation from the energy profile of Figure 1B would produce, after splicing the remaining segments, composite CASPT2 energy profiles topologically similar to the CASSCF profiles of Figure 1A. Finally, we find that differences in the CI structures at the CASSCF and CASPT2 level (and especially in the CIBLA structure) leads to a different shape of the CI seam at the two levels of theory.
4.4.2. Dynamic electron correlation effects on the CASSCF reference PES

In this section, we perform MRCI, MRPT2, and EOM-CC single point calculations along the composite CASSCF path comprising MEP\textsubscript{CIS}, CI seam, and MEP\textsubscript{TRANS}. Figure 4.8 displays the energy profiles along the composite path (we display only one variant of each method that performs better than the rest). We find that dynamic electron correlation stabilizes the S\textsubscript{1} state along the entire path, and also stabilizes the S\textsubscript{0} energy of all structures relative to the equilibrium \textit{cis}- and \textit{trans}-PSB3 structures. Near the \textit{cis}-PSB3 and \textit{trans}-PSB3 FC regions (see left and right insets in Figure 4.8), we notice that the clearly barrierless CASSCF S\textsubscript{1} path becomes either flatter upon introduction of dynamic electron correlation, or even develops an S\textsubscript{1} barrier, forming a minimum. This is most noticeable at the CASPT2(IPEA=0.25) level of theory, but the effect is present with other MRPT2, MRCISD+Q, and EOM-CC methods as well.
Figure 4.8. The CASSCF (red), MRCISD+Q (black), QD-NEVPT2 (orange), XMCQDPT2 / F (Γₘₙ) (green), CASPT2 (IPEA=0.25) (blue) and SF-CCSD(dT) (violet) energy profiles along the composite CASSCF path comprising MEPₗ₡ₐₜ, CI seam, and MEPₜₐₜ₉ₐ₅. The energies are relative to the trans-PSB3 S₀ energy. The insets show an expansion of the 10 points nearest to each of the cis- and trans-PSB3 structures, and of the CI seam.
Along the CI seam (Figure 4.8, center inset), we notice that dynamic electron correlation lifts the degeneracy between the $S_0$ and $S_1$ states along the seam (to a different degree depending on the method). The only exception is CASPT2(IPEA=0.25) where the $S_0$ and $S_1$ states remain degenerate along the seam, except at CI_{BLA} where the degeneracy splits suddenly. This behavior of CASPT2(IPEA=0.25) can be explained by looking at the electronic character of the underlying CASSCF wavefunction shown in the top of Figure 4.5.B. We find that the $S_0$ and $S_1$ wavefunctions are strongly mixed along this path, except near CI_{BLA} (and a little also near CI_{TRANS}) where we see sudden variations in the wavefunction. Due to an already documented problematic behavior of CASPT2, these variations in the CASSCF wavefunction near a CI can result in sudden variations of the CASPT2 energy, resulting in artifacts such as those observed here. In fact, the MS-CASPT2(IPEA=0.25) profile is significantly different along the CI seam, leading to an overestimation of the energy gap (as documented in the previous chapter).

In order to more accurately compare the different methods, we present in Table 4.1 a more quantitative analysis of their performance along the composite CASSCF path. Namely, we present the $S_0$-$S_1$ energy gaps ($\Delta E_{S0:S1}$) at the equilibrium structures and the CI structures used to construct the CI seam. We also report NPEs along the composite path and the CI seam. MRCISD +Q is considered here to be the most accurate method and, therefore, is the reference against which NPEs are computed, and to which energy gaps are compared.
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<th>ΔE_{S0-S1} at trans-PSB3</th>
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<th>ΔE_{S0-S1} at Cl(_{bla})</th>
<th>ΔE_{S0-S1} at Cl(_{trans})</th>
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</tbody>
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Table 4.1. The S\(_0\)-S\(_1\) energy gaps (ΔE\(_{S0-S1}\)) at cis-PSB3, trans-PSB3, Cl\(_{cis}\), Cl\(_{bla}\), and Cl\(_{trans}\), as well as the NPEs for various methods along the composite CASSCF path. All values are reported in kcal/mol. NPEs were computed along the composite path which combines MEP\(_{cis}\), the CI seam, and MEP\(_{trans}\), as well as just along the CI seam. The reference values (i.e. those belonging to MRCISD+Q) are highlighted in blue, while the values obtained at the level of theory with which the paths were generated are highlighted in orange.
In the following, we will discuss the energy gaps at the *cis*- and *trans*-PSB3 geometries. MRCISD+Q has energy gaps (101.4 and 104.4 kcal/mol for *cis*- and *trans*- PSB3, respectively) that are ca. 9-10 kcal/mol smaller than those of CASSCF. In fact, we find that dynamic electron correlation consistently reduces the energy gap at the equilibrium structures. While MRCISD without the Davidson correction and EOM-CC methods give larger energy gaps than MRCISD +Q, all MRPT2 methods underestimate them. In the case of XMCQDPT2, while the classical version gives energy gaps that are 7-8 kcal/mol smaller than MRCISD+Q, the diagonal fit and $f'(\Gamma_{ns})$ variants yield a very good (and in the case of diagonal fit; excellent) agreements with MRCISD+Q. QD-NEVPT2 also performs very well in reproducing the MRCISD+Q energy gaps. As for CASPT2 and MS-CASPT2, we find that the energy gaps computed without the IPEA shift are largely red-shifted by 9-10 kcal/mol, but the introduction of the IPEA shift significantly improves the agreement with MRCISD+Q. Both with and without the IPEA shift, MS-CASPT2 performs slightly better than CASPT2. The effect of the IPEA, as well as the geometry optimization, was already explored in the previous chapter addressing the ground state PES. In that chapter, it was found that for *cis*- and *trans*-PSB3, when a modest (6-31G*) basis set is used with a CASSCF optimized geometry, it appears to be more accurate to use CASPT2 without an IPEA shift rather than with the default IPEA, as this allows for better agreement due to a cancellation of errors (including differences in optimized geometries, IPEA values and modest basis sets). This is important for cases where large basis sets and CASPT2 optimizations may not be affordable. Finally, we notice in Table 4.1 that SF-CCSD with the triples correction (dT) reproduces the MRCISD+Q energy gaps very well.
From the energy gaps at the CI\textsubscript{CIS}, CI\textsubscript{BLA}, and CI\textsubscript{TRANS} geometries we find that MRCISD +Q splits the degeneracy that exists at the CASSCF level of theory at these structures, as we already saw in Figure 4.8 along the entire CI seam. The degree of splitting of the degeneracy is different for different methods. This is due to the CI being displaced to different geometries along the BLA coordinate, as already we already saw in Chapter III. At the XMCQDPT2 level, the energy splitting at the three CI geometries is in reasonable agreement with MRCISD+Q, especially when the diagonal fit and $f(\Gamma_{ns})$ variants are used. QD-NEVPT2 overestimates the energy gap by ca. 4-5 kcal/mol. This is due to the overestabilization of the S\textsubscript{0} energy in that region of the PES, a property of QD-NEVPT2 that has already been documented\cite{footnote} and was found to be due to the limit in the zero-order CASSCF wave function. This issue could be resolved by using a larger active space for the underlying CASSCF wavefunction.\cite{footnote} In the case of CASPT2, we find that these CI structures present a particular challenge due to the degeneracy of the CASSCF S\textsubscript{0} and S\textsubscript{1} states and strong mixing of the corresponding wavefunctions. As a result, we notice that that two states remain almost degenerate along the entire CI seam at the CASPT2(IPEA=0) level, as well as with CASPT2(IPEA=0.25) level (especially at CI\textsubscript{CIS} where the mixing of states is largest (Figure 4.5.B top). This artifact also leads to a large overestimation of the energy gaps at the MS-CASPT2(IPEA=0) and MS-CASPT2(IPEA=0.25) levels of theory as the available implementations overcorrect for the artifactual degeneracy.\cite{footnote} Finally, we notice that while the energy gap at the CI structures remains small with SF-CCSD, the triples correction again improves the agreement with MRCISD+Q.

The NPE values provide a good indication of the overall performance of each method along the composite CASSCF path. We also present in Table 4.1 the NPE value along just the CI
seam, since this region may be of particular interest to the photochemistry of the system. In fact, a low NPE would imply that the CI seam has the same shape along the PES, but is displaced to a different region (e.g. could be displaced along the BLA value). On the other hand, a large NPE would indicate that the shape of the CI seam changes with the method, and therefore this would indicate a different dependence of the energy gap on the geometrical coordinates. In fact, we find from Table 4.1 that the NPE values along the CASSCF CI seam are very small, confirming that CI seams computed at one level of theory at least have a reasonably correct shape, at least in the case of PSB3. The largest NPE value observed is for CASSCF, but this value is still not significantly large. This is followed by CASPT2 and MS-CASPT2, but this can be easily explained by artifacts along the CI seam region arising from the variations in the underlying CASSCF wavefunctions, especially at CI\textsubscript{BLA} and CI\textsubscript{TRANS}. Also, for QD-NEVPT2, while the NPE for the S\textsubscript{1} state is very small, it is larger for S\textsubscript{0}. Again, this could possibly be remedied by improving the zero-order CASSCF wave function (i.e. expanding the active space).

Along the composite CASSCF path, we naturally start to see larger NPE values, indicating that there are fewer methods capable of describing the full path. For example, along the CASSCF path we find NPE values of 11.4 and 6.0 kcal/mol for the S\textsubscript{0} and S\textsubscript{1} states, respectively. The effect is large enough to be visibly seen in Figure 4.8, where we find that the MRCISD+Q profiles along both the S\textsubscript{0} and S\textsubscript{1} states are significantly flatter than those of CASSCF. On the other hand, XMCQDPT2 displays a relatively negligible NPE for both S\textsubscript{0} and S\textsubscript{1} along the composite path, in particular with the f(\Gamma\textsubscript{ns}) variant which performs remarkably well relative to MRCISD+Q. QD-NEVPT2 has a larger NPE value than XMCQDPT2, but this is mainly due to the CI seam region where it deviated from the MRCISD+Q CI seam. If we ignore
the CI seam and two nearby structures from each side of the seam, we would obtain a NPE of 3.0 kcal/mol for $S_0$ and 1.7 kcal/mol for $S_1$. This means that QD-NEVPT2 is able to describe most of the PSB3 PES mapped in this work and only suffers from over-stabilizing one of the states when the molecule is completely twisted (particularly, the state with charge transfer character as documented in the previous chapter). We also see a reasonably good NPE value for CASPT2. The IPEA shift has small effect on the NPE value, indicating that the shift only moves up the entire PES, but does not change its topology significantly. However, MS-CASPT2 displays very large NPEs, comparable with CASSCF, but this is again due to the CI seam region where artifacts occur and cause a large splitting in the energy degeneracy. In fact, if the CI seam and nearby regions were ignored when computing the NPEs, the NPE value would decrease. Finally, while SF-CCSD has a relatively large NPE value (especially for the $S_1$ state), the triples correction significantly improves the agreement with MRCISD+Q. In Figure 4.8, we notice that along most of the path SF-CCSD(dT) remains very close to the MRCISD+Q curve.
Figure 4.9. The CASSCF (red), MRCISD+Q (black), QD-NEVPT2 (orange), XMCQDPT2 / F ($\Gamma_\text{ns}$) (green), CASPT2 (IPEA=0.25) (blue) and SF-CCSD(dT) (violet) energy profiles along the composite CASPT2(IPEA=0.25) path comprising MEP cis, CI seam, and MEP trans. The energies are relative to the trans-PSB3 S0 energy. The insets show an expansion of the 12 points nearest to each of the cis- and trans-PSB3 structures, and of the CI seam.
4.4.3. Dynamic electron correlation effects on the CASPT2 reference PES

In **Figure 4.9** we present the energy profiles for several methods along the composite CASPT2(IPEA=0.25) path (the same methods presented in **Figure 4.8**). There are several notable differences between **Figures 4.8** and 4.9. First, we find that all methods display a barrierless path along the single bond isomerization, with the exception of CASSCF (see left and right insets in **Figure 4.9**). Therefore, while CASSCF predicts a completely barrierless double bond isomerization and a barrier along the single bond isomerization, correlated methods display a barrierless single bond isomerization. Therefore PSB3 presents an interesting example of a system where single and double bond isomerizations are both possible on \( S_1 \), and different methodologies may predict a different reactivity. On the other hand it has to be again stressed that the non-adiabatic reaction leading to double bond isomerization is qualitatively invariant and displays the same mechanistic features (i.e. a BLA change precedes the torsional motion typical of a two-states two-modes mechanism) for both the CASSCF and CASPT2 levels. In contrast the single bond conformational change (i.e. an s-trans to s-cis change) is an adiabatic process occurring entirely on the \( S_1 \) PES and exclusively at the CASPT2 level which, therefore, predicts the formation of two distinct \( S_1 \) intermediates (i.e. corresponding to the MIN\(_{\text{CIS}}\) and MIN\(_{\text{TRANS}}\) structures). As for the the CI seam (see center inset), we see a substantially different picture than that along the CASSCF CI seam. In fact, at the CI\(_{\text{BLA}}\) geometry, most methods (including MRCISD+Q) display a small or vanishing energy gap, indicating that this point is a CI for these methods (in fact, these methods displayed in **Figures 4.8** and 4.9 generally appear to be more accurate than their other variants, and therefore reproduce the position of the MRCISD+Q CI well, as documented in Chapter III). However, this does not appear to be the case for CI\(_{\text{CIS}}\) and
CI\textsubscript{TRANS}, which, while being CI points at the CASPT2(IPEA=0.25) level of theory, do not remain so at the MRCISD+Q level. Therefore, to quantify such differences, we present in Table 4.2 again energy gaps at select structures and NPE values.

<table>
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<tr>
<th>Method</th>
<th>$\Delta E_{S0-S1}$ at MIN\textsubscript{CIS}</th>
<th>$\Delta E_{S0-S1}$ at MIN\textsubscript{TRANS}</th>
<th>TS\textsubscript{CIS} barrier</th>
<th>TS\textsubscript{TRANS} barrier</th>
<th>$\Delta E_{S0-S1}$ at CI\textsubscript{CIS}</th>
<th>$\Delta E_{S0-S1}$ at CI\textsubscript{BLA}</th>
<th>$\Delta E_{S0-S1}$ at CI\textsubscript{TRANS}</th>
<th>composite path S\textsubscript{0} NPE</th>
<th>composite path S\textsubscript{1} NPE</th>
<th>CI seam S\textsubscript{0} NPE</th>
<th>CI seam S\textsubscript{1} NPE</th>
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<td>MRCISD+Q</td>
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<td>47.9</td>
<td>7.4</td>
<td>7.1</td>
<td>4.4</td>
<td>0.5</td>
<td>5.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
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<tr>
<td>MRCISD</td>
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<td>48.8</td>
<td>7.9</td>
<td>8.0</td>
<td>4.0</td>
<td>2.8</td>
<td>4.4</td>
<td>3.7</td>
<td>1.8</td>
<td>2.6</td>
<td>0.3</td>
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<td>50.7</td>
<td>8.3</td>
<td>9.1</td>
<td>3.5</td>
<td>8.7</td>
<td>3.7</td>
<td>8.2</td>
<td>4.6</td>
<td>7.4</td>
<td>2.2</td>
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<td>48.7</td>
<td>3.1</td>
<td>2.9</td>
<td>2.8</td>
<td>3.0</td>
<td>3.6</td>
<td>4.3</td>
<td>6.2</td>
<td>1.4</td>
<td>2.8</td>
</tr>
<tr>
<td>XMCQDPT2 / diagonal fit</td>
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<td>51.8</td>
<td>5.1</td>
<td>4.6</td>
<td>4.9</td>
<td>0.3</td>
<td>5.6</td>
<td>3.8</td>
<td>4.1</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>XMCQDPT2 / $f(\Gamma_{ns})$</td>
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<td>52.6</td>
<td>5.5</td>
<td>5.0</td>
<td>4.8</td>
<td>0.6</td>
<td>5.5</td>
<td>4.9</td>
<td>3.1</td>
<td>1.3</td>
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<td>6.5</td>
<td>9.7</td>
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<td>44.6</td>
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<td>2.1</td>
<td>3.0</td>
<td>5.1</td>
<td>3.6</td>
<td>4.0</td>
<td>10.7</td>
<td>1.3</td>
<td>4.8</td>
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<td>CASPT2 (IPEA=0.25)</td>
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<td>44.6</td>
<td>3.4</td>
<td>2.6</td>
<td>15.6</td>
<td>5.1</td>
<td>16.4</td>
<td>8.8</td>
<td>12.1</td>
<td>5.1</td>
<td>1.7</td>
</tr>
<tr>
<td>MS-CASPT2 (IPEA=0)</td>
<td>47.9</td>
<td>48.2</td>
<td>5.4</td>
<td>4.4</td>
<td>0.0</td>
<td>0.3</td>
<td>0.8</td>
<td>2.8</td>
<td>3.9</td>
<td>1.1</td>
<td>3.2</td>
</tr>
<tr>
<td>MS-CASPT2 (IPEA=0.25)</td>
<td>47.9</td>
<td>48.2</td>
<td>5.7</td>
<td>4.7</td>
<td>14.1</td>
<td>0.3</td>
<td>14.7</td>
<td>6.1</td>
<td>7.6</td>
<td>6.1</td>
<td>3.9</td>
</tr>
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<td>SF-CCSD</td>
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<td>17.1</td>
<td>16.7</td>
<td>3.4</td>
<td>7.2</td>
<td>3.4</td>
<td>11.1</td>
<td>10.3</td>
<td>3.8</td>
<td>5.3</td>
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<tr>
<td>SF-CCSD (dt)</td>
<td>44.0</td>
<td>43.7</td>
<td>10.8</td>
<td>10.3</td>
<td>1.1</td>
<td>0.5</td>
<td>2.9</td>
<td>3.1</td>
<td>5.2</td>
<td>2.6</td>
<td>3.0</td>
</tr>
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Table 4.2. The S\textsubscript{0}-S\textsubscript{1} energy gaps ($\Delta E_{S0-S1}$) at MIN\textsubscript{CIS}, MIN\textsubscript{TRANS}, CI\textsubscript{CIS}, CI\textsubscript{BLA}, and CI\textsubscript{TRANS}, as well as TS\textsubscript{CIS} and TS\textsubscript{TRANS} energy barriers and NPEs for various methods along the composite CASPT2 path. All values are reported in kcal/mol. The TS\textsubscript{CIS} and TS\textsubscript{TRANS} barriers are computed as the energy difference between the transition state (TS\textsubscript{CIS} or TS\textsubscript{TRANS}) and the corresponding MIN\textsubscript{CIS} or MIN\textsubscript{TRANS} intermediate. NPEs were computed along the composite path which combines MEP\textsubscript{CIS}, the CI seam, and MEP\textsubscript{TRANS}, as well as just along the CI seam. The reference values (i.e. those belonging to MRCISD+Q) are highlighted in blue, while the values obtained at the level of theory with which the paths were generated are highlighted in orange.
First, we discuss the energy gap at the MIN\textsubscript{CIS} and MIN\textsubscript{TRANS} structures. These structures are almost completely twisted around the C1-C2 single bond, resulting in a nitrogen-containing radical moiety and a conjugated radical cation moiety (see Figure 4.2; such an electronic character is also supported by the geometry of these minima which display pyramidalization of the nitrogen and C1 carbon, as well as a small pyramidalization of the C2 carbon). The energy gaps at both MIN\textsubscript{CIS} and MIN\textsubscript{TRANS} are very similar to each other. The CASSCF gaps are not very different from MRCISD+Q, differing by 2.8-3.4 kcal/mol. Moreover, we find in this case that XMCQDPT2 is in very good agreement, but, oddly, this agreement decreases with the diagonal fit and $J(\Gamma_{ns})$ variants (the origin of this disagreement is in the description of the S\textsubscript{0} energy, and not S\textsubscript{1}, which actually improves upon using the diagonal fit and $J(\Gamma_{ns})$ variants as shown in Figure 4.9). QD-NEVPT2 is also in reasonable agreement with MRCISD+Q, as well as CASPT2 (especially when the IPEA shift is used, where the results become virtually identical to MRCISD+Q). MS-CASPT2 results are identical to CASPT2. Finally, SF-CCSD significantly underestimates the energy gap, but the agreement with MRCISD+Q is improved upon introducing the triples correction, as expected.

In Table 4.2 we also present the energy barriers of TS\textsubscript{CIS} and TS\textsubscript{TRANS} with respect to MIN\textsubscript{CIS} and MIN\textsubscript{TRANS}, respectively. Such a calculation would be expected to be challenging for any method, since it would require a correct description of the intermediate electronic structure (diradicaloid with a positive charge on one side) as well as the transition state electronic character (where the electronic character is in between that of the S\textsubscript{1} intermediate and the mixed diradical/charge transfer electronic character of the double-bond twisted S\textsubscript{1} species). Indeed, we see a larger variation in the performance of different methods. All MRPT2 methods give a lower
barrier than MRCISD+Q, with QD-NEVPT2 being the closest. Meanwhile, SF-CCSD significantly overestimates the barrier, while SF-CCSD(dT) still yields a high barrier but in closer agreement with MRCISD+Q.

In Table 4.2 we also present the energy gaps at CI\_CIS, CI\_BLA, and CI\_TRANS, as well as the NPE value along the CI seam for the CASPT2 paths. CI\_BLA has degenerate S\(_0\) and S\(_1\) states at the MRCISD+Q, XMCQDPT2 / diagonal fit, XMCQDPT2 / \(f(\Gamma\_ns)\), CASPT2 (IPEA=0.25), MS-CASPT2(IPEA=0.25), and SF-CCSD(dT). These are the same methods that have an energy profile similar to MRCISD+Q along the BLA path in Chapter III, and therefore have a similar geometry of the CI. Meanwhile, XMCQDPT2 has an energy gap comparable with uncorrected MRCISD. QD-NEVPT2, as before (see Chapter III), overestimates the energy gap, requiring a larger active space to properly describe this region, while CASPT2(IPEA=0), MS-CASPT2 (IPEA=0), and SF-CCSD appear more similar to CASSCF than MRCISD+Q. On the other hand, at the CI\_CIS and CI\_TRANS geometries, only CASPT2(IPEA=0.25) energies are degenerate. This may again be attributed to the absence of interaction between perturbed states in CASPT2, which results in large differences between CASPT2 and MS-CASPT2. So, while we find that CASPT2 degeneracy is maintained along the entire CI seam (see Figure 4.9), it is unique in this regard since other methods do not share the same CI seam shape, resulting in significantly larger NPE values in Table 4.2 than those found in Table 4.1.

Finally, a look at the NPE values along the composite CASPT2 path confirms the same main conclusions as those derived from the CASSCF path. Namely, we find that (1) CASSCF yields a large NPE value, but not as large as along the CASSCF path, (2) XMCQDPT2 performs relatively well, but the diagonal fit and \(f(\Gamma\_ns)\) variants perform better than the classical version in
general, (3) QD-NEVPT2 has a larger NPE due to the CI seam region which, when ignored in computing the NPE, would reduce the NPE to 2.1 and 3.1 kcal/mol for $S_0$ and $S_1$, respectively, (4) CASPT2(IPEA=0) and MS-CASPT2(IPEA=0) have a large NPE, partially due to artifacts in the CI seam region, but the NPE decreases when using the IPEA shift, and (5) SF-CCSD gives a large NPE, even larger than along the CASSCF path (mainly due to the inability of SF-CCSD to accurately describe PES region reached by single bond twisting), but the NPE value is reduced again when triples correction is included.

### 4.5. Conclusions and Perspectives

PSB3 has been a very popular reduced model system for rPSB, the chromophore of visual pigments.\textsuperscript{1-4,12,18,47,49} The photochemistry of rPSB in visual pigments has been and still is the focus of many experimental and computational studies not only due to its basic importance in photobiology (e.g. photoisomerization of rPSB in visual pigments, microbial sensory and ion-pumping pigments), but also due to the technology that appears to be connected with its properties (e.g. as actuators and probes in optogenetics\textsuperscript{50,51} and models for the development of molecular devices\textsuperscript{52}). PSB3 has proven to be a very useful model for probing the photochemical properties of rPSB,\textsuperscript{1,2,4,53,54} and also as a benchmark for understanding which methods are best for describing the spectroscopy, photochemistry, or even thermal properties of visual pigments.\textsuperscript{12,18,47} as we also saw in Chapter III.
Figure 4.10. Schematic representation of the geometric deformations driven by the $S_1$ force field of PSB3 as revealed by our composite CASPT2 paths. The located stationary points and conical intersections are indicated by circles. The arrows indicate the direction of the force along the paths which are described by bold black curves. Left. Structure of the three dimensional configuration space defined by the BLA, HOOP and C2=C3 double bond torsion. Such a space is proposed to contain a two-dimensional cross-section of the $S_1/S_0$ intersection space spanning a combination of the torsional and HOOP modes. The cross-section must contain the mapped $S_1/S_0$ intersection seam (see the bold red curve). The proportions (e.g. in Å and degrees) are not respected to facilitate inspection. Right. Structure of the two dimensional configuration space defined by the BLA and C1-C2 single bond torsion modes. The balloon diagrams at the top and bottom provide information on the evolution of the charge distribution for $CI_{BLA}$ along the MEP$_{TRANS}$ path (The charge evolution along MEP$_{CIS}$ is similar to than along MEP$_{TRANS}$ and is not shown). A numerical value is given for -CH-, -CH2 and -NH2 groups with a total charge equal or larger than 0.1 in absolute value. Consistently with the charge transfer data in Figure 2B and 4B the positive charge resides almost completely on the -C2H=C3H-C4H=C5H2 and =C3H-C4H=C5H2 moieties in the MIN$_{TRANS}$/MIN$_{CIS}$ and $CI_{BLA}$ highly twisted structures respectively.
In the current chapter, we expand the present knowledge on the excited state force field structure of PSB3 and benchmark the performance of MPRT2 and EOM-CC methods for such systems to understand the possible sources of inaccuracy when studying its photoisomerization. Accordingly, we have mapped the relevant $S_1$ paths at both the CASSCF and CASPT2 levels of theory and documented the differences in the $S_1$ PES of CASSCF and CASPT2. The resulting $S_1$ CASPT2 geometric deformations driven by the force field is pictorially summarized in Figure 4.10 where it is apparent that four different molecular modes (BLA, HOOP, double bond torsion and single bond torsion) control the relaxation dynamics of the system. One remarkable difference is the existence of LE regions on the CASPT2 $S_1$ PES centered on single bond twisted structures. Such regions need to be overcome or avoided in order for the double bond photoisomerization to occur. However, the results presented in this work have to be interpreted with caution. The differences between CASSCF and CASPT2 in PSB3 are not necessarily present in rPSB in the gas-phase or in rPSB embedded in a protein environment. This is especially true when considering that the increased conjugation in the full chromophore would have an important effect on the size and stability of such an LE region, as well as the opsin environment and presence of counterions and polarizing residues. Furthermore, the pre-twisting of the chromophore in the opsin environment might help to remove the chromophore from the LE region and prepare it for immediate isomerization around the double bond. In this sense, the CASSCF PES of PSB3 may provide a better model of the rPSB PES because it is more consistent with its observed ultrafast isomerization mechanism, which would be expected to be along a barrierless $S_1$ path. In fact, while one study has successfully located a LE region and an $S_1$ barrier in the PES of 11-cis locked rPSB, such a barrier was not located for natural rPSB.\textsuperscript{40} In
summary, the current model system indicates that such regions do, in principle, exist, and further QM/MM studies on the full rPSB chromophore embedded in a protein environment can help us to better understand the significance of the environment on the relative stabilities of these regions with respect to the rest of the $S_1$ PES. On the other hand, the potential interest of these regions for the control and engineering of rPSB photophysics appears to be high. Further stabilization of the LE region, perhaps via single bond twisting and/or via a suitable electrostatic potential imposed by the protein cavity, may lead to long lived fluorescent states and, consequently, to the engineering of rhodopsin-based genetically encodable fluorescent probes.

The performance of MRPT2 and EOM-CC methods along the paths presented in this study confirm and extend the results of Chapter III. We find that MRPT2 methods perform well when compared against MRCISD+Q. The performance of XMCQDPT2 is good but further improved by using the diagonal fit and $f(\Gamma_{ns})$ variant, while QD-NEVPT2 performs very well along most regions of the PES with the exception of structures twisted along the double bond, where a larger active space is most likely required to improve the quality of the zero-order CASSCF wavefunction. CASPT2 and MS-CASPT2 also perform well in most regions of the PES, with the exception of regions of strong mixing of the CASSCF wavefunction (near degeneracies) where artifact may arise. The use of the IPEA parameter in CASPT2 and MS-CASPT2 improves their agreement with MRCISD+Q. Finally, while SF-CCSD provides a qualitatively accurate description of the PSB3 PES, the triples correction (dT) is needed to get more quantitatively accurate profiles which are in good agreement with MRCISD+Q.
4.6. References


(51) Pastrana, E. *Nat. Methods*. 2011, 8, 447-.


5.1. Abstract

Spontaneous electrical signals in the retina's photoreceptors impose a limit on visual sensitivity. Their origin is attributed to a thermal, rather than photochemical, activation of the transduction cascade. While the mechanism of such a process is under debate, the observation of a relationship between the maximum absorption wavelength ($\lambda_{\text{max}}$) and the thermal activation kinetic constant ($k$) of different visual pigments (the Barlow correlation) indicates that the thermal and photochemical activations are related. Here we show that a quantum chemical model of the bovine rod pigment provides a molecular-level understanding of the Barlow correlation. The transition state mediating thermal activation has the same electronic structure as the photoreceptor excited state, thus creating a direct link between $\lambda_{\text{max}}$ and $k$. Such a link appears to be the manifestation of intrinsic chromophore features associated with the existence of a conical intersection between its ground and excited states.

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1 This chapter is partially based on results presented in the following article:
5.2. Introduction and background to the problem

Vision in vertebrates is possible due to visual pigments lining the membrane shelves of cone photoreceptors (responsible for color vision) and rod photoreceptors (responsible for dim-light vision) found in the retina of the eye. Here, we focus on Bovine rhodopsin (Rh), a prototypal G-protein coupled receptor and visual pigment of rod photoreceptors. Rh consists of an opsin apoprotein and the 11-cis retinal protonated Schiff base chromophore (rPSB), which is covalently linked to a lysine side chain in the opsin core (see Figure 5.1). When light is absorbed in Rh, an electronic transition occurs in rPSB from the ground state ($S_0$) to the first electronic excited state ($S_1$). This transition then triggers the isomerization of rPSB which makes the 11-cis double bond to become all-trans (AT-rPSB), leading to the formation of the metastable intermediate bathorhodopsin (bathoRh).

![Figure 5.1](90x166 to 522x304)

**Figure 5.1.** The structure of the 11-cis-retinal protonated Schiff base (11-cis-rPSB) of bovine rhodopsin, which is covalently linked to the Lys296 amino acid residue.
The mechanism for the primary process in vision currently supported by both spectroscopic\textsuperscript{2-5} and computational\textsuperscript{6,7} studies can be schematically represented by the solid black lines and arrows shown in Figure 5.2. Briefly, excitation of the rPSB chromophore leads to a transfer of part the positive charge (which is originally almost fully localized on the Schiff base side of the chromophore) to the $\beta$-ionone side of the chromophore. This leads to a weakening of the double bond character in the chromophore, which then follows a barrierless path on the $S_1$ potential energy surface and isomerizes around the 11-cis double bond. In the prototypical pigment Rh, the isomerization is ultrafast, occurring on a femtosecond timescale.\textsuperscript{2-4} The additional observation of $S_0$ vibrational coherence\textsuperscript{5} supports a direct transfer of the excited-state ($S_1$) population to the photoproduct along a downhill path passing through a conical intersection (CI). Such a path has been located along the Rh potential energy surfaces using hybrid multiconfigurational quantum chemical / molecular mechanical (QM/MM) calculations,\textsuperscript{6-8} and has been spectroscopically supported by probing in the infrared.\textsuperscript{8} The CI is ca. 90° twisted around the isomerizing bond, and so is almost in the middle of the reaction path between 11-cis and all-trans rPSB\textsuperscript{6,7} (see Figure 5.2).
Figure 5.2. A schematic representation of the photochemical (solid arrows) and thermal (dashed arrows) isomerization paths of rPSB. The photochemical path proceeds via a conical intersection (CI) which is located energetically above the transition state (TS). The thermal isomerization, on the other hand, proceeds entirely on the ground state and via a TS that is assumed here to have diradical character. The $S_0$ and $S_1$ Mulliken charge distributions along the backbone of rPSB found using QM/MM calculations are represented with bubble diagrams on the left of the graph. The labels indicate the maximum and minimum values for the charges. The bond-line formulas represent the dominant electronic configurations (wavefunctions) of the corresponding states.

In contrast to light activation, the mechanism of thermal activation of visual pigments has not been established. Thermal activation of Rh is responsible for reducing visual sensitivity by increasing background noise levels,$^9,10$ and especially since the brain cannot distinguish these thermally triggered signals from photochemically triggered ones.$^{11}$

A number of different mechanisms have been proposed to explain the origin of thermal noise in visual pigments. A milestone for the understanding of thermal noise was the measurement of the Arrhenius activation barrier of the visual pigments in rod cells of *Bufo marinus*. The unexpectedly low ca. 22 kcal/mol barrier for thermal activation suggested that the
photochemical activation is controlled by a distinct mechanism, since in Rh the first photocycle intermediate bathorhodopsin (bathoRh) is 32 kcal/mol higher in energy than Rh,\textsuperscript{12} a quantity significantly higher than the measured thermal barrier in toads. This has prompted several research groups to propose thermal activation mechanisms which differ from the canonical isomerization of the opsin embedded rPSB chromophore since it was believed that thermal isomerization must somehow bypass bathoRh production. In 1993, Barlow et al.\textsuperscript{13} presented an experimental and computational study indicating that the rPSB thermal isomerization takes place in a limited molecular population of non-protonated retinal Schiff bases. While the model did successfully reproduce the experimental activation energy barrier, later studies have found that a pH dependence does not exist in the thermal isomerization rates of toad rhodopsin and salamander L cones, which meant that while the mechanism proposed by Barlow et al. might be true for Limulus, it cannot be generalized\textsuperscript{14,15}.

More recently, and based on a study showing that the thermal hydrolysis and isomerization of rPSB are slower in D\textsubscript{2}O, Liu et al. suggested that thermal activation may be controlled by simultaneous hydrolysis and isomerization.\textsuperscript{16} This study was followed by a complementary study demonstrating that the hydrogen-bonding network near the active site plays an important role in lowering the rate of thermal noise\textsuperscript{17}. Indeed, it was shown that the E181Q and S186A mutants of Rh (featuring mutations of residues involved in the hydrogen-bonding network) causes an increase of the rPSB thermal isomerization rate by 1-2 orders of magnitude.
In another attempt to address the discrepancy between the thermal and photo-activation energies, Bókkon et al. suggested that “thermal activation” is in fact not thermal, but it is due to bioluminescence near the retina\(^\text{18}\). Since the rate of bioluminescence itself is temperature dependent, it was proposed that this could be the origin of the observed activation.

A study published by Lorenz-Fonfria et al.\(^\text{19}\) proposes that fluctuations in the protein structure might help in lowering the barrier for thermal activation. The study suggests that the opsin can exist in an “open state”, where rPSB has a reduced activation energy.

In addition to the above experimental studies, a hybrid quantum-mechanics/molecular-mechanics (QM/MM) computational study\(^\text{20}\) of bovine Rh and bathoRh based on published x-ray crystal structures of Rh reported a transition state (TS) located 22-23 kcal/mol above the reactant. While this value is in agreement with the measured toad rhodopsin thermal activation barrier\(^\text{11}\) Khrenova et al. also found that the computed value for the bathoRh storage energy is 16 kcal/mol and thus considerably smaller than the observed value\(^\text{12}\). The TS reported by Khrenova et al. has a diradical character. In a more recent DFT and TD-DFT study\(^\text{21}\) it was suggested that the excited state of Rh could be accessed by the thermal isomerization.

The overview above of some of the research efforts targeting thermal isomerization reveals that the origin of thermal noise remains a topic of much debate. However, there is evidence pointing to the fact that the thermal isomerization in visual pigments must be related to the photochemical one. First, the signal triggered by thermal activation is indistinguishable from that caused by light\(^\text{11}\). Second, the thermal process leads to the isomerization of the retinal at the C11=C12 bond without significantly changing the secondary structure of the opsin, as is the case
with photoisomerization. Finally, a study published in 2004 by Ala-Laurila et al. has collected results from different measurements and found experimental evidence for the existence of a relationship between the maximum absorption wavelength ($\lambda_{\text{max}}$, a photophysical property) and the thermal activation kinetic constant of different visual pigments ($k$, a thermal property). We refer to this relationship between $\lambda_{\text{max}}$ and $k$ as “the Barlow correlation” since it was first predicted by the British neuroscientist Horace B. Barlow in 1957. The Ala-Laurila et al. study received more recent support by Luo et al. in 2011, where the authors provided further evidence for the relation between the thermal and photochemical mechanisms. To explain this correlation, Luo et al. proposed that the pigment activation by light and heat must go through the same ground-state isomerization energy barrier. However, this explanation is unlikely in the light of the well-established CI-driven photochemical mechanism which occurs on a femtosecond timescale, and depicted in Figure 5.2. Following previous considerations, Luo et al. also suggested that the measured 22 kcal/mol thermal activation barrier is inaccurate because it is derived on the basis of Boltzmann statistics, which are considered by the authors to be inadequate for describing a unimolecular process occurring in a reactant with many vibrational degrees of freedom such as rPSB in rhodopsin.

From the studies mentioned above there appear to be two main irreconcilable schools of thought about thermal isomerization in Rh. One advances the idea that thermal isomerization in Rh must proceed via some non-canonical pathway unrelated to the photochemical path, since the thermal isomerization barrier is reported at 22 kcal/mol while the photochemical activation energy is reported at 40-50 kcal/mol and the primary photoprodut is 32 kcal/mol above Rh.
related, especially with the observation of a Barlow correlation which the aforementioned mechanisms have yet to explain.

In the following, we present a hypothesis where we show that it may be possible to predict the Barlow correlation based on just theoretical considerations. We then test this hypothesis by employing a hybrid QM/MM of the prototypal Rh pigment in order to investigate the electronic and geometric properties of the transition state(s) controlling its thermal isomerization. Furthermore, we also construct QM/MM models of a series of 11 related systems in order to simulate the Barlow correlation in these systems. Such QM/MM models allow us to model the entire protein and at the same time study at the ab initio multiconfigurational quantum chemical level the geometric and electronic structure of the chromophore imbedded within.

5.3. Hypothesis

We attempt to present a hypothesis that can at least partially reconcile the two schools of thought above. Our hypothesis holds that the thermal isomerization of rPSB in Rh should follow entirely an $S_0$ pathway and is controlled by an energy barrier ($E_{aT}$) corresponding to an $S_0$ TS (see dashed lines and arrows in Figure 5.2). Therefore, in contrast to the hypothesis of Luo et al., we propose that the thermal isomerization mechanism must be qualitatively different and distinct from the photochemical isomerization (also shown in Figure 5.2 with solid lines and arrows). However, the existence of the Barlow correlation, which establishes a relationship between $k$ and $\lambda_{\text{max}}$ such that $-\log k$ is directly proportional to $1/\lambda_{\text{max}}$, strongly suggests that the thermal
mechanism must be related to the photochemical one even though the mechanisms are different. Therefore, the explanation for the Barlow correlation must lie in the electronic properties of opsin-embedded rPSB.

The $S_0$ potential energy surface of rPSB features two TSs that control the thermal isomerization. The explanation for the existence of these two TSs lies in the existence of a CI between them, as explained below.

At a CI the $S_1/S_0$ degeneracy can be lifted by changing the chromophore geometry along two specific modes, the gradient difference vector (GDV) and the derivative coupling vector (DCV). As reported for rPSB and illustrated in Figure 5.3 (top), the DCV corresponds to a stretching that inverts the double-bond and single-bond positions along the backbone, the bond length alternation (BLA) mode, while the GDV corresponds to the torsional deformation driving the isomerization, the reaction coordinate (RC). Indeed, in ref. 28, an $S_0$ potential energy scan along a loop lying on the plane defined by DCV (corresponding to BLA) and the GDV (corresponding to RC) and centered on the CI displayed two minima and two maxima (see Figure 5.3 bottom). The minima reflect the existence of two valleys developing in opposite directions along RC and leading to Rh and bathoRh respectively. The maxima indicate the presence of ridges developing in opposite directions along BLA and separating the valleys. Each ridge may host a TS featuring the same torsional deformations of the CI but different bond length alternations and, as explained below, different electronic structures.
Figure 5.3. Energy and electronic structure changes along a loop lying on the branching plane of rPSB. The loop is constructed by using the derivative coupling vector (mostly parallel to the BLA mode) and the gradient difference vector (mostly parallel to the RC mode). The angle $\alpha$ (labeled in the upper scheme) corresponds to the $0-2\pi$ coordinate defining the position along the circular loop. The diagram at the bottom reports the CASPT2//CASSCF $S_0$ energy profile (dashed black line) as well as the total charge on the $\beta$-ionone moiety (solid red line) as a function of $\alpha$ for a loop of 0.09 Å radius. All regions associated with a dominant covalent/diradical ($\psi_{\text{DIR}}$) and charge-transfer ($\psi_{\text{CT}}$) electronic configuration are show in green and brown, respectively. This figure is based on the results reported in ref. 28.
In 1963 Herzberg and Longuet-Higgins demonstrated the existence of a geometric phase in the electronic wavefunction of the states crossing at a CI\(^{29}\). Their theorem holds that when moving along a small loop encircling a single CI point, the wavefunctions of the states exchange character. This behavior has been documented along the loop considered above for rPSB. As revealed in Figure 5.3 (bottom), the charge distribution changes twice along the loop and divides the \(S_0\) potential energy surface into two regions centered on different ridges. In the first region (100° to 300°, shown in green) the chromophore has the positive charge localized on the Schiff base segment and has a wavefunction (represented by the wavefunction \(\psi_{\text{DIR}}\)) similar to that of Rh in its \(S_0\) state (see Figure 5.2). In the second region (300° to 100°, shown in brown) the charge is located on the opposite segment and the wavefunction (represented by the wavefunction \(\psi_{\text{CT}}\)) is similar to that of Rh in \(S_1\) (see Figure 5.2).\(^{30}\)

![Figure 5.4](image)

**Figure 5.4.** A schematic representation of the two-dimensional \(S_0\) potential energy surface driving the thermal Rh to bathoRh isomerization. The part of the potential energy surface associated with the charge-transfer electronic structure (\(\Psi_{\text{CT}}\)) is shown in brown while the rest of the green surface represents a covalent/di-radical electronic structure (\(\Psi_{\text{DIR}}\)). The bond-line formulas represent the dominant electronic configurations (wavefunctions) of the corresponding regions where each of the two TSs is hosted.
The CI analysis revised above is consistent with a structure of the S$_0$ potential energy surface reported in Figure 5.4 that features two thermal isomerization paths defined by TSs with different BLA values (TS$_{\text{DIR}}$ and TS$_{\text{CT}}$). The Herzberg and Longuet-Higgins theorem indicates that TS$_{\text{DIR}}$ and TS$_{\text{CT}}$ would differ in electronic structure. Accordingly, TS$_{\text{DIR}}$ would display a diradical character (described by $\psi_{\text{DIR}}$) and TS$_{\text{CT}}$ a charge-transfer character (described by $\psi_{\text{CT}}$). These two TSs have already been reported in cis-locked rPSB models$^{31,32}$, 5-double bond protonated Schiff base reduced models of rPSB (PSB5)$^{32}$ as well as in the penta-2,4-dieniminium cation (PSB3), as discussed in Chapter III. However, only one of the two transition states (TS$_{\text{DIR}}$) has been reported for Rh.$^{20}$

From Figure 5.2, we recall that vertical excitation of rPSB in Rh results in ca. 30% of the positive charge to be transferred from the Schiff base moiety to the $\beta$-ionone side of the chromophore. Therefore, TS$_{\text{CT}}$, which also has a charge transfer character, in fact has an electronic character close to that of the S$_1$ state of Rh. This creates a direct link between excitation energy ($\Delta$E$_{(S1-S0)}$ in Figure 5.2) and TS stability ($E_a^T$ in Figure 5.2), and, in turn, a direct link between $-\log k$ and $1/\lambda_{\text{max}}$. Therefore, if TS$_{\text{CT}}$ controls the thermal isomerization barrier (i.e. if it is lower in energy than the other transition state, TS$_{\text{DIR}}$), in principle this could provide a natural explanation for the observed Barlow correlation. This will be further explained in Section 5.5.
5.4. Methods

A QM/MM method\textsuperscript{33} with a link-atom (LA) approach\textsuperscript{34} was used to describe the rPSB chromophore at the \textit{ab initio} complete active space self consistent field (CASSCF) level of theory\textsuperscript{35} while taking into account the electrostatic and steric effects imposed by the opsin, which is treated at the molecular mechanics (MM) level using the AMBER force field.\textsuperscript{36} The CASSCF method is a multiconfigurational quantum chemical method offering suitable flexibility for an unbiased description (i.e. with no empirically derived parameters and avoiding single-determinant wavefunctions) of the electronic and geometrical structures of the ground and excited states of a molecule. Furthermore, the CASSCF wavefunction can be used for subsequent multiconfigurational second-order perturbation theory\textsuperscript{37} computations (CASPT2) ultimately allowing for an evaluation of energy barriers on a single electronic state and gap between different electronic states\textsuperscript{35} in a balanced way as required by the present investigation. For the visual pigment of bovine rhodopsin, its isomers bathoRh and isorhodopsin and two point mutants, the observed $\lambda_{\text{max}}$ values were reproduced with an error of $<$30 nm (3 kcal/mol in excitation energy).\textsuperscript{6,38,39} These results have been confirmed and extended by different groups.\textsuperscript{40-44} Similar accuracies were documented for the Archea proton pump bacteriorhodopsin\textsuperscript{45} and the Eubacteria sensory rhodopsin from the fresh water cyanobacterium Anabaena (Nostoc) sp. PCC7120\textsuperscript{46} and for a number of different chromophore containing proteins (see Figure 2.6 in Chapter II). While similar studies have been carried out with different quantum chemical methods,\textsuperscript{47,48} the CASPT2/CASSCF remains an affordable method allowing a description of spectra, excited state reaction paths, CIs, and trajectories consistently.\textsuperscript{7,49-51} The same QM/MM
method has also been used to compute classical and semiclassical trajectories that yield excited state lifetimes in qualitative agreement with the experiment. Furthermore, different studies have shown that the chromophore gas-phase absorption can be computed in agreement with experiment, within a few kcal/mol. This indicates that when excluding other factors (i.e. the accuracy of the opsin model) such a protocol can be used for simulating spectroscopic and photochemical data.

The model of wild type bovine rhodopsin was constructed on the basis of the 1U19 PDB crystal structure. Hydrogens were introduced and relaxed using Tinker. In cases where the ionization of a residue was unknown, we made simple assumptions: the carboxylic residues located in the region near the cytoplasmic and extracellular side (far from the chromophore) were considered deprotonated if a counterion is in their vicinity. If not, these are kept protonated. For the internal Glu113, Asp83, Glu181 and Glu122 residues we used the same strategy. Glu113 is the retinal counterion and has a deprotonated state (forming a salt bridge with the protonated Schiff base). The Glu122 residue near the retinal region could potentially have His211 as a counterion but Fourier Transform IR (FTIR) experiments suggest that Glu122 is protonated. Accordingly, His211 and Glu122 have been taken as neutral in our model. Finally, all histidine residues have been defined as neutral with the only exception of His195 that is in close contact with Glu197. Asp83 was kept neutral since no counterion can be identified in its vicinity.

Notice that we use a protonated Glu181 side-chain in our model, consistently with Strambi et al. and Frutos et al. While early experimental evidence indicates a protonated state, more recent studies are unconverging. In a recent contribution, we maintained a protonated Glu181 but also produced a second model where the chromophore was optimized.
with a deprotonated Glu181. This leads to ca. 55 nm blue-shift in the excitation energy, reducing
the agreement with experiment. This is consistent with other QM/MM studies where
deprotonation of the Glu181 leads to a blue-shift, including Sandberg et al.,\textsuperscript{58} Frahmke et al.,\textsuperscript{60}
and Tomasello et al.\textsuperscript{62} Notice, that the study by Tomasello et al.\textsuperscript{62} has concluded that models with
neutral or charged Glu181 can both be utilized to study the primary photoisomerization event
and its mechanism successfully. This is further supported by the fact that Rh excited state
trajectories computed with a deprotonated Glu181\textsuperscript{8} and with a protonated Glu181\textsuperscript{7} yield the same
reaction timescale and mechanism.

In our models, two crystallographic waters (from the 1U19 PDB) were included in the
retinal binding cavity and treated with the TIP3P model. One of these waters is near the Glu113
counterion, while the other is near Glu181. All other waters were considered far from the
chromophore and were removed. To get a globally neutral model, a chloride ion was added near
Arg147.

\begin{figure}
\centering
\includegraphics[width=0.3\textwidth]{schiff_base.png}
\caption{A schematic representation of the Schiff base between Lysine and a retinal model.
The link atoms was introduced between Cδ and Ce. The labeled dihedrals were parameterized in
our QM/MM model.}
\end{figure}
The QM/MM frontier (i.e. the frontier between the QM and MM subsystems) is placed at the Cε-Cδ bond of Lys296, far enough from the π-conjugated backbone to avoid significant effects on the chromophore properties. The hydrogen LA cap is placed on the QM subsystem Cε atom (See Figure 5.5 for Lys296 atom labels). Reparameterization of the Lys296 was necessary to reflect its QM/MM status. The net charge of the MM part of lysine is set to 0 (since the positive charge is carried by the QM fragment). Therefore the charges on Lys296 were redistributed as shown in Table 5.1.

<table>
<thead>
<tr>
<th>Atom</th>
<th>N</th>
<th>Cα</th>
<th>C carbonyl</th>
<th>HN</th>
<th>O carbonyl</th>
<th>Ha</th>
</tr>
</thead>
<tbody>
<tr>
<td>Charge</td>
<td>-0.3981</td>
<td>-0.2400</td>
<td>0.6840</td>
<td>0.2246</td>
<td>-0.6396</td>
<td>0.1426</td>
</tr>
<tr>
<td>Atom</td>
<td>Cβ</td>
<td>Hβ</td>
<td>Cγ</td>
<td>Hγ</td>
<td>Cδ</td>
<td>Hδ</td>
</tr>
<tr>
<td>Charge</td>
<td>-0.0094</td>
<td>0.0362</td>
<td>0.0187</td>
<td>0.0103</td>
<td>0.0000</td>
<td>0.0621</td>
</tr>
</tbody>
</table>

Table 5.1. A table showing the re-parameterized MM charges for Lysine 296, which is covalently bound to retinal. Since the positive charge is carried by the QM moiety, the total charge for the MM moiety is 0.

The charge of the frontier MM carbon atom Cδ was set to 0 to ensure that the QM wavefunction is not overpolarized near the LA. The Cδ point charge originally assigned by AMBER is small, and so changing it to 0 does not introduce a large imbalance. This also made possible the use of the standard MM bonded potentials (stretching, bending, etc.) for the description of the geometry at the frontier.

The van der Waals atomic parameters for retinal and the C15-N-Cε-Cδ torsion potential do not exist in AMBER. These missing parameters have been determined in such a way as to reproduce the ground state (S₀) and first excited state (S₁) CASSCF torsional energy profiles.
relative to the N-C$_\varepsilon$-C$_\delta$-C$_\gamma$ and C15-N-C$_\varepsilon$-C$_\delta$ dihedral angles of the model system shown in Figure 5.5. The resulting van der Waals parameters were (R*=1.87 Å, $\varepsilon$=0.0860 kcal/mol) for an sp$^2$ carbon atom of the retinal $\pi$-system, (R*=1.87 Å, $\varepsilon$=0.1094 kcal/mol) for an sp$^3$ carbon atom of retinal and (R*=0.92 Å, $\varepsilon$=0.0157 kcal/mol) for the hydrogen atom of retinal. The C15-N-C$_\varepsilon$-C$_\delta$ torsion potential was given by: $0.750 \left[1 + \cos(\varphi - 0) \right]$.\textsuperscript{64}

During the QM/MM optimization, all cavity side-chains were relaxed together with the two TIP3P waters, the chromophore, and the connected Lys296 side-chain. The cavity side-chains are those with at least one atom less than 4Å away from any atom of the chromophore in either its 11-cis or all-trans configuration (see green residues in Figure 5.6). All other residues were kept frozen at the initial 1U19 crystallographic structure. In total, 28 opsin side-chains were relaxed in all models. The optimization was performed with microiterations, such that the MM atoms were minimized by Tinker between each QM/MM optimization step.\textsuperscript{65} The QM/MM interactions were described with electrostatic potential fitting (ESPF).\textsuperscript{66} However, during the microiterations, Mulliken charges were used because ESPF tends to give electrostatic interactions that are too large during microiterations.
Figure 5.6. QM/MM model of Rh. The rPSB chromophore and part of the attached lysine side-chain are treated quantum mechanically (yellow), while the rest of the protein is treated with molecular mechanics. Frozen residues are in grey, while side-chains that are relaxed during the optimization are shown in green.

In this work, we produce QM/MM models for 12 different systems. Those comprise the wild-type Rh (WT), five single point mutants (A269T, E113D, F261Y, A292S, and T118A, all shown in Figure 5.7), and six derivatives based on the former six, but incorporating a modified chromophore, 3,4-dehydro-rPSB (see Figure 5.7), instead of rPSB (these derivatives are labeled A2-WT, A2-A269T, A2-E113D, A2-F261Y, A2-A292S, and A2-T118A).
Figure 5.7. Top. Bond-line structure of the two chromophores used in our models (rPSB and 3,4-dehydro-rPSB). Bottom. The 5 mutations considered in our models, and their positions relative to the chromophore. Mutations in red are red-shifting, while those in blue are blue-shifting. The two chromophores and 6 possible opsin sequences (one wild-type and 5 mutants) allow us to model 12 different systems.
The bovine rhodopsin structure (WT) provided a starting structure for building the corresponding A2-WT model. In all cases the full π-system was incorporated in the active space that comprised 12-electron in 12-orbital for WT and 14-electron in 14-orbital for A2-WT. The corresponding minima and TS\textsubscript{CT} and TS\textsubscript{DIR} structures were optimized using the two-root state-average CASSCF/6-31G*/AMBER level. The obtained WT, A2-WT and their TS\textsubscript{CT} and TS\textsubscript{DIR} models were used as templates to generate the corresponding mutant structures. These were generated manually using Molden\textsuperscript{67} and relaxed using the protocol described above. In a few cases different conformers were optimized and the lowest energy conformer was chosen. For the evaluation of excitation energies and activation barriers, CASPT2 single point calculations over a three-root state-average CASSCF wavefunction were performed with an imaginary shift of 0.2 to remove intruder states. Activation energies were computed with the default IPEA of 0.25,\textsuperscript{68} while excitation energies at the minima were computed without an IPEA shift, consistently with the benchmark results of Chapter III (see Section 3.4.7).

All QM/MM calculations were performed using the protocol distributed with the MOLCAS program\textsuperscript{69} which works in conjunction with Tinker.\textsuperscript{55} For seven mutants it was possible to validate the models by comparing the predicted with the observed\textsuperscript{70-74} excitation energies. We compare the computed excitation energies with the experimental ones (expressed as S\textsubscript{1}-S\textsubscript{0} energy gap) in Figure 5.8. We find that we have a maximum absolute error below 5 kcal/mol and a maximum relative error of 2 kcal/mol. On average, we have a systematic blue shift of ca. 3.2 kcal/mol for the excitation energies.
Figure 5.8. Correlation between computed and observed excitation energies for bovine Rh (WT), 5 of its mutants and one derivative featuring the A2-chromophore (A2-WT). The energies have been computed at the CASPT2//CASSCF//6-31G*/AMBER level with IPEA set to 0 (here we assume that the same cancellation of errors will be in effect as those observed for PSB3 in Chapter III). The experimental data are from refs. 70-74.

TSs were optimized using the restricted-step rational-function-optimization method.75 Since QM/MM frequency analysis has not yet been implemented in MOLCAS, initial attempts to optimize a TS had to rely on a guess Hessian computed at a suitable guess structure. The quality of the Hessian is evaluated by looking at the reaction vector, thus making sure that it describes the expected isomerization motion connecting the reactant (Rh) and the product (bathoRh). The selected guess Hessian was saved and read by the program in all subsequent TS optimizations. The optimizations were considered completed after convergence to a stationary point (i.e. when the analytical gradients are below the default thresholds) and the corresponding final updated transition vector represents the correct bicycle-pedal motion. The resulting single TSs are considered acceptable representations of the average TS operating in the protein environment.
The protocol we use is generally accepted for the optimization of TSs in conformationally inflexible protein environments e.g. in enzyme catalysis (see refs. 76-78) and where the x-ray crystallographic structure already provides an acceptable representation of the protein backbone. On the other hand, our work is not concerned with reproducing the absolute value of the potential energy barriers (which, according to the adopted protocol, may be upper limits), but rather with the barrier differences (e.g. $T_{\text{CT}}-T_{\text{DIR}}$) and the barrier changes across the different models. These are less sensitive to the details of the methodology used.

Since we cannot verify the TSs by frequency calculations, we use instead classical trajectory calculations to verify that $T_{\text{CT}}$ and $T_{\text{DIR}}$ correspond to saddle points driving the isomerization. The results are presented in Section 5.5. For each stationary point, two trajectories were launched in the positive and negative direction of the reaction vector. We used the trajectory module DYNAMIX,$^{52}$ which is part of the MOLCAS software package and which uses the Verlet algorithm to propagate the Newton equations of motion.$^{79}$ The trajectories were run with a timestep of 1 fs, and with small initial velocities parallel to the reaction vector.

Out of the 24 TSs, only one could not be successfully optimized. This is the $T_{\text{CT}}$ for A2-A292S (the most blue-shifted and high energy of all considered A2-WT mutant). We speculate that this TS could not be optimized because it ceases to exist at the CASSCF level due to a change in the shape of the CI from peaked to sloped where only one TS exists.$^{25}$
5.5. Results and Discussion

In order to test the hypothesis presented earlier in this report, we first optimized the two transition states, TS\textsubscript{CT} and TS\textsubscript{DIR}, in WT Rh. The geometries and electronic structure (represented by the charge distribution) of the two TSs are presented in Figure 5.9. This data supports the potential energy surface of Figure 5.4, since we can see that the geometries of the TS\textsubscript{CT} and TS\textsubscript{DIR} structures differ along the BLA coordinate but have similar dihedrals (i.e. are ca. 90° twisted along the C11=C12 double bond). The two TSs also display a very different charge distribution, consistent with the fact that they lie in different regions of the S\textsubscript{0} potential energy surface dominated by a different wavefunction. In order to demonstrate that TS\textsubscript{CT} and TS\textsubscript{DIR} lie on opposite sides of the CI, we computed the excited and ground state energies along series of structures produced by a linear interpolation/extrapolation of the coordinates of TS\textsubscript{DIR} and TS\textsubscript{CT} (similar to how the PSB3 BLA scan was generated in Chapter III). As expected, these generated structures had different BLAs but otherwise had similar geometries. The energy profile along this scan is shown in Figure 5.10. Indeed, we see that the two TSs lie on a minimum along this scan (which we assume is mostly parallel to a BLA coordinate), and that they are separated by a CI point.
Figure 5.9. Electronic (left) and geometric (right) structure of TS\textsubscript{CT} and TS\textsubscript{DIR} structures in WT Rh. The $S_0$ Mulliken charges along the backbone of the chromophore are represented with bubble diagrams. The labels indicate the maximum and minimum values for the charges. The bond-line formulas represent the dominant electronic configurations (wavefunctions) of the corresponding states. The labels on the geometric structures show the main geometrical parameters (numbers in red are dihedral angles in degrees, while numbers in black are bond lengths in Angstroms).

Figure 5.10. $S_0$ CASPT2 energies along a scan produced by a linear interpolation/extrapolation of coordinates between the two optimized TS geometries. The color of the curve represents the dominant electronic character of the wavefunction (green = $\psi_{\text{DIR}}$, brown = $\psi_{\text{CT}}$). The energies are shown relative to the energy of the Rh equilibrium structure. The CASPT2 energies were computed with the default IPEA value of 0.25, which is more accurate for computing energies along such a BLA scan as we show in Chapter III.
To verify that both TSs mediate cis to trans thermal isomerization, in Figure 5.11, we show the energy profiles along CASSCF trajectories released from each of the optimized TS\textsubscript{CT} and TS\textsubscript{DIR} structures with a small velocity in the positive (towards bathoRh) and negative (towards Rh) directions. For each TS, the trajectories going towards Rh were reversed and conjoined with the trajectory towards bathoRh. Therefore, the four trajectories are paired up to give two trajectories going from Rh to bathoRh, one trajectory passing through TS\textsubscript{DIR} and the other passing through TS\textsubscript{CT} (as shown in Figure 5.11). In the same figure we also plot the charge transfer character along these trajectories. As expected, we find in both cases that the trajectories lead to the expected cis reactant (corresponding to Rh) and trans product (corresponding to bathoRh) via a downhill path. Also, while the TS\textsubscript{CT} and TS\textsubscript{DIR} trajectories appear energetically similar, we see a clear difference in the charge transfer profile. Indeed, the TS\textsubscript{CT} trajectory passes through a region with significant charge transfer character, whereas the region near TS\textsubscript{DIR} has a reduced charge transfer character with respect to the rest of the trajectory. These results, together with the results of Figure 5.10, demonstrate that the two TSs are minima along the BLA coordinate, maxima along the RC (isomerization) coordinate, and have different electronic characters, therefore supporting the general shape of the S\textsubscript{0} potential energy surface presented in Figure 5.4.
Figure 5.11. CASSCF energy profiles (black lines) and charge transfer character (orange area) along trajectory calculations simulating the thermal isomerization from Rh to bathoRh via TS\textsubscript{CT} (top) and TS\textsubscript{DIR} (bottom). Two trajectories were launched from each TS with small velocities directing them towards the reactant or product. The backward trajectory (towards Rh) was reversed and conjoined with the forward trajectory (towards bathoRh) for each case in order to produce the displayed trajectory path. Charge transfer character is computed by summing the Mulliken charges on the $\beta$-ionone-containing moiety, as in Figure 5.3.
TS_{DIR} features the structure expected for a homolytically broken double bond with two radical centers delocalized along orthogonal p-systems and corresponds to the TS of Figure 5.2. As expected, its charge distribution correlates with that of the S_0 rPSB reactant, with a +0.98 e charge localized in the Schiff base region. In contrast, TS_{CT} has most of its charge (+0.90 e) located on the β-ionone region and thus more closely resembles the charge distribution of vertically excited S_1 rPSB (compare the corresponding bubble diagrams in Figures 5.2 and 5.9).

We find that TS_{CT} has a computed activation energy which lies ca. 10.8 kcal/mol in energy below TS_{DIR} at the CASPT2//CASSCF/6-31G*/AMBER level of theory with IPEA=0.25 (see Figure 5.9). Therefore, TS_{CT} has full control of the thermal isomerization.

**Figure 5.12.** CASPT2//CASSCF/6-31G*/AMBER computed E_a^T values vs. 1/λ_{max} in the 12 QM/MM models of Rh and its mutants and A2 derivatives described in Figure 5.7. Linear regressions are shown separately for sequences incorporating the A1 chromophore (rPSB) and the A2 chromophore (3,4-dehydro-rPSB), and for the different transition states (TS_{CT}, in red, or TS_{DIR} in blue).
The close electronic characters of $\text{TS}_{\text{CT}}$ and of the opsin-embedded $S_1$ rPSB (i.e., a positive charge preferentially localized on the $\beta$-ionone region) provide a direct link between $E_{aT}$ and $\Delta E_{(S_1 - S_0)}$ and, in turn, $-\log k$ and $1/\lambda_{\text{max}}$. Accordingly, any opsin red-shifting the absorption would simultaneously decrease $E_{aT}$. The opposite behavior, not consistent with the Barlow correlation, would be observed if the isomerization were controlled by $\text{TS}_{\text{DIR}}$. In order to support these conjectures, we display in Figure 5.12 the $E_{aT}$ versus $1/\lambda_{\text{max}}$ correlation computed by modeling the full set of 12 pigments. We note that $1/\lambda_{\text{max}}$ is directly proportional $\Delta E_{(S_1 - S_0)}$ due to the relationship:

$$\Delta E_{(S_1 - S_0)} = \frac{hc}{\lambda_{\text{max}}}$$

where $h$ is Planck’s constant and $c$ is the speed of light. The QM/MM-derived correlation supports the conclusion that $\text{TS}_{\text{CT}}$ and $S_1$ rPSB have similar charge distributions in all 12 pigments, displaying a clear positive slope exclusively for the $\text{TS}_{\text{CT}}$ barriers. This is in agreement with the observed Barlow correlation that holds that red-shifted visual pigments have lower thermal isomerization barriers. On the other hand, $\text{TS}_{\text{DIR}}$ barriers display a negative slope as a function of $1/\lambda_{\text{max}}$. 
Figure 5.13. VB state ($\Phi_{\text{DIR}}$ and $\Phi_{\text{CT}}$) correlation diagram along the TS$\text{CT}$ path (panels I and II) and TS$\text{DIR}$ path (panels III and IV). Panels II and IV display the result of stabilizing $\Phi_{\text{CT}}$ relative to panels I and IV (i.e. by a red-shifting mutation, shown as red lines compared to the original blue lines). The structures represent the charge distribution, and the numbers represent the value of the total charge on the two moieties defined by cutting the C11=C12 bond (i.e. the charge transfer character).

The linear dependence of $E_a^T$ on $1/\lambda_{\text{max}}$ in both TS$\text{CT}$ and TS$\text{DIR}$ may also be understood with Valence Bond (VB) theory. In Figure 5.13 we have constructed a two-state VB mixing diagram for both reaction paths of Figure 5.4. Here, we temporarily abandon our earlier use of $\psi_{\text{CT}}$ and $\psi_{\text{DIR}}$, and instead represent the wavefunctions of the two states as $\psi_{S0}$ and $\psi_{S1}$ instead, corresponding to ground and excited state wavefunctions, respectively. These states are the result
of mixing of two different resonance formulas displaying inverted rPSB electron-pairing, where one is a pure charge transfer configuration ($\Phi_{\text{CT}}$) with respect to the other which is a pure covalent diradical configuration ($\Phi_{\text{DIR}}$). These two configurations feature a full localization of the positive charge in one of the two halves of the chromophore framework.

In the case of TS$_{\text{CT}}$, the explanation for the linear dependence is relatively straightforward. The VB states ($\Phi_{\text{CT}}$ and $\Phi_{\text{DIR}}$) cross along the TS$_{\text{CT}}$ path such that, consistently with our calculations, at TS$_{\text{CT}}$ the $\psi_{S0}$ wavefunction is dominated by $\Phi_{\text{CT}}$ (hence throughout this chapter we refer to it as $\psi_{\text{CT}}$). A mutation that stabilizes $\Phi_{\text{CT}}$ would decrease $\Delta E_{(S1 - S0)}$, but would also decrease $E_{aT_{\text{CT}}}$ (the effect of such a red-shifting mutation could be understood by comparing panels I and II of Figure 5.13).

On the other hand, the computed charge distribution indicates that while the Rh $S_0$ wavefunction ($\psi_{S0}$) is dominated by $\Phi_{\text{DIR}}$, it contains a contribution of $\Phi_{\text{CT}}$. Conversely, the $\psi_{S1}$ is dominated by $\Phi_{\text{CT}}$ but contains a contribution of $\Phi_{\text{DIR}}$. Meanwhile, $\psi_{S0}$ at TS$_{\text{DIR}}$ is almost completely dominated by $\Phi_{\text{DIR}}$ with almost no contribution from $\Phi_{\text{CT}}$ (the two configurations do not mix along this central part of the path). Therefore, a mutation that stabilizes $\Phi_{\text{CT}}$ would decrease $\Delta E_{(S1 - S0)}$, but would still stabilize the $\psi_{S0}$ at Rh more than at TS$_{\text{DIR}}$, and accordingly (see panels III and IV in Figure 5.13) would result in an relative destabilization of TS$_{\text{DIR}}$ relative to the reactant, decreasing $E_{aT_{\text{DIR}}}$. Therefore, TS$_{\text{DIR}}$ behaves very different compared to TS$_{\text{CT}}$, and displays an inverse proportionality with respect to $1/\lambda_{\text{max}}$ (as we see in Figure 5.12).

In the above, we explain the correlation between $\Delta E_{(S1 - S0)}$, the excitation energy, and $E_{aT}$, the thermal activation barrier. This is sufficient to understand the experimentally observed Barlow correlation, which is a correlation between the log of the kinetic rate constant (-log $k$)
and the inverse absorption maximum ($1/\lambda_{\text{max}}$). However, in order to simulate the Barlow correlation itself, we must adopt a kinetic model to compute the kinetic rate constant from $E_{aT}$. While this can be attempted by using TS theory, such a calculation is impractical for molecules of the size of visual pigments. Therefore, here we start by assuming the validity of the same Hinshelwood kinetic model originally proposed by Ala-Laurila et al.\textsuperscript{22} and adopted in Luo et al.\textsuperscript{24}

**Figure 5.14.** Simulation of the -log $k$ vs. $1/\lambda_{\text{max}}$ relationship. By using the set of our computed TSCT $E_{aT}$ values and the same Hinshelwood pre-exponential factor used in Luo et al. (with $m=45$)\textsuperscript{24} it is possible to successfully simulate the Barlow relation for 11 rod pigments using the log($k$) = log($A(E_{aT}, T)-E_{aT}/RT$) expression (open circles). Note that by “pre-exponential factor” $A(E_{aT}, T)$ we meant the full set of terms preceding the “exponential factor” $e^{-E_{aT}/RT}$ in the expression of the rate constant $k$. In the case of the Hinshelwood model this factor depends on $E_{aT}$, $T$, and $m$. The computed relation is compared with experimental data (crosses), which are collected in ref. 22. The positive slope of the -log $k$ vs. $1/\lambda_{\text{max}}$ relation computed with a constant pre-exponential factor ($m=1$, dashed line) clearly shows that the Hinshelwood pre-exponential factor modulates (decreases) the slope of the relation. In the bottom right corner, we show the effect of the parameter $m$ on the slope of the graph.
By using the set of our computed TS\textsubscript{CT} $E_a^T$ values and the Hinshelwood kinetic model it is possible to successfully simulate the Barlow relation for 11 rod pigments using the Hinshelwood expression:

$$\log k = \log A(E_a^T, T) - \frac{E_a^T}{RT}$$

where:

$$A(E_a^T, T) \propto \sum_{1}^{m} \frac{1}{(m-1)!} \left( \frac{E_a^T}{RT} \right)^{m-1}$$

We plot the resulting -log $k$ values against $1/\lambda_{\text{max}}$ Figure 5.14 (open circles). Here we use $m=45$ as in ref. 22. Notice that the pre-exponential factor in this equation, i.e. the $A(E_a^T, T)$ factor, depends on $E_a^T$, $T$, and $m$. The computed relation is compared with experimental data resulting from various measurements of thermal kinetic constants (crosses in Figure 5.14), which are collected in ref. 22. The positive slope of the -log $k$ vs. $1/\lambda_{\text{max}}$ relation computed with a constant pre-exponential factor ($m=1$, corresponding to a simple Boltzmann model and shown as a dashed line in Figure 5.14) clearly shows that the Hinshelwood pre-exponential factor decreases the slope of the relation. However, we also show in the inset of Figure 5.14 (bottom right corner) the effect of the value of $m$ on the slope of the curve. We find that the slope decreases as a function of the number of modes $m$ but this will never result into a change of the slope from positive to negative. We find that any $m$ value between 40 and 50 reproduces the experimental slope satisfactorily.
The Barlow correlation appears to be generally valid for experimentally tested visual pigments, so we conclude that in these experimentally tested systems, TS\textsubscript{CT}, rather than TS\textsubscript{DIR}, controls the thermal isomerization. However, the data of Figure 5.12 predict that TS\textsubscript{DIR} will control thermal isomerization for $\lambda_{\text{max}}$ below 470 nm (after accounting for a systematic $\sim$3.2 kcal/mol blue-shift error in our computed excitation energy, as we saw in Figure 5.8). In more blue-shifted pigments, we would expect to observe an anti-Barlow correlation, seen as a negative slope in the diagram of Figure 5.12. The same results provide evidence that the rate-determining step controlling the thermal noise must be the canonical rPSB isomerization, in agreement with the Luo et al.\textsuperscript{24} hypothesis. As discussed in Luo et al.,\textsuperscript{24} the observed correlation is not necessarily incompatible with the previously reported 22 kcal/mol apparent activation energy because, in that case, an energy- and temperature-independent pre-exponential factor was assumed.\textsuperscript{11} Our conclusions are also not incompatible with the recently proposed noise model based on protein fluctuations\textsuperscript{19} provided that rPSB isomerization remains the kinetic bottleneck and takes place inside the protein cavity. Finally, the hypothesis that the Barlow correlation is a chromophore property is supported by similar relationships observed for the 13-cis chromophore in solution and in bacterioRh\textsubscript{1},\textsuperscript{81,82} and by earlier computational studies showing the existence of a charge transfer region on the S\textsubscript{0} potential energy surface\textsuperscript{42,83}. 
5.6. Conclusion and Perspective

The results above provide an explanation for the fact that thermal noise decreases when the $\lambda_{\text{max}}$ is shifted to shorter wavelengths (i.e. the Barlow correlation). This apparently technical fact has basic consequences for the biology of vision. Related to the Barlow correlation is the so-called Purkyné effect, which refers to the fact that in the dark (e.g. at dusk or night) bluer objects are more clear than red objects, which may appear black (see Figure 5.15). In fact, our finding that maximum visual sensitivity can be achieved at the green-blue end of the spectrum, beyond which no further noise decrease can be attained (due to the existence of $\text{TS}_{\text{DIR}}$ which can control thermal isomerization at that point in an anti-Barlow way) is highly consistent with the fact that rod rhodopsins typically absorb at green-blue wavelengths, and therefore with the Purkyné effect.

![Day, Dusk, Night](image)

**Figure 5.15.** Photographic reconstruction of the blue-shift of perceived color under diminishing levels of illumination. This picture is adapted from derivative work by Dick Lyon of original by Lewis Collard.
It can be concluded that the key to understand the origin of the dark noise is the existence of two electronically different transition states (two reaction bottlenecks) with the lower displaying the same charge transfer character of the excited state of Rh. This is a mere consequence of the geometric phase: a fundamental property of the chromophore electronic wavefunction in the region of the CI\textsuperscript{25,29}. Therefore the Barlow correlation represents a manifestation of the existence of a CI in Rh and complements the evidence provided by spectroscopic studies. It connects the biology of vision to molecular physics at a fundamental level. In fact, without this CI high visual sensitivity would be achieved at the red edge of the visible spectrum rather than the blue. Further evidence supporting this theory could be provided by the observation of an “anti-Barlow” correlation (i.e. a decrease of -log \( k \) as a function of 1/\( \lambda_{\text{max}} \)) for a series of pigments absorbing radiations shorter than 470 nm.
5.7. References


