

chapter two

METHODS



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SUMMARY

A background is given on the computational methods used in this thesis, ranging from quantum chemical methods like Density Functional Theory (DFT), via classical mechanics to classical Molecular Dynamics (MD) simulations. Also the concepts of geometry optimizations and hybrid QM/MM, which are frequently used in this thesis, are explained.

Chemistry deals with molecules and atoms¹¹⁰. If one wants to get an accurate description of chemical processes, one should treat the atom by its own constituents: a small nucleus and a large electron cloud. At this point, it is no longer valid to treat the particles in a classical manner, i.e. Newton's classical equations of motion no longer hold. In Section 2.2, we shall return to the classical description and get an indication to what extent the classical picture is valid in providing a reliable description for the system under study.

The fundamental equation which states explicitly why the classical picture no longer holds, is Heisenberg's uncertainty principle:

$$\delta p \delta r \geq \frac{1}{2} \hbar \quad (1)$$

It simply states that at any point in time, it is not possible in any way to get at the same time both the position and momentum of an electron exactly; the uncertainty of the products is at least as large as the quantity \hbar (Planck's constant, $6.6 \cdot 10^{-34}$ Js, divided by 2). The fact that this quantity is so small compared to our normal day world is the main reason for to the correctness of Newtonian mechanics for describing macroscopic phenomena. However, the dimensions of an electron are comparable to the size of the atom to which it belongs, and the uncertainty principle can no longer be ignored. Therefore, the electron can no longer be treated as a particle, but rather a wavefunction should be used to indicate the probability that an electron is located at a certain position in space. That is, we enter the field of quantum mechanics^{1,111,112} and we have to use the Schrödinger equation:

$$\hat{H}\psi = i\hbar \frac{\partial \psi}{\partial t} \quad (2)$$

This time-dependent equation when solved, produces the wavefunction ψ for a number of electrons that can be used to get a probability distribution for the electrons in space. It also gives the energy associated with that particular state, or electron distribution. The Hamiltonian \hat{H} consists of the following parts:

$$\hat{H} = \hat{T}_e + \hat{T}_N + \hat{V}_{eN} + \hat{V}_{ee} + \hat{V}_{NN} \quad (3)$$

Here, T_e and T_N stand for the kinetic energy of the electrons and nuclei respectively, V_{eN} for the nuclear attraction, V_{ee} for the electron-electron repulsion and V_{NN} the nuclear repulsion. Since the difference in mass between the electrons and nuclei is so large (electrons are of the order of magnitude of 10^5 times lighter than nuclei), the Schrödinger equation is normally split up into an electronic and a nuclear part (Born-Oppenheimer approximation). It is then solved only for the electronic part, keeping the positions of the nuclei as external parameters in solving it. Even then, the Schrödinger equation can be solved exactly only for systems containing *one* electron only; for all other systems, approximations have to be used.

The conventional way of solving Schrödinger's equation starts with Hartree-Fock theory^{1,111,112}. In this method, electron correlation (interaction between electrons with different spins) is not present explicitly; this correlation has to do with the Coulomb hole. This hole is connected to the interaction of two unlike spins, while the so-called Fermi hole is connected to two like spins. As electrons are Fermi particles, two electrons with the same spin can not be present at the same time at the same position (Fermi hole). Two electrons with differing spin (one alpha, the other beta) *can* be present at the same time at the same place, but are less likely to do so due to electrostatic repulsion (Coulomb hole). As the wavefunction is built up of Slater determinants, the Fermi hole is correctly taken care of. But the Coulomb hole is in the Hartree-Fock theory only taken into account in an average way. The way to improve upon this, is to include determinants where electrons are "promoted" from occupied to unoccupied orbitals, and optimize the weight with which these additional determinants should be added. The two most simple methods to improve upon Hartree-Fock, are Configuration Interaction (CI) and Møller-Plesset perturbation theory. Although these methods usually improve upon Hartree-Fock results, they are not nearly as accurate as more sophisticated methods like Multi-Configurational Self Consistent Field {CASSCF/CASPT2}, Coupled Cluster {CCSD(T)} or Multi-Reference CI. However, the more accurate the method, the higher the computational cost; for instance, while Hartree-Fock scales formally as N^4 (where N is the number of particles), CCSD(T) scales formally as N^7 . Therefore, these methods can be applied only to relatively small molecular systems.

A radically different approach is the development of Density Functional Theory (DFT)^{1,113}. Already in the early 60's, Hohenberg and Kohn¹¹⁴ have proven that the ground-state electronic energy is determined completely by the electron density ρ . That is, there exists a one-to-one correspondence between the electron density of a system and the energy. This does not seem that surprising, as indicated by the well-known spectroscopist Bright-Wilson who stood up at a conference in 1965¹¹⁵, and shared his thoughts about these new ideas with the audience: "*well, the total electron density defines the number of electrons in the system; the cusps in the density define the nuclear coordinates; the derivative of the density at a cusp defines the nuclear charge at that cusp and thus the configuration of the elements in the molecule; therefore, the system is fully defined*". Real life however is not that simple, because although the Hohenberg/Kohn-theorem states that there should exist one density functional that describes the connection between density and energy, it does not provide the functional as such. Even though the fundamentals of DFT had already been completed in 1965 with the Kohn-Sham theory¹¹⁶, the standard use on a large scale emerged at the end of the 80's and beginning of the 90's, when more accurate exchange-correlation potentials had been developed. Eventually, one of the founders of the theory (Walter Kohn) was awarded the Nobel prize for it; at that time (1998) and from then on, DFT has overtaken the use of conventional quantum chemistry due to its efficiency. As it scales formally as N^3 , it enables to study larger systems in a reasonable time with an accuracy that could not be reached with for instance Hartree-Fock theory. In recent years, several DFT codes have successfully implemented special techniques^{117,118}, which make the method scale linearly with the number of particles; this enables one to treat even larger system (up to hundreds or even thousands of atoms) at an accurate level in a reasonable time.

In Density Functional Theory, the point of view changes with respect to the wavefunction based methods. Taking Hartree-Fock theory as an example for the latter, in wavefunction based methods the wavefunction governs everything, and the charge density results from it. In DFT, the picture is reversed. The charge density governs everything and it can be represented by molecular orbitals (the Kohn-Sham orbitals). The Hamiltonian now is built up in another way also; this is coming directly from the Kohn-Sham theory, which states that there exists a system of non-interacting electrons that produces *exactly* the same density as a system of interacting electrons. The kinetic energy T_S of the

non-interacting electrons is known exactly from the molecular orbitals, and forms together with the nuclear attraction energy U_{ne} , the Coulomb energy U_c and the exchange-correlation energy U_{xc} the components of the total DFT energy U_{dft} :

$$U_{dft} = T_s + U_{ne} + U_c + U_{xc} \quad (4)$$

The nuclear attraction energy U_{ne} is the attractive interaction between the nuclei and the electrons and the Coulomb energy U_c the repulsive interaction between electrons; the exchange-correlation energy U_{xc} is the sum of the exchange and correlation energy, as well as a correction term for the kinetic energy for the system of interacting electrons. By equating U_{dft} to the exact energy, this expression can be used to define U_{xc} , which is coming from the “exact” exchange-correlation potential, if it were known. Unfortunately it is not, and approximations have to be made.

Several exchange-correlation potentials are currently available. The simplest of them is the Local Density Approximation (LDA), that results directly from the description of the uniform electron gas. The most commonly found implementation for this potential is represented by Vosko, Wilk and Nusair (VWN)¹¹⁹. However, as this uses only the local density and as such underestimates the interactions due to other atoms, the generalized gradient approximations have been introduced in the mid 80’s. They take also the derivative of the density into account, and generally give a better description for a wide range of phenomena than LDA. The most popular *pure* DFT potential is probably the combination of the Becke exchange¹²⁰ and the Perdew correlation¹²¹ potentials, which has successfully been applied to predicting geometries and other properties, and was constructed on physical grounds.

However, it can not be left unnoticed that the most popular DFT-potential at this point in time is the B3LYP potential¹²². It combines *pure* DFT potentials like BLYP with a portion of exact Hartree-Fock (HF) exchange, where the amount of mixing is based on empirical grounds, i.e. fitted to improve some properties for a certain set of molecules. Although the physical motivation for this choice may be lacking, it is shown that this combination improves the performance of the BLYP potential (see also Section 4.2), but gives results comparable to the *pure* Becke-Perdew potential. Recently, both the B3LYP and the Becke-Perdew potentials have been improved (by the Becke97¹²³ and the PKZB potential^{124,125} respectively), where again the former was based on fitting to some properties of some molecules. The PKZB potential instead is one of the Meta-GGA’s where not only the first but also the second density derivative is used, and has been constructed again on physical grounds. Therefore, if one is interested in the properties the B3LYP or Becke97 was optimized for, and one wants to apply it to a molecule similar to the ones in the set used for fitting the parameters, it is probably safe to use them. However, it seems more reasonable to study the molecule with *pure* DFT potentials as these give not only comparable accuracies for the properties the B3LYP/Becke97 were optimized for, but also provide a better description for properties or molecules that were not used in the B3LYP/Becke97 set for obtaining the parameters.

To get an idea of the accuracy that can be obtained with DFT with currently available exchange-correlation potentials, one is referred to Chapters 4 and 5, where DFT has been applied to study several chemically interesting properties or systems, like for instance molecular polarizabilities of organic molecules, geometries of molecules or even the complete reaction path of an aminothioli/aminoalcohol catalyzed addition of dialkylzinc to benzaldehyde.

In Section 2.1, the electrons and nuclei were the particles around which everything evolves. In this section, the interactions are treated at the atomic level. That is, instead of solving Schrödinger's equation for obtaining the energy, the interactions are given by classical energy expressions, or force fields. Many force fields are available, each with their own set of force field parameters that can be applied to either a certain class of molecules (for instance proteins or organic molecules) or to any molecule, that can be built from any atoms. Example of the former are the AMBER95¹²⁶ and GROMOS96¹²⁷ force field, that were designed for applications on biochemical systems like proteins; the latter are exemplified by for instance the Universal Force Field (UFF)¹²⁸ or the MM2/MM3¹²⁹ force fields. The interactions within any force field can normally be divided into two parts: bonding and non-bonding.

Bonding potentials

These potentials deal with bonding interactions between close-by atoms, that are normally connected by a chemical bond; as such they replace for a large part the interactions which should normally be treated at the quantum chemical level. The bonding interactions are a function of bonds, angles and dihedral angles between different atoms. The simplest way to describe the interactions for these *internal* coordinates is by the harmonic approximation:

$$U(X) = \frac{1}{2} K_X (X - X_{eq})^2 \quad (1)$$

In this equation, the energy changes quadratically as the *internal* coordinate changes from its equilibrium value. The K_X parameter is called the force constant and determines how much the energy changes if the *internal* coordinate changes; a small value defines a flexible coordinate, while a large value indicates a rather stiff coordinate. In Section 3.2, a new method (IntraFF) is introduced that can be used to obtain values for force constants that are not present in the force field one is using. There are of course many other ways to treat the bonding interactions; for instance, in the GROMOS96¹²⁷ force field a quartic potential is used instead of the harmonic potential. It was introduced to avoid the square root operation in the calculation of the energy and the forces working on the atoms. In practice however, the difference between the quartic and the harmonic potential is rather small; the differences are substantial only at larger deviations from the equilibrium value, which are in practice never reached in the calculations.

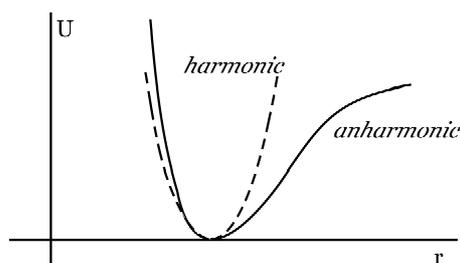


FIGURE 2.2.1 ANHARMONIC POTENTIALS

Other potentials that differ to a large extent from the harmonic potential are the Morse¹³⁰ or Frost^{131,132} potentials; both are anharmonic potentials for bonds, and give an improved description of the energy profile as the bond is stretched. That is, if the atoms making up the bond are pushed close to each other, the energy increases enormously, while if they are stretched really far apart, eventually the system is treated as two non-interacting free atoms, as would be expected. The well-known Morse potential has three parameters: the equilibrium distance, the dissociation energy and the anharmonic constant; the less well known Frost potential only has two, and is based on theoretical grounds. Both methods are described in more detail in Section 3.2, where it is shown also how to obtain the parameters for the Frost potential.

Dihedral potential

For the dihedral angles, one normally uses a periodic function with minima at either 120, 180 or 240 degrees apart (depending on the number of atoms connected to the central atoms on either side). The most convenient way to treat this situation is by assuming a (combination of) cosine potential(s) for the dihedral angle connecting the four atoms involved:

$$U_{ijkl}^{dihedral} = K_{\phi} \left[1 + \cos(n\phi_{khif}) \right] \quad (2)$$

This assumes that there is no preferential minimum involved; i.e., it is based on the case of ethane, which has a favorable (*staggered*) and an unfavorable (*eclipsed*) conformation, while there is no preference for two hydrogen atoms to have a dihedral angle of either 60, 180 or 300°. However, this is in general not valid, but a properly chosen combination of dihedral angle functions will give a proper representation of the potential energy surface again.

Non-bonding interactions in non-polarizable force fields

The interactions between molecules that are not involved directly in bonding interactions, are called the non-bonding interactions, and comprise normally electrostatic and van der Waals interactions. The former is represented by the interactions of point charges, which should be properly chosen to give a good representation of the charge distribution within a certain molecule. The latter interactions are in non-polarizable force fields normally represented by a Lennard-Jones potential, which consists of a short range repulsion term and a longer range attraction term (due to dispersion terms):

$$U^{Lennard-Jones} = \frac{C_{12}}{r^{12}} - \frac{C_6}{r^6} \quad (3)$$

Each of the force fields usually has its own set of Lennard-Jones (or vdW) parameters, where the particular choices made for them depend to a large extent on the parameters chosen for other parts of the force field. For instance, as polarization effects are absent in these force fields, for several force fields it was chosen to mimic these effects by increasing the charges on the atoms. Other force fields have been developed without such an increase, and the effect of polarization interactions has been introduced in an effective way by changing the vdW parameters.

Electrostatic interactions are a special kind of interaction, as they have a long range influence; two unit point charges at a distance of 100 Bohr (~53 Angstroms) still have an electrostatic interaction of approximately 6 kcal/mol! This is of the same order of magnitude as van der Waals interactions between two atoms that are very close (~3 Å). Therefore, treating these interactions properly is of the utmost importance; fortunately, this large interaction between distant atoms is only present if the

atoms are isolated. In condensed matter, the electrostatic interactions are screened by the atoms inbetween, which reduce the interactions by a factor of 2 to 80, depending on the kind of matter.

Non-bonding interactions in a polarizable force field

Polarization effects arise when two molecules approach each other and start to feel the presence of the other molecule; depending on the particular orientation of the two molecules and their respective electrostatic molecular multipole moments, the charge distribution within each of the two molecules will start to differ from their monomeric distributions. When the system of the two molecules are treated with quantum chemical methods, these polarization effects are treated properly. In a non-polarizable force field, these effects can be treated only in an effective way, as described earlier.

The polarization effects can however be treated properly also at a classical level by using the (*atomic* or *molecular*) polarizability. This property is related to the change in dipole moment of the molecule in an electrostatic field; this field can then be either an external macroscopic field or the field of a neighboring molecule with permanent electrostatic multipole moments. It can be even the field at molecule A of the dipoles induced in molecule B by the field of the permanent multipole moments of molecule A!

For a set of atomic polarizabilities in an electrostatic field, the *induced* dipole moment in each of the polarizable points p or q is given by the polarizability times the total field; this total field consists of the electrostatic field E_O (from all the permanent dipole moments and/or an external electrostatic field) plus the dipole field T_{pq} of all other *induced* dipoles:

$$\alpha_{p,induced} = \alpha_p E_{static} + \sum_{q \neq p} T_{pq} \alpha_{q,induced} = \alpha_p E_{total} \quad (4)$$

This can be rewritten in a matrix formulation, leading to a matrix that should be inverted to get the relay matrix (see also Section 4.1). This $3N \times 3N$ relay matrix then gives the response of the molecule to a given electrostatic field, and as such represents the molecular polarizability (in a $3N \times 3N$ representation). It can be reduced to a 3×3 form to retain the normal molecular polarizability tensor (see Section 4.1).

In the Direct Reaction Field (DRF) approach¹³³⁻¹⁴², extensive use has been made of this polarizable nature of molecules, in combination with a screening function that accounts for overlapping charge densities. In principle, the approach has been primarily used for QM/MM purposes (see Section 2.3), but also a classical force field has been developed. In this polarizable force field, the non-bonding interactions are split up into six terms: *electrostatic*, *dispersion*, *repulsion*, *induction*, *field energy* and *long-range electrostatics* (through either a dielectric continuum description or an image charge method; in both cases, the Poisson-Boltzmann equation is solved). The *electrostatic* interactions are similar to the ones used in non-polarizable force fields, except that the interactions are screened for overlapping charge densities. The *repulsion* and *dispersion* interactions can be thought of as similar to the Lennard-Jones potential, except that again the dispersion interactions are in the DRF approach screened for overlapping charge densities, and it is normally obtained as the anisotropic interaction between two polarizability tensors. The Lennard-Jones treatment of isotropic interactions is generally speaking only valid for isolated atoms, not for atoms within a molecule. Moreover, unlike most non-polarizable force fields, the parameters needed for describing the interactions are coming directly from properties of the isolated molecule itself (see also Section 4.1). The *induction* interactions are obtained directly from the interaction of the polarizabilities with the electrostatic field:

$$U^{induction} = \frac{1}{2} E_{static} \cdot \mu_{induced} = \frac{1}{2} E_{static} \cdot E_{total} \quad (5)$$

The induced dipole moments can be obtained either from the relay matrix times the electrostatic fields, or from an iterative scheme. The former needs a matrix inversion of a matrix of size $3N \times 3N$, which takes a long time, especially for larger systems; therefore, the iterative scheme is much preferred, as it is much faster and due to the form of the relay matrix, extremely stable. The *field energy* treats the direct interaction of a permanent molecular moment with an external field, and is only present if an external field is present. Finally, the *long range electrostatic interactions* can be treated in two different ways, which both invoke a dielectric continuum environment of the system. The first way to deal with this is by employing an enveloping surface of Boundary Elements^{133,135-138,143-145}, representing a solution to the Poisson-Boltzmann equation. However, this method can be applied only to moderately sized systems (like a solvated organic molecule, not for an entire protein), as the size of the relay matrix is of the order of $3 \times N + N_{bem}$, where N stands for the number of polarizable points and N_{bem} for the number of Boundary Elements. A more convenient solution to the Poisson-Boltzmann equation can then be obtained by employing Friedman's image charge method¹⁴⁶, or 3D finite difference methods¹⁴⁷.

In the Direct Reaction Field approach¹³³⁻¹⁴², the correct physics is employed at all levels. Going from the quantum chemical system to the classical polarizable system to the dielectric continuum, everything is consistently coupled. In principle one can treat two atoms either by a full quantum chemical description, a mixed QM/MM description (see Section 2.3) putting either one of the two in the classical system, or both in the classical system, and end up with the same total picture for the interaction, energy and charge distribution. However, apart from some isolated instances, the approach has not been applied systematically to describe the interactions within proteins, which is one of the major goals for the near future.

Hybrid QM/MM methods

Combining quantumchemical and classical systems

In Section 2.1, the energy was obtained for a pure quantum chemical system, while in Section 2.2 it was obtained for a purely classical system. However, in many cases it is more advantageous to have a system that is described partially quantum chemically and partially classically. In order to do this, one has to divide the total system into two subsystems: a QM system that is described by quantum chemistry and a MM system that is described by a classical description. The interactions between these two systems are then usually treated at the same level as the interactions in the MM system itself.

One of the distinguishing features between the different ways of combining QM and MM systems is concerned with polarization effects. One would like to have a system that can be polarized in all regions, like in quantum chemical calculations. However, for regions described by a non-polarizable force field this is not possible. They can polarize the QM regions, but not be polarized themselves. As the force field parameters are obtained for use in a non-polarizable (MM) system, it is more appropriate to treat the interactions between the QM and MM system at the same level. Therefore, it is better to treat the electrostatic interaction at the classical level and not let the QM system be polarized by the MM system. In the application of a non-polarizable force field in QM/MM calculations on copper proteins (see Chapter 9), the QM and MM systems are therefore coupled through classical (non-polarizable) electrostatic interactions.

For a polarizable force field like the Direct Reaction Field¹³³⁻¹⁴² approach, the opposite is true, as the MM system is polarized already by itself, and as such should also polarize the QM system. In the past, this approach has been primarily used for solvent effects on organic molecules, reactions, excited state lifetimes and charge distributions, or excitation energies. In those applications, wavefunction based methods have been used for the QM description; progress has been made to couple the Direct Reaction Field approach also with Density Functional Theory¹⁴⁸.

A very important issue related to QM/MM calculations is the treatment of the QM/MM boundary region¹⁴⁹. For solvent effects on organic molecules, the division in a QM and a MM system is straightforward and doesn't cause any problems. However, for a protein this no longer holds; in order to make a division in a QM and a MM system, one has to cut through covalent bonds (see Figure 2.3.1).

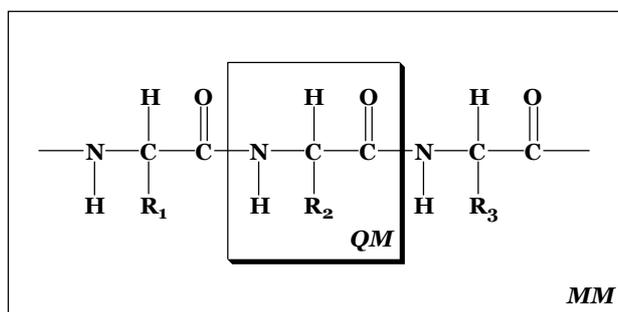


FIGURE 2.3.1. CUTTING THROUGH BONDS IN PROTEINS IN QM/MM SYSTEMS

For instance, if amino acid residue 2 (consisting of NHCHR_2CO) is supposed to be put in the QM system and residues 1 and 3 in the MM system, residue 2 is left with dangling bonds. For the MM

system this poses no problem, as the interaction with the QM system is treated on a MM level in which the QM system can be treated as if it were a MM system.

Several options are available to circumvent the problem with the dangling bonds of the QM system (see ref 149). One option, that is preferred if one uses plane-wave basissets, is to use pseudo-potentials¹⁵⁰. However, for regular QM calculations with atomic basisfunctions this is less straightforward to implement. Another solution is to use localized orbitals, but the most commonly chosen way to solve the problem is to use link atoms. In this method, *capping* (link) atoms are added to the QM system in order to fulfil the valency of the system, which are normally chosen to be hydrogens. The QM calculation is then performed on the *capped* QM system, while the *capping* atoms are not involved in the interaction between the QM and MM systems. In the first paper describing a study with link atoms, no constraint was put on the position of the *capping* atoms, thereby leading to an increased number of degrees of freedom for the total system. In later implementations (like ONIOM¹⁵¹⁻¹⁵³, QMPot¹⁵⁴ or IMOMM/ADF¹⁵⁵⁻¹⁵⁷), these unwanted additional degrees of freedom were removed by putting constraints on the positions of the atoms involved in such link bonds. Although the number of degrees of freedom is corrected, sometimes this is achieved by removing them for the wrong atom. For instance in the IMOMM/ADF¹⁵⁵⁻¹⁵⁷ implementation, the real classical atom is “following” the *capping* atom; therefore, the degrees of freedom of the artificial *capping* atom are kept, while those of the real classical atom are removed. In Section 9.1, a new link model (AddRemove¹⁵⁸) is presented that doesn’t have this unwanted behavior; furthermore, the introduction of the artificial *capping* atom is corrected for afterwards, and in principle its introduction should have no influence.

In Chapter 9, QM/MM calculations will be applied to obtain active site geometries of copper proteins in the presence of the complete protein and a layer of solvent molecules surrounding the protein. As the computer resources are not unlimited, treating the complete protein by Density Functional Theory is *at present* out of reach. Moreover, by doing so one would waste valuable computer resources as one uses a high-quality description for a large region that can be treated very well by classical interactions. Therefore, only the site of interest (the active site) has been placed in the QM system, while the rest of the protein and the solvent have been put in the MM system. It is shown that including the protein is necessary for obtaining correct active site geometries.

Optimizations and simulations

Obtaining equilibrium geometries and sampling the degrees of freedom of the system

In the previous sections, only the energies were discussed but not it was not shown how the geometries needed for the calculations can be obtained or improved. One can imagine that the geometry may have a large influence on the energy and molecular properties like the multipole moments. Also the dynamical behavior of molecules at room temperature was not discussed yet. In this section, the basics for methods involved in these two important matters will be discussed, while the applications of the methods are given in later chapters.

Geometry optimizations

The energy profile for a molecule can be visualized as in Figure 2.4.1, where the energy is given as function of some intrinsic coordinate of the molecule. This intrinsic coordinate consists of a combination of all relevant coordinates of the molecule, but can be visualized most easily for a diatomic molecule. In that case, the intrinsic coordinate consists of the distance between the two atoms.

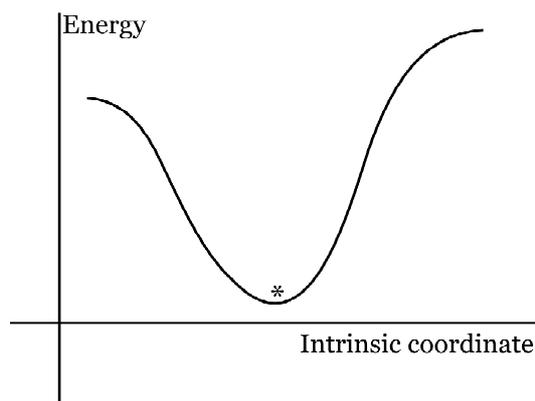


FIGURE 2.4.1. ENERGY PROFILE FOR A MOLECULE

The equilibrium geometry, indicated in Figure 2.4.1 by the star, is found where the energy is lowest. In a geometry optimization, one aims to find this equilibrium geometry. One could use energy values only, but as this requires lots of energy evaluations it is useful only for methods in which the evaluation of the energy does not take a long time (as in the case of semi-empirical or molecular mechanics methods).

A more efficient approach is to use also the gradient g , which is a vector containing the first derivatives of the energy U with respect to the Cartesian coordinates of the atoms \mathbf{r}_i :

$$\mathbf{g}_i = \frac{\partial U}{\partial \mathbf{r}_i} \quad (1)$$

In Figure 2.4.2, the gradient is sketched that belongs to the energy profile of Figure 2.4.1 as function of the intrinsic coordinate. In the equilibrium geometry, it is zero.

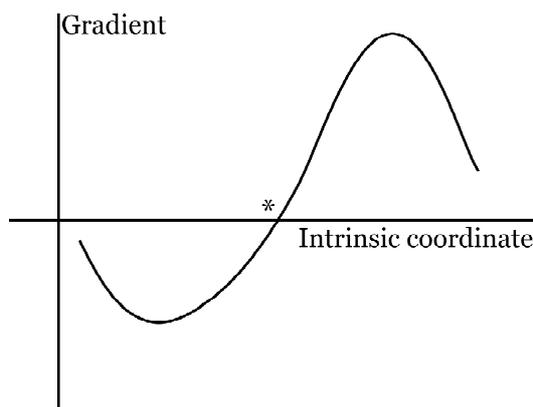


FIGURE 2.4.2. GRADIENT PROFILE

On both sides of the equilibrium, a force is working on the molecule to push it back towards equilibrium. This can be used in geometry optimizations to obtain the equilibrium geometry; one has to find the geometry where the gradient is zero.

The most simple algorithm takes the negative gradient as step in the geometry optimization (*steepest descent*)¹. This requires lots of optimization steps as the convergence is usually slow. It can be improved upon by using the *conjugate gradient* method. This method constructs its steps by taking a mixture of the current negative gradient and the previous search direction, thereby making it *conjugate* to the previous step. This will improve the convergence to some extent, but the convergence remains slow.

A further improvement of optimization techniques can be obtained by including the Hessian H , which is a matrix containing the second derivatives of the energy U with respect to the atomic coordinates r_i :

$$H_{ij} = \frac{\partial^2 U}{\partial r_i \partial r_j} \quad (2)$$

One could compute this matrix after every geometry optimization step and use it to construct a new step in the optimization, but as the calculation of the Hessian matrix may be very time-consuming, the use of approximate Hessians may be worthwhile. These approximate Hessians are not re-calculated after every geometry optimization cycle, but simply *updated* based on the gradient change. One of the most popular and powerful update schemes is the Broyden-Fletcher-Goldfarb-Shanno (BFGS)¹ scheme. In this scheme, the Hessian is updated from H_0 to H_+ by using the step vector s and the gradient difference Δg :

$$H_+ = H_0 + \frac{\Delta g \Delta g^T}{\Delta g^T s} - \frac{(H_0 \cdot s)(H_0 \cdot s)^T}{s^T H_0 \cdot s} \quad (3)$$

In this equation, the tensor product has been used:

$$(a \otimes b)_{ij} = a_i b_j \quad (4)$$

The BFGS scheme has, under certain weak conditions on the step vector s , the property of non-negative definiteness. This property is useful as it ensures that the quadratic model has a minimum, or put otherwise, that the eigenvalues of the Hessian are all larger than or equal to zero.

The BFGS is less appropriate for use in a *transition state* optimization. This *transition state* (\ddagger) is defined as the highest point on the energy curve along a reaction coordinate connecting reactants R and products P (see Figure 2.4.3).

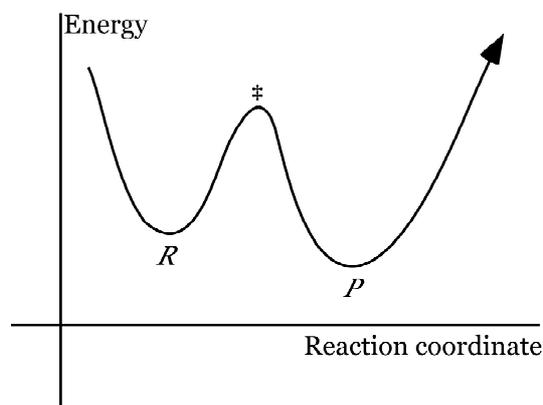


FIGURE 2.4.3. ENERGY PROFILE ALONG A REACTION COORDINATE

At the *transition state*, the energy goes downhill if a step is taken along the reaction coordinate and uphill for all other directions. It is therefore characterized by a Hessian that has one *and only one* negative eigenvalue. A Hessian update scheme that uses non-negative definiteness is therefore not appropriate for finding such a transition structure. The *Powell-symmetric-Broyden* (PSB)¹ update scheme has, like the BFGS scheme, the property that if H_0 is symmetric then also H_+ is. However, the PSB updated Hessian is not positive definite and is therefore better suited for *transition state* optimizations. The updated Hessian is obtained as:

$$H_+ = H_0 + \frac{(s \cdot s)[T \quad s] + (s \cdot s)[s \quad T] - [(T \cdot s)[s \quad s]]}{(s \cdot s)^2} \quad (5)$$

with the T -vector:

$$T = -g - H \cdot s \quad (6)$$

For the geometry optimizations described in this thesis, the BFGS update scheme was used for *equilibrium structure* optimizations of molecules and the PSB scheme for *transition structures*. In Section 4.2, accuracies of optimized geometries obtained by Density Functional Theory are discussed.

Molecular simulations

The statistical or dynamical behavior of molecules at room temperature can have some marked effects on properties one is interested in; thermodynamic properties like equilibria, redox potentials and protein stabilities are determined by probability distributions at a certain temperature, while for dynamic properties like reaction rates also time correlation functions play a role. To enable a comparison between calculations and experiments, one therefore has to do the calculations at a certain temperature and follow the (dynamics of the) system for a certain time. The time scale on which the

system should be followed depends on the property one is interested in. This thesis deals with *atomic* and *molecular* properties; therefore, the dynamical behavior should be handled by using Molecular Dynamics¹⁵⁹⁻¹⁶¹.

In Molecular Dynamics simulations, the system is followed for a certain time, in which the coordinates of the atoms/molecules are updated at regular intervals (with a timestep that is normally of the order of 1 fs). At every timestep, the energy and the forces working on the atoms are calculated, and the atomic velocities and coordinates updated. Several properties like the energy, the pressure or total dipole moment of the system may be monitored during the simulation, which results in statistical averages for these properties during the simulations. An appropriate total simulation time depends on the system and on the properties one is interested in. For solvent effects on organic reactions, a total simulation time of 50-100 ps seems appropriate, while for the stability check of a protein a few ns may be more appropriate. The simulations generate a certain ensemble, which is determined by the system properties that are constrained during the simulation. In the *canonical* (NVT) ensemble, the total number of atoms, the volume and the temperature are kept constant, while in the *isothermal-isobaric* (NpT) ensemble the pressure is constrained instead of the volume. Several methods have been proposed to keep the temperature (and/or pressure) constant. It is achieved for instance by extending the Hamiltonian (Nosé-Hoover¹⁶²⁻¹⁶⁵ dynamics) or by velocity/pressure scaling (Berendsen¹⁶⁶ dynamics). Also, there are several schemes available for updating the atomic/molecular coordinates, the simplest of which is the Verlet scheme. More detailed information about the art of Molecular Dynamics simulations can be obtained elsewhere¹⁵⁹⁻¹⁶¹.

In Section 6.4, Molecular Dynamics simulations are used to check the force field that is obtained for the active site of Azurin in Sections 6.1 and 6.2. Vibrational frequencies from either Density Functional Theory, the copper force field or MD simulations are compared with each other and with experimental data.