**GENERAL INTRODUCTION**

Heme based enzymes are very important to nature. They participate in various reaction associated with the human body such as O₂ transport and storage (haemoglobin & myoglobin), O₂ reduction (Cytochrome c oxidase), substrate oxidation (cytochrome P450), electron transfer (cytochrome c) etc. The basic scaffolds of these above enzymes are same; a heme active site with different spatial arrangements and the coordinating ligands. These spatial conformatons of the peptide linkage as well as the co-ordinating ligands are responsible for various different reactivities of these enzymes. Oxygen is the most abundant in the earth’s crust. The oxygen reduction reaction is the most important process in life cycle. Unlike biological respiration is completely depends on ORR. Similarly energy converting system such as fuel cells are also depends on ORR. Oxygen reduction occurs in two ways, directly 4e⁻ reduction pathway from O₂ to H₂O and another 2e⁻ reduction pathway from O₂ to H₂O₂ in aqueous solvent. Again in organic solvent, 1e⁻ reduction pathway from O₂ to superoxide (O₂⁻) can also possible. These two processes can be discussed in the thesis. Recently Iron-porphyrins are used as O₂ reduction catalyst. Several groups are trying to mimic these reactivities by synthesizing model heme active site for different mettaloenzymes. Few of the groups are able to synthesize some of the heme active site model such as Cytochrome c oxidase (CcO), Nitric oxide Reductase (NOR), Hemoglobin, Cytochrome P450 etc. World’s leading scientist James P. Collmann & co-workers was able to synthesize a synthetic Cytochrome C oxidase active site model. They have developed this active site model by dedicated research and successfully attached this model on the surface to measure the electron transfer rate and the O₂ reduction reactivity; thus successfully mimicking the functions of a native Cytochrome C oxidase enzyme. They also showed the effect of Tyrosine for mimicking the cytochrome c Oxidase. Similarly Karlin & co-workers had been able to synthesize a series of cytochrome c Oxidase active site models and isolated intermediates associated with the reaction mechanism. Naruta & co-workers also synthesized a series of cytochrome c Oxidase models. They were also able to isolate and characterize the iron-Oxy intermediate species by resonance Raman spectroscopy. To study the catalytic behaviour of metalloenzymes, Hirobe & co-workers synthesized cytochrome P450 active site model to show small molecule hydroxylation.

Apart from iron porphyrins, scientists are also working with the other transitional metals such as Cobalt, Zinc, Ruthenium, Rhodium etc. as core elements in porphyrins or
substituted porphyrins and have been showing catalytic or electrocatalytic processes.\textsuperscript{16-19} Zinc based porphyrins are very important for the photo-voltic processes.\textsuperscript{20, 21, 22} Nocera & co-workers have showed water oxidation by hangman porphyrins which are cobalt based substituted porphyrins.\textsuperscript{16}

Similarly porphyrins are used for the photodynamic therapy in medical study.\textsuperscript{23} For PDT, porphyrins with photoactive metals are used as photosensitiser for cancer therapy. Depending on the mode of treatment, the photosensitizing agents is either injected into the bloodstream of the vein or put on the skin. Over a certain time, some of the photosensitizing drug is absorbed by the affected cells. Then the specific wavelength of light is treated on the affected area. The light causes the drug to react with oxygen that generates chemicals which kill the affected cells. Recently several scientists have been working on this field. They have synthesized and developed new photosensitisers for better results.

In most of the transitional metal active sites that catalyze the catalytic processes requires electrons and protons. The electrons are generally stored at the electron transfer sites such as reductase component of methane mono-oxygenase, type I copper site in multicopper oxidases, Nitrogenase, Nirite reductase etc.\textsuperscript{24-26} It is well established that electron transfer properties (both thermodynamic and kinetic) of these sites play a major role in catalysis.\textsuperscript{27-30} It is also well known that the reduction potential of an active site can be tuned by hundreds of millivolts such that it can participate in high potential as well as low potential processes without altering its geometry significantly.\textsuperscript{31,32} Several factors are known to affect the reduction potential of these electron transfer sites e.g. solvation, hydrogen bonding, local dielectric.\textsuperscript{33-35}

We have design a ferrocene substituted metal porphyrins that can have electron donating sites attached to the distal site of the porphyrin. This iron based models are successfully reduced O\textsubscript{2} to water when physiabsored on both the slow and fast SAM with a 2\textsuperscript{nd} order rate constant is the order of 10\textsuperscript{4}. The electron donating ferrocene functional groups are responsible for O\textsubscript{2} reduction. When O\textsubscript{2} reduction was done inorganic solvent, the iron porphyrin complex was unable to do so for the high redox potential for Fc\textsuperscript{+}/Fc couple. Therefore we also observed the solvent selectivity which is completely described in chapter 2.

Cytochrome P450 represents the one of the most important metalloenzymes from the oxygenase family which is derived by name because of its hallmark absorption band at 450 nm
when binds to the CO.\textsuperscript{36, 37} The active site contains cystine thiolate residue as its fifth coordinated ligand to the iron centre. In case haemoglobin, the fifth ligand is histidine residue instead of cystine thiol and it act as O\textsubscript{2} carrier protein. Cytochrome P450 has the unique ability to oxidise very strong C-H bonds even inert also using molecular O\textsubscript{2}. In course of catalytic oxygenation processes, a highly reactive intermediate is generated called compound I, \textsuperscript{38-40} which is best described by Fe(IV)-oxo radical species which is responsible for oxygenation process. The reactive species is common for all other heme enzymes such as peroxidases, catalases, haemoglobin, myoglobin.\textsuperscript{41-45} Other heme based metalloenzymes generate compound I in presence of H\textsubscript{2}O\textsubscript{2} but in presence of cysteine thiolate ligand for cytochrome P450, it generates compound I in presence of molecular oxygen. The axial anionic thiolate ligand of P450 exerts a “push effect” to drive the O-O bond cleavage as opposed the conventional “pull effect” associated with the peroxidases.\textsuperscript{46} The “push effect” of thiolate has been proposed to be responsible for several unique functional attributes of P450.\textsuperscript{47} It is proposed to lower the reduction potential of the active site, increase the pKa of the trans axial H\textsubscript{2}O ligand, lower the binding constants of other axial ligands, activate O-O bond cleavage and stabilize the high oxidizing compound I species.\textsuperscript{48-51} Mutational studies on P450 have clearly indicated the quintessential role played by this axial thiolate ligand.\textsuperscript{48,52}

Inspired from the biological relevance, we have able to design our own models of heme active site and also mimic some of the metalloenzyme active site model.

Attention to the active site model of cytochrome P450 enzyme, we designed our first simplest synthetic analogue of cyt-P450 using tetraaminophenyl porphyrin (TAPP) and chlorovaleryl chloride as our basic scaffolds. Surprisingly the aliphatic model found to be air stable for several months. We named it as PPSR. The thiolate moiety co-ordinated to iron centre is protected by the bulky pivolyl groups. Due to this air stability, we also studied the oxygen reduction reactivity in pH 7 buffer. The 2\textsuperscript{nd} order rate constant is quite higher than the iron ferrocene complex i.e. is the order of 10\textsuperscript{6}. The comparative study was also done with a imidazole based iron porphyrin model (PIM).

However till date O\textsubscript{2} reduction by these thiolate bound iron porphyrin complexes have not been investigated where there are some reports of O\textsubscript{2} reduction by electrodes bearing cytochrome P450 enzyme. Comparison of the physical and chemical properties of these active site models with the native enzymes leads to deeper understanding for the structure function relationships. However several studies using the native enzymes have been
These reports reveal the various physico-chemical properties but using the synthetic models, these are limited. So we are designed a cytochrome P450 that can bear an alkyne functional groups as terminal. Using this terminal alkyne to the cyt.P450 synthetic model, we are successfully attached the synthetic model the surface covalently by “Click Chemistry”. This is the first report of attachment of cyt-P450 active site to the surface. Analysis of the steady state electrochemical kinetics reveals that this catalyst can selectively reduce O2 to H2O with a 2nd order $k_{\text{cat}} \sim 10^7$ M$^{-1}$s$^{-1}$ at pH 7. An analogous phenolate bound iron porphyrin complex is also synthesized which reduces O2 with a 2nd order rate constant of $10^5$ M$^{-1}$s$^{-1}$ under the same conditions. The anionic ligand bound iron porphyrin complexes catalyze ORR faster than any known synthetic heme analogues. The kinetic parameters of O2 reduction of this synthetic thiolate bound complex, which is devoid of any 2nd sphere effects present in protein active sites, provide fundamental insight into the role of the protein environment (dielectric and hydrogen bonding) in tuning its reactivity.

We have recorded cyclic voltammetry of the axially ligated iron-porphyrin (thiolate and phenolate model) in various solvents having different dielectric constants to investigate the solvent dependency. The data indicate that the Fe$^{III/II}$ potential shifts to more negative values as the solvent dielectric constant is lowered. These potentials vary over ~300 mV and show a linear correlation with the inverse of solvent dielectric constant (1/ε). DFT calculations reproduce the trend in the experimental data as the linear dependence of $E^0$ vs 1/ε and further show that the dependence of $E^0$ with solvent dielectric is opposite for a neutral imidazole ligand bound iron porphyrin complexes i.e. the $E^0$ shifts to more positive values as the solvent dielectric is lower. These data and computation results are rationalized using differences in the solvation energies of oxidized and reduced states of these complexes. The $E^0$ measured in an aqueous medium (under heterogeneous conditions) of the thiolate and phenolate bound complexes are ~300 mV and ~200 mV more positive, respectively, than the values expected from the dielectric constant of water alone. This additional shift in potential in an aqueous medium is due to hydrogen bonding between the water molecules and the metal bound axial ligand which is reproduced quantitatively in DFT calculation. The details study was discussed in chapter 5.

Lastly we have also developed the valence tautomerism effect on cytochrome P450 synthetic active site model. cyt-P450s have a cysteine-bound heme cofactor which, in its as-isolated resting (oxidized) form, can be conclusively described as a ferric thiolate species. Unlike the native enzyme, most synthetic thiolate-bound ferric porphyrins are unstable in air.
unless the axial thiolate ligand is sterically protected. Spectroscopic investigations on a series of synthetic mimics of cyt-P450 indicate that a thiolate-bound ferric porphyrin coexists in organic solutions at room temperatures (RT) with a thyl-radical bound ferrous porphyrin i.e. its valence tautomer. The ferric thiolate state is favored by greater enthalpy and is air stable. The ferrous thyl state is favored by entropy, populates at RT and degrades in air. These ground states can be reversibly interchanged at RT by the addition or removal of water to the apolar medium. It is concluded that hydrogen bonding and local electrostatics protect the resting oxidized cyt-P450 active site from degradation in air by stabilizing the ferric thiolate ground state in contrast to its synthetic analogues.

REFERENCES