# Computationally Designing a Fluorescent Probe to Detect Lipid Membrane Phase Changes 

Max Winslow<br>School of Science and Technology<br>Nottingham Trent University

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#### Abstract

Lipid membranes are responsible for the regulation of many key cellular processes. Some of these processes, such as signalling events, are thought to be induced by the presence of lipid rafts. Observation of rafts in vivo has therefore been a key area of research to gain a better insight to the existence and function of membrane rafts. One promising technique is the use of fluorescent molecular probes designed to report on phase changes within a lipid membrane. In this work, a computational protocol to investigate a novel probe of membrane phase has been developed. In this approach, spin-flip time-dependent density functional theory is carefully calibrated and employed to investigate conical intersections to understand the restriction of intramolecular movement mechanism, whereby the fluorescence of a molecule is dependent upon the freedom of rotation around a bond responsible for the access of non-radiative decay pathways.

The probes tested in this study are methyl derivatives of 1,6-diphenylhexatriene and applied to quantum mechanics/molecular mechanics simulations of raft and non-raft environments, along with a preliminary study of molecules which could act as delivery vessels in vivo. It was found that the key dihedral angle of the 2-methyl derivative (2Me) could rotate sufficiently for non-radiative decay in the liquid-disordered non-raft environment whereas rotation was inhibited in the liquid-ordered raft environment promoting fluorescence making $\mathbf{2 M e}$ an ideal candidate for a simple phase sensitive fluorescent membrane probe.


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## Chapter 1. Introduction

### 1.1. Lipid Membranes

Phospholipids are the most common class of lipids found in cell membranes. These lipids have a typical structure consisting of a hydrophilic, polar head group and two hydrophobic hydrocarbon tails, giving the lipids an amphiphilic property. One type of phospholipid are the phosphoglycerides which are built upon a three-carbon glycerol backbone, Figure 1.1. The two long chain, aliphatic, hydrocarbon tails attach to two neighbouring carbon atoms in the glycerol, each linked through an ester bond, whilst the final carbon attaches to the phosphate group. The phosphate group can then link to a head group, such as choline. Different combinations of hydrophobic tails and head groups generate different types of phospholipids, some common examples and their components are given in Figure 1.2.

Another type of phospholipid are sphingolipids, which have a sphingosine structure rather than a glycerol backbone. Sphingosine is comprised of an 18-carbon chain capped with an amino group and two hydroxyl groups. In this case, another hydrocarbon tail is added to the amino group whilst the terminal hydroxyl group is linked to the phosphate of a head group (Figure 1.2d).

The hydrocarbon tails of phospholipids are typically fatty acids that can differ in length, usually ranging from 14-24 carbon atoms. ${ }^{1}$ One of these tails tends to be completely saturated whilst the other would contain one or more double bonds allowing for a cis conformation, creating a kink in the tail.

The amphiphilic nature of lipids means that they spontaneously self-assemble to form a lipid bilayer in aqueous environments due to the hydrophobic effect, the lowering in energy of a system by reducing the amount of contact between hydrophobic molecules and polar solvents, such as water. This effect results in the polar, hydrophilic headgroups aligned next to each other pointing towards the water molecules and the hydrophobic tails pointing inwards to each other.


Figure 1.1 Chemical structure of phosphatidylcholine. The blue box displays the choline part of the head group. The green box displays the phosphate part of the head group. The orange box displays the glycerol part of the head group.


Figure 1.2 Common phospholipids: a) phosphatidylethanolamine, b) phosphatidylserine, c) phosphatidylcholine, d) sphingomyelin.

Lipid bilayers play an important role in biology as they not only form a barrier to protect the internal components of a cell but are also involved in the transportation of materials entering and leaving the cell by altering its fluidity. ${ }^{2,3}$

Lipids have a characteristic phase transition temperature, $\mathrm{T}_{\mathrm{m}}$, which allows them to exist in different physical state depending on the temperature. Below $T_{m}$ the lipids in a bilayer become rigid and well-ordered resulting in gel phase termed solid ordered, $\mathrm{S}_{\mathrm{o}}$, which subsequently reduces the fluidity and permeability of the bilayer. Temperatures above $\mathrm{T}_{\mathrm{m}}$ causes the lipids to exist in a liquid disordered phase, $L_{d}$, therefore increasing the bilayer fluidity and permeability.

Another important component of lipid bilayers is cholesterol, Figure 1.3. Cholesterol is a sterol comprised of a ring structure attached to a polar hydroxyl group at one end and a short hydrocarbon chain at the other. Cholesterol also has amphiphilic properties and aligns in the same direction as the lipids in the bilayer. Cholesterol itself does not form a bilayer structure; however due to its smaller size it can insert itself into spaces formed by the kinked fatty acid chains of the lipids. When a bilayer is in the $L_{d}$ phase, the presence of cholesterol decreases the membrane fluidity and at high enough molar concentration this leads to formation of a liquid-ordered phase, $\mathrm{L}_{\mathrm{o}}$.


Figure 1.3 Molecular structure of cholesterol.

It is possible within a lipid membrane for there to be small, short-lived regions ${ }^{4}$ existing in a different phase to the surrounding bilayer; these regions are known as lipid rafts. ${ }^{5}$ These domains are enriched in cholesterol and sphingomyelin and display an increased thickness compared to the surrounding bilayer. ${ }^{6,7}$ A schematic diagram of a lipid raft within a membrane bilayer is shown in Figure 1.4. Lipid rafts are also thought to be involved in cell signalling events, ${ }^{8}$ however much is still unknown about their formation and function ${ }^{9}$ with many experimental studies failing to produce results always in agreement with each other due to issues such as the use of detergents, which may be responsible for changing the composition of the extracted membrane, or the use of labels to track raft associated molecules affecting the diffusion of those molecules. ${ }^{10}$


Figure 1.4 Schematic diagram of a lipid bilayer membrane. (1) Liquid disordered phase, $\mathrm{L}_{\mathrm{d}}$. (2) Lipid raft, consisting of liquid ordered phase, $\mathrm{L}_{0}$. (3) Raft-associated protein, such as transmembrane glycoproteins and GPI-anchored proteins. ${ }^{7}$ (4) Non-raft associated protein. (5) Cholesterol.

Klymchenko et al. reviewed the works of fluorescent membrane probes designed to identify lipid rafts, each with their own pitfalls such as requiring a high concentration, having low photosensitivity or having limited staining due to their structure being different to those naturally present in cell membranes. ${ }^{11}$

Therefore, the aim of this research is to computationally design a simple fluorescent probe that can report on membrane phase changes, thereby identifying lipid rafts and allowing for a greater insight into their role, lifetime, and frequency.

### 1.2. Fluorescence

Fluorescence is a radiative process which occurs when a molecule releases excess energy as a photon. A molecule can be promoted from the ground state to an electronically excited state by absorbing a photon of sufficient energy, resulting in an excitation of an electron, typically from a bonding or non-bonding orbital into an antibonding orbital creating a rapid redistribution of electron density. The nuclei then begin to vibrate and collide with any surrounding molecules to dissipate energy. When the remaining energy is too large to be removed non-radiatively, the molecule will relax to its ground state by emitting a photon. This loss of energy through vibrational relaxation means that the emitted photon is of a lower frequency than the absorbed photon. Figure 1.5 is of a Jablonski diagram displaying the events leading to fluorescent emission.


Figure 1.5 Jablonski diagram of fluorescence.

A molecule with a conjugated $\pi$ system can give rise to fluorescence occurring in the visible part of the electromagnetic spectrum. This is due to the molecular orbitals lying
close together in energy, reducing the gap between the highest occupied molecular orbital (HOMO) and the lowest unoccupied molecular orbital (LUMO) which in turn reduces the required energy of a photon to be absorbed which also subsequently reduces the energy of the photon released in emission.

### 1.3. The ACQ and AIE Effect

Fluorescent molecules are utilised in many different areas, including organic light-emitting diodes (OLEDs), ${ }^{12,13}$ bioimaging techniques, ${ }^{14}$ chemosensors ${ }^{15}$ and dyes. ${ }^{16}$ An obstacle often faced when using fluorescent molecules is the aggregation-caused quenching (ACQ) effect, whereby the fluorescence of a molecule decreases when it is in solid ${ }^{17}$ or aggregated states due to $\pi-\pi$ stacking interactions or the formation of excimers. These types of molecules are termed ACQphores. In 2001, Tang et al. discovered that the silole derivative 1-methyl-1,2,3,4,5-pentaphenylsilole (Figure 1.6) exhibited enhanced fluorescence upon aggregation. ${ }^{18,19}$ This phenomenon was coined the aggregation-induced emission (AIE) effect, with molecules exhibiting this effect described as AIEgens. Since its discovery there have been other compounds found to possess this phenomenon, including 8,8a-dihydrocyclopenta[a]indene derivatives, ${ }^{20}$ tetraphenylethene, ${ }^{21}$ THBDBA ${ }^{22}$ and DCMP derivatives. ${ }^{23}$


Figure 1.6 Molecular structure of 1-methyl-1,2,3,4,5-pentaphenylsilole.

One proposed mechanism for the AIE effect is the restriction of intramolecular movement (RIM). When in dilute solutions, the motor groups of AIEgens, such as phenyl and methyl substitutions, are free to rotate thereby allowing access to radiationless decay pathways, such as a conical intersection (ConInc), resulting in a loss of fluorescence. When the AIEgens begin to aggregate, the steric hinderance of the motor groups increase therefore preventing them from rotating. This blocks the non-radiative decay pathway resulting in fluorescence.

Conlncs occur when the potential energy surface (PES) of an electronically excited state becomes degenerate with a lower electronic state allowing for a non-radiative relaxation, a schematic diagram of a ConInc is shown in Figure 1.7. The accessibility of a ConInc therefore affects the fluorescence emission of a molecule. To computationally optimise a ConInc is challenging and often requires high levels of theory, incurring a significant computational cost. Therefore, to design a fluorescent membrane probe will also require a suitable approach to identify Conlncs.


Figure 1.7 Schematic representation on a ConInc on a PES.

Bhongale et al. demonstrated that making simple substitutions to an ACQphore can convert it into an AIEgen. ${ }^{24}$ In their work they took the ACQphore of 1,4-di[(E)-styryl]benzene and substituted the $\alpha$-olefinic hydrogens with methyl groups
(Figure 1.8). This resulted in the new molecule displaying no fluorescence when in dilute solutions but becoming highly emissive upon aggregate formation.



Figure 1.8 (left) 1,4-di[(E)-styryl]benzene and (right) its dimethyl derivative.

The fluorescent membrane probe in this work will utilise the RIM mechanism of the AIE effect so that a non-radiative decay pathway will be accessible in a non-raft $L_{d}$ phase of a membrane which will become blocked in the $L_{\circ}$ phase of a membrane raft resulting in fluorescent emission.

### 1.4. Using POMs to Transport the Probe to a Membrane

The versatility of polyoxometalates (POMs) and their ability to be modified, thereby changing their properties, makes them a viable candidate for the transportation of the probe developed in this work for cellular uptake.

POMs are a group of photoactive, polynuclear anionic molecular metal-oxide clusters comprised of early transition metals in their highest oxidation states; some common examples are $\mathrm{V}^{\mathrm{V}}, \mathrm{Mo}^{\mathrm{VI}}$ and $\mathrm{W}^{\mathrm{V} \mathrm{V}}$. POMs have attracted a lot of attention due to their reversible redox processes as well as their stability and tunability. ${ }^{25}$ The ability to attach organic groups onto a POM structure to change their properties has proved to be a desired feature in applications to medicine. ${ }^{26,27}$ POMs have also been shown to have biological activity due to their interactions with proteins. ${ }^{28,29}$ Stephan et al. conducted a review which looked at strategies for the biofunctionalization of POMs, ${ }^{30}$ stating the possibilities to graft bioorganic compounds onto a POM framework to then be transported to cells.

### 1.5. Outline

Chapter 2 provides background theory to the computational methods used throughout this work. Chapter 3 details the collaborative project concerned with the function of POMs as photocatalysts. Chapter 4 then compares different computational approaches in their ability to identify Conlncs. Chapter 5 provides details of the probes to be investigated in this work and their performance in a gas phase environment. Finally, Chapter 6 applies the probes to membrane raft and non-raft simulations to determine whether they can report on membrane phase changes.

## Chapter 2. Computational Background

The time-independent Schrödinger equation, eqn(2.1), is used to describe the electronic properties of a molecule.

$$
\begin{equation*}
\widehat{H}\left|\Psi_{a}\right\rangle=E_{a}\left|\Psi_{a}\right\rangle \tag{2.1}
\end{equation*}
$$

In eqn(2.1), $\Psi$ is the wavefunction which contains the positions of the particles in the system, $E$ is the energy of the system, $a$ denotes the electronic state of interest and $\widehat{H}$ is the molecular Hamiltonian operator acting on the wavefunction (in atomic units):

$$
\begin{equation*}
\widehat{H}=-\sum_{i} \frac{1}{2} \nabla_{i}^{2}-\sum_{A} \frac{1}{2 M_{A}} \nabla_{A}^{2}-\sum_{i} \sum_{A} \frac{Z_{A}}{r_{i A}}+\sum_{i<j} \frac{1}{r_{i j}}+\sum_{A<B} \frac{Z_{A} Z_{B}}{R_{A B}} \tag{2.2}
\end{equation*}
$$

The first term represents the kinetic energy of the $i$ th electron, the second term is the kinetic energy of nucleus $A$, the third term is the potential energy of attraction between electron $i$ and nucleus $A$, the fourth term is the potential energy of repulsion between electrons $i$ and $j$, and the final term represents the repulsion between nuclei $A$ and $B$.

The energy of a system using eqn(2.1) is obtained by pre-multiplying by the complex conjugate of $\Psi_{a}$, and rearranging to find $E$, resulting in

$$
\begin{equation*}
E=\frac{\left\langle\Psi_{a}\right| \widehat{H}\left|\Psi_{a}\right\rangle}{\left\langle\Psi_{a} \mid \Psi_{a}\right\rangle} \tag{2.3}
\end{equation*}
$$

The Schrödinger equation can only be solved exactly for a one electron system, the solutions of which are the atomic orbitals $s, p, d$, $f$ and higher angular momentum orbitals. For this equation to be used in systems containing more than one electron requires approximations to be made.

Electrons have a much smaller mass than protons and neutrons, such that they have a high momentum in comparison. Therefore, it can be assumed that the electrons move around fixed nuclei. If the nuclei are in fixed positions, then their kinetic energy is zero. This means the second term of the Hamiltonian can be removed and the last term becomes a constant turning the molecular Hamiltonian into the electronic Hamiltonian,

$$
\begin{equation*}
\widehat{H}=-\sum_{i} \frac{1}{2} \nabla_{i}^{2}-\sum_{i} \sum_{A} \frac{Z_{A}}{r_{i A}}+\sum_{i<j} \frac{1}{r_{i j}} \tag{2.4}
\end{equation*}
$$

The wavefunction of the electronic Schrödinger equation is now dependent upon the electronic positions for a given set of nuclear coordinates. This is known as the Born-Oppenheimer approximation, which gives rise to the concept of a potential energy surface (PES).

Another approximation which can be made to solve the electronic Schrödinger equation is the Hartree-Fock (HF) model which assumes that electrons move independently of each other. Each electron can be described by a one electron wavefunction, an orbital, allowing the overall wavefunction to be evaluated as the product of the individual wavefunctions. However, this description of wavefunction does not account for the spin of the electrons, $\phi(X)$ where $X$ includes the variables of space, $r$, and spin, $s$, meaning the system does not satisfy the Pauli exclusion principle - it is not antisymmetric if the coordinates of two electrons are swapped. To combat this, the wavefunction can be written as a Slater determinant, eqn(2.5), a matrix containing the combinations of each electron distributed between each spin orbital. If two electrons were to have the same spin and coordinates, meaning that two rows or columns of the Slater determinant are equivalent, the determinant will equal zero, which also satisfies the Pauli exclusion principle.

$$
\left|\Psi\left(X_{1} X_{2} X_{3} \ldots X_{N}\right)\right\rangle=\frac{1}{\sqrt{N!}}\left|\begin{array}{cccc}
\phi_{i}\left(X_{1}\right) & \phi_{j}\left(X_{1}\right) & \cdots & \phi_{k}\left(X_{1}\right)  \tag{2.5}\\
\phi_{i}\left(X_{2}\right) & \phi_{j}\left(X_{2}\right) & \cdots & \phi_{k}\left(X_{2}\right) \\
\vdots & \vdots & \vdots & \vdots \\
\phi_{i}\left(X_{N}\right) & \phi_{j}\left(X_{N}\right) & \cdots & \phi_{k}\left(X_{N}\right)
\end{array}\right|
$$

The potential energy surfaces produced from the Born-Oppenheimer approximation show that each electronic state has its own wavefunction and associated energy, meaning the wavefunctions have no overlap with each other; they are orthogonal. When evaluating wavefunctions it is also necessary for them to be normalised. This condition of orthonormality can be expressed using the Kronecker delta function;

$$
\delta_{i j}=\left\langle\Psi_{i} \mid \Psi_{j}\right\rangle=\left\{\begin{array}{l}
0 \text { if } i \neq j  \tag{2.6}\\
1 \text { if } i=j
\end{array}\right.
$$

The expectation value in eqn(2.3) can be reduced to

$$
\begin{equation*}
E=\langle\Psi| H|\Psi\rangle \tag{2.7}
\end{equation*}
$$

by employing eqn(2.6).

The form of the electronic Hamiltonian contains two different electron operators, one-electron operators, and two-electron operators. The first category describes the kinetic energy of each electron and the potential energy of attraction between each electron and nuclei. There is then a two-electron operator for the potential energy of repulsion between each pair of electrons. The one-electron operators can be combined into a single term, for example for an electron $i$

$$
\begin{equation*}
\hat{h}\left(\mathbf{r}_{i}\right)=-\frac{1}{2} \nabla_{i}^{2}-\sum_{A}^{n u c l e i} \frac{Z_{A}}{r_{i A}} \tag{2.8}
\end{equation*}
$$

and the two-electron operator for electrons $i$ and $j$ is

$$
\begin{equation*}
\frac{1}{r_{i j}} \tag{2.9}
\end{equation*}
$$

The energy of a system can be obtained using a Slater determinant and substituting eqn(2.8) and eqn(2.9) into eqn(2.7). For example, a system containing $N$ electrons with a Slater determinant of $N$ spin orbitals

$$
\begin{equation*}
|\Psi\rangle=\left|\phi_{i} \phi_{j} \phi_{k} \phi_{l} \cdots\right\rangle \tag{2.10}
\end{equation*}
$$

would yield

$$
\begin{align*}
\langle\Psi| H|\Psi\rangle & =\left\langle\phi_{i} \phi_{j} \phi_{k} \phi_{l} \cdots\right| H\left|\phi_{i} \phi_{j} \phi_{k} \phi_{l \cdots}\right\rangle \\
& =\sum_{i}^{N} h_{i i}+\sum_{i<j}^{N}[(i i \mid j j)-(i j \mid j i)] \tag{2.11}
\end{align*}
$$

Whereby $h_{i i}$ is the one-electron operator, $(i i \mid j j)$ and $(i j \mid j i)$ are the two-electron Coulomb and exchange integrals respectively. The exchange integral differs from the Coulomb integral in that the coordinates of $\phi_{i}$ are swapped with the coordinates $\phi_{j}$ due to the antisymmetry of the wavefunction, this term vanishes if the orbitals have a different spin.

The chemists' notation is used for the two electron integrals (as opposed to the physicists' notation which uses angled brackets). Inside the parenthesis, the left-hand side corresponds to the orbitals of electron 1 and those to the right refer to the orbitals of electron 2

$$
\begin{equation*}
\left\langle\phi_{i} \phi_{k}\right| \frac{1}{r_{12}}\left|\phi_{j} \phi_{l}\right\rangle=\left\langle\phi_{i} \phi_{k} \mid \phi_{j} \phi_{l}\right\rangle=\left(\phi_{i} \phi_{j} \mid \phi_{k} \phi_{l}\right)=(i j \mid k l) \tag{2.12}
\end{equation*}
$$

The notation used in eqn(2.11) can be simplified further by using $J_{i j}$ to represent the coulomb integral $(i i \mid j j)$ and $K_{i j}$ to represent the exchange integral ( $\left.i j \mid j i\right)$.

Using these notations, the HF energy of a closed shell $N$ electron wavefunction expressed as a Slater determinant is given as

$$
\begin{equation*}
E_{0}=\left\langle\Psi_{0}\right| H\left|\Psi_{0}\right\rangle=2 \sum_{i}^{N / 2} h_{i i}+\sum_{i j}^{N / 2}\left[2 J_{i j}-K_{i j}\right] \tag{2.13}
\end{equation*}
$$

The factor of 2 before the one electron operator occurs because it has no dependence on the spin of the orbitals meaning that $h_{i i}=h_{\bar{l}}$. The coulomb integral is also multiplied by 2 as each electron in $\phi_{i}$ feels the repulsion of both electrons in $\phi_{j}$.

HF theory varies the orbitals to minimise the energy of the system

$$
\begin{equation*}
\phi_{i} \rightarrow \phi_{i}+\delta \phi_{i} \tag{2.14}
\end{equation*}
$$

This variation is under the constraint of the orbitals remaining orthonormal which is written in the form

$$
\begin{equation*}
\left\langle\phi_{i} \mid \phi_{j}\right\rangle-\delta_{i j}=0 \tag{2.15}
\end{equation*}
$$

This is inserted into eqn(2.13) and optimised by making the first order variation equal to zero. By collecting terms and noting the symmetry of indices the equation becomes

$$
\begin{equation*}
\delta E_{0}=2 \sum_{i}^{\frac{N}{2}}\left\langle\delta \phi_{i}\right| h+\sum_{j}^{\frac{N}{2}} 2 J_{j}-K_{j}\left|\phi_{i}\right\rangle+2 \sum_{i}^{\frac{N}{2}}\left\langle\phi_{i}\right| h+\sum_{j}^{\frac{N}{2}} 2 J_{j}-K_{j}\left|\delta \phi_{i}\right\rangle \tag{2.16}
\end{equation*}
$$

This can be simplified further by introducing the Fock operator which collects the one and two electron operators

$$
\begin{gather*}
\hat{F}=\hat{h}+\sum_{j}^{\frac{N}{2}} 2 \hat{J}_{j}-\widehat{K}_{j}  \tag{2.17}\\
\delta E_{0}=2 \sum_{i}^{\frac{N}{2}}\left[\left\langle\delta \phi_{i}\right| F\left|\phi_{i}\right\rangle+\left\langle\phi_{i}\right| F\left|\delta \phi_{i}\right\rangle\right] \tag{2.18}
\end{gather*}
$$

When applied to a set of orbitals $\{\phi\}$, the HF equations can be written in matrix form, producing the canonical HF equation

$$
\begin{equation*}
\mathbf{F} \boldsymbol{\Phi}=\boldsymbol{\Phi} \boldsymbol{\varepsilon} \tag{2.19}
\end{equation*}
$$

where $\boldsymbol{\varepsilon}$ is a diagonal matrix containing the energy of each orbital.

### 2.1. Basis Sets

To apply these equations requires the use of a basis set. A basis set is a collection of $m$ basis functions, $\chi$, used to describe the molecular orbitals along with a set of coefficients, $c_{\mu i}$, to determine the contribution of each atomic basis function to a molecular orbital.

Each orbital is described as a linear combination of atomic orbitals (LCAO)

$$
\begin{equation*}
\phi_{i}(r)=\sum_{\mu}^{m} c_{\mu i} \chi_{i} \tag{2.20}
\end{equation*}
$$

A large number of basis functions need to be selected to improve the accuracy of the calculation however this can become computationally inefficient. Slater-type basis functions, Slater type orbitals (STO), can be used on one-electron systems such as the hydrogen atom to solve the Schrödinger equation exactly and have a radial dependence of $e^{-\zeta r}$, where $\zeta$ is an exponent describing how steeply the function reduces away from the nucleus, and $r$ is the distance from the nucleus. Although the STO displays a cusp at the nucleus and provides the correct asymptotic form moving away from the nucleus, the Slater functions require calculation of integrals that cannot be done analytically making their use computationally expensive. Gaussian functions have a radial dependence of
$e^{-\alpha r^{2}}$ where $\alpha$ is the exponent of a gaussian type orbital. This dependence results in a smoothed off peak at the nucleus and drops off too quickly moving away from the nucleus; however, the integrals can be solved analytically. The computational efficiency of Gaussian type orbitals means it is more beneficial to combine primitive functions together creating a contracted basis set resulting in a performance similar to a Slater function. An example are the STO- $n \mathrm{G}$ basis sets where, $n$ is the number of primitive Gaussian functions used to construct the function.

A basis set using one contracted Gaussian function for each occupied atomic orbital in a neutral atom is known as a minimal basis set. For example, a minimal basis set for water would contain 7 basis functions, 1 for the 1 s orbitals of each hydrogen atom and 5 functions for the $1 \mathrm{~s}, 2 \mathrm{~s}, 2 \mathrm{p}_{\mathrm{x}}, 2 \mathrm{p}_{\mathrm{y}}$ and $2 \mathrm{p}_{\mathrm{z}}$ orbitals of the oxygen atom. The STO-nG basis sets are examples of a minimal basis set. These demonstrate poor quantitative results, as the orbitals have fixed sizes which do not allow for orbital breathing, the change in shape of an orbital due to the number of electrons contained within it. A double-zeta (DZ) basis set can be employed to account for this effect, in which two functions are used for each atomic orbital, one with a larger exponent to describe a more compact orbital and another with a smaller exponent to describe a more diffuse orbital. A DZ basis set for water would then contain 14 basis functions. This breathing effect is more prevalent in the valence orbitals meaning it is only necessary to use two functions for the valence orbitals which leads to the split-valence (SV) basis sets where one function is used for core orbitals and two or more functions for valence orbitals. An SV-DZ basis set for water contains 13 basis functions; the 1s orbital of oxygen would only be described using one basis function, whilst all other orbitals would be described using two basis functions. An example of an SV-DZ basis set is the Pople 6-31G basis set, in which the core orbitals are described by one Gaussian orbital comprised of a contraction of 6 primitive functions and the valence orbitals are described by two Gaussian orbitals, one made of a contraction of 3 Gaussian functions and the other described by a single contracted Gaussian function.

These basis sets do not account for orbital polarisation, for example in $\pi$ bonding, where the orbitals can deviate from their aligned axis therefore, it is useful to describe orbitals with an extra function of higher angular momentum. In the Pople basis sets these functions are represented by a '*' or (d) when polarisation functions are added to non-hydrogen atoms, or by '**' or ( $\mathrm{d}, \mathrm{p}$ ) when polarisation functions are added to both hydrogen and non-hydrogen atoms. It is also possible to add diffuse functions to the basis set for systems containing anions or electronegative atoms. These functions have small exponents and allow the orbital to expand, thereby minimising the repulsion between electrons. A '+' is used to represent diffuse functions on non-hydrogen atoms whereby additional $s$ and $p$ functions are added and ' ++ ' denotes extra $s$ and $p$ functions on non-hydrogen atoms and an extra s function on hydrogen atoms.

### 2.1.1. Effective Core Potentials

When performing calculations on molecules with a large number of electrons, such as metals, it is often preferable to use an effective core potential (ECP) to model the behaviour of the core electrons. This is because the core electrons, typically taken to be all electrons not residing in the two outermost shells, do not play a direct role in the chemical behaviour of these atoms, yet their explicit inclusion adds to the computational cost. The ECP therefore is a pseudopotential around the nucleus which mimics the effect of the core electrons on the surrounding valence electrons. ${ }^{31,32}$

### 2.2. Self-Consistent Field Procedure

The introduction of basis sets leads to the Roothaan-Hall equations which can be used to solve the HF equations

$$
\begin{equation*}
\mathrm{FC}=\mathrm{SC} \varepsilon \tag{2.21}
\end{equation*}
$$

where $\mathbf{F}$ is the Fock matrix, $\mathbf{C}$ is a matrix within which each column contains the molecular orbital coefficients and $\mathbf{S}$ is an overlap matrix formed because, unlike molecular orbitals,
the basis functions used to describe the orbitals are not orthogonal. To solve this first requires transforming $\mathbf{S}$ to an orthonormal basis set therefore turning the equation into an eigenvalue equation, which once solved, can be transformed back to its original basis.

The Fock operator is a pseudo one-electron operator in that its solution, a single orbital, is dependent on itself through the two electron terms. Thus, the Roothaan-Hall equation must be solved iteratively through the self-consistent field (SCF) method. This is achieved by choosing an initial guess of a density matrix, $\mathbf{P}$, which is used to construct $\mathbf{F}$. This can then be transformed to the orthonormal basis and diagonalised to obtain $\mathbf{C}$, also in the transformed basis, and $\boldsymbol{\varepsilon}$. The first set of molecular orbitals can then be obtained by transforming C back into the original basis. These coefficients can then be used to guess a new $\mathbf{P}$ for the process to be started again. The electronic energy is evaluated at each iteration and the process is stopped when the energy and the elements of the density matrix have converged. The total energy of the system is then calculated by adding the nuclear repulsion energy.

The use of the HF equations and orbital coefficients rely on the form of the spin orbitals. A system of $N$ electrons separated into $N / 2$ doubly occupied orbitals can be classed as a closed shell system and be treated using restricted Hartree-Fock (RHF) as two electrons of opposite spin occupy the same spatial orbital. If there are systems with an odd number of electrons or a high-spin configuration, then an open shell approach is needed. It is assumed that any unpaired electrons carry an a spin. The first approach is the restricted open shell Hartree-Fock (ROHF) approach which simply assigns the unpaired electron to its own spatial orbital. Another approach is to use the unrestricted Hartree-Fock (UHF) method which allows each electron to have its own spatial orbital. The only requirement of this method is that the orbitals associated with $\alpha$ spin orbitals are orthonormal to each other and so are those associated with $\beta$ spin orbitals

$$
\begin{align*}
& \left\langle\phi_{i}^{\alpha} \mid \phi_{j}^{\alpha}\right\rangle=\delta_{i j}  \tag{2.22}\\
& \left\langle\phi_{i}^{\beta} \mid \phi_{j}^{\beta}\right\rangle=\delta_{i j} \tag{2.23}
\end{align*}
$$

But there are no requirements for the spatial parts of an $\alpha$ spin orbital to be orthonormal to a $\beta$ spin orbital. The treatment of orbitals in this way leads to an effect called spin contamination as the wavefunction may not correspond to a pure spin state. For a restricted set of orbitals, the eigenvalue of the squared spin operator, $\hat{S}^{2}$, is given by $S(S+1)$, where $S$ is the total spin of the system ( $\alpha=+\frac{1}{2}$ and $\beta=-\frac{1}{2}$ ). A system with no unpaired electrons (a singlet state), one unpaired electron (doublet) or two unpaired electrons (triplet) will have an $\hat{S}^{2}$ value of $0,0.75$ and 2 respectively. For unrestricted orbitals, the eigenvalue of $\hat{S}^{2}$ is given by

$$
\begin{equation*}
\left\langle\hat{S}^{2}\right\rangle=\left(\frac{N_{\alpha}-N_{\beta}}{2}\right)\left(\frac{N_{\alpha}-N_{\beta}}{2}+1\right)+N_{\beta}-\sum_{i}^{N_{\alpha}} \sum_{j}^{N_{\beta}}\left|S_{i j}^{\alpha \beta}\right|^{2} \tag{2.24}
\end{equation*}
$$

where $N_{\alpha}$ and $N_{\beta}$ are the number of $\alpha$ and $\beta$ spin electrons respectively and $S_{i j}^{\alpha \beta}$ is the overlap integral

$$
\begin{equation*}
S_{i j}^{\alpha \beta}=\left\langle\Phi_{\mathrm{i}}^{\alpha} \mid \Phi_{\mathrm{j}}^{\beta}\right\rangle \tag{2.25}
\end{equation*}
$$

This equation indicates that if the deviation between the spatial parts of the sets of orbitals is small then so will be the spin contamination, a large deviation however will cause a large spin contamination. To calculate the energy using UHF means that two Fock operators are now needed, one for each spin, and the coulomb and exchange operators are associated with a specific spin.

An advantage of using UHF is that it can describe the dissociation of a molecule better than a restricted method, an example of this is shown in Figure 2.1. In RHF the description of orbitals means that at dissociation it is possible for a molecule to incorrectly possess ionic character, leading to an increase in energy at large internuclear distances. Since the spin orbitals are allowed to have different spatial forms in UHF, the ionic character can be removed and only a radical form is allowed.


Figure 2.1 PES of $\mathrm{H}_{2}$ using RHF (orange) and UHF (blue) with the $6-31 \mathrm{G}(\mathrm{d}, \mathrm{p})$ basis set.

### 2.3. Variational Principle

Another property of the HF method is that it is variational, that is the energy obtained is an upper bound to the exact ground state. This is because the exact Hamiltonian is used but the wavefunction is approximate, meaning that by optimising the wavefunction will reduce the energy with the minimum corresponding to the exact ground state. To demonstrate this, assuming real wavefunctions, consider that the exact wavefunction, $|\Phi\rangle$, can be expanded in a set of orthonormal functions $\left\{\Psi_{i}\right\}$,

$$
\begin{equation*}
|\Phi\rangle=\sum_{i} c_{i}\left|\Psi_{i}\right\rangle \tag{2.26}
\end{equation*}
$$

The normalisation of $|\Phi\rangle$ means that

$$
\begin{align*}
\langle\Phi \mid \Phi\rangle=1 & =\sum_{i}\left\langle\Psi_{i}\right| c_{i} \sum_{j} c_{j}\left|\Psi_{j}\right\rangle \\
& =\sum_{i j} c_{i} c_{j}\left\langle\Psi_{i} \mid \Psi_{j}\right\rangle \\
& =\sum_{i} c_{i}^{2} \tag{2.27}
\end{align*}
$$

These conditions mean that the energy associated with $|\Phi\rangle$ is

$$
\begin{align*}
\langle\Phi| H|\Phi\rangle & =\sum_{i j}\left\langle c_{i} \Psi_{i}\right| H\left|c_{j} \Psi_{j}\right\rangle \\
& =\sum_{i j} c_{i} c_{j}\left\langle\Psi_{i}\right| H\left|\Psi_{j}\right\rangle \\
& =\sum_{i j} c_{i} c_{j} E_{j} \delta_{i j} \\
& =\sum_{i} c_{i}^{2} E_{i} \tag{2.28}
\end{align*}
$$

Since $c_{i}^{2} \geq 0$, and whilst the values of the energies and coefficients are unknown, only the exact wavefunction will provide the lowest (exact) energy, $E_{i} \geq E_{\text {Exact }}$, therefore,

$$
\begin{equation*}
E_{\text {Exact }} \leq E_{i}=\frac{\langle\Phi| H|\Phi\rangle}{\langle\Phi \mid \Phi\rangle} \tag{2.29}
\end{equation*}
$$

### 2.4. Configuration Interaction

The difference between the exact energy and that obtained from HF is the correlation energy. HF only accounts for correlation of electrons with the same spin through the exchange operator $\widehat{K}$. One way to improve upon HF is to use configuration interaction (CI) theory, where more Slater determinants are included therefore improving the many-electron wavefunction. These extra Slater determinants are made based upon excitations of electrons from the HF occupied orbitals into the vacant orbitals. If the HF wavefunction is taken to be $\Psi_{0}$ with occupied orbitals $i, j, k, l \ldots$, and unoccupied orbitals $a, b, c, d \ldots$, then a substituted wavefunction could have the form $\Psi_{i}^{a}$ indicating that an electron from orbital $i$ has been substituted into orbital $a$. The exact wavefunction would then be produced by including every combination of substitutions within the orbitals produced from the basis set used. The inclusion of all possible determinants is known as full configuration interaction (FCI), this is the limiting case for Cl and can only be applied computationally to systems containing a few electrons. To make CI more applicable to large systems, using a suitable basis set, requires limiting the types of substitutions that can be made. The first instance of this is CI singles, CIS , whereby only the HF
wavefunction and all single substitutions are considered. This however does not improve the energy obtained compared to just using $\Psi_{0}$ due to Brillouin's theorem, see Section 2.5.

Cl doubles, CID , is the smallest form of Cl that leads to an improvement on the HF energy by including all double substitutions. It is also possible to include higher levels of substitutions, such as triple and quadruple, or to combine different levels for example, CISD includes all single and double substitutions. Whilst singly substituted wavefunctions do not interact with the HF wavefunction, they do interact with the doubly substituted wavefunctions therefore CISD provides a better energy than CID. All forms of CI obey the variational principle due to the use of approximate wavefunctions with an exact Hamiltonian. The overriding issue of using a form of Cl , other than FCl , is that they are not size consistent. A method is size consistent when the energy calculated for two systems is the same as when they treated in the same system but separated so there is no interaction between them. For example, a CID calculation of two identical atoms containing two electrons in two orbitals would produce the correct electron configurations when treated individually but, when treated in the same system and infinitely separated, would not be able to describe a case where both atoms are doubly substituted at the same time as this would be classed as a quadruple substitution (Figure 2.2c).


Figure 2.2 a) CID configuration on an atom containing two electrons and two orbitals. b) CID configuration on two identical non-interacting atoms. c) Quadruple substitution not accounted for in a CID calculation.

### 2.5. Brillouin's Theorem

Brillouin's theorem states that an optimised HF reference wavefunction, $\Psi_{0}$, will not directly interact with a determinant that differs by a single substitution. The substituted wavefunction, $\Psi_{i}^{a}$, is built from the orbitals of the reference wavefunction, meaning that the interaction is equivalent to an off-diagonal element of the Fock matrix

$$
\begin{equation*}
\left\langle\Psi_{0}\right| F\left|\Psi_{i}^{a}\right\rangle \tag{2.30}
\end{equation*}
$$

Which is equal to zero when the orbitals have been optimised, as the Fock matrix is diagonalised during the SCF procedure.

### 2.6. Multi-Configurational Methods

The previous post-HF methods all use a single Slater determinant, built upon the HF wavefunction, to calculate the energy. The HF wavefunction does not give a good description of non-dynamical electronic correlation and therefore can be qualitatively incorrect, for example when there is a small HOMO-LUMO gap, indicating that a substituted determinant(s) provide a significant contribution to the overall wavefunction requiring the system to be described by more than one determinant. This type of method is called multi-configurational self-consistent field (MCSCF) and involves variationally optimising the coefficients of the MCSCF wavefunction, namely the Cl and orbital coefficients, simultaneously. Complete active space self-consistent field (CASSCF) is an MCSCF method in which the orbitals are separated into three sections, shown in Figure 2.3. One section is the inactive space, all orbitals chosen in this space will always be doubly occupied. Another section is the virtual space within which the orbitals will remain unoccupied. The final section is termed the active space, the orbitals in this space are allowed to have varying occupancy. FCl is then performed for all electrons within the active space generating all possible distributions of the electrons in the orbitals. The coefficients of each determinant are simultaneously optimised to generate the wavefunction. The main complication with using this method is the choice of active space.

It needs to be large enough so that the determinants generated accurately describe the wavefunction but small enough so that the calculation does not become too computationally expensive.


Virtual
space


Active space


Inactive space

Figure 2.3 Orbital spaces used in a CASSCF calculation.

Whilst MCSCF approaches provide a better wavefunction than single determinant reference wavefunctions, they still do not fully account for dynamical correlation. This can be recovered by using a multi-reference perturbation theory (MRPT) method, where all of the determinants created by CASSCF are then subject to further substitutions outside of the active space. One example of MRPT is complete active space perturbation theory at the second order (CASPT2), in which the second-order energy is obtained by performing double substitutions between the different orbital spaces:

$$
\begin{aligned}
i-i & \rightarrow a-a \\
i-i & \rightarrow a-v \\
i-i & \rightarrow v-v \\
i-a & \rightarrow a-a \\
i-a & \rightarrow a-v \\
i-a & \rightarrow v-v \\
a-a & \rightarrow a-a \\
a-a & \rightarrow a-v \\
a-a & \rightarrow v-v
\end{aligned}
$$

For a CASSCF wavefunction, the $a-a \rightarrow a-a$ term is fully accounted for by FCI.

Whilst CASPT2 offers an improvement over CASSCF, it still may produce errors when used in the vicinity of a ConInc since the configurations are state-specific. To alleviate this, a multistate approach was developed which allowed mixing of wavefunctions of different states using the diagonal elements of a generalised Fock matrix of the zeroth-order Hamiltonian. However, this method often fails to produce smooth or continuous PESs in the region of a ConInc. To overcome this problem, an extended multistate (XMS) method was proposed, which also includes the off-diagonal elements of the Fock matrix, ensuring the wavefunctions are invariant to unitary rotations of the reference wavefunctions. ${ }^{33-36}$

### 2.7. Density Functional Theory

The previous methods all rely on creating a many electron wavefunction to solve the Schrödinger equation. A wavefunction of this form requires four coordinates, three spatial and one spin, for each electron. The Hohenberg-Kohn theorems show that it is possible to calculate the electronic ground state energy and properties of a system using a functional of the one-electron density, $\rho(\mathbf{r})$, meaning that only three spatial and one spin coordinates are needed for a system of any size. Integration of the electron density gives the number of electrons in a system,

$$
\begin{equation*}
N=\int \rho(\mathbf{r}) d \mathbf{r} \tag{2.31}
\end{equation*}
$$

Within DFT, the nuclear attraction term of the electronic Hamiltonian is treated as an external potential

$$
\begin{equation*}
\hat{V}_{\mathrm{ext}}\left(\mathbf{r}_{i}\right)=-\sum_{A}^{\text {nuclei electrons }} \sum_{i}^{\frac{Z_{A}}{r_{i A}}} \tag{2.32}
\end{equation*}
$$

The first theorem states that the external potential is uniquely determined by the ground state electron density through proof by contradiction.

Assuming the theorem is not true, then if there are two systems, each with an exact energy and exact associated wavefunctions, that only differ in the external potential

$$
\begin{aligned}
\text { (1) } E & =\langle\Psi| H|\Psi\rangle \\
\text { (2) } E^{\prime} & =\left\langle\Psi^{\prime}\right| H^{\prime}\left|\Psi^{\prime}\right\rangle
\end{aligned}
$$

Then under the variational principle the Hamiltonian of system (1) acting on system (2) must result in a higher energy

$$
\begin{equation*}
E<\left\langle\Psi^{\prime}\right| H\left|\Psi^{\prime}\right\rangle \tag{2.33}
\end{equation*}
$$

The Hamiltonian of (1) is considered as

$$
\begin{equation*}
H=H^{\prime}+\left(H-H^{\prime}\right) \tag{2.34}
\end{equation*}
$$

Then eqn(2.33) can be rewritten as

$$
\begin{align*}
& E<\left\langle\Psi^{\prime}\right| H^{\prime}\left|\Psi^{\prime}\right\rangle+\left\langle\Psi^{\prime}\right| H-H^{\prime}\left|\Psi^{\prime}\right\rangle \\
& E<E^{\prime}+\left\langle\Psi^{\prime}\right| H-H^{\prime}\left|\Psi^{\prime}\right\rangle \tag{2.35}
\end{align*}
$$

As the Hamiltonians of (1) and (2) are only different by their external potential and that eqn(2.32) is a one electron operator, then eqn(2.35) can be written in terms of the ground state density

$$
\begin{equation*}
E<E^{\prime}+\int \rho(\mathbf{r})\left[V_{\mathrm{ext}}(\mathbf{r})-V_{\mathrm{ext}}^{\prime}(\mathbf{r})\right] d \mathbf{r} \tag{2.36}
\end{equation*}
$$

This same process can be followed for the Hamiltonian of (2) acting on system (1) resulting in

$$
\begin{equation*}
E^{\prime}<E+\int \rho(\mathbf{r})\left[V_{\text {ext }}^{\prime}(\mathbf{r})-V_{\text {ext }}(\mathbf{r})\right] d \mathbf{r} \tag{2.37}
\end{equation*}
$$

The addition of eqn(2.36) and eqn(2.37) results in

$$
\begin{equation*}
E+E^{\prime}<E^{\prime}+E \tag{2.38}
\end{equation*}
$$

This shows that the assumption that the external potential is not uniquely determined by the ground state density is not true. Therefore, a given density determines the number of electrons, eqn(2.31), and the external potential of a system which in turn determines the Hamiltonian and wavefunction. This means the ground state energy is a functional of electron density

$$
\begin{equation*}
E_{\text {Exact }}=E_{V}[\rho] \tag{2.39}
\end{equation*}
$$

The second theorem states that DFT obeys the variational principle in that a trial density, $\rho_{\text {trial }}(\mathbf{r})$, that is non-negative throughout space and integrates to the correct number of electrons, $N$, provides an upper bound to the exact energy, that is

$$
\begin{equation*}
E_{\text {Exact }} \leq E_{V}\left[\rho_{\text {trial }}\right] \tag{2.40}
\end{equation*}
$$

Whilst the theorems show that a functional of the density can be used to find the ground state energy, they do not provide a form of the functional. To combat this, Kohn and Sham developed a model within which the electrons are not interacting but have the same density of the fully interacting system. The Hamiltonian can then be written as

$$
\begin{equation*}
\widehat{H}(\lambda)=\widehat{T}+\widehat{V}_{\mathrm{ext}}(\lambda)+\lambda \hat{\mathrm{v}}_{\mathrm{e}} \tag{2.41}
\end{equation*}
$$

where $\hat{T}$ and $\hat{V}_{\text {ee }}$ are the kinetic and electron repulsion operators respectively and $\lambda$ is a scaling parameter, taking values of $0 \leq \lambda \leq 1$, so that the exact Hamiltonian is obtained when $\lambda=1$. The external potential is also dependent on the value of $\lambda$ so that the exact ground state density is always obtained.

In a similar way to HF theory, a determinant can be produced from a set of orbitals, known as Kohn-Sham orbitals, where each occupied orbital only contains one electron allowing for the kinetic energy of a non-interacting system, $\widehat{T}_{\text {ni }}$, to be obtained. The kinetic energy of the fully interacting system can then be defined as

$$
\begin{equation*}
\widehat{T}=\widehat{T}_{\mathrm{ni}}+\Delta \widehat{T} \tag{2.42}
\end{equation*}
$$

where $\Delta \hat{T}$ is an unknown. The electron repulsion term for a fully interacting system can also be separated into the classical coulomb repulsion added to the remaining repulsion terms which are also unknown

$$
\begin{equation*}
\hat{V}_{\mathrm{ee}}=\hat{J}+\Delta \hat{V}_{\mathrm{ee}} \tag{2.43}
\end{equation*}
$$

The energy of a system using DFT with Kohn-Sham orbitals is now given by

$$
\begin{equation*}
E[\rho(\mathbf{r})]=\widehat{T}_{\mathrm{ni}}[\rho(\mathbf{r})]+\Delta \widehat{T}[\rho(\mathbf{r})]+\hat{V}_{\mathrm{ext}}[\rho(\mathbf{r})]+J+\Delta \hat{\mathrm{V}}_{\mathrm{ee}}[\rho(\mathbf{r})] \tag{2.44}
\end{equation*}
$$

The two unknown terms, $\Delta \hat{T}$ and $\Delta \widehat{V}_{\text {ee }}$, are often combined to create an exchange-correlation functional,

$$
\begin{equation*}
E_{\mathrm{XC}}[\rho(\mathbf{r})]=\Delta \hat{T}[\rho(\mathbf{r})]+\Delta \hat{V}_{\mathrm{ee}}[\rho(\mathbf{r})] \tag{2.45}
\end{equation*}
$$

This functional has a small but significant contribution to the description of a system therefore approximations are needed to define it.

The earliest approximation is the local density approximation (LDA), which uses the uniform electron gas model which assumes that there are infinite electrons evenly distributed over a system that is electrically neutral with an infinite volume; thus, the electron density is constant throughout the system. $E_{\mathrm{XC}}[\rho(\mathbf{r})]$ can be treated in two parts, an exchange part and a correlation part. Within LDA, the exchange functional has the form of Dirac exchange and the correlation functional is produced using the functional, VWN, produced by Vosko, Wilk and Nusair. ${ }^{37}$ Whilst LDA gives a form for the $E_{\mathrm{Xc}}[\rho(\mathbf{r})]$, it is based on a poor model. The uniform electron gas model does not give a good description of molecular systems as molecules have non-uniform electron densities, for example, a system containing atoms with differing electronegativities will not have a uniform distribution of electrons. LDA also assumes a slow variation of density with regard to position. This can be improved by using the generalised gradient approximation, GGA, which accounts for the gradient of the density. Becke's exchange functional, B88, ${ }^{38}$ is a type of GGA and can be added to Perdew's GGA correlation functional, P86, ${ }^{39}$ to form the BP86 functional. A further improvement can be made to GGAs by including the second derivative of the density, $\nabla^{2} \rho(\mathbf{r})$. This form of exchange correlation functional is known as meta-GGAs. Although they often perform better than GGAs they are more computationally expensive.

### 2.7.1. The Adiabatic Connection

The form of the Hamiltonian in eqn(2.41) makes use of the coupling strength, $\lambda$, whereby a value of 0 represents a non-interacting system and 1 represents a fully interacting system. This is achieved by applying this to the electron repulsion term so that

$$
\begin{equation*}
\hat{V}_{\mathrm{ee}}(\lambda)=\lambda \sum_{i<j}^{N} \frac{1}{r_{i j}} \tag{2.46}
\end{equation*}
$$

This means that for a non-interacting system $(\lambda=0)$ there are no electron-electron interactions. The difference in energy between a non-interacting system $(\lambda=0)$ and a fully interacting system $(\lambda=1)$ is then the contribution of the Coulomb, exchange and correlation energy.

Becke found that, through the adiabatic connection, the exchange-correlation functional could be written in a form including exact exchange, which has the form of HF exchange, and that including this term improved the performance of functionals. These are known as hybrid functionals. The B3 exchange hybrid functional was created by fitting 3 parameters to determine how much HF exchange should be included, in this case $20 \% .{ }^{40}$

Exchange-correlation functionals are often constructed as corrections to the LDA approach. This induces an effect known as the self-interaction error due to the form of the classical coulomb repulsion used

$$
\begin{equation*}
J[\rho]=\frac{1}{2} \iint \frac{\rho\left(\mathbf{r}_{1}\right) \rho\left(\mathbf{r}_{2}\right)}{r_{12}} d \mathbf{r}_{1} d \mathbf{r}_{2} \tag{2.47}
\end{equation*}
$$

Meaning that an electron in the space of $\mathbf{r}_{1}$ is able to interact with itself in the space of $\mathbf{r}_{2}$ which is an incorrect description, therefore correlation functionals tend to also include a correction for the self-interaction error. The LYP correlation functional was developed by Lee, Yang and Parr ${ }^{41}$ in a different way to other GGAs to completely remove the self-interaction error for one electron systems. The LYP correlation functional is often used in conjunction with the B3 exchange functional creating the B3LYP exchange-correlation functional. ${ }^{42}$

The accuracy and computational cost of these exchange-correlation functionals can be expressed using Jacob's ladder (Figure 2.4) where each rung moving up is an improvement on the previous approach in terms of accuracy however also results in an increase of computational cost


Figure 2.4 Jacob's ladder of density functionals.

### 2.8. Geometry Optimisation

The preceding sections focus on obtaining the lowest energy of a system for a given set of coordinates. Often it is desirable to find a structure relating to a stationary point of a potential energy surface, usually an energy minimum. One way of achieving this is by selecting one or two degrees of freedom within a molecule to vary in a range of values to plot a 2D or 3D potential energy surface. This method is only useful when optimising a structure containing a few degrees of freedom or when studying a particular coordinate. When optimising entire structures, it is possible to use the gradient of the energy to determine the next change in coordinates to lower the energy. This method is known as the steepest decent. This works by first calculating the energy and energy gradient at the initial coordinates, $R_{0}$. A line search is then performed whereby the lowest energy structure along a path, determined by the initial coordinates and gradient direction, is then
obtained to be used as the starting point for the next iteration. This process is repeated until the change in energy, gradients and structure are below a threshold value. Whilst this method can lead to structures sufficiently close to the minimum, it can require many steps to complete. This method can be improved upon by employing the Newton-Raphson equation, eqn(2.48), which uses the Hessian matrix (H), containing elements representing the second derivative of energy (eqn(2.49)), to improve the estimate for the next structure thereby reducing the number of steps taken.

$$
\begin{align*}
& R_{1}=R_{0}+\Delta R=R_{0}-\mathbf{g H}^{-1}  \tag{2.48}\\
& H_{i j}=\frac{\partial^{2} E\left(R_{o}\right)}{\partial R_{i} \partial R_{j}} \tag{2.49}
\end{align*}
$$

where $\mathbf{g}$ is the gradient vector and $\mathbf{H}^{-1}$ is the inverse of the Hessian matrix. A step parameter can also be added to the final term to control the size of the descent similar to the steepest descent approach.

To determine the elements of a Hessian matrix is computationally expensive, therefore methods tend to make an approximation of the Hessian which updates with each iteration. One such approximation is the BFGS method, developed by Broyden, ${ }^{43}$ Fletcher, ${ }^{44}$ Goldfarb ${ }^{45}$ and Shanno, ${ }^{46}$ which guesses and updates $\mathbf{H}^{-1}$ based on the gradient from the previous steps.

### 2.9. Time Dependent Density Functional Theory

The methods discussed so far have been concerned with obtaining and optimising the lowest energy wavefunction of a system, i.e. the ground state. It is necessary in photochemistry to explore excited state potential energy surfaces as well. Some of the wavefunction based methods discussed, such as $\mathrm{CI}, \mathrm{CASSCF}$ and CASPT2, achieve this through electron substitutions between molecular orbitals of the ground state Slater determinant, see Section 2.6. The issues with these are that although CIS is a relatively simple method, it is often qualitatively and quantitatively incorrect, whereas CASSCF and

CASPT2 methods provide accurate results at a large computational cost. DFT is also able to explore excited states by solving the time dependent Schrödinger equation

$$
\begin{equation*}
\widehat{H}(\mathbf{r}, \mathbf{R}, t) \left\lvert\, \Psi(\mathbf{r}, \mathbf{R}, t\rangle=i \frac{\delta \mid \Psi(\mathbf{r}, \mathbf{R}, t\rangle}{\delta t}\right. \tag{2.50}
\end{equation*}
$$

resulting in the time-dependent density functional theory (TDDFT) method which can provide a middle ground to the two extremes of wavefunction based methods. In matrix form, the TDDFT equations can be written as ${ }^{47}$

$$
\left[\begin{array}{cc}
\mathbf{A} & \mathbf{B}  \tag{2.51}\\
\mathbf{B}^{*} & \mathbf{A}^{*}
\end{array}\right]\left[\begin{array}{l}
\mathbf{X} \\
\mathbf{Y}
\end{array}\right]=\omega\left[\begin{array}{cc}
-1 & 0 \\
0 & 1
\end{array}\right]\left[\begin{array}{l}
\mathbf{X} \\
\mathbf{Y}
\end{array}\right]
$$

where the matrix A contains the energy difference of the occupied and unoccupied orbitals involved in the single electron substitution along with an exchange-correlation functional, matrix B contains exchange-correlation elements, $\omega$ represents the excitation energies and the matrices X and Y contain the excitation and de-excitation amplitudes respectively. The Tamm-Dancoff approximation can be made by neglecting the B matrix, resulting in ${ }^{48}$

$$
\begin{equation*}
\mathbf{A X}=\omega \mathbf{X} \tag{2.52}
\end{equation*}
$$

### 2.10. Polarisable Continuum Model

In some cases, it is preferable to study the effects a solvent can have on a molecular system or to see how a single molecule behaves in a solvent rather than in the gas phase. In such instances, a different approach known as the polarisable continuum model (PCM) can be used instead of using periodic boundary conditions. In this model the solvent molecules aren't included explicitly or even as a force-field, instead the solvent is accounted for as a uniform dielectric medium, characterised by a dielectric constant for the solvent of interest, surrounding a cavity which contains the molecule, or solute, being studied.

In PCM the shape of the cavity is determined by the surface created from the overlapping atom centred van der Waals radii of each atom in the molecule. These radii are typically
taken from a set of crystallographic data ${ }^{49}$ and increased by approximately $20 \%$ so that the surface prevents the solvent molecules from directly approaching the solute atoms. Inside this cavity, the dielectric constant is the same as in a vacuum. ${ }^{50}$

Partial charges are then assigned around different areas of the cavity to replicate the dipole moment of the solute. This dipole will then cause the solvent around the cavity to be polarised forming a reaction field. The surface charge is created around the solute using a conductor like PCM (C-PCM) which first assumes the dielectric constant is infinite before applying a scaling factor to reduce the constant. ${ }^{51}$ The electrostatic interaction between the cavity and reaction field is solved self consistently using the self-consistent reaction field (SCRF).

### 2.11. Molecular Dynamics

The procedures discussed so far have been concerned with obtaining the lowest energy for a given structure or system in a gas phase environment. It is often desirable to see how a molecule will react in a certain environment, for example in this research in a solvent (Chapter 3) or a lipid membrane (Chapter 6). Whilst it would be possible to include a large number of molecules to the gas phase environment to replicate the effects of a solvent, it is not a feasible approach as this would dramatically increase the computational cost. Instead, the environment is often treated at a classical molecular mechanics level. A calculation of this form requires the use of force-fields.

A force-field is a collection of terms used to describe the forces acting on a molecule or atom in a system. The movement within individual molecules can be described using force constants and reference values of the equilibrium structure. Eqns(2.51) - (2.55) provide the energy expressions for bond stretching, angles and dihedrals respectively.

$$
\begin{equation*}
V_{\text {stretch }}=k_{\text {stretch }}\left(l-l_{0}\right)^{2} \tag{2.53}
\end{equation*}
$$

where $k_{\text {stretch }}$ is a force constant, $l$ is the current bond length and $l_{0}$ is the reference bond length corresponding to the equilibrium bond length in the structure.

The energy expression for bond angles is similar to that of eqn(2.53),

$$
\begin{equation*}
V_{\text {bend }}=k_{\theta}\left(\theta-\theta_{0}\right)^{2} \tag{2.54}
\end{equation*}
$$

where $k_{\theta}$ is a force constant and $\theta$ and $\theta_{0}$ are the current and equilibrium reference bond angles respectively. An extra parameter can also be used for bond angles using the Urey-Bradley term

$$
\begin{equation*}
V_{\mathrm{UB}}=k_{\mathrm{UB}}\left(d-d_{0}\right)^{2} \tag{2.55}
\end{equation*}
$$

where $k_{\mathrm{UB}}$ is a force constant, $d$ is the distance between the first and third atoms creating the bond angle and $d_{0}$ is the distance between those atoms in an equilibrated structure. When $V_{\mathrm{UB}}$ is employed then the total bond angle potential energy is

$$
\begin{equation*}
V_{\text {angle }}=V_{\text {bend }}+V_{\mathrm{UB}} \tag{2.56}
\end{equation*}
$$

For dihedral angles formed between four consecutively bonded atoms (a proper dihedral), the following equation is used

$$
\begin{equation*}
V_{\text {dihedral }}=k_{\text {dihedral }}[1+\cos (n(\omega-\gamma))] \tag{2.57}
\end{equation*}
$$

In eqn(2.57), $k_{\text {dihedral }}$ is a force constant, $\omega$ is the current dihedral angle, $n$ is an integer used to describe the periodicity of the dihedral and $\gamma$ is the phase shift of a sinusoidal wave.

It is also necessary to describe the attractive and repulsive interaction between molecules in the system, this is achieved by using the Lennard-Jones potential

$$
\begin{equation*}
V_{\mathrm{LJ}}\left(r_{A B}\right)=4 \varepsilon\left[\left(\frac{\sigma_{A B}}{r_{A B}}\right)^{12}-\left(\frac{\sigma_{A B}}{r_{A B}}\right)^{6}\right] \tag{2.58}
\end{equation*}
$$

where the term to the power of 12 describes the repulsion between an atom on molecule $A$ and an atom on molecule $B$ and the term to the power of 6 describes their potential energy of attraction, $\varepsilon$ is a constant to describe the strength of interaction between the two atoms and $\sigma_{A B}$ is related to the equilibrium distance between the two atoms. Electrostatic interactions can also be accounted for, when using polar or ionic molecules, by assigning partial charges $(q)$ to each atom and applying Coulomb's law

$$
\begin{equation*}
V_{\text {Coulomb }}=\sum_{A=1}^{N} \sum_{B=i+1}^{N} \frac{q_{A} q_{B}}{4 \pi \varepsilon_{0} r_{A B}} \tag{2.59}
\end{equation*}
$$

where $q_{A}$ and $q_{B}$ are the partial charges on atoms $A$ and $B$ respectively, $\varepsilon_{0}$ is the dielectric constant of the medium used in the simulation and $r_{A B}$ is the distance between atoms $A$ and $B$.

The use of force constants and reference values for each structure means that many parameters are required for a force-field. To keep these at a manageable level, force-fields make use of atom types. This is where atoms or bonds that are repeated within a structure and have similar properties can be assigned the same parameters.

Computational efficiencies can be achieved using cut offs to neglect interactions at large distances, which will only contribute a small amount to the overall energy. One consequence of this is that it may cause a step in the potential at the cut off distance resulting in energy discontinuities. One way of minimising this effect is to use a shifted force potential which smooths off the energy rather than creating a step. This is achieved by using two thresholds for the cut off distance, between which a smoothing function is used to gradually decrease the energy.

When using molecular mechanics (MM), it is often required for the system to possess bulk behaviour. This requires a large number of molecules to be included in the simulation, resulting in a large computational cost. This issue can be alleviated through the use of periodic boundary conditions.

The periodic boundary approach treats the system being studied as a unit cell and builds a larger system by surrounding the central cell with replicas of itself. A cut off distance is then chosen to be less than half the length of the unit cell and interactions of molecules or atoms are then taken to be those between the nearest real atom or replica, whichever is closest. This means that the periodic boundary approach does not increase the amount of parameterisation compared to a single unit cell whilst creating a system that possesses bulk behaviour.

Whilst force-fields are useful in mimicking the behaviour of the environment it is still often required to get detailed information of a molecule being studied in that environment. One way to model this type of system is to use a hybrid approach of quantum mechanics and molecular mechanics (QM/MM), where the molecule of interest is treated at a higher level of theory, such as quantum chemical approaches, whilst the rest of the system is treated at the MM level. In this approach it is also necessary to describe the interaction between the QM and MM regions, for example, the energy of a system can be calculated as

$$
\begin{equation*}
V^{\mathrm{QM} / \mathrm{MM}}\left(R_{\mathrm{C}}, R_{\mathrm{E}}\right)=V^{\mathrm{QM}}\left(R_{\mathrm{C}}\right)+V^{\mathrm{MM}}\left(R_{\mathrm{C}}, R_{\mathrm{E}}\right)-V^{\mathrm{MM}}\left(R_{\mathrm{C}}\right) \tag{2.60}
\end{equation*}
$$

where $R_{\mathrm{C}}$ is the atoms of the core region under investigation to be treated at the QM level and $R_{\mathrm{E}}$ is the coordinates of the atoms making up the surrounding environment. The first term on the RHS of eqn(2.60) is the energy of the core region treated at the QM level, the second term is the energy of the entire system treated at the MM level and the final term is the energy of the core region treated at the MM level. Electrostatic embedding is also often used where the MM point charges are included in the one electron Hamiltonian of the QM region. The QM calculation also calculates the electrostatic potential (ESP), from the electron density of the QM region, at each coordinate of the MM atoms. The MM region is then able to account for the QM region as a classical potential.

## Chapter 3. POMs

Polyoxometallates (POMs) have a wide variety of bioapplications. In this work, the possibility of POMs being used to transport a molecular probe to a membrane by selectively catalysing reactions to sever bonds within raft associated proteins to covalently attach the fluorescent probe have been investigated.

A collaboration was formed with the Newton group within the School of Chemistry at the University of Nottingham were studying the effects of organofunctionalisation on a POMs photocatalytic properties. The experimental research of this work is still ongoing therefore the biological application of the POMs has not yet been investigated.

The work reported in this chapter is related to the mixed metal substitution effect on the electronic properties of a POM and has been published in the European Journal of Inorganic Chemistry, ${ }^{52}$ in which I contributed the computational study.

### 3.1. Introduction

Polyoxometalates (POMs) are a class of metal oxide clusters typically formed of early transition metals with high oxidation states such as $\mathrm{V}^{\mathrm{V}}, \mathrm{Mo}^{\mathrm{VI}}$ and $\mathrm{W}^{\mathrm{VI}}$. These clusters can exist in a wide range of stable structures ${ }^{27}$ and possess electronic properties allowing for reversible redox processes. ${ }^{25}$ These processes are photoactive through the excitation of an oxygen-to-metal ligand-to-metal charge transfer (LMCT) band resulting in the employment of POMs as photocatalysts. ${ }^{53-55}$ This excitation usually occurs within the UV region of the spectrum. The physical and chemical properties of POMs can be modified through the attachment of organic moieties resulting in an organic-inorganic hybrid POM. ${ }^{56}$ These hybrids allow the HOMO-LUMO gap to be varied, potentially allowing for photoactivation with visible light. ${ }^{57,58}$ The properties of a POM can be tuned further by creating a mixed-metal POM formed by substituting a different metal into the structure. ${ }^{59}$ 61

In this work the combination of using mixed-metals and organo-functionalisation on the electronic properties of POMs are to be investigated by comparing a Wells-Dawson structured mixed-metal hybrid POM of $\mathrm{K}_{6}\left[\mathrm{P}_{2} \mathrm{~W}_{15} \mathrm{Mo}_{2} \mathrm{O}_{61}\left(\mathrm{POC}_{6} \mathrm{H}_{5}\right)_{2}\right]$ (1), with its single metal parent structure $\mathrm{K}_{6}\left[\mathrm{P}_{2} \mathrm{~W}_{17} \mathrm{O}_{61}\left(\mathrm{POC}_{6} \mathrm{H}_{5}\right)_{2}\right]$ (2), see Figure 3.1.


Figure 3.1 Mixed-metal hybrid POM 1 (left) and its all-tungsten analogue 2 (right).
Carbon atoms are grey, oxygen atoms are red, phosphorous atoms are orange, tungsten atoms are light blue and molybdenum atoms are light green.

### 3.2. Computational Details

The geometries of $\mathbf{1}$ and $\mathbf{2}$ were optimised using DFT employing the BP86 functional with the CRENBL basis set and ECP. ${ }^{62-64}$

PCM was used to account for solvation effect of dimethylformamide (DMF), to be consistent with the experimental data, by employing a dielectric constant of 36.7 with standard van der Waals (VDW) radii for all atoms, provided by Q-Chem, except for W and Mo which were manually assigned values of $2.1 \AA$ and 2.09 Å respectively.

Atomic spin densities were obtained using the Mulliken procedure.

### 3.3. Results and Discussion

### 3.3.1. Experimental Analysis

The effect on the electronic structure of substituting two W atoms for Mo was investigated through use of cyclic voltammetry (CV) and UV-Vis spectroelectrochemical analysis. The CV analysis showed that both $\mathbf{1}$ and $\mathbf{2}$ possess a series of redox processes closely overlapping, Figure 3.2. Square wave voltammetry was the performed to provide a better resolution of the reductions. It was shown from this that $\mathbf{1}$ displays its first redox process at a more positive potential compared to $\mathbf{2}$, indicating that the Mo substitutions lead to a lowering of the LUMO energy.


Figure 3.2 CV plots of $\mathbf{1}$ (blue) and $\mathbf{2}$ (green) conducted in DMF.

The oxidising ability of common addenda atoms is ordered as $\mathrm{W}^{\mathrm{VI}}>\mathrm{Mo}^{\mathrm{VI}}>\mathrm{V}^{\mathrm{V}}$ meaning that, in this work, it is expected that a single electron $\left(e^{-}\right)$, reduction of $\mathbf{1}$ to be attributed to the reduction of $\mathrm{Mo}^{\mathrm{VI}}$ to $\mathrm{Mo}^{\mathrm{V}}$. UV-Vis absorption spectroelectrochemistry was used in conjunction with electron paramagnetic resonance (EPR) spectroscopy to investigate this. UV-Vis spectra related to reduced POMs display characteristic bands caused by inter-valence charge transfer (IVCT) between metal centres. ${ }^{65}$ The absorption spectrum
of [1] ${ }^{-}$shows a broad peak between $800-900 \mathrm{~nm}$ (Figure 3.3a), whilst the spectrum of [2]- has a broad band, associated with IVCT, with a peak at 837 nm (Figure 3.3c). This difference in absorption profiles thus indicates that the reduction does occur, at least in part, at the Mo centres. With regards to the EPR spectra, Figure 3.3b and d, [1] ${ }^{-}$displays a series of small features indicative of Mo hyperfine coupling whereas [2] ${ }^{-}$possesses features consistent with W hyperfine coupling. ${ }^{66,67}$


Figure 3.3 UV-Vis spectra of a) $\mathbf{1}$ and c) $\mathbf{2}$ in DMF with lines representing the (blue) $1^{\text {st }}$, (red) $2^{\text {nd }}$, (black) $3^{\text {rd }}$, (purple) $4^{\text {th }}$ reduced species. EPR spectra of b) [1] $]^{-}$and d) [2]-in DMF.

### 3.3.2. Computational Analysis

The geometry optimisations of $\mathbf{1}$ and $\mathbf{2}$ allowed for the frontier orbitals to be determined, thus identifying the HOMO-LUMO energy gap $(\Delta \mathrm{E})$. However, for these compounds, the HOMOs are generally located around the phosphonate ligands therefore the selected HOMO is not taken to be the 'true' HOMO but rather the HOMO possessing a desirable amount of POM-centred orbitals, for both $\mathbf{1}$ and $\mathbf{2}$ this was taken to be the HOMO-4 level.

The LUMO of $\mathbf{1}$ lies lower in energy than the LUMO of $\mathbf{2}$ (Figure 3.4), which is consistent with the experimental CV findings of the first reduction potentials. The spin density plot of [1] ${ }^{-}$(Figure 3.5) shows that the reduced electron is likely to reside on an Mo centre which is also consistent with the experimental results. The distribution of the LUMO orbital is also different between the two POMs. The LUMO of $\mathbf{1}$ displays some orbital character around the Mo centres whereas the LUMO of $\mathbf{2}$ evenly spread across the POM core. This is also consistent with the experimental finding as IVCT tends to occur between identical, neighbouring metal centres, hence the LUMO distribution toward the Mo centres in 1. Whilst the LUMO of $\mathbf{1}$ is lower in energy compared to the LUMO of 2, the HOMO is higher than that of $\mathbf{2}$. This can be attributed to a lower mixing of orbitals due to the varying nuclei in the structure, therefore the HOMO of $\mathbf{2}$ allows for more electron delocalisation resulting in a more stabilised HOMO. Overall this results in $\mathbf{1}$ having a smaller $\Delta \mathrm{E}$ which is to be expected from POMs containing Mo centres. ${ }^{67}$


Figure 3.4 Calculated HOMO and LUMO orbitals of (left) $\mathbf{1}$ and (right) 2.


Figure 3.5 Calculated spin density of [1] ${ }^{-}$. Carbon atoms are grey, oxygen atoms are red, phosphorous atoms are orange, tungsten atoms are light blue and molybdenum atoms are light green.

### 3.4. Conclusion

In this work the effect of altering the metal centres of an organofunctionalized hybrid POM on its electronic structure was investigated. This was achieved by preparing and comparing a mixed addenda POM, containing two Mo centres, with a POM containing only W through spectrochemical analysis and DFT. It was found that the mixed addenda POM displayed quasi-reversible redox processes, involving both Mo and W centres. The substitution of two W for Mo in the POM structure contributes to the electron withdrawing effect of the phosphonate ligand in altering the energy gap thus allowing for the POM to be photoactive at longer wavelengths. This example of a stable mixed metal hybrid POM with redox processes creates more opportunities in the development of photocatalysts and tuneable energy materials.

## Chapter 4. Searching for Conical Intersections

The following work has been published in the Journal of Chemical Theory and Computation. ${ }^{68}$

This chapter discusses the first stage of developing a fluorescent molecular probe which is finding a suitable method to identify Conlncs.

### 4.1. Introduction

In Chapter 1 it was proposed that a phase sensitive probe could utilise an effect similar to the AIE mechanism of RIM to control the accessibility of Conlncs for radiationless decay. This chapter compares the use of different computational approaches in locating ConIncs.

A ConInc is formed when two or more electronic states become energetically degenerate. This causes the Born-Oppenheimer approximation to break down due to the importance of nuclear motion in this region, ${ }^{69,70}$ which is neglected in the approximation, eqn(2.4). Whilst it is preferable to use high levels of theory to investigate these pathways, it is not always possible to use these approaches due to their computational cost. Therefore, a range of studies have been conducted comparing the use of TDDFT with various multireference approaches, such as CASSCF and CASPT2. Minezawa et al. compared the use of spin flip TDDFT (SF-TDDFT) with CASPT2 in locating the minimum energy crossing points (MECPs) of ethylene. ${ }^{71}$ Their study showed that the SF variation of DFT could successfully identify the desired Conlncs, providing geometries and energies in good agreement with the CASPT2 results. The same group then applied SF-TDDFT, with the penalty constraint optimisation, to examine the photoisomerization of stilbene to support experimental findings. ${ }^{72}$ Zhang et al. also studied the use of SF-TDDFT for identifying the MECPs of 9 H -adeninde ${ }^{73}$ and compared them to MR-CIS results obtained by Barbatti et al. ${ }^{74}$ again showing good agreement between the two levels of theory.

Boggio-Pasqua et al. ${ }^{75}$ compared the use of DFT based methods with CASSCF and CASPT2 on the photophysical radiationless decay pathways of polycyclic aromatic hydrocarbon radicals. Whilst their work shows that SF-TDDFT results compare well with the multiconfigurational results, they only use a penalty function optimisation with the B3LYP functional for locating MECPs. When identifying MECPs, it has been found that employing the BHHLYP hybrid functional has obtained the best results in various studies. ${ }^{76-78}$ It has also been shown that when using CASPT2 that it can be beneficial to include either multistate $(\mathrm{MS})^{79}$ or extended multistate $(\mathrm{XMS})^{33,80}$ formalisms to account for dynamical correlation effects to evaluate energies within the vicinity of a ConInc.

This chapter compares the performance of SF-TDDFT in identifying MECPs occurring between the $\mathrm{S}_{1}$ and $\mathrm{S}_{0}$ states of medium sized molecules against the high-level theory approaches of CASSCF and XMS-CASPT2. Three approaches of SF-TDDFT are employed, one of which includes the explicit calculation of nonadiabatic coupling terms.

### 4.2. Method Background

### 4.2.1. Branching Planes

In the case where two electronic states, $J$ and $K$, become degenerate they form two subspaces. The first is the seam space defined by the $N_{\text {int }}-2$ dimensions, where $N_{\text {int }}$ is the internal degrees of freedom, within which the two states are degenerate. The other two dimesons form the branching space where the degeneracy of the two Born-Oppenheimer surfaces are lifted by an infinitesimal shift in nuclear coordinates. The branching space is covered by two vectors, $\mathbf{g}_{J K}$ and $\mathbf{h}_{J K}$, the first of which is defined by the difference in gradient vectors of the two Born-Oppenheimer electronic states

$$
\begin{equation*}
\mathbf{g}_{J K}=\frac{\partial E_{J}}{\partial \mathbf{R}}-\frac{\partial E_{K}}{\partial \mathbf{R}} \tag{4.1}
\end{equation*}
$$

The second is the nonadiabatic coupling vector which is defined as

$$
\begin{equation*}
\mathbf{h}_{J K}=\left\langle\Psi_{J}\right| \frac{\partial}{\partial \mathbf{R}}\left|\Psi_{K}\right\rangle \tag{4.2}
\end{equation*}
$$

This can then be used to calculate the derivative coupling vector

$$
\begin{equation*}
\mathbf{d}_{J K}=\frac{\mathbf{h}_{J K}}{E_{J}-E_{K}} \tag{4.3}
\end{equation*}
$$

The topology of the seam space is then determined by the relative orientation and magnitude of the $\mathbf{g}_{J K}$ and $\mathbf{h}_{J K}$ vectors.

The topography of the PESs around a Conlnc can be categorised as either peaked or sloped where a peaked topology is thought to provide a better transition. ${ }^{81}$

The scaled projection parameters are defined as

$$
\begin{equation*}
s^{x}=\frac{\left(\mathbf{s}_{J K} \cdot \mathbf{g}_{J K}\right)}{g^{2}} \tag{4.4}
\end{equation*}
$$

and

$$
\begin{equation*}
s^{y}=\frac{\left(\mathbf{s}_{J K} \cdot \mathbf{h}_{J K}\right)}{h^{2}} \tag{4.5}
\end{equation*}
$$

where $g=\left\|\mathbf{g}_{J K}\right\|, h=\left\|\mathbf{h}_{J K}\right\|$ and $\mathbf{s}_{J K}$ is a vector

$$
\begin{equation*}
\boldsymbol{s}_{J K}=\frac{1}{2}\left(\frac{\partial E_{J}}{\partial \mathbf{R}}+\frac{\partial E_{K}}{\partial \mathbf{R}}\right) \tag{4.6}
\end{equation*}
$$

If both parameters are close to zero then the topology is peaked around the ConInc, otherwise it is classed as sloped.

As the seam space of a Conlnc can span a range of coordinates, the optimisation methods used in this work locate the MECP within the ConInc.

### 4.2.2. Multireference Methods

The CASSCF and XMS-CASPT2 approaches, outlined in Section 2.6, can both calculate the $\mathbf{g}_{J K}$ and $\mathbf{h}_{J K}$ vectors analytically making them the ideal choice to search for ConIncs. However, these methods are computationally expensive and therefore can only be used for small molecules.

### 4.2.3. TDDFT Methods

Due to the expensive cost of the multireference approaches it would be ideal to use TDDFT for the search of ConIncs. Whilst TDDFT can be used for Conlncs formed between two excited states, it does not provide the correct topology of a Conlnc involving the ground state ${ }^{71,82}$ due to Brillouin's theorem. The Hamiltonian of a Conlnc is given by ${ }^{81}$

$$
\mathbf{H}=\left[\begin{array}{cc}
H_{J J}(\mathbf{R}) & H_{J K}(\mathbf{R})  \tag{4.7}\\
H_{K J}^{*}(\mathbf{R}) & H_{K K}(\mathbf{R})
\end{array}\right]
$$

For a CIS calculation, the off-diagonal terms must be zero when evaluated meaning that the matrix elements of vector $\mathbf{h}_{J K}$ vanish, causing the branching space to be one dimensional. One way to alleviate this is by using SF-TDDFT, whereby a reference triplet state is generated from the ground state by promoting an electron and changing its spin. This means the ground state can now be treated as an excited state and the correct topology can be obtained.

The MECP optimisation method using SF-TDDFT with calculation of nonadiabatic couplings, as described in Section 4.2.1, using a gradient projection algorithm developed by Bearpark et al. ${ }^{83}$ is defined as the NAC method from here.

### 4.2.4. Penalty Function Optimisation

When $\mathbf{h}_{J K}$ cannot be calculated, or in some cases may be too expensive to obtain, more approximate methods need to be used. The first considered in this work is the penalty constrained optimisation (herein defined as PC) algorithm developed by Martínez et al. ${ }^{84}$ In this approach the MECP is located by minimising an objective function

$$
\begin{equation*}
F_{\sigma}(\mathbf{R})=\frac{\left[E_{J}(\mathbf{R})+E_{K}(\mathbf{R})\right]}{2}+\sigma\left(\frac{\left[E_{K}(\mathbf{R})-E_{J}(\mathbf{R})\right]^{2}}{E_{K}(\mathbf{R})-E_{J}(\mathbf{R})+\alpha}\right) \tag{4.8}
\end{equation*}
$$

where $\alpha$ is a parameter included to avoid singularities and $\sigma$ is a Langrange multiplier.

The minimisation process is solved iteratively for increasing values of $\sigma$.

### 4.2.5. Branching Plane Update

The other approximate approach used in this work is the branching plan update method (herein defined as BP) developed by Morokuma et al. ${ }^{85}$ The mean energy gradient is given as

$$
\begin{equation*}
\mathbf{G}_{\text {mean }}=\frac{\left(\mathbf{G}_{J}+\mathbf{G}_{K}\right)}{2} \tag{4.9}
\end{equation*}
$$

and a normalised difference gradient is defined as

$$
\begin{equation*}
\mathbf{G}_{\mathrm{diff}}=\frac{\mathbf{G}_{K}-\mathbf{G}_{J}}{\left|\mathbf{G}_{K}-\mathbf{G}_{J}\right|} \tag{4.10}
\end{equation*}
$$

Eqn(4.9) and eqn(4.10) can then be used to create a projection vector

$$
\begin{equation*}
\mathbf{P}=1-\mathbf{G}_{\text {diff }} \mathbf{G}_{\text {diff }}^{\mathrm{T}}-\mathbf{G}_{\text {orth }} \mathbf{G}_{\text {orth }}^{\mathrm{T}} \tag{4.11}
\end{equation*}
$$

where $\mathbf{G}_{\text {orth }}$ is a vector orthogonal to $\mathbf{G}_{\text {diff }}$ and is an approximation to the derivative coupling vector.

The gradient of the objective function is then defined as

$$
\begin{equation*}
\mathbf{G}=\mathbf{P} \mathbf{G}_{\text {mean }}+2\left(E_{K}-E_{J}\right) \mathbf{G}_{\text {diff }} \tag{4.12}
\end{equation*}
$$

### 4.3. Computational Details

The molecules in this work were chosen as they are representative of molecular structures commonly found in molecular probes for biological environments. Figure 4.1 shows the skeletal structures of the molecules studied along with the numbering system used throughout this work, whilst Table 4.1 gives the basis sets used for the calculation of each molecule as well as the active spaces used in the multireference approaches. The first number given in the parenthesis is the number of electrons in the active space and the second number is the number of orbitals making up the active space. For CASSCF, the $S_{0}, S_{1}$ and $S_{1} / S_{0}$ MECP geometries were optimised using an average over the first two singlet excited states (in $\mathrm{C}_{1}$ symmetry). For XMS-CASPT2 optimisations, a real shift of 0.2 a.u was applied. Density fitting was used employing the TZVPP-JKFIT density
fitting basis set for all molecules except fulvene, where the cc-pVDZ-JKFIT set was employed. MECP geometries were calculated using the same gradient projection algorithm as for SF-TDDFT. ${ }^{83}$ The basis sets were chosen to replicate the ones used in references ${ }^{86-93}$, also given in Table 4.1.

When starting the MECP optimisations, the optimised $\mathrm{S}_{1}$ geometry was used as an initial guess structure. For instances when this did not lead to a converged MECP geometry, the initial structure was modified by adding a small kink. This helped lead to convergence as it avoided limiting symmetry constraints, for example, a planar ring structure would become puckered by moving an atom out of the ring plane by $0.1 \AA$.

Along with comparing different methods of locating the MECP of each molecule, this work also compares the use of two functionals for the SF-TDDFT approaches: BHHLYP and wB97X. The BHHYLP functional was chosen as it contains 50\% HF exchange with 50\% B88 exchange which has previously been shown to be successful when using SF based approaches due to its larger fraction of HF exchange. ${ }^{71,76,78}$ The $\omega$ B97X functional was chosen as an example of a contemporary range separated hybrid GGA functional that has been used for a wide variety of applications. ${ }^{94}$ Range-separated functionals help to improve the self-interaction error, see Section 2.7.1, by treating the exact exchange in two components, one for short range and one for long range.

$$
\begin{equation*}
\frac{1}{r_{12}}=\frac{\operatorname{erfc}\left(\omega r_{12}\right)}{r_{12}}+\frac{\operatorname{erf}\left(\omega r_{12}\right)}{r_{12}} \tag{4.13}
\end{equation*}
$$

The first term on the RHS is the short-range component, where erfc is the complementary error function; the second term is the long-range component, where erf is the error function.

Throughout this work all XMS-CASPT2 calculations were performed using the BAGEL software, ${ }^{95}$ whilst all the CASSCF MECP calculations used the Molpro 2015.1 software. ${ }^{96}$ The DFT and SF-TDDFT were performed using the Q-Chem 5.0 software suite. ${ }^{97}$

| Molecule | Active space | Basis set |
| :---: | :---: | :---: |
| Fulvene $^{86}$ | $(6,6)$ | cc-pVDZ |
| $4 \mathrm{ABN}^{87}$ | $(10,9)$ | $6-31+\mathrm{G}(\mathrm{d})$ |
| $5 \mathrm{FC}^{88}$ | $(8,7)$ | $6-31 \mathrm{G}(\mathrm{d})$ |
| 9 H -adenine |  |  |
| $2,4,6-$ octatriene $^{80}$ | $(12,10)$ | $6-31 \mathrm{G}(\mathrm{d}, \mathrm{p})$ |
| Azomethane $^{91}$ | $(6,6)$ | $6-31+\mathrm{G}(\mathrm{d})$ |
| Azoxymethane $^{91}$ | $(6,4)$ | $6-31 \mathrm{G}(\mathrm{d})$ |
| Phenol $^{92}$ | $(6,4)$ | $6-31 \mathrm{G}(\mathrm{d})$ |
| SMAC $^{93}$ | $(8,7)$ | $6-31 \mathrm{G}(\mathrm{d}, \mathrm{p})$ |

Table 4.1 Molecules considered in this work with the active spaces used for multireference approaches and the basis sets employed.


Fulvene


4ABN


5FC


9H-adenine


2,4,6-octatriene


Azoxymethane


Phenol


Azomethane


Figure 4.1 Skeletal structures of the molecules studied in this work and the numbering system used throughout.

In all cases, the purpose of this work is to compare the performance of each SF-TDDFT approach, using the two different functionals, to the multireference approaches of CASSCF and XMS-CASPT2 for previously identified MECPs, not to locate new ones.

### 4.4. Results and Discussion

This section will be broken down to give a brief comparison of the MECPs obtained from the different approaches for each molecule before giving a discussion of the overall performance of the SF-TDDFT methods Section 4.2. Where possible, the parameters for the geometries obtained using CASSCF have been taken from the values reported in the corresponding references.

### 4.4.1. Fulvene

Fulvene has been frequently used as a benchmark for characterising Conlncs between the $\mathrm{S}_{0}$ and $\mathrm{S}_{1}$ electronic states. ${ }^{86}$ The structure obtained by Robb et al. and labelled as the minimum on the intersection seam ${ }^{86}$ is the geometry our results are replicating. Table 4.2 provides the selected geometrical parameters obtained by the different SF-TDDFT approaches (where NAC, PC and BP are the nonadiabatic coupling, penalty constrained and branching plane approaches respectively).

Each of the methods show good qualitative agreement with each other. The reported CASSCF bond lengths are all within $0.01 \AA$ of the XMS-CASPT2 structure. In the case of the SF-TDDFT methods, the largest deviation in bond length from the XMS-CASPT2 structure is $0.04 \AA$. This occurs from the C4-C5 bond length calculated in most of the methods. Figure 4.2 shows the MECP geometries obtained using XMS-CASPT2 and NAC-BHHLYP with Figure A1 highlighting structural differences.

Table 4.3 gives the vertical excitation (VE), $0-0$, and the $\mathrm{S}_{1} / \mathrm{S}_{0} \mathrm{MECP}$ energies calculated using each method. The energies obtained by each approach follow the same trend as the XMS-CASPT2 results. Comparing the MECP energies, the BHHLYP approaches are
all within 0.55 eV of the of the XMS-CASPT2 MECPs, whilst the approaches employing the $\omega B 97 \mathrm{X}$ functional resulted in energies at least 0.72 eV higher.

|  | BHHLYP |  |  | WB97X |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCF | XMS-CASPT2 |
| C1-C2 | 1.396 | 1.395 | 1.395 | 1.388 | 1.389 | 1.415 | 1.410 | 1.417 |
| C1-C4 | 1.473 | 1.473 | 1.475 | 1.485 | 1.483 | 1.480 | 1.461 | 1.471 |
| C3-C5 | 1.474 | 1.473 | 1.473 | 1.484 | 1.483 | 1.484 | 1.460 | 1.471 |
| C4-C5 | 1.340 | 1.340 | 1.339 | 1.344 | 1.345 | 1.358 | 1.371 | 1.377 |
| C2-C6 | 1.464 | 1.464 | 1.464 | 1.476 | 1.475 | 1.458 | 1.481 | 1.477 |
| H7-C-C-C3 | -76.9 | -75.5 | -76.1 | -68.0 | -67.8 | -76.8 | -58.7 | -67.5 |
| H7-C-C-H8 | 179.1 | 180.0 | 179.2 | 180.0 | 179.8 | 173.4 | 171.3 | 180.0 |

Table 4.2 Selected geometrical parameters of the fulvene MECP. ${ }^{a}$ taken from reference 86


Figure 4.2 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP of fulvene: (left) XMS-CASPT2, (right) NAC-BHHLYP.

|  |  |  | BHHLYP |  |  | wB97X |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| VE | 3.39 | 4.07 |  | 3.70 |  | 3.61 |  |  |
| $0-0$ | 2.44 | 2.60 |  | 3.13 |  |  | 3.24 |  |
| S $_{1} / \mathrm{S}_{0}$ | 2.41 | 2.46 | 2.95 | 2.94 | 2.95 | 3.14 | 3.13 | 3.18 |

Table 4.3 Energies of fulvene relative to $\mathrm{S}_{0}$. Values given in eV .

### 4.4.2. 4ABN

4-aminobenzonitrile, 4ABN, and its N-dimethyl derivatives are usually studied for the effect of an $S_{2} / S_{1}$ ConInc on the fluorescent behaviour in solvents, with 4ABN showing single fluorescence in both polar and non-polar solvents but its derivatives displaying dual fluorescence only in polar solvents. ${ }^{87,98}$ However, this work is focused on the Conlnc occurring between the ground state and first electronically excited state, $\mathrm{S}_{1} / \mathrm{S}_{0}$. Table 4.4 provides the selected geometrical parameters of this structure.

|  | BHHLYP |  |  | WB97X |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCF | XMS-CASPT2 |
| C1-N | 1.394 | 1.400 | 1.367 | 1.379 | 1.400 | 1.333 | 1.345 | 1.362 |
| C4-C | 1.421 | 1.422 | 1.421 | 1.406 | 1.422 | 1.436 | 1.421 | 1.398 |
| C-N | 1.143 | 1.143 | 1.142 | 1.161 | 1.143 | 1.165 | 1.152 | 1.191 |
| C2-C3 | 1.430 | 1.439 | 1.442 | 1.462 | 1.439 | 1.366 | 1.347 | 1.357 |
| C5-C6 | 1.348 | 1.348 | 1.344 | 1.340 | 1.348 | 1.345 | 1.347 | 1.346 |
| C2-C3-C4 | 108.4 | 108.5 | 108.6 | 107.2 | 108.5 | 114.7 | 112.5 | 110.2 |
| C3-C4-C5 | 116.0 | 115.9 | 115.7 | 114.1 | 115.9 | 110.3 | 118.9 | 117.8 |
| C4-C5-C6 | 110.1 | 110.1 | 110.5 | 112.5 | 110.1 | 114.5 | 112.5 | 110.1 |
| H-C3-C4-C | 80.5 | 68.5 | 75.0 | 61.9 | 68.5 | 104.6 | 34.3 | 59.2 |
| H-N-C1-C2 | -21.3 | -22.0 | -22.1 | -17.8 | -22.0 | -11.6 | -20.7 | -21.9 |

Table 4.4 Selected geometrical parameters for the MECP of 4ABN.

The multireference approaches both converge on a boat like structure, with the cyano and amine groups both pointing up, clearly out of the plane of the ring. In contrast, each of the SF-TDDFT methods provide structures where the amine group is almost planar
with the ring. In the case of the BP-wB97X approach, the amine group is still planar with the ring as shown in Figure 4.3 and Figure A2.


Figure 4.3 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0} \mathrm{MECP}$ of 4ABN: (left) XMS-CASPT2, (centre) NAC- $\omega \mathrm{B97X}$, (right) BP- $\omega \mathrm{B} 97 \mathrm{X}$.

The relative energetics are given in Table 4.5. The SF-TDDFT MECP relative energies, apart from PC-BHHLYP, are within 0.8 eV of the XMS-CASPT2 energy. However, all of them have a smaller deviation than the CASSCF energy, which is over 1 eV larger.

|  |  |  | BHHLYP |  |  | $\omega B 97 X$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| VE | 4.06 | 4.68 |  | 5.32 |  | 5.08 |  |  |
| $0-0$ | 3.95 | 4.09 |  | 5.02 |  |  | 4.94 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 4.07 | 5.15 | 4.87 | 5.04 | 4.88 | 4.84 | 4.83 | 4.94 |

Table 4.5 Calculated energies of 4ABN relative to $S_{0}$. Values given in eV .

### 4.4.3. 5FC

The nonradiative decay of 5 -flurocytosine was studied by Blancafort et al., identifying ConIncs between the $S_{2}$ and $S_{1}$ state as well as one located between the $S_{1}$ and $S_{0}$ state..$^{88}$ Table 4.6 gives the geometrical parameters of the $\mathrm{S}_{1} / \mathrm{S}_{0} \mathrm{MECP}$.

The structures obtained from the multireference approaches are in reasonable agreement with each other, the main difference being that the CASSCF structure has the $\mathrm{C} 6-\mathrm{H}$ and $\mathrm{N} 1-\mathrm{H}$ planar to the ring whereas the XMS-CASPT2 geometry shows them out of plane with the ring. In terms of the SF-TDDFT approaches, the structures tend to differ to that
obtained using XMS-CASPT2, most obviously seen in the conformation of the ring. Of the SF-TDDFT approaches, the NAC method gives the most qualitatively correct structure, however, quantitatively the C2-O bond length is too long for each method. These structures are shown in Figure 4.4 and Figure A3.

|  | BHHLYP |  |  | wB97X |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCF | XMS-CASPT2 |
| C4-N | 1.383 | 1.383 | 1.383 | 1.396 | 1.357 | 1.372 | 1.389 | 1.389 |
| C2-O | 1.471 | 1.471 | 1.471 | 1.484 | 1.471 | 1.494 | 1.414 | 1.411 |
| N1-H | 1.001 | 1.001 | 1.001 | 1.009 | 1.018 | 1.003 | 0.997 | 1.016 |
| C5-F | 1.353 | 1.353 | 1.353 | 1.368 | 1.379 | 1.348 | 1.343 | 1.373 |
| H-N-H | 112.0 | 112.0 | 112.0 | 110.8 | 118.4 | 114.6 | 111.8 | 111.6 |
| H-N-C4-C5 | -17.1 | -17.1 | -17.1 | -7.6 | -8.7 | -18.4 | -21.2 | -18.2 |
| H-N1-C6-H | 45.6 | 45.6 | 45.6 | 53.2 | 97.8 | 3.8 | 55.0 | 48.7 |

Table 4.6 Selected geometrical parameters of the 5FC $\mathrm{S}_{1} / \mathrm{S}_{0} \mathrm{MECP}$.


Figure 4.4 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP of 5FC: (left) XMS-CASPT2, (centre) CASSCF, (right) NAC-BHHLYP

Table 4.7 provides the relative energetics from each method for 5 FC. The methods using the $\omega$ B97X functional all give MECP energies higher relative to $S_{0}$ than BHHLYP. Due to the qualitatively different structures obtained from PC and BP, only the NAC approach can be compared to the XMS-CASPT2 results, which is larger by 0.4 eV when the BHHLYP functional is employed.

|  |  |  | BHHLYP |  |  | $\omega B 97 X$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| VE | 4.37 | 5.31 |  | 5.36 |  | 5.30 |  |  |
| $0-0$ | 3.75 | 3.93 |  | 4.78 |  |  | 4.85 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 4.55 | 4.55 | 4.95 | 4.81 | 4.95 | 6.00 | 5.71 | 5.99 |

Table 4.7 Energies of 5FC relative to $\mathrm{S}_{0}$. Values given in eV .

### 4.4.4. 9H-Adenine

Fast radiationless decay pathways are thought to be responsible for the photostability of DNA bases. Perun et al. investigated the nonradiative decay pathway of 9 H -adenine and found there to be two ConIncs between the $S_{1}$ and $S_{0}$ surfaces: one occurring from the intersection of a $\pi \pi^{*}\left({ }^{1} L_{b}\right)$ state with the ground state and another from a $n \pi^{*}$ state. ${ }^{89}$ The parameters of these MECPs are given in Table 4.8 and Table 4.9 respectively.

With regards to the $\pi \pi^{*}\left({ }^{1} L_{b}\right) S_{1} / S_{0}$ MECP, the CASSCF geometry gives large differences in comparison to the XMS-CASPT2 structure, as does the SF-TDDFT NAC and PC approaches when using the BHHLYP functional as shown in Figure 4.5 and Figure A4.


Figure 4.5 Calculated geometries for the $\pi \pi^{*} / \mathrm{S}_{0}$ MECP of 9H-adenine: (left) XMS-CASPT2, (right) NAC-BHHLYP.

| Parameter | BHHLYP |  |  | $\omega \mathrm{B97X}$ |  |  | CASSCF $^{\text {a }}$ | XMS-CASPT2 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | NAC | PC | BP | NAC | PC | BP |  |  |
| $\mathrm{N} 3-\mathrm{C} 2$ | 1.277 | 1.368 | 1.390 | 1.396 | 1.363 | 1.393 | 1.285 | 1.396 |
| $\mathrm{C} 2-\mathrm{N} 1$ | 1.404 | 1.504 | 1.288 | 1.295 | 1.322 | 1.298 | 1.402 | 1.319 |
| C6-N1-C2-N3 | 68.1 | 53.6 | 64.9 | 66.0 | 67.2 | 65.3 | 66.0 | 31.1 |
| C6-N1-C2-H | -139.9 | -157.0 | -165.4 | -166.2 | -165.8 | -167.0 | -142.3 | -164.7 |

Table 4.8 Selected geometrical parameters for the $\pi \pi^{*} S_{1} / S_{0}$ MECP of $9 H$-adenine. ${ }^{\text {a }}$ taken from reference ${ }^{89}$.

| Parameter | BHHLYP |  |  | $\omega \mathrm{B97X}$ |  |  | CASSCF ${ }^{\text {a }}$ | XMS-CASPT2 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | NAC | PC | BP | NAC | PC | BP |  |  |
| N3-C2 | 1.446 | 1.470 | 1.470 | 1.489 | 1.517 | 1.449 | 1.407 | 1.435 |
| $\mathrm{C} 2-\mathrm{N} 1$ | 1.426 | 1.444 | 1.444 | 1.417 | 1.400 | 1.439 | 1.390 | 1.413 |
| C6-N1-C2-N3 | 74.3 | 83.9 | 83.8 | 74.4 | 70.8 | 63.7 | 67.6 | 64.4 |
| C6-N1-C2-H | -171.2 | -171.2 | -171.2 | -176.2 | 179.5 | 176.1 | -84.1 | -77.4 |

Table 4.9 Selected geometrical parameters for the $n \pi^{*} S_{1} / S_{0}$ MECP of $9 H$-adenine. ${ }^{\text {a }}$ taken from reference ${ }^{89}$.

The structures obtained for the $n \pi^{*} S_{1} / S_{0}$ MECP all give good quantitative agreement with the XMS-CASPT2 structure except for PC-wB97X which gives larger bond lengths.

Overall, each of the SF-TDDFT methods give qualitatively correct structures compared to XMS-CASPT2, except for the orientation of the C2-H bond, an example of this is shown in Figure 4.6 and Figure A5.


Figure 4.6 Calculated XMS-CASPT2 geometry for the $n \pi^{*} / \mathrm{S}_{0} \mathrm{MECP}$ of 9 H -adenine: (left) XMS-CASPT2, (right) BP-BHHLYP.

The $\pi \pi^{*}\left({ }^{1} L_{b}\right)$ and $n \pi^{*}$ energetics are provided in Table 4.10 and Table 4.11, respectively.

|  |  |  | BHHLYP |  |  | $\omega B 97 X$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| VE | 5.13 | 5.43 |  | 4.80 |  | 4.92 |  |  |
| $0-0$ | 4.39 | 4.63 |  | 5.06 |  |  | 4.89 |  |
| S $_{1} / \mathrm{S}_{0}$ | 4.56 | 4.46 | 5.33 | 5.24 | 5.26 | 5.27 | 5.26 | 5.30 |

Table 4.10 9H-adenine $\pi \pi^{*}$ MECP energetics, given in eV , relative to $\mathrm{S}_{0}$.

|  |  |  | BHHLYP |  |  | $\omega$ B97X |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| VE | 5.73 | 6.36 |  | 5.73 |  |  | 5.73 |  |
| $0-0$ | 4.51 | 5.12 |  | 5.07 |  |  | 4.89 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 4.12 | 4.59 | 5.75 | 7.33 | 7.33 | 6.26 | 7.14 | 6.85 |

Table 4.119H-adenine $n \pi^{*}$ MECP energetics, given in eV , relative to $\mathrm{S}_{0}$.

For both Conlncs, the CASSCF approach gives the closest MECP energy gap to XMS-CASPT2. For the $\pi \pi^{*}\left({ }^{1} L_{b}\right)$ MECPs the SF-TDDFT approaches give differences larger than 0.7 eV .

The energies obtained for the $n \pi^{*}$ MECPs from the SF-TDDFT approaches also tend to be much larger than that of XMS-CASPT2 with the closest one being achieved by NAC-BHHLYP which is still larger by more than 1.5 eV .

In both cases the XMS-CASPT2 energies show the MECPs being lower than the vertical excitation energy, whereas the SF-TDDFT approaches calculate them lying above or close to the respective vertical excitation energy.

### 4.4.5. 2,4,6-Octatriene

The photoisomerization process of the all trans form of 2,4,6-octatriene was studied by Chattopadhyay et al. ${ }^{90}$ where a $\mathrm{S}_{1} / \mathrm{S}_{0}$ ConInc was located using CASSCF. Table 4.12 provides geometrical parameters obtained from the methods used in this study.

The CASSCF structure gives good agreement with that of XMS-CASPT2 with a maximum deviation within $0.02 \AA$ A for the C3-C4 bond. Most of the SF-TDDFT approaches also provide good qualitative and quantitative agreement with the XMS-CASPT2 structure, which also deviate by no more than 0.02 Å. However, the PC-wB97X approach does not perform as well resulting in a qualitatively different structure. It was attempted to alleviate this in a number of ways, one of which was using a larger basis set, $6-31 \mathrm{G}(\mathrm{d}, \mathrm{p})$, and using this converged geometry as the starting point with the correct basis set; another approach attempted was to use the converged XMS-CASPT2 geometry as the initial guess structure. In each attempt the PC- $\omega \mathrm{B} 97 \mathrm{X}$ method provided the same incorrect structure shown in Figure 4.7 and Figure A6.

|  | BHHLYP |  |  | $\omega$ WB97X |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCF | XMS-CASPT2 |
| C1-C2 | 1.494 | 1.494 | 1.494 | 1.494 | 1.560 | 1.494 | 1.504 | 1.499 |
| C2-C3 | 1.444 | 1.441 | 1.444 | 1.441 | 1.419 | 1.441 | 1.464 | 1.453 |
| C3-C4 | 1.404 | 1.399 | 1.406 | 1.399 | 1.445 | 1.399 | 1.427 | 1.408 |
| C4-C5 | 1.445 | 1.448 | 1.446 | 1.448 | 1.351 | 1.448 | 1.466 | 1.464 |
| C5-C6 | 1.407 | 1.417 | 1.407 | 1.417 | 1.452 | 1.417 | 1.427 | 1.419 |
| C6-C7 | 1.359 | 1.364 | 1.357 | 1.364 | 1.340 | 1.364 | 1.365 | 1.378 |
| C7-C8 | 1.489 | 1.496 | 1.485 | 1.496 | 1.494 | 1.496 | 1.501 | 1.497 |
| C1-C2-C3 | 119.4 | 119.0 | 119.0 | 119.0 | 108.5 | 119.0 | 119.5 | 119.0 |
| C1-C2-C3-C4 | -100.8 | -98.2 | -102.0 | -98.2 | -57.0 | -98.2 | -103.9 | -107.7 |
| C2-C3-C4-C5 | -127.4 | -130.2 | -126.9 | -130.2 | -177.3 | -130.2 | -118.2 | -125.3 |
| C3-C4-C5-C6 | 111.8 | 107.7 | 112.4 | 107.7 | 177.7 | 107.7 | 102.3 | 102.7 |

Table 4.12 Selected geometrical parameters for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP of 2,4,6-octatriene.


Figure 4.7 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP of 2,4,6-octatriene: (left) XMS-CASPT2, (right) PC- $\omega B 97 X$.

Table 4.13 provides the vertical excitation (VE), 0-0, and the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP energies calculated using each method. The CASSCF result for the MECP energy most closely matches the one obtained from XMS-CASPT2. Of the SF-TDDFT approaches the NAC- $\omega$ B97X most closely resembles the XMS-CASPT2 energy, differing by 0.3 eV . Each method using the BHHLYP functional gives a similar energy to each other and are within 0.6 eV of the XMS-CASPT2 energy.

|  |  |  | BHHLYP |  |  | $\omega B 97 X$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| VE | 5.78 | 5.58 |  | 4.83 |  |  | 5.04 |  |
| $0-0$ | 5.51 | 5.21 |  | 4.83 |  |  | 4.89 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 3.99 | 3.72 | 4.55 | 4.54 | 4.56 | 4.28 | 4.47 | 5.00 |

Table 4.13 Energetics of 2,4,6-octatriene relative to $\mathrm{S}_{0}$. Values given in eV .

### 4.4.6. Azomethane

Ghosh et al. ${ }^{91}$ studied the internal conversion pathway from the first electronically excited state to the ground state, through the $\mathrm{S}_{1} / \mathrm{S}_{0} \mathrm{MECP}$, of trans-azomethane using CASSCF. Table 4.14 shows the selected geometrical parameters of azomethane.

Each method compared in this study reproduced the XMS-CASPT2 geometry with reasonable accuracy, with deviations no larger than $0.03 \AA$ for the CASSCF structure and each of the SF-TDDFT approaches, all occurring from the N2-C2 bond length an example of this is shown in Figure 4.8 and Figure A7.


Figure 4.8 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP of azomethane: (left) XMS-CASPT2, (right) NAC-BHHLYP.

Table 4.15 gives the vertical excitation (VE), 0-0, and the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP energies calculated using each method. The MECP energy from CASSCF is very accurate compared to XMS-CASPT2 for azomethane. The energies obtained when the $\omega$ B97X functional is employed are all higher compared to BHHLYP; however, NAC-wB97X most closely matches these results. Each method provides the same energy when using the BHHLYP functional and deviate by 0.21 eV from the XMS-CASPT2 MECP energy.

|  | BHHLYP |  |  | $\omega$ WB97X |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCFa | XMS- <br> CASPT2 |
| C1-N1 | 1.458 | 1.458 | 1.458 | 1.458 | 1.458 | 1.458 | 1.433 | 1.438 |
| N1-N2 | 1.254 | 1.254 | 1.254 | 1.254 | 1.254 | 1.254 | 1.287 | 1.271 |
| N2-C2 | 1.458 | 1.458 | 1.458 | 1.458 | 1.458 | 1.458 | 1.462 | 1.488 |
| C1-N1-N2 | 120.2 | 120.2 | 120.2 | 120.2 | 120.2 | 120.2 | 130.9 | 114.4 |
| N1-N2-C2 | 120.4 | 120.4 | 120.4 | 120.4 | 120.4 | 120.4 | 114.8 | 136.5 |
| C1-N1-N2-C2 | 87.7 | 87.7 | 87.7 | 87.7 | 87.7 | 87.7 | 94.2 | 93.5 |

Table 4.14 Geometrical parameters of the $\mathrm{S}_{1} / \mathrm{S}_{0} \mathrm{MECP}$ of azomethane. ${ }^{\text {a }}$ taken from reference ${ }^{91}$.

|  |  |  | BHHLYP |  |  | $\omega B 97 X$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| VE | 3.23 | 3.45 |  | 3.30 |  | 3.43 |  |  |
| $0-0$ | 2.87 | 3.06 |  | 2.89 |  |  | 2.91 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 2.76 | 2.82 | 2.97 | 2.97 | 2.97 | 3.05 | 3.23 | 3.24 |

Table 4.15 Azomethane energetics relative to $\mathrm{S}_{0}$. Values given in eV .

### 4.4.7. Azoxymethane

In the same piece of research discussed in Section 4.4.6, Ghosh et al. ${ }^{91}$ also studied the photoisomerization pathway of azoxymethane. Table 4.16 provides the selected geometrical parameters obtained from the methods compared in this study.

The CASSCF and XMS-CASPT2 structures are in good agreement with each other.
Whilst overall the SF-TDDFT approaches provide qualitatively correct structures, there are some noticeable differences. The first is the N1-O1 bond length, which is the largest deviation recorded for most approaches, shown in Figure 4.9 and Figure A8, except for NAC-BHHLYP which closely replicates the N1-O1 bond length. The second difference concerns the C1-N1-N2-C2 dihedral angle. The XMS-CASPT2 structure has a dihedral of $172^{\circ}$ whereas PC-BHHLYP, BP-BHHLYP, BP- $\omega$ B97X and NAC- $\omega$ B97X all give dihedral angles of $155^{\circ}$ (Table 4.16).

|  | BHHLYP |  |  | $\omega$ B97X |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCF | XMS- <br> CASPT2 |
| C1-N1 | 1.453 | 1.459 | 1.459 | 1.459 | 1.465 | 1.459 | 1.452 | 1.466 |
| N1-N2 | 1.378 | 1.404 | 1.404 | 1.404 | 1.447 | 1.404 | 1.335 | 1.367 |
| N1-O1 | 1.401 | 1.328 | 1.328 | 1.328 | 1.483 | 1.328 | 1.419 | 1.395 |
| N2-C2 | 1.389 | 1.430 | 1.430 | 1.430 | 1.436 | 1.430 | 1.456 | 1.460 |
| C1-N1-N2 | 109.1 | 112.0 | 112.0 | 112.0 | 106.1 | 112.0 | 114.2 | 112.3 |
| N1-N2-C2 | 109.2 | 109.6 | 109.6 | 109.6 | 106.1 | 109.6 | 114.4 | 111.1 |
| C1-N1-O1 | 107.4 | 112.6 | 112.6 | 112.6 | 95.4 | 112.6 | 112.6 | 110.3 |
| C1-N1-N2-C2 | 172.5 | 155.1 | 155.1 | 155.1 | 163.3 | 155.1 | 178.6 | 172.1 |
| C1-N1-O1-N2 | 116.3 | 117.4 | 117.4 | 117.4 | 108.9 | 117.4 | 118.7 | 115.6 |

Table 4.16 Geometrical parameters of the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP of azoxymethane. ${ }^{\text {a }}$ taken from reference ${ }^{91}$.


Figure 4.9 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP of azoxymethane: (left) XMS-CASPT2, (right) PC-BHHLYP.

Table 4.17 provides the vertical excitation (VE), $0-0$, and the $\mathrm{S}_{1} / \mathrm{S}_{0} \mathrm{MECP}$ energies calculated using each method. In each case, use of the BHHLYP functional results in more accurate MECP energies. Although PC-BHHLYP and BP-BHHLYP are almost identical to the XMS-CASPT2 MECP energy, their structures are quantitatively different. The NAC-BHHLYP energy is 0.5 eV lower than the XMS-CASPT2 energy and is closer than the one obtained using CASSCF.

|  |  |  | BHHLYP |  |  | wB97X |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| VE | 4.66 | 4.45 |  | 4.09 |  |  | 4.21 |  |
| $0-0$ | 3.88 | 2.95 |  | 3.30 |  |  | 2.93 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 3.87 | 2.91 | 3.37 | 3.84 | 3.86 | 4.15 | 4.63 | 4.38 |

Table 4.17 Energetics of azoxymethane relative to $\mathrm{S}_{0}$. Values given in eV .

### 4.4.8. Phenol

Phenol has previously been studied for the role of ConIncs between the $S_{2}$ and $S_{0}$ states as well as between the $\mathrm{S}_{2}$ and $\mathrm{S}_{1}$ states in the O-H photodissociation mechanism. ${ }^{92}$ However, in this work only ConIncs between $\mathrm{S}_{1}$ and $\mathrm{S}_{0}$ are considered. Table 4.18 gives the selected calculated geometrical parameters for phenol.

The CASSCF geometry shows good qualitative and quantitative agreement with the XMS-CASPT2 structure, with deviations less than 0.03 Å. Whilst all the SF-TDDFT
approaches show qualitatively correct structures, only the BP-BHHLYP approach does not give a quantitively correct geometry showing multiple differences in bond length, around $0.20 \AA$, compared to the XMS-CASPT2 geometry as shown in Figure 4.10 and Figure A9.

The use of the $\omega$ B97X functional gave higher energies than the BHHLYP functional for each MECP method (Table 4.19). Of the three SF-TDDFT approaches, the PC approach resulted in the highest energy for each functional. The NAC-BHHLYP and BP-BHHLYP both give energies that are approximately 0.5 eV higher than that given by XMS-CASPT2; however, as noted above, the BP-BHHLYP approach was the only one that did not give a quantitatively correct structure.


Figure 4.10 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0} \mathrm{MECP}$ of phenol: (left) XMS-CASPT2, (right) BP-BHHLYP.

|  | BHHLYP |  |  | wB97X |  |  |  | NAC |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCF | XMS <br> CASPT2 |
| C1-O | 1.327 | 1.350 | 1.400 | 1.335 | 1.350 | 1.350 | 1.350 | 1.363 |
| O-H | 0.958 | 0.943 | 0.952 | 0.966 | 0.943 | 0.943 | 0.943 | 0.967 |
| C1-C2 | 1.445 | 1.455 | 1.619 | 1.441 | 1.455 | 1.455 | 1.455 | 1.450 |
| C2-C3 | 1.421 | 1.456 | 1.264 | 1.446 | 1.456 | 1.456 | 1.456 | 1.453 |
| C3-C4 | 1.492 | 1.455 | 1.518 | 1.464 | 1.455 | 1.455 | 1.455 | 1.453 |
| C4-C5 | 1.435 | 1.461 | 1.679 | 1.433 | 1.461 | 1.461 | 1.461 | 1.453 |
| C2-C3-C4 | 84.2 | 84.5 | 101.8 | 81.9 | 84.5 | 84.5 | 84.5 | 83.0 |
| H-O-C1-C2 | 162.9 | 165.9 | 152.2 | 174.9 | 165.9 | 165.9 | 165.9 | 168.5 |
| O-C1-C2-H | -18.5 | -31.1 | -43.0 | -27.4 | -31.1 | -31.1 | -31.1 | -28.5 |
| C1-C2-C3-H | -176.4 | -169.6 | -176.2 | -169.9 | -169.6 | -169.6 | -169.6 | -170.4 |

Table 4.18 Selected geometrical parameters for the $S_{1} / S_{0}$ MECP of phenol.

|  |  |  | BHHLYP |  |  | $\omega B 97 X$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| VE | 4.82 | 4.93 |  | 5.50 |  |  | 5.26 |  |
| $0-0$ | 4.64 | 4.73 |  | 5.55 |  |  | 5.40 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 4.95 | 5.45 | 5.46 | 5.65 | 5.46 | 5.63 | 5.95 | 5.63 |

Table 4.19 Energies of phenol relative to $\mathrm{S}_{0}$. Values given in eV .

### 4.4.9. SMAC

Zhao et al. investigated the photoisomerization process of the aromatic Schiff base 2-(1-(methylamino)methyl)-6-chlorophenol, SMAC, using CASSCF and CASPT2. They located five Conlncs between the $S_{1}$ and $S_{0}$ state which were categorised into three
types: one ConInc belonged to the excited state intramolecular proton transfer (ESIPT) process, the remaining Conlncs belonged to two categories defined by a bond rotation. ${ }^{93}$

The first Conlnc to be reproduced is the one due to the ESIPT pathway, Table 4.20 contains the selected geometrical parameters. The CASSCF structure qualitatively matches the XMS-CASPT2 structure, with reasonable quantitative agreement, the largest deviation of the CASSCF approach overestimates the O-H bond length by 0.11 Å.

Qualitatively, both PC approaches and the NAC-BHHLYP methods match the XMS-CASPT2 geometry; however, the length of the O-H bond is not well reproduced, differing up to $0.5 \AA$. The NAC- $\omega \mathrm{B} 97 \mathrm{X}$ and both BP methods struggle to reproduce the orientation of the C2-N-C3 substituent. In the XMS-CASPT2 geometry this group is perpendicular to the plane of the ring whereas BP-BHHLYP has an angle of $\sim 45^{\circ}$ and the BP- $\omega \mathrm{B} 97 \mathrm{X}$ and NAC- $\omega \mathrm{B} 97 \mathrm{X}$ approaches show it as almost in plane with the ring, seen in Figure 4.11 and Figure A10.


Figure 4.11 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ ESIPT MECP of SMAC: (left) XMS-CASPT2, (centre) NAC-BHHLYP, (right) BP- $\omega$ B97X.

Table 4.21 provides the vertical excitation (VE), 0-0, and the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP energies calculated using each method. The CASSCF and SF-TDDFT methods give similar energy differences for the MECPs where the structures match and are within 0.8 eV of the XMS-CASPT2 result. Although the PC- $\omega$ B97X approach gives a qualitatively correct structure, the energy is not well reproduced.

|  | BHHLYP |  |  | 1 B97X |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCF | XMS-CASPT2 |
| C1-C2 | 1.471 | 1.446 | 1.540 | 1.419 | 1.489 | 1.419 | 1.460 | 1.468 |
| C2-N | 1.384 | 1.301 | 1.390 | 1.360 | 1.395 | 1.358 | 1.366 | 1.381 |
| C5-Cl | 1.723 | 1.722 | 1.727 | 1.777 | 1.711 | 1.776 | 1.733 | 1.722 |
| O-H | 3.624 | 3.318 | 4.191 | 4.928 | 3.614 | 4.879 | 3.307 | 3.193 |
| N-H | 1.003 | 1.012 | 1.006 | 1.013 | 1.014 | 1.018 | 0.995 | 1.012 |
| C4-C1-C2-N | 107.2 | 102.1 | 147.3 | 174.5 | 109.2 | 163.4 | 85.3 | 81.4 |
| C1-C2-N-C3 | -170.0 | -167.9 | -162.8 | -177.3 | -169.3 | -175.9 | -154.1 | -145.0 |
| H-O-C4-C1 | -36.5 | -36.5 | -33.0 | -11.1 | -33.8 | -19.1 | -37.7 | -38.8 |

Table 4.20 Selected geometrical parameters for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ ESIPT MECP of SMAC. ${ }^{\text {a }}$ taken from reference ${ }^{93}$.

|  |  |  | BHHLYP |  |  | $\omega B 97 X$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| $0-0$ | 2.72 | 4.46 |  | 3.61 |  |  | 3.68 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 2.35 | 3.08 | 3.10 | 3.08 | 4.22 | 5.03 | 3.78 | 5.05 |

Table 4.21 Energetics of SMAC relative to $\mathrm{S}_{0} . \mathrm{S}_{1} / \mathrm{S}_{0}$ refers to the ESIPT MECP. Values given in eV .

The following Conlncs (shown in Figure 4.12) are formed from the rotation around the $\mathrm{C} 2-\mathrm{N}$ bond. Here the original authors nomenclature for the Conlncs is retained and labelled as TWin1, TWin2, TWout1, TWout2, where TW denotes the twist (rotation) motion, in and out refer to whether the $\mathrm{O}-\mathrm{H}$ is pointing towards or away from the N atom respectively. Finally, the 1 and 2 indicate whether the rotation is anti-clockwise or clockwise, respectively.


TWin1


TWout1


TWin2


Figure $4.12 \mathrm{~S}_{1} / \mathrm{S}_{0}$ MECPs of SMAC characterised by a rotation around the $\mathrm{C} 2-\mathrm{N}$ bond: (top left) TWin1, (top right) TWin2, (bottom left) TWout1, (bottom right) TWout2.

Table 4.22 provides the geometrical parameters for the TWin1 MECP. The XMS-CASPT2 and CASSCF procedures both produce concurrent structures with a largest deviation of $0.05 \AA$ in the O-H bond length. Each of the SF-TDDFT approaches give geometries that are in good qualitative and quantitative agreement with the XMS-CASPT2 structure, an example is shown in Figure 4.13 and Figure A11. The largest deviation comes from the $\mathrm{C} 2-\mathrm{N}$ bond length of the BP-wB97X approach being 0.08 Å longer than the XMS-CASPT2 structure.

The methods that use the $\omega$ B97X functional each give an MECP energy gap larger than when the BHHLYP functional is used (Table 4.23). NAC-BHHLYP and PC-BHHLYP give the closest energy gaps compared to XMS-CASPT2, but are still larger by 1.1 eV ; these both perform better than the CASSCF method.

|  | BHHLYP |  |  | WB97X |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCF | XMS- <br> CASPT2 |
| C1-C2 | 1.411 | 1.402 | 1.399 | 1.368 | 1.399 | 1.393 | 1.416 | 1.440 |
| C2-N | 1.412 | 1.438 | 1.433 | 1.427 | 1.468 | 1.471 | 1.399 | 1.391 |
| C5-Cl | 1.740 | 1.736 | 1.738 | 1.738 | 1.738 | 1.741 | 1.767 | 1.731 |
| O-H | 0.959 | 0.958 | 0.959 | 0.966 | 0.966 | 0.965 | 0.945 | 0.991 |
| C4-C1-C2-N | -6.3 | -7.6 | -5.3 | -2.1 | -16.5 | 4.7 | -0.9 | -9.0 |
| C1-C2-N-C3 | -91.0 | -86.6 | -88.7 | -89.2 | -76.5 | -95.4 | -92.3 | -88.2 |
| H-O-C4-C1 | -31.1 | -22.7 | -34.3 | -42.1 | -41.1 | -61.1 | -31.5 | -8.0 |

Table 4.22 Selected geometrical parameters for the $S_{1} / S_{0}$ TWin1 MECP of SMAC. ${ }^{\text {a }}$ taken from reference ${ }^{93}$.


Figure 4.13 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ TWin1 MECP of SMAC: (left) XMS-CASPT2, (right) NAC-BHHLYP.

|  |  |  | BHHLYP |  |  | $\omega$ B97X |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| $0-0$ | 2.72 | 4.46 |  | 3.61 |  |  | 3.68 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 2.15 | 3.62 | 3.25 | 3.24 | 3.52 | 3.75 | 4.59 | 4.77 |

Table 4.23 Energetics of SMAC relative to $\mathrm{S}_{0} . \mathrm{S}_{1} / \mathrm{S}_{0}$ refers to the TWin1 MECP. Values given in eV .

Table 4.24 provides the geometrical parameters for the TWin2 MECP. Each of the SF-TDDFT approaches give geometries that are in good qualitative and quantitative agreement with the XMS-CASPT2 structure, shown in Figure 4.14 and Figure A12, with the largest deviation occurring in the $\mathrm{C} 2-\mathrm{N}$ bond length of the BP- $\omega \mathrm{B} 97 \mathrm{X}$ approach.

Table 4.25 provides the vertical excitation (VE), $0-0$, and the $\mathrm{S}_{1} / \mathrm{S}_{0} \mathrm{MECP}$ energies calculated using each method. The energies calculated here are identical to those of TWin1, Table 4.23, with an identical trend of energies for each approach with the use of BHHLYP functional performing better than the use of the $\omega$ B97X functional and the CASSCF method.

| Parameter | BHHLYP |  |  | $\omega \mathrm{B97X}$ |  |  | CASSCF $^{\text {a }}$ | XMSCASPT2 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | NAC | PC | BP | NAC | PC | BP |  |  |
| C1-C2 | 1.409 | 1.381 | 1.395 | 1.368 | 1.411 | 1.392 | 1.416 | 1.440 |
| $\mathrm{C} 2-\mathrm{N}$ | 1.408 | 1.449 | 1.346 | 1.429 | 1.470 | 1.469 | 1.399 | 1.392 |
| $\mathrm{C} 5-\mathrm{Cl}$ | 1.739 | 1.751 | 1.738 | 1.738 | 1.737 | 1.738 | 1.767 | 1.731 |
| $\mathrm{O}-\mathrm{H}$ | 0.959 | 1.170 | 0.957 | 0.966 | 0.967 | 0.966 | 0.945 | 0.991 |
| C4-C1-C2-N | 7.3 | -1.3 | 2.0 | 4.0 | 21.8 | -6.1 | 0.9 | 9.2 |
| $\mathrm{C} 1-\mathrm{C} 2-\mathrm{N}-\mathrm{C} 3$ | 88.7 | 93.2 | 91.2 | 88.1 | 72.3 | 95.4 | 92.3 | 88.1 |
| H-O-C4-C1 | 31.1 | 34.8 | 40.5 | 40.7 | 34.6 | 58.7 | 31.3 | 8.1 |

Table 4.24 Selected geometrical parameters for the $S_{1} / S_{0}$ TWin2 MECP of SMAC. ${ }^{a}$ taken from reference ${ }^{93}$.


Figure 4.14 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ TWin2 MECP of SMAC: (left) XMS-CASPT2, (right) NAC-BHHLYP.

|  |  |  | BHHLYP |  |  | $\omega B 97 X$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| $0-0$ | 2.72 | 4.46 |  | 3.61 |  |  | 3.68 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 2.15 | 3.62 | 3.25 | 3.24 | 3.54 | 3.75 | 4.59 | 4.76 |

Table 4.25 Energetics of SMAC relative to $\mathrm{S}_{0} . \mathrm{S}_{1} / \mathrm{S}_{0}$ refers to the TWin2 MECP. Values given in eV .

Table 4.26 provides the geometrical parameters for the TWout1 MECP. The geometry obtained from the CASSCF approach is in good agreement with the XMS-CASPT2 structure. Whilst all the SF-TDDFT geometries qualitatively match the XMS-CASPT2 geometry, there are some quantitative differences, a comparison of structures are shown in Figure 4.15 and Figure A13. The PC-BHHLYP, PC- $\omega$ B97X and BP- $\omega$ B97X approaches all differ by up to $0.11 \AA$ in the C2-N bond length.


Figure 4.15 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ TWout1 MECP of SMAC: (left) XMS-CASPT2, (centre) NAC-BHHLYP, (right) BP-wB97X.

Of the methods that obtain quantitatively correct structures, the BHHLYP functional provides better energies, with NAC-BHHLYP giving an energy gap that is within 0.8 eV of XMS-CASPT2, better than the one obtained from using CASSCF, see Table 4.27.

|  | BHHLYP |  |  | WB97X |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCF | XMS-CASPT2 |
| C1-C2 | 1.409 | 1.380 | 1.397 | 1.380 | 1.376 | 1.380 | 1.438 | 1.454 |
| C2-N | 1.369 | 1.468 | 1.397 | 1.389 | 1.473 | 1.468 | 1.374 | 1.361 |
| C5-Cl | 1.753 | 1.751 | 1.752 | 1.751 | 1.752 | 1.751 | 1.752 | 1.745 |
| O-H | 0.957 | 0.965 | 0.957 | 0.967 | 0.965 | 0.965 | 0.944 | 0.969 |
| C4-C1-C2-N | 10.2 | 17.7 | 7.1 | 7.7 | 6.5 | 17.7 | 8.9 | 14.5 |
| C1-C2-N-C3 | -98.5 | -104.0 | -96.9 | -99.8 | -92.8 | -104.0 | -97.2 | -98.5 |
| H-O-C4-C1 | -177.5 | -176.5 | -177.5 | -176.4 | -176.6 | -176.5 | -176.1 | -178.2 |

Table 4.26 Selected geometrical parameters for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ TWout1 MECP of SMAC. ${ }^{a}$ taken from reference ${ }^{93}$.

|  |  |  | BHHLYP |  |  | $\omega B 97 X$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| $0-0$ | 2.72 | 4.46 |  | 3.61 |  |  | 3.68 |  |
| $\mathrm{~S}_{1} \mathrm{~S}_{0}$ | 2.38 | 3.66 | 3.16 | 3.15 | 3.48 | 3.59 | 4.59 | 4.71 |

Table 4.27 Energetics of SMAC relative to $\mathrm{S}_{0} . \mathrm{S}_{1} / \mathrm{S}_{0}$ refers to the TWout1 MECP. Values given in eV.

Table 4.28 provides the geometrical parameters for the TWout2 MECP. The structure obtained from CASSCF is in good qualitative and quantitative agreement with that of XMS-CASPT2. With regards to the SF-TDDFT methods, BP-BHHLYP and the methods using the $\omega$ B97X functional all provide qualitatively correct structures with deviations in the bond lengths less than 0.09 Å. However, PC-BHHLYP and NAC-BHHLYP do not provide even qualitatively correct structures. These geometries exhibit a puckered ring structure with C1 out of plane of the ring as shown in Figure 4.16 and Figure A14.


Figure 4.16 Calculated geometries for the $\mathrm{S}_{1} / \mathrm{S}_{0}$ TWout2 MECP of SMAC: (left) XMS-CASPT2, (centre) NAC-wB97X, (right) PC-BHHLYP.

The high MECP energy gaps from NAC-BHHLYP and PC-BHHLYP can be attributed to the qualitatively incorrect structures. The BP-BHHLYP approach gives the closest energy to XMS-CASPT2 but the gap is 1.1 eV larger (Table 4.29).

|  | BHHLYP |  |  | WB97X |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Parameter | NAC | PC | BP | NAC | PC | BP | CASSCF | XMS-CASPT2 |
| C1-C2 | 1.404 | 1.404 | 1.399 | 1.382 | 1.384 | 1.381 | 1.419 | 1.454 |
| C2-N | 1.282 | 1.286 | 1.391 | 1.388 | 1.444 | 1.450 | 1.379 | 1.361 |
| C5-Cl | 1.719 | 1.719 | 1.752 | 1.751 | 1.751 | 1.751 | 1.778 | 1.745 |
| O-H | 0.956 | 0.956 | 0.957 | 0.966 | 0.966 | 0.966 | 0.943 | 0.969 |
| C4-C1-C2-N | -31.6 | 31.6 | -4.9 | -7.1 | -1.7 | -1.3 | -11.1 | -14.5 |
| C1-C2-N-C3 | 178.3 | 178.5 | 92.7 | 98.8 | 87.4 | 87.2 | 98.8 | 98.5 |
| H-O-C4-C1 | 163.8 | 161.5 | 173.6 | 176.9 | 173.9 | 173.8 | 175.8 | 178.2 |

Table 4.28 Selected geometrical parameters for the $S_{1} / S_{0}$ TWout2 MECP of SMAC. ${ }^{\text {a }}$ taken from reference ${ }^{93}$.

|  |  |  | BHHLYP |  |  | $\omega$ B97X |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  | XMS-CASPT2 | CASSCF | NAC | PC | BP | NAC | PC | BP |
| $0-0$ | 2.72 | 4.46 |  | 3.61 |  |  | 3.68 |  |
| $\mathrm{~S}_{1} / \mathrm{S}_{0}$ | 2.38 | 3.66 | 5.62 | 5.61 | 3.48 | 3.59 | 4.60 | 4.62 |

Table 4.29 Energetics of SMAC relative to $\mathrm{S}_{0} . \mathrm{S}_{1} / \mathrm{S}_{0}$ refers to the TWout2 MECP. Values given in eV.

### 4.4.10. Discussion

In many cases the methods struggled to converge on a MECP using the selected basis sets; notable examples were the optimisation of 4ABN and azomethane. In these instances the issue was alleviated by increasing the basis set to $6-31 \mathrm{G}(\mathrm{d}, \mathrm{p})$. This quickly led to a converged structure which could then be used as the initial guess for the optimisation using the original $6-31 \mathrm{G}(\mathrm{d})$ basis set. This issue suggests that the difficulty of convergence lies with getting to the region of a Conlnc rather than the choice of method used to get there.

One instance where this still did not work was for the convergence of 2,4,6-octatriene when using PC-wB97X, see Section 4.4.5. Instead, it was attempted to aid convergence by using the XMS-CASPT2 geometry, although the method still did not converge on a comparable structure. These issues mainly occurred when using approaches that did not include nonadiabatic coupling (the BP and PC approaches) or when the $\omega$ B97X functional was used, such was the case for the SMAC EISPT MECP with NAC-wB97X. These points agree with the work of Herbert et al. ${ }^{81}$ who recommend the use of BHHLYP and nonadiabatic coupling terms to efficiently locate MECPs using SF-TDDFT.

Spin contamination, see Section 2.2, can often be the cause of poor convergence when using SF-TDDFT. ${ }^{73,81}$ It is not a contributing factor in the cases described above, as the $\left\langle S^{2}\right\rangle$ value remained below the chosen threshold (set at 1.20, as recommended in the Q-Chem manual for convergence of a singlet state) ${ }^{99}$ This further supports our findings that the incorrect converged structure was caused by the choice of method and functional rather than the use of SF-TDDFT methodology.

Segarra-Marti et al. identified the importance of employing dynamical correlation when locating ConIncs through the use of XMS-CASPT2. ${ }^{80}$ In this work there were a couple of cases where the CASSCF MECP differed from the XMS-CASPT2 structure: some bond orientations for 5FC, Section 4.4.3, the overall structure of the $\pi \pi^{*}\left({ }^{1} L_{b}\right)$ MECP of 9 H -adenine, Table 4.8, and the elongation of the O-H bond length in the SMAC ESIPT MECP, Table 4.20. One drawback of using multireference approaches is their dependence on the choice of active space. This is apparent for Perun et al., studying 9 H -adenine in 2005, where the program being used limited their active space to $(6,6)$ rather than their desired choice of $(12,10) .{ }^{89}$

The overall performance of the CASSCF and SF-TDDFT approaches in replicating the XMS-CASPT2 structures has been provided in Table 4.30 where the mean deviation mean unsigned error (MUE) and maximum errors for the geometrical parameters have been calculated.

|  |  | CASSCF | BHHLYP |  |  | $\omega \mathrm{B97X}$ |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
|  |  | NAC | PC | BP | NAC | PC | BP |
| $\begin{aligned} & \infty \\ & 0 \\ & 0 \\ & 0 \end{aligned}$ | Mean deviation |  | -0.006 | -0.008 | 0.001 | 0.001 | -0.004 | 0.005 | -0.001 |
|  | MUE | 0.019 | 0.024 | 0.032 | 0.034 | 0.024 | 0.032 | 0.026 |
|  | Maximum | 0.111 | 0.119 | 0.185 | 0.226 | 0.105 | 0.113 | 0.107 |
|  | Mean deviation | 0.9 | -1.8 | -1.0 | 0.6 | -1.5 | -3.8 | -0.4 |
|  | MUE | 2.0 | 3.2 | 2.9 | 4.5 | 3.4 | 6.4 | 4.3 |
|  | Maximum | 5.6 | 16.1 | 16.1 | 18.8 | 16.1 | 16.1 | 16.1 |
|  | Mean deviation | 1.3 | 1.2 | 1.7 | -0.8 | 1.5 | 1.6 | -0.7 |
|  | MUE | 7.3 | 13.9 | 13.9 | 13.2 | 13.9 | 18.4 | 18.3 |
|  | Maximum | 34.9 | 93.8 | 93.8 | 93.8 | 98.8 | 102.1 | 98.7 |

Table 4.30 Mean deviation, MUE and maximum error of the CASSCF and SF-TDDFT approaches considered in this work compared to the corresponding XMS-CASPT2 structures.

The MUE is the more realistic metric of performance and further indicates that the NAC-BHHLYP approach results in structures closely resembling those produced by using XMS-CASPT2. The maximum errors occur from the instances previously mentioned where qualitatively concurrent structures could not be obtained.

It was determined from the $s^{x}$ and $s^{y}$ tilt parameters calculated that each MECP structure had a peaked topology.

The energies of the MECPs obtained from the SF-TDDFT approaches are mostly within 1.0 eV of those obtained from XMS-CASPT2 when the structures quantitatively match and the BHHLYP functional is employed. However, when considering the MECP energies relative to the vertical excitation energy the qualitative trend is correct but is not always quantitatively accurate, especially in the cases of 9 H -adenine and 2,4,6-octatriene.

### 4.5. Conclusions

This study has compared the use of three different SF-TDDFT methods, each using two different functionals, to XMS-CASPT2 in optimising Conlnc for a range of molecules. It was found that the NAC-BHHLYP approach was the most reliable SF-TDDFT method providing the most concurrent MECP structures with XMS-CASPT2, although it should be noted that the BP-BHHLYP and PC-BHHLYP also perform reasonably well and at a lower computational cost.

With regards to energetics, the NAC-BHHLYP approach performs best in replicating the relative energy trend of XMS-CASPT2. The PC method, with either functional, provides a better energetic picture than the BP approach. Therefore, it is recommended that when using SF-TDDFT for MECP optimisation to employ the NAC-BHHLYP approach.

This work has been published in the Journal of Chemical Theory and Computation ${ }^{68}$ and has since lead to citations in works either identifying Conlncs and MECPs or review papers. ${ }^{100-109}$

## Chapter 5. AIE Molecules in the Gas Phase

In the previous chapter it was shown that SF-TDDFT employing the NAC method with the BHHLYP functional provided a suitable way of locating MECPs in comparison to the use of the higher computational method of XMS-CASPT2. In the following chapter, any MECPs are located using this application of SF-TDDFT.

The results of this work have been published in Physical Chemistry Chemical Physics. ${ }^{109}$

### 5.1. Introduction

The work in this chapter focuses on the gas phase properties of the potential probes to be used in detecting phase changes in a membrane environment.

The molecule 1,6-diphenyl-1,3,5-hexatriene (DPH), Figure 5.1, is commonly used as a fluorescent probe for lipid membrane environments. ${ }^{110,111}$ Whilst DPH is not selective to the different phases which occur in a membrane with regards to emission wavelength or intensity, it does display fluorescence anisotropy between different phases. ${ }^{112,113}$

The mechanism to be utilised by the probes in this work is the restriction of intramolecular movement (RIM) mechanism responsible for the AIE effect ${ }^{114}$ where a freely rotating molecule does not fluoresce but when sterically hindered access through the ConInc is restricted therefore causing fluorescent emission. ${ }^{115}$

Bhongale et al. ${ }^{24}$ demonstrated that it is possible to induce AIE-like properties by making substitutions on a molecule. They took the ACQphore molecule of 1,4-Distyrylbenze and replaced two hydrogen atoms with methyl groups. This substitution changed the fluorescent properties of the molecule, making it an AIEgen. This was attributed to the change in packing structure upon aggregation. 1,4-Distyrylbenze exhibited a face-to-face overlap structure when aggregated allowing for $\pi-\pi$ interactions thus reducing the fluorescence emission. In comparison, the substituted molecule has a twisted conformation caused by the steric hindrance of the two methyl groups. This reduced the
occurrence of $\pi-\pi$ interactions therefore resulting in fluorescence upon aggregation. In dilute solutions, when the molecules are isolated, 1,4-Distyrylbenze was fluorescent whereas the substituted molecule was not due to the intramolecular rotations allowing for non-radiative decay. ${ }^{116}$

Itami et al. conducted a similar study of 1,4-Distyrylbenze, in which phenyl groups were used in the substitution rather than methyl groups, resulting in the same effect. ${ }^{117}$

Shimizu et al. were able to induce an AIE effect onto the DPH molecule through phenyl substitutions along the polyene chain as well as substitutions at the para- positions of those rings, ${ }^{118}$ also shown to alter the fluorescent emission. ${ }^{19,120}$

In this work, derivatives of DPH are generated by either singly- or doubly-substituting methyl groups along the polyene chain. Each derivative is shown in Figure 5.2 with a corresponding naming scheme, whilst Figure 5.1 displays the atom numbering scheme for DPH and its derivatives, both of which are used throughout this work. These simple substitutions are made in the hope of altering the planarity of the molecule in gaseous and solvated environments thereby making it susceptible to phase changes within a lipid membrane.

The ground state, $\mathrm{S}_{0}$, first electronically excited state, $\mathrm{S}_{1}$, and the $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP of each molecule is located using DFT, TDDFT and SF-TDDFT respectively. Ab initio molecular dynamics (AIMD) is then used to determine if the MECPs are thermally accessible from the $S_{1}$ state.

A successful probe should possess an energetically accessible Conlnc, thus displaying no or low fluorescence when in an unrestricted environment. Ideally the amount of rotation required to access the $S_{1} / S_{0}$ Conlnc from the $S_{1}$ state geometry is large enough that a restricted environment will sterically hinder the rotation, therefore blocking the ConInc pathway, thus causing the molecule to fluoresce.


Figure 5.1 Molecular structure of DPH with atom numbering scheme used throughout this work.










3-Me


Figure 5.2 Molecular structures of DPH derivatives along with abbreviated names used throughout this work.

### 5.2. Computational Details

The molecules of DPH and each of its derivates shown in Figure 5.2 were studied in the gas phase. The $\mathrm{S}_{0}$ states were optimised using DFT with the BHHLYP functional and $6-31 \mathrm{G}(\mathrm{d}, \mathrm{p})$ basis set. The $\mathrm{S}_{1}$ geometries were optimised using TDDFT also using the BHHLYP functional and 6-31G(d,p) basis set. The MECP between the $S_{1}$ and $S_{0}$ states of each molecule were located using SF-TDDFT employing the NAC method, ${ }^{68}$ as outlined in Chapter 4, using the same combination of functional and basis set as the ground and excited states. In each case, the initial guess structure for the MECP optimisation was
taken from the optimised $\mathrm{S}_{1}$ geometry where atom(s) of the polyene chain which had been substituted were displaced by $0.1 \AA$ out of plane with the rest of the chain to aid convergence

AIMD simulations were performed on each molecule, using the same functional and basis set as above, in the $\mathrm{S}_{1}$ state at 298 K for 5000 steps with a timestep of 0.484 fs . Initial velocities were taken from a random sampling of a Maxwell-Boltzman distribution. A Fock matrix extrapolation scheme was used to provide a guess for the Fock matrix at the current iteration whereby the 12 previous Fock matrices are extrapolated using a sixth-order polynomial. ${ }^{121,122}$

### 5.3. Results and Discussion

The $\mathrm{S}_{0}, \mathrm{~S}_{1}$ and $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP geometries for each DPH derivative were optimised using the methods described above. The optimised MECPs for each molecule shared a common feature in that there was a distortion from planarity occurring at, or near, a site containing a methyl substituent. This was to be expected as it was in this region the starting geometries were distorted to aid convergence. In each case the Conlnc displayed a peaked topology.

The DPH derivatives must exhibit no, or low, fluorescence as an isolated molecule in an unrestricted environment to potentially act as a phase dependent membrane probe. This requirement indicates that the MECP geometry must be energetically accessible.

AIMD simulations were used to determine the accessibility of the MECP for each structure from the $S_{1}$ state by observing the critical dihedral angles, those display the most distortion from planarity in the optimised MECP geometry. The values of the critical dihedrals of the $S_{0}, S_{1}$ and MECP optimised structures, along with the average value and standard deviation obtained from the AIMD simulation for each critical dihedral of each derivative are given in Tables 5.1-5.11. In each instance the numbering scheme used for the dihedrals are taken from Figure 5.1.

|  | Dihedral angle / ${ }^{\circ}$ |  |
| :---: | :---: | :---: |
|  | C8-C9-C10-C11 | C11-C12-C13-C18 |
| $\mathrm{S}_{0}$ | 0.0 | 0.0 |
| $\mathrm{~S}_{1}$ | 0.0 | 0.0 |
| MECP | 34.3 | 18.2 |
| AIMD | $4.7 \pm 3.1$ | $5.5 \pm 23.0$ |

Table 5.1 Critical dihedral angles of DPH.

|  | Dihedral angle ${ }^{\circ}$ |  |  |
| :---: | :---: | :---: | :---: |
|  | C14-C13-C12-C11 | C1-C6-C7-C8 | C8-C9-C10-C11 |
| $\mathrm{S}_{0}$ | 0.0 | 31.3 | 0.1 |
| $\mathrm{~S}_{1}$ | 0.2 | 14.2 | 0.1 |
| MECP | 18.9 | 29.7 | 34.5 |
| AIMD | $9.7 \pm 6.9$ | $12.3 \pm 8.1$ | $4.5 \pm 3.4$ |

Table 5.2 Critical dihedral angles of 1 Me .

|  | Dihedral angle $/^{\circ}$ |  |  |
| :---: | :---: | :---: | :---: |
| C9-C10-C11-C12 | C1-C6-C7-C8 | C6-C7-C8-C9 |  |
| $\mathrm{S}_{0}$ | 0.4 | 37.0 | 1.4 |
| $\mathrm{~S}_{1}$ | 0.8 | 16.1 | 6.7 |
| MECP | 3.3 | 2.4 | 42.0 |
| AIMD | $3.6 \pm 2.6$ | $17.0 \pm 6.0$ | $6.8 \pm 4.4$ |

Table 5.3 Critical dihedral angles of $1,3 \mathrm{Me}$.

|  | C8-C9-C10-C11 | C1-C6-C7-C8 | C6-C7-C8-C9 |
| :---: | :---: | :---: | :---: |
| $\mathrm{S}_{0}$ | 0.3 | 33.0 | 0.7 |
| $\mathrm{~S}_{1}$ | 1.2 | 16.7 | 0.5 |
| MECP | 11.0 | 48.2 | 44.0 |
| AIMD | $5.5 \pm 3.9$ | $15.5 \pm 5.7$ | $5.2 \pm 4.1$ |

Table 5.4 Critical dihedral angles of $1,4 \mathrm{Me}$.

|  | Dihedral angle $/^{\circ}$ |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | C7-C8-C9-C10 | C1-C6-C7-C8 | C11-C12-C13-C14 | C10-C11-C12-C13 |
| $\mathrm{S}_{0}$ | 1.3 | 31.4 | 39.6 | 1.9 |
| $\mathrm{~S}_{1}$ | 1.9 | 17.1 | 15.2 | 6.2 |
| MECP | 1.8 | 28.8 | 48.0 | 36.6 |
| AIMD | $4.4 \pm 3.1$ | $15.5 \pm 6.0$ | $13.6 \pm 7.7$ | $5.8 \pm 3.9$ |

Table 5.5 Critical dihedral angles of $1,5 \mathrm{Me}$.

|  | Dihedral angle / ${ }^{\circ}$ |  |
| :---: | :---: | :---: |
|  | C1-C6-C7-C8 | C6-C7-C8-C9 |
| $\mathrm{S}_{0}$ | 38.2 | 2.1 |
| $\mathrm{~S}_{1}$ | 14.5 | 6.5 |
| MECP | 47.4 | 36.9 |
| AIMD | $30.0 \pm 19.5$ | $48.3 \pm 27.0$ |

Table 5.6 Critical dihedral angles of 2 Me .

|  | C8-C9-C10-C11 | Dihedral angle ${ }^{\circ}$ <br> C6-C7-C8-C9 | C1-C6-C7-C8 |
| :---: | :---: | :---: | :---: |
| $\mathrm{S}_{0}$ | 1.4 | 2.6 | 40.5 |
| $\mathrm{~S}_{1}$ | 3.8 | 12.6 | 18.9 |
| MECP | 3.8 | 37.0 | 39.8 |
| AIMD | $7.3 \pm 4.8$ | $7.8 \pm 5.7$ | $19.2 \pm 10.2$ |

Table 5.7 Critical dihedral angles of $2,3 \mathrm{Me}$.

|  | Dihedral angle $/^{\circ}$ |  |  |
| :---: | :---: | :---: | :---: |
|  | C11-C12-C13-C14 | C1-C6-C7-C8 | C6-C7-C8-C9 |
| $\mathrm{S}_{0}$ | 6.4 | 33.5 | 0.7 |
| $\mathrm{~S}_{1}$ | 0.9 | 12.3 | 5.8 |
| MECP | 13.0 | 4.9 | 45.1 |
| AIMD | $4.0 \pm 3.3$ | $15.2 \pm 4.8$ | $6.0 \pm 3.4$ |



|  | Dihedral angle $/^{\circ}$ |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | C11-C12-C13-C14 | C1-C6-C7-C8 | C6-C7-C8-C9 | C10-C11-C12-C13 |
| $\mathrm{S}_{0}$ | 35.5 | 38.3 | 2.3 | 2.3 |
| $\mathrm{~S}_{1}$ | 12.9 | 14.9 | 7.7 | 7.7 |
| MECP | 31.4 | 46.3 | 36.2 | 4.5 |
| AIMD | $14.4 \pm 8.4$ | $15.0 \pm 8.5$ | $9.6 \pm 7.1$ | $10.1 \pm 6.8$ |

Table 5.9 Critical dihedral angles of $2,5 \mathrm{Me}$.

|  | Dihedral angle $/{ }^{\circ}$ |  |
| :---: | :---: | :---: |
|  | C10-C11-C12-C13 | C8-C9-C10-C11 |
| $\mathrm{S}_{0}$ | 0.0 | 0.0 |
| $\mathrm{~S}_{1}$ | 0.1 | 1.0 |
| MECP | 8.7 | 38.0 |
| AIMD | $4.9 \pm 3.7$ | $4.5 \pm 3.2$ |

Table 5.10 Critical dihedral angles of 3 Me .

|  | Dihedral angle $/ \circ^{\circ}$ C10-C11-C12-C13 |  |  |
| :---: | :---: | :---: | :---: |
| C6-C7-C8-C9 |  |  |  |$\quad$ C7-C8-C9-C10

Table 5.11 Critical dihedral angles of 3,4Me.

The DPH probe does not possess AIE-like properties as suggested by the lack of rotation around the C8-C9-C10-C11 when in the $\mathrm{S}_{1}$ state (Table 5.1) indicating that the DPH molecule is likely to remain planar and fluoresce regardless of the environment.

The data above suggests that the $\mathbf{2 M}$ derivative of DPH could be an ideal candidate for a phase sensitive membrane probe. Although the AIMD simulations show that a few
molecules can access an MECP from the $S_{1}$ state, in most cases the $S_{1}$ and MECP dihedrals are only a few degrees different meaning that only a small rotation is needed to facilitate non-radiative decay. 2Me differs from this trend because the $\mathrm{S}_{1}$ and MECP dihedrals are well separated meaning a large rotation is needed to access this pathway. The large standard deviation of the dihedral angle around its average position in the $\mathrm{S}_{1}$ AIMD simulation shows that there is enough rotation for non-radiative decay to occur (Table 5.6). This indicates that the MECP of $\mathbf{2 M e}$ is energetically accessible in an unrestricted environment and that a sufficiently restricted environment could prevent access to a non-radiative decay pathway.

Figure 5.3 and Figure 5.4 show the 2Me PES of the lowest two singlet states as a function of the dihedrals $\mathrm{C} 1-\mathrm{C} 6-\mathrm{C} 7-\mathrm{C} 8$ and $\mathrm{C} 6-\mathrm{C} 7-\mathrm{C} 8-\mathrm{C} 9$ respectively. These were obtained by optimising the molecule with a constrained dihedral at set intervals. The constrained optimisations failed to converge when employing SF-TDDFT therefore, it was decided to optimise the structures using TDDFT to then be used in single-point SF-TDDFT energy calculation. This means the plots of Figures 5.3 and 5.4 are not relaxed PESs at the SF-TDDFT level, hence the presence of discontinuities however they still provide an approximation to the accessibility of the Conlnc. The scan for the C1-C6-C7-C8 shows a wide range of angles where the $S_{1}$ and $S_{0}$ state are close to degeneracy suggesting that the MECP can be accessed from a dihedral angle of $30-60^{\circ}$. The scan of the C6-C7-C8-C9 dihedral on the other hand displays a much narrower range of angles which are near degeneracy indicating that only when the dihedral is around $37^{\circ}$ can the MECP be accessed.

The C1-C6-C7-C8 dihedral of $\mathbf{2 M}$ 放 at $34^{\circ}$ when optimised in the $\mathrm{S}_{0}$ state (Figure 5.5 a ), while in the $S_{1}$ state the dihedral is at $14.5^{\circ}$ (Figure $5.5 b$ ) suggesting that emission is likely to occur from a near planar geometry. Whilst the emission energy increases with an increased dihedral angle (Figure 5.5c), the intensity of the emission decreases (Figure $5.5 \mathrm{~d})$.


Figure 5.3 PES of the $\mathrm{S}_{0}$ (blue) and $\mathrm{S}_{1}$ (orange) states of $\mathbf{2 M e}$ as a function of the C1-C6-C7-C8 dihedral obtained using SF-TDDFT.


Figure 5.4 PES of the $\mathrm{S}_{0}$ (blue) and $\mathrm{S}_{1}$ (orange) states of $\mathbf{2 M e}$ as a function of the C6-C7-C8-C9 dihedral obtained using SF-TDDFT.

When optimised in the $\mathrm{S}_{0}$ state, the C6-C7-C8-C9 dihedral of 2Me is near planar (Figure 5.6a). It can be seen from Figure 5.6b that emission from the $S_{1}$ state would preferentially occur when the C6-C7-C8-C9 is around $10-20^{\circ}$. Figure 5.6 c and d show that the emission is at its maximum energy and intensity when the dihedral is around $0^{\circ}$ and decreases as the dihedral angle increases.


Figure 5.5 Calculated parameters of $\mathbf{2 M e}$ as a function of the $\mathrm{C} 1-\mathrm{C} 6-\mathrm{C} 7-\mathrm{C} 8$ dihedral. a) PES of the $\mathrm{S}_{0}$ state obtained using DFT, b) PES of the $\mathrm{S}_{1}$ state obtained using TDDFT, c) emission energy obtained using TDDFT and d) Oscillator strength obtained using TDDFT.


Figure 5.6 Calculated parameters of $\mathbf{2 M e}$ as a function of the $\mathrm{C} 6-\mathrm{C} 7-\mathrm{C} 8-\mathrm{C} 9$ dihedral. a) PES of the $\mathrm{S}_{0}$ state obtained using DFT, b) PES of the $\mathrm{S}_{1}$ state obtained using TDDFT, c) emission energy obtained using TDDFT and d) Oscillator strength obtained using TDDFT.

### 5.4. Conclusion

This work used gas phase calculations as a starting point to determine whether a molecule could be used as a phase sensitive, fluorescent probe for lipid membranes. The molecule considered in this work were all derivatives of DPH which had methyl groups substituted along the polyene chain. It was determined from AIMD simulations that the 2-methyl derivative ( $\mathbf{2 M e}$ ) shows the most promise of being a phase sensitive probe due to it possessing an energetically accessibly MECP which requires a large rotation around a dihedral to be reached.

Energetic analysis of the PESs as a function of these dihedrals indicate that emission of 2Me would preferentially occur from a near planar geometry and that increasing these dihedrals leads to a shift in energy with a decrease in intensity. Applied to a membrane environment it would be expected that $\mathbf{2 M e}$ would be fluorescent when restricted to a planar geometry in a raft like environment and be non-fluorescent, or at least decrease in intensity, when allowed to rotate in the non-raft environment.

## Chapter 6. AIE Molecules in Membranes

The preceding chapter used gas phase calculations on a selection of molecules, derived from the DPH fluorescent membrane probe, to measure metrics which could be used to determine their functionality as a phase sensitive probe in lipid bilayers. Of the molecules considered, shown in Figure 5.2, the 2Me derivative of DPH displayed the most promise of being a successful phase sensitive probe as it possesses a dihedral angle that can freely rotate, therefore accessing a non-radiative decay pathway, in an unrestricted environment. This chapter uses molecular dynamics (MD) and hybrid QM/MM to analyse each molecules behaviour in non-raft and raft like membrane environments.

The results of this work were published alongside the work of Chapter 5. ${ }^{109}$

### 6.1. Introduction

Within a lipid membrane it is possible for a high concentration of cholesterol and sphingolipids to assemble forming a region of liquid order ( $\mathrm{L}_{0}$ ) known as a lipid raft. These regions have a short lifetime ${ }^{4}$ and display an increased thickness compared to the rest of the bilayer, ${ }^{6,7}$ and can form in sizes ranging from 10-200 nm in diameter. ${ }^{5}$ The presence of a lipid raft can also be characterised by a low tilt angle of cholesterol. ${ }^{123}$ Membrane rafts are thought to play a role in cell signalling events and membrane trafficking, however, their function in living cells are difficult to observe. ${ }^{9}$

Recently the Matile group have developed the use of mechanosensitive probes termed 'flippers' based on a dithienothiophene structure (Figure 6.1), sometimes attached to a polar head group to aid its positioning within a bilayer. ${ }^{124-134}$ They found that rotation around a dihedral angle linking the thiophene moieties is responsible for a conformational change between regions of $L_{o}$ and $L_{d}$, thereby altering the emission spectra. ${ }^{126} \mathrm{Kim}$ et al. have developed a two-photon probe that displays a higher intensity fluorescence in raft regions compared to non-raft regions. ${ }^{135}$ More recently, Yue et al. have developed a large fluorescent probe, also with AIE characteristics, to detect lipid rafts. ${ }^{136}$ Their probe is built
upon a central tetraphenylethylene unit, a typical AIEgen, attached to cholesterol and triethylene glycol to anchor the molecule into the cell membrane. They found that a shift in fluorescent wavelength was observed upon the lipid membrane phase changes.


Figure 6.1 Dithienothiophene moiety investigated by Matile et al. ${ }^{126}$ Numbered atoms are those involved in the key dihedral rotation.

This chapter builds upon the work performed in Chapter 5 by monitoring the rotation around key dihedral angles to determine whether the probes are phase sensitive by performing $\mathrm{QM} / \mathrm{MM}$ simulations in raft and non-raft environments. It was found that in an unrestricted (gas phase) environment, performed using AIMD, that the 2Me derivative of DPH possessed thermally accessible Conlncs with freely rotating dihedrals. Therefore, it is hoped that a non-raft environment will still allow enough rotational freedom for radiationless decay pathways to be accessed whereas a raft environment will be too restrictive thereby forcing the molecule to fluoresce.

### 6.2. Computational Details

### 6.2.1. Classical Dynamics

As described in Chapter 2, force-fields are required to perform molecular dynamics simulations. Whilst there are force-fields readily available for the molecules which make up the lipid bilayer, parameters for each DPH derivative need to be generated. This is achieved using CHARMM General Force-Field (CGENFF) ${ }^{137,138}$ in which a penalty is
assigned to each parameter, any parameter with a score higher than 10 needs to be optimised further to produce a better-quality parameter. It was found that the parameters with scores higher than 10 were mostly dihedral terms. A force-matching algorithm developed by Claridge et al. ${ }^{139}$ was used to reoptimise these terms by minimising an objective function. For a set of parameters, $[p]$, with penalty scores larger than 10 , the objective function is given as

$$
\begin{equation*}
O([p])=\sqrt{\frac{1}{3 M N} \sum_{i=1}^{M} \sum_{j=1}^{N}\left|\mathbf{f}_{i j}^{D F T}-\mathbf{f}_{i j}^{M D}\right|^{2}} \tag{6.1}
\end{equation*}
$$

Where $N$ is the number of atoms in the molecule, $M$ is the number of reference structures (for this work 15 reference structures were sampled, taken from equal timesteps of the AIMD simulations used in Chapter 5), and $\mathbf{f}_{i j}$ are the forces of atom $i$ of structure $j$ from DFT or MD.

The descent direction for each parameter in set $[p]$ is numerically obtained from the gradient of the objective function

$$
\begin{equation*}
G_{i} \approx \frac{O\left(p_{1}, \ldots, p_{i}+\Delta p_{i}, \ldots, p_{n}\right)-O\left(p_{1}, \ldots, p_{i}, \ldots, p_{n}\right)}{\Delta p_{i}} \tag{6.2}
\end{equation*}
$$

Each parameter in set $[p]$ is then changed using the negative of the gradient

$$
\begin{equation*}
p_{\mathrm{i}}^{\text {new }}=p_{\mathrm{i}}-\alpha G_{i} \tag{6.3}
\end{equation*}
$$

where $\alpha$ is a scaling parameter used to determine the step size of the descent.

The optimised force-fields for the DPH derivative probes, provided in Appendix B, were used in conjunction with those needed to create the lipid membrane environment and equilibrated using molecular dynamics. The CHARMM-GUI web service ${ }^{140}$ was used alongside the CGENFF to create a lipid bilayer membrane with a single probe molecule inserted into it. The bilayer was comprised of 200 lipids per leaflet; for the non-raft system, there was a total of 400 SSM (d18:1/18:0), whereas the raft-like system contained 30 molar\% of cholesterol, consistent with known $L_{o}$ membrane phases, resulting in a total of 280 SSM (d18:1/18:0) and 120 cholesterol molecules equally
distributed between the leaflets. Water molecules were added above each leaflet to a minimum thickness of 22.5 Å generating a total of 12500-12900 water molecules for the two systems. Potassium and chloride ions were added at a concentration of 0.15 M .

Once the systems were constructed, the CHARMM-GUI Membrane Builder ${ }^{141-144}$ was employed to minimise the system for 10000 timesteps for a total of 1875 ps over six steps which gradually removed force constraints initially applied to prevent the water molecules from entering the hydrophobic section of the membrane, Table 6.1.

| Selection | Step 1 | Step 2 | Step 3 | Step 4 | Step 5 | Step 6 |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| Carbon atoms of DPH derivative | 10.0 | 5.0 | 2.5 | 1.0 | 0.5 | 0.1 |
| Water | 2.5 | 2.5 | 1.0 | 0.5 | 0.1 | 0.0 |
| Lipid tails | 2.5 | 2.5 | 1.0 | 0.5 | 0.1 | 0.0 |
| Lipid heads | 2.5 | 2.5 | 1.0 | 0.5 | 0.1 | 0.0 |
| lons | 10 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 |

Table 6.1 Force constants (kcal mol${ }^{-1} \AA^{-2}$ ) applied at each equilibration step.

After the Membrane Builder procedure was complete, three sets of production dynamics were carried out at different temperatures ( $310 \mathrm{~K}, 320 \mathrm{~K}$ and 330 K ) for 100 ns , with a 2 fs timestep, to produce a fully equilibrated system. The temperature was set using the NPT ensemble. Constant pressure was maintained using the Nosé-Hoover Langevin-piston algorithm ${ }^{145,146}$ whilst constant temperature was achieved through Langevin dynamics. Long-range electrostatics were described using the Particle Mesh Ewald method, ${ }^{147}$ using $6{ }^{\text {th }}$-order spline interpolation. Lennard-Jones interactions were used to describe van der Waals' interactions, with a force-switching function acting in the range of 10-12 Å. ${ }^{148}$ The CHARMM36 lipid force-field ${ }^{149-151}$ and TIP3P water model ${ }^{152,153}$ were used.

Electron density profiles were constructed for each equilibrated system, at each temperature, by calculating the average electron density over the MD trajectory using a slab thickness of $0.8 \AA$. Each system was repositioned so that the centre of the bilayer was at $Z=0 \AA$.

Deuterium order parameters were calculated using

$$
\begin{equation*}
-\mathrm{S}_{\mathrm{CD}}=\frac{1}{2}\left\langle 3 \operatorname{Cos}^{2} \theta-1\right\rangle \tag{6.4}
\end{equation*}
$$

where $\theta$ is the angle between a given $\mathrm{C}-\mathrm{H}$ vector and the membrane normal.

### 6.2.2. QM/MM Simulations

The fully equilibrated system from the classical MD simulations were used as the start point for the QM/MM simulation. The DPH derivative was treated at the QM level, in its $\mathrm{S}_{0}$ and $S_{1}$ state, whilst the rest of the system was treated at the MM level. The simulations were repeated 5 times, each consisting of 16000 steps with a 2 fs timestep. At the QM level, the BHHLYP functional was employed with the $6-31 \mathrm{G}(\mathrm{d}, \mathrm{p})$ basis set for consistency with the gas phase calculations produced in Chapter 5.

The oscillator strengths are calculated using

$$
\begin{equation*}
\left.f=\frac{2}{3}\left(E_{S_{1}}-E_{S_{0}}\right)\left|\left\langle\Psi_{S_{1}}\right| \mu\right| \Psi_{S_{0}}\right\rangle\left.\right|^{2} \tag{6.5}
\end{equation*}
$$

where $\mu$ is the transition dipole moment for the transition from the $S_{0}$ to the $S_{1}$ electronic state.

The NAMD software suite ${ }^{154,155}$ was used for both classical and QM/MM sets of MD simulations. The QM level of treatment was performed using Q-Chem. ${ }^{97}$ The electron density profiles and deuterium order parameters were produced using CHARMM. ${ }^{156}$

### 6.3. Results and Discussion

### 6.3.1. Classical Molecular Dynamics

Electron density profiles were constructed of the membrane and probe molecule in each simulation to determine the thickness of the membrane, by measuring the distance between the peaks in Figures 6.2 - 6.5, and the position of the probe in each environment. At each temperature the thickness of the bilayer was larger in the raft environment compared to the non-raft environment, Table 6.2. ${ }^{157}$

| Temperature / <br> K | Non-raft bilayer <br> thickness / $\AA$ | Raft bilayer <br> thickness / $\AA$ | Non-raft bilayer <br> thickness / $\AA$ | Raft bilayer <br> thickness / $\AA$ |
| :---: | :---: | :---: | :---: | :---: |
| 320 | 41.6 | 46.4 | 41.6 | 46.4 |
| 330 | 42.4 | 44.8 | 41.6 | 45.6 |

Table 6.2 Calculated bilayer thicknesses of each simulation.


Figure 6.2 Calculated electron density profile from the MD simulation of the non-raft environment containing the DPH probe.


Figure 6.3 Calculated electron density profile from the MD simulation of the raft environment containing the DPH probe.


Figure 6.4 Calculated electron density profile from the MD simulation of the non-raft environment containing the $\mathbf{2 M e}$ probe.


Figure 6.5 Calculated electron density profile from the MD simulation of the raft environment containing the $\mathbf{2 M e}$ probe.

The electron density profile of the probes are shown in Figures 6.6-6.9. In most cases the $\mathbf{2 M e}$ probe is located in the upper leaflet of the bilayer, the only exception is the raft simulation at 330 K where the molecule crosses to the lower leaflet in the early steps of the simulation, remaining there throughout. On the other hand, DPH resides in the lower leaflet for each simulation expect for the non-raft simulation at 330 K where the molecule remains in the upper leaflet throughout.


Figure 6.6 Calculated electron density profile of $\mathbf{2 M e}$ from the MD simulation of the raft environment.


Figure 6.7 Calculated electron density profile of $\mathbf{2 M e}$ from the MD simulation of the non-raft environment.


Figure 6.8 Calculated electron density profile of DPH from the MD simulation of the raft environment.


Figure 6.9 Calculated electron density profile of DPH from the MD simulation of the non-raft environment.

Deuterium order parameters for the C-H bonds in both environments at each temperature are shown in Figures 6.10 - 6.13. At 330 K , the average order parameter is 0.30 when in the raft environment and 0.22 in the non-raft environment. These results are in good agreement with Wang et al. ${ }^{158}$ and Sodt et al. ${ }^{159}$ who have previously studied $L_{o}$ phases of lipid bilayers using MD.


Figure 6.10 Calculated deuterium order parameter for the sphingosine tail of SSM from the MD simulation at 320 K .


Figure 6.11 Calculated deuterium order parameter for the stearoyl tail of SSM from the MD simulation at 320 K .


Figure 6.12 Calculated deuterium order parameter for the sphingosine tail of SSM from the MD simulation at 330 K .


Figure 6.13 Calculated deuterium order parameter for the stearoyl tail of SSM from the MD simulation at 330 K .

These results confirm that the membrane raft simulations are indeed in the $L_{o}$ phase and that the non-raft simulations are in fact in the $L_{d}$ phase before being used in $Q M / M M$ calculations.

### 6.3.2. QM/MM Simulations

The average dihedral angles of the probes, in the $\mathrm{S}_{1}$ state, in a raft-like environment at 310 K are given in Tables 6.3-6.13.

|  | Dihedral angle $/{ }^{\circ}$ |  |
| :---: | :---: | :---: |
|  | C8-C9-C10-C11 | C11-C12-C13-C18 |
| $\mathrm{S}_{0}$ | 0.0 | 0.0 |
| $\mathrm{~S}_{1}$ | 0.0 | 0.0 |
| MECP | 34.3 | 18.2 |
| $\mathrm{~S}_{1}$ Raft | $6.2 \pm 4.7$ | $8.7 \pm 6.6$ |

Table 6.3 Critical dihedral angles of DPH. $\mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $\mathrm{S}_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

|  | ${\text { Dihedral angle } /^{\circ}}$ |  |  |
| :---: | :---: | :---: | :---: |
| C14-C13-C12-C11 | C1-C6-C7-C8 | C8-C9-C10-C11 |  |
| $\mathrm{S}_{0}$ | 0.0 | 31.3 | 0.1 |
| $\mathrm{~S}_{1}$ | 0.2 | 14.2 | 0.1 |
| MECP | 18.9 | 29.7 | 34.5 |
| $\mathrm{~S}_{1}$ Raft | $36.2 \pm 26.9$ | $26.0 \pm 8.2$ | $8.3 \pm 5.7$ |

Table 6.4 Critical dihedral angles of $1 \mathrm{Me} . \mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $S_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

|  | Dihedral angle $/ \circ^{\circ}$ |  |  |
| :---: | :---: | :---: | :---: |
|  | C9-C10-C11-C12 | C1-C6-C7-C8 |  |$\quad$ C6-C7-C8-C9

Table 6.5 Critical dihedral angles of $1,3 \mathrm{Me}$. $\mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $S_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

|  | Dihedral angle $/ \circ^{\circ}$ |  |  |
| :---: | :---: | :---: | :---: |
|  | C8-C9-C10-C11 | C1-C6-C7-C8 |  |$\quad$ C6-C7-C8-C9

Table 6.6 Critical dihedral angles of $\mathbf{1 , 4 M e}$. $\mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $\mathrm{S}_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

|  | Dihedral angle / ${ }^{\circ}$ |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | C7-C8-C9-C10 | C1-C6-C7-C8 | C11-C12-C13-C14 | C10-C11-C12-C13 |
| $\mathrm{S}_{0}$ | 1.3 | 31.4 | 39.6 | 1.9 |
| $\mathrm{~S}_{1}$ | 1.9 | 17.1 | 15.2 | 6.2 |
| MECP | 1.8 | 28.8 | 48.0 | 36.6 |
| $\mathrm{~S}_{1}$ Raft | $28.7 \pm 10.8$ | $46.1 \pm 12.1$ | $40.6 \pm 13.4$ | $6.8 \pm 4.7$ |

Table 6.7 Critical dihedral angles of $1,5 \mathrm{Me}$. $\mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $S_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

|  | Dihedral angle ${ }^{\circ}$ |  |
| :---: | :---: | :---: |
|  | C1-C6-C7-C8 | C6-C7-C8-C9 |
| $\mathrm{S}_{0}$ | 38.2 | 2.1 |
| $\mathrm{~S}_{1}$ | 14.5 | 6.5 |
| MECP | 47.4 | 36.9 |
| $\mathrm{~S}_{1}$ Raft | $18.4 \pm 10.2$ | $9.0 \pm 6.9$ |

Table 6.8 Critical dihedral angles of $\mathbf{2 M e} . \mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $S_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

|  | ${\text { Dihedral angle } /^{\circ}}$ |  |  |
| :---: | :---: | :---: | :---: |
| C8-C9-C10-C11 | C6-C7-C8-C9 |  |  |$\quad$ C1-C6-C7-C8

Table 6.9 Critical dihedral angles of $2,3 \mathrm{Me} . \mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $\mathrm{S}_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

|  | ${\text { Dihedral angle } /^{\circ}}$ |  |  |
| :---: | :---: | :---: | :---: |
| C11-C12-C13-C14 | C1-C6-C7-C8 | C6-C7-C8-C9 |  |
| $\mathrm{S}_{0}$ | 6.4 | 33.5 | 0.7 |
| $\mathrm{~S}_{1}$ | 0.9 | 12.3 | 5.8 |
| MECP | 13.0 | 4.9 | 45.1 |
| $\mathrm{~S}_{1}$ Raft | $28.2 \pm 11.0$ | $61.3 \pm 13.8$ | $8.6 \pm 5.4$ |

Table 6.10 Critical dihedral angles of $\mathbf{2 , 4 M e} . \mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $S_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

|  | Dihedral angle/゚ |  |  |  |
| :---: | :---: | :---: | :---: | :---: |
|  | C11-C12-C13-C14 | C1-C6-C7-C8 | C6-C7-C8-C9 | C10-C11-C12-C13 |
| $\mathrm{S}_{0}$ | 35.5 | 38.3 | 2.3 | 2.3 |
| $\mathrm{~S}_{1}$ | 12.9 | 14.9 | 7.7 | 7.7 |
| MECP | 31.4 | 46.3 | 36.2 | 4.5 |
| $\mathrm{~S}_{1}$ Raft | $30.9 \pm 17.5$ | $14.2 \pm 8.5$ | $12.5 \pm 7.6$ | $9.4 \pm 6.5$ |

Table 6.11 Critical dihedral angles of $2,5 \mathrm{Me}$. $\mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $\mathrm{S}_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

|  | Dihedral angle $/{ }^{\circ}$ |  |
| :---: | :---: | :---: |
|  | C10-C11-C12-C13 | C8-C9-C10-C11 |
| $\mathrm{S}_{0}$ | 0.0 | 0.0 |
| $\mathrm{~S}_{1}$ | 0.1 | 1.0 |
| MECP | 8.7 | 38.0 |
| $\mathrm{~S}_{1}$ Raft | $6.9 \pm 5.1$ | $27.9 \pm 10.6$ |

Table 6.12 Critical dihedral angles of $3 \mathrm{Me} . \mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $\mathrm{S}_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

|  | ${\text { Dihedral angle } /^{\circ}}$ |  |  |
| :---: | :---: | :---: | :---: |
|  | C10-C11-C12-C13 | C6-C7-C8-C9 |  |$\quad$ C7-C8-C9-C10

Table 6.13 Critical dihedral angles of $\mathbf{3 , 4 \mathrm { Me }} \mathrm{S}_{0}, \mathrm{~S}_{1}$ and MECP values are from optimised geometries in the gas phase whereas $S_{1}$ raft values are the average dihedrals, with standard variation, taken from a QM/MM simulation at 310 K .

The equilibrated systems from the production dynamics were viewed using VMD. ${ }^{160}$ It was found that at 310 K both raft and non-raft environments displayed a gel phase membrane, this is likely due to SSM having a melting temperature around $314 \mathrm{~K} .{ }^{110,161}$ To obtain the correct phase behaviour the simulations were repeated at 320 K and 330 K . To save on computational effort this was only done for DPH and 2Me. Although other molecule's critical dihedrals are restricted enough in the gel phase to block access to the nonradiative pathway, such as the C9-C10-C11-C12 dihedral of $\mathbf{1 , 3 M e}$ and the C7-C8-C9-C10 dihedral of $\mathbf{1 , 5 M e}$, Table 6.5 and Table 6.7 respectively, there is not much freedom of rotation around those angles in the AIMD simulations, compared to those of 2Me (Chapter 5). Results herein only refer to those collected from the simulations performed at 320 K and 330 K .

### 6.3.3. 2Me as a Probe

In a raft, low tilt angles of the lipid components should be observed. Table 6.14 provides the tilt angle of the $\mathbf{2 M}$ e probe, both of the SSM tails and cholesterol from the QM/MM simulation performed at 330 K . The low tilt angle, and low standard deviation, of cholesterol are characteristic of a raft membrane ${ }^{123}$ also indicating that the simulation is in the correct $L_{0}$ phase.

| Component | Tilt angle $/{ }^{\circ}$ |
| :---: | :---: |
| 2Me | $9.1 \pm 3.3$ |
| SSM d18:1 | $17.5 \pm 0.3$ |
| SSM d18:0 | $19.4 \pm 0.3$ |
| Cholesterol | $17.2 \pm 0.4$ |

Table 6.14 Tilt angles of the $\mathbf{2 M e}$ probe, cholesterol and both tails of SSM from the 330 K QM/MM raft simulation. Angles were estimated from the C6-C13 vector of $\mathbf{2 M e}, \mathrm{C} 3-\mathrm{C} 17$ vector of cholesterol and the C5-C13 vectors of both SSM tails against the membrane normal. See Figure 5.1 and Figure 6.14 for atom numbering.



Figure 6.14 Atom numbering used to calculate tilt angles for (top) SSM and (bottom) cholesterol.

In the non-raft region, at 330 K , the $\mathbf{2 M e}$ probe displays a tilt angle of $13.1 \pm 5.1^{\circ}$. Assuming that the $\mathbf{2 M e}$ probe possesses the same anisotropic features as the parent DPH molecule, then it can be suggested that the fluorescent anisotropy would increase in $\mathrm{L}_{d}$ regions should the probe still be fluorescent.

Table 6.15 - Table 6.18 provide the average key dihedral angles of $\mathbf{2 M e}$ and DPH from each QM/MM environment.

| C1-C6-C7-C8 dihedral / $\circ$ |  | Temperature / K |  |
| :---: | :---: | :---: | :---: |
| Probe electronic state | Membrane environment | 320 | 330 |
| S $_{0}$ | Non-raft | $40.0 \pm 13.2$ | $44.4 \pm 14.6$ |
| $S_{1}$ | Non-raft | $20.3 \pm 11.9$ | $25.6 \pm 10.6$ |
| $S_{0}$ | Raft | $49.5 \pm 13.8$ | $46.7 \pm 15.8$ |
| $S_{1}$ | Raft | $16.9 \pm 10.5$ | $16.3 \pm 10.3$ |

Table 6.15 Average dihedral angle for the C1-C6-C7-C8 dihedral of $\mathbf{2 M e}$ from the QM/MM simulations.

| C6-C7-C8-C9 dihedral/ $\circ$ |  | Temperature / K |  |
| :---: | :---: | :---: | :---: |
| Probe electronic state | Membrane environment | 320 | 330 |
| $\mathrm{~S}_{0}$ | Non-raft | $5.6 \pm 4.2$ | $5.7 \pm 4.4$ |
| $\mathrm{~S}_{1}$ | Non-raft | $8.0 \pm 5.9$ | $8.1 \pm 5.9$ |
| $\mathrm{~S}_{0}$ | Raft | $6.1 \pm 4.4$ | $6.4 \pm 4.8$ |
| $\mathrm{~S}_{1}$ | Raft | $9.0 \pm 6.6$ | $9.4 \pm 6.8$ |

Table 6.16 Average dihedral angle for the C6-C7-C8-C9 dihedral of $\mathbf{2 M e}$ from the QM/MM simulations.

| C8-C9-C10-C11 dihedral/ ${ }^{\circ}$ |  | Temperature / K |  |
| :---: | :---: | :---: | :---: |
| Probe electronic state | Membrane environment | 320 | 330 |
| $\mathrm{~S}_{0}$ | Non-raft | $5.8 \pm 4.5$ | $5.8 \pm 4.4$ |
| $\mathrm{~S}_{1}$ | Non-raft | $7.1 \pm 5.3$ | $7.1 \pm 5.4$ |
| $\mathrm{~S}_{0}$ | Raft | $6.3 \pm 4.7$ | $6.9 \pm 5.0$ |
| $\mathrm{~S}_{1}$ | Raft | $7.3 \pm 5.5$ | $7.7 \pm 5.7$ |

Table 6.17 Average dihedral angle for the C8-C9-C10-C11 dihedral of DPH from the QM/MM simulations.

| C11-C12-C13-C18 dihedral / ${ }^{\circ}$ |  | Temperature / K |  |
| :---: | :---: | :---: | :---: |
| Probe electronic state | Membrane environment | 320 | 330 |
| $\mathrm{~S}_{0}$ | Non-raft | $16.8 \pm 11.9$ | $45.3 \pm 58.1$ |
| $\mathrm{~S}_{1}$ | Non-raft | $10.2 \pm 7.5$ | $10.3 \pm 7.8$ |
| $\mathrm{~S}_{0}$ | Raft | $14.9 \pm 10.6$ | $16.2 \pm 11.3$ |
| $\mathrm{~S}_{1}$ | Raft | $10.0 \pm 7.3$ | $10.0 \pm 7.4$ |

Table 6.18 Average dihedral angle for the C11-C12-C13-C18 dihedral of DPH from the QM/MM simulations.

In the case of DPH, it can be seen from Table 6.17 and Table 6.18 that there is no phase selectivity. The C8-C9-C10-C11 is fairly restricted in its freedom of rotation in each environment in both states, at no point does it rotate to a degree close enough to access the MECP located at $34.3^{\circ}$, indicating that it remains fluorescent at all times.

The C11-C12-C13-C18 dihedral of DPH is identical in the non-raft and raft environments when in the $S_{1}$ state again indicating that its emission is not phase dependent. The absence of membrane phase selectivity with regards to emission is consistent with experimental findings. ${ }^{112,113}$

Just like the DPH dihedrals, the C6-C7-C8-C9 dihedral of 2Me also does not display phase sensitivity as the angles do not approach that required to access the MECP in either environment.

On the other hand, the C1-C6-C7-C8 dihedral does possess the desired properties of a phase sensitive fluorescent probe. In the non-raft environment the dihedrals are within the region required to access the non-radiative pathway, shown in Figure 5.3, when in the $S_{1}$ state. Along with the data shown in Figure 5.5d, it can be assumed that there would be no fluorescence, or at least a decrease in intensity, when excited to the $S_{1}$ state in a non-raft environment. However, when $\mathbf{2 M e}$ is in a membrane raft environment, the average $\mathrm{C} 1-\mathrm{C} 6-\mathrm{C} 7-\mathrm{C} 8$ dihedral is around $16^{\circ}$, at both temperatures. This is below the required rotation needed to access the MECP suggesting that the molecule will remain fluorescent in the $L_{o}$ phase.

### 6.4. Conclusions

This chapter has looked at the potential use of DPH derivatives as phase sensitive probes in a membrane environment using $\mathrm{QM} / \mathrm{MM}$ simulations. A membrane environment was created using SSM to mimic the $L_{d}$ phase of a non-raft region whilst an SSM/cholesterol mix was used to create a $L_{o}$ raft like environment. It was found that the 2-methyl derivative ( $\mathbf{2 M e}$ ) of DPH is likely to display no, or low intensity, fluorescence in a non-raft environment but would remain fluorescent in a raft environment, the key characteristics of a phase sensitive fluorescent membrane probe.

## Chapter 7. Conclusions and Future Work

The aim of this research project was to computationally design a fluorescent probe able to report on phase changes within a lipid membrane thereby identifying the presence of lipid rafts.

Our attention was first focused on the use of POMs as a means of transporting the probe to the membrane bilayer. However, the progress of experimental work prevented this being investigated. The research did demonstrate that the use of mixed metal addenda with organofunctionalisation provides a tuneable approach to lowering the POMs HOMO-LUMO gap, thus allowing them to be used for visible light activated photooxidation catalysis.

This research also provided a comprehensive comparison of SF-TDDFT approaches with XMS-CASPT2, finding that inclusion of nonadiabatic coupling with the BHHLYP functional provided reasonable geometric and energetic results when identifying MECPs between the $S_{1}$ and $S_{0}$ electronic states.

For the design of the molecular probe, the commonly used DPH fluorescent probe was used as a parent molecule, creating derivatives by substituting methyl groups along the hexatriene chain to try and induce a RIM mechanism to be exploited in making the molecule sensitive to membrane phase changes. This work showed that the $\mathbf{2 M e}$ derivative possesses a ConInc which is energetically accessible in the gas phase, even though it requires a large rotation away from the $\mathrm{S}_{1}$ geometry. Through $\mathrm{QM} / \mathrm{MM}$ studies, it was found that the ConInc could still be accessed in a $L_{d}$ non-raft environment, indicating that the molecule would display no, or at least low, fluorescence. However, in a $\mathrm{L}_{\mathrm{o}}$ raft environment the rotations are prohibited thereby blocking access to the Conlnc, indicating that the molecule would fluoresce. These properties demonstrate the potential advantages of simple substitutions on a molecule's fluorescent properties.

The work conducted in this study was solely computational, although we are now collaborating with an organic synthesis group to synthesise $\mathbf{2 M e}$ in the hope that the experimental work will validate our results.

The membrane environments used in this project only contained SSM for the non-raft membrane and an SSM/cholesterol mix for the raft-like membrane. Whilst this provided the desired phase environments to test the RIM mechanism utilised in this study, living cell membranes contain a variety of other lipids and proteins which can also diffuse across the membrane. Future studies may include these factors to study the interactions they may have with the probe and whether they alter the molecules fluorescent characteristics.

It may also be desirable to modify the probe molecule further by adding groups onto the terminal rings to either alter the fluorescent emission of the probe or to add a polar group to anchor the molecule to the bilayer.

The force-fields used in this work were created using CGENFF and refined using a force-matching algorithm. Whilst that approach was suitable for this work, it has been found that the Force-Field Toolkit (ffTK), implemented in VMD, ${ }^{160}$ can create a more specific force-field for individual molecules. ${ }^{126,162}$ Therefore, in future studies of this nature it would be desirable to compare the force-fields to see if ffTk offers any improvement.

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## Appendix A

The following figures are superimposed images of the molecules studied in Chapter 4 to show the similarities and differences of the structures obtained using various approaches.


Figure A1 Overlap of the fulvene $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP structures obtained using (red) XMS-CASPT2 and (blue) NAC-BHHLYP.


Figure A2 Overlap of the 4ABN $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP structures obtained using (red) XMS-CASPT2, (blue) BP- $\omega$ B97X and (green) NAC- $\omega$ B97X.


Figure A3 Overlap of the 5FC $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP structures obtained using (red) XMS-CASPT2, (green) CASSCF and (blue) NAC-BHHLYP.


Figure A4 Overlap of the 9 H -adenine $\pi \pi^{*} / \mathrm{S}_{0}$ MECP structures obtained using (red) XMS-CASPT2 and (blue) NAC-BHHLYP.


Figure A5 Overlap of the 9 H -adenine $n \pi^{*} / \mathrm{S}_{0}$ MECP structures obtained using (red) XMS-CASPT2 and (blue) BP-BHHLYP.


Figure A6 Overlap of the 2,4,6-octatriene $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP structures obtained using (red) XMS-CASPT2 and (blue) PC-wB97X.


Figure A7 Overlap of the azomethane $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP structures obtained using (red) XMS-CASPT2 and (blue) NAC-BHHLYP.


Figure A8 Overlap of the azoxymethane $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP structures obtained using (red) XMS-CASPT2 and (blue) PC-BHHLYP.


Figure A9 Overlap of the phenol $\mathrm{S}_{1} / \mathrm{S}_{0}$ MECP structures obtained using (red) XMS-CASPT2 and (blue) BP-BHHLYP.


Figure A10 Overlap of the SMAC $\mathrm{S}_{1} / \mathrm{S}_{0}$ ESIPT MECP structures obtained using (red) XMS-CASPT2, (blue) NAC-BHHLYP and (green) BP-wB97X.


Figure A11 Overlap of the SMAC $\mathrm{S}_{1} / \mathrm{S}_{0}$ TWin1 MECP structures obtained using (red) XMS-CASPT2 and (blue) NAC-BHHLYP.


Figure A12 Overlap of the SMAC $\mathrm{S}_{1} / \mathrm{S}_{0}$ TWin1 MECP structures obtained using (red) XMS-CASPT2 and (blue) NAC-BHHLYP.


Figure A13 Overlap of the SMAC $\mathrm{S}_{1} / \mathrm{S}_{0}$ TWout1 MECP structures obtained using (red) XMS-CASPT2, (blue) NAC-BHHLYP and (green) BP- $\omega$ B97X.


Figure A14 Overlap of the SMAC $\mathrm{S}_{1} / \mathrm{S}_{0}$ TWout1 MECP structures obtained using (red) XMS-CASPT2, (blue) NAC-wB97X and (green) PC-BHHLYP.

## Appendix B

Force-field parameters used for the DPH derivative probe molecules. In each case the force-fields were generated by CGENFF and modified using the procedure outlined in Section 6.2.

## 1,3Me

| RESI AIE |  | 0.000 |
| :---: | :---: | :---: |
| GROUP | ! | CHARGE |
| ATOM C1 | CG2R61 | -0.115 |
| ATOM C2 | CG2R61 | -0.115 |
| ATOM C3 | CG2R61 | -0.115 |
| ATOM C4 | CG2R61 | -0.115 |
| ATOM C5 | CG2R61 | -0.004 |
| ATOM C6 | CG2R61 | -0.115 |
| ATOM H1 | HGR61 | 0.115 |
| ATOM H2 | HGR61 | 0.115 |
| ATOM H3 | HGR61 | 0.115 |
| ATOM H4 | HGR61 | 0.115 |
| ATOM H5 | HGR61 | 0.115 |
| ATOM C7 | CG2DC1 | 0.002 |
| ATOM C8 | CG2DC1 | -0.140 |
| ATOM C9 | CG2DC2 | -0.006 |
| ATOM C10 | CG2DC2 | -0.164 |
| ATOM C11 | CG2DC1 | -0.131 |
| ATOM C12 | CG2DC1 | -0.150 |
| ATOM C13 | CG331 | -0.270 |
| ATOM C14 | CG331 | -0.270 |
| ATOM H6 | HGA4 | 0.150 |
| ATOM H7 | HGA4 | 0.150 |
| ATOM C15 | CG2R61 | -0.007 |
| ATOM H8 | HGA4 | 0.150 |
| ATOM C16 | CG2R61 | -0.115 |
| ATOM C17 | CG2R61 | -0.115 |
| ATOM C18 | CG2R61 | -0.115 |
| ATOM C19 | CG2R61 | -0.115 |
| ATOM C20 | CG2R61 | -0.115 |
| ATOM H9 | HGR61 | 0.115 |
| ATOM H10 | HGR61 | 0.115 |
| ATOM H11 | HGR61 | 0.115 |
| ATOM H12 | HGR61 | 0.115 |
| ATOM H13 | HGR61 | 0.115 |
| ATOM H14 | HGA3 | 0.090 |
| ATOM H15 | HGA3 | 0.090 |
| ATOM H16 | HGA3 | 0.090 |
| ATOM H17 | HGA3 | 0.090 |
| ATOM H18 | HGA3 | 0.090 |
| ATOM H19 | HGA3 | 0.090 |
| ATOM H20 | HGA4 | 0.150 |
| BOND H14 | C13 |  |
| BOND H15 | C13 |  |
| BOND H18 | C14 |  |
| BOND H3 | C4 |  |
| BOND H5 | C3 |  |
| BOND C4 | C3 |  |


| BOND | C4 | C5 |
| :--- | :--- | :--- |
| BOND | C13 | C7 |
| BOND | C13 | H16 |
| BOND | C3 | C2 |
| BOND | C7 | C5 |
| BOND | C7 | C8 |
| BOND | C5 | C6 |
| BOND | H7 | C11 |
| BOND | C14 | C9 |
| BOND | C14 | H19 |
| BOND | C14 | H17 |
| BOND | H10 | C20 |
| BOND | H12 | C19 |
| BOND | C9 | C8 |
| BOND | C9 | C10 |
| BOND | C20 | C19 |
| BOND | C20 | C16 |
| BOND | C19 | C18 |
| BOND | C8 | H20 |
| BOND | H9 | C16 |
| BOND | C16 | C15 |
| BOND | C11 | C10 |
| BOND | C11 | C12 |
| BOND | C18 | H11 |
| BOND | C18 | C17 |
| BOND | C10 | H6 |
| BOND | C15 | C17 |
| BOND | C15 | C12 |
| BOND | C17 | H13 |
| BOND | C12 | H8 |
| BOND | C2 | H4 |
| B0ND | C2 | C1 |
| BOND | C6 | C1 |
| BOND | C6 | H1 |
| B0ND | C1 | H2 |

END

BONDS

| ANGLES |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| CG2DC1 | CG2DC1 | CG2R61 | 29.00 | 122.00 |  |  |
| CG2R61 | CG2DC1 | CG331 | 48.00 | 113.00 |  |  |
|  |  |  |  |  |  |  |
| DIHEDRALS |  |  |  |  |  |  |
| CG2DC1 | CG2DC1 | CG2R61 | CG2R61 | 0.7500 | 2 | 180.00 |
| CG2DC1 | CG2DC1 | CG2R61 | CG2R61 | 0.1900 | 4 | 0.00 |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 0.103516 | 1 | 180 |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 8.776453 | 2 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | HGA4 | 6.016354 | 2 | 180 |
| CG331 | CG2DC1 | CG2R61 | CG2R61 | 1.268181 | 2 | 180 |
| CG2R61 | CG2DC1 | CG331 | HGA3 | 0.074396 | 3 | 180 |

## 1,4Me

| RESI AIE |  | 0.000 |
| :--- | ---: | ---: |
| GROUP | ! | CHARGE |
| ATOM C1 | CG2R61 | -0.115 |
| ATOM C2 | CG2R61 | -0.115 |
| ATOM C3 | CG2R61 | -0.115 |
| ATOM C4 | CG2R61 | -0.115 |
| ATOM C5 | CG2R61 | -0.004 |
| ATOM C6 | CG2R61 | -0.115 |


| ATOM | H1 | HGR61 | 0.115 |
| :---: | :---: | :---: | :---: |
| ATOM | H2 | HGR61 | 0.115 |
| ATOM | H3 | HGR61 | 0.115 |
| ATOM | H4 | HGR61 | 0.115 |
| ATOM | H5 | HGR61 | 0.115 |
| ATOM | C7 | CG2DC1 | -0.001 |
| ATOM | C8 | CG2DC1 | -0.138 |
| ATOM | C9 | CG2DC2 | -0.159 |
| ATOM | C10 | CG2DC2 | -0.011 |
| ATOM | C11 | CG2DC1 | -0.133 |
| ATOM | C12 | CG2DC1 | -0.147 |
| ATOM | C13 | CG331 | -0.270 |
| ATOM | H6 | HGA4 | 0.150 |
| ATOM | H7 | HGA 4 | 0.150 |
| ATOM | C14 | CG331 | -0.270 |
| ATOM | H8 | HGA4 | 0.150 |
| ATOM | C15 | CG2R61 | -0.007 |
| ATOM | H9 | HGA4 | 0.150 |
| ATOM | C16 | CG2R61 | -0.115 |
| ATOM | C17 | CG2R61 | -0.115 |
| ATOM | C18 | CG2R61 | -0.115 |
| ATOM | C19 | CG2R61 | -0.115 |
| ATOM | C20 | CG2R61 | -0.115 |
| ATOM | H10 | HGR61 | 0.115 |
| ATOM | H11 | HGR61 | 0.115 |
| ATOM | H12 | HGR61 | 0.115 |
| ATOM | H13 | HGR61 | 0.115 |
| ATOM | H14 | HGR61 | 0.115 |
| ATOM | H15 | HGA3 | 0.090 |
| ATOM | H16 | HGA3 | 0.090 |
| ATOM | H17 | HGA3 | 0.090 |
| ATOM | H18 | HGA3 | 0.090 |
| ATOM | H19 | HGA3 | 0.090 |
| ATOM | H20 | HGA3 | 0.090 |
| BOND | H3 | C4 |  |
| BOND | H5 | C3 |  |
| BOND | H15 | C13 |  |
| BOND | H20 | C14 |  |
| BOND | C4 | C3 |  |
| BOND | C4 | C5 |  |
| BOND | H11 | C20 |  |
| BOND | H10 | C16 |  |
| BOND | C3 | C2 |  |
| BOND | H16 | C13 |  |
| BOND | C20 | C16 |  |
| BOND | C20 | C19 |  |
| BOND | C16 | C15 |  |
| BOND | C13 | C7 |  |
| BOND | C13 | H17 |  |
| BOND | H7 | C9 |  |
| BOND | H8 | C11 |  |
| BOND | C7 | C5 |  |
| BOND | C7 | C8 |  |
| BOND | H13 | C19 |  |
| BOND | C19 | C18 |  |
| BOND | C5 | C6 |  |
| BOND | C9 | C8 |  |
| BOND | C9 | C10 |  |
| BOND | C8 | H6 |  |
| BOND | C11 | C10 |  |
| BOND | C11 | C12 |  |
| BOND | C15 | C12 |  |
| BOND | C15 | C17 |  |

```
BOND C10 C14
BOND C2 H4
BOND C2 C1
BOND C12 H9
BOND C14 H18
BOND C14 H19
BOND C18 C17
BOND C18 H12
BOND C17 H14
BOND C6 C1
BOND C6 H1
BOND C1 H2
END
BONDS
ANGLES
CG2DC1 CG2DC1 CG2R61 29.00 122.00
CG2R61 CG2DC1 CG331 48.00 113.00
DIHEDRALS
CG2DC1 CG2DC1 CG2R61 CG2R61 0.7500 2 180.00
CG2DC1 CG2DC1 CG2R61 CG2R61 0.1900 4 0.00
CG2R61 CG2DC1 CG331 HGA3 0.3000 3 180.00
CG2DC2 CG2DC1 CG2DC1 CG2R61 0.074976 180
CG2DC2 CG2DC1 CG2DC1 CG2R61 9.105853 180
CG2R61 CG2DC1 CG2DC1 HGA4 5.917803 2 180
CG331 CG2DC1 CG2R61 CG2R61 1.361484 2 180
CG2R61 CG2DC1 CG331 HGA3 0.121966 3 180
```

1,5Me

| RESI AIE |  | 0.000 |
| :---: | :---: | :---: |
| GROUP | ! | CHARGE |
| ATOM C1 | CG2R61 | -0.115 |
| ATOM C2 | CG2R61 | -0.115 |
| ATOM C3 | CG2R61 | -0.115 |
| ATOM C4 | CG2R61 | -0.115 |
| ATOM C5 | CG2R61 | -0.004 |
| ATOM C6 | CG2R61 | -0.115 |
| ATOM H1 | HGR61 | 0.115 |
| ATOM H2 | HGR61 | 0.115 |
| ATOM H3 | HGR61 | 0.115 |
| ATOM H4 | HGR61 | 0.115 |
| ATOM H5 | HGR61 | 0.115 |
| ATOM C7 | CG2DC1 | -0.001 |
| ATOM C8 | CG2DC1 | -0.143 |
| ATOM C9 | CG2DC2 | -0.149 |
| ATOM C10 | CG2DC2 | -0.154 |
| ATOM C11 | CG2DC1 | 0.010 |
| ATOM C12 | CG2DC1 | -0.157 |
| ATOM H6 | HGA 4 | 0.150 |
| ATOM H7 | HGA 4 | 0.150 |
| ATOM C13 | CG2R61 | -0.002 |
| ATOM C14 | CG2R61 | -0.115 |
| ATOM C15 | CG2R61 | -0.115 |
| ATOM C16 | CG2R61 | -0.115 |
| ATOM C17 | CG2R61 | -0.115 |
| ATOM C18 | CG2R61 | -0.115 |
| ATOM H8 | HGR61 | 0.115 |
| ATOM H9 | HGR61 | 0.115 |
| ATOM H10 | HGR61 | 0.115 |
| ATOM H11 | HGR61 | 0.115 |
| ATOM H12 | HGR61 | 0.115 |

```
\begin{tabular}{llr} 
ATOM C19 & CG331 & -0.270 \\
ATOM H13 & HGA4 & 0.150 \\
ATOM H14 & HGA4 & 0.150 \\
ATOM C20 & CG331 & -0.270 \\
ATOM H15 & HGA3 & 0.090 \\
ATOM H16 & HGA3 & 0.090 \\
ATOM H17 & HGA3 & 0.090 \\
ATOM H18 & HGA3 & 0.090 \\
ATOM H19 & HGA3 & 0.090 \\
ATOM H20 & HGA3 & 0.090
\end{tabular}
BOND H9 C18
BOND H8 C14
BOND H5 C3
BOND H3 C4
BOND C18 C14
BOND C18 C17
BOND C14 C13
BOND H19 C20
BOND C3 C4
BOND C3 C2
BOND C4 C5
BOND H15 C19
BOND H4 C2
BOND H16 C19
BOND C2 C1
BOND H11 C17
BOND C17 C16
BOND C5 C7
BOND C5 C6
BOND C13 C12
BOND C13 C15
BOND C7 C8
BOND C7 C19
BOND C8 H14
BOND C8 C9
BOND C12 H13
BOND C12 C11
BOND H6 C9
BOND C9 C10
BOND H7 C10
BOND C19 H17
BOND C10 C11
BOND C11 C20
BOND C20 H20
BOND C20 H18
BOND C1 C6
BOND C1 H2
BOND C6 H1
BOND C16 C15
BOND C16 H10
BOND C15 H12
END
BONDS
```

```
ANGLES
```

ANGLES
CG2DC1 CG2DC1 CG2R61 29.00 122.00
CG2DC1 CG2DC1 CG2R61 29.00 122.00
CG2R61 CG2DC1 CG331 48.00 113.00
CG2R61 CG2DC1 CG331 48.00 113.00
DIHEDRALS
DIHEDRALS
CG2DC1 CG2DC1 CG2R61 CG2R61 0.7500 2 180.00
CG2DC1 CG2DC1 CG2R61 CG2R61 0.7500 2 180.00
CG2DC1 CG2DC1 CG2R61 CG2R61 0.1900 4 0.00

```
CG2DC1 CG2DC1 CG2R61 CG2R61 0.1900 4 0.00
```

| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 0.857374 | 1 | 180 |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 5.836502 | 2 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | CG331 | 0.589424 | 1 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | CG331 | 7.206005 | 2 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | HGA4 | 5.077691 | 2 | 180 |
| CG331 | CG2DC1 | CG2R61 | CG2R61 | 1.513733 | 2 | 180 |
| CG2R61 | CG2DC1 | CG331 | HGA3 | 0.214945 | 3 | 180 |

## 2,3Me

| SI AIE |  | 0.000 |
| :---: | :---: | :---: |
| GROUP | ! | CHARGE |
| ATOM C1 | CG2R61 | -0.115 |
| ATOM C2 | CG2R61 | -0.115 |
| ATOM C3 | CG2R61 | -0.115 |
| ATOM C4 | CG2R61 | -0.115 |
| ATOM C5 | CG2R61 | -0.002 |
| ATOM C6 | CG2R61 | -0.115 |
| ATOM H1 | HGR61 | 0.115 |
| ATOM H2 | HGR61 | 0.115 |
| ATOM H3 | HGR61 | 0.115 |
| ATOM H4 | HGR61 | 0.115 |
| ATOM H5 | HGR61 | 0.115 |
| ATOM C7 | CG2DC1 | -0.154 |
| ATOM C8 | CG2DC1 | 0.013 |
| ATOM C9 | CG2DC2 | -0.008 |
| ATOM C10 | CG2DC2 | -0.161 |
| ATOM C11 | CG2DC1 | -0.131 |
| ATOM C12 | CG2DC1 | -0.150 |
| ATOM C13 | CG331 | -0.270 |
| ATOM C14 | CG331 | -0.270 |
| ATOM H6 | HGA4 | 0.150 |
| ATOM H7 | HGA4 | 0.150 |
| ATOM C15 | CG2R61 | -0.007 |
| ATOM C16 | CG2R61 | -0.115 |
| ATOM C17 | CG2R61 | -0.115 |
| ATOM C18 | CG2R61 | -0.115 |
| ATOM C19 | CG2R61 | -0.115 |
| ATOM C20 | CG2R61 | -0.115 |
| ATOM H8 | HGR61 | 0.115 |
| ATOM H9 | HGR61 | 0.115 |
| ATOM H10 | HGR61 | 0.115 |
| ATOM H11 | HGR61 | 0.115 |
| ATOM H12 | HGR61 | 0.115 |
| ATOM H13 | HGA4 | 0.150 |
| ATOM H14 | HGA4 | 0.150 |
| ATOM H15 | HGA3 | 0.090 |
| ATOM H16 | HGA3 | 0.090 |
| ATOM H17 | HGA3 | 0.090 |
| ATOM H18 | HGA3 | 0.090 |
| ATOM H19 | HGA3 | 0.090 |
| ATOM H20 | HGA3 | 0.090 |
| BOND H3 | C4 |  |
| BOND H5 | C3 |  |
| BOND H19 | C14 |  |
| BOND C3 | C4 |  |
| BOND C3 | C2 |  |
| BOND C4 | C5 |  |
| BOND H9 | C20 |  |
| BOND H8 | C16 |  |
| BOND C20 | C16 |  |
| BOND C20 | C19 |  |

```
BOND H17 C13
BOND C16 C15
BOND H13 C7
BOND C14 H20
BOND C14 C9
BOND C14 H18
BOND H11 C19
BOND H4 C2
BOND C2 C1
BOND H7 C11
BOND C5 C7
BOND C5 C6
BOND C19 C18
BOND C7 C8
BOND C15 C12
BOND C15 C17
BOND C11 C12
BOND C11 C10
BOND C9 C10
BOND C9 C8
BOND C12 H14
BOND C10 H6
BOND C8 C13
BOND C18 C17
BOND C18 H10
BOND C17 H12
BOND C13 H15
BOND C13 H16
BOND C6 C1
BOND C6 H1
BOND C1 H2
END
BONDS
\begin{tabular}{lllllll} 
ANGLES & & & & & \\
CG2DC1 & CG2DC1 & CG2R61 & 29.00 & 122.00 & & \\
& & & & & & \\
DIHEDRALS & & & & & \\
CG2DC1 & CG2DC1 & CG2R61 & CG2R61 & 0.7500 & 2 & 180.00 \\
CG2DC1 & CG2DC1 & CG2R61 & CG2R61 & 0.1900 & 4 & 0.00 \\
CG2DC2 & CG2DC1 & CG2DC1 & CG2R61 & 0.096978 & 1 & 180 \\
CG2DC2 & CG2DC1 & CG2DC1 & CG2R61 & 11.415094 & 2 & 180 \\
CG2R61 & CG2DC1 & CG2DC1 & CG331 & 0.193584 & 1 & 180 \\
CG2R61 & CG2DC1 & CG2DC1 & CG331 & 5.531836 & 2 & 180 \\
CG2R61 & CG2DC1 & CG2DC1 & HGA4 & 6.61659 & 2 & 180 \\
CG331 & CG2DC1 & CG2DC2 & CG331 & 0.693951 & 1 & 180 \\
CG331 & CG2DC1 & CG2DC2 & CG331 & 1.317678 & 2 & 180
\end{tabular}
```

2,4Me

| RESI AIE |  | 0.000 |
| :--- | :--- | :--- |
| GROUP | ! | CHARGE |
| ATOM C1 | CG2R61 | -0.115 |
| ATOM C2 | CG2R61 | -0.115 |
| ATOM C3 | CG2R61 | -0.115 |
| ATOM C4 | CG2R61 | -0.115 |
| ATOM C5 | CG2R61 | -0.002 |
| ATOM C6 | CG2R61 | -0.115 |
| ATOM H1 | HGR61 | 0.115 |
| ATOM H2 | HGR61 | 0.115 |
| ATOM H3 | HGR61 | 0.115 |
| ATOM H4 | HGR61 | 0.115 |


| ATOM | H5 | HGR61 | 0.115 |
| :---: | :---: | :---: | :---: |
| ATOM | C7 | CG2DC1 | -0.157 |
| ATOM | C8 | CG2DC1 | 0.015 |
| ATOM | C9 | CG2DC2 | -0.161 |
| ATOM | C10 | CG2DC2 | -0.008 |
| ATOM | C11 | CG2DC1 | -0.133 |
| ATOM | C12 | CG2DC1 | -0.147 |
| ATOM | H6 | HGA 4 | 0.150 |
| ATOM | C13 | CG331 | -0.270 |
| ATOM | C14 | CG2R61 | -0.007 |
| ATOM | C15 | CG2R61 | -0.115 |
| ATOM | C16 | CG2R61 | -0.115 |
| ATOM | C17 | CG2R61 | -0.115 |
| ATOM | C18 | CG2R61 | -0.115 |
| ATOM | C19 | CG2R61 | -0.115 |
| ATOM | H7 | HGR61 | 0.115 |
| ATOM | H8 | HGR61 | 0.115 |
| ATOM | H9 | HGR61 | 0.115 |
| ATOM | H10 | HGR61 | 0.115 |
| ATOM | H11 | HGR61 | 0.115 |
| ATOM | H12 | HGA4 | 0.150 |
| ATOM | H13 | HGA 4 | 0.150 |
| ATOM | C20 | CG331 | -0.270 |
| ATOM | H14 | HGA4 | 0.150 |
| ATOM | H15 | HGA3 | 0.090 |
| ATOM | H16 | HGA3 | 0.090 |
| ATOM | H17 | HGA3 | 0.090 |
| ATOM | H18 | HGA3 | 0.090 |
| ATOM | H19 | HGA3 | 0.090 |
| ATOM | H20 | HGA3 | 0.090 |
| BOND | H3 | C4 |  |
| BOND | H5 | C3 |  |
| BOND | H20 | C13 |  |
| BOND | C4 | C3 |  |
| BOND | C4 | C5 |  |
| BOND | C3 | C2 |  |
| BOND | H12 | C7 |  |
| BOND | C7 | C5 |  |
| BOND | C7 | C8 |  |
| BOND | H18 | C13 |  |
| BOND | C5 | C6 |  |
| BOND | C2 | H4 |  |
| BOND | C2 | C1 |  |
| BOND | H10 | C18 |  |
| BOND | H8 | C19 |  |
| BOND | C18 | C19 |  |
| BOND | C18 | C17 |  |
| BOND | C19 | C15 |  |
| BOND | H9 | C17 |  |
| BOND | C17 | C16 |  |
| BOND | C15 | H7 |  |
| BOND | C15 | C14 |  |
| BOND | H17 | C20 |  |
| BOND | C16 | C14 |  |
| BOND | C16 | H11 |  |
| BOND | C14 | C12 |  |
| BOND | H14 | C11 |  |
| BOND | C12 | C11 |  |
| BOND | C12 | H13 |  |
| BOND | C11 | C10 |  |
| BOND | C13 | C10 |  |
| BOND | C13 | H19 |  |
| BOND | H6 | C9 |  |


| BOND | C10 | C9 |
| :--- | :--- | :--- |
| BOND | C9 | C8 |
| BOND | C8 | C20 |
| BOND | C6 | C1 |
| BOND | C6 | H1 |
| BOND | C1 | H2 |
| BOND | C20 | H15 |
| BOND | C20 | H16 |

END

BONDS

| ANGLES |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| CG2DC1 | CG2DC1 | CG2R61 | 29.00 | 122.00 |  |  |
| DIHEDRALS |  |  |  |  |  |  |
| CG2DC1 | CG2DC1 | CG2R61 | CG2R61 | 0.7500 | 2 | 180.00 |
| CG2DC1 | CG2DC1 | CG2R61 | CG2R61 | 0.1900 | 4 | 0.00 |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 0.094644 | 1 | 180 |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 9.162901 | 2 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | CG331 | 1.244941 | 1 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | CG331 | 10.107544 | 2 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | HGA4 | 6.709147 | 2 | 180 |

## 2,5Me

| RESI AIE |  | 0.000 |
| :--- | :--- | ---: |
| GROUP |  | CHARGE |
| ATOM C1 | CG2R61 | -0.115 |
| ATOM C2 | CG2R61 | -0.115 |
| ATOM C3 | CG2R61 | -0.115 |
| ATOM C4 | CG2R61 | -0.115 |
| ATOM C5 | CG2R61 | -0.002 |
| ATOM C6 | CG2R61 | -0.115 |
| ATOM H1 | HGR61 | 0.115 |
| ATOM H2 | HGR61 | 0.115 |
| ATOM H3 | HGR61 | 0.115 |
| ATOM H4 | HGR61 | 0.115 |
| ATOM H5 | HGR61 | 0.115 |
| ATOM C7 | CG2DC1 | -0.157 |
| ATOM C8 | CG2DC1 | 0.010 |
| ATOM C9 | CG2DC2 | -0.151 |
| ATOM C10 | CG2DC2 | -0.151 |
| ATOM C11 | CG2DC1 | 0.010 |
| ATOM C12 | CG2DC1 | -0.157 |
| ATOM H6 | HGA4 | 0.150 |
| ATOM H7 | HGA4 | 0.150 |
| ATOM C13 | CG2R61 | -0.002 |
| ATOM C14 | CG2R61 | -0.115 |
| ATOM C15 | CG2R61 | -0.115 |
| ATOM C16 | CG2R61 | -0.115 |
| ATOM C17 | CG2R61 | -0.115 |
| ATOM C18 | CG2R61 | -0.115 |
| ATOM H8 | HGR61 | 0.115 |
| ATOM H9 | HGR61 | 0.115 |
| ATOM H10 | HGR61 | 0.115 |
| ATOM H11 | HGR61 | 0.115 |
| ATOM H12 | HGR61 | 0.115 |
| ATOM H13 | HGA4 | 0.150 |
| ATOM H14 | HGA4 | 0.150 |
| ATOM C19 | CG331 | -0.270 |
| ATOM C20 | CG331 | -0.270 |
| ATOM H15 | HGA3 | 0.090 |
| HOM |  |  |

```
\begin{tabular}{lll} 
ATOM H16 & HGA3 & 0.090 \\
ATOM H17 & HGA3 & 0.090 \\
ATOM H18 & HGA3 & 0.090 \\
ATOM H19 & HGA3 & 0.090 \\
ATOM H20 & HGA3 & 0.090
\end{tabular}
BOND H9 C18
BOND H5 C3
BOND H8 C14
BOND H3 C4
BOND C18 C14
BOND C18 C17
BOND C3 C4
BOND C3 C2
BOND C14 C13
BOND C4 C5
BOND H19 C20
BOND H17 C19
BOND H11 C17
BOND H4 C2
BOND C17 C16
BOND C2 C1
BOND C5 C7
BOND C5 C6
BOND C13 C12
BOND C13 C15
BOND C7 H13
BOND C7 C8
BOND C12 H14
BOND C12 C11
BOND H6 C9
BOND C11 C10
BOND C11 C20
BOND C8 C9
BOND C8 C19
BOND C9 C10
BOND H7 C10
BOND C20 H20
BOND C20 H18
BOND C19 H15
BOND C19 H16
BOND C1 C6
BOND C1 H2
BOND C16 C15
B0ND C16 H10
BOND C6 H1
BOND C15 H12
END
BONDS
\begin{tabular}{llllllll} 
ANGLES & & & & & & & \\
CG2DC1 & CG2DC1 & CG2R61 & 29.00 & 122.00 & & & \\
\\
DIHEDRALS & & & & & & & \\
CG2DC1 & CG2DC1 & CG2R61 & CG2R61 & 0.7500 & 2 & 180.00 & \\
CG2DC1 & CG2DC1 & CG2R61 & CG2R61 & 0.1900 & 4 & 0.00 & \\
CG2DC2 & CG2DC1 & CG2DC1 & CG2R61 & 0.04648 & 1 & 180 & \\
CG2DC2 & CG2DC1 & CG2DC1 & CG2R61 & 12.977052 & 2 & 180 \\
CG2R61 & CG2DC1 & CG2DC1 & CG331 & 0.7836 & 1 & 180 & \\
CG2R61 & CG2DC1 & CG2DC1 & CG331 & 7.926638 & 2 & 180
\end{tabular}
```


## 3,4Me

| SI | AIE |  | 0.000 |
| :---: | :---: | :---: | :---: |
| GROUP |  | ! | CHARGE |
| ATOM | C1 | CG2R61 | -0.115 |
| ATOM | C2 | CG2R61 | -0.115 |
| ATOM | C3 | CG2R61 | -0.115 |
| ATOM | C4 | CG2R61 | -0.115 |
| ATOM | C5 | CG2R61 | -0.007 |
| ATOM | C6 | CG2R61 | -0.115 |
| ATOM | H1 | HGR61 | 0.115 |
| ATOM | H2 | HGR61 | 0.115 |
| ATOM | H3 | HGR61 | 0.115 |
| ATOM | H4 | HGR61 | 0.115 |
| ATOM | H5 | HGR61 | 0.115 |
| ATOM | C7 | CG2DC1 | -0.147 |
| ATOM | C8 | CG2DC1 | -0.128 |
| ATOM | C9 | CG2DC2 | -0.017 |
| ATOM | C10 | CG2DC2 | -0.017 |
| ATOM | C11 | CG2DC1 | -0.128 |
| ATOM | C12 | CG2DC1 | -0.147 |
| ATOM | C13 | CG331 | -0.271 |
| ATOM | C14 | CG331 | -0.271 |
| ATOM | C15 | CG2R61 | -0.007 |
| ATOM | C16 | CG2R61 | -0.115 |
| ATOM | C17 | CG2R61 | -0.115 |
| ATOM | C18 | CG2R61 | -0.115 |
| ATOM | C19 | CG2R61 | -0.115 |
| ATOM | C20 | CG2R61 | -0.115 |
| ATOM | H6 | HGR61 | 0.115 |
| ATOM | H7 | HGR61 | 0.115 |
| ATOM | H8 | HGR61 | 0.115 |
| ATOM | H9 | HGR61 | 0.115 |
| ATOM | H10 | HGR61 | 0.115 |
| ATOM | H11 | HGA 4 | 0.150 |
| ATOM | H12 | HGA 4 | 0.150 |
| ATOM | H13 | HGA 4 | 0.150 |
| ATOM | H14 | HGA 4 | 0.150 |
| ATOM | H15 | HGA3 | 0.090 |
| ATOM | H16 | HGA3 | 0.090 |
| ATOM | H17 | HGA3 | 0.090 |
| ATOM | H18 | HGA3 | 0.090 |
| ATOM | H19 | HGA3 | 0.090 |
| ATOM | H20 | HGA3 | 0.090 |
| BOND | H16 | C13 |  |
| BOND | H20 | C14 |  |
| BOND | H3 | C4 |  |
| BOND | H8 | C18 |  |
| BOND | H5 | C3 |  |
| BOND | H17 | C13 |  |
| BOND | C13 | C9 |  |
| BOND | C13 | H15 |  |
| BOND | H11 | C7 |  |
| BOND | C4 | C3 |  |
| BOND | C4 | C5 |  |
| BOND | H10 | C17 |  |
| BOND | C18 | C17 |  |
| BOND | C18 | C19 |  |
| BOND | C3 | C2 |  |
| BOND | H14 | C11 |  |
| BOND | H9 | C19 |  |
| BOND | C7 | C5 |  |
| BOND | C7 | C8 |  |
| BOND | C17 | C15 |  |

```
BOND C5 C6
BOND C19 C20
BOND C9 C8
BOND C9 C10
BOND C8 H13
BOND C11 C10
BOND C11 C12
BOND C2 H4
BOND C2 C1
BOND C15 C12
BOND C15 C16
BOND C10 C14
BOND C6 C1
BOND C6 H1
BOND C20 C16
BOND C20 H7
BOND C12 H12
BOND C1 H2
BOND C16 H6
BOND C14 H18
BOND C14 H19
```

END

BONDS

| ANGLES |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :---: | :---: | :---: |
| CG2DC1 | CG2DC1 | CG2R61 | 29.00 | 122.00 |  |  |
|  |  |  |  |  |  |  |
| DIHEDRALS |  |  |  |  |  |  |
| CG2DC1 | CG2DC1 | CG2R61 | CG2R61 | 0.7500 | 2 | 180.00 |
| CG2DC1 | CG2DC1 | CG2R61 | CG2R61 | 0.1900 | 4 | 0.00 |
| CG331 | CG2DC2 | CG2DC2 | CG331 | 10.0000 | 2 | 180.00 |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 0.08265 | 1 | 180 |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 9.758027 | 2 |  |
| CG2R61 | CG2DC1 | CG2DC1 | HGA4 | 6.169531 | 2 | 180 |


| 1Me |  |  |
| :--- | :--- | ---: |
| RESI AIE |  | 0.000 |
| GROUP |  | CHARGE |
| ATOM H1 | HGR61 | 0.115 |
| ATOM C1 | CG2R61 | -0.115 |
| ATOM C2 | CG2R61 | -0.115 |
| ATOM H2 | HGR61 | 0.115 |
| ATOM C3 | CG2R61 | -0.115 |
| ATOM H3 | HGR61 | 0.115 |
| ATOM C4 | CG2R61 | -0.115 |
| ATOM H4 | HGR61 | 0.115 |
| ATOM C5 | CG2R61 | -0.115 |
| ATOM H5 | HGR61 | 0.115 |
| ATOM C6 | CG2R61 | -0.004 |
| ATOM C7 | CG2DC1 | -0.001 |
| ATOM H6 | HGR61 | 0.115 |
| ATOM C8 | CG2R61 | -0.115 |
| ATOM C9 | CG2R61 | -0.115 |
| ATOM H7 | HGR61 | 0.115 |
| ATOM C10 | CG2R61 | -0.007 |
| ATOM C11 | CG2R61 | -0.115 |
| ATOM H8 | HGR61 | 0.115 |
| ATOM C12 | CG2R61 | -0.115 |
| ATOM H9 | HGR61 | 0.115 |

```
ATOM C13 CG2R61 -0.115
ATOM H10 HGR61 0.115
ATOM C14 CG2DC1 -0.143
ATOM C15 CG2DC2 -0.152
ATOM C16 CG2DC2 -0.157
ATOM C17 CG2DC1 -0.136
ATOM C18 CG2DC1 -0.150
ATOM C19 CG331 -0.270
ATOM H11 HGA4 0.150
ATOM H12 HGA4 0.150
ATOM H13 HGA4 0.150
ATOM H14 HGA4 0.150
ATOM H15 HGA4 0.150
ATOM H16 HGA3 0.090
ATOM H17 HGA3 0.090
ATOM H18 HGA3 0.090
BOND H18 C19
BOND H5 C5
BOND H4 C4
BOND H16 C19
BOND C5 C4
BOND C5 C6
BOND H6 C8
BOND C4 C3
BOND H7 C9
BOND C19 C7
BOND C19 H17
BOND H12 C15
BOND C8 C9
BOND C8 C13
BOND C9 C10
BOND H14 C17
BOND C7 C6
BOND C7 C14
BOND C15 C14
BOND C15 C16
BOND C6 C1
BOND C14 H11
BOND C17 C16
BOND C17 C18
BOND C16 H13
BOND H10 C13
BOND C13 C12
BOND C10 C18
BOND C10 C11
BOND C3 H3
BOND C3 C2
BOND C18 H15
BOND C12 C11
BOND C12 H9
BOND C11 H8
BOND C1 C2
BOND C1 H1
BOND C2 H2
END
BONDS
ANGLES
CG2DC1 CG2DC1 CG2R61 29.00 122.00
CG2R61 CG2DC1 CG331 48.00 113.00
```

| DIHEDRALS |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :---: | :---: |
| CG2DC1 | CG2DC1 | CG2R61 | CG2R61 | 0.7500 | 2 | 180.00 |
| CG2DC1 | CG2DC1 | CG2R61 | CG2R61 | 0.1900 | 4 | 0.00 |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 0.161071 | 1 | 180 |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 8.51945 | 2 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | HGA4 | 4.73837 | 2 | 180 |
| CG331 | CG2DC1 | CG2R61 | CG2R61 | 1.590478 | 2 | 180 |
| CG2R61 | CG2DC1 | CG331 | HGA3 | 0.461611 | 3 | 60 |

## 2Me

| RESI AIE |  | 0.000 |
| :--- | :--- | ---: |
| GROUP |  | CHARGE |
| ATOM H1 | HGR61 | 0.115 |
| ATOM C1 | CG2R61 | -0.115 |
| ATOM C2 | CG2R61 | -0.115 |
| ATOM H2 | HGR61 | 0.115 |
| ATOM C3 | CG2R61 | -0.002 |
| ATOM C4 | CG2DC1 | -0.157 |
| ATOM C5 | CG2R61 | -0.115 |
| ATOM H3 | HGR61 | 0.115 |
| ATOM C6 | CG2R61 | -0.115 |
| ATOM H4 | HGR61 | 0.115 |
| ATOM C7 | CG2R61 | -0.115 |
| ATOM H5 | HGR61 | 0.115 |
| ATOM H6 | HGR61 | 0.115 |
| ATOM C8 | CG2R61 | -0.115 |
| ATOM C9 | CG2R61 | -0.115 |
| ATOM H7 | HGR61 | 0.115 |
| ATOM C10 | CG2R61 | -0.115 |
| ATOM H8 | HGR61 | 0.115 |
| ATOM C11 | CG2R61 | -0.115 |
| ATOM H9 | HGR61 | 0.115 |
| ATOM C12 | CG2R61 | -0.115 |
| ATOM H10 | HGR61 | 0.115 |
| ATOM C13 | CG2R61 | -0.007 |
| ATOM C14 | CG2DC1 | 0.010 |
| ATOM C15 | CG2DC2 | -0.154 |
| ATOM C16 | CG2DC2 | -0.154 |
| ATOM C17 | CG2DC1 | -0.136 |
| ATOM C18 | CG2DC1 | -0.150 |
| ATOM H11 | HGA4 | 0.150 |
| ATOM C19 | CG331 | -0.270 |
| ATOM H12 | HGA4 | 0.150 |
| ATOM H13 | HGA4 | 0.150 |
| ATOM H14 | HGA4 | 0.150 |
| ATOM H15 | HGA4 | 0.150 |
| ATOM H16 | HGA3 | 0.090 |
| ATOM H17 | HGA3 | 0.090 |
| ATOM H18 | HGA3 | 0.090 |


| BOND H17 | C19 |
| :--- | :--- |
| BOND H1 | C1 |
| BOND H2 | C2 |
| BOND H18 | C19 |
| BOND C1 | C2 |
| BOND C1 | C7 |
| BOND C2 | C3 |
| BOND H10 | C12 |
| BOND C19 | C14 |
| BOND C19 | H16 |
| BOND H9 | C11 |
| BOND H13 | C16 |


| BOND | C12 | C11 |
| :--- | :--- | :--- |
| BOND | C12 | C13 |
| BOND | H15 | C18 |
| BOND | C11 | C10 |
| BOND | C16 | C15 |
| BOND | C16 | C17 |
| BOND | C14 | C15 |
| BOND | C14 | C4 |
| BOND | C18 | C17 |
| BOND | C18 | C13 |
| BOND | C15 | H12 |
| BOND | C17 | H14 |
| BOND | C13 | C8 |
| BOND | H5 | C7 |
| BOND | C7 | C6 |
| BOND | C4 | C3 |
| BOND | C4 | H11 |
| BOND | C3 | C5 |
| BOND | C10 | H8 |
| BOND | C10 | C9 |
| BOND | C8 | C9 |
| BOND | C8 | H6 |
| BOND | C9 | H7 |
| BOND | C6 | C5 |
| BOND | C6 | H4 |
| BOND | C5 | H3 |

END

BONDS

| ANGLES |  |  |  |  |  |  |
| :--- | :--- | :--- | :--- | :--- | :--- | :--- |
| CG2DC1 | CG2DC1 | CG2R61 | 29.00 | 122.00 |  |  |
| DIHEDRALS |  |  |  |  |  |  |
| CG2DC1 | CG2DC1 | CG2R61 | CG2R61 | 0.7500 | 2 | 180.00 |
| CG2DC1 | CG2DC1 | CG2R61 | CG2R61 | 0.1900 | 4 | 0.00 |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 1.318489 | 1 | 180 |
| CG2DC2 | CG2DC1 | CG2DC1 | CG2R61 | 3.914232 | 2 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | CG331 | 0.126094 | 1 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | CG331 | 5.227654 | 2 | 180 |
| CG2R61 | CG2DC1 | CG2DC1 | HGA4 | 5.810909 | 2 | 180 |


| 3Me |  | 0.000 |
| :--- | :--- | ---: |
| RESI AIE |  | 0.000 |
| GROUP |  | CHARGE |
| ATOM H1 | HGR61 | 0.115 |
| ATOM C1 | CG2R61 | -0.115 |
| ATOM C2 | CG2R61 | -0.115 |
| ATOM H2 | HGR61 | 0.115 |
| ATOM C3 | CG2R61 | -0.007 |
| ATOM C4 | CG2DC1 | -0.150 |
| ATOM C5 | CG2R61 | -0.115 |
| ATOM H3 | HGR61 | 0.115 |
| ATOM C6 | CG2R61 | -0.115 |
| ATOM H4 | HGR61 | 0.115 |
| ATOM C7 | CG2R61 | -0.115 |
| ATOM H5 | HGR61 | 0.115 |
| ATOM C8 | CG2DC1 | -0.131 |
| ATOM C9 | CG2DC2 | -0.164 |
| ATOM C10 | CG2DC2 | -0.011 |
| ATOM C11 | CG2DC1 | -0.133 |
| ATOM C12 | CG2DC1 | -0.147 |

```
ATOM C13 CG2R61 -0.007
ATOM C14 CG2R61 -0.115
ATOM C15 CG2R61 -0.115
ATOM C16 CG2R61 -0.115
ATOM C17 CG2R61 -0.115
ATOM C18 CG2R61 -0.115
ATOM H6 HGA4 0.150
ATOM H7 HGA4 0.150
ATOM H8 HGA4 0.150
ATOM C19 CG331 -0.270
ATOM H9 HGA4 0.150
ATOM H10 HGA4 0.150
ATOM H11 HGR61 0.115
ATOM H12 HGR61 0.115
ATOM H13 HGR61 0.115
ATOM H14 HGR61 0.115
ATOM H15 HGR61 0.115
ATOM H16 HGA3 0.090
ATOM H17 HGA3 0.090
ATOM H18 HGA3 0.090
BOND H18 C19
BOND H12 C15
BOND H13 C16
BOND H4 C6
BOND H3 C5
BOND C15 C16
BOND C15 C13
BOND C16 C17
BOND C6 C5
BOND C6 C7
BOND C5 C3
BOND H5 C7
BOND C13 C12
BOND C13 C14
BOND C7 C1
BOND C12 H10
BOND C12 C11
BOND C17 H14
BOND C17 C18
BOND H16 C19
BOND H7 C8
BOND C3 C4
BOND C3 C2
BOND C11 C10
BOND C11 H9
BOND C10 C19
BOND C10 C9
BOND C8 C9
BOND C8 C4
BOND C19 H17
BOND H8 C9
BOND C4 H6
BOND C1 C2
BOND C1 H1
BOND C2 H2
BOND C14 C18
BOND C14 H11
BOND C18 H15
END
BONDS
```

```
ANGLES
CG2DC1 CG2DC1 CG2R61 29.00 122.00
DIHEDRALS
CG2DC1 CG2DC1 CG2R61 CG2R61 0.7500 2 180.00
CG2DC1 CG2DC1 CG2R61 CG2R61 0.1900 4 0.00
CG2DC2 CG2DC1 CG2DC1 CG2R61 0.08811 1 180
CG2DC2 CG2DC1 CG2DC1 CG2R61 8.721226 180
CG2R61 CG2DC1 CG2DC1 HGA4 5.688978 2 180
```

