QUANTUM MECHANICAL STUDY OF MODELLED/BIOMIMETIC HEME AND NON-HEME TYPE METALLOENZYMES

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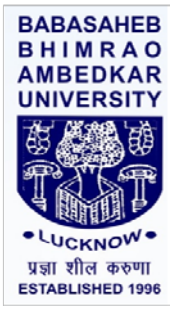

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Quantum Mechanical Study of Modelled/Biomimetic Heme and Non-Heme Type Metalloenzymes

Chapter 1: Introduction

Enzymes are known as biological catalysts which are responsible for performing chemical reactions inside living beings. The enzymes that involve metal at their active site are termed as metalloenzymes or metalloproteins. Metal ions can easily bind to the protein as they easily lose electron to become ions and enzymes are electron rich species. Transition metals exhibit multiple oxidation states hence they can assist in electron/ proton transfer reactions, and thus are present in large number of enzymes at active centre. Metals are regarded inevitable through enzymes as without them reactions catalyzed will be rather slow. Metalloenzymes account for nearly half of enzymes in nature. Various metabolic functions play key role in all living forms, e.g., methane hydroxylation used in methanophiles (bacteria), desaturation of fatty acids (plants), DNA and RNA repairs, β-lactam antibiotics biosynthesis (animals). In these metabolic functions, controlled oxidation of organic substrates takes place by the activation of dioxygen (O_2) and it is mediated by transition metals. This attribute of enzyme finds important applications in industry, to be efficiently used in oxidation reactions. Thus, researchers are interested in understanding mechanism of dioxygen activation in different heme and non-heme metalloenzymes. There are diverse types of active sites in enzymes like mononuclear, dinuclear, hetro-dinuclear regardless of this a common mechanistic hypothesis may also be fruitful in understanding dioxygen activation.

Nature utilizes enzymes to speed up the biochemical transformations in regioselective and stereospecific manner. Iron availability in earth crust is maximum hence most of the enzymes comprise iron at their active site. Besides iron there are numerous other metal containing enzymes (transition as well as non-transition) like copper, vanadium, and molybdenum. Also, in few of the metalloenzymes two or more metals are present at their active centers, for example ribonucleotide reductase is a diiron enzyme or in photosystem II there is a multi-center cluster.

On the basis of mode of binding of the metal to the rest of the protein, enzymes can be classified as heme and non-heme. Two most well studied examples of each of these categories are: cytochrome P450 and taurine/α-ketoglutarate dioxygenase. The heme group is connected to the rest of the protein via thiolate group of cyteinate residue (Cys439). This axial ligand is found responsible in fine-tuning the electronic properties of the enzyme (oxidant) and also in attributing its functional properties to behave as monooxygenases. Peroxidases and catalases are two other varieties of heme enzymes which mainly differ due to type of axial ligand. In peroxidases hystidine is the axial ligand while in catalases tyrosinate group. Here iron metal is bound through a facial triad consisting of, two histidine (His) and an aspartate amino acid (Asp). This enzyme is a non-heme enzyme and is involved in the biodegradation of taurine and also acts as a sulphur source. The intermediates involved during the reaction mechanism are highly short-lived and hence are difficult to be detected and characterized. This necessitates theoretical studies to be used in investigation of the mechanism and further validation of the experimental findings.

A few decades ago, theoretical modeling played a minor role in understanding redox-active reactions of metalloenzymes. The theoretical methods were underdeveloped, due to this, either result was not accurate enough or the processing time was too long. But in today's scenario, the situation is changed with the development of methods and as well as insights from the applications. Development of density functional theory (DFT) has reached at the stage where their accuracy is not far from most accurate. The breakthrough was due to incorporation of density gradient terms for the exchange part and of fractions of exact exchange. During first year of its applications in transition-metal complexes, it was clear that results were quite accurate. Decades of experience gained from the study of small models of transition metal containing complexes, gave ideas for further improvements in method to be used and how to address mechanism studies of large organometallic complexes, as surprisingly, small model methods were quite helpful in gaining insights in the action of mechanism of biomolecules. Transition-state structures as well as individual reaction steps turned not to be entirely dependent of the size of the model for understanding of reaction mechanism.

There are two originally different approaches in the study of enzymatic systems. First is the cluster model approach which uses small or truncated model system. This small model approach had a potential to elucidate main features of mechanism. First study using this model was done in 1997 on methane monoogygenase enzyme (MMO). Second approach was based on treating small core active site of the enzyme with extensive quantum mechanical (QM) methods using DFT, while rest of the system is described by molecular mechanics (MM); hence this approach is called as QM/MM model Warshel and Levitt in 1976. The first application of QM/MM on the mechanism of galactose

oxidase was made in 2000. Both approaches have been developed over the years from their original form. Nowadays, with improvements in computer technology, QM cluster models can handle quite big models i.e. with more than 200 atoms, and even larger QM core can be used in the QM/MM approach.

Today theoretical model calculation can be regarded of equal importance in determining mechanism of metalloenzymes. Experimental methods have the advantage that they are being studied on the actual system, but spectroscopically guarding of short-lived species, electron transfer and interpretation of results is quite troublesome. In both approaches accuracy of the results is the key factor. In theoretical modeling, accuracy of the results depends on the accurate choice of the method and real system under consideration. More than two decades of experience in this area has made the understanding of limitations and applicability on different models to reach a mature stage.

Computational modeling has emerged to be very useful in assisting experimentalist by analyzing various fundamental properties and effects of ligand substitution on the reactivity and catalytic features of enzymes.

Chapter 2: Methodology

In reaction mechanism studies based on the DFT modeling, particularly in catalysis that is based upon transition state theory, the key objective is characterization and prediction of all these structures. This is usually attained through geometry optimization algorithms which are inbuilt in software employed (Gaussian, jaguar etc). Various algorithms for finding these structures have been developed. They can be broadly classified into two categories i.e. first-order methods or second-order methods. The first-order involves only

an analytical first-order derivative and the second-order method employ Hessian matrix and first-order derivatives to build a quadratic model for optimization.

There are various types of such basis sets that are nowadays available in various computational chemistry softwares. Double zeta (DZ) basis set uses two basis functions for each type of minimal basis functions for two atoms with a variation of orbital exponent ζ. Double Zeta type basis set are usually sufficient in geometry optimization. The various hybrid density functional used in present work are previously benchmarked by various scientific groups for such systems. The calculations for coefficient of hybrid density functional are benchmarked by experimental values using calculations that utilize double-ζ basis functions. Split valence double-ζ basis set is a slight variation above discussed method and is useful in non-isotropic calculations. This method utilizes double basis functions for valence electrons and single basis functions for core electrons. Other basis set of such type are triple-ζ, quadrupole-ζ etc. which necessarily may not hold great variations in results.

Charge around atom of a molecule is slightly perturbed than isolated atom itself. To account the perturbation of electronic charges in a molecule polarization functions are used in basis sets. They use higher angular momentum orbitals and are indicated by the sign '*'. They enhance the wave function flexibility to change shape. Those molecular systems which have an electronic density situated far away from the nucleus like (anions, lone pairs, highly electronegative atoms etc.) diffuse basis functions are used in the basis set and these are represented by symbol '+' in the basis set representation. They basically involve small orbital exponent that results in larger spread of Gaussian functions. The effect of addition of diffuse functions to the basis set results in the change of relative

energies of these molecular systems. These basis functions are also called as augmented basis sets. Charge around atom of a molecule is slightly perturbed than isolated method itself. To account the perturbation of electronic charges in a molecule polarization functions are used in basis sets. They use higher angular momentum orbitals and are indicated by the sign '*'. They enhance the wave function flexibility to change shape. Those molecular systems which have an electronic density situated far away from the nucleus like (anions, lone pairs, highly electronegative atoms etc.) diffuse basis functions are used in the basis set and these are represented by symbol '+' in the basis set representation. They basically involve small orbital exponent that results in larger spread of Gaussian functions. The effect of addition of diffuse functions to the basis set results in the change of relative energies of these molecular systems. These basis functions are also called as augmented basis sets.

Like minimal basis set, another basis set is Pople basis set, denoted by 6-31G and usually popular for organic molecules. It indicates that each core orbital is described by a single contraction of six GTO primitives which describe each core orbital and two contractions, of which one with three primitives and another with one primitive describe each valence shell orbital. It is modified by the addition of single asterisk and double asterisks sign. Single asterisk (*) means addition of d primitives to the atoms excluding hydrogen whereas two asterisks (**) means addition of p primitives to atoms including hydrogen. Pople basis set can also be modified with the addition of plus '+' and double '++' sings. The single (+) simply means addition of diffused functions to all atoms other than hydrogen and double (++) indicates addition of diffuse functions to all atoms including hydrogen.

There are various special basis sets that are used in computational chemistry calculations for transition metals. They utilizes effective core potential (ECP) for all the electrons. As core electrons do not take part in a chemical reaction the orbitals are replaced by the electric potential in Hamiltonian in the ECP treatment. Also relativistic effect can be incorporated to improve energies. For iron, typical ECP containing basis sets are from Los Alamos type, eg., LACVP or LANL2DZ. In our work, triple-ζ basis set (LACV3P+) has also been used that employs diffuse and polarization functions on metal. Kumar *et al* have used two different basis sets, namely BS1 (LACVP on iron and 6-31G on the rest of the atoms) and BS2 (LACV3P+ on iron and 6-311+G* on the rest of the atoms) in a test calculation on substrate hydroxylation potential energy profile by a Cpd I model of cytochrome P450. This resulted in very little changes in optimized geometries and virtually identical relative energies along a reaction profile. As such, geometries are usually optimized using double-ζ basis set followed by calculation by single point energy calculation with a triple-ζ basis set. A subsequent analytic frequency calculation characterized the structures as local minima (with real frequencies) and transition state presence was marked with large single imaginary frequency for the correct mode.

Chapter 3: Modeling the Hydroxylation of Estragole via Human Liver Cytochrome P450

Natural compounds derived from plants are generally regarded safe and devoid of adverse effects. However, there are individual ingredients that possess toxic, genotoxic and carcinogenic activities. These compounds when exposed at specific level become hazardous to health. Estragole (1-allyl-4-methoxybenzene) is a common component of

spice plants. Its toxicity gets activated with the hydroxylation at C1 position by P450s present in human liver. Present study grounds to explore the reaction mechanism of conversion of estragole to hydroxylated metabolite using computational methodology. Density functional theory (DFT) based calculations were employed to explore the cytochrome P450 catalyzed mechanism at C1 position aliphatic hydroxylation of estragole to explore the overall reaction energy profile and obtain the electronic and 3D structure of all intermediates, transition states and product complexes formed during the reaction along with their free energies.

The calculations provided in the present study were computed using Gaussian 09 software and implemented DFT method. To support our results from previous studies B3LYP hybrid density functional method has been chosen, using LACVP (Los Almos) type basis set on iron that uses double ζ- core potential along with 6-31 G basis set on the rest of the atoms (Basis set BS1). Optimization of geometry and scans were performed at B3LYP/BS1 level of the theory. Geometry scan maxima is used for the transition state searches along with frequency calculations that confirm structures to be first order saddle point depicting single imaginary frequency for the correct mode. Full geometry optimization at same level of the theory has been performed, followed with frequency calculations that confirmed structures to be local minima and transition states to be first order saddle point. Cpd I used in present investigation was modeled as iron embedded in protoporphyrin IX, side chains were removed to make calculations less extensive, also replacement of side chain will not greatly affect the energies of high-lying occupied and low lying virtual orbitals of chemical system. Similary, for simplification of the substrate

structure 3-4 methoxyestragole is replaced by 4-methoxy substituent to reduce computation cost. The model and the reaction scheme is shown in Figure 1.

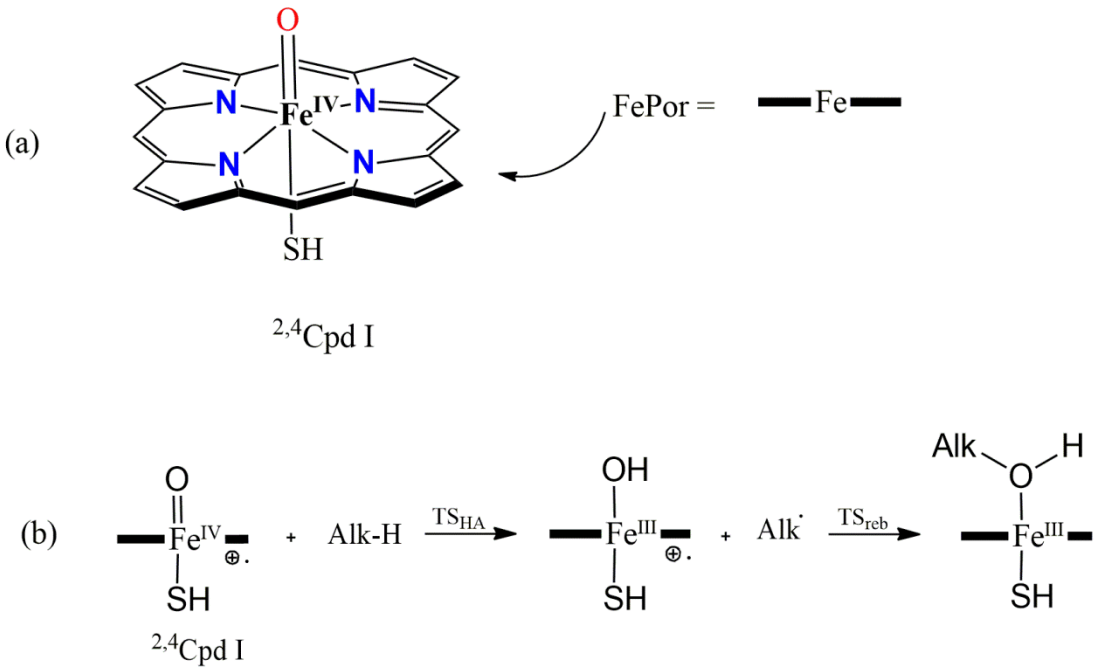

Figure 1: (a) Schematic Structure of Compound 1 (Cpd I) along with porphyrin ring representation in right (b) Schematic Two-state rebound mechanism used by P450 for aliphatic hydroxylation.

In accordance with previously calculated and benchmarked studies, we investigated our reaction mechanism with modeled active site complex of cytochrome P450 i.e. Cpd I with substrate. We are focused at C1 position aliphatic hydroxylation of estragole and it starts with hydrogen abstraction step via transition state TS_H to generate a radical intermediate INT. This radical intermediate rebounds to generate product complex PC crossing rebound transition state (TS_{reb}). The potential energy surface of the reaction mechanism is shown in Figure 2.

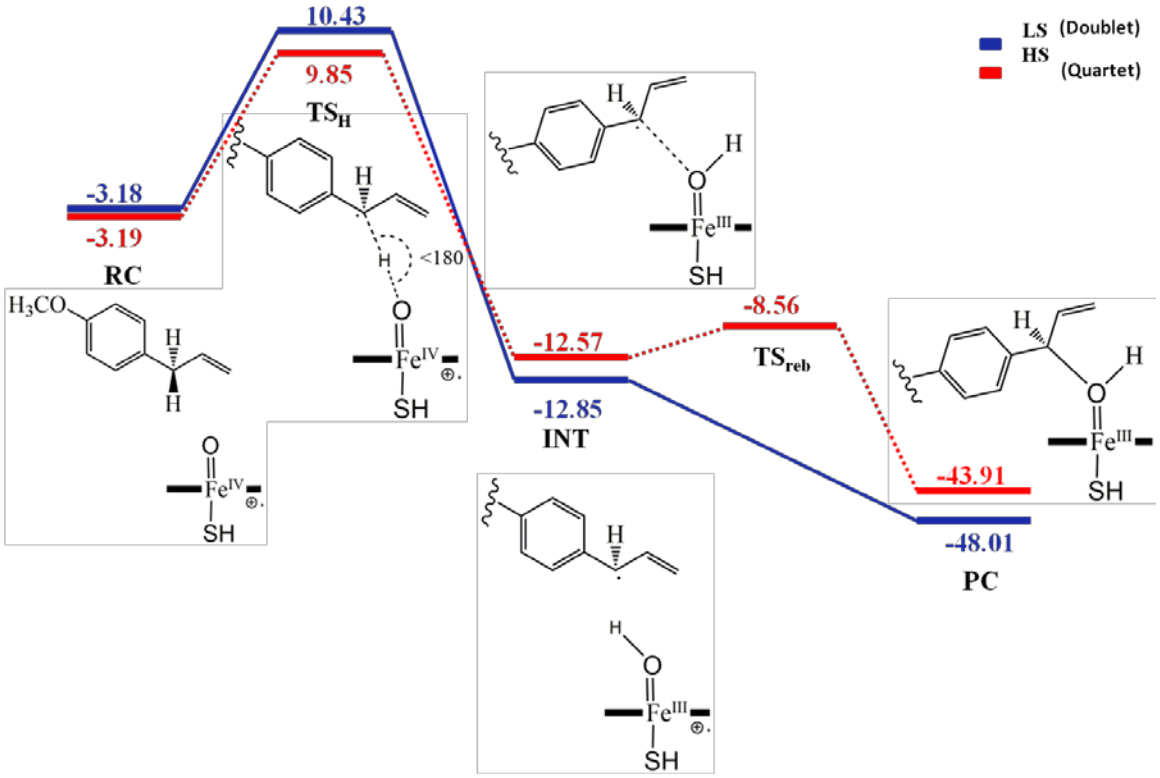

Figure 2: Potential energy profile for aliphatic hydroxylation at benzylic position of estragole calculated using DFT methodology at B3LYP/BS1 level of the theory, all energies here are reported in kcal/mol, the bond lengths in angstrom (Å), bond angles in degree (°) and frequencies in wavenumber (cm^{-1}).

The theoretical investigation revealed that a Two State Reactivity (TSR) mechanism is followed for both HS and LS. The reaction is throughout exothermic and the LS surface offers easier pathway for the product formation. The rate limiting step was found to be H-abstraction step with 9.85 kcal/mol and 10.43 kcal/mol for quartet and doublet spin state respectively. It can be asserted from above discussed results that C1 position hydroxylation of estragole with Cpd I of P450 is rebound mechanism for HS surface and

concerted for LS surface. The intermediates are highly short-lived and product formation directly occurs from intermediate on LS surface, although possibility of stereochemical scrambling is present on HS.

Chapter 4: Biotransformation of Bisphenol and their Analogues by Cytochrome P450 using DFT.

Metabolites formed during the biotransformation mediated by CYP450 may turn toxic. Investigations upon the mechanism involved in the formation of such toxic byproducts are important in analyzing their potential threat and also in elucidating the pathway for their removal. Bisphenols belong to the class of omnipresent phytochemicals. Eventually this leads to their higher exposure to humans. Therefore, preventions for the toxicity in BPA (2, 2-bis (4-hydroxyphenyl propane) has been of utmost priority. However, the mechanisms involved in their biotransformation are still a domain of topical interest.

Metabolism largely effects the toxicity of bisphenols. Present work focuses on investigating aromatic hydroxylation reaction mechanism mediated by P450 to produce catechol (o-OH BPA or 3-OH-BPA) and two of the substitutes of BPA which are used in industry viz BPF and BPZ, along with the epoxidation of BPA mediated by P450 has also been thoroughly explored.

Hydroxylation of BPA through P450 results in the formation of catechol (3-OH-BPA), this show weakly endrogenic and weakly antiandrogenic activities. Further oxidation of 3-OH-BPA produces ortho quinone i.e. BPA 3-4-quinone, it is found to from adducts with DNA. Likewise epoxides are highly unstable and hence reactive; they have potency to readily react with the DNA bases and amino acids of protein to form adducts that are

comparably stable. Hence, epoxidation reactions have raised serious concern owing to their toxicological effects. To investigate energy barriers, overall reaction profiles, electronic structure of reactant, transition states, intermediates and product during the formation of hydroxylated metabolite catechol density functional theory (DFT) calculations have been used in present study. Two spin states is an attribute of P450 which is responsible for different co-products and reaction intermediates on different spins, so we have investigated the reaction mechanism on both spin states (quartet and doublet). Catalytic cycle of P450 leads to the formation high valent iron (IV) oxo species (compound I), this is an active oxidant responsible for substrate activation. We modelled complexes of compound I with BPA and its analogs (BPF, BPZ) to elucidate the plausible reaction mechanism. Optimization of structures was performed in gas phase using B3LYP functional. Split basis set were utilized for better results. Iron atom was subjected to LANL2DZ basis set along with double zeta effective core potential (ECP), and for remaining of the atoms 6-31G basis set was used (referred to as BS1)

Investigation of the reaction mechanism for aromatic hydroxylation of BPA at *ortho* position to elucidate full reaction potential energy profile Figure 3. After the formation of reactant complex on both spin surfaces doublet and quartet virtual degeneracy can be observed. The reaction is stepwise with electrophilic attack of oxo group of Cpd I to the *ortho* carbon atom of the BPA to form tetrahedral Meisenheimer intermediate complex **I 1** as the first step, by crossing the C-O bond formation barrier TS1. The π- activation barrier ^{4,2}**TS1** is observed as 15.69 and 12.92 kcal mol^{-1} for high spin (HS)/ low spin (LS) respectively and aromaticity of the ring gets distorted with the formation of C-O bond.

The difference of HS over LS is nearly 3 kcal mol^{-1} and hence we can see the preference of aromatic activation to LS surface.

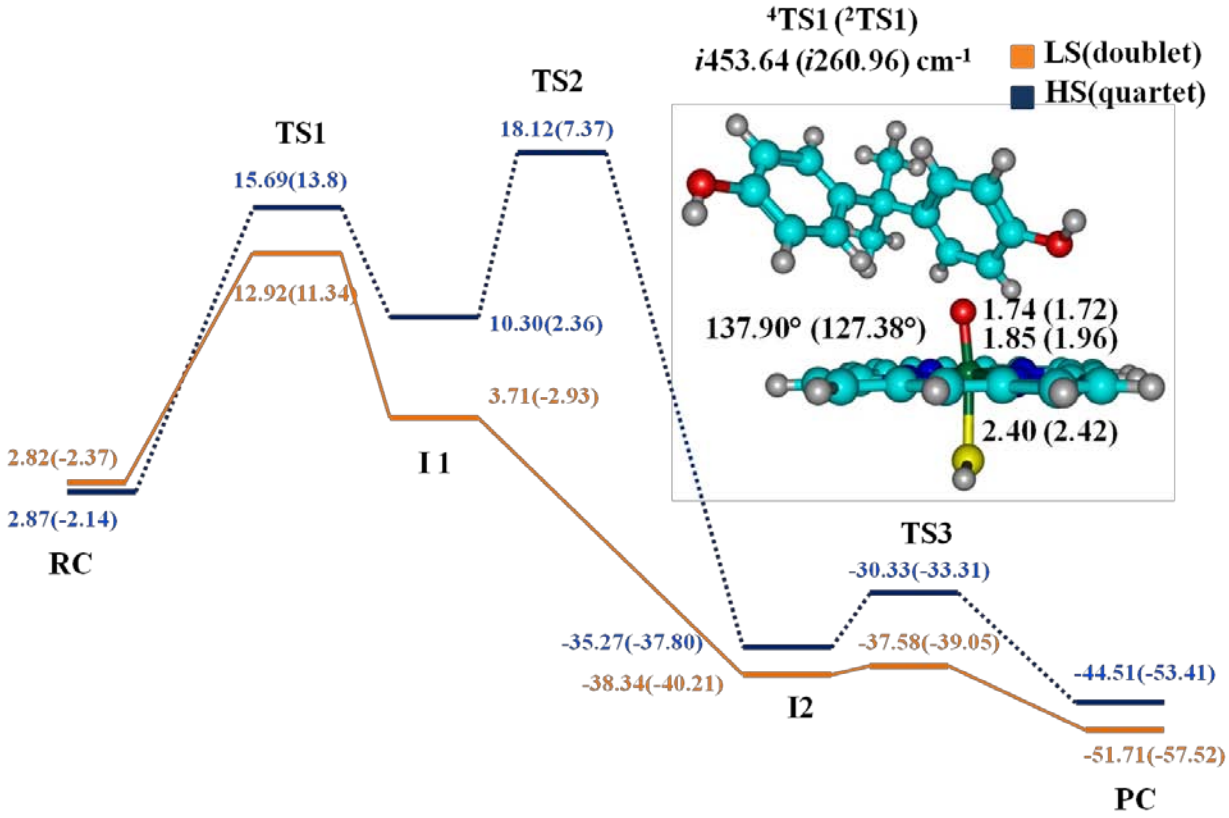

Figure 3: Represents potential energy surface of hydroxylation of BPA by active oxidant Cpd I of P450 for HS and LS, all energies are expressed in kcal/mol and imaginary frequencies in cm^{-1}. The free energies are obtained by optimization at BS1//BS2 level of the theory for the reactant, transition states, intermediates and product.

Thereafter, we calculated the full reaction profile for BPA analogs Bisphenol F (BPF) and Bisphenol (BPZ), shown in Figure 4. The landscape of BPZ shows similar patterns and energies of activation were in same range. Similar to the BPA data reported above the ^{2}**TS1** was below ^{4}**TS1** and rate determining step was π activation.

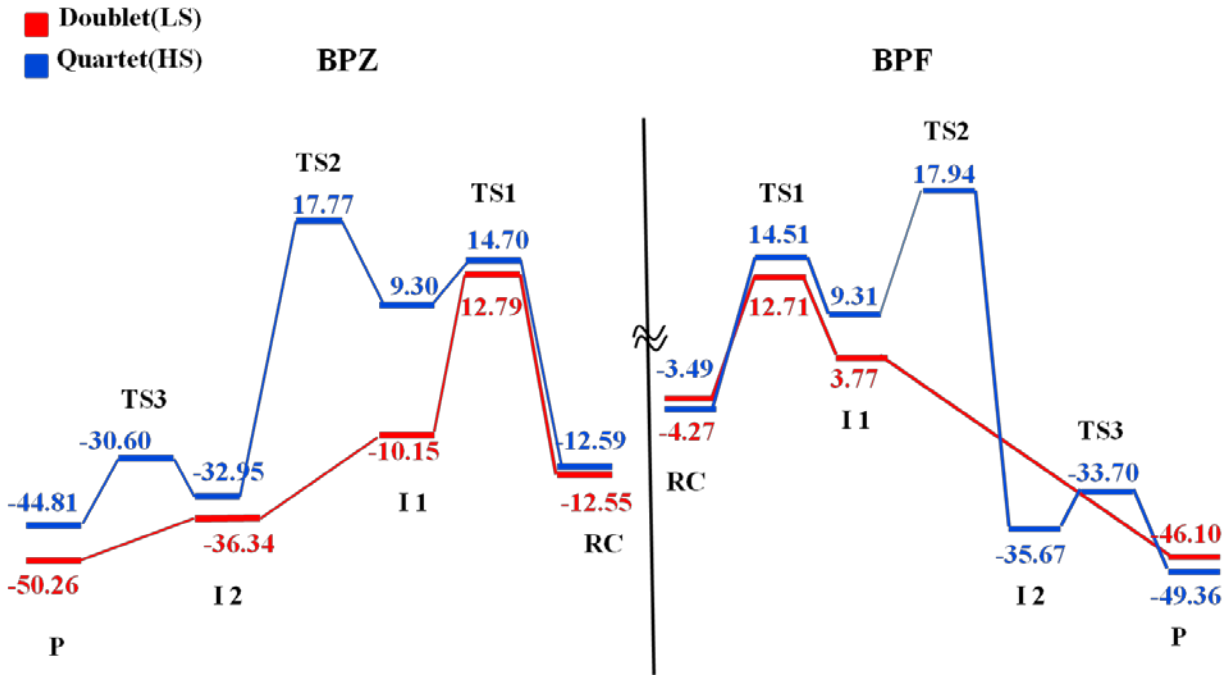

Figure 5: Above Figure represents schematic energy landscape of BPZ and BPF for two spin states (doublet and quartet) optimized at BS1 level of the theory. All energies reported above are in kcal/mol.

The energy profile for epoxidation mechanism of BPA with modeled Cpd I (SH) of P450 is illustrated in Figure 6. After the formation of reactant complex **(^{2,4}RC)**, the reaction proceeds with the attack of Cpd I Fe=O moiety on the π system of the BPA to give O-addition radical intermediate (**^{2,4}Int)** via transition state TS_{CO}. Intermediated formation is further accompanied by ring-closure barrier **(^{2,4}TSrc)** to generate epoxide product **(^{2,4}P)**. Mostly the LS surface offers lower or no ring-closure barrier whereas HS surface provide substantial barrier. The mechanism is stepwise just like aliphatic hydroxylation which offers initial rate determining H abstraction barrier.

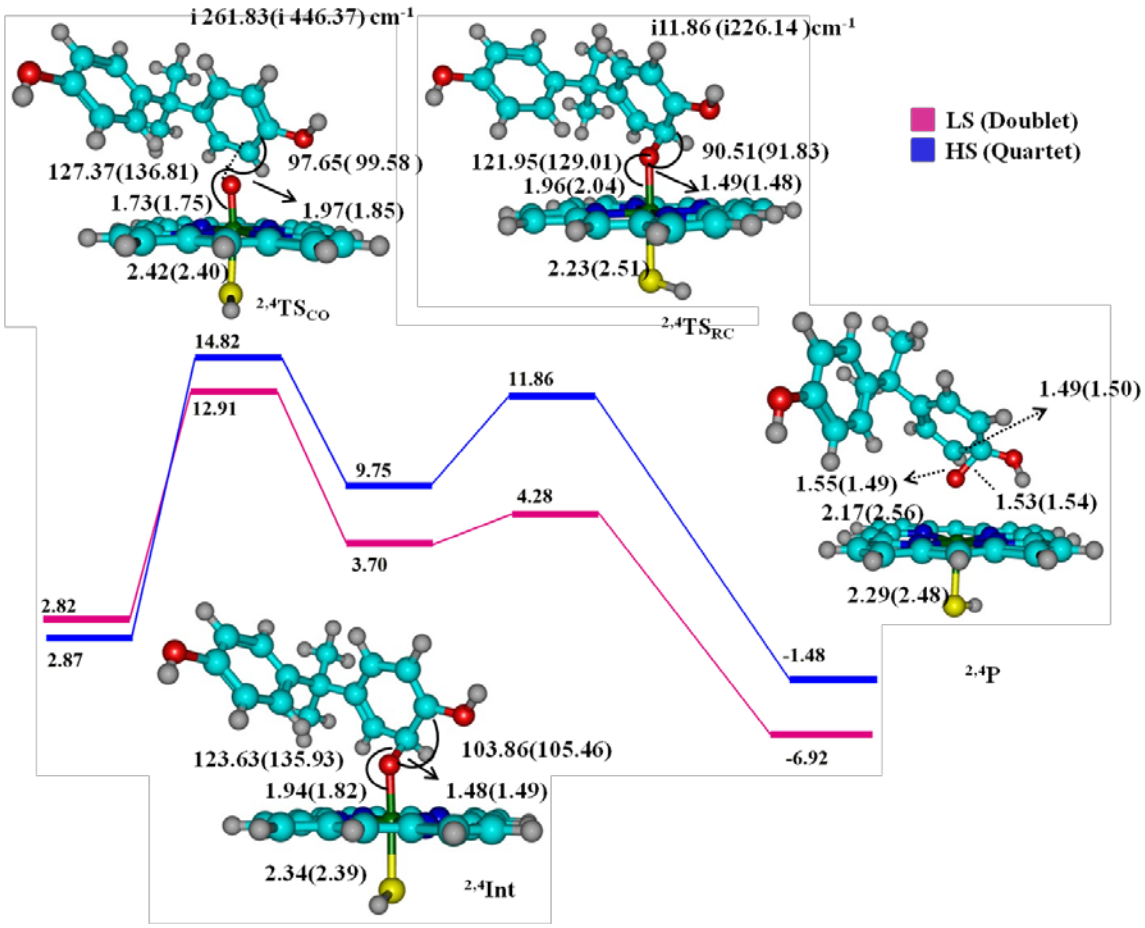

Figure 6: Free energy landscape of epoxidation mediated by P450 all energies reported are in kcal/mol, bond lengths in Å, bond angles in degree (°), and frequencies in wavenumber units. The energies depicted in Figure reported as LS (doublet)/HS (quartet) respectively. The energy is calculated using B3LYP/BS1 level of the theory.

Intermediate formation is followed by ring closure that takes place towards hydroxyl group containing carbon i.e. C4 carbon with barrier of 11.86/4.28 kcal/mol for HS/LS respectively. The ring closure barrier offered negligible barrier of >1kcal/mol on LS profile whereas large barrier is encountered on HS surface, homologous to previously reported results on epoxidation. The product formed was exothermic in nature with

respective energy values of -1.48/-6.92 kcal/mol for HS/LS. The results are in unison with previously reported results on C=C epoxidation.

Chapter 5: Equatorial Ligand Effects on the Rate of Hydrogen Abstraction Barriers by Iron (IV)=Oxo Species of N4py

In order to completely understand functional properties of these metal containing enzymes (active sites), synthetic models that mimic the actual enzyme (known as biomimetic models) are developed. These models comprises of metal centre embedded inside coordination environment which resembles with actual enzyme and are dissolved in organic solvents. These biomimetic models provide vital information regarding the catalytic cycle and operational mode of the biological systems.

One such N5 based pentadentate ligand has been extensively studied is N4PY {N, N-bis (2-pyridylmethyl)-N bis (2-pyridyl) methylamine}. In its iron (IV)-oxo form, i.e. $[FeIV(O)(N4Py)]^{2+}$ complex have four nitrogen donar atoms (N_{eq}) which are perpendicular to the iron(IV)-oxo axis and axially ligated nitrogen atom of amine which is trans to the oxo group. This ligand framework offers bowl like cavity for the iron atom as well as to the substrates involved in the reaction mechanism. In successive years, Fe (IV)-oxo complex of N4PY (1b) have been successfully characterized and well studied which makes it a suitable candidate to study the effect of ligand substitution on the electronic properties and its reactivity.

All the complexes (biomimetic catalyst) used in the present study are discussed below and are shown in the Figure 7. The unsubstituted ligand N4PY is **[**N, N-bis (2-pyridylmethyl)-N bis (2-pyridyl) methylamine], its active intermediate responsible for the

hydrogen atom abstraction (HAA) and oxygen atom transfer (OAT) reactions is $[FeIV(O)(N4Py)]^{2+}$, this catalyst is referred in present investigation as **1b**. Substitution changes are performed on the 6^{th} position of the pyridine ring systems of the N4PY ligand framework. The two pyridine rings which are connected through the methylene carbon are substituted by the methyl group, this ligand framework is named as 2(6-^{Me}Py)N_{ax}2Py or MeN4Py. The active oxidant is $[FeIV(O)(^{Me}N4Py)]^{2+}$, this is referred as **2b**. The **3b** complex consists of substitution on the two other pyridine rings which are connected with the two methine carbon, this sort of engineering is rarely reported in the literature. This ligand framework is named as 2PyN_{ax}2(6-^{Me}Py) or $N4Py^{Me}$, its active intermediate is $[FeIV(O)(N4Py^{Me})]^{2+}$.

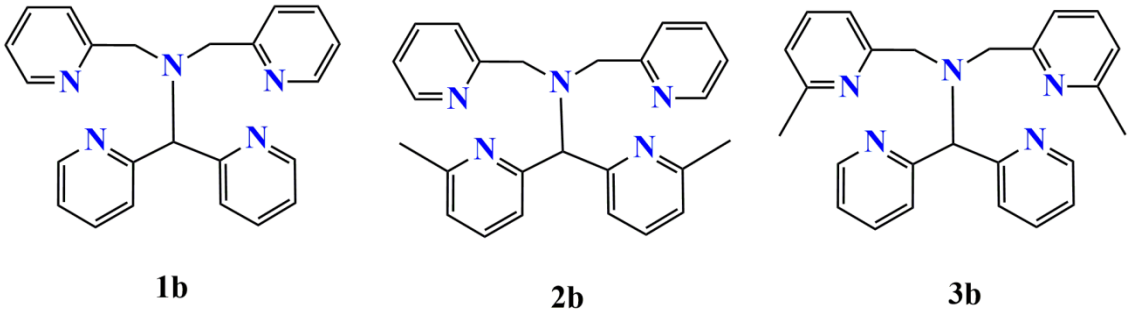

Figure 7: Schematic models of 1b, 2b and 3b oxidants that are used in the present study.

The calculations were performed using DFT functional B3LYP along with split basis set. For iron atom LAND2Z basis set with double zeta effective core potential was used and for the remaining of the atoms (C, H, N,O and S) 6-31 G basis set was used i.e. (BS1). All the structures were optimized in gas phase and relaxed potential energy scans (PES) were set for finding hydrogen abstraction rates for three models viz 1b, 2b and 3b. To

ascertain structures as minima and saddle point analytic frequency calculations were also subjected. Corresponding to transition state single imaginary with correct mode was obtained and real frequencies were found for reactants and intermediates for all models with ethylbenzene as substrate.

Investigation of the HAA step from ethyl benzene as a substrate for all three complexes **1b**, **2b** and **3b** depicts that the **3b** complex is the better oxidant of all and offers fast hydrogen transfer with a lower energy barrier. Substrate approach towards iron(IV)-oxo species is changed in both the substituted systems. In case of ^{3,5}2b the substrate tries to access the oxo group from left whereas in ^{3,5}3b substrate enters from right hand side. All the respective reaction energy profile for 1b, 2b and 3b are shown in Figure 8, 9 and 10.

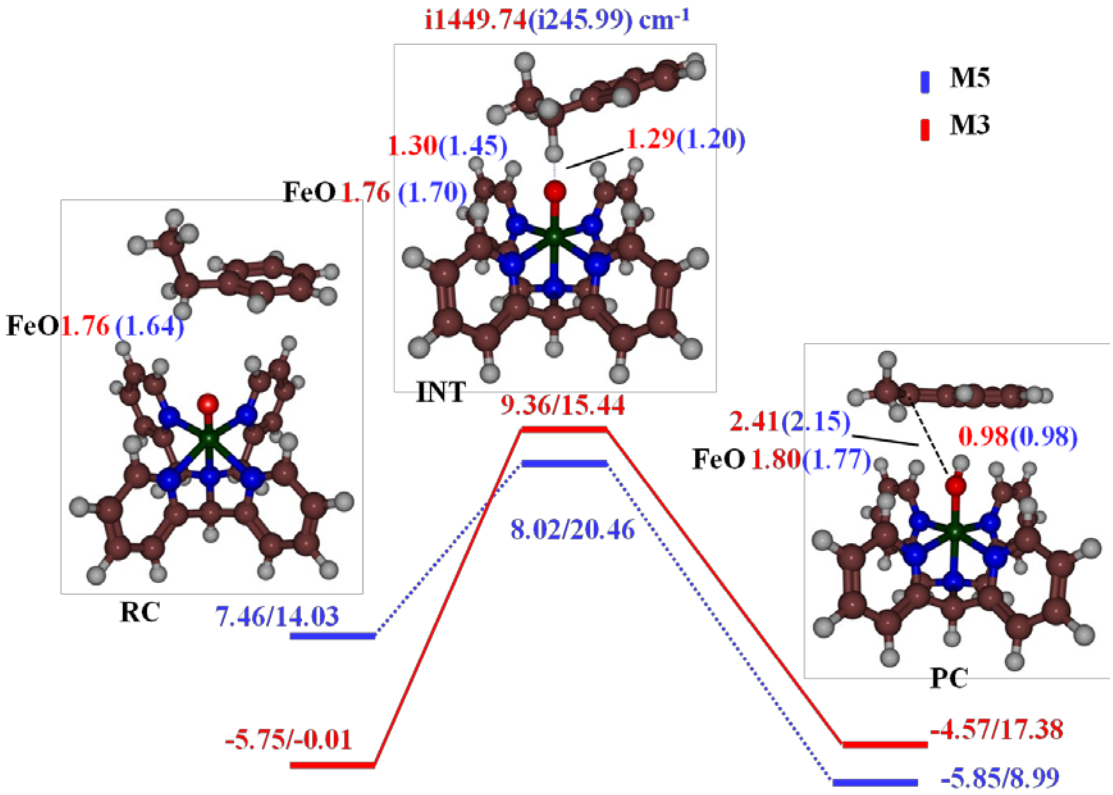

Figure 8: Potential energy landscape of H-abstraction barrier heights from ethyl benzene by **1b** for triplet/quintet spin states. The energy are calculated at the B3LYP/BS1//B3LYP/BS1(solv) level of the theory. All energy values are reported are in kcal/mol, frequencies in wavenumber (cm^{-1}), and bond lengths in Å.

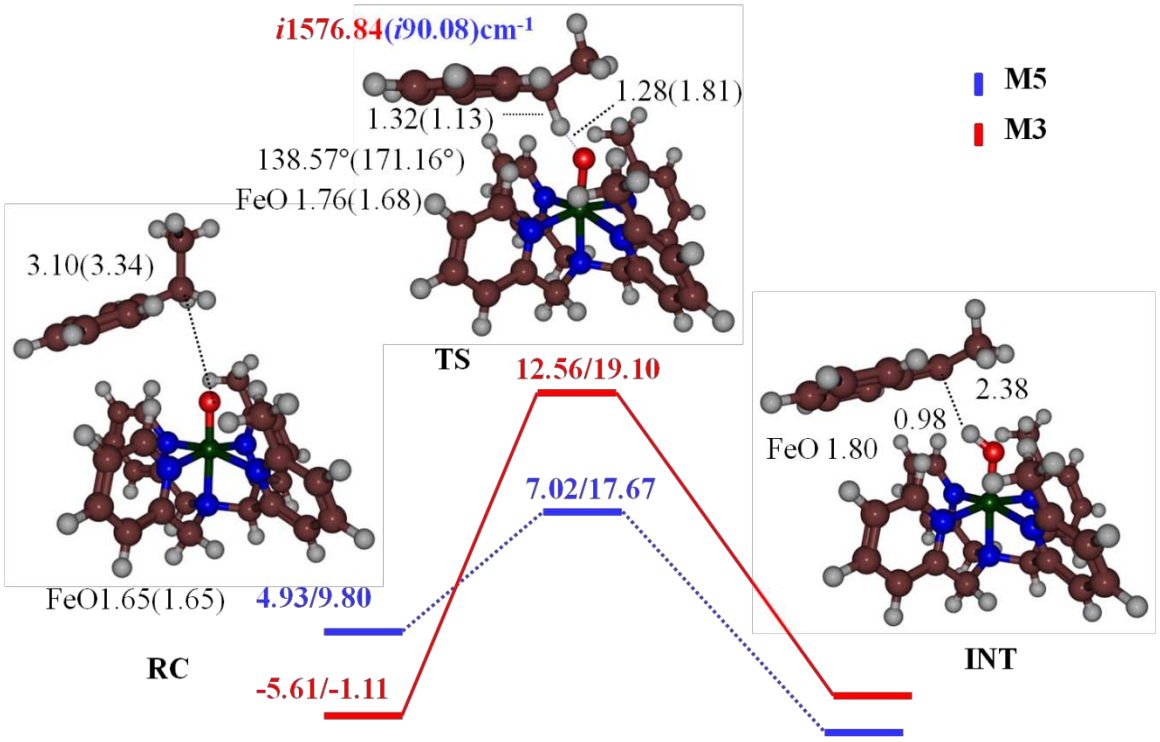

Figure 9: Potential energy landscape of H-abstraction barrier heights from ethyl benzene by **2b** for triplet/quintet spin states. The energy are calculated at the B3LYP/BS1//B3LYP/BS1(solv) level of the theory. All energy values are reported are in kcal/mol, frequencies in wavenumber (cm^{-1}), and bond lengths in Å.

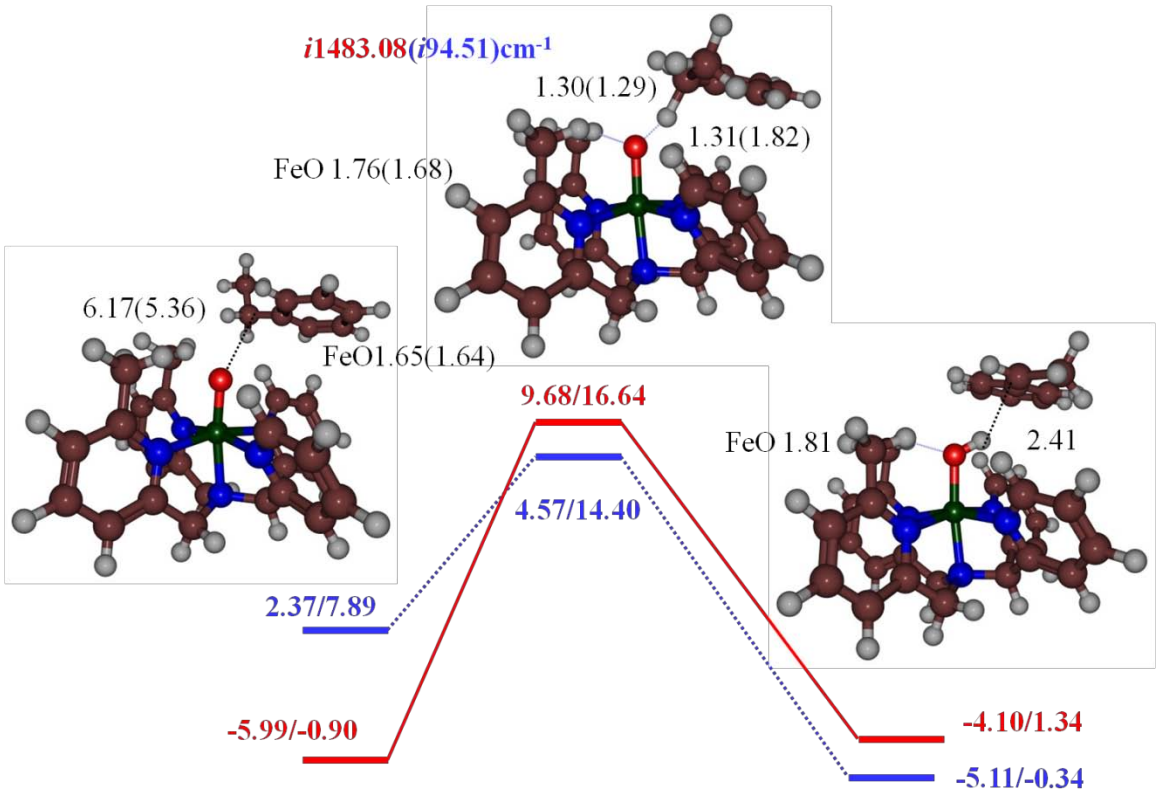

Figure 10: Potential energy landscape of H-abstraction barrier heights from ethyl benzene by **3b** for triplet/quintet spin states. The energy are calculated at the B3LYP/BS1//B3LYP/BS1(solv) level of the theory. All energy values are reported are in kcal/mol, frequencies in wavenumber (cm^{-1}), and bond lengths in Å.

The theoretical calculations on the N4PY and its substituted scaffolds allowed through understanding of the intricate behavior raised due to steric and electronic effects in details. With the substitution of methyl groups on the mononuclear nonheme iron (IV)-oxo model systems in an octahedral environment, changes in the reactivity are prominent from the above investigation. Engineered oxidant 3b shows lower HAA barrier compared from 2b and 1b complexes. Detailed investigation of the electronic organization points

out that the enhanced reactivity is not an outcome of electronic features in all the complexes. Rather geometrical features which occur due to the substitution could be the cause for the possible reactivity. The substitution causes steric factors on the forefront and which brings better positing of the substrate with respect of the oxidant. In real enzymatic systems residues help in the positing of the substrate and easy electron flow. The present investigation suggests that substitutional effects can also channelize the approach of the substrate which can thereby enhance the reactivity and selectivity of the reaction.

Chapter 6: Conclusions

The present thesis highlights following points as general conclusions:

- Theoretical model calculations are now regarded equally important in determining mechanism of metalloenzymes. Experimental methods have the advantage that they are studied on the actual (real) system, but spectroscopically guarding of short-lived species, electron transfer and interpretation of results is quite troublesome. Theoretical modeling can easily assist experimental studies with good accuracy due to both the development of the theory and the decades of experience in this area. The accuracy of the system depends on the choice of the method and the real system under consideration.

- The present thesis work helped in exploring the reactions mechanism of various oxygen atom transfer (OAT) reactions which includes reactions like, aromatic and aliphatic hydroxylation, olefin epoxidation, of transition metal containing

complexes. The research characterized the active oxidant in the reaction processes and the rate determining step in the mechanism. Moreover, in several cases models were devised that rationalize reaction processes and barrier heights that can be used to predict rate constants of processes.

- Present work also devised models for non-heme iron(IV)-oxo species of N4PY that investigated in lowering the reaction barrier of hydrogen transfer by equatorial substitution of ligands. The theoretical calculations revealed lower activation barrier in H-atom transfer from the substituted systems.

- The studies present in this thesis work are expected to bridge the computational and experimental work being carried out in biomimetic/enzymatic reactions. Also they are expected to meet challenges of drug synthesis (pharmaceutical reactions), hydroxylation and oxidation of common toxic compounds both natural and artificial.