Fluctuations and Instantons in Complex Landscapes: From Ligand Unbinding to Proton Transfer

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Fluctuations and Instantons in Complex Landscapes: From Ligand Unbinding to Proton Transfer

A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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Abstract

FLUCTUATIONS AND INSTANTONS IN COMPLEX LANDSCAPES: FROM LIGAND UNBINDING TO PROTON TRANSFER

By Justin E. Elenewski

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Biophysical entities are complex systems systems with strong environmental coupling, dominated by fluctuations on a hierarchy of timescales. These properties confound simulation of ligand binding and catalysis, inflating the scale of the problem to one tractable only with a considerable outlay of resources. In an attempt to ameliorate this restriction, several techniques are developed to accelerate biomolecular simulations while collaterally lending physical insight. The first segment of this dissertation is concerned with directed simulations of ligand binding in a model system. Using the serum retinol binding protein as a prototype, the potential of mean force associated with ligand binding is calculated and dissected. Desolvation is sufficient to drive formation of an intermediate binding state; however, a combination of electrostatic and van der Waals interactions pull the intermediate into a stable configuration. Association is accompanied by a change in the conformational flexibility of the portal domains of sRBP and subsequent “stiffening” of the holo sRBP, reflecting
an order–disorder transition in the protein. The third and fourth chapters of this
dissertation entail *ab initio* molecular dynamics (AIMD) and quantum Monte Carlo
methods (QMC) for computational enzymology. An ideal system for the application of
AIMD, are the cytochromes P450 (CYP450s). Most AIMD calculations are performed
using plane–wave (PW) density functional theory as an electronic structure method;
conversely, computational enzymology is generally performed using calculations with
Gaussian basis sets. In this scenario, no benchmark exists to comparison of PW
calculations with experimental data. To clarify this situation, benchmark PW calcu-
lations are performed on CYP450 Compound I, the iron–oxo species operant in
these enzymes. Finally, lattice QMC methods are developed to characterize tunneling
in mean–field backgrounds. Using AIMD simulations, a potential of mean force is
constructed in the limit of classical nuclei. A framework for path integral Monte
Carlo is introduced in which the Euclidean functional integral is discretized on a
lattice, permitting calculations of correlation functions and ultimately the action of
the system. As the action is quenched, instanton solutions and their contribution to
degeneracy splitting are obtained. This technique is demonstrated for malonaldehyde,
a system in which proton tunneling is critical.
Introduction

In this chapter, the theoretical notions underpinning subsequent developments are outlined. The central theme of this dissertation comprises the refinement and application of molecular dynamics simulation techniques to condensed phase systems. Molecular dynamics, in short, is a methodology that evolves the mechanistic dynamics of a classical or quantum system computationally by simulating the equations of motion in the presence of stochastic noise.

In the second chapter, classical molecular dynamics simulations are exploited in the presence of a biasing force in order to characterize ligand unbinding from a host–guest system, the retinol / retinol binding protein complex. As a collateral result, this technique defines trajectories along which the free energy surface for association may be calculated and dissected to determine physical underpinnings of complexation and ligand recognition. These two free energy methods, based on the Jarzynski equality and adaptive biasing force method, are outlined in this chapter, along with foundations of the biasing method, termed steered molecular dynamics.

The two subsequent chapters develop the theoretical machinery necessary to characterize an enzymatic reaction in which proton tunneling plays a prominent role, specifically hydrogen atom abstraction by cytochrome P450 Compound I. This process is best explored from within the framework of \textit{ab initio} molecular dynamics, in particular the Car—Parrinello method. Accordingly, the third chapter comprises a systematic benchmarking of plane–wave density functional theory for this system, with
reference to both previously reported localized–basis density functional theory (DFT) calculations and experimental data. DFT itself is the driving electronic structure technique underlying Car—Parrinello molecular dynamics, and the foundations for both techniques are delineated in this chapter.

The final chapter addresses proton tunneling in an effective framework by exploiting \textit{ab initio} molecular dynamics and two free energy sampling techniques, metadynamics and blue–moon ensemble sampling, to construct a free energy surface for proton transfer. This surface encapsulates a free energy profile for migration of the proton as a classical nucleus in the mean–field background defined by \textit{ab initio} molecular dynamics sampling. Nuclear quantum effects are incorporated by exploring this landscape using path integral Monte Carlo calculations discretized on a space–time lattice. Within this approximation, the migration of a proton can be treated without the overhead associated with either path integral molecular dynamics or all—atom path integral Monte Carlo calculations. These methods are applied to a model system, comprising proton transfer during keto–enol tautomerism in malonaldehyde. The metadynamics and blue–moon ensemble free energy sampling methodologies are likewise developed in the following.

1.1 Molecular Dynamics

Consider a nonrelativistic system comprising electronic and nuclear degrees of freedom. The most general Hamiltonian for such a system is of the form
\[
\hat{H} = -\sum_i \frac{\hbar^2}{2M_I} \nabla_i^2 - \sum_i \frac{\hbar^2}{2m_e} \nabla_i^2 + \frac{1}{4\pi\varepsilon_0} \sum_{i<j} \frac{\varepsilon^2}{|\vec{r}_i - \vec{r}_j|^2} - \frac{1}{4\pi\varepsilon_0} \sum_{i,j} \frac{e^2 Z_I}{|\vec{R}_i - \vec{R}_j|} + \frac{1}{4\pi\varepsilon_0} \sum_{i<j} \frac{e^2 Z_I Z_J}{|\vec{R}_i - \vec{R}_j|} \]
\[
= -\sum_i \frac{\hbar^2}{2M_I} \nabla_i^2 + \hat{H}_e(\vec{r}_i, \vec{R}_i) \tag{1.3}
\]

where \(\vec{r}_i\) and \(\vec{R}_i\) correspond to the cartesian electronic and nuclear coordinates, respectively. In this case, \(M_I\) and \(Z_I\) are the the mass and atomic number of the I-th nucleus, \(m_e\) is the electron mass, \(\varepsilon_0\) is the permittivity of free space, and \(-\varepsilon\) is the fundamental unit of electronic charge. The eigenfunctions of this Hamiltonian are described by a joint electronic–nuclear wavefunction \(\Psi(\vec{r}_i, \vec{R}_i, t)\). Using a successive series semiclassical approximations, several molecular dynamics methods naturally emerge from this Hamiltonian \([1]\).

As a first step, assume that the timescale for nuclear motion is sufficiently longer than that for electronic motion. In this case, the nuclei may be treated as frozen at their initial coordinates and the Schrodinger equation solved in this fixed nuclear configuration. The nuclear gradient term in \([1.1]\) then vanishes and the problem reduces to solution of the time–independent Schrodinger equation

\[
\hat{H}_e \Psi(\{\vec{r}_i\}, \{\vec{R}_i\}) = E_k \Psi(\{\vec{r}_i\}, \{\vec{R}_i\}) \tag{1.4}
\]

where \(\Psi(\{\vec{r}_i\}, \{\vec{R}_i\})\) is the wavefunction for the electronic subsystem in the presence of free electronic coordinates \(\{\vec{r}_i\}\) and frozen nuclear coordinates \(\{\vec{R}_i\}\). The full wavefunction may be written in a separable form through the so–called Born ansatz, an expansion into a superposition of the electronic and nuclear terms.
\[
\Phi(\{\vec{r}_i\}, \{\vec{R}_I\}, t) = \sum_k \Psi_k(\{\vec{r}_i\}, \{\vec{R}_I\}) \Xi_k(\{\vec{R}_I\}, t)
\] (1.5)

where \(\Xi(\{\vec{R}_I\}, t)\) is the many-body nuclear wavefunction. Inserting this expression into the time-independent Schrödinger equation

\[
\imath \hbar \frac{\partial}{\partial t} \Phi(\{\vec{r}_i\}, \{\vec{R}_I\}, t) = \hat{H} \Phi(\{\vec{r}_i\}, \{\vec{R}_I\}, t)
\] (1.6)

and substituting the electronic eigenvalues of \(\hat{H}_e\) affords

\[
-\imath \hbar \Psi_l \frac{\partial}{\partial t} \Xi_l = - \sum_{l} \frac{\hbar^2}{2M_l} \left( \Psi_l \nabla^2 \Xi_l + 2 (\nabla \Psi_l) \cdot (\nabla \Xi_l) + \Xi_l \nabla^2 \Psi_l \right) + \Psi_l \Xi_l E_l
\] (1.7)

To find an equation of motion for the complete system it is necessary to project the electronic subsystem onto its eigenvalues by taking the inner product with respect to another electronic state vector. Multiplying this expression by \(\Psi_l^*\) and integrating over all \(\{\vec{r}_i\}\) affords

\[
\imath \hbar \frac{\partial}{\partial t} \Xi_l = \left[ \sum_{k} \frac{\hbar^2}{2M_l} \nabla^2 + E_k \right] \Xi_k + \sum_{l} \int d\vec{r} \Psi_k^* \left( -\sum_{l} \frac{\hbar}{2M_l} \nabla^2 \right) \Psi_l \] (1.8)

\[
\quad + \sum_{l} \sum_{l} \left( \int d\vec{r} \Psi_k^* \left[ -\imath \hbar \nabla_l \right] \Psi_l \right) \left( -\imath \hbar \nabla_l \Xi_l \right)
\] (1.9)

\[
\quad = \left[ \sum_{l} \frac{\hbar^2}{2M_l} \nabla^2 + E_k \right] \Xi_k + \sum_{l} C_{kl} \Xi_l.
\] (1.10)

The matrix \(C_{kl}\) represents the nonadiabatic coupling between states of the system.

The first term in this approximation is associated with the coupling of the kinetic energy operator of the nuclei to the electronic subsystem. Conversely, the second
term is associated with the overlap between the nuclear momentum operator and the
electronic states, and the nuclear momentum. The diagonal terms of $C_{kl}$ depend only
on a single adiabatic wavefunction. Retaining only these terms affords the so called
“adiabatic approximation:”

$$C_{kk} = - \sum_l \int d^2 \Psi_k^* \nabla^2 \Psi_k$$

which effectively decouples the differential equations corresponding to the nuclear
and electronic degrees of freedom. Within this scenario, the nuclear motion evolves
without perturbing the electronic state of the system, and the adiabatic coupling term
may be treated as a correction to the eigenvalue of the electronic Hamiltonian. Given
these considerations, the wavefunction assumes the separable form

$$\Phi(\{r_i\}, \{\vec{R}_i\}, t) = \Psi(\{r_i\}, \{\vec{R}_i\}) \Xi(\{\vec{R}_i\}, t)$$

A successive degree of simplification is afforded by the Born—Oppenheimer approxi-
mation, in which $C_{kk} = 0$ and the nuclear Schrodinger equation becomes

$$\left[ - \sum_l \frac{\hbar}{2M_l} \nabla^2_l + E_k(\vec{R}_l) \right] \Xi_k = i\hbar \frac{\partial}{\partial t} \Xi_k.$$

This approximation underlies the remainder of physical processes discussed in this work.
It should be noted that condition need not be enforced, and non-Born—Oppenheimer
dynamics schemes do exist which permit simulation of nonadiabatic processes such as
photochemistry, charge transfer, and other surface hopping scenarios [2]. Nonetheless,
none of these methods have been utilized in this work and hence no further remarks
will be made.
Semiclassical Approximations

Both classical and \textit{ab initio} molecular dynamics may be derived by taking successive semiclassical approximations to the Born—Oppenheimer scheme. Let us adopt a polar representation for the nuclear coordinate, in terms of an amplitude $A_k(\{\vec{R}_I\})$ and a phase $S_k(\{\vec{R}_I\})$ factor

$$
\Xi_k(\{\vec{R}_I\}, t) = A_k(\{\vec{R}_I\}, t) \exp \left[ \frac{i}{\hbar} S_k(\{\vec{R}_I\}, t) \right]
$$

(1.14)

with the provision that $A_k > 0$ for all configurations $\{\vec{R}_I\}$ and times $t$. Substituting this representation into (1.13) and collecting the real and complex terms provides evolution equations for the amplitude and the phase:

$$
\frac{\partial S_k}{\partial t} + \sum_I \frac{1}{2M_I} (\nabla_I S_k)^2 + E_k - \sum_I \frac{\hbar^2}{2M_I} \nabla^2 A_k
= 0
$$

(1.15)

$$
\frac{\partial A_k}{\partial t} + \sum_I \frac{1}{M_I} (\nabla_I A_k) \cdot (\nabla_I S_k) + \sum_I \frac{1}{2M_I} (\nabla^2 S_k)
= 0
$$

(1.16)

These equations may be recast in a hydrodynamic formulation. Multiplying both sides of (1.16) by $2A_k$ and using the identities $2A_k \frac{\partial}{\partial t} A_k = \frac{\partial}{\partial t} (A_k^2)$ and $\nabla_I \cdot (A_k^2 \nabla_I S_k) = 2A_k (\nabla_I A_k) \cdot (\nabla_I S_k) + A_k^2 (\nabla^2 S_k)$, the evolution equation for the amplitude $A_k$ becomes:

$$
\frac{\partial}{\partial t} (A_k)^2 + \sum_I \frac{1}{M_I} \nabla_I \cdot (A_k^2 \nabla_I S_k) = 0
$$

(1.17)

The nuclear probability density $\rho_k = \Xi_k^* \Xi_k$ may be identified with $A_k^2$ since the complex phase factors cancel under conjugation. Consequently (1.17) may be cast in the form of a continuity equation.
\[ \frac{\partial \rho_k}{\partial t} + \sum_i \nabla_i J_k = 0 \]  

(1.18)

where the nuclear probability current density is defined as \( J_k = \frac{1}{M_i} A_k^2 (\nabla I S_k) \). The phase equation \([1.15]\) may be expanded in powers of \( \hbar \), each corresponding to a different level of semiclassical approximation. In the limit \( \hbar \to 0 \) the case of classical nuclei is recovered, in which case the phase evolves according to

\[ \frac{\partial S_k}{\partial t} + \sum_i \frac{1}{2M_i} (\nabla I S_k)^2 + E_k = 0 \]  

(1.19)

where the missing term \( Q = -\sum_i \frac{\hbar^2}{2M_i} \frac{\nabla^2 A_k}{A_k} \) encapsulates the quantum features of the system. This quantity, the quantum potential, is nonvanishing at all points in \( \mathbb{R}^3 \) and is responsible for nonlocality.

Now, equation \([1.19]\) is simply the Hamilton–Jacobi equation for a classical particle moving in the potential determined by \( E_k \). Accordingly, we can make the identification

\[ \frac{\partial S_k}{\partial t} + H_k(\{ \tilde{R}_I \}, \nabla I S_k) = 0 \]  

(1.20)

with the classical Hamiltonian \( H_k(\{ \tilde{R}_I \}, \nabla I S_k) = T(\tilde{P}_I) + V_k(\{ \tilde{R}_I \}) \) where \( T(\tilde{P}_I) \) and \( V_k(\{ \tilde{R}_I \}) \) correspond to the classical kinetic and potential energy, respectively. Identifying \( \tilde{P}_I = \nabla I S_k = M_i J_k/\rho_k \) as the momentum canonically conjugate to the nuclear coordinates \( \tilde{R}_I \), we may involve Hamilton’s equations \( \dot{R} = \partial H(P, R, t)/\partial P \) and \( \dot{P} = -\partial H(P, R, t)/\partial R \) to obtain \( \dot{\tilde{P}}_I = -\nabla I E_k \). This can be written more suggestively as

\[ M_i \ddot{\tilde{R}}_I = -\nabla I V_{BO}(\tilde{R}_I) \]  

(1.21)

where \( V_{BO} \) is the potential defining the so–called Born—Oppenheimer potential energy
While the Born—Oppenheimer framework affords a dynamics comprising classical nuclei accompanied by quantum mechanical electronic degrees of freedom, the electronic parameters may be neglected to afford a dynamics which is governed entirely by the behavior of classical ions. In this case, the electronic potential is taken to be a series of empirically parameterized functions, termed a force field, which approximate the Born—Oppenheimer surface as $V_{FF}$, an effective potential:

$$V_{FF} = \sum_{I=1}^{N} V_1(R_I) + \sum_{I<J}^{N} V_2(R_I, R_J) + \sum_{I<J<K}^{N} V_3(R_I, R_J, R_K) + \ldots$$  \hspace{1cm} (1.22)

The force field approximation is generates the entire spectrum of dynamical processes in a classical molecular dynamics simulation.

### 1.2 Classical Molecular Dynamics

Classical molecular dynamics [3,5] is a numerically efficient approximation scheme for simulation of condensed phase systems, in particular those for which inherently quantum processes such as chemical reactivity and most polarization effects may be neglected. The scope of this method is best appreciated by excluding what it cannot do, leaving the remainder under its purview. More specifically, within a classical molecular dynamics simulation, physical processes involving bond scission or formation are neglected as a matter of course. This includes common phenomena such as acid–base and solvent proton transfer; hence the contribution of these effects must be accommodated through the mean–field formulation of molecular dynamics parameters. Likewise, electronic charge transfer may not be treated in classical molecular dynamics, as the charge on a given atom in the system is fixed through force field parameters.
at run–time. Most hydrogen bonds are also treated on equal footing, as shifts in electron density due to proximity effects are neglected during a simulation. While some efforts have been made to circumvent these limitations to the classical framework, such as through reactive and polarizable force fields, these techniques remain in their infancy. Nonetheless, outside of these limitations, the only remaining restrictions are largely hardware–based. The aforementioned sacrifices made to utilize this method are nonetheless small for problems falling within the scope of classical molecular dynamics, especially for biomolecular systems. Simulations have grown from tens of amino acids in the first protein molecular dynamics simulation of the Bovine Pancreatic Trypsin Inhibitor (BPTI) \textsuperscript{6} to calculations for activation \textsuperscript{7} of, and unguided ligand binding to, transmembrane systems \textsuperscript{8} on microsecond timescales.

Let us make these notions concrete. Within our context, \textit{classical molecular dynamics} refers to a molecular dynamics scheme in which nuclei are propagated under Newtonian dynamics, with a potential energy term defined by a force field $V_{FF}(\ldots)$. Neglecting the details of the force field for a moment, consider a set of $N$ point particles with positions $\{ \vec{r}_i \}$. Furthermore, assume that the force on a given particle $\vec{F}_i$ is derived from the gradient of the force field potential with respect to the coordinate frame of the $i$–th particle $\vec{F}_i = -\nabla_i V_{FF}(\vec{r}_1, \ldots, \vec{r}_N)$.

The most fundamental consideration underlying a molecular dynamics simulation is how dynamics will be generated and propagated from an initial static configuration of atoms. In the continuum limit, the trajectory of the $i$–th particle is afforded by the integrals of motion

$$\vec{r}_i(t) = \vec{r}_0 + \vec{v}_i t + \frac{1}{2} \vec{a}_i t^2$$  \hfill (1.23)

This elementary relation may be discretized to afford an algorithm propagating the
motion of a particle under molecular dynamics simulation. Assuming a discrete timestep \( \Delta t \) between updates of the trajectory, the simulated motion may be obtained by expanding

\[
\vec{r}_i(t + \Delta t) \approx \vec{r}_i(t) + \Delta t \vec{v}_i(t) + \frac{(\Delta t)^2}{2} \vec{\ddot{r}}_i(t) + \mathcal{O}((\Delta t)^3)
\]

\[
= \vec{r}_i(t) + \Delta t \vec{v}_i(t) + \frac{(\Delta t)^2}{2m_i} \vec{F}_i(t)
\]

(1.24)

(1.25)

where \( m_i \) is the mass of the \( i \)-th particle, \( \vec{v}_i(t) \) its velocity vector, and \( \vec{F}_i = \vec{F}_i/m_i \) the force acting on this particle. Writing a relation for the positions in the preceding timestep

\[
\vec{r}_i(t - \Delta t) = \vec{r}_i(t) - \Delta t \vec{v}_i(t) + \frac{(\Delta t)^2}{2m_i} \vec{F}_i(t)
\]

(1.26)

and adding (1.26) to (1.24) affords

\[
\vec{r}_i(t + \Delta t) = 2\vec{r}_i(t) - \vec{r}_i(t - \Delta t) + \frac{(\Delta t)^2}{2m_i} \vec{F}_i(t)
\]

(1.27)

which defines the integration scheme known as the Verlet algorithm [6]. In practice, it is desirable to obtain particle positions and velocities simultaneously. A simple scheme to accomplish this is obtained by using \( \vec{r}_i(t + \Delta t) \) and \( \vec{v}_i(t + \Delta t) \) to evolve backward to \( \vec{r}_i(t) \) through

\[
\vec{r}_i(t) = \vec{r}_i(t + \Delta t) - \Delta t \vec{v}_i(t + \Delta t) + \frac{(\Delta t)^2}{2m_i} \vec{F}_i(t)
\]

(1.28)

\[
= \vec{r}_i(t + \Delta t) - \Delta t \vec{\ddot{r}}_i(t + \Delta t) + \frac{(\Delta t)^2}{2m_i} \vec{F}_i(t)
\]

(1.29)
Substituting this expression into (1.24) and solving for $\vec{v}_i(t + \Delta t)$ gives

$$
\vec{v}_i(t + \Delta t) = \vec{v}_i(t) + \Delta t \frac{F_i(t) + F_i(t + \Delta t)}{2m_i}
$$

(1.30)

which, together with (1.24), constitute the velocity Verlet scheme [10] for integrating the equations of motion. These integration schemes only explicitly make reference to the force $\vec{F}_i$ acting on a given particle in the simulation. While this derivation assumes that the force arises from a classical force field, no modification is necessary if the force is obtained from a suitable electronic structure method. Accordingly, similar integration schemes are exploited for the classical nuclear propagation conducted in both Born—Oppenheimer and Car—Parrinello molecular dynamics [11,12]. It should be noted that other integration schemes also exist, including those exploiting the symplectic geometry of the underlying dynamical equations. Furthermore, the integration may be extended to include constraints through one of several algorithms [13] such as SHAKE [14] or RATTLE [15]. The velocity Verlet scheme nonetheless remains the most widely employed integration method (Figure 1.1).

1.2.1 Force Fields

As illustrated in (1.22), a force field is a parameterized approximation of the Born—Oppenheimer surface in the vicinity of local extrema [16]. The prototypical example, and that used in this work, is that of the CHARMM (Chemistry at Harvard Molecular Mechanics) force field [17,18]. The CHARMM potential function is defined as follows:
Initialize positions, velocities.

\[ \vec{r}_i(t + \Delta t) = \vec{r}_i(t) + \vec{v}_i \Delta t + \frac{\vec{F}_i(t)}{2m} (\Delta t)^2 \]

Calculate new position using velocities, force from Langevin equation.

\[ \vec{v}_i(t + \Delta t) = \vec{v}_i(t) + \frac{(\vec{F}_i(t + \Delta t) + \vec{F}_i(t))}{2m} (\Delta t)^2 \]

Calculate new velocities using old velocities, force at past and present positions.

\[ t \rightarrow t + \Delta t \]

Repeat process at updated timestep.

Figure 1.1: Flowchart and summary of the velocity Verlet integration algorithm.

\[
\begin{align*}
\vec{F}_i(t) &= -\nabla_i V_{\text{CHARMM}}(\vec{r}_i(t)) \quad (1.31) \\
V_{\text{CHARMM}} &= \sum_{\text{bonds}} k_b (x - x_0)^2 + \sum_{\text{angles}} k_\theta (\theta - \theta_0)^2 + \\
&+ \sum_{\text{dihedrals}} k_\phi [1 + \cos(n\phi - \delta)] + \sum_{\text{impr}} k_\omega (\omega - \omega_0)^2 + \\
&+ \sum_{\text{Urey-Bradley}} k_u (u - u_0)^2 + \\
&+ \sum_{\text{Nonbonded}} \epsilon \left[ \left( \frac{R_{\text{minij}}}{r_{ij}} \right)^{12} - \left( \frac{R_{\text{minij}}}{r_{ij}} \right)^{6} \right] + \sum_{i \neq j} \frac{q_i q_j}{\epsilon r_{ij}} \quad (1.35)
\end{align*}
\]

where each summation is taken over all applicable atoms or groups of atoms in the simulation. The first term in the summation is simply the harmonic approximation of a bond stretching mode, where \(k_b\) is the bond spring constant and \(x_0\) is the equilibrium bond position. By analogy, the second term corresponds to a small-angle harmonic approximation for the angle bending energy of a set of three bonded atoms, where \(k_\theta\)
denotes the bending spring constant and $\theta_0$ the equilibrium angle. A more complicated approximation is utilized for dihedral angles in the third term, as each minimum on the potential energy surface of a rotamer must be parameterized. For this term, $k_\phi$ is the dihedral spring constant and $\delta$ a parameter fixing the location of the dihedral minima. Improper dihedral angles are treated in the fourth term, which correspond to out of plane deformations present in planar and conjugated systems. Yet again, a harmonic approximation is employed. The Urey—Bradley term is associated with 1,3–interactions, which are treated as a virtual, harmonic bond between atoms involved in a given angular configuration with spring constant $k_u$ and equilibrium position $u_0$. The last two terms correspond to nonbonded interactions. The first of these is a Leonard—Jones potential for dispersive, Van der Waals type coupling, and the second term is the usual Coulombic attraction or repulsion between atoms in the simulation. The parameters for this particular force field are generally derived from quantum chemical calculations at the B3LYP/6–31G(d) level or higher. Other widely–used force fields, such as AMBER [19], and OPLS [20][21] exist, and generally offer comparable computational resolution with particular refinements and spectrum of limitations unique to each scheme [22].

1.2.2 Langevin Dynamics

If the Newtownian evolution of a molecular system is simulated using a Verlet–like integration procedure, the behavior of the system is inherently deterministic as proscribed by the initial velocity distribution. Nonetheless, the true physical system is highly stochastic, being subject to heat transfer into the system from the surrounding environment and heat loss from the system through collisional processes. Accordingly, if no further accommodations are made for the purposes of simulation, the model will bear little resemblance to a true system in the thermodynamic limit. A first order
correction to the molecular dynamics scheme is accomplished by adding a combination of damping and random driving forces to the equations of motion. In practice, this problem has been thoroughly studied and a traditional model from the theory of stochastic processes, the *Langevin equation* is employed:

\[
m\ddot{r}_i(t) = -\nabla V(\vec{r}_i(t)) - \gamma m_i \dot{r}_i(t) + R(t)
\]  

(1.36)

where \(\gamma\) is an empirically-derived frictional coupling coefficient and \(R(t)\) is a Gaussian stationary process satisfying \(\langle R(t) \rangle = 0\) and \(\langle R(t)R(t') \rangle = 2\gamma k_BT m_i \delta(t - t')\), where the brackets \(\langle \cdot \rangle\) denote an ensemble average. The first of these restrictions on \(R(t)\) is simply the constraint that the average fluctuations of a Brownian particle in a thermal bath cancel in the long-time regime. The second restriction imposed on the random variable is more interesting, and it is a statement of the fundamental fluctuation–dissipation theorem for Brownian motion. This requirement ensures that the mean square velocity of the particle is determined by equipartition of energy. In particular, imposing the constraint

\[
\lim_{t \to \infty} \langle \dot{\vec{r}}^2 \rangle = \frac{F}{2\gamma}
\]

(1.37)

specifies the random force \(F = 2\gamma k_BT/m\) in a fixed association with the dissipation coefficient \(\gamma\). This, in turn, implies that any initial configuration for the system will evolve into one in thermal equilibrium with the surrounding bath at temperature \(T\) given sufficient simulation time. Accordingly, a balance is established in which energy is drawn out of the system by the viscous coupling \(\gamma\) and replenished by random ‘kicks’ from the thermal force. This thermalization proceeds on a timescale dictated by the solution to the Langevin equation.
\[ \langle \dot{r}^2 \rangle = \frac{k_B T}{m} = \left( \frac{\langle \dot{r}^2 \rangle_0}{k_B T} - \frac{k_B T}{m} \right) e^{-2\gamma t} \]  

(1.38)

An additional benefit to utilizing Langevin equations of motion is the elimination of systematic energy drifts which are observed due to cumulative discretization errors in the rudimentary Newtonian scheme.

1.2.3 Thermostats

While an isolated system will evolve in the microcanonical (NVE) ensemble, we generally wish to simulate a system coupled to a thermal bath and hence study physical phenomena in the canonical (NVT) ensemble. One algorithmic approach to this problem is to add an additional term to the Hamiltonian which controls the kinetic energy of the system and to rescale the momentum degrees of freedom accordingly \[23\][24]. By modulating this parameter, the calculated temperature of the system may be fixed about a given value, simulating the effect of an infinite, thermally rigid bath. This procedure is known as thermostating.

Assume that our simulation system comprises an \( N \)-body classical Hamiltonian

\[ H_N = \sum_{i=1}^{N} \frac{\vec{p}_i^2}{2m_i s^2} + V(\vec{r}_1, \ldots, \vec{r}_N) + \frac{\vec{p}_s^2}{2Q} + gk_B T \ln s \]  

(1.39)

where \((\vec{p}_i, \vec{r}_i, m_i)\) represent the momenta, positions, and masses of the \( i \)-th particle in the system, and \((p_s, s, Q)\) are the fictitious momentum, position, and mass as introduced as components of the thermostatting scheme. As a consequence of this modification, the phase space dimensionality for the canonical distribution is expanded from \( 2dN \) to \( 2dN + 2 \), where \( d \) is the cartesian dimensionality of the simulation space and \( N \) is the number of particles in the system. The last term appearing in (1.39) has the form of a potential of mean force arising from the distribution \( s \), with the
parameter $g$ chosen such that the microcanonical distribution in the extended $2dN + 2$ dimensional phase space determines the canonical distribution in the $2dN$ phase space.

That is, the microcanonical distribution in the $2dN + 2$ space is utilized to mimic a canonical ensemble in a $2dN$-dimensional subset of phase space. \[25\] \[29\].

The partition function arising from the extended Hamiltonian is

\[
\mathcal{Z} = \int d^N \vec{r} d^N \vec{\bar{p}} ds d\bar{s} s^{dN} \delta \left( \sum_{i=1}^{N} \frac{\bar{p}_i^2}{2m_i} + V(\vec{r}) + \frac{\bar{p}_s^2}{2Q} + gKT \ln s - E \right) \quad (1.40)
\]

\[
= \int d^N \vec{r} d^N \vec{\bar{p}} ds d\bar{s} s^{dN} \delta \left( H_{\text{phys}}(\vec{\bar{p}}, \vec{r}) + \frac{\bar{p}_s^2}{2Q} + gKT \ln s - E \right) \quad (1.41)
\]

where $\bar{p}_i = \bar{p}_i / s$ is the rescaled momentum and the definition of $H_{\text{phys}}(\vec{\bar{p}}, \vec{r})$, the physical Hamiltonian, is apparent from the context. Now, given a function $f : \mathbb{R} \rightarrow \mathbb{R}$, with a single zero at $s_0$, the Dirac delta function $\delta(f(s))$ satisfies the identity $\delta(f(s)) = \delta(s - s_0)/|f'(s_0)|$. Accordingly, if $f(s) = H(\vec{r}, \vec{\bar{p}}) + \bar{p}_s^2/2Q + gKT \ln s - E$, we seek solutions such that $f(s_0) = 0$ and hence

\[
s_0 = \exp \left[ \left( E - \frac{\bar{p}_s^2}{2Q} - H(\vec{\bar{p}}, \vec{r}) \right) / gKT \right] \quad (1.42)
\]

Furthermore, $f'(s) = gKT / s$ and hence

\[
\frac{1}{|f(s_0)|} = \frac{1}{gKT} \exp \left[ \left( E - \frac{\bar{p}_s^2}{2Q} - H(\vec{\bar{p}}, \vec{r}) \right) / gKT \right] \quad (1.43)
\]

Accordingly, the partition function becomes
\[ Z = \int d^N \tilde\varphi d^N \tilde p \, ds \, dp_s \, s \, \frac{d^N}{f(s_0)} \delta(s - s_0) \]  

(1.44)

\[ = \int d^N \tilde\varphi d^N \tilde p \, ds \, dp_s \, s \, \frac{d^N}{gKT} \times \]  

(1.45)

\[ \exp \left[ \frac{1}{gKT} \left( E - \frac{p_s^2}{2Q} - H(\tilde p, \tilde r) \right) \right] \delta(s - s_0) \]  

(1.46)

\[ = \frac{1}{gKT} \int d^N \tilde\varphi d^N \tilde p \, dp_s \exp \left[ \frac{d^N + 1}{gKT} \left( E - H(\tilde p, \tilde r) \right) \right] \times \]  

(1.47)

\[ \exp \left[ (dN + 1) \frac{p_s^2}{2QgKT} \right] \]  

(1.48)

where, in the second step, the integral over \( s \) forces the system onto the \( s_0 \) shell. Setting \( g = dN + 1 \) and utilizing the Gaussian identity \( \int_{-\infty}^{\infty} a \exp \left[ -(x + b)^2/c^2 \right] dx = a|c|\sqrt{\pi} \), the \( p_s \) integral is readily performed to afford

\[ Z = \frac{\sqrt{2\pi QKT}}{(dN + 1)KT} \exp \left[ E/KT \right] \int d^N \tilde p \, d^N \tilde r \exp \left[ -\frac{H(\tilde p, \tilde r)}{KT} \right] \]  

(1.49)

The resultant partition function has the form of a canonical distribution, and hence the microcanonical Nosé Hamiltoninan is effectively equivalent to a canonical physical Hamiltonian, via the additional degrees of freedom. Applying Hamilton’s equations of motion to \[ \{1.39\} \], we obtain

\[ \dot{\tilde r}_i = \frac{\tilde p_i}{2m_i s^2} \]  

(1.50)

\[ \dot{\tilde p}_i = -\frac{\partial}{\partial \tilde r_i} U(\tilde r_1, \ldots, \tilde r_N) = \vec{\vec{F}}_i \]  

(1.51)

\[ \dot{s} = \frac{p_s}{Q} \]  

(1.52)

\[ \dot{p}_s = \sum_{i=1}^{N} \frac{p_i^2}{2m_is^2} - \frac{gKT}{s} = \frac{1}{s} \left( \sum_{i=1}^{N} \frac{p_i^2}{2m_is^2} - gKT \right) \]  

(1.53)
These equations may be recast in a more familiar ‘physical’ form, as first suggested by Hoover \[30\] through the change of variables $\tilde{p}_i^t = p_i/s$, $p'_s = p_s/s$, and $dt' = dt/s$ to yield

\[
\begin{align*}
\frac{d\tilde{r}_i}{d\tilde{t}} &= \frac{\tilde{p}_i^t}{m_i} \\
\frac{dp_i^t}{dt} &= \bar{F}_i^t - \frac{s p'_s}{Q} p'_i \\
\frac{ds}{d\tilde{t}} &= -\frac{s^2 p'_s}{Q} \\
\frac{dp'_s}{dt} &= \frac{1}{s} \left[ \sum_{i=1}^{N} \frac{(\tilde{p}_i)^2}{m_i} - gKT \right] - \frac{s (p'_s)^2}{Q}
\end{align*}
\]

which is notable in that these equations no longer define a Hamiltonian dynamical system.

As a consequence of ergodicity, a molecular dynamics simulation employing a Nosé thermostat should be capable of sampling the entire canonical distribution. Nonetheless, non-ergodic behavior is observed in several systems, including those containing simple harmonic oscillator potentials \[30\] This condition arises as a consequence of additional conservation laws and, accordingly, conserved quantities, which have been introduced to the Hamiltonian system. To circumvent this limitation, a given Nosé thermostat may itself be thermostated by an additional thermostat degree of freedom. This auxiliary thermostat may be coupled to another thermostat in turn, and the process repeated to construct a Nosé—Hoover chain thermostat with two additional phase space dimensions for each conservation law \[31\].
1.3 Car—Parrinello Molecular Dynamics

The Car—Parrinello (CP) molecular dynamics \[^{32}\] method, and other similar extended-Lagrangian methods, \[^{33}\] were developed to incorporate electronic structure theory into a molecular dynamics framework with an efficiency exceeding that of Born—Oppenheimer molecular dynamics (BOMD). Similar to Born—Oppenheimer calculations, forces acting on the nuclei are calculated using an electronic structure method, generally taken to be density functional theory. However, as opposed to diagonalizing the system at each timestep, the Car—Parinello scheme introduces a fictitious classical dynamics for the electronic degrees of freedom. The net effect of this modification is that the system can be evolved from timestep to timestep, with the Lagrangian constraints restricting the configuration to the Born—Oppenheimer surface. Accordingly, the electronic structure problem need not be solved at every step, thereby drastically reducing both memory consumption and computational overhead. While this formulation appears empirical or \textit{ad hoc}, a rigorous mathematical foundation has been rigorously established \[^{34}\,^{37}\].

Consider a configuration consisting of \(N_e\) electrons and \(N\) nuclei, in which the nuclear and electronic degrees of freedom are decoupled under the adiabatic approximation. This system partitions into either a set of \(n = N_e/2\) or \(n = N_e\) orbitals, corresponding to a closed– or open–shell electronic configuration, respectively. Denoting the electronic wavefunction for the \(i\)-th orbital at position \(\vec{r}\) and time \(t\) by \(\psi_i(\vec{r}, t)\) and the position of the \(I\)-th nucleus by \(\vec{R}_I(t)\), the CP Lagrangian may be written:
\[
\mathcal{L} = Q \sum_{i=1}^{n} \int d\vec{r} \dot{\psi}_i^* (\vec{r}, t) \dot{\psi}_i (\vec{r}, t) + \frac{1}{2} \sum_{i=1}^{N} M_i \ddot{R}_i(t)^2 \\
- E \left[ \{ \psi (\vec{r}, t) , \bar{R}(t) \} \right] + \sum_{ij} \Lambda_{ij} \left( \int d\vec{r} \psi_i^* (\vec{r}, t) \psi_j (\vec{r}, t) - f_i \delta_{ij} \right)
\]

(1.58)

(1.59)

where \( Q \) is the mass associated with the fictitious electronic degree of freedom, \( M_i \) is the mass of the \( I \)-th nucleus, \( E \left[ \{ \psi (\vec{r}, t) , \bar{R}(t) \} \right] \) is taken to be the Kohn—Sham energy functional, \( f_i \) is the occupancy of the \( i \)-th orbital, and \( \Lambda_{ij} \) is a Lagrange multiplier. The first term in (1.58) is a fictitious ‘kinetic energy’ term for the electronic subsystem, with the provision that \( \psi_i(\vec{r}, t) \) is treated as a complex scalar field. It should be noted that this kinetic energy has no relation to any physical observable. The “mass” parameter, which has units of energy \( \times \) time\(^2\), is employed to explicitly enforce the adiabatic decoupling of the electronic system from the ionic degrees of freedom. Conversely, the second term in the Car—Parrinello Lagrangian corresponds to the familiar physical kinetic energy of the nuclear degrees of freedom. The last term is a holonomic constraint utilized to enforce orthornormality of the orbital scheme. Note that this particular constraint term is only relevant to electronic structure theories described by an effective single—body Hamiltonian, such as density functional theory [38,39]. Applying the variational principle to the Car—Parrinello Lagrangian through Euler—Lagrange equations

\[
\frac{d}{dt} \frac{\delta \mathcal{L}}{\delta \dot{\psi}_i^* (\vec{r}, t)} - \frac{\delta \mathcal{L}}{\delta \psi_i^* (\vec{r}, t)} = 0
\]

(1.60)

\[
\frac{d}{dt} \frac{\delta \mathcal{L}}{\delta \ddot{R}_i(t)} - \frac{\delta \mathcal{L}}{\delta \dot{R}_i(t)} = 0
\]

(1.61)
affords equations of motion for the electronic and nuclear degrees of freedom:

\[
Q \ddot{\psi}_i(\vec{r}, t) = -\frac{\delta E}{\delta \psi^*_i(\vec{r}, t)} + \sum_j \Lambda_{ij} \psi_j(\vec{r}, t) \tag{1.62}
\]

\[
M_I \ddot{R}_I(t) = -\frac{\delta E}{\delta R_I(t)} \tag{1.63}
\]

It is apparent from (1.62) that the electronic and nuclear systems evolve collectively under the influence of the Kohn—Sham energy, thereby coupling the two collective degrees of freedom. The electronic evolution equation, however, differs in that it contains a constraint force term arising from the Lagrange multiplier constraint term in the Lagrangian. A further provision of this construction is that variation of the energy with respect to the electronic degree of freedom (electronic wavefunction) affords a direct relation to the Hamiltonian, \( \hat{H} \) of the corresponding electronic structure method:

\[
\frac{\delta E}{\delta \psi^*(\vec{r}, t)} = -f_i \hat{H} \psi_i(\vec{r}, t) \tag{1.64}
\]

which evolves under the influence of the Kohn—Sham energy.

1.3.1 Constraints and Invariants of the CP Equations.

Assume that the energy functional appearing in the CP Lagrangian is of the Kohn—Sham form appearing in density functional theory
\[ E \left[ \{ \psi_i(\vec{r}), \vec{R}_I(t) \} \right] = \sum_i f_i \int d\vec{r} \, \psi_i^* \left( -\frac{1}{2} \nabla^2 \right) \psi_i(\vec{r}) + \frac{1}{2} \int \int d\vec{r}_1 \, d\vec{r}_2 \frac{\rho(\vec{r}_1)\rho(\vec{r}_2)}{|\vec{r}_1 - \vec{r}_2|} \] 
\[ + \int d\vec{r} \, E_{xc} [\rho(\vec{r})] \rho(\vec{r}) + E_{\text{ext}} \left( [\rho], \{ \vec{R}_I \} \right) + \frac{1}{2} \sum_{i \neq j} Z_i Z_j |\vec{R}_I - \vec{R}_J| \] 

where the explicit \( t \)-dependence of \( \psi(\vec{r}, t) \) has been suppressed. Note that, in this case, \( E_{\text{ext}} \) is the electron–nuclear interaction term and \( E_{xc} \) is the LDA or GGA representation of the exchange and correlation energy. In terms of the orbital wavefunctions, the density functional assumes the form \( \rho(\vec{r}) = \sum_i f_i \psi_i^*(\vec{r})\psi_i(\vec{r}) \), and hence represents an orbital occupation density. By virtue of equation (1.60), variation with respect to \( \psi_i(\vec{r}) \) yields the Kohn–Sham Hamiltonian:

\[ \hat{H} = -\frac{1}{2} \nabla^2 + \int d\vec{r} \frac{\rho(\vec{r})}{|\vec{r} - \vec{r}'|} + E_{xc} [\rho(\vec{r})] + \rho(\vec{r}) \frac{dE_{xc}}{d\rho(\vec{r})} + \frac{\delta E_{\text{ext}}}{\delta \rho(\vec{r})}. \] 

It is prudent to make some observations regarding this framework. In analogy to the behavior of a classical molecular dynamics simulation, there is a conserved energy [11] for the CP system:

\[ E_c = Q \sum_i \int d\vec{r} \, \dot{\psi}_i(\vec{r}) \dot{\psi}_i(\vec{r}) + \frac{1}{2} \sum_i M_i \dot{\vec{R}_I^2} + E \left[ \{ \psi_i(\vec{r}), R_I \} \right] \] 

To demonstrate this it is necessary to show that \( E_c \) is stationary in time. Accordingly,
differentiate each term of (1.70) with respect to time to yield

\[
\frac{d}{dt} \left[ Q \sum_i \int d\vec{r} \dot{\psi}^*_i(\vec{r}) \psi_i(\vec{r}) \right] = Q \sum_i \int d\vec{r} \left[ \dot{\psi}^*_i(\vec{r}) \psi_i(\vec{r}) + \ddot{\psi}^*_i(\vec{r}) \dot{\psi}_i(\vec{r}) \right] \quad (1.71)
\]

\[
\frac{d}{dt} \left[ \frac{1}{2} \sum_I M_I \dot{r}_I^2 \right] = \sum_I M_I \dot{r}_I \ddot{r}_I \quad (1.72)
\]

While the energy term appearing in (1.70) has the total time derivative

\[
\frac{d}{dt} E[\psi^*, \psi, \dot{\vec{r}}] = \sum_i \int d\vec{r} \left[ \frac{\delta E}{\delta \psi^*_i(\vec{r}, t)} \dot{\psi}^*_i(\vec{r}, t) + \frac{\delta E}{\delta \psi_i(\vec{r}, t)} \dot{\psi}_i(\vec{r}, t) \right] + \sum_I \frac{\partial E}{\partial \dot{R}_I} \ddot{R}_I = 0 \quad (1.73)
\]

The time derivative of the first term appearing in (1.70), given by (1.71), can be rewritten using the equation of motion for the CP fields (suppressing functional dependence on \((\vec{r}, t)\)):

\[
Q \int d\vec{r} \dot{\psi}^*_i \dot{\psi}_i = \int d\vec{r} \left[ \dot{\psi}^*_i \left( -\frac{\delta E}{\delta \psi^*_i} + \sum_j \Lambda_{ij} \psi_j \right) \right] + \int d\vec{r} \left[ \dot{\psi}_i \left( -\frac{\delta E}{\delta \psi_i} + \sum_j \Lambda_{ij} \psi^*_j \right) \right] \quad (1.74)
\]

\[
= \int \left[ -\frac{\delta E}{\delta \psi^*_i} \dot{\psi}^*_i - \frac{\delta E}{\delta \psi_i} \dot{\psi}_i \right] d\vec{r} + \sum_j \lambda_{ij} \int \left[ \dot{\psi}^*_i \psi_j + \psi^*_i \dot{\psi}_j \right] d\vec{r} \quad (1.75)
\]

Note that the first term appearing in the final equality will cancel against the corresponding term in the total derivative (1.73). A bonus constraint is derived from
the $t = 0$ normalization condition employed in the CP scheme. Differentiating the orthnormality condition with respect to time

$$\frac{d}{dt} \int d\vec{r} \psi_i^*(\vec{r}) \psi_j(\vec{r}) = \frac{d}{dt} \delta_{ij}$$

provides the identity

$$\int d\vec{r} \left[ \dot{\psi}_i^*(\vec{r}) \psi_j(\vec{r}) + \psi_i^*(\vec{r}) \dot{\psi}_j(\vec{r}) \right] = 0$$

and thus demonstrating that the last term in (1.74) is identically zero. The term appearing in (1.72) affords, using the nuclear equation of motion $M_I \ddot{\vec{R}}_I = -\partial E/\partial \vec{R}_I$, a direct expression

$$\sum_i M_I \dddot{\vec{R}}_I = \sum_i \ddot{\vec{R}}_I \left( -\frac{\partial E}{\partial \vec{R}_I} \right)$$

which again cancels with the last remaining term in (1.73), thereby demonstrating that the sum of time derivatives of (1.70) vanish and hence that the energy $E_c$ is a conserved quantity.

A second and essential consideration in CP simulations is enforcement of the orthonormality constraint for the electronic degrees of freedom. This condition is necessary for the system to remain on the Born—Oppenheimer surface, and hence to avoid diagonalizing the Hamiltonian at every timestep or for each nuclear configuration of the system. To see how this is done, note that the variational principle for Kohn—Sham density functional theory postulates that
\[
\frac{\delta E}{\delta \psi_i^*(\vec{r}, t)} = -f_i \dot{\hat{H}} \psi_i(\vec{r}, t)
\]  

(1.82)

and hence the orbital equation of motion for the electronic degrees of freedom may be written as

\[
Q|\ddot{\psi}_i(t)\rangle = -f_i \hat{H}_{KS}|\psi_i(t)\rangle + \sum_j \Lambda_{ij}|\psi_j(t)\rangle.  
\]  

(1.83)

To enforce the constraint, it is necessary to utilize the second time derivative of the constraint equation

\[
\frac{d^2}{dt^2} \int d\vec{r}(\psi^i)^* \psi_j = \frac{d}{dt} \int d\vec{r} \left( \dot{\psi}_i^* \psi_j + \psi_i^* \dot{\psi}_j \right) = \int d\vec{r} \left( \ddot{\psi}_i^* \psi_j + 2\dot{\psi}_i^* \dot{\psi}_j + \psi_i^* \ddot{\psi}_j \right) = 0. 
\]  

(1.84) \hspace{1cm} (1.85) \hspace{1cm} (1.86)

It is more convenient to rewrite the integrals in this expression as inner produces through bra–ket notation as

\[
\langle \ddot{\psi}_i | \psi_j \rangle + 2\langle \dot{\psi}_i | \dot{\psi}_j \rangle + \langle \psi_j | \ddot{\psi}_j \rangle = 0.
\]  

(1.87)

Inserting \([1.87]\) into \([1.83]\) these inner products may be expressed explicitly in terms of the dynamical orbital equations.
\[ \langle \ddot{\psi}_i | \psi_j \rangle = -\frac{f_i}{Q} \langle \psi_i | \hat{H} | \psi_j \rangle + \sum_k \Lambda_{ik} \langle \psi_k | \psi_j \rangle \] (1.88)

\[ \langle \psi_i | \ddot{\psi}_j \rangle = -\frac{f_j}{Q} \langle \psi_i | \hat{H} | \psi_j \rangle + \sum_k \Lambda_{jk} \langle \psi_i | \psi_k \rangle \] (1.89)

which simplify by using the normalization condition \( \langle \psi_i | \psi_j \rangle = \delta_{ij} \)

\[ \langle \ddot{\psi}_i | \psi_j \rangle = -\frac{f_i}{Q} \langle \psi_i | \hat{H} | \psi_j \rangle + \Lambda_{ij} \] (1.90)

\[ \langle \psi_i | \ddot{\psi}_j \rangle = -\frac{f_j}{Q} \langle \psi_i | \hat{H} | \psi_j \rangle + \Lambda_{ij} \] (1.91)

where we have exploited the fact that \( \Lambda_{ij} \) is symmetric. At this point the Lagrange multiplier constraint has been formally introduced by construction. From the preceding relations it is clear that (1.87) is identical to

\[ \Lambda_{ij} = \frac{f_i + f_j}{2} \langle \psi_j | \hat{H} | \psi_i \rangle - Q \langle \dot{\psi}_j | \dot{\psi}_j \rangle \] (1.92)

which, when substituted into (1.83) provides an equation which may be practically used to generate constrained electronic dynamics during a CP calculation

\[ Q | \ddot{\psi}_i \rangle = -f_i \hat{H} | \psi_i \rangle + \sum_k f_{jk} |\psi_k \rangle \langle \psi_k | \hat{H} | \psi_i \rangle - Q \sum_k |\psi_k \rangle \langle \dot{\psi}_k | \dot{\psi}_i \rangle \] (1.93)

where the constraint matrix is defined as \( f_{ij} = (f_i + f_j)/2 \). In practice, this scheme is far more efficient than directly enforcing the Lagrange multipliers which are formally present in the CP Lagrangian.
1.3.2 Electronic Thermostatting

While the nuclear degrees of freedom in the CP scheme may be thermostatted using classical Nose—Hoover chains, as the nuclear dynamics are inherently classical, thermostatting of electronic degrees of freedom requires additional care [11]. In practice, this is done by modifying the CP equations of motion to include a fractional damping term

\[
Q |\dot{\psi}\rangle = -f_i \dot{H} |\psi_i\rangle + \sum_j \Lambda_{ij} |\psi_j\rangle - Q \dot{\eta} |\psi_i\rangle \tag{1.94}
\]

\[
M_i \ddot{R}_i = \ddot{F}_i - M_i \ddot{\xi} \ddot{R}_i \tag{1.95}
\]

where we have written the electronic equation of motion in terms of a formal expression containing the Lagrange multiplier constraint constraint for brevity. In this case, the electronic and classical thermostat variables are denoted by \( \eta \) and \( \xi \), respectively. The electronic thermostat evolves under the equation of motion

\[
Q_e \ddot{\eta} = 2 \left[ Q \sum_i \langle \psi_i | \dot{\psi}_i \rangle - E_e \right] \tag{1.96}
\]

where \( Q_e \) is the electronic thermostat mass. The first term on the right hand side is effectively the electronic kinetic energy for the fictitious degree of freedom, and the second term is the target kinetic energy chosen for this subsystem. The nuclear equations of motion have a similar structure

\[
Q_R \ddot{\xi} - \left[ \sum_i M_i \dddot{R}_i^2 - g k_b T \right] \tag{1.97}
\]

where \( Q_R \) is the classical thermostat mass, the first term on the right side is the
classical ionic kinetic energy, and the second is the effective energy of the ionic thermal bath. Ultimately, the entire scheme affords a kinetic energy which is constructed to give the appropriate equations of motion and invariants under variation, as discussed in the preceding section:

\[
E = Q \sum_i \langle \dot{\psi}_i | \dot{\psi}_i \rangle + \frac{1}{2} \sum_i M_i \ddot{R}_i^2 + E \left[ \{ \psi_i \}, \{ \tilde{R}_i \} \right] + \frac{1}{2} Q_e \dot{\eta}^2 + \frac{1}{2} Q_R \dot{\xi}^2 + 2 E_e \eta + g k_b T \xi
\]  
(1.98)

If greater numerical stability is required, these may be extended to chain thermostats as in the classical case. It should be noted that, an ideal choice of mass parameters should have a maximal overlap of their power spectra with that of the thermostated subsystems. This imposes the relation \( Q = g k_b T / \omega_n^2 \), where \( \omega_n \) is a typical vibrational frequency for the nuclear subsystem [11][12][10].

1.3.3 Control of a Car—Parrinello Simulation

The Car—Parrinello method is more sensitive than classical molecular dynamics calculations, largely due to complications introduced by the electronic degrees of freedom [1][11]. In addition to the conserved energy \( E_e \) that was introduced earlier, there are several other interesting quantities including a physical energy \( E_{\text{phys}} \), an electronic ‘potential’ energy \( V_e \), and the electronic ‘kinetic’ energy \( T_e \)

\[
E_{\text{phys}} = \sum_i \frac{1}{2} M_i \ddot{R}_i^2 + \langle \Psi_0 | \hat{H} | \Psi_0 \rangle = E_{\text{cons}} - T_e
\]  
(1.100)

\[
V_e = \langle \Psi_0 | \hat{H} | \Psi_0 \rangle
\]  
(1.101)

\[
T_e = Q \sum_i \langle \dot{\psi}_i | \dot{\psi}_i \rangle
\]  
(1.102)
In a functional CP simulation, the conserved energy $E_c$ will generally remain stable with extremely small relative variations and little to no drift of the mean value. The potential energy $V_e$ is found to fluctuate in time, with at least some consistent oscillatory behavior to phonon modes in the system. For all intents and purposes the physical energy $E_{phys}$ will remain constant on the simulation timescale, and accordingly this parameter may be used to characterize the energetic state of the system. The most highly variable terms are those associated with the electronic degree of freedom, in particular its kinetic energy $T_e$. This value should oscillate during the course of the simulation, however, it should remain fixed with oscillation about a specific value. A drift in this parameter indicates that the adiabatic separation between electronic and ionic degrees of freedom has been broken, and accordingly that energy transfer is occurring between these subsystems.

The adiabatic separation, and hence restriction to the Born—Oppenheimer surface, is accomplished through a judicious choice of the electronic mass parameter, $Q$. Expanding the orbital vibrational spectrum to second order about the ground state minimum affords a relation between the occupied—unoccupied orbital gap of Kohn—Sham orbitals and the eigenfrequencies $\omega_{ij}$

$$\omega_{ij} = \left(\frac{2(E_i - E_j)}{Q}\right)^{1/2}$$  \hspace{1cm} (1.103)

where $E_j$ is the occupied and $E_i$ the unoccupied energy eigenvalues. The lowest possible such frequency is given by the splitting $E_{gap}$ between the highest occupied molecular orbital and the lowest unoccupied molecular orbital

$$\omega_{e\text{min}} \propto \left(\frac{E_{gap}}{Q}\right)^{1/2}. \hspace{1cm} (1.104)$$

To maintain adiabatic decoupling, the gap between electronic and nuclear phonon
frequencies, \( \omega_n^{\text{min}} - \omega_n^{\text{max}} \) should be as large as possible. While \( E_{\text{gap}} \) and \( \omega_n^{\text{max}} \) are fixed by the system under consideration, the mass parameter \( Q \) remains tunable. As the fictitious mass is decreased, thereby increasing the adiabatic gap, the maximum timestep likewise decreases as

\[
\Delta t^{\text{max}} \propto \left( \frac{Q}{E_{\text{cut}}} \right)^{1/2} \tag{1.105}
\]

where \( E_{\text{cut}} \) is the planewave cutoff. While the mass value may be taken to be small, thereby isolating each degree of freedom, places a natural restriction on the simulation timescale which may be accessed for a given degree of computational effort. An additional concern occurs for metallic systems, in which case \( E_{\text{gap}} = 0 \) and the frequency gap vanishes, thereby eliminating adiabicity. Nonetheless, this limitation may be overcome with the use of Nose—Hoover chain thermostats [1][41].

1.4 Free Energy Methods

In each segment of this dissertation, it is necessary to calculate free energy surfaces for direct characterization of a physical process or for the parameterization of subsequent calculations. The choice of a particular method is highly context-dependent, and accordingly several distinct techniques have been exploited. These techniques are briefly reviewed in this section.

1.4.1 Steered Molecular Dynamics and the Jarzynski Equality

Steered molecular dynamics (SMD) is a simulation methodology in which a given atom or set of atoms is ‘pulled’ by a virtual spring during a molecular dynamics simulation. As a consequence, physical processes and conformational changes which are not typically observable on equilibrium molecular dynamics timescales may be
probed. Furthermore, the force on the spring may be observed, providing a estimator for the work performed during this process. Through use of the Jarzynski equality, this nonequilibrium work profile may be exploited to calculate the corresponding free energy change for the process. The Jarzynski equality itself may be derived from a minimum of physical assumptions, and will feature prominently in the subsequent steered molecular dynamics calculations.

Let $f(\vec{x}, t)$ represent a classical phase–space distribution function, such that $f(\vec{x}, t)$ is a solution to the Liouville equation

$$\frac{\partial}{\partial t} f(\vec{x}, t) = \hat{L}_t f(\vec{x}, t)$$

(1.106)

where $\vec{x}$ is a phase space volume element $x(q_1, q_2, \ldots, q_{N/2}, p_{N/2+1}, \ldots, p_N)$. For a diffusive dynamical process in which the generalized forces on the system are given by the gradient of a potential $F_i = - (\partial / \partial x_i) V(x_i)$, the Liouville evolution operator $\hat{L}_t$ assumes the form $\hat{L}_t = D \nabla \exp[-\beta V(x, t)] \nabla \exp[\beta V(x, t)]$, where $D$ is a diffusion constant. If we take the distribution function $f(\vec{x}, t)$ to be probability distribution $p(x, t|x_0, 0)$ giving the amplitude for evolution of the phase space volume element along a streamline from $(x_0, 0) \rightarrow (x, t)$, the Liouville equation becomes the Smoluchowski equation for a diffusive system. A convenient choice of distribution, which will be relevant to all subsequent discussion, is that of the Boltzmann distribution

$$p(x, t) = \frac{\exp[-\beta H(x, t)]}{\int dx' \exp[-\beta H(x', 0)]}$$

(1.107)

This distribution is readily verified to be a stationary point of the Liouville operator, and hence $\hat{L}_t p(x, t) = 0$. Taking the partial derivative of $p(x, t)$ with respect to time and inserting this null quantity affords the sink equation
\[
\frac{\partial}{\partial t} p(x, t) = -\beta \frac{\partial H(x, t)}{\partial t} p(x, t) \quad (1.108)
\]

\[
= \mathcal{L}_t p(x, t) - \beta \frac{\partial H(x, t)}{\partial t} p(x, t) \quad (1.109)
\]

This property is particularly important, as this condition is sufficient to asserts that the distribution may be expressed as an Euclidean path integral via the Feynman—Kac theorem:

\[
p(x, t) = \frac{\exp[-\beta H(x, t)]}{\int dx' \exp[-\beta H(x', 0)]} \quad (1.110)
\]

\[
= \langle \delta(x - x') \exp \left[ -\beta \int_0^t \frac{\partial H}{\partial t'} (\vec{x}_{t'}, t') dt' \right] \rangle \quad (1.111)
\]

As usual, the average \( \langle \cdot \rangle \) is taken over an ensemble of trajectories branching from \((x_0, 0) \rightarrow (x_t, t)\). Note that the argument of the exponential function is simply the net, external work performed along one of these trajectories

\[
W(t) = \int_0^t \frac{\partial H}{\partial t'} (\vec{x}_{t'}, t') dt' \quad (1.112)
\]

Substituting this quantity into (1.110) and integrating over both sides of the equation, we obtain

\[
\exp[-\beta \Delta A(t)] = \frac{\int \exp[-\beta H(x, t) dx]}{\int \exp[-\beta H(x, 0) dx']} \quad (1.113)
\]

\[
= \langle \exp[-\beta W(t)] \rangle \quad (1.114)
\]
This equality, denoted the Jarzynski equality (JE), relates the exponentially weighted work performed along a trajectory $W(t)$ to the free energy change between endpoints, $\Delta A(t)$. Note that this equality holds true for an equilibrium or a nonequilibrium process, as well as for both Hamiltonian and non-Hamiltonian dynamical systems. This latter fact is particularly important, as most thermostatting schemes employed in molecular dynamics simulation do not maintain the volume of a phase space element under time evolution. These properties are not apparent from the preceding derivation, based on the work of Hummer and Szabo, however, this derivation underscores the mathematical relationship of this technique to the others exploited in this work.

Since equilibrium and nonequilibrium processes are placed on equal footing, JE calculations are particularly amenable to simulations such as steered molecular dynamics (SMD). During constant velocity SMD (cvSMD) simulations, steered atoms are constrained in the direction of the reaction coordinate $\xi$ by applying a harmonic potential between their center of mass and a fictitious particle (Figure 1.2). The fictitious particle is pulled along the reaction coordinate at constant velocity, $v$, and the resultant
force profile measured. Accordingly, a series of cvSMD calculations provide an ideal ensemble for free energy calculations using the JE estimator, with the corresponding work readily calculated from the force profile along the trajectory.

In this case, the Hamiltonian for the constrained system is given by

\[ H(\vec{r}, t) = H_0(\vec{r}) + \frac{k}{2} \left[ (\vec{r}(t) - \vec{r}_0) \cdot \hat{\xi} - \nu t \right]^2 \]  

(1.115)

where \( H_0(\vec{r}) \) is the Hamiltonian in the absence of the external potential, \( k \) is the spring constant for the harmonic constraint, \( \vec{r}_0 \) is the initial position of the constrained atoms, \( \vec{r}(t) \) is the position at time \( t \), and \( \hat{\xi} \) a unit vector along the reaction coordinate. In the course of the simulation, the net force \( \vec{F} \) on the center of mass is measured and the net external work calculated by integration along the reaction coordinate

\[ W(t) = \int_0^t \vec{F}(t') \cdot \vec{v} dt'. \]

This net work contains a contribution due to the harmonic constraint from the spring; however, we are interested in the energy landscape in the absence of the biasing force. Accordingly, the quantity of interest is the unperturbed work, defined as the difference between the net work and the biasing potential for the mass–spring system, \( W'(t) = W(t) - \frac{k}{2} \left[ (\vec{r}(t) - \vec{r}_0) \cdot \hat{\xi} - \nu t \right]^2 \). This quantity may be used directly in JE calculations.

Due to the exponential average, the JE is highly sensitive to small values of the work, substantially biasing the calculation in the case of limited numerical precision. If the work distribution is highly Gaussian, as for a sufficiently stiff spring (high \( k \)), the JE may be expanded (exactly) as a second–order cumulant\[48\]:

...
\[
\Delta A = -\frac{1}{\beta} \log \left[ \frac{1}{N} \sum_{i=1}^{N} e^{-\beta W'(t)} \right] \\
= -\frac{1}{\beta} \log \langle e^{-\beta W'(t)} \rangle \\
\approx \langle W'(t) \rangle - \frac{\beta}{2} \left[ \langle W'(t)^2 \rangle - \langle W'(t) \rangle^2 \right].
\]

Furthermore, it has been shown that the PMF obtained from SMD simulation is more accurate when computed from a smaller number of trajectories with a smaller steering velocity, versus a large number of trajectories with a large steering velocity \cite{48,49}. In this manner, it is possible to circumvent nonequilibrium effects, which require relaxation on timescales greater than the simulation.

1.4.2 Thermodynamic Integration, The Blue Moon Ensemble, and Adaptive Biasing Force Calculations

A second family of free energy estimators are based on the notion of thermodynamic integration. This technique affords the work performed in a thermodynamic process when the the mean mechanical force acting on the system is known at several intermediate steps. Two specific variants of thermodynamic integration are developed, in particular the adaptive biasing force method and blue moon ensemble sampling.

Potentials of Mean Force and Thermodynamic Integration

Assume that we are simulating a thermodynamic system consisting of \( N \) particles within the canonical (NVT) ensemble. In this case, the partition function for the system may be written
\[ \mathcal{Z} = N \int d^{3N} p_i d^{3N} q_i \exp \left[ -\beta \left( \sum_i \frac{p_i^2}{2m_i} + V(\xi, q_1, \ldots q_N) \right) \right] \]  

(1.119)

where the coordinate \( \xi \) has been distinguished as a parameter along which the thermodynamic properties of the system will be calculated. Restriction along this parameter, termed a 'reaction coordinate,' 'order parameter,' or 'collective variable' is accomplished by imposing either holonomic constraints or constraint forces within the simulation. This is equivalent to confining the system to sample a constant \( \xi \) region of phase space. This partition function may be associated with a free energy \( \mathcal{A}(\xi) \) through the usual prescription

\[ \mathcal{A}(\xi) = -\frac{1}{\beta} \log \mathcal{Z} \]  

(1.120)

and hence \( \mathcal{A}(\xi) \) is an effective free energy within this restricted phase–space volume. Taking the partial derivative of \( \mathcal{A}(\xi) \) with respect to \( \xi \) gives

\[ \frac{\partial}{\partial \xi} \mathcal{A}(\xi) = -\frac{1}{\beta} \frac{\partial \mathcal{Z}}{\partial \xi} \]  

(1.121)

\[ = -\frac{1}{\beta} \mathcal{Z} N \int d^{3N} p_i d^{3N} q_i \left( -\beta \frac{\partial V}{\partial \xi} \right) \times \]  

(1.122)

\[ \exp \left[ -\beta \left( \sum_i \frac{p_i^2}{2m_i} + V(\xi, \ldots) \right) \right] \]  

(1.123)

\[ = \left\langle \frac{\partial V}{\partial \xi} \right\rangle \]  

(1.124)

where in the last line the expression is written as an average of the potential energy in the canonical ensemble. Integrating from \( \xi_0 \) to \( \xi_1 \) along the constrained reaction coordinate affords the effective free energy change \( \Delta \mathcal{A}_{0 \to 1} \) associated with evolution through this constrained thermodynamic process.
\[ \Delta A_{0\rightarrow1} = \int_{\xi_0}^{\xi_1} \frac{dA(\xi)}{d\xi} d\xi. \]  
(1.125)

(1.126)

Within the context of a molecular dynamics simulation, it is possible for define a force \( F_\xi \) acting on a particle (or set of particles) at a given point along the reaction coordinate, \( \xi \). Provided that the force is defined in terms of a potential \( V(\xi) \) from the molecular dynamics force field, \( F_\xi = \partial V(\xi)/\partial \xi \). If the system is then sorted into bins along \( \xi \) during the simulation and an ensemble average performed over this force, it is readily demonstrated that

\[ F_\xi = \frac{dA_\xi}{d\xi} = \langle \frac{\partial V(\xi)}{\partial \xi} \rangle. \]  
(1.127)

Integrating from \( \xi_0 \) to \( \xi_1 \) along the reaction coordinate affords the effective free energy change \( \Delta A_{0\rightarrow1} \) associated with evolution of the system in terms of an observable simulation parameter

\[ \Delta A_{0\rightarrow1} = \int_{\xi_0}^{\xi_1} \frac{dA(\xi)}{d\xi} d\xi \]  
(1.128)

\[ = \int_{\xi_0}^{\xi_1} \langle F_\xi \rangle d\xi. \]  
(1.129)

This procedure is known as thermodynamic integration (TI). While this method affords an effective free energy \( \Delta A \) for the \( \xi_0 \rightarrow \xi_1 \) process, termed a potential of mean force, there are several caveats. First, the imposition of constraints which restrict the simulation to lie along \( \xi \) reduce the phase space volume which is sampled during the
simulation. Accordingly, phase space averages are performed over a restricted subset of the total phase space, and hence observable thermodynamic parameters may not be accurately calculated. Furthermore, since these constraints restrict the phase space trajectories which may be sampled during the simulation, kinetically competent paths between regions may be ignored. As a consequence, the significant regions of phase space may be unaccessible with reasonable simulation timescales provided a given set of initial conditions.

Adaptive Biasing Force Calculations

The *adaptive biasing force* (ABF) method \[50\, \text{[51]}\] was developed to circumvent the shortcomings inherently present in thermodynamic integration from its restrictive underlying approximations. Within the context of ABF calculations, define the potential of mean force \( \mathcal{A}(\xi) \) along a reaction coordinate \( \xi \) as

\[
\mathcal{A}(\xi) = -\frac{1}{\beta} \log P(\xi) + \mathcal{A}_0
\]

(1.130)

where \( P(\xi) \) is the probability density for observing the system at \( \xi \) in phase space and \( \mathcal{A}_0 \) is a constant fixed by boundary conditions. The change of notation for the distribution function is intentional. When deriving TI, it is assumed that the partition function is of a form in which potential and kinetic terms are separated. However, in a numerical simulation, it is likely that the sampled partition function is not a separable partition function of the canonical ensemble. In particular, it is possible that the distribution could be of the form

\[
P_\xi = \mathcal{N} \int \delta [\xi - \xi(\vec{x})] \exp \left[ -\beta H(\vec{x}, \vec{p}_x) \right] d\vec{x} d\vec{p}_x
\]

(1.131)
where $\vec{x}$ and $\vec{p}_x$ are cartesian simulation coordinates. In this case, the kinetic and potential terms are interwoven through our definition of the coordinate system. It is always possible to perform a change of basis to generalized coordinates, where the transformation is given by the Jacobian $J_{ij} = \partial x_i / \partial y_j$, where $y = (\xi, q_1, \ldots, q_{3N-1})$.

With this transformation, the distribution function may be brought into separable form

$$P_\xi = \mathcal{N} \left( \int dq_i \det(\hat{J}) \exp[-\beta V(\xi, q_i)] \right) \left( \int dp_x \exp[-\beta T(p_x)] \right)$$

(1.132)

Following [1.121] it may be shown that

$$\frac{\partial}{\partial \xi} P_\xi = \mathcal{N} \left( \int dq_i \left[ -\beta \det(\hat{J}) \frac{\partial V(\xi, q_i)}{\partial \xi} + \frac{\partial}{\partial \xi} \det(\hat{J}) \right] \exp[-\beta V(\xi, q_i)] \right)$$

$$\times \left( \int dp_x \exp[-\beta T(p_x)] \right).$$

(1.133)

As in the formulation of thermodynamic integration, it is clear that the kinetic term vanishes in computation of the averages. Accordingly, this term will be suppressed in further steps. Introducing the dummy variable $\xi^*$ through a delta function and integration

$$\frac{\partial}{\partial \xi} P_\xi = \mathcal{N} \int dq_i \int d\xi^* \left[ -\beta \det(\hat{J}) \frac{\partial V(\xi, q_i)}{\partial \xi} + \frac{\partial}{\partial \xi} \det(\hat{J}) \right] \times$$

$$\delta(\xi - \xi^*) \exp[-\beta V(\xi, q_i)]$$

(1.134)

(1.135)

and subsequently transforming to cartesian coordinates affords
\[ \frac{\partial}{\partial \xi} P_\xi = N \int \bar{x}_i \int d\xi^* \left[ -\beta \frac{\partial V(\bar{x})}{\partial \xi} + \frac{1}{\det(J)} \frac{\partial}{\partial \xi^c} \det(J) \right] \times \delta(\xi - \xi(\bar{x})) \exp \left[ -\beta V(\bar{x}) \right] \] (1.136)

(1.137)

where the dummy integration has effectively knocked out explicit dependence on \( \xi(\bar{x}) \).

Again noting that this is a canonical ensemble average affords an explicit expression for the \( \xi \) derivative of the PMF

\[ \frac{dA_\xi}{d\xi} = \langle \frac{\partial V(\bar{x})}{\partial \xi} - \frac{1}{\beta \det(J)} \rangle_\xi = -\langle F_\xi \rangle_\xi. \] (1.138)

Note the appearance of a term depending on the Jacobian of the coordinate transformation. This term is especially important in a system for which a more complex collective variable is defined, such as a dihedral angle, contact number, center of mass distance, \( \alpha \)–helical tendency, or other simulation constraint parameter. Accordingly, a TI–like procedure will work for an arbitrary collective variable subspace, provided that the mean force observed along the reaction coordinate is observant of the coordinate transformation. A similar derivation may be utilized in the case of dynamics in the presence of a generalized constraint along a reaction coordinate, affording the force in terms of the constraint

\[ \langle F_\xi \rangle = \frac{\langle Z^{-1/2}[\lambda - k_b T G] \rangle_\xi}{\langle Z^{-1/2} \rangle_\xi} \] (1.139)

where \( Z \) and \( G \) are scaling factors for the constraint and \( \lambda \) is the Lagrange multiplier implementing the constraint. In the case of a one–dimensional distance constraint, this reduces to \( F_\xi = \langle \lambda \rangle_\xi \). Accordingly, a free energy surface may be constructed by fixing a collective variable \( \xi \) at several values and calculating the mean force during simulations.
at each value via (1.139). Thermodynamic integration may then be performed to generate a free energy surface for evolution along $\xi$. This prescription is termed the Blue Moon Ensemble as it captures the potential of mean force ‘once in a blue moon’ events.

Explicitly, the ABF method utilizes the instantaneous, local free energy gradient $\nabla_\xi \tilde{A}(\xi)$ as an estimate of the force $F_{\text{ABF}} = \nabla_\xi \tilde{A}(\{\xi\})$ required to overcome free energy barriers experienced by a defined set of atoms. Here $\nabla_\xi$ denotes the gradient over a local collective variable subspace and $\{\xi\}$ is a particular ensemble of linearly independent collective variables. In practice, the force $F_{\text{ABF}}$ is calculated at a given timestep and added to the net force acting on the set of atoms during the successive integration step, effectively reducing the depth of the potential well in which the atoms are confined. This process is repeated in a cyclic fashion during consecutive simulation steps. As a consequence, the system ultimately converges to a limit in which the net force along $\xi$ is zero. In this case, motion of the atoms is diffusion limited, thereby facilitating unrestricted exploration of conformational space and drastically enhancing counting statistics for the system. As a collateral result, the time average of this force projected along a particular collective variable $\langle F_\xi \rangle_\xi = -\nabla_\xi A(\xi)$ yields the PMF along $\xi$ through thermodynamic integration

$$A(\xi) = \int_{\xi_0}^{\xi} \left( \frac{\partial A(\xi')}{\partial \xi'} \right) d\xi' = -\int_{\xi_0}^{\xi} \langle F(\xi') \rangle d\xi'. \quad (1.140)$$

The advantage of this method over equilibrium MD with thermodynamic integration is thus manifest.
1.4.3 Metadynamics

The *metadynamics* (MTD) technique \[53\] bears a superficial resemblance to the adaptive biasing force method. In short, during a metadynamics calculation, the potential sampled during the MD simulation is filled by a history-dependent counter-potential. Assuming that the system starts in a given basin during the simulation, the time-dependent bias gradually fills the well, allowing the system to escape to other minima. The process proceeds again, until all minima are filled as the simulation reaches a diffusion limited regime on a featureless potential energy hypersurface. In this limit, the potential complementary to the bias filling the basins reflects the initial landscape of the system, thereby affording a free-energy estimate.

To make these notions explicit, let $s = (s_1, \ldots, s_d)$ denote a set of collective variables defining a trajectory, where $d$ is the dimension of the collective variable subspace necessary to capture the dynamics of the system. Furthermore, assume that the equilibrium distribution of these collective variables is determined by a probability distribution

$$P(s) = \frac{\exp[-\beta \mathcal{A}(s)]}{\int ds \exp[-\beta \mathcal{A}(s)]} \quad (1.141)$$

that is weighted by a free energy

$$\mathcal{A}(s) = -\frac{1}{\beta} \log \left[ \int dx \exp[-\beta V(x)] \delta(s - S(x)) \right] \quad (1.142)$$

where $S(x) = (S_1(x), \ldots, S_d(x))$ is the value assumed by the collective variable for a cartesian coordinate $x$. For practical purposes $x(t)$ is the trajectory emerging from a given molecular dynamics simulation.

Similar to the ABF method, the exploration of the free energy surface is guided by
an estimate of the forces \( F_i = -\partial A(s)/\partial s_i(t) \) acting on the collective variable at a given time during the trajectory. Metadynamics differs from ABF in that sampling is not performed in the reaction coordinate domain, but in the temporal simulation domain. In practice, metadynamics is implemented as an extended Lagrangian method, in which the simulation Lagrangian is augmented by a term \( \sum_{i=1,\ldots,d} \lambda_i (s_i - s_i(t)) \), where the \( \{\lambda_i\} \) are a set of Lagrange multipliers. It may be readily shown that derivatives of the free energy, and hence the forces, \( F_i \), are given by the trajectory average of the Lagrange multipliers \( F_i(t) = \langle \lambda_i \rangle \). Subsequently, these forces are utilized to rescale the collective variables

\[
s_i^{t+1} = s_i^t + (\delta s) \frac{\tilde{F}_i^t}{|F(t)|}
\]

where \( |\cdot| \) denotes the norm of \( F(t) \) in the collective variable subspace. The term \( \tilde{F}_i(t) \), denoted the \textit{metadynamics force}, consists of the observed simulation force and a sum of Gaussian terms centered on all points of the CV subspace explored to time \( t \)

\[
\tilde{F}_i(t) = F_i(t) - W \frac{\partial}{\partial s_i} \sum_{t' \leq t} \exp \left[ -\frac{|s - s(t')|^2}{2(\delta s)^2} \right].
\]

Here \( \delta s \) is the width of the gaussian terms and \( W \) is their height. After rescaled forces and collective variables have been calculated, a new collective variable ensemble is generated and the simulation is performed again to calculate new thermodynamic forces \( F_i(t+1) \).

In summation, during a metadynamics calculation, an ensemble of collective variables \( \{s_i(t)\} \) is chosen, from which a set of thermodynamic forces \( \{F_i\} \) is calculated. These forces are then used to calculate a set of metadynamics forces \( \{\tilde{F}_i(t)\} \) and hence rescale the collective variables to new values \( \{s_i(t + 1)\} \) as a function of the history–dependent Gaussian bias. In effect, this bias is added to the potential at the
point defined by the collective variable. The new ensemble is then utilized to calculate a new series of thermodynamic forces \( \{F_i(t + 1)\} \) and hence rescale the collective variables yet again. Accordingly, the potential well is gradually filled with Gaussians which are progressively shifted by the preceding metadynamics steps. Each such point depositing Gaussian functions in the potential landscape and exploring the collective variable space is termed a *walker*. Ultimately, in the long timescale limit, the free energy landscape will be completely filled with Gaussians. In this case, the resultant free energy profile is simply

\[
A(s, t) = -W \sum_{t' \leq t} \exp \left[ -\frac{|s - s(t')|^2}{2(\delta s)^2} \right].
\]  

This method is denoted *discrete metadynamics*, and was the initial formulation of the metadynamics method. The assumption that the free energy will converge to an equilibrium value from a series of nonequilibrium simulations is the key underlying approximation of the metadynamics method.

In practice, it is more convenient and numerically stable to employ an algorithm in which transformations occur continuously in the collective variable space. This contrasts with the jumps that that occur in the discrete algorithm through every collective variable update. In this case, Gaussian functions are added at every molecular dynamics step. In this case the additional metadynamics force on the collective variable generated by the walker’s exploration is

\[
\tilde{F}_i(t) = F_i(t) - \frac{\partial}{\partial x} \tau \int_0^t dt' \exp \left( -\frac{(s(x) - s(x(t')))^2}{2\delta s^2} \right)
\]

where the parameter \( w/\tau \) controls the height of the Gaussians deposited along the trajectory. The resultant free energy is then
\[ A(s, t) = -\frac{w}{\tau} \int_0^t dt' \exp \left( -\frac{(s(x) - s(x(t')))^2}{2\delta s^2} \right) \]  

(1.147)

The metadynamics technique may be extended to thermostated systems through Lagrangian metadynamics \[ \text{[54]} \]. The implementation of metadynamics for Car—Parrinello scheme is itself an extended Lagrangian method

\[ \mathcal{L} = \mathcal{L}_{\text{CP}} + \frac{1}{2} \sum_{i=1}^{N} \left[ M_i \dot{s}_i^2 - k_i (S_i(\{\vec{R}\}) - s_i)^2 \right] - V(t, \{s_i\}) \]  

(1.148)

where the collective variables are assumed to be defined in terms of the nuclear coordinates \( \{\vec{R}_i\} \). In this context, the additional dynamical variables \( \dot{s}_i \) are coupled to the collective variables through a harmonic potential with coupling \( k_i \) and inertia installed through the effective masses \( M_i \). The mass and coupling constant determine the rate at which the metadynamics degrees of freedom evolve with respect to those of the Car—Parrinello simulation, and allow them to be decoupled from the simulation variables. These additional degrees of freedom are accompanied by the potential \( V(t, \{s_i\}) \) which is generated through the metadynamics algorithm. The particular from of the potential exploited in this case is

\[ V(t, \{s_i\}) = W \sum_{t_{i+1} < t} \exp \left( -\frac{s - s_i}{2\delta s^\perp} \right) \exp \left( -\frac{[(s_{i+1} - s_i)(s - s_i)]^2}{2(\delta s^\parallel)^2} \right) \]  

(1.149)

where \( \delta s^\perp \) defines the width of the Gaussians perpendicular to the collective variable and \( \delta s^\parallel \) the width along the collective variable. In practice, this method can exhibit numerical instabilities when utilized with a single collective variable; a difficulty which may be circumvented through the addition of a dissipative, Langevin term to the equations of motion \[ \text{[55],[56]} \].
A more comprehensive overview of these techniques and their extensions may be found in the standard references \[57, 58\].

1.5 Density Functional Theory

The Car—Parrinello requires an electronic structure method to drive calculations. In the following, the most conventional choice of technique is made, specifically density functional theory (DFT) \[38, 39\]. The central assumption underlying DFT is that the many-body wavefunction utilized in the Hartree—Fock method (c.f. \[59\]) may be effectively replaced by the electronic density, while retaining a comparable degree of theoretical accuracy. Each electron evolves as a single particle in a background mean-field electron density defined collectively by all other independent electrons in the system. Accordingly, the variational problem is transformed from a minimization in the space of all possible many-body wavefunctions to the minimization a set of mutually orthogonal, single-particle functions. The net effect of this approximation is to reduce the dimensionality of the problem from one existing in \(\mathbb{R}^{3N}\) to one in \(\mathbb{R}^{3}\), which is inherently an enormous computational simplification. In principle DFT will converge to an exact solution provided that the correct form of the density functional, in particular the correct exchange correlation potential, is known. While no such ‘universal’ DFT functional affording an exact result for every system has been constructed, the method nonetheless affords a high resolution, often rivaling or exceeding Hartree—Fock calculations in terms of numerical accuracy. A brief overview is provided here, while a more detailed exposition can be found in references \[60, 61\].

By reference to \[(1.1)\], consider a Hamiltonian \(\hat{H}\) comprising an electron kinetic energy operator \(\hat{T}\), an electron–electron interaction potential term \(\hat{V}_{el-el}\), and an external potential \(\hat{V}_{ext}\) which includes nuclear–nuclear and electron–nuclear interactions,
\[ \hat{H} = \hat{T} + \hat{V}_{\text{el-el}} + \hat{V}_{\text{ext}}. \]  

(1.150)

Furthermore, let us introduce a density operator \( \rho(\vec{r}) \) for our \( N \) electron system in terms of the many–particle wavefunction \( \Psi(\vec{r}_1s_1, \ldots, \vec{r}_Ns_N) \) such that

\[
\rho(\vec{r}) = N \sum_{s_1, \ldots, s_N} \int d\vec{r}_1 \ldots d\vec{r}_N |\Psi(\vec{r}_1s_1, \ldots, \vec{r}_Ns_N)|^2 \\
= \sum_i f_i \psi_i^*(\vec{r}) \psi_i(\vec{r})
\]

(1.151)

(1.152)

where \( \vec{r}_i \) and \( s_i \) are the position and spin state of the \( i \)–th particle, and \( f_i \) is the occupancy of the orbital \( \psi_i(\vec{r}) \). Note that the density operator has the property that

\[ \int \rho(\vec{r}) \, d\vec{r} = N, \]

where the integral is taken over all space. The Hamiltonian (1.150) affords the Kohn—Sham energy functional

\[
E \left[ \{ \psi_i(\vec{r}), \vec{R}_I(t) \} \right] = \sum_i f_i \int d\vec{r} \psi_i^* \left( -\frac{1}{2} \nabla^2 \right) \psi_i(\vec{r}) \\
+ \frac{1}{2} \int d\vec{r}_1 d\vec{r}_2 \frac{\rho(\vec{r}_1) \rho(\vec{r}_2)}{|\vec{r}_1 - \vec{r}_2|} \\
+ \int d\vec{r} E_{\text{xc}}[\rho(\vec{r})] \rho(\vec{r}) + E_{\text{ext}} \left[ [\rho], \{ \vec{R}_I \} \right] \\
+ \frac{1}{2} \sum_{i \neq j} \frac{Z_iZ_j}{|\vec{R}_I - \vec{R}_J|}.
\]

(1.153)

(1.154)

(1.155)

(1.156)

This is the starting point for the DFT variational problem.

The Kohn—Sham equations are solved through a self–consistent variational procedure to produce the converged density, DFT orbitals, and energy eigenvalues. Using an initial density guess, \( \rho^{(0)}(\vec{r}) \), the Kohn—Sham Hamiltonian is constructed.
\[ \hat{H}^{KS} = \left[ -\frac{\hbar^2}{2m} \nabla^2 + \hat{V}_H[\rho(\vec{r})] + \hat{V}_{xc}[\rho(\vec{r})] + \hat{V}_{\text{ext}} \right] \]  

(1.157)

and diagonalized through a unitary transformation to afford a solution to the eigenvalue problem

\[ H^{KS} \psi_k^{(1)}(\vec{r}, s) = E_k \psi_k^{(1)}(\vec{r}, s). \]  

(1.158)

The resulting set of orbitals are used to calculate a new density

\[ \rho^{(1)}(\vec{r}) = \sum_s \sum_{k=1}^{N} \left( \psi_k^{(1)}(\vec{r}, s) \right)^* \psi_k^{(1)}(\vec{r}, s) \]  

(1.159)

which, in turn, is exploited to generate a new Hamiltonian (1.157) and afford new orbital eigenfunctions and eigenvalues (1.158). By construction, DFT possesses an additional computational advantage in that the Hartree potential \( \hat{V}_H \) may be calculated directly from the density through Poisson’s equation \( \nabla^2 V_H(\vec{r}) = -4\pi \rho(\vec{r}) \), and hence the integral corresponding to the Hartree term need not be performed. This loop is repeated as long as \( \rho^{(n)}(\vec{r}) \neq \rho^{(n-1)}(\vec{r}) \). Once this condition fails, the algorithm is terminated and convergence of the DFT scheme is assumed.

While the aforementioned protocol generates a converged electronic state for the system and any associated observables, the method may be extended. In particular, the DFT procedure may be coupled to a scheme for geometry optimizations, in which each converged electronic configuration is exploited to calculate the force \( F_i \) acting on the \( i \)-th nucleus:
\[ F_i = -\frac{\partial E_{\text{KS}}}{\partial R_i} \]
\[ = \sum_i f_i \langle \psi_i \rangle \left( \frac{\partial V_i}{\partial R_i} \right) |\psi_i\rangle. \]

The resulting set of forces are utilized to update the nuclear positions, and the KS equations are solved again for the new nuclear configuration. This process repeats until a given threshold is met for the maximal variation in force between successive optimization steps. The uniqueness of the converged solution is assured by the Hohenberg—Kohn theorem, which posits that two external potentials differing by more than a constant cannot afford the same ground-state density \[38\].

1.5.1 Density Functionals

All terms in expressed within the Kohn—Sham functional \([1.153]\) may be evaluated exactly from first principles, excepting the exchange correlation \(E_{\text{xc}}[\rho(\vec{r})]\). The formulation of DFT only guarantees existence of such a term, however no prescription is made for the form of this expression. A judicious choice of this term is particularly important, as all many-body effects within the DFT scheme are treated through the exchange correlation. The simplest approximation is to assume that the exchange correlation function is well-modeled by that of a homogeneous electron gas. Accordingly, this term is a function of the density at a single point in space and hence is inherently a local quantity. This approach is termed the *Local Density Approximation* (LDA) for a closed-shell system \([39]\), or *Local Spin-Density Approximation* (LSDA) in the case of a spin-unrestricted calculation\([62]\). For the latter case, the corresponding term in the Kohn—Sham energy is of the form
\[ \int d\vec{r} E_{xc}[\rho_\alpha(\vec{r}), \rho_\beta(\vec{r})] \rho(\vec{r}) \]  

(1.162)

where \( \rho_\alpha(\vec{r}) \) and \( \rho_\beta(\vec{r}) \) are the corresponding densities for the \( \alpha \) and \( \beta \) spin populations, respectively. In practice, this may be implemented in terms of the *spin difference density* \( \rho_\alpha(\vec{r}) - \rho_\beta(\vec{r}) \), which gives the spin excess at a given point in space. The integral of this quantity over the simulation volume affords the total number of unpaired electrons in the calculation.

Nonetheless, when utilized in practical calculations, the LDA/LSD scheme fails to accurately reproduce bond distances and energies for many systems, and furthermore fails to do so in a sporadic manner. A successive refinement to the LDA / LSDA scheme entails extending the exchange correlation to include a functional dependence on the gradient of the electronic density \( \nabla \rho(\vec{r}) \), thereby permitting inhomogeneities in the electron gas distribution \cite{63, 64}. In this case, our functional becomes

\[ \int d\vec{r} E_{xc}[\rho_\alpha(\vec{r}), \nabla \rho_\alpha(\vec{r}), \nabla^2 \rho_\alpha(\vec{r}) \rho_\beta(\vec{r}), \nabla \rho_\beta(\vec{r}), \nabla^2 \rho_\beta(\vec{r})] \rho(\vec{r}) \]  

(1.163)

which is denoted the *Generalized Gradient Approximation* (GGA). In most cases, the GGA exchange correlation is split into separate exchange and correlation terms \( E_{xc} = E_x + E_c \), and each term specified by a different functional for a given calculation. Commonly used examples of GGA functionals include the BP functional, comprising the 1988 Becke \cite{65} exchange and Perdew \cite{63} correlation interactions, the BLYP functional, combining the 1988 Becke exchange \cite{65, 66} with Lee–Yang–Parr correlation \cite{67}, and the the Perdew–Burke–Ernzerhof (PBE) functional \cite{68}.

A further degree of refinement entails including some degree of exact exchange (from Hartree–Fock theory) into the functional, giving rise to the so called *hybrid functionals*. While the GGA scheme corrects many of the shortcomings of LDA/LSDA for energetic
and geometric parameters, the hybrid functionals lend further flexibility to cases where
the GGA approximation fails. This effect is particularly pronounced for complex
systems with a dense, low–lying state manifold. In practice, the most commonly
utilized hybrid methods are the B3LYP \cite{65,67} and PBE0 \cite{69} functionals. Nonetheless,
there are universal limitations to DFT. In particular, all straight DFT schemes perform
poorly for systems in which weak, dispersive interactions are important, such as van
der Waals solids \cite{60,61}.

1.5.2 Plane–Wave Bases

A choice of density functional is only one–half of the DFT recipe. To generate any
numerical result, a specific basis must be chosen to represent the orbitals \( \psi_k(\vec{r}, s) \) from
which the electron density is constructed. For most calculations presented herein, this
basis is chosen to be a plane—wave basis. In a plane–wave calculation, the simulation
is taken to be periodic, in which case the electronic subsystem may be expanded in
terms of Bloch states. For a given wavevector \( \vec{k} \) in the first Brioullin zone, we define
the plane–wave eigenstates through the Fourier expansion of wavefunctions in the
momentum representation

\[
\langle \vec{r} | \vec{k} + \vec{G} \rangle = \frac{1}{\sqrt{N\Omega}} \exp \left[ i \left( \vec{k} + \vec{G} \right) \cdot \vec{r} \right]
\]  

(1.164)

where \( N \) is the number of periodic cells in the supercell of the system, \( \Omega \) is the cell
volume, and \( \vec{G} \) is a vector in the Bravais lattice generating translations between cells
(for an overview of solid–state concepts, including reciprocal lattices, see \cite{70} or \cite{71}).

Accordingly, an arbitrary Bloch state \( \psi_k(\vec{r}) \) may be expanded as

\[
\psi_k(\vec{r}) = \frac{1}{\sqrt{N\Omega}} \exp[i\vec{k} \cdot \vec{r}] \sum_G c_G^k \exp[i\vec{G} \cdot \vec{r}]
\]  

(1.165)
where $c_G^k$ is an expansion coefficient. Conversely, the position space wavefunction may be reproduced for a given plane–wave basis through the inverse Fourier expansion

$$|\psi_i\rangle = \sum_G c_{i,k+G} |\vec{k} + \vec{G}\rangle$$

where the coefficients $c_{i,k+G}$ are given by

$$c_{i,k+G} = (\vec{k} + \vec{G} |\psi_i\rangle = \frac{1}{\sqrt{N\Omega}} \int \psi_i(\vec{r}) e^{-i(\vec{k}+\vec{G})\cdot\vec{r}} d\vec{r}$$

It is precisely this property, the ability to cycle rapidly between position and momentum space representations using fast Fourier transforms, that makes the plane–wave technique numerically advantageous. In all further calculations, it is assumed that we are working at band–center ($\Gamma$) within the periodic cell, and hence $\vec{k} = 0$, simplifying the expansion. This approximation works well for isolated systems, however, for a band structure calculation on a periodic solid, restriction to the $\Gamma$ point insufficiently samples $\vec{k}$ space. Nonetheless, with these provisions several quantities appearing in the Kohn–Sham variational problem, including the density, kinetic energy, and Hartree energy terms, assume the form of simple summations.

In a plane wave DFT calculation, the basis size is determined by a momentum cutoff. This term, $E_{\text{cut}}$, represents a kinetic energy cutoff four the plane waves propagating under a free–particle Hamiltonian. That is, the basis is filled with all plane waves of the form (1.164) subject to the restriction

$$\frac{k^2}{2m} |\vec{k} + \vec{G}|^2 \leq E_{\text{cut}}.$$ 

From a simple counting argument on the Fermi sphere, it may be shown that the number of plane waves needed to fill to a given cutoff $E_{\text{cut}}$ is given by
\[ N \approx \frac{\Omega}{6\pi^2} E_{\text{cut}}^{3/2} \]  

where \( \Omega \) is the volume of the system cell.

1.5.3 Pseudopotentials

Since the number of plane waves for a system grows as a power of the plane-wave cutoff and likewise linearly with the cell volume, the number of plane waves needed for a realistic problem becomes intractably large \( (\sim 10^6 \) plane waves). Furthermore, the cutoff cannot be decreased significantly to compensate for this effect, as the many core states contain a large number of ‘wiggles,’ which accordingly necessitate a large number of plane waves to synthesize through Fourier transformation. To circumvent this problem, the pseudopotential notion is introduced, which are smooth, effective potentials for the core region that reproduce the effect of the nucleus and core electrons on the valence electrons. In this case, only the valence electron wavefunctions need be retained and the volume of the calculation shrinks considerably. The caveat of this scheme is that pseudopotentials must be parameterized for a given physical configuration in order to reproduce known physical data (band gap, ionization potential, etc.), and hence much be generated for each element and, occasionally, elemental configuration.

The first class of pseudopotentials that will be considered are the norm-conserving pseudopotentials (NCPPs). Let \( \psi^{AE}_l(r) \) denote the all electron, radial, atomic valence wavefunction for a given atom. In this case, the core pseudowavefunction \( \psi^{PS}_l(r) \) is a nodeless function such that the energy eigenfunctions satisfy

\[ E^{PS}_l = E^{AE}_l \]  

(1.170)
for a given angular momentum state \( l \). The two wavefunctions are constrained to coincide outside of the core radius

\[
\psi_l^{\text{PS}}(r) = \psi_l^{\text{AE}}(r), \quad \text{for } r > r_c
\]

and that the norms of the wavefunctions are forced to overlap within the core region

\[
\int_{r<r_c} |\psi_l^{\text{PS}}(r)|^2 r^2 dr = \int_{r<r_c} |\psi_l^{\text{AE}}(r)|^2 r^2 dr
\]

hence the term \textit{norm-conserving}. The NCPPs have the advantage that they replicate the scattering properties of the all electron system while retaining only a single potential per angular momentum channel (termed a \textit{nonlocal} pseudopotential, \( V^{\text{PS}} = \sum_i V_i(\vec{r} | l \rangle \langle l |) \)). This is ensured by construction, as the NCPPs are designed to reproduce the logarithmic derivatives which appear in scattering calculations for the full potential terms. The consistency in scattering properties enhances the \textit{transferability} of the pseudopotential, and hence its applicability for the same element under conditions outside of its initial electronic parameterization. Nonetheless, the NCPPs also tend to exhibit a property termed ‘hardness’ as pseudopotentials, meaning that they may require large electronic cutoffs to adequately describe certain physical situations. This problem is particularly pronounced for atoms with strongly-oscillating pseudowavefunctions in the core region such as first row elements and those with 3d or 4f valence. Consequently, a relatively high plane wave cutoff is required to adequately compensate for the fluctuations in this region. The default NCPP scheme utilized in this work is the Martins—Troullier pseudopotential scheme [72].

An alternative is to generate a ‘soft’ pseudopotential, embodying highly nonlocal parameters. To achieve ideal computational efficiency such a pseudopotential would be fully nonlocal, and accordingly separable. More specifically, a pseudopotential
typically splits into a local part proportional to $-Ze^2/r$ as $r \to \infty$, which remains semilocal in terms of spherical harmonics near the nucleus. From this observation, Vanderbilt \cite{73} derived a protocol to produce such pseudopotentials, which exhibit high computational efficiency when integrated into DFT and Car–Parrinello molecular dynamics schemes \cite{74}. In this protocol, a local potential is generated such that $V_{\text{loc}}(r) = V(r)$ for $r > r_L$, where $r_L$ is the locality radius and $V(r)$ the full potential, and such that $V_{\text{loc}}$ is any smooth regular function when $r < r_L$. From this, a series of atomic plane waves $|\phi_i\rangle$ are generated to solve the Kohn–Sham equations for the corresponding energy eigenvalue $E_i$, with several possible waves permitted per angular momentum channel. These wavefunctions are then used to produce pseudowaves such that $\tilde{\phi}_i(r) = \phi_i(r)$ for $r > r_{L,i}$, and core waves satisfying $|\psi_i\rangle = (E_i - T - V_{\text{loc}})|\tilde{\phi}_i\rangle$.

This method is then extended through the addition of a term projecting the core functions onto each other, termed an ultrasoft potential

$$\hat{V}_{\text{US}} = V_{\text{loc}}(r) + \sum_{lm} D_{lm} |\beta_i\rangle \langle \beta_m|$$  \hspace{1cm} (1.173)$$

where the core projectors are defined as

$$|\beta_i\rangle = \sum_j (B^{-1})_{ij} |\psi_j\rangle$$  \hspace{1cm} (1.174)$$

with the overlap matrix $B_{ij} = \langle \tilde{\phi}_i | \psi_j \rangle$ and such that $\langle \beta_i | \tilde{\phi}_j \rangle = \delta_{ij}$. The ultimate effect of this procedure is to mimic the effect of a smoothed core potential, homogenizing the charge distribution in the core region

$$\rho(r) = \sum_i |\phi_i(r)|^2 + \sum_i \sum_{lm} \langle \phi_i | \beta_i \rangle Q_{lm}(r) \langle \beta_m | \phi_i \rangle$$  \hspace{1cm} (1.175)$$

where $Q_{lm}$ is termed an augmentation charge. This protocol allows plane–wave DFT
calculations to proceed at comparable accuracy with a far lower plane wave cutoff than required for NCPPs, and hence reduced computational expense.

1.5.4 Gaussian Basis Sets

While plane waves afford a delocalized basis scheme well suited to periodic systems, this method can become quite costly for isolated molecules. For methods such as CP dynamics, this cost is offset by the decreased overhead associated with performing Fourier transforms. Nonetheless, with many non–dynamic electronic structure methods, this is not the case, and a localized basis scheme is preferable. The most natural choice for such a basis follows from the hydrogen atom, where the basis wavefunctions assume the form

\[ \psi_{jnm}(r, \theta, \phi) \propto r^{n-1}Y_{lm}(\theta, \phi)e^{-\zeta_j r} \]  

(1.176)

with \((r, \theta, \phi)\) the standard spherical coordinates, \(Y_{lm}(\theta, \phi)\) the Legendre polynomials, and such that \(\zeta_j\) is an adjustable parameter which is varied to reproduce experimentally known atomic properties. This basis scheme, known as the Slater–type orbitals (STOs), are computationally inefficient as the two electron integrals containing a \((r_1 - r_2)^{-1}\) term cannot be solved in a closed form.

Accordingly, it is necessary to find a function with more well–regulated convergence properties. A natural choice is that of the Gaussian–type orbitals (GTOs)

\[ \psi_{jnm}(\vec{r}) \propto x^l y^m z^n e^{-\zeta_j r^2} \]  

(1.177)

which posses the satisfying property that the product of any two Gaussians is also a single Gaussian. Since we have that \((r_1 - r_2)^{-1} = (2/\sqrt{\pi}) \int_0^\infty d\alpha \exp[-\alpha^2 (r_1 - r_2)^2]\), the formerly ill–behaved terms may also be written as Gaussians and hence possess an
analytic solution. Gaussian basis sets are numerous, and the approximation schemes for their evaluation equally so — discussion of such considerations is beyond the scope of this discussion. The default scheme utilized in this work is the def2-TZVP basis [75].
Ligand Unbinding and the Retinol Binding Protein

2.1 Introduction

The serum retinol binding protein (sRBP) is a monomeric, 21 kDa member of the lipocalin family responsible for shuttling the hydrophobic ligand all-trans-retinol through the bloodstream to target organs. (Figure 2.1) Lipocalins possess a highly-conserved global fold, consisting of an eight stranded antiparallel β-barrel containing the ligand binding site and a three–loop portal domain mediating ligand entry and egress\[\text{76,77}\]. To prevent glomerular filtration, lipocalins circulate in association with accessory proteins. In the case of sRBP this is the tetrameric protein transthyretin, which oligomerizes with the sRBP in a region containing the portal loops\[\text{78}\]. Ligand binding is apparently driven by hydrophobic contacts between the substrate and nonpolar residues in the binding cavity, with the C15 hydroxy group establishing contacts either to solvent or to polar residues in the portal loops. (Figure 2.1)

From a structural and functional perspective, lipocalins are similar to conventional host–guest systems. Small molecule host–guest complexes, such as cyclodextrins, calixarenes, cucurbiturils, and cryptophanes, have traditionally provided models for biological ligand recognition. Recent theoretical studies of these systems have dissected the potential of mean force driving host–guest complexation into constituent intermolecular\[\text{80,84}\] and thermodynamic\[\text{85}\] parameters. These calculations indicate

Reproduced in part with permission from *J. Phys. Chem. B* 2010, 114, 11315 Copyright 2010 American Chemical Society. All energetic quantities expressed in this chapter are understood to possess an uncertainty of at least \(\pm k_B T = \pm 0.60 \text{ kcal mol}^{-1}\), as estimated at \(T = 300 \text{ K}\).
Figure 2.1: (A) Retinol / sRBP complex from the crystal structure 1RBP\(^7\) as utilized for simulations. A hydrophobic cup consisting of aromatic residues encapsulates the \(\beta\)-ionone ring system of retinol (blue) while the sidechain is flanked by portal–gating residues (yellow). Note the location of residues flanking the structural water (pink) and the the portal loop domain (\(\beta1 - \beta2\), \(\beta3 - \beta4\) and \(\beta5 - \beta6\) loops). (B) Structure of retinol, with carbon numbering convention indicated.
that small molecule complexation is driven primarily by desolvation and the formation of van der Waals contacts. Conversely, biological systems provide a broad spectrum of intermolecular interactions and hence the opportunity for more elaborate recognition processes. Furthermore, direct extrapolation of theoretical results for small molecule complexes to the biological domain is precluded by the comparative simplicity of these supramolecular assemblies. The conformational free energy landscapes of biological macromolecules are rugged, containing a spectrum of nearly–degenerate states which cluster in local minima separated by high potential barriers. Accordingly, host–guest complexation becomes a convoluted many–body problem characterized by disparate time and energy scales for conformational rearrangement. If energetic behavior analogous to small molecule host–guest chemistry exists, it is unclear how these would manifest for protein–small molecule interactions.

To characterize the free energy landscape for a biological host–guest analogue, the sRBP was adopted as a model system. Steered molecular dynamics simulations (SMD) and free energy methods were employed to calculate the potential of mean force (PMF) for unbinding of retinol from the retinol / sRBP complex. Using an ensemble of SMD simulations as input, Jarzynski’s equality (JE) was utilized to establish characteristics of the free energy profile shared between members of the ensemble. A representative SMD simulation was chosen for subsequent adaptive biasing force (ABF) calculations, permitting a high–resolution determination of the unbinding PMF. Since the ABF calculations permit decomposition of the PMF into contributions from constituent interactions, the forces driving host–guest complexation were readily dissected and quantified.
2.2 Theory and Methods

2.2.1 Free Energy Calculations

Both adaptive biasing force (ABF) \([50]\) and Jarzynski equality (JE) \([44,45]\) based methods have been employed to calculate the potential of mean force for unbinding of retinol from the retinol / sRBP complex. The JE technique utilizes an ensemble of unbinding trajectories from SMD simulations as input. Calculations using the JE have been exploited to characterize ligand unbinding \([86,87]\), transport \([88,89]\) and channel permeation \([90,91]\). This method is highly sensitive to both the size of the sample ensemble and nonequilibrium effects manifesting during SMD simulation. \([48,49,92]\).

For large ligands these nonequilibrium effects can lead to physically inadmissible free-energy profiles, since the system is unable to relax on the short simulation timescale \([93]\). Nonetheless, qualitative features of these landscapes, such as the positions of maxima and minima, generally remain similar to those obtained during more robust ABF simulations \([90,94]\). Accordingly, the JE method can be used as a guide to verify that our ABF calculations produce a PMF representative of the ensemble.

2.2.2 Adaptive Biasing Force Calculations

2.2.3 sRBP System Construction and Equilibration

The 2.0 Å resolution structure of holo sRBP from *Homo sapiens* was used as the starting point for subsequent calculations (PDB Entry: 1RBP) \([79]\). The system was stripped of all waters except the conserved structural water in the M87–H104–Y133 triad. (Figure 2.1) Protonation states of amino acids were assigned using Poisson–Boltzmann based PropKa calculations (pH = 7.0) \([95]\). The system was solvated with
a 15 Å layer of TIP3P water in the x, y, and z directions. Sodium and chloride ions were added to an ionic strength of 0.15 M, thereby ensuring electrical neutrality.

Molecular dynamics procedures were performed using the NAMD 2.6 [96] code and the CHARMM22 force field [17,18]. All simulations were conducted with Langevin dynamics at 300 K (γ = 5 ps⁻¹). Bonds between hydrogens and heavy atoms were constrained to their equilibrium lengths using the ShakeH algorithm, thereby permitting a 2 fs timestep [97]. Nonbonded interactions were calculated every timestep and a full electrostatic calculation performed every other timestep. Calculations involving nonbonded interactions were terminated at a 12 Å cutoff, with a switching function initiated at 10 Å and 13.5 Å cutoff for nonbonded pair–list exceptions. All simulations were performed in a periodic cell with wrap–around boundary conditions. The particle mesh Ewald summation [98] was employed to handle periodic electrostatics. Pressure control in the isobaric–isothermal (NPT) ensemble was achieved using the Langevin piston method [99,100] (target pressure 1.01325 bar, decay period 100 fs, piston temperature 300K) with anisotropic cell fluctuations permitted in all directions.

Simulations were preceded by a 5000 step conjugate gradient (CG) minimization to eliminate unfavorable interatomic contacts. Water and ions were equilibrated around the protein using a 200 ps simulation in the NVT ensemble, with protein and retinol atoms fixed at their initial coordinates. Next, the protein was equilibrated with the solvent by performing a 5000 step CG minimization and 200 ps equilibration in the NPT ensemble, with the protein backbone and retinol atoms harmonically constrained (k = 5.0 kcal mol⁻¹ Å⁻²). Finally, all constraints on the system were released and a final NPT equilibration performed for 3 ns to ensure solvation of the system.
2.2.4 Steered Molecular Dynamics Simulations

Constant velocity steered MD simulations were initiated following the final phase of NPT equilibration. A moving harmonic constraint was attached to the retinol center of mass and pulled with a velocity of $1.25 \times 10^{-5} \, \text{Å fs}^{-1}$ toward the center of the sRBP binding cavity entrance. Parameters were optimized by performing SMD simulations with harmonic constraint force constants of 150 pN Å$^{-1}$, 300 pN Å$^{-1}$, 500 pN Å$^{-1}$, and 700 pN Å$^{-1}$, with a constraint of 700 pN Å$^{-1}$ assumed for production simulations. Alpha carbons at the base of the sRBP beta barrel were harmonically constrained (600 kcal mol$^{-1}$ Å$^{-2}$) to the coordinates assumed following NPT equilibration (G22, V47, M53, D79, K85, T109, Y114, S138) to prevent translation of the sRBP/retinol system. The force on the harmonic constraint was recorded every 500 fs. SMD simulations were performed for a total of 3 ns and repeated 10 times to generate an ensemble for PMF calculations.

2.2.5 Adaptive Biasing Force Calculations

The vector utilized during SMD simulations was adopted as the reaction coordinate for ABF calculations. Simulations were initiated by subdividing the coordinate into eight 2 Å wide nonoverlapping segments and selecting a retinol / sRBP complex lying at the beginning of each segment from a representative SMD run. Each complex was subjected to a 5000 step CG minimization and 1 ns NPT simulation with retinol harmonically restrained to its initial position (20 kcal mol$^{-1}$ Å$^{-2}$) to equilibrate the protein around the initial ligand conformation. Other simulation conditions were identical to those utilized for free NPT simulations. Next, a 6 ns ABF calculation in the NVT ensemble was performed independently in each segment and the PMF was then calculated (initial pressure set by dimensions of cell from NPT simulation). Simulations
were resumed in 2 ns steps until the overall PMF was observed to converge, defined as a mean PMF difference of less than 0.3 kcal mol\(^{-1}\) for three consecutive iterations. ABF data was accrued in bins 0.1 Å wide. Furthermore, since the biasing force is rapidly varying, no bias was applied during the first 500 steps of ABF simulation to avoid perturbation by nonequilibrium effects.

2.3 Results and Discussion

2.3.1 Potential of Mean Force Analysis Along \(\xi\)

Convergence of the unbinding PMF was attained within 16 ns of simulation time. (Figure 2.2) The PMF landscape consists of three distinct regions, corresponding to bound and unbound states of retinol as well as a partially–solvated binding intermediate. In the bound state, retinol is buried deep within the sRBP, the \(\beta\)-ionone ring flanked by a cluster of hydrophobic residues (I41, A43, A55, A57, F45, V74, F77, M88, F135, F137), and retinol / solvent contacts found only at the polar hydroxy headgroup. (Figure 2.1) As retinol emerges from the sRBP, the isoprene tail of retinol becomes solvated while the \(\beta\)-ionone ring remains buried within a hydrophobic cluster consisting of F36, M72, and Y90. The PMF barrier proceeding from the bound state to the intermediate state \(\Delta A_{B\rightarrow I}\) is 11.0 kcal mol\(^{-1}\), corresponding to the initial stage of the unbinding process. Conversely, the barrier for the reverse, binding, process \(\Delta A_{I\rightarrow B}\) is 1.4 kcal mol\(^{-1}\). The ratio of Arrhenius factors for binding to unbinding \(\exp(-\Delta A_{I\rightarrow B}/RT)/\exp(-\Delta A_{B\rightarrow I}/RT) = 9.82 \times 10^6\), suggesting that retinol is kinetically trapped upon formation of the retinol / sRBP complex. The difference in these energies \(\Delta A_{I\rightarrow B} - \Delta A_{B\rightarrow I} = -9.6\) kcal mol\(^{-1}\) reflects the relative energetics of the bound and intermediate states of the retinol / sRBP complex. The final region of the landscape consists of the unbound state, separated from the intermediate by a
barrier of $\Delta A_{I \rightarrow U}$ of 4.8 kcal mol$^{-1}$. This state is characterized by a fully solvated retinol, disengaged from the loop domains of the sRBP. The observed intermediate unbinding energy $\Delta A_{I \rightarrow U}$ reflects a straight–line reaction coordinate. This may not reflect the minimum energy for this process, as a ‘cone’ of paths exist by which retinol may disengage from the sRBP. Conversely, egress from bound to intermediate states is well described by a straight–line reaction coordinate, and hence variations this choice would not be expected alter $\Delta A_{I \rightarrow B} - \Delta A_{B \rightarrow I}$. As a consequence, this energetic difference is the most robust component of the total unbinding PMF given by $\Delta A_{B \rightarrow U} = \Delta A_{I \rightarrow B} - \Delta A_{B \rightarrow I} + \Delta A_{I \rightarrow U}$, and hence the quantity most appropriate for comparison with experimental data. Thermochemical studies indicate a free energy difference of $\Delta G^\circ = -10.3$ kcal mol$^{-1}$ for release of retinol into water by the bovine protein at 20 °C $^{101}$, similar to the -9.6 kcal mol$^{-1}$ PMF between intermediate and bound states of the retinol / sRBP complex. Furthermore, the scale is consistent with the observed dissociation constants ($K_d = 4.0 \times 10^{-8}$ M at 20 °C and $K_d = 1.9 \times 10^{-7}$ M at 27 °C) for the retinol / human sRBP complex$^{102,103}$.

2.3.2 Jarzynski Estimates of the Potential of Mean Force

While ABF simulations provide a potential of mean force for one member of the SMD conformational ensemble, it is unclear if these results are representative of the entire ensemble. Due to the high computational cost of ABF calculations, as–generated SMD data was utilized as input for PMF calculations using Jarzynski’s equality. The Jarzynski method is generally slow to converge and provides an unphysical energy scale for large ligands $^{93}$. Nonetheless, the general location of local minima and maxima in the PMF tend to be reflected between ABF and JE–based calculations. Accordingly, the potential of mean force was calculated through direct application of the JE (Eqn. $^{1.113}$) and through the second–order cumulant expansion (Eqn. $^{1.116}$). (Figure 2.3)
Figure 2.2: PMF along the reaction coordinate $\xi$ for retinol unbinding from sRBP. The landscape is characterized by three general regions corresponding to the bound retinol / sRBP complex, a binding intermediate in which retinol is partially solvated, and the unbound, solvated retinol. Dashed lines denote stages of convergence for the PMF, starting from 6 ns (top) to 16 ns (bottom, thick line) in 2 ns intervals.

The PMF from both the JE and the cumulant expansion show features reflecting the bound, intermediate, and unbound states from ABF calculations. In all cases the energy scale is several times that of the PMF arising from ABF calculations and well exceeds experimental data. The JE PMF contains local minima between $\xi = -4$ Å and $\xi = 4$ Å corresponding to an intermediate–state like configuration. Nonetheless, this region exhibits a double–well feature, perhaps reflecting an exaggeration of the local minimum at $\xi = -5.0$ Å in the ABF landscape. The local minima disappear for values of $\xi$ greater than $\xi = 4$ Å, corresponding to the unbound state of the retinol / sRBP complex. Conversely, below $\xi = -4$ Å the PMF is characterized by a deep well reflective of the bound state. While quantitative agreement is not achieved with the PMF calculated with the JE method, the qualitative features of the JE–based PMF suggest that ABF calculations reflect features seen throughout the set of SMD simulations.
Figure 2.3: PMF estimates for an ensemble of SMD trajectories \((k = 700 \text{ pN \text{ Å}^{-2}})\) calculated from the net work \(<W>\), the Jarzynski equality, and the second-order cumulant expansion. Regions corresponding to bound, intermediate, and unbound states as determined by ABF calculations are indicated.

2.3.3 Conformational Rearrangement During Retinol Unbinding

SMD simulations reveal distinct behavior as the ligand traverses each region of the PMF landscape. In the bound state, \(\xi = -8\), the \(\beta\)-ionone ring of retinol is flanked by the aforementioned cluster of hydrophobic residues. (Figure 2.4 A1) The isoprene units are flanked by a ring of residues, F36, M73, and Y90, located at the top of the \(\beta\) barrel or in the portal loop domains. (Figure 2.4 A2) Direct contact with solvent is present only at the terminal hydroxyl group of retinol, with protein preventing other solvent contacts. As retinol is withdrawn from the binding site, partial solvation begins at \(\xi = -2 \text{ Å}\) as retinol enters the intermediate state. (Figure 2.4 B1) Solvation is concomitant with conformational rearrangement of the F36–M73–Y90 ring, with F36 and its \(\beta_1–\beta_2\) loop deflected outward to expand the entrance to the binding cavity. (Figure 2.4 B2) During this transit, the \(\beta\)-ionone ring of retinol rotates with respect to the isoprene chain, bringing methyl groups on the sidechain and \(\beta\)-ionone ring into a \textit{cis} conformation. Conformations were assumed in which the C18–C19, C16–C19 and
C17–C19 pairs in retinol became proximate. (Figure 2.1) Upon progression into the unbound state, $\xi = 6$, the ligand becomes fully solvated. (Figure 2.4 C1) The torsional displacement found in the intermediate state relaxes and the ligand assumes the trans configuration observed in the bound state. Rearrangement occurs in the portal loops, the $\beta_1$–$\beta_2$ loop falling inward toward the $\beta_5$–$\beta_6$ loop and F36 assuming an interstitial role within the channel, occluding the binding site in conjunction with M73 and Y90. (Figure 2.4 C2) This configuration remains stable during further simulation, rapidly equilibrating to a mean distance of 7.9 Å between the F36 centroid and the $\alpha$–carbon of M73 and 9.4 Å between the centroid and the $\alpha$–carbon of Y90. The mean value remains constant over an additional 8 ns of unrestrained MD simulation. (Figure 2.5)

Relative motion of the $\beta_1$–$\beta_2$ and $\beta_5$–$\beta_6$ loops was reported in previous MD
Figure 2.5: Distance between the F36 sidechain centroid and the α-carbons of M73 and Y90 from a representative SMD simulation. Occupation of the bound, intermediate, and unbound regions by retinol is denoted by the pink, purple, and teal bars, respectively.

simulations [104, 105], however this result may be spurious due to the short (70 ps) timescale of these calculations. Additionally, essential dynamics (ED) simulations of apo and holo sRBP support concerted motion of the β1–β2, β3–β4, and β7–β8 loops and suggest that the conformational changes upon retinol unbinding are small [106]. These calculations are limited by the timescale (600 ps) utilized for ED sampling. The current simulations exceed this scale by more than an order of magnitude, permitting observation of events inaccessible in these early studies. These calculations reveal that, upon retinol unbinding, the β1–β2 and β5–β6 loops collapse toward each other while the β3–β4 and β7–β8 loops move apart, pinching the binding site shut in a stable configuration (Figure 2.6).

2.3.4 Componentwise Decomposition of the Potential of Mean Force

While the net unbinding PMF describes relative energetics for distinct states along the reaction coordinate, the physical interactions underlying this behavior are not apparent. To clarify this, it is necessary to quantify the contributions of electrostatic, van der Waals, and solvation energy terms to the global PMF. Conveniently, the net force $\vec{F}(\vec{x})$
Figure 2.6: sRBP conformations from crystallographic and simulation data. (A) Comparison of the apo sRBP structure (PDB Entry: 1BRQ; black), apo structure generated by removing retinol and performing free MD simulation (MD; teal), and the apo structure from SMD simulation followed by free MD equilibration (SMD; blue). (B) Comparison of the holo conformation assumed following MD simulation (MD; red) and the crystallographic data characterizing the holo form (PDB Entry: 1BRP; black). (C) Comparison of SMD–derived apo sRBP and holo conformation from MD simulation (MD; red). (D) Overlay of crystallographic holo (1BRP; green) and apo (1BRQ; purple) sRBP conformations. All simulation–derived structures represent the mean structure averaged over the last nanosecond of simulation.
between the ligand and the binding protein may be decomposed into electrostatic and van der Waals components, \( \vec{F}(\vec{x}) = \vec{F}_E(\vec{x}) + \vec{F}_V(\vec{x}) \). These components are readily calculated from ABF simulation trajectories. These components are then projected onto the reaction coordinate \( \vec{F}_{E,V}(\xi) = \vec{F}_{E,V}(\vec{x}) \cdot \frac{\vec{\xi}}{||\vec{\xi}||} \) and averaged in bins 0.1 Å in width along \( \xi \) to give the mean force component \( \langle F_{E,V}(\xi) \rangle \). The PMF for each component is obtained directly by integration:

\[
A_{E,V}(\xi) = \int \langle \frac{\partial A(\xi)}{\partial \xi} \rangle d\xi = -\int \langle F_{E,V}(\xi) \rangle d\xi
\]  

(2.1)

This decomposition reveals drastically different behavior for \( A_V(\xi) \) and \( A_E(\xi) \) along the reaction coordinate. The electrostatic component \( A_E(\xi) \) exhibits simple, monotonically decreasing behavior during the progression from unbound (\( \xi = 8 \)) to bound (\( \xi = -8 \)) states. Furthermore, this profile exhibits a kink between \( \xi = 2 \) Å and \( \xi = 3.75 \) Å and a rapid downturn in the potential occurring when \( \xi = -3.5 \) Å and proceeding into the bound state. (Figure [2.7]) Inspection of the van der Waals component \( A_V(\xi) \) of the PMF reveals substantially more complex behavior. In particular, the shallow valley appearing between \( \xi = -4 \) Å and \( \xi = 2 \) Å in the overall PMF appears to be the consequence of a broader dip in the van der Waals component, occurring between \( \xi = -4.25 \) Å and \( \xi = 4.2 \) Å with a minimum at \( \xi = 1.1 \) Å. A rapid downturn is also observed for \( A_V(\xi) \) near the bound state, with a slight upturn as \( \xi \) approaches the bound state from above at \( \xi = -5.1 \) Å followed by a rapid decrease to the bound value. The sum of these interactions, \( \Delta A_E + \Delta A_V \), has a shape identical to that of the full PMF.

The PMF term \( \Delta A_{\text{cplx}} \), describing interactions between the retinol / sRBP complex and the solvent / ion component, exhibits a profile complementary to \( \Delta A_E + \Delta A_V \). While the solvent profile exhibited oscillatory behavior out to 24 ns as a consequence
Figure 2.7: Decomposition of the PMF into constituent interactions. Here the electrostatic and van der Waal’s components of the PMF for interaction of retinol and sRBP are $\Delta A_E$ and $\Delta A_V$, respectively. The overall PMF for intramolecular interactions between retinol and the sRBP is simply $\Delta A_E + \Delta A_V$. The PMF for both electrostatic and van der Waal’s interactions between the solvent/ions and the retinol/sRBP complex is $\Delta A_{cplx}$. The overall PMF for the unbinding process is shown for reference.

of ion motion and structural rearrangement, the relative energetics and location of maxima and minima were constant. The mean PMF encapsulated in $\Delta A_{cplx}$ remains approximately constant while retinol is fully solvated (unbound), dropping once the ligand enters the binding intermediate state and looses part of its solvation shell. The minimum of $\Delta A_{cplx}$ coincides with the barrier between bound and intermediate states in the full PMF; however, the solvation profile does not decrease past this point. Accordingly, a picture emerges in which the energetic gain of desolvation carries retinol into the intermediate state. The protein conforms to retinol in the intermediate state, staging the system for the last step in which the rapid drop in the PMF between retinol and sRBP traps retinol in the fully bound state.
2.3.5 Rigidity of Apo Versus Holo sRBP

To characterize the effect of ligand unbinding on conformational stability of the retinol / sRBP complex, unrestrained NPT simulations were performed for 8 ns on both the equilibrated structure used to initiate SMD simulations and on the terminal simulation point from an unbinding trajectory. Isotropic temperature factors $B_i$ were calculated by averaging the root mean square displacements $\langle r_i^2 \rangle$ for each atom $i$ over the last 3 ns of the simulation to give $B_i = 8\pi^2\langle r_i^2 \rangle$. Restriction of sampling to late timesteps ensures that the calculated temperature factors are representative of an equilibrated conformational ensemble. Convergence was assessed by performing MD simulation until temperature factors remained constant over the terminal sampling window of the calculation. Simulations were extended for an additional 6 ns beyond convergence to ensure stability of the system. Inspection of these values indicates approximately two-fold increase in flexibility for the $\beta_1-\beta_2$ and $\beta_3-\beta_4$ and $\beta_5-\beta_6$ loops in the portal domain for simulations of apo-sRBP versus the holo form. (Figure 2.8 A, D) A similar increase in flexibility is observed for the $\beta_2-\beta_3$ loop on the anterior side of the $\beta$-barrel. Conversely, motion of the $\beta_8-\alpha$ loop spanning between the $\beta$-barrel and the sole $\alpha$-helical domain is attenuated in apo-sRBP. This behavior is consistent with the variation in temperature factors observed between apo and holo crystal structures of human [107] and bovine sRBP [108] as well as with conformational changes implicated in pH-dependent unbinding [109]. To ascertain if the observed shift in flexibility occurs in the absence of SMD biasing, a second simulation was performed in which retinol was removed from the initial holo form of the protein. The system was subjected to equilibration and an 8 ns unconstrained NPT simulation with identical parameters. Temperature factors were calculated in the same manner as the SMD simulations. These results differ substantially from the biased simulations.
In particular, a shift in conformational flexibility between apo and holo is observed only in the $\beta_1-\beta_2$ and $\beta_3-\beta_4$ loops, inconsistent with the crystallographic data. Furthermore, the predicted shift for the $\beta_3-\beta_4$ loop is substantially larger than indicated by experiment. The use of SMD simulation appears to bias the system into a conformational ensemble capable of reproducing the experimentally observed fluctuation behavior. In contrast, free MD simulation is unable to produce the requisite conformational state spectrum for observation of these phenomena. This limitation emerges from the long timescales required to surmount the PMF barriers involved in conformational relaxation upon unbinding. In this case, the biased simulation circumvents these paths in the free energy landscape, permitting observation of physical phenomena that would be inaccessible through MD simulation alone.

Nonetheless, simulations do exhibit some deviations from the experimental data. Crystallographic B–values indicate variation in flexibility between apo and holo sRBP for amino acids near the N–terminus, a feature absent in calculations. Furthermore, the predicted B–value depression around residue 140 is not experimentally observed. These anomalies occur in the terminal domains or near the seven C–terminal amino acids absent in the crystal structure. Accordingly, these variations can be ascribed to a combination of crystal packing forces, the radically different environment experienced by the protein in the solvated simulation system, and deficiencies in the structural data utilized as simulation input.

2.3.6 Comparison With Other Host–Guest Systems

Our calculations reveal a surprising mechanism of host–guest complexation for the retinol / sRBP system. While desolvation effects are sufficient to drive formation of an intermediate retinol / sRBP state, a combination of electrostatic and van der Waals interactions between the retinol and sRBP pull the intermediate state into
Figure 2.8: (A) Difference in computed temperature factors for apo and holo sRBP, projected onto the apo sRBP and viewed from two different perspectives. Green text denotes regions of enhanced conformational flexibility and red text regions of decreased conformational flexibility. Regions in black text remain unchanged between apo and holo forms. Mean residue-wise temperature factor computed for the apo (B) and holo (C) forms of sRBP. Subtracted \( B_{\text{apo}} - B_{\text{holo}} \) temperature factors (D) for apo and holo forms, with respect to the temperature factors of apo sRBP. Subtracted temperature factors for the apo structure generated by deleting retinol at the onset of simulation are provided for reference (E). The blue dashed line represents the backbone B-factor difference between apo (1BRQ) and holo (1BRP) sRBP crystal structures [107].
the stable complex. This coupling is associated with a change in the conformational flexibility of the portal domains of sRBP and subsequent 'stiffening' of the sRBP in the holo form. Comparison of our results with thermochemical and crystallographic data reveals good agreement, suggesting that our observations and mechanistic proposals are physically plausible. Studies of sterol inclusion in cyclodextrin–based host guest complexes revealed PMFs characterized by two local minima corresponding to partial and complete inclusion of the sterol in the complex \[80,81\]. In these systems, full inclusion is generally preferred by approximately \(1 - 2 \text{ kcal mol}^{-1}\), with complexation driven by desolvation of the sterol coupled with the burial of hydrophobic surface area \[80,81\]. More recent simulations also indicate that the interaction between the cyclodextrin complexes and solvent drive the conformational changes in cyclodextrin / rotaxane molecular switches \[84\]. Our simulations support that, in the retinol / sRBP system, binding is driven by a similar process, with the aforementioned intermediate state playing an essential role in ligand binding. Our decomposition procedure does not permit direct quantification of the entropic and enthalpic contributions to binding. Nonetheless, our mechanistic proposal for retinol recognition and encapsulation suggests a process that would necessarily be entropic in nature as indicated by herein unreported variable–temperature ABF calculations.

NMR studies of other hydrophobic carrier proteins such as the cellular retinoic acid binding protein I (CRABP I) \[110\], cellular retinol binding protein I (CRBP I) \[111\] and II (CRBP II) \[112\] reveal softening of loop portal domains upon ligand unbinding. While these proteins are not close structural homologues, it appears that a conformational softening of the portal region is a hallmark of the apo form of retinoid carrier proteins. NMR suggests that large scale fluctuations in the portal regions occur on the microsecond to second timescale. While the timescale for analogous fluctuations in human sRBP is unclear, our molecular dynamics simulations suggest
that this same behavior does occur. Such conformational fluctuations may alter the interaction of these proteins with accessory proteins, in this case transthyretin, and modulate the formation of carrier / receptor complexes. Conformational changes in the loop portal domain driven by the small molecule sRBP ligand A1120 have been shown to do precisely this, disrupting coupling between the sRBP / A1120 complex and transthyretin [113].

2.3.7 Conclusion

Taken together, these observations suggest that complexation in sRBP–like biological host guest systems is promoted by the same general desolvation effects as for small molecule host–guest complexes. Nonetheless, the complex environment present in the biological domain provides the opportunity for novel complexation behavior, such as the trapping mechanism suggested between intermediate and bound states of the retinol / sRBP complex. Furthermore, biological host–guest complexes are capable of undergoing concerted conformational changes, such as the shielding of the retinol binding site by portal residues and loops in the apo form of sRBP. Thus, the entropic cost of solvating a large hydrophobic cavity can be compensated in these systems, permitting the formation of larger, higher–affinity host–guest complexes than observed in the small molecule realm. In addition, binding–related changes in both protein conformations and in the conformational spectrum can disrupt the formation of protein–protein complexes. This serves to modulate, in essence, a higher order form of host–guest complexation.
Cytochrome P450 Compound I and Plane–Wave Density Functional Theory

3.1 Introduction

In the most generic sense, Compound I (Cpd I) refers to one of several oxyferryl porphyrin cation radical (Figure 3.2) species. This potent oxidizing agent is the putative, critical catalytic intermediate in a variety of enzymes, including the catalases, peroxidases, and the cytochromes P450 (P450s). Experimental and computational studies suggest that the ground–state electronic structure of Cpd I is either a \( S = 1/2 \) or \( S = 3/2 \) triradicaloid configuration. In the former case, two electrons of parallel spin are localized on the Fe(IV)\=O moiety, with an additional electron of antiparallel spin delocalized over the porphyrin cycle and the axial ligand. Conversely, in the \( S = 3/2 \) case, the spatial distribution of spin is identical, however, all three spins are aligned in parallel. In each enzymatic system, the axial ligand coordinated to the heme in Cpd I is distinct. Within the catalases, this ligand is a phenolate anchored in the peptide backbone, whereas for peroxidase the ligand is the imidazole comprising a histidine sidechain. P450 Cpd I is likewise unique, in that the corresponding axial ligand is a cysteine thiolate.

The active–site architecture for the prototypical substrate–hydroxylating P450, cytochrome P450cam (CYP101) from \textit{Pseudomonas putida}, has been the subject of thorough experimental characterization (Figure 3.1) \cite{14}. Within this system, Cpd I occurs within an iron protoporphyrin IX cycle, which is locked into the active site
through two propionate sidechains as well as the aforementioned cysteine–thiolate axial ligand. The substrate of this enzyme, camphor, is oxidized to 5-<i>exo</i>–hydroxycamphor through hydrogen atom abstraction by Cpd I, followed by hydroxyl radical rebound to the substrate \[115\][116]. Two additional residues in the active site, Asp251 and Thr252, are responsible for proton shuttling to the active site during catalytic turnover leading to the formation of Cpd I. Throughout the P450 catalytic cycle, the enzyme active site tunes the electronic state in a concerted manner to each catalytic intermediate \[117\][118]. Nonetheless, only a single reactive intermediate, Cpd I, is considered within this chapter.

Cpd I and analogous systems have been the subject of extensive theoretical characterization in both the gas phase and in quantum mechanical / molecular
Figure 3.2: Compound I model systems (A) employed in GTO, MT, and VDB USPP calculations, and representative geometries for $L = \text{--SH}$ (B) and $L = \text{--SCH}_3$ (C) models, as calculated with the PBE functional and Vanderbilt ultrasoft pseudopotentials at a 30 Ry cutoff.
mechanical (QM/MM) simulations. To date, most gas-phase electronic structure calculations and QM/MM simulations have been performed using either density functional theory (DFT) or correlated, post Hartree–Fock (HF) methods with Gaussian-type orbital (GTO) basis sets \[115\]. The exceptions to this rule are several recent Car–Parrinello (CP) \[32\] molecular dynamics simulations of catalase and peroxidase Cpd I using a plane–wave (PW) pseudopotential basis in a QM/MM framework \[119\,124\]. Similar electronic structure calculations have likewise been performed with other porphyrin–based species, with an emphasis on solid–state applications \[125\,132\]. The CP formalism facilitates simulations which provide a high degree of \textit{a priori} unstructured physical insight, allowing proton flow and reaction dynamics to be studied without the imposition of prior mechanistic restraints. Extending these studies to the CYP450s would afford an invaluable perspective on molecular oxygen activation, proton transport both into and within the catalytic center, the electronic structure of reaction intermediates, and the competition between hopping and tunneling in proton abstraction. Nonetheless, all existing CP studies of Cpd I have been performed for systems with a histidine– or tyrosine–based axial ligand.

Characterization of a cysteine thiolate Cpd I using DFT and PW pseudopotential basis is essential before meaningful CP simulations can be performed for the CYP450s. Accordingly, electronic structure calculations for CYP450 Cpd I model systems containing either an isolated thiolate (−SH) or methylthiolate (−SCH\(_3\)) axial ligand are presented within this framework. The model axial ligands emulate the effect of a protein–bound cysteine sidechain, with the choice of ligand subtly manipulating the radical character localized on the respective porphyrin cycle. These calculations were executed for three different generalized gradient approximation (GGA) density functionals. Furthermore, multiple plane–wave basis sets were employed, including Martins—Troullier scheme, which is most frequently employed in hybrid AIMD/MM
Figure 3.3: Representative curve for determination of appropriate cell parameters in Martins—Troullier pseudopotential scheme, using PBE functional with nonlinear core correction and $L = -\text{SCH}_3$ in $S = 1/2$ state.

calculations, and the Vanderbilt ultrasoft pseudopotential scheme, which is known to give excellent performance for transition–metal systems [133]. For comparison, an identical set of calculations were performed using the same density functionals and a moderately–sized GTO basis set, which has been thoroughly validated for Cpd I and other P450 catalytic intermediates in previous calculations.

3.2 Theoretical Methods

Electronic structure calculations were performed using DFT in a PW pseudopotential framework with either a norm–conserving pseudopotential (NCPP) or ultrasoft pseudopotential (USPP) basis. NCPP calculations employed the CPMD 3.13.2 [134] code, and USPP calculations were conducted using both CPMD and the PWSCF module of the QuantumESPRESSO 4.2 suite [135]. All calculations were executed in
Figure 3.4: Representative curve for determination of reasonable cutoff energy for Martins–Troullier pseudopotential scheme using PBE functional with nonlinear core correction and $L = -SCH_3$ in $S = 1/2$ state.

the gas phase ($\epsilon = 1$) using a model system for Cpd I comprising either a thiolate ($L_1 = -SH$) or methylthiolate ($L_2 = -SCH_3$) axial ligand. Systems were embedded in generously–sized orthorhombic supercells, measuring 17.0 Å $\times$ 17.0 Å $\times$ 12.5 Å (System $L_1$) and 17.0 Å $\times$ 17.0 Å $\times$ 17.0 Å (System $L_2$). Convergence with respect to cell volume was assessed by manipulating supercell dimensions and assessing any change in the total energy or net forces acting on the system (Figure 3.3). The volume minimizing the total energy was adopted for simulation parameters. Similarly, convergence with respect to cutoffs for the PW expansion was determined by calculating the energy of the optimized system as cutoffs were successively increased (Figure 3.4 and 3.5).

The isolated, non–periodic nature of these systems eliminates the need for broad $k$–point sampling. Accordingly all physical quantities were calculated at the $\Gamma$ point
Figure 3.5: Representative curve for determination of reasonable cutoff energy for 
Vanderbilt ultrasoft pseudopotential scheme using PBE functional with nonlinear core 
correction and \( L = -S \text{CH}_3 \) in \( S = 1/2 \) state.

in \( k \)-space for the periodic system (band center). Electronic states were converged to 
within \( 1 \times 10^{-6} \) eV for both geometry optimizations and for pure SCF calculations. 
Geometry optimizations were performed using either the ODIIS optimizer \([130]\) in 
CPMD or the CG/BFGS optimizer in PWSCF until the force on each atom was less 
than \( 0.005 \text{ eV } \text{Å}^{-1} \). A series of density functionals within the generalized gradient 
approximation (GGA) were adopted and utilized in the local spin density (LSD) 
approximation to DFT. These functionals included a combination of the 1988 Becke\([65]\) 
exchange and Perdew\([63]\) correlation interactions (BP), the Perdew–Burke–Ernzerhof 
(PBE) functional \([68]\), and an additional functional combining the 1988 Becke exchange 
\([65,66]\) with Lee–Yang–Parr correlation \([67]\) (BLYP). In all calculations, the gradient 
correction was neglected when the electronic density was less than \( 1 \times 10^{-6} \text{ e} \text{a}_0^{-3} \). 
For the NCPP case, pseudopotentials generated in the Martins—Troullier (MT)
scheme \cite{72} were utilized alongside the Kleinman—Bylander \cite{137} approximation to treat core–level electrons. For MT calculations, valence electrons were expanded in a plane wave basis with cutoff of 70 Ry. To negate Kleinman—Bylander ghost states in MT systems, calculations were performed with local potentials on the $s$ and $p$ pseudopotential channels as well as on the $d$ channel. USPP calculations were performed using Vanderbilt pseudopotentials \cite{73}, with a PW kinetic cutoff of 30 Ry and a charge–density cutoff at 240 Ry.

Three distinct approximations were employed to accommodate correlation between core and valence densities at the transition metal center. First, a nonlinear core correction \cite{138} (NLCC) was utilized in conjunction with either MT or Vanderbilt pseudopotentials. In the NLCC scheme, the exchange–correlation energy is calculated in terms of a modified density which is split into core and valence contributions. The net result is a correction of errors resulting from linearization of the exchange–correlation energy term, which is particularly important in a system with strong overlap between core and valence densities. In the second case, semicore electronic states were included with Vanderbilt pseudopotentials. Unlike the NLCC, this scheme circumvents errors arising from linearization of the exchange–correlation by treating states in the overlap between core and valence density explicitly, at greater computational expense.

Reference calculations using a GTO scheme were performed with both ligands. All GTO calculations for GGA functionals were conducted using the ORCA 2.7 package \cite{139}. Calculations were executed for the same GGA functionals employed in the PW schemes (BP, BLYP, PBE). A def2-TZVP basis \cite{75} was utilized on all atoms. Additional calculations using the hybrid B3LYP \cite{65, 67} and PBE0 \cite{69} functionals were likewise performed using the def2-TZVP basis and the Turbomole 5.10 \cite{140} package, as ORCA was unable to converge the $S = 1/2$ systems to their proper triradicaloid ground state.
Atomic spin densities for both PW and GTO cases were determined using volumetric spin difference density profiles and Voronoi triangulation as implemented in the Bader 0.27 code [141,143]. All volumetric data was calculated using a mesh with resolution equal to the number of grid points comprising the finest Fourier grid in the corresponding PW calculations (180 × 180 × 128 real space mesh points).

3.3 Results and Discussion

3.3.1 Gaussian–Type Orbitals

Calculations employing GTOs were executed using the three GGA functionals most frequently encountered in PW electronic structure and CPMD calculations (BP, BLYP, PBE). These results were supplemented with additional calculations employing two hybrid functionals frequently exploited in gas-phase and QM/MM GTO studies as a higher–level reference set (B3LYP, PBE0). All five functionals afford consistent predictions for the magnitude of calculated geometric parameters, deviating primarily in terms of spin–state ordering and level–spacing (Tables 3.1 and 3.2).

The scaling behavior for key distances with respect to spin–state is conserved among all three GGA functionals (Tables 1 and 2). In particular, the Fe=O distance \(d_{\text{Fe=O}}\) follows a nonlinear trend with increasing spin

\[
d_{\text{Fe=O}}(S = 3/2) > d_{\text{Fe=O}}(S = 5/2) > d_{\text{Fe=O}}(S = 1/2)
\]

whereas the Fe–S distance \(d_{\text{Fe–S}}\) scales linearly with increasing spin

\[
d_{\text{Fe–S}}(S = 5/2) > d_{\text{Fe–S}}(S = 3/2) > d_{\text{Fe–S}}(S = 1/2)
\]

for both ligands. Furthermore, a high degree of consistency among geometric parame-
ters exists between GGA functionals, with the highest degree of uniformity observed between the Fe=O distances. The mean Fe=O distance, averaged over all GGA functionals, is \( d_{\text{Fe}=\text{O}}(S = 1/2) = 1.643 \text{ Å} \), while \( d_{\text{Fe}=\text{O}}(S = 3/2) = 1.667 \text{ Å} \) when the spin is increased, and \( d_{\text{Fe}=\text{O}}(S = 5/2) = 1.657 \text{ Å} \) in the highest considered spin state, all for \( L = -\text{SH} \). The same degree of homogeneity is observed with calculations for a methylthiolate axial ligand, in which case the mean \( d_{\text{Fe}=\text{O}}(S = 1/2) \) is 1.646 Å, while the mean \( d_{\text{Fe}=\text{O}}(S = 3/2) \) is 1.665 Å, and \( d_{\text{Fe}=\text{O}}(S = 5/2) \) is 1.655 Å. The electronic state ordering is likewise paralleled among all three GGA functionals. In particular, the \( S = 1/2 \) doublet is consistently predicted to be the ground state, with the \( S = 3/2 \) quartet lying an average of 0.169 eV above the doublet when \( L = -\text{SH} \) and 0.154 eV above the doublet when \( L = -\text{SCH}_3 \). Conversely, the \( S = 5/2 \) state is the highest in energy, lying an average of 1.063 eV above the \( S = 1/2 \) state when \( L = -\text{SH} \) and 1.046 eV above the \( S = 1/2 \) state when \( L = -\text{SCH}_3 \).

Geometric parameters calculated using the B3LYP and PBE0 hybrid density functionals exhibit an even higher degree of homogeneity between states than those generated from the GGA functionals. The Fe=O distances between states are equivalent, however, the Fe—S distance \( d_{\text{Fe—S}} \) exhibits a more pronounced, increasing behavior with increasing spin

\[
d_{\text{Fe—S}}(S = 5/2) > d_{\text{Fe—S}}(S = 3/2) > d_{\text{Fe—S}}(S = 1/2)
\]  \quad (3.3)

in a manner identical to that observed with GGA functionals, excepting \( L = -\text{SH} \) with the B3LYP functional. The variation in distance between successive \( d_{\text{Fe—S}} \) for \( S = 1/2 \) and \( S = 3/2 \) spin states is small, differing by 0.001 Å for B3LYP and 0.002 Å for PBE0 when \( L = -\text{SH} \), and by 0.018 Å for B3LYP and 0.011 Å for PBE0 when \( L = -\text{SCH}_3 \). A small to nonexistent dependence of \( d_{\text{Fe}=\text{O}} \) on spin multiplicity
<table>
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<th>Fe—S (Å)</th>
<th>Energy (eV)</th>
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Table 3.1: Geometric parameters for several density functionals with $L = \text{—SH}$ and GTO type orbitals.
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<th>Fe–S (Å)</th>
<th>Energy (eV)</th>
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Table 3.2: Geometric parameters for several density functionals with $L = \text{–SCH}_3$ and GTO type orbitals.
Figure 3.6: Representative spin difference density plots for Cpd I with \( L = -\text{SH} \) as calculated by the PBE density functional in the (A) \( S = 1/2 \) doublet, (B) \( S = 3/2 \) quartet, and (C) \( S = 5/2 \) sextet states (contour = \( \pm 0.001 \text{ au} \)).

is found, consistent with previously reported results for UB3LYP calculations with a LACVP-6-31G basis set and an effective core potential on Fe [144]. For model systems with a thiolate ligand, \( d_{\text{Fe}=\text{O}}(^{2}\text{A}_{2u}) \) and \( d_{\text{Fe}=\text{O}}(^{4}\text{A}_{2u}) \) are reported to differ by only 0.003 Å. Likewise, these calculations indicate that \( d_{\text{Fe}--\text{S}}(^{2}\text{A}_{2u}) \) and \( d_{\text{Fe}--\text{S}}(^{4}\text{A}_{2u}) \) should vary by 0.019 Å, consistent with the 0.023 Å difference for B3LYP and 0.025 Å difference for PBE0 reported here [145] [147]. These variations are readily attributed to the specific form of the density functional and the choice of basis functions. Likewise, with respect to the \( S = 3/2 \) state, the calculated \( d_{\text{Fe}=\text{O}}(\text{L} = -\text{SH}) \) exceeds \( d_{\text{Fe}=\text{O}}(\text{L} = -\text{SCH}_{3}) \) by 0.005 Å, whereas \( d_{\text{Fe}--\text{S}}(\text{L} = -\text{SH}) \) lags \( d_{\text{Fe}--\text{S}}(\text{L} = -\text{SCH}_{3}) \) by 0.067 Å, again consistent with the trend established in prior studies [148]. This variation is generally attributed to differential distribution of the third spin between porphyrin and ligand for different axial ligands. Furthermore, the geometric parameters predicted by hybrid functionals are comparable to those of the GGAs, with the hybrids affording slightly shorter Fe=O distances and slightly longer Fe–S distances.

The most prominent, discriminating characteristic observed between GGA and hybrid functionals is the relative energetics and populations of higher spin states (Tables 3 and 4). For both axial ligands, the GGA functionals predict a triradicaloid
$S = 1/2$ ground state. Electronically, this corresponds to an average of 1.48 spins on the Fe=O unit for $L = -\text{SH}$, with an opposing antiparallel spin population partitioned between the ligand and the porphyrin cycle (Figure 3.6). The net spin density on the Fe=O unit is larger when $L = -\text{SCH}_3$, corresponding to an average of 1.80 spins when averaging over all GGA functionals. The exact partition of spin density between ligand and porphyrin cycle is a convenient parameter for characterization of electronic structure for a given configuration. In the case of $S = 1/2$ and $L = -\text{SH}$, the ratio of ligand to porphyrin spin density is 1.48, which increases approximately twofold to 2.20 spins when $L = -\text{SCH}_3$ (Tables 3.3 and 3.4). This trend is seen as a general characteristic paralleled between ligands, with the methylthiolate favoring a stronger ligand–centered radical character. Taken together, the computational results for the methylthiolate are consistent with recent EPR data on cryogenically trapped Cpd I from CYP119, which indicate ligand–centered character for the ground–state radical [149]. The $S = 3/2$ state is likewise a triradicaloid configuration, lying an average of 0.169 eV above the $S = 1/2$ state when $L = -\text{SH}$, with a comparable gap averaging 0.154 eV for $L = -\text{SCH}_3$. The state gap is largest for the PBE functional, with a slightly smaller value assumed for BP and BLYP functionals. In contrast to the $S = 1/2$ case, three parallel spins are distributed between the Fe=O unit and the porphyrin / axial ligand. The Fe=O unit retains an average of approximately two spins (2.27 for $L = -\text{SH}$; 2.25 for $L = -\text{SCH}_3$), while the third spin is delocalized over the remainder of the system. In this case, the mean ratio of ligand to porphyrin spin is 1.11 when $L = -\text{SH}$ and 1.93 for $L = -\text{SCH}_3$, corresponding to a slightly more homogeneous partition of excess spin population. Finally, the $S = 5/2$ state is a parallel pentaradicaloid arrangement lying an average of 1.063 eV over $S = 1/2$ when $L = -\text{SH}$ and 1.046 eV when $L = -\text{SCH}_3$. Within this high spin configuration, the majority of the spin population is distributed on the porphyrin with a mean ratio
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<th>Spin State</th>
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<th>Porphyrin</th>
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Table 3.3: Spin-density analysis from Voronoi triangulation with $L = —SH$ and a GTO scheme.

of ligand to porphyrin spin of 0.46 when $L = —SH$ and 0.61 when $L = —SCH_3$. Nonetheless, for both ligands, the predominant spin density accumulation continues to occur on the Fe=O unit.

In contrast to the GGA case, the hybrid B3LYP and PBE0 functionals predict a near degeneracy of $S = 1/2$ and $S = 3/2$ states, with a slight preference to a $S = 1/2$ ground states. In particular, the mean $S = 1/2 \rightarrow S = 3/2$ gap is 0.009 eV for $L = —SH$ and 0.006 eV when $L = —SCH_3$. The $S = 1/2$ ground state configuration is again characterized by an antiparallel triradicaloid configuration, with an average ratio of ligand to porphyrin spin of 1.42 when $L = —SH$ and 2.09 when $L = —SCH_3$.  

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Table 3.4: Spin-density analysis from Voronoi triangulation with $L = -\text{SCH}_3$ and a GTO scheme.
Accordingly, the spin accumulation on the axial ligand is substantially larger for the hybrid functionals with methylthiolate ligand than for GGA functionals. As the spin is increased to \( S = 3/2 \), a nominally higher energy state, the spin accumulation on the ligand increases slightly for \( L = -\text{SH} \), bringing the ligand to porphyrin spin ratio to an average of 1.64. In the case of the methylthiolate, the increase in ligand spin accumulation is more drastic, the ratio increasing to a mean value of 4.80. The highest spin state considered, \( S = 5/2 \), is lower–lying than in the case of GGA functionals, with the \( S = 1/2 \rightarrow S = 5/2 \) gap averaging 0.42 eV for both \( L = -\text{SH} \) and \( L = -\text{SCH}_3 \). The spin partition between ligand and porphyrin is less extreme in this case, with a mean ligand to porphyrin spin density ratio of 0.82 for the thiolate and 1.52 for the methylthiolate. Taken together, these results indicate that the incorporation of exact exchange in hybrid functionals increases the sensitivity of the electronic structure to the nature of the axial ligand. In particular, the degeneracy of low lying states, the decreased gap for high spin states, and the stronger spin partitioning characteristic of the methylthiolate ligand are unlike any behavior observed with the GGA functionals. Nonetheless, correspondence to GGAs in terms of geometric parameters, overall spin distribution (Fe=O versus ligand + porphyrin), and electronic state ordering suggest that the GGA functionals nonetheless afford a reasonable picture of Cpd I structure. Given that our hybrid and GGA calculations are both internally consistent and consistent with existing literature [115]. Accordingly, the hybrid calculations are taken to canonically afford a high–level representative model system for Cpd I, with the GGA functionals benchmarking the minimum, necessary behavior which must be reproduced in a plane wave pseudopotential framework.
3.3.2 Martins–Troullier Scheme

The Martins–Troullier (MT) pseudopotential scheme is that most frequently employed in CPMD–based QM/MM studies. More specifically, this scheme has been exclusively utilized for any CPMD QM/MM calculations in which an oxyferryl species is present \cite{119, 124, 126, 127}. In order to accommodate overlap between core and valence electron densities, the PW basis is supplemented with a nonlinear core–correction on the Fe pseudopotential. This correction partially mitigates error in calculation of kinetic and exchange correlation energies, and hence enhances transferability of the pseudopotential. In addition, local s and p channels are included on the Fe pseudopotential to avoid the proliferation of ghost states in the Kleinman—Bylander scheme. All calculations presented below were conducted at a kinetic cutoff of 70 Ry, which is a reasonable value for practical Car—Parinello calculations on contemporary hardware. The predicted energetic and geometric parameters obtained with MT pseudopotentials were found to have a weaker correspondence to GTO calculations than those obtained with their Vanderbilt USPP counterparts.

Despite a high degree of internal consistency within the MT scheme, the absolute values of predicted geometric parameters exhibit several notable deviations from those obtained through GTO calculations (Table \text{3.5}). Only minute variations are observed in Fe=O bond lengths in the GTO case, specifically a maximum variation between ±0.035 Å between two states for any functional. The variation observed with MT pseudopotentials is comparable, with a maximum shift of 0.039 Å. Nonetheless, there exists a discrepancy in relative bond lengths between GTO and MT schemes. In particular, when averaging over spin states, the Fe=O distance is found to deviate from that of GTO calculations by a mean value of 0.034 Å for BP, by 0.033 Å for BLYP, and by 0.033 Å for the PBE functional when L = −SH. This deviation is
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<th>Fe–S (Å)</th>
<th>Energy (eV)</th>
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Table 3.5: Geometric parameters for several density functionals with L = —SH and L = —SCH$_3$ and several GGA functionals using a Martins–Troullier basis.
comparable to that obtained in the case where \( L = -\text{SCH}_3 \) assuming mean values of 0.033 Å for BP, 0.034 Å for BLYP, and of 0.036 Å for the PBE functional when \( L = -\text{SH} \). The situation is more severe for Fe–S distances, in which case the mean values are 0.095 Å for BP, with 0.116 Å for BLYP, and 0.103 Å for the PBE functional when \( L = -\text{SH} \). This discrepancy persists when \( L = -\text{SCH}_3 \) assuming mean values of values of 0.108 Å for BP, with 0.151 Å for BLYP, and 0.106 Å for the PBE when \( L = -\text{SCH}_3 \). Using a MT basis, the Fe=O distance is observed to satisfy:

\[
d_{\text{Fe=O}}(S = 3/2) > d_{\text{Fe=O}}(S = 5/2) > d_{\text{Fe=O}}(S = 1/2)
\] (3.4)

between states. The mean values of these distances are consistent between ligands, with the mean length \( d_{\text{Fe=O}}(S = 1/2) \) assuming a value of 1.671 Å, the mean \( d_{\text{Fe=O}}(S = 3/2) \) measuring 1.702 Å, and the mean \( d_{\text{Fe=O}}(S = 1/2) \) calculated to be 1.694 Å when \( L = -\text{SH} \). Once again, comparable values are obtained for calculations in which \( L = -\text{SCH}_3 \), where the mean \( d_{\text{Fe=O}}(S = 1/2) \) is 1.677 Å, while the mean \( d_{\text{Fe=O}}(S = 3/2) \) is 1.701 Å, and the mean \( d_{\text{Fe=O}}(S = 5/2) \) is 1.692 Å. With respect to the ligand itself, the scaling of \( d_{\text{Fe–S}} \) with spin state parallels the trend observed for calculations employing GTO basis:

\[
d_{\text{Fe–S}}(S = 5/2) > d_{\text{Fe–S}}(S = 3/2) > d_{\text{Fe–S}}(S = 1/2)
\] (3.5)

A high degree of consistency between ligands is likewise observed for \( d_{\text{Fe–S}} \), with a comparable magnitude of elongation with increasing spin. In particular, for \( L = -\text{SH} \), the mean value of \( d_{\text{Fe–S}}(S = 1/2) \) is 2.286 Å, that of \( d_{\text{Fe–S}}(S = 3/2) \) is 2.379 Å, and that of \( d_{\text{Fe–S}}(S = 5/2) \) is 2.412 Å, whereas when \( L = -\text{SCH}_3 \) the mean value of \( d_{\text{Fe–S}}(S = 1/2) \) is 2.279 Å, that of \( d_{\text{Fe–S}}(S = 3/2) \) is 2.374 Å, and that of \( d_{\text{Fe–S}}(S = 5/2) \) is 2.426 Å.
The state level spacings between MT and GTO schemes exhibit relatively large deviations when considering the first spin state gap when considering $L = \text{SH}$. In particular, the $S = 1/2 \rightarrow S = 3/2$ gap deviates by 0.047 eV for BP and 0.082 eV for the PBE functional when exploiting the nonlinear core correction. The BLYP functional is not included for comparison, as the states assume a different energetic ordering than in the GTO case. Similar deviations are seen for the case in which $L = \text{SCH}_3$, excepting a comparable value for the PBE functional. Specifically, when $L = \text{SCH}_3$, the $S = 1/2 \rightarrow S = 3/2$ gap varies from GTO values by 0.071 eV for the BP functional, 0.053 eV for BLYP, and 0.015 eV for PBE with the nonlinear core correction. This translates to a mean deviation of 0.065 eV between schemes for the $L = \text{SH}$ and 0.046 eV for $L = \text{SCH}_3$. In the case of the $S = 1/2 \rightarrow S = 5/2$ gap, the observed deviations are smaller, varying by 0.028 eV for BP and by 0.054 eV for PBE with the nonlinear core correction when $L = \text{SH}$. When $L = \text{SCH}_3$, these deviations comprise 0.023 eV for BP, with 0.018 eV for both BLYP and PBE functionals with the nonlinear core correction.

Excepting configurations with the BLYP functional, the MT scheme predicts a triradicaloid $S = 1/2$ ground state, with an average of 1.44 spins belonging to the Fe=O unit between functionals when $L = \text{SH}$ and 1.53 spins on the Fe=O when $L = \text{SCH}_3$ (Table 3.6). A remaining antiparallel spin fraction accumulates on the porphyrin cycle and on the axial ligand. The $S = 3/2$ is likewise a triradicaloid configuration, however, in this case all spins are in a parallel arrangement. The spin accumulation on the Fe=O unit is more substantial than in the $S = 1/2$ case, averaging 2.51 spins over all functionals when $L = \text{SH}$ and 2.44 spins when $L = \text{SCH}_3$. In the extreme case, the $S = 5/2$ system is characterized by a spin parallel pentaradicaloid configuration, with an average of 3.77 spins on Fe=O when $L = \text{SH}$ and 3.76 spins when $L = \text{SCH}_3$. 

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<th>Porphyrin</th>
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Table 3.6: Spin–density analysis from Voronoi triangulation with $L = \text{—SH}$ and $L = \text{—SCH}_3$ and several GGA functionals using a Martins—Troullier basis.
The choice of axial ligand and spin state has a marked effect on the partition of spin between ligand and porphyrin centered radicals. When \( L = -\text{SH} \), the ligand accumulates an average of 1.65 times the spin of the porphyrin cycle for the BP, 1.36 spins for BLYP, and a 34 fold accumulation for the PBE functional, placing nearly all spin density on the axial ligand. As the net spin is increased for the \( S = 3/2 \) case, the ligand retains 1.88 times the spin of the porphyrin for the BP functional, while BLYP and PBE functionals retain an average of 0.96 times the spin. In the highest spin state, \( S = 5/2 \), porphyrin radical character dominates, with the ligand retaining an average fraction of 0.37 times the porphyrin spin content over all functionals. When \( L = -\text{SCH}_3 \) this partition becomes more drastic, the ligand accruing an average of 4.30 times the spin density of the porphyrin for BP, 4.56 times for PBE, and 3.00 times the spin density for the BLYP functional in the \( S = 1/2 \) state. Shifting to the \( S = 3/2 \) case, the anisotropy increases. Under these conditions, the BP functional affords a 5.00 fold spin excess and PBE a 4.67 fold spin excess favoring the ligand. The partition calculated using the BLYP functional is smaller, providing only a 2.96 fold accumulation. In the \( S = 5/2 \) case, the \( L = -\text{SCH}_3 \) systems likewise shift to a porphyrin centered radical, however, the deficit on the ligand is comparable between functionals, averaging 0.67 times that of the integrated porphyrin spin density. Accordingly, the methylthiolate favors a roughly two-fold accumulation of spin on the ligand compared to the excess favored by the the thiolate systems. Irrespective of state or functional, the corresponding spin distribution between ligand and porphyrin is substantially larger than that of the corresponding GGA with a GTO basis. Furthermore, the sporadic deviations observed between functionals suggests that care must be taken when choosing a model to represent the electronic structure of a given system.
3.3.3 Vanderbilt Ultrasoft Pseudopotentials

The Vanderbilt (VDB) ultrasoft pseudopotential (USPP) scheme affords the most accurate description of CYP450 Cpd I within a plane-wave pseudopotential framework. Kinetic cutoffs for the Vanderbilt USPP scheme generally fall far below those employed in the Martins-Troullier scheme at a comparable level of accuracy. An optimal kinetic cutoff of 30 Ry was ascertained for the Vanderbilt USPPs; this value of the cutoff was employed in all subsequent calculations (Figure 3.3).

The geometric parameters for the Vanderbilt USPPs closely parallel those obtained using the GTO scheme (Table 3.7). In particular, the Fe=O bond lengths universally obey the state dependence

\[ d_{\text{Fe=O}}(S = 3/2) > d_{\text{Fe=O}}(S = 5/2) > d_{\text{Fe=O}}(S = 1/2) \] (3.6)

identical to GGA functionals in the the GTO case. Likewise, the Fe—S bond lengths \( d_{\text{Fe—S}} \) obey the relation:

\[ d_{\text{Fe—S}}(S = 5/2) > d_{\text{Fe—S}}(S = 3/2) > d_{\text{Fe—S}}(S = 1/2) \] (3.7)

further underscoring the parallel between Vanderbilt USPPs and the GTO basis. The similarity in this ordering is significant, as the mean deviation of geometric parameters from those predicted by the GTO scheme is small. In particular, when \( L = -\text{SH} \), the mean deviation for the Fe=O distance from that of the corresponding GTO calculation for any state is 0.028 Å for BP, 0.007 Å for BLYP, 0.005 Å for PBE with a semilocal approximation, and 0.005 Å for PBE with the nonlinear core correction. This variation is comparable in the case of the methylthiolate ligand, in which case the deviation is an average of 0.003 Å for BP, 0.000 Å for BLYP, 0.005 Å
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Table 3.7: Geometric parameters for several density functionals with $L = -$SH and $L = -$SCH$_3$ and several GGA functionals using a Vanderbilt ultrasoft pseudopotential basis.
for PBE with a semilocal approximation, and 0.005 Å for PBE with the nonlinear core correction. These deviations are approximately one order of magnitude smaller than those observed when using the Martins—Troullier psuedopotential scheme. The deviation for Fe—S distances is larger but still trivial, averaging 0.014 Å for BP, 0.007 Å for BLYP, 0.008 Å for PBE with a semilocal approximation, and 0.016 Å for PBE with the nonlinear core correction when $L = \text{—SH}$. The values assumed in the case of $L = \text{—SCH}_3$ are slightly larger, averaging 0.007 Å for BP, 0.029 Å for BLYP, 0.013 Å for PBE with a semilocal approximation, and 0.019 Å for PBE with the nonlinear core correction. It is notable that these deviations from GTO–based results are again one order of magnitude smaller than those observed when comparing the MT scheme to GTO calculations. Given that these variations lie near the limit of experimentally accessible measurements, the VDB USPP calculations at 30 Ry cutoff may be taken as equivalent to the GTO calculations for structural purposes.

Within the Vanderbilt USPP scheme, most functionals predict a $S = 1/2$ ground state, followed by the $S = 3/2$ and $S = 5/2$ states in succession. A weak exception to this rule is afforded by the BP functional, which presents nearly degenerate $S = 1/2$ and $S = 3/2$ states, with the $S = 3/2$ state lying 0.059 eV lower in energy. The state level spacings between USPP and GTO schemes are highly comparable for the PBE functional with a semilocal approximation, PBE with the nonlinear core correction, and the BLYP functional when considering the first spin state gap. In particular, the $S = 1/2 \rightarrow S = 3/2$ gap deviates 0.005 eV for BLYP and 0.004 eV for the PBE functional with a semilocal approximation, while do deviation is obtained for PBE with the nonlinear core correction when $L = \text{—SH}$. In the case of $L = \text{—SCH}_3$, the deviation is 0.036 eV for BP, 0.03 eV for BLYP, 0.007 eV for PBE functional with a semilocal approximation, and 0.023 eV for PBE with the nonlinear core correction. This translates to a mean deviation of 0.003 eV between schemes for the thiolate
and 0.021 eV for the methylthiolate. In the case of the $S = 1/2 \rightarrow S = 5/2$ gap, a dependence on the specific functional is apparent. The deviations from the GTO scheme are larger than in the preceding case, varying by 0.375 eV for BP, 0.254 eV for BLYP, 0.147 eV for PBE in the semilocal approximation, and 0.026 eV for PBE with the nonlinear core correction when $L = -\text{SH}$.

When $L = -\text{SCH}_3$, these deviations comprise 0.223 eV for BP, 0.265 eV for BLYP, 0.147 eV for PBE in the semilocal approximation, and 0.065 eV for PBE with a nonlinear core correction. It is appropriate to note that overall the best agreement with the GTO scheme is obtained with the PBE functional, irrespective of the correction method. Interestingly, these results are juxtaposed with those for the MT scheme. With Vanderbilt USPPs, the $S = 1/2 \rightarrow S = 3/2$ gap is most consistent with the GTO results, while the MT pseudopotentials perform relatively poorly. Conversely, the MT pseudopotentials afford comparatively better performance when calculating the $S = 1/2 \rightarrow S = 5/2$ gap, while in this case the Vanderbilt USPPs perform poorly. Nonetheless, most relevant Cpd I–initiated chemistry is believed to occur in the first two spin states. Accordingly, the relatively tight performance afforded by the Vanderbilt USPPs with respect to low–lying spin state energetics makes this pseudopotential scheme a prudent choice for most applications.

As a final point of comparison, the spin density distributions for the VDB USPP system likewise exhibit excellent similarity to the GTO scheme (Table 3.8). In particular, the VDB USPP ground state is a $S = 1/2$ triradicaloid for all three functionals, with 1.5 to 2.0 spins of parallel spin localized to the Fe=O unit, and a third antiparallel spin distributed between the porphyrin ring and the axial ligand. The $S = 3/2$ state is likewise a triradicaloid, however in this case slightly more spin accumulates on the Fe=O unit, with a parallel spin distributed among the porphyrin and axial ligand. The deviation from the spin distribution on the Fe=O unit in
GTO calculations is small. Averaging over all spin states for a given functional, this corresponds to a mean variation of 0.09 spins for BP, of 0.12 spins for BLYP, 0.08 spins for PBE with the nonlinear core correction, and 0.09 spins for PBE with the semilocal approximation when \( L = -\text{SH} \). In the case of \( L = -\text{SCH}_3 \), the mean variation is 0.09 spins for BP, 0.10 spins for BLYP, 0.12 spins for PBE with the nonlinear core correction, and 0.11 for PBE with the semilocal approximation. It is notable that in all cases the VDB USPPs tend to distribute more spin density onto the axial ligand than their GTO counterparts. Once again, the PBE functional provides good agreement with its GTO counterpart.

Similar to the MT case, the Vanderbilt USPPs predict a an antiparallel triradicaloid \( S = 1/2 \) ground state, excepting the aforementioned ambiguity arising from state degeneracy in the case of the BP functional when \( L = -\text{SH} \). In the \( S = 1/2 \) state, a mean value of 1.55 spins accumulate on the Fe=O unit when \( L = -\text{SH} \), averaging among the BP, BLYP, and PBE functional with nonlinear core correction, and 1.67 spins when \( L = -\text{SCH}_3 \). As the spin state is increased to \( S = 3/2 \), the system again assumes a parallel triradicaloid configuration with an average of 2.35 spins on Fe=O when \( L = -\text{SH} \) and 2.29 spins when \( L = -\text{SCH}_3 \). Increasing to the parallel pentaradicaloid in the \( S = 5/2 \) state, an average of 3.76 spins accumulate on Fe=O for \( L = -\text{SH} \) and 3.74 spins on Fe=O in the case that \( L = -\text{SCH}_3 \). Note that the PBE functional with the semilocal approximation was omitted from these statistics to avoid biasing numerical values in favor of the PBE functional. These quantities are comparable to trends observed within the MT and GTO schemes.

In contrast to the MT scheme, the partition of spin density between the axial ligand and the porphyrin is generally more consistent with calculations corresponding to GGA functionals in the GTO framework. When \( L = -\text{SH} \), the ligand accumulates an average of 1.44 times the spin of the porphyrin cycle for the BLYP and PBE
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<th>Ligand</th>
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<th>Spin State</th>
<th>Fe=O</th>
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<th>Porphyrin</th>
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<td>—SH</td>
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Table 3.8: Spin–density analysis from Voronoi triangulation with $L = —SH$ and $L = —SCH_3$ and several GGA functionals using a Vanderbilt ultrasoft pseudopotential basis.
functionals, and a 15.00 fold accumulation favoring the ligand for the BP functional. As the net spin is increased in the $S = 3/2$ case, the ligand retains 1.00 times the spin of the porphyrin when averaging over GGA functionals. In the highest spin state, $S = 5/2$, the porphyrin radical character overshadows that of the ligand, with the ligand retaining an average fraction of 0.42 times the porphyrin spin content. Taken together, the Vanderbilt USPP scheme affords a more symmetric distribution of spin for the case in which $L = -\text{SH}$. In the $L = -\text{SCH}_3$ case, the spin partition becomes more strongly biased in favor of the ligand. Nonetheless, this occurs in a manner less substantial than that observed within the MT scheme. In particular, the mean spin excess on the ligand is a 3.15 fold accumulation over the porphyrin for $S = 1/2$ and 3.15 fold accumulation for $S = 3/2$. Furthermore, this excess is calculated to decrease in the highest spin state considered, $S = 5/2$, depleting the spin population on the ligand to 0.78 times that of the porphyrin cycle, averaging over functionals. Variations exist between functionals in terms of the nuances of these spin distributions, however, no trend is concrete enough to warrant further commentary. Inspection of GGA data in the GTO framework reveals a high degree of similarity to spin distribution trends observed with the Vanderbilt USPPs. Furthermore, the trend observed with the methylthiolate ligand again echoes that of previous computational and EPR studies favoring a ligand–centered radical character for Cpd I [149].

In a final attempt to contact experiment and afford methodological validation, the Vanderbilt USPPs were exploited to calculate EPR $g$–tensors for Cpd I. For all functionals and spin states decent agreement is found with respect to the experimental values ($g_x = 1.72$, $g_y = 1.86$, $g_z = 2.00$, $g_{iso} = 1.94$) recently observed in Cyp119 [149]. The tightest comparison between computation and experiment exists in the $S = 1/2$ state for the BP and PBE functionals with both ligands (Tables 3.9 and 3.10). Of these, the most exceptional is the $S = 1/2$ state with the PBE functional and $L =$
—\text{SCH}_3$, which is expected to be the most representative model for the Cpd I ground state.

3.3.4 Conclusions

The Vanderbilt USPP scheme most accurately reproduces the GTO reference calculations within a plane–wave pseudopotential framework. In contrast to the MT psuedopotential scheme, the Vanderbilt USPPs afford geometric parameters which, in many cases, coincide almost exactly with GGA / GTO results. Furthermore, the energetic state spacing has a far tighter correspondence to that observed using GTO–based calculations, and the spin density distribution and partition is likewise strongly paralleled. While most calculations involving oxyferryl species and a plane wave framework, to date, have utilized the MT scheme, our results suggest that it is advisable and advantageous to preferentially exploit the Vanderbilt USPP scheme. Beyond corresponding geometric and electronic parameters, the increased stability of Vanderbilt USPPs with transition metal systems and the higher computational speed afforded by the use of lower kinetic cutoffs make this method particularly prudent for use in future calculations.
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Table 3.9: Electronic g-tensor components from DFT calculations with a def2–TZVP basis and L = SH. Experimental values are from EPR spectroscopy of CYP119 Cpd I [149].
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Table 3.10: Electronic $g$–tensor components from DFT calculations with a def2–TZVP basis and $L = -\text{SCH}_3$. Experimental values are from EPR spectroscopy of CYP119 \[149\].
4.1 Introduction

A frequent approximation in AIMP methods is the classical description of nuclear degrees of freedom. Nonetheless, this assumption fails for many biological, chemical, and solid-state systems of practical interest, in which zero-point corrections to nuclear motion and nuclear quantum tunneling play a nontrivial role. One approach to circumvent these limitations is path integral molecular dynamics (PIMD) [1][51][53]. PIMD exploits an isomorphism between the quantum partition function and a ring-polymer comprising harmonically coupled replicas of the quasiclassical system in order to compute ensemble properties of the full quantum system. Convergence to the quantum limit is obtained as the number of replicas is increased, at the cost of simulating many copies at the level of AIMP. The expense of this approach quickly becomes prohibitive for large systems or for simulations with poor counting statistics in the corresponding AIMP calculations. The existence of such methods would augment

Note bene: Unless otherwise explicitly indicated, Hartree atomic units are adopted throughout this chapter, in which \( \hbar = e = m_e = 1/(4\pi\epsilon_0) = 1 \), corresponding to Planck’s reduced constant, the fundamental unit of electric charge, the electron mass, and Coulomb’s constant, where \( \epsilon_0 \) is the permittivity of free space. Within this scheme, energies are measured in Hartree (1 Ha = 27.2113386 eV = 627.5094706 kcal mol\(^{-1}\) = 2625.4996251 kJ mol\(^{-1}\)) and distances in units of the Bohr radius \( a_0 = 0.52917720859 \) Å. All numerical values are adopted from the CODATA 2006 physical and chemical data set[150].
studies of biological importance in which tunneling occurs, in particular CYP450 Compound I catalyzed hydrogen atom abstraction.

An alternative approach is presented for systems in which (i) a high barrier exists to the sampling of a given process and in which (ii) nuclear dynamics may be described in an ensemble-averaged effective background. Examples of these processes may include hydrogen–atom abstraction in enzyme active sites, reactions in quenched backgrounds at cryogenic temperatures, and proton migration in disordered solids. Using Car–Parrinello molecular dynamics and a free energy sampling method, a potential of mean force is constructed to describe the quantum process in the limit of classical nuclei. This free–energy landscape for nuclear motion is consequently exploited to generate an effective landscape for quantum Monte Carlo simulations. Inspired by methods from quantum chromodynamics, we utilize an alternative framework for path integral Monte Carlo in which the Euclidean functional integral is discretized on a lattice, thereby permitting calculations of correlation functions and ultimately the action of the system. As the action of the system is quenched through Monte Carlo moves, instanton solutions and their contribution to the degeneracy splitting may be obtained within the collective variable subspace, and the effective potential corresponding to the instanton corrections identified. This technique is demonstrated for malonaldehyde, a model system in which hydrogen tunneling is critical. This hitherto unreported combination of free energy methods and Monte Carlo calculation represents a novel extension to ab initio simulation methodologies.
4.2 Theoretical Methods

4.2.1 Functional Integrals

The formal basis for subsequent calculations is the Feynman path integral representation of quantum mechanics [157]. Since the numerical methods exploited in tunneling calculations are intimately bound to the construction of this method, a brief overview is presented. Consider a simple quantum-mechanical point particle moving in a potential \( \hat{V} \). In this case, the Hamiltonian for the system is

\[
\hat{H} = \frac{1}{2m} \hat{p}^2 + \hat{V}
\]

(4.1)

where \( \hat{p} = -i\hbar \partial / \partial x \) is the momentum operator. Within the Heisenberg picture of quantum mechanics, the amplitude for this particle to propagate from an initial position eigenstate \( |x_i, t_i\rangle \) to a final state \( |x_f, t_f\rangle \) is typically calculated by applying the unitary time evolution operator \( \hat{U}(t_f, t_i) = \exp[-\hat{H}(t_f - t_i)/\hbar] \) to the initial state and taking the inner product. An entirely equivalent formulation is obtained in the Feynman prescription by taking the sum of a phase factor \( \exp[iS[x]/\hbar] \) over all possible worldlines progressing from \( x(t_i) = x_i \) to \( x(t_f) = x_f \) in time \( |t_f - t_i| \)

\[
\mathcal{G}(x_f, x_i; t_f - t_i) = \langle x_f | e^{-i\hat{H}(t_f - t_i)/\hbar} | x_i \rangle
\]

(4.2)

\[
= \mathcal{N} \int \mathcal{D}x \exp[iS[x(t)]/\hbar]
\]

(4.3)

where \( \mathcal{N} \) is a normalization factor. Within this context, the notation \( \int \mathcal{D}x \) specifies an integral over paths in the aforementioned worldline space, and the quantity \( S[x] \) is the classical action.
\[ S[x] = \int_{t_i}^{t_f} \mathcal{L}(x(t)) \, dt \] (4.4)

and where, for the case of a point–particle, the Lagrangian is

\[ \mathcal{L}(x(t)) = \frac{1}{2m} \left( \frac{dx(t)}{dt} \right)^2 - V(x(t)) \] (4.5)

for motion in one dimension.

While exact solutions have been derived for several model potentials \( V(x) \), it is nonetheless necessary to numerically evaluate the path integral for most physically interesting systems. Unfortunately, in practice, the complex argument of the exponential function causes the integral to oscillate wildly when numerical evaluation is attempted thus necessitating large number of path samples for convergence. This instability may be circumvented by shifting to a Euclidean prescription by analytic continuation via Wick rotation \( t \rightarrow \tau = it \), in which case the Lagrangian becomes

\[ \mathcal{L}(x(\tau)) = -\frac{1}{2m} \left( \frac{dx(\tau)}{d\tau} \right)^2 - V(x(\tau)) \] (4.6)

and similarly the functional integral

\[ Z = \mathcal{N} \int_0^T Dx \exp[-S[x(\tau)]/\hbar] \] (4.7)

In this case, the weighting factors \( \exp[-S[x(\tau)]/\hbar] \) are exponentially damped, hence affording better numerical stability and hence convergence properties. Furthermore, the propagator is transformed into the partition function \( Z \) of a \( d + 1 \) statistical theory, which may be exploited to calculate a variety of physical observables. The quantum formulation may likewise be obtained from the Euclidean prescription through a second Wick rotation \( \tau \rightarrow t = -i\tau \). Nonetheless, the Euclidean functional integral
reflects the ground state properties of the quantum mechanical theory as $T \to \infty$
(equivalent to $1/\hbar$ as $\hbar \to 0$).

4.2.2 Instantons

Instantons were first constructed as a framework to characterize the decay of metastable vacuum in quantum field theory [158][159]. These solutions, which are the functional integral representation of tunneling processes, have found application to systems ranging from high–energy and nuclear physics [155][160] to condensed matter problems [161][163] to chemical physics, and feature as a prominent nonperturbative method in quantum field theory, in particular for strongly–coupled gauge theories [164][166]. Assume that a point particle is trapped in a double–well potential of the form $V(x) = \lambda (x^2 - \eta^2)^2$ so that the Lagrangian for the system corresponds to an anharmonic oscillator

$$\mathcal{L} = \frac{1}{2m} \left( \frac{dx(t)}{dt} \right)^2 - \lambda (x(t)^2 - \eta^2)^2. \quad (4.8)$$

This potential clearly admits both bound and scattering state solutions. The bound state solutions, corresponding to particles with energy $E < \lambda \eta^4$, mix under the potential and ‘leak’ between wells. For a sufficiently large barrier, the harmonic oscillator eigenstates corresponding to the left and right vacua at $x = \pm \eta$ combine in a simple manner, generating a composite wavefunction which is a direct superposition of the left $\psi_L(x)$ and right $\psi_R(x)$ well eigenfunctions

$$\psi_{\pm} = \frac{1}{\sqrt{2}} (\psi_L(x) \pm \psi_R(x)). \quad (4.9)$$

The objective of the instanton formalism is to address this mixing process from a particle–oriented viewpoint. In particular, this methodology affords insight into the
manner through which tunneling between vacua begets the superposition of states characterizing the full anharmonic oscillator potential.

The Lagrangian (4.8) may be exploited to write the transition amplitude from the position eigenstate \(|-\eta\rangle\) to \(|\eta\rangle\) as

\[
\langle \eta | \exp[-i\hat{H}t/\hbar] | -\eta\rangle = \int \mathcal{D}x \exp \left[\frac{i}{\hbar} S[x] \right] \tag{4.10}
\]

While this expression may be evaluated exactly, a convenient trick is to transform to the Euclidean action by the Wick rotation \(t \rightarrow \tau = it\) so that (suppressing \(\hbar\) for notational simplicity)

\[
Z = \int \mathcal{D}x \exp[-S_E[x]]
\]

\[
= \int \mathcal{D}x \exp \left[ - \int_0^T d\tau \left( \frac{1}{2m} \left( \frac{dx(\tau)}{d\tau} \right)^2 + \lambda (x(\tau)^2 - \eta^2)^2 \right) \right] \tag{4.11}
\]

where the sign of the potential has been inverted in the Euclidean Lagrangian \(L_E[x(\tau)]\).

While tunneling governed the Lagrangian \(L[x(t)]\) corresponds to a barrier penetration process interpolating between vacua \(x = -\eta\) and \(x = \eta\), the equivalent solution for \(L_E[x(\tau)]\) corresponds to a particle ‘rolling’ between two hills with maxima at the same positions. The classical solution interpolating between \(x = -\eta\) and \(x = \eta\) within the Euclidean functional integral is termed an instanton (Figure 4.1).

A unique property of instantons is that the action of these solutions is bounded. This behavior is a direct consequence of the boundary conditions placed on the instanton. In the trivial case, the instanton solution \(X(\tau)\) will remain stationary at the top of a well \(X(\tau) = \pm \eta\), and hence the action is invariant as the Euclidean time interval grows \(T \rightarrow \infty\). Any nontrivial solution must also retain this invariance. One
Figure 4.1: Propagation trajectories between position eigenstates $|x_i\rangle$ to $|x_f\rangle$ for a particle in a double–well potential $V(x(t))$. The Minkowski–space paths (A) correspond to either activated hopping over the barrier (green) or tunneling through the barrier (pink). Instantons comprise the classical trajectories (pink) interpolating between maxima of the inverted potential $-V(x(t))$ in the Euclidean–space functional integral (B).

possibility is to seek solutions such that the particle begins motion at $X(-\tau/2) = -\eta$, traverses the well of the inverted potential, and comes to rest at $X(\tau/2) = \eta$. This case corresponds to a classical solution for the motion of a particle on a hill; a mechanical process in which the net energy of the system is nil. While application of the Euler–Lagrange equations to $\mathcal{L}_E[x(\tau)]$ yields

$$m \frac{d^2 X(\tau)}{d\tau^2} = 2\lambda X(\tau) (X(\tau)^2 - \eta^2)$$  \hspace{1cm} (4.13)

it is far simpler to utilize conservation of energy to equate the potential and ‘kinetic’ terms to write (taking the negative root)

$$\frac{dX(\tau)}{d\tau} = \sqrt{2\lambda m} \left( X(\tau)^2 - \eta^2 \right)$$  \hspace{1cm} (4.14)

which separates and integrates trivially to afford the solution
\[ X(\tau) = \eta \tanh \left[ \sqrt{2m\lambda\eta}(\tau - \tau_c) \right] \]  \hspace{1cm} (4.15)

This particular trajectory is termed an *instanton*, and \( \tau_c \) is an arbitrary parameter fixing the center of the instanton. Conversely, the corresponding return solution interpolating from \( \eta \rightarrow -\eta \) is known as an *antiinstanton*. Not only is the action of an instanton finite, but it is also quantized

\[
S_0 = \int_{-\infty}^{\infty} d\tau \left( \frac{dX(\tau)}{d\tau} \right)^2 \\
= \int_{-\eta}^{\eta} \frac{dX(\tau)}{d\tau} dX \\
= \int_{-\eta}^{\eta} \left[ -\sqrt{2\lambda m}(X(\tau)^2 - \eta^2) \right] dX \\
= \frac{\omega^3}{12\lambda m} \hspace{1cm} (4.19)
\]

where in the last line the frequency \( \omega^2 = 8\lambda m\eta^2 \) has been introduced, consistent with the notational convention in literature [164].

The instanton solutions for the anharmonic oscillator are only one class of instanton solutions; those for which a return antiinstanton trajectory is possible. Nonetheless, in the original formulation, solutions were considered which represent escape from a vacuum into a lower energy vacuum [158,159]. Within a chemical context, this would correspond to a tunneling process such as hydrogen–atom abstraction, while the anharmonic oscillator–like scenario distinctly embodies resonant phenomena. Nonetheless, these developments will be sufficient for the purposes of future calculations within this section. In particular, the presence and nature of instanton solutions afford insight into the tunneling processes for a given system.
4.2.3 Physical Observables

The simplest quantity to calculate in the functional integral formalism is the expectation value of a given observable $O$

\[
\langle O \rangle = \frac{1}{Z} \int_0^T d\tau \mathcal{D}x \exp[-S[x]]
\]  

(4.20)

Correlation functions are only slightly less trivial to compute [167]. By augmenting the action through addition of a scalar current $J(\tau)$ to afford the generating functional

\[
\mathcal{Z}[J] = \int \mathcal{D}x \exp \left[ - \int_0^\beta \left[ \mathcal{L}(x(\tau)) + J(\tau)x(\tau) \right] \right]
\]  

(4.21)

the $N$–point correlator $\Gamma^N = \langle x(\tau_N)x(\tau_{N-1}) \ldots x(\tau_0) \rangle$ is readily obtained as the $N$–point functional derivative

\[
\Gamma^{(N)} = \left[ \frac{\partial}{\partial J(\tau_N)} \frac{\partial}{\partial J(\tau_{N-1})} \cdots \frac{\partial}{\partial J(\tau_0)} \mathcal{Z}[J] \right]_{J=0}
\]  

(4.22)

Similarly, the $N$–point connected correlation functions are obtained through functional derivatives of the logarithm of the generating functional

\[
\Gamma_c^{(N)} = \left[ \frac{\partial}{\partial J(\tau_N)} \frac{\partial}{\partial J(\tau_{N-1})} \cdots \frac{\partial}{\partial J(\tau_0)} \log(\mathcal{Z}[J]) \right]_{J=0}
\]  

(4.23)

\[
= \left[ \frac{\partial}{\partial J(\tau_N)} \frac{\partial}{\partial J(\tau_{N-1})} \cdots \frac{\partial}{\partial J(\tau_0)} \mathcal{Z}[J] \right]_{J=0}
\]  

(4.24)

which itself corresponds to the correlation function arising from a sum over connected Feynman diagrams.

For the purposes of contacting experiment, the primary item of interest is the connected 2–point correlator
\[ \Gamma_c^{(2)} = \langle x(\tau_1)x(\tau_2) \rangle - \langle x(\tau_1) \rangle \langle x(\tau_2) \rangle. \]  

The long-time limit of this quantity is particularly fortuitous, as the logarithmic derivative of the connected 2-point function affords the degeneracy splitting \( \Delta E_{0\rightarrow1} \) for tunneling processes under a given potential. This property is most readily demonstrated in the equivalent state vector representation of Hamiltonian quantum statistical mechanics \( [168] \), adopting the notation of \( [154] \). Within this context, the generating function is

\[ \mathcal{Z}[J] = \text{Tr} \exp \left[ -\frac{T}{\hbar} \left( \hat{H} + \sum_{i=1}^{N} J_i x_i \right) \right] \]  

such that the trace is defined as

\[ \mathcal{Z} = \text{Tr}[\exp[-\hat{H}T/\hbar]] = \int_{-\infty}^{\infty} dx \langle x| \exp[-\hat{H}T/\hbar]|x \rangle. \]  

Consequently, the expectation value of an operator \( \hat{O} \) becomes

\[ \langle \hat{O} \rangle = \mathcal{Z}^{-1} \text{Tr}[\exp[-\hat{H}T/\hbar]\hat{O}]. \]  

In the large \( T \) limit, the expectation values reduce to those of the traditional quantum mechanical picture:

\[ \langle \hat{O} \rangle = \lim_{T \to \infty} \sum_{n} \langle n|\hat{O}|n \rangle \exp[-E_n T/\hbar] = \langle 0|\hat{O}|0 \rangle \]  

where \( E_n \) are the energy eigenvalues \( \hat{H}|n \rangle = E_n|n \rangle \).

Having written a discrete representation for \( x_i \) and \( J_i \), correlation functions may be obtained through the preceding proscription. This estimator is particularly fortuitous
for large $T$. Inserting an intermediate set of states and writing $x(\tau)$ in terms of the time evolution operator

\[
\lim_{T \to \infty} \Gamma_c^{(2)} = \langle 0| x(0)x(\tau)|0 \rangle - \langle 0|x(0)|0 \rangle^2 \quad (4.30)
\]

\[
= \langle 0|x(0) \exp[-\hat{H}\tau/\hbar]x(0) \exp[\hat{H}\tau/\hbar]|0 \rangle \quad (4.31)
\]

\[
- \langle 0|x(0)|0 \rangle^2 \quad (4.32)
\]

\[
= \sum_n \langle 0|x(0)|n \rangle \langle n|x(0)|0 \rangle \exp\left[-\frac{\tau}{\hbar}(E_n - E_0)\right] \quad (4.33)
\]

\[
- \langle 0|x(0)|0 \rangle^2 \quad (4.34)
\]

\[
= \sum_{n \neq 0} |\langle n|x(0)|0 \rangle|^2 \exp\left[-\frac{\tau}{\hbar}(E_n - E_0)\right] + \quad (4.35)
\]

\[
(\langle 0|x(0)|0 \rangle)^2 - \langle 0|x(0)|0 \rangle^2 \quad (4.36)
\]

\[
= \sum_{n \neq 0} |\langle n|x(0)|0 \rangle|^2 \exp\left[-\frac{\tau}{\hbar}(E_n - E_0)\right]. \quad (4.37)
\]

This is the standard spectral representation for the two point Green’s function. For large $\tau$, the first energy gap is readily obtained by taking the ratio of two correlation functions

\[
\lim_{\tau \to \infty} \frac{\Gamma_c^{(2)}(\tau')}{\Gamma_c^{(2)}(\tau)} = \exp\left[-(E_1 - E_0)(\tau' - \tau)/\hbar\right] \quad (4.38)
\]

This quantity in turn may be formally written as a limit
\[ \Delta E_{0 \rightarrow 1} = E_1 - E_0 = \hbar \lim_{T \rightarrow \infty} \left[ -\frac{1}{\tau' - \tau} \log \left( \frac{\Gamma^{(2)}(\tau')}{\Gamma^{(2)}(\tau)} \right) \right] \]

\[ = \hbar \lim_{T \rightarrow \infty} \left[ -\frac{1}{\tau' - \tau} \left[ \log \left( \Gamma^{(2)}(\tau') \right) - \log \left( \Gamma^{(2)}(\tau) \right) \right] \right] \]

\[ = \hbar \lim_{\tau \rightarrow \infty} \frac{d}{d\tau} \log \left[ \Gamma^{(2)}(\tau) \right] \]

Since the correlation function is readily calculated from Monte Carlo ensemble averages, this quantity is trivial to extract from simulation.
4.2.4 Discretization and Numerical Implementation

For the purpose of simplicity and practical implementation, the following discussion will pertain to a one-dimensional system. Nonetheless, these considerations extrapolate trivially to any spatial dimensionality. Formally, the Feynman path integral is defined as a sum over worldlines in Minkowski space

$$\mathcal{G}(x_F, x_I; t_F, t_I) = \sum \exp \left[ \frac{iS[x]}{\hbar} \right]$$

(4.42)

The domain of this problem may be subdivided into discrete sets of time \(\{t_i\}\) and space \(\{x_i\}\) points, which comprise a two-dimensional spacetime lattice given by the Cartesian product \(\{t_i\} \times \{x_i\}\). Any such \(x_i \in \mathbb{R}\) is spatially unbounded so \(-\infty < x < \infty\), however, the times are constrained to lie within an interval \(t \in [0, t_N] \subset \mathbb{R}\) and satisfy the property that \(t_i < t_{i+1}\). The sum in the path integral is then a discrete series of \(N\) points such that \(x(t_0) = x_i\) and \(x(t_N) = x_N\), where \(x(t_i) = x_i\) for all \(x \in \mathbb{N}\) with \(i < N\). In the following, time times are assumed to be monotonically discretized such that any two consecutive points in time are separated by a fixed interval \(\epsilon = t_{i+1} - t_i\) (Figure 4.2).

The Euclidean functional integral is then defined through the substitution \(a = i\epsilon\). In this case, the path integral measure is well-defined

$$\int \mathcal{D}x = \int_{-\infty}^{\infty} \prod_{i=1}^{N-1} dx_i$$

(4.43)

leading to the definition of the functional integral

$$\mathcal{Z} = \lim_{N \to \infty} \frac{1}{N} \int_{-\infty}^{\infty} \prod_{i=1}^{N-1} dx_i \exp \left[ -\frac{1}{\hbar} S[x_i] \right].$$

(4.44)

The corresponding discrete action is then afforded by noting \(d\tau = a\) and \(dx/d\tau = \ldots\)
\[(x_i - x_{i-1})/a^2 \text{ hence}

\[
S[x] = \sum_{i=1}^{N} \left[ \frac{m}{2a} (x_i - x_{i-1})^2 + aV(x_i) \right]
\] (4.45)

This formulation naturally lends to the definition of the Euclidean temperature \(T = Na\). Within this context, the Euclidean partition function implies the existence of a free energy for the spacetime lattice \(154\) such that

\[
F = -\frac{1}{\beta} \log Z
\] (4.46)

where \(\beta = 1/h\). This definition then naturally extends to a free–energy density \(f\) so that \(F = fT\), where \(T\) is the time volume given by \(T = Na\). In this case,

\[
Z = \exp[-\beta Tf] = \exp[-Tf/h]
\] (4.47)

thereby drawing a connection to the ‘inverse temperature’ \(T\) appearing in the partition functions of the preceding section. It is this formulation that underlies the simulation methods.

4.3 Monte Carlo Methods

Lattice Monte Carlo is a technique in which the Euclidean path integral is discretized into a series of time slices comprising a spatial configuration of points. The resulting configuration resembles a lattice of spins, begetting the name of the method. Each configuration is updated in a pairwise manner using Monte Carlo sampling, and the resulting samples exploited to calculate observables of the Euclidean theory. This section elucidates this method, in particular the implementation exploited in
subsequent numerical calculations. For a more comprehensive overview of Monte Carlo methods, including lattice Monte Carlo, see \[169,170\].

4.3.1 Importance Sampling

The category of importance sampling techniques consists of methods, in which a probability distribution function is utilized to systematically sample phase space in a biased manner. A judicious choice of phase space points is particularly important, as certain regions tend to dominate contributions to a given integral. To enhance the rate at which a given Monte Carlo calculation converges, it is desirable to sample in the vicinity of these heavily-weighted points. A physically intuitive approach to this problem is to choose the form of the Boltzmann distribution, where the probability measure is

\[
p(x) dx = \frac{\exp[-S(x)] dx}{\int dx \exp[-S(x)]}
\]  

(4.48)

where the distribution is weighted by the action \(-S(x)\), as opposed to the more traditional energetic weights \(-E/k_bT\). In this configuration, the Monte Carlo estimate for a given physical observable then reduces to an arithmetic mean over Monte Carlo iterations

\[
\langle A \rangle \approx \bar{A} = \frac{1}{N} \sum_{i=1}^{N} A(x_i)
\]  

(4.49)

where \(N\) is the number of configurations sampled in the Monte Carlo sequence. Given these considerations, the primary obstacle is generation of the lattice configurations \(\{x_i\}\). In particular, it is essential to generate a series of configurations so that as \(N \rightarrow \infty\), the Markov chain affords a distribution of the form (4.48).
4.3.2 Markov Chains

Let $W_{ij}$ be a matrix such that the $ij$-th element corresponds to the probability that a system in state $i$ will propagate to state $j$ under a given process occurring in one unit of time. As a probability, the matrix must satisfy the property that $W_{ij} > 0$ for all $i, j \in \mathbb{N}$, and $\sum_{ij} W_{ij} = 1$. Hence, all possible exchanges $i \rightarrow j$ are spanned by $W_{ij}$.

Extrapolating to a continuous spectrum, $W_{ij}$ is promoted to a function $W(x, x')$ for the process $x \rightarrow x'$, subject to the normalization condition $\int dx' W(x, x') = 1$ for all $x \in \mathbb{N}$. It may be shown that the $W(x, x')$ form a semigroup. In particular, under the process $x \rightarrow x' \rightarrow x''$ there is a product rule for two elements

$$W^{(2)}(x, x'') = \int dx' W(x, x')W(x', x'')$$  \hspace{1cm} (4.50)

The preceding expression defines the notation that $W^{(2)}$ corresponds to the composition of two elements of $\{W(\cdot, \cdot)\}$, and similarity $W^{(n)}$ the composition of $n$ elements. This property descends to matrix multiplication in the case of a discrete system.

The behavior of a statistical system is recovered in the limit that $n \rightarrow \infty$, in which case $W^{(n)}$ becomes the probability $P(x')$ for observing an element $x'$ of an ensemble

$$\lim_{n \rightarrow \infty} W^{(n)} = P(x')$$  \hspace{1cm} (4.51)

The probability $P(x')$ is a stationary element of $W$ and hence

$$P(x') = \int dx P(x)W(x, x')$$  \hspace{1cm} (4.52)

The set of all $P$ form a probability distribution and hence
\[ \int dx' P(x') = 1. \]  

(4.53)

For a given ensemble, \( E \), the evolution \( W \) may be applied repeatedly to yield a new ensemble

\[ E \xrightarrow{W} E^{(1)} \xrightarrow{W} E^{(2)} \xrightarrow{W} \ldots \xrightarrow{W} E^{(n)} \]  

(4.54)

and in the long-time limit this reproduces the equilibrium ensemble

\[ \lim_{n \to \infty} E^{(n)} = E^{eq} \]  

(4.55)

While these considerations illustrate the requisite properties for a Markov process to reproduce the equilibrium ensemble \( E^{eq} \), they do not specify any particular form for \( W(x, x') \). Note that, for any realistic system in equilibrium, the system will satisfy the detailed balance condition for forward and backward transition probabilities

\[ \frac{W(x, x')}{W(x', x)} = \frac{P(x')}{P(x)} \]  

(4.56)

This condition guarantees that such a \( W \) will be an eigenvector of \( W(x, x') \)

\[
P(x') = \int dx P(x)W(x, x') \tag{4.57}
\]

\[
= \int dx P(x)W(x', x) \frac{P(x')}{P(x)} \tag{4.58}
\]

\[
= \int dx W(x, x') P(x') \tag{4.59}
\]

\[
= P(x') \tag{4.60}
\]
From this point, it is apparent that an action-weighted Boltzmann distribution will satisfy these properties \cite{154,156}

\[
\frac{W(x, x')}{W(x', x)} = \frac{\exp[-S(x')]}{\exp[-S(x)]}
\] (4.61)

Furthermore, this is inherently local as \(S(x)\) only depends on the element \(x\) of a lattice and its nearest neighbors. Accordingly, the Boltzmann weights proposed for importance sampling are capable of reproducing the equilibrium ensemble for long times \cite{154}.

4.3.3 Metropolis Algorithm

The Metropolis Algorithm \cite{169,171} is a particular importance sampling method in which the exchange probability between two Monte Carlo configurations is biased by Boltzmann–type distribution. In this case, the propagation probability between configurations \(x\) and \(x'\) are dictated by a function \(\Delta S(x', x) = S(x') - S(x)\), which is the difference in action between two lattice configurations. The Metropolis algorithm is executed as follows:

1. Choose a lattice site \(j\).

2. Choose a configuration \(x'_j\) with uniform probability.

3. If the action is lowered by the choice of \(x'_j\), then set the variable at site \(j\) to \(x'_j\).

4. If \(\Delta S(x', x) \geq 0\), then generate a random variable \(r \in [0, 1]\) with uniform probability. Update if \(\exp(-\Delta S) > r\), otherwise leave site \(j\) unchanged.

5. Advance to lattice site \(j + 1\).

This algorithm forms the basis for all Monte Carlo sampling methods employed in this section. Furthermore, the lattice configurations generated in the course of Metropolis evaluations may be utilized to calculate multi-point correlation functions and hence provide energy estimates for simulation. The method may also be adopted to quench onto instanton trajectories, which are solutions to the classical equations of motion, by only accepting Monte Carlo moves which lower the action of the system.

4.4 Malonaldehyde

Malonaldehyde is a well-characterized model for intermediate–barrier proton transfer in which tunneling imbues a substantive correction to the ground–state electronic structure. Within this system, a hydrogen atom is observed to transfer between two oxygens in a concerted process concomitant with keto–enol tautomerism. The hydrogen atom transfer mechanism itself occurs through a combination of activated and tunneling processes, with the physical regime tunable through the temperature of the system (Figure 4.3). The extensive experimental data for this system firmly establish a ground state degeneracy splitting 21.58 cm\(^{-1}\), as obtained from rotovibrational far–infrared and microwave rotovibrational spectroscopy \cite{172,173}. Numerous computational efforts have been made to characterize this splitting, ranging from semiclassical dynamics \cite{174} to the nuclear–electronic orbital method \cite{175} to diffusion Monte Carlo calculations \cite{176} to multireference time–dependent Hartree estimates \cite{177}, and afford tight agreement with experimental values albeit at very heavy computational cost. Despite firm knowledge of the tunneling splitting, the exact barrier for hydrogen atom transfer itself is not known. Physically plausible computational estimates for this barrier have been shown to range between 3.1 to 4.6 kcal mol\(^{-1}\)\cite{26,174,178,181}, however no direct and reliable experimental value for this barrier has been reported.
Taken together, these considerations make malonaldehyde an ideal testing ground for the aforementioned Monte Carlo method. The hydrogen atom transfer in malonaldehyde should be rapid with respect to skeletal relaxation, and hence any transverse vibrational modes would likely be accommodated through a mean–field potential energy surface. While no precise barrier is known for this surface, an excellent reference point for comparison with experimental data comes from vibrational spectroscopy.

To see how this arises, consider a double–well potential formed in the vicinity of the two oxygens of malonaldehyde. Let \( \phi_A \) and \( \phi_B \) denote the left– and right–well wavefunctions, with corresponding free Hamiltonians \( \hat{h}_A \) and \( \hat{h}_B \) for the isolated particle Hamiltonians in each well. When the system is allowed to undergo hydrogen atom transfer through a barrier, the system experiences a potential \( \hat{V} \), which we take to be constant \( \hat{V}_{A/B} = V \) in this toy picture. Accordingly, the full Hamiltonian for each well becomes \( \hat{H}_{A/B} = \hat{h}_{A/B} + V \). This corresponds to a simple eigenvector problem, with eigenvalues of the interacting system provided through solution of the system

\[
\begin{pmatrix}
\hat{h}_A & V \\
V & \hat{h}_B
\end{pmatrix}
\begin{pmatrix}
\phi_A \\
\phi_B
\end{pmatrix}
= \epsilon
\begin{pmatrix}
\phi_A \\
\phi_B
\end{pmatrix}
\]  

(4.62)

and hence \( \epsilon_\pm = \epsilon_0 \pm V \), where \( \epsilon_0 \) is the ground–state energy of each individual noninteracting well. The associated eigenfunctions are then \( \phi_\pm = \frac{1}{\sqrt{2}}(\phi_A \pm \phi_B) \). This simple picture affords a point of contact with experiment. In particular, the proximity of left– and right– states cause the electronic state to split under perturbation by the potential \( V \), leading to an energy gap \( \Delta E = \epsilon_+ - \epsilon_- = 2V \), which is termed the degeneracy splitting. This low–energy gap has an excellent estimator afforded by the \( \Delta E_{0 \to 1} \) estimator in the Monte Carlo scheme. While this picture is highly simplistic, and assumes the the potential responsible for the tunneling coupling will
Figure 4.3: Snapshots of malonaldehyde conformations obtained via Car—Parrinello metadynamics calculations. Configurations (A) and (C) represent each of the keto–enol tautomers, while (B) corresponds to the transition state structure for hydrogen atom transfer.

act only along the reaction coordinate, this is a good approximation for the system at hand. Any off–coordinate vibrational modes would have a dissipative effect through bath–coupling and could be added post–hoc.

To construct a potential energy surface for hydrogen atom transfer Car—Parrinello molecular dynamics simulations were performed on a gas—phase model of malonaldehyde, and the hydrogen atom transfer free–energy surface calculated using a combination of blue–moon ensemble sampling and metadynamics calculations. The resulting surface was then utilized as input for Monte Carlo calculations of tunneling dynamics and degeneracy splitting.

4.4.1 Computational Methods

All subsequent calculations were performed using CPMD 3.13.2 [134]. Malonaldehyde was embedded in a 15.00 Å × 15.00 Å × 15.00 Å supercell employing CPMD’s molecular isolation algorithm. Geometry optimizations were executed using the BP [63,65], BLYP [65,67], and PBE [68] density functionals in conjunction with the Vanderbilt ultrasoft pseudopotential scheme [73] at a plane–wave kinetic energy cutoff of 30 Ry. Electronic states were converged to within 1 × 10^{-6} eV for both
geometry optimizations and for pure SCF calculations. Geometry optimizations were performed using either the ODIIS optimizer in CPMD until the net force on each atom was less than 0.005 eV Å$^{-1}$. The density functional gradient correction was neglected when the electronic density was less than $1 \times 10^{-6} \text{ e} a_0^{-3}$. To construct a zero–temperature surface for hydrogen atom transfer a reaction coordinate was defined as the difference between both proton–oxygen distances $\xi = d_{O1-H} - d_{O2-H}$. Optimizations were performed for both the unconstrained system and for values of the constraint ranging from 0.00 Å to 1.00 Å in steps of 0.05 Å.

Car–Parrinello simulations were initiated from each constrained geometry optimization, retaining the constraint, as well as from an unconstrained global minimum geometry. Calculations were initiated at 300.0 K with a timestep of 0.0343 fs and a fictitious electron mass of 900 a.u. while using Vanderbilt USPPs. Temperature control of the ions was maintained using a massive Nose–Hoover thermostat with a target temperature of 300K and a coupling frequency of 2500.00 cm$^{-1}$. The electronic degree of freedom was likewise thermostatted with a target temperature of 0.007 a.u. and a coupling constant of 15000.0 cm$^{-1}$. Simulations were equilibrated for 1 ps, and continued for an additional 2 ps to sample the phase space distribution of malonaldehyde for the thermodynamic integration in the blue moon ensemble.

Additional free–energy calculations were executed using the metadynamics scheme, as this is the most frequently exploited method for exploration of biomolecular reaction dynamics. Metadynamics was performed along the collective variable $\xi$, which was coupled to the CP Lagrangian with a unit scaling parameter, harmonic coupling parameter of 0.5 a.u. and fictitious mass of 60 a.m.u. Gaussians functions of width $\Delta s = 0.1$ collective variable units and height 0.13 kcal mol$^{-1}$ were added after a minimum of 10 CP–MD steps provided that the displacement along the collective variable was at least 0.100 units. If this condition was not met, sampling continued up to 100 CP–MD
steps, after which addition of the Gaussian was forced by the walker. The walker was coupled to the thermostatting scheme at 300.0 K with a tolerated fluctuation of ±200.0 K. To circumvent numerical instability in the thermostatting scheme with a single collective variable, the system was augmented with a dissipative Langevin term with dissipation parameter $\gamma = 0.001$ \cite{55}.

![Figure 4.4: Potential energy surface scans for hydrogen atom transfer in malonaldehyde, as obtained from zero–temperature geometry optimizations at fixed values of $\xi$.](image)

4.5 Numerical Results

4.5.1 Hydrogen Atom Transfer Surfaces

The free energy surfaces for malonaldehyde hydrogen atom transfer, as obtained from restrained potential energy surface scans, differ substantially for the three density
functionals employed in this calculation (Figure 4.4). The half-surfaces for the BP and PBE calculations exhibit similar minima along $\xi = d_{01\text{-H}} - d_{02\text{-H}}$, occurring at $\xi = 0.50$ Å and $\xi = 0.45$ Å, respectively. The maximal barrier at $\xi = 0$ is likewise comparable between these functionals, assuming a value of 0.879 kcal mol$^{-1}$ for the BP functional and 0.662 kcal mol$^{-1}$ for the PBE functional. Conversely, the BLYP functional exhibits a substantially broader and higher barrier, with a maximum of 1.921 kcal mol$^{-1}$ at $\xi = 0$ and a minimum at $\xi = 0.65$ Å. Both barriers rapidly increase in energy as $\xi$ increases past the minimum and the hydrogen atom concomitantly approaches the backbone oxygen. Given that the scan produced by the BLYP functional coincides most closely with that indicated by previous hydrogen atom transfer barrier calculations $^{26,174,178,181}$, this functional was utilized as a starting point for subsequent Car—Parrinello metadynamics and blue-moon ensemble free energy calculations.

The free energy surface calculated using the blue-moon ensemble method and the BLYP functional exhibits the same qualitative features as the zero-temperature scans (Figure 1.139). The PMF obtained at 300K exhibits a more pronounced hydrogen atom transfer barrier of 2.239 kcal mol$^{-1}$ while retaining a minimum at $\xi = 0.65$ Å. Accordingly, this surface would have a minimum–to–minimum distance of 1.20 Å for hydrogen transfer between oxygen atoms. The barrier arising from the BLYP functional nonetheless remains small with respect to values reported in literature. Previous calculations affording a higher barrier were obtained with either hybrid density functional or coupled-cluster based methods, which are not readily amenable to Car—Parrinello dynamics, or using a GGA functional combining Becke exchange $^{65}$ in conjunction with the Perdew—Zunger $^{182}$ functional and Martins—Troullier pseudopotentials $^{26,183}$. At this juncture, a decision was made to explore the metadynamics methodology, which is not only more applicable to calculation of
potential energy surfaces in \textit{ab initio} simulation \cite{121,123,184}, but is also a more ‘exploratory’ technique in terms of parameter space sampling.

Car—Parrinello metadynamics calculations using the BLYP functional exhibit a far more pronounced barrier, averaging 3.824 kcal mol$^{-1}$ over several independent metadynamics runs. Nonetheless, these calculations tend to exhibit a slight asymmetry between sides of the well, as the oxygens must be held fixed from an initial starting frame during Car—Parrinello simulation (Figure 4.6). Each such simulation then samples a distinct ensemble and hence converges to a slightly different free energy surface. To circumvent this limitation, twenty distinct metadynamics runs were initiated from different frames of an equilibrium Car—Parrinello calculation, and the surfaces averaged. The resulting configuration was surprisingly similar, exhibiting
Figure 4.6: Snapshot of a single metadynamics calculation, illustrating features corresponding to hydrogen atom transfer along the reaction coordinate.

minima an average of 0.650 Å from the maximal barrier, in excellent agreement with the more simplistic blue–moon ensemble method (4.7). While this value falls into the desired range, it is unclear exactly why this estimate is larger than that of the corresponding blue–moon calculation. It is tempting to attribute this deviation to improved phase–space sampling as well as a fortuitous cancellation of errors. In conjunction with the sensitivity of the metadynamics method to the choice of reaction coordinate and the large number of adjustable parameters, these considerations seem
sufficient to account for the discrepancy. Irrespective of the origin, the surface so obtained possesses a barrier in the target region, and hence this will be exploited in subsequent Monte Carlo calculations. It is notable that the potential obtained in this manner is well-fit by that of the anharmonic oscillator in the barrier region (Figure 4.7).

![Figure 4.7](image_url)

Figure 4.7: Metadynamics potential energy surface obtained from ensemble average of individual metadynamics calculations (pink) and anharmonic oscillator potential fit $y = \lambda(x^2 - \eta^2)^2$ to the metadynamics result ($\lambda = 21.6185$ kcal mol$^{-1}$ Å$^{-4}$, $\eta = 0.6500$ Å).

4.5.2 Lattice Monte Carlo Calculations

Lattice path integral Monte Carlo calculations were performed using Euclidean time intervals ranging between $T = 1200$ to $T = 100,000$ units. In this case, the longer the Euclidean time interval, the more strongly the Monte Carlo calculations reflect the ground–state configuration of the corresponding quantum mechanical theory.
Calculations were run for 500,000 Monte Carlo cycles, with an step displacement chosen such that an acceptance ratio of $0.5 \pm 0.05$ was obtained between moves. Simulations were equilibrated for 10,000 Monte Carlo cycles from a spread of points ranging $\pm 0.3$ Å about one of the potential minima before accumulation of statistics was initiated.

In order to ascertain that the Monte Carlo method was indeed reproducing the physics of a double well potential, the method was tested for both harmonic and anharmonic oscillators, as well as for an asymmetric potential profile obtained from metadynamics calculations (Figure 4.6). The resultant calculation was utilized to assemble a histogram of spatial occupancies which, in turn, reflects the underlying probability distribution $|\psi(x_i)|^2$ for the quantum system [154]. One such representative distribution exhibits the skewed, asymmetric behavior of the individual metadynamics profile, suggesting that the calculation is indeed reproducing occupancies following real-world data (Figure 4.8).

![Figure 4.8: Configurations of malonaldehyde obtained through Car—Parrinello metadynamics calculations.](image)
Correlation functions were calculated from a set of 50 data points selected in 50 sets of samples per Monte Carlo move. The error estimator is based on the fluctuation in a given observable \( \mathcal{O} \) such that \( \Delta \langle \mathcal{O} \rangle = ((\langle \mathcal{O}^2 \rangle - \langle \mathcal{O} \rangle^2)/N)^{1/2} \), where \( N \) is the number of configurational samples and the brackets denote ensemble averaging. The degeneracy splitting gap estimated for the unquenched configuration in the free Monte Carlo samples is \( 0.0690 \pm 0.0011 \text{ kcal mol}^{-1} \), as obtained from the logarithmic derivative of \( \langle x(\tau)x(0) \rangle \). This value is in excellent agreement with results from experimental (0.0615 kcal mol\(^{-1}\)) 172, diffusion Monte Carlo (0.0644 kcal mol\(^{-1}\)) 176, and multiconfigurational time-dependent Hartree (0.0667 kcal mol\(^{-1}\)) calculations 177.

![Figure 4.9: Convergence of the topological charge, or net instanton population, with number of Monte Carlo cooling sweeps.](image)

The ground state instanton content was extracted from calculations by accepting moves which only reduce the action of the system. This is performed using the same pairwise Monte Carlo method as for the unquenched calculation. A quench
was performed every 250 Monte Carlo moves for a minimum of 50 steps, ensuring that the system was converged on an instanton configuration (Figure 4.9). The net instanton / antiinstanton crossings, or topological charge, may be estimated either from counting trajectory passes across the well, or from the action of a single instanton $S_0$ in the corresponding anharmonic oscillator potential (Equation 4.16). One such multi-instanton configuration obtained at a lower Euclidean temperature clearly illustrates the manner in which the dominant quantum fluctuations ‘wash out’ to afford the semiclassical tunneling trajectories (Figure 4.10). The instanton is observed to perform a periodic orbit, interpolating between vacua at ±0.650 Å. The net effect of the ‘cooling’ is to smooth the trajectory into a classical–looking orbit. The logarithmic derivative of the quenched correlation function from this configuration affords an energetic estimate of $0.06337 \pm 0.0010 \text{ kcal mol}^{-1}$. The similarity of this value to that of the full quantum calculation suggests that the instanton plays a critical role in the keto–enol tautomerism of malonaldehyde.

Interestingly, the result of this calculation coincides closely with that of a similar instanton–based technique which employed a barrier of 3.81 kcal mol$^{-1}$ from a CCSD(T)/aug-cc-PVTZ surface and obtained a degeneracy splitting of 0.0606 kcal mol$^{-1}$ [186,187]. Within the scope of this method, the results of this section are thus not particularly surprising. Nonetheless, the method presented herein offers greater computational efficiency at the cost of substantially reduced generality with respect to other instanton–based techniques [188,189]. In particular, the restriction to a one–dimensional potential energy surface is limiting for many applications. However, the outlined scheme is sufficient for characterization of enzymatic tunneling processes, particularly in a QM/MM setting.
Figure 4.10: Unquenched (blue) and quenched (pink) instanton trajectories for malonaldehyde.
Bibliography
Bibliography


