IN SILICO IDENTIFICATION AND MODELING OF CYTOCHROME C OXIDASE SUBUNIT 1PARTIAL (MITOCHONDRION) OF *Labeo rohita* AND DOCKING STUDY WITH CARDIOLIPIN

A Thesis Submitted

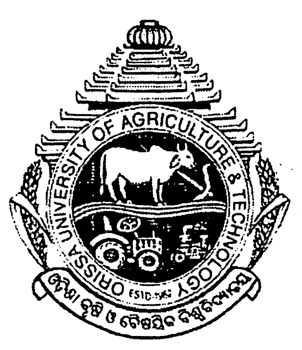
in partial fulfillment of the requirements for the award of the Degree of

MASTER OF SCIENCE IN BIOINFORMATICS

BY

ABHIPSHA BISWAL

ADM. NO.: 04BI/12



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BHUBANESWAR – 751003

2014

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TO

MY BELOVED PARENTS

CENTRAL INSTITUTE OF FRESHWATER AQUACULTURE
केन्द्रीय मीठाजल जीवपालन अनुसंधान संस्थान
(An ISO 9001:2008 Certified Institute)

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Scientist (Senior Scale)

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This is to certify that thesis entitled "***In Silico* Identification, Modelling and Docking Study of Cytochrome c Oxidase Subunit 1 partial (mitochondrion) of *Labeo rohita* with Cardiolipin**" submitted for award for the degree of **Integrated Master of Science** in the subject of bioinformatics embodies a four months research work carried out by Abhipsha Biswal under my guidance & supervision.

I further certify that any help or information received during the course of investigation have been duly acknowledged by her.

Place: Bhubaneswar
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CERTIFICATE-II

This is to certify that the thesis entitled **"*In Silico* Identification and Modelling of Cytochrome c Oxidase Subunit 1partial (mitochondrion) of *Labeorohita* and Docking study with Cardiolipin"** submitted by **Miss Abhipsha Biswal (Adm. No. 04BI/12)** to Orissa University of Agriculture and Technology, Bhubaneswar in partial fulfillment of the requirements for the award of the **degree Master of Science in Bioinformatics** has been approved by the student advisory committee after an oral examination of the same in collaboration with external examiner.

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ABSTRACT

Labeo rohita is considered as a model species among Indian Major Carps and has the high demand due to its food value. The cytochrome c oxidase (CcO) is one of three protein complexes of OxPhos building up a proton gradient across the inner mitochondrial membrane, which is ultimately used by the ATP synthase to produce ATP. Cardioilipins (CLs) being able to carry protons our results strongly support an involvement of CLs in the proton delivery machinery to CcO. The ubiquitous nature of CL interactions with the components of the OxPhos suggests that this delivery mechanism might extend to the other respiratory complexes. So here the binding of CL to COX1 (Cytochrome c oxidase subumit 1) have been associated with the regulation of the electron activity of the enzyme.Furthermore, dysfunctional cytochrome c oxidase in the cells leads to a compromised mitochondrial membrane potential, a decreased ATP. Interestingly, suppression of COX1 expression also sensitizes the cells to apoptosis and Asphyxiation. The identified COX1 is a 215 amino acid length polypeptide (GenBank ID ACF34406.1).Despite its major importance in Electron Transfer Chain, the three dimensional (3D) structure of COX1 in many aquaculture species including Indian carps has not yet been reported of fish. This is the first report on predicting a 3D structure for CcO of *Labeo rohita*.

A combined strategy of homology modeling and Docking study has been applied to predict a reasonable structure of COX1 and to understand its dynamic behavior. The Validated 3D structure has been further used in structure based drug designing where the conserved domain of COX1 derived and can be used to bind with cardiolipin for its full activity and oxidative phoshorylation. The final model was assessed to be reliable using PROCHECK, Verify3D and ERRAT programs. This overall study provides possible electron transfer pathways from via water molecule & through binding with cardiolipin.

Keywords: Modeller 9.12, Autodock 4.2,Cytochrome c oxidase, Cytochrome c oxidase subunit1,Cardiolipin,Oxidative Phosphorylation, cardiolipin.

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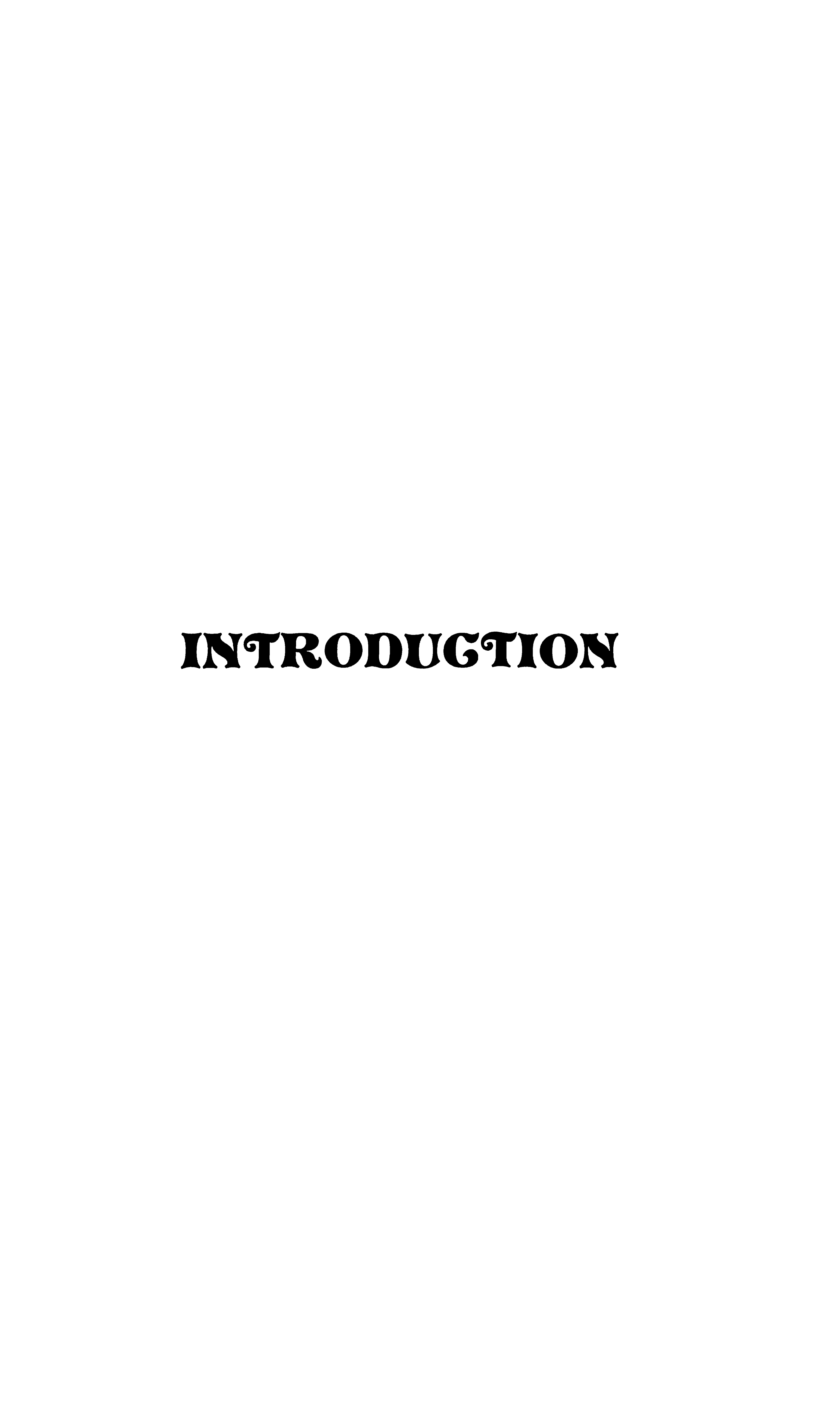
ABBREVIATIONS

CcO	:	Cytochrome c Oxidase
COX1	:	Cytochrome c Oxidase Subunit 1
CL	:	Cardiolipin
Cyt c	:	Cytochrome c
ATP	:	Adenosine Triphosphate
ADP	:	Adenosine Diphosphate
ROS	:	Reactive Oxygen Species
ETC	:	Electron Transport Chain
NCBI	:	National Center for Biotechnology Information
PDB	:	Protein Data Bank
BLAST	:	Basic Local Alignment Search Tool
SOPMA	:	Self-Optimized Prediction Method with Alignment
PSIPRED	:	Psi-blast based secondary structure prediction



INTRODUCTION

INTRODUCTION

India is the second largest fish producer in the world inland aquaculture after China. The Indian freshwater aquaculture is mainly based on Indian major carps (*Labeo rohita, Catla catla* and *Cirrhinnus mrigala*), contributing more than 80% of the total national aquaculture production (FAO 2005). Among these carps, *L. rohita* (rohu) is the most economical freshwater fish species (Berman *et al.*, 2003). India is rich with inland freshwater fish, with about 940 species known from its rivers, lakes and estuaries. Freshwater aquaculture in India is mainly carp-based and accounts for a considerable proportion of total aquaculture production. *Labeo rohita* is an important freshwater fish species normally cultured in Asia particularly in the Indian subcontinent. Rohu (*Labeo rohita*) is the most important among the three Indian major carp species used in carp polyculture systems. It is a fact that growth of fish is a plastic process that can change considerably in response to internal factors of the fish.

Cytochrome-c oxidase (COX-1), an enzyme which is central to energy metabolism and particularly relevant for developing nervous systems. In healthy cells, the protein is found in the mitochondrial inter membrane space; it is associated with the inner mitochondrial membrane cardiolipin (CL), a lipid which comprises approximately 9 weight) of that membrane.CL is also required for full activity of cyt c oxidase, and it has been suggested that cyt c-CL interactions may potentially recruit cyt c to this redox enzyme. The long-awaited structure of the integral membrane respiratory complex cytochrome c oxidase will now allow more precisely targeted studies designed to understand the mechanism of redox-linked proton pumping. All members of this superfamily are redox-driven proton pumps that generate a cross-membrane proton gradient that is subsequently used by ATPases to catalyze ATP synthesis. The role of the lipids is still largely investigated, mainly through reconstitution experiments. Kinetic studies of electron transfer between cytochrome c and cytochrome-c oxidase lead to a single catalytic site model to account for the multiphasic kinetics. Cytochrome c oxidase controls the last step of food oxidation. At this point, the atoms themselves have all been removed and all that is left are a few of the electrons from the food molecules. Cytochrome c oxidase takes these electrons and attaches them to an oxygen molecule.

Cytochrome c oxidase subunit I (CO1 or MT-CO1) is one of three mitochondrial DNA (mtDNA) encoded subunits (MT-CO1,MT-CO2, MT-CO3) of respiratory complex IV. Complex IV is the third and final enzyme of the electron transport chain of mitochondrial oxidative phosphorylation. Cytochrome c oxidase (COX) is one of only four known bigenomic proteins, with three mitochondria-encoded subunits and 10 nucleus-encoded ones derived from nine different chromosomes. Cytochrome-c oxidase is a highly regulated enzyme which is believed to be the pacesetter for mitochondrial oxidative metabolism and ATP synthesis. The structure and function of the enzyme are affected in a wide variety of diseases including cancer, neurodegenerative diseases, myocardial ischemia/reperfusion, bone and skeletal diseases, and diabetes. COX-1 could play essential roles in developing cells and in stem cells of the eye periphery. Both ATP and ADP binds with cytochrome c oxidase. In cytochrome c oxidases, the subunit contains a Cu center (Cu_A) with 2 Cu atoms, which is thought to be the immediate electron acceptor from cytochrome c. Zinc ions are shown to be an efficient inhibitor of mitochondrial cytochrome c oxidase activity, both in the solubilized and the liposome-reconstituted enzyme. Zinc presumably blocks the entrance of the D-protonic channel opening into the inner aqueous phase. Nitric oxide is capable of rapidly and reversibly inhibiting the mitochondrial respiratory chain or cytochrome c oxidase and may be implicated in the cytotoxic effects of nitric oxide in the CNS and other tissues which gives Implications for neurodegenerative diseases.

Cytochrome c Oxidase deficiency is a very rare inherited metabolic disorder characterized by deficiency of the enzyme cytochrome C oxidase (COX), an essential enzyme that is active in the subcellular structures that help to regulate energy production (mitochondria). Deficiency of COX may be limited (localized) to the tissues of the skeletal muscles or may affect several tissues, such as the heart, kidney, liver, brain, and/or connective tissue (fibroblasts); in other cases, the COX deficiency may be generalized (systemic).

Cardiolipin (IUPAC name "1,3-bis(sn-3'-phosphatidyl)-sn-glycerol") is an important component of the inner mitochondrial membrane, where it constitutes about 20% of the total lipid composition and also helps to build quaternary structure. Since cardiolipin is an important phospholipid found almost exclusively in the inner mitochondrial membrane and very essential in maintaining mitochondrial function, it is suggested that abnormalities in CL can impair mitochondrial function and bioenergetics.

If cardiolipin is mixed into the outer mitochondrial membrane near intermembrane contact sites, cyt c might contribute to its own escape from mitochondria during apoptosis. Cyt c is often studied as a model of protein folding, and several groups have used a fluorescent tag covalently bound to the protein as a reporter for differently-folded states.

Interactions of cytochrome c (cyt c) with cardiolipin (CL) are important for both electron transfer and apoptotic functions of this protein. A sluggish peroxidase in its native state, when bound to CL, cyt c catalyzes CL peroxidation, which contributes to the protein apoptotic release. Phospholipid fraction is extremely critical for the larval growth of *Labeo rohita.*

COX also engages in proton pumping in the establishment of an electrochemical gradient for the synthesis of ATP. COX is a complex of 13 subunits; the largest three are encoded in the mitochondrial DNA and the remaining 10 are encoded in the nuclear genome. Four different gases, nitric oxide (NO), carbon monoxide (CO), hydrogen sulfide (H2S), and hydrogen cyanide bind to CcO and invariably inhibit the enzyme activity. Cyanide, sulfide, azide, and carbon monoxide all bind to cytochrome c oxidase, thus competitively inhibiting the protein from functioning, which results in chemical asphyxiation of cells. Methanol in methylated spirits is converted into formic acid, which also inhibits the same oxidase system.

Cytochrome c oxidase deficiency is a genetic condition that can affect several parts of the body, including the muscles used for movement (skeletal muscles), the heart, the brain, or the liver. Cytochrome c oxidase deficiency is caused by mutations in one of at least 14 genes. The genes associated with cytochrome c oxidase deficiency are involved in energy production in mitochondria through a process called oxidative phosphorylation. The gene mutations that cause cytochrome c oxidase deficiency affect an enzyme complex called cytochrome *c* oxidase, which is responsible for one of the final steps in oxidative phosphorylation.

Cytochrome c oxidase is made up of two large enzyme complexes called holoenzymes, which are each composed of multiple protein subunits. Three of these subunits are produced from mitochondrial genes; the rest are produced from nuclear genes. Many other proteins, all produced from nuclear genes, are involved in assembling these subunits into holoenzymes. A lack of functional cytochrome c oxidase disrupts the last step of oxidative phosphorylation, causing a decrease in energy production.

Researchers believe that impaired oxidative phosphorylation can lead to cell death by reducing the amount of energy available in the cell. Certain tissues that require large amounts of energy, such as the brain, muscles, and heart, seem especially sensitive to decreases in cellular energy. Cell death in other sensitive tissues may also contribute to the features of cytochrome c oxidase deficiency.

Defects involving genetic mutations altering cytochrome c oxidase (COX) functionality or structure can result in severe, often fatal metabolic disorders. Among the many classified mitochondrial diseases, those involving dysfunctional COX assembly are thought to be the most severe. The vast majority of COX disorders are linked to mutations in nuclear-encoded proteins referred to as assembly factors, or assembly proteins. These assembly factors contribute to COX1 structure and functionality, and are involved in several essential processes, including transcription and translation of mitochondrion-encoded subunits, processing of preproteins and membrane insertion, and cofactor biosynthesis and incorporation.

The origin of low cardiolipin content strongly correlated with the overproduction of inositol, an intrinsic phenotype of this mutation. Overall, our results show that adequate regulation of phospholipid synthesis is essential for the maintenance of mitochondrial function.

OBJECTIVES

The objective of this thesis includes

1. Molecular Modeling of protein cytochrome c oxidase subunit 1 of *Labeo rohita.*
2. Docking interaction study of cytochrome c oxidase with cardiolipin.
3. Role of cytochrome c oxidase in oxidative phosphorylation

REVIEW
OF
LITERATURE

REVIEW OF LITERATURE

Freshwater aquaculture in India is dominated by carp (*Labeo rohita, Catla catla and Cirrhinus mrigala*) (Cyprinidae), which contribute about 87% of the total freshwater production (ICLARM, 2001). *L. rohita* is a major carp, widely cultured throughout India owing to its high commercial value.. The compatibility of rohu with other carps like catla (*Catla catla)* and mrigal (*Cirrhinus mrigala*) made it an ideal candidate for carp polyculture systems. Its high growth potential, coupled with high consumer preference, have established rohu as the most important freshwater species cultured in India, Bangladesh and other adjacent countries in the region. *Labeo rohita* (rohu) is a major carp and an annual breeder. Based on observations of wild fish, it attains maturity in the end of second year of life (Jhingran and Pullin 1985). *Labeo rohita*, because of its higher growth rate (Reddy *et al.,* 2002), is also used in single species aquaculture. Programs on stock improvement s through breeding and selection conducted at the Central Institute of Freshwater Aquaculture, Bhubaneswar, India, on *Labeo rohita* showed a growth increase of 34% (Mahapatra *et al.*, 2000; Reddy *et al.,* 2002).

2.1-CYTOCHROME C OXIDASE AND ITS FUNCTION

The nature, specificity and functional consequences of lipid-protein interactions .The nature, specificity and functional consequences of lipid-protein interactions remain fundamental problems in membrane biology. To date, most investigators have focused their attention on the influence of membrane fluidity on protein function, with the precept in mind that the composition and physical state of local 'annular' lipids may play functional regulatory roles.

Cytochrome c oxidase is the principle terminal oxidase of high affinity oxygen in the aerobic metabolism of all animals, plants, yeasts, and some bacteria. Cytochrome c oxidase is a multisubunit metalloenzyme which has as its main function catalysis of the reduction ofmolecular oxygen to water: the enzyme also functions as a proton pump (Wikstrom, M *et al.*) and therefore contributes directly towards the synthesis of ATP via oxidative phosphorylation (Mitchell, P. (1966)). The electrons for these two processes, which are postulated to be coupled (Babcock, G. & Callahan, P. (1983)), are derived from cytochrome c, another metalloprotein.

$$4CytC(Fe^{2+}) + O_2 + 4H+ \xrightarrow{\text{CytC oxidase}} 4CytC\ (Fe^{3+}) + 2H_2O$$

$$ADP + PO_4^{2-} \longrightarrow ATP$$

Fig 1: Shows Scheme of the Reaction Resulting in the production of ATP and of Water Molecules from Oxygen

The enzyme is present in mitochondria of the more highly developed cells and in the cytoplasmic membrane of bacteria. This enzyme is probably unique in providing the energy for the cell by coupling of the electron transport through the cytochrome chain with the process of oxidative phosphorylation.

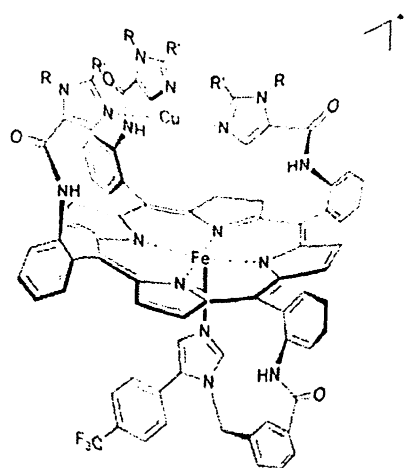


Fig.2: Shows the active site (heme a_3 and Cu_B) of cyrochrome c oxidase

Cytochrome c oxidase is located on the inner mitochondrial membrane that divides the mitochondrial matrix from the intermembrane space and it has been used for many years as a marker for this membrane. Cytochrome c oxidase is where over 90% of oxygen is consumed. The relationship between Cytochrome c oxidase activity and mitochondrial proteins, which provides a guide to understanding the mechanisms in primary mitochondrial disorders, has been determined by histochemistry (activity) and immunohistochemistry in serial sections. Though Cytochrome c oxidase is the terminal enzyme of the mitochondrial electron transport chain, without which oxidative metabolism cannot be carried to completion. The holoenzyme is made up of three mitochondrial-encoded and ten nuclear-encoded subunits in a 1:1

stoichiometry. The ten nuclear subunit genes are located in nine different chromosomes. The coordinated regulation of such a multi subunit, multichromosomal, bigenomic enzyme poses a challenge. It is especially so for neurons, whose mitochondria are widely distributed in extensive dendritic and axonal processes, resulting in the separation of the mitochondrial from the nuclear genome by great distances. Neuronal activity dictates COX activity that reflects protein amount, which, in turn, is regulated at the transcriptional level. All 13 COX transcripts are up- and downregulated by neuronal activity. The ten nuclear COX transcripts and those for Tfam and Tfbms important for mitochondrial COX transcripts are transcribed in the same transcription factory. Bigenomic regulation of all 13 transcripts is mediated by nuclear respiratory factors 1 and 2 (NRF-1 and NRF-2). NRF-1, in addition, also regulates critical neurochemicals of glutamatergic synaptic transmission, thereby ensuring the tight coupling of energy metabolism and neuronal activity at the molecular level in neurons.

2.2-COX1 AND CYTOCHROME C IN ETC

Cytochrome c (Cyt*c*) and cytochrome c oxidase (COX) catalyze the terminal reaction of the mitochondrial electron transport chain (ETC), the reduction of oxygen to water. This irreversible step is highly regulated, as indicated by the presence of tissue-specific and developmentally expressed isoforms, allosteric regulation, and reversible phosphorylations, which are found in both Cyt*c* and COX. The crucial role of the ETC in health and disease is obvious since it, together with ATP synthase, provides the vast majority of cellular energy, which drives all cellular processes. However, under conditions of stress, the ETC generates reactive oxygen species (ROS), which cause cell damage and trigger death processes. Regulation of Cyt*c* and COX1 with a focus on cell signaling pathways, including cAMP/protein kinase A and tyrosine kinase signaling has been also evolved. Based on the crystal structures it has been highlighted all identified phosphorylation sites on Cyt*c* and COX, and it present a new phosphorylation site. It concluded with a model that links cell signaling with the phosphorylation state of Cyt*c* and COX.

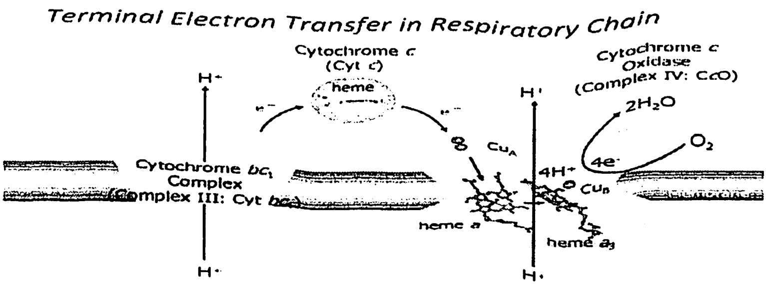


Fig 3: Shows terminal electron transfer in respiratory chain

This in turn regulates their enzymatic activities, the mitochondrial membrane potential, and the production of ATP and ROS. This model is discussed through two distinct pathologies, acute inflammation as seen in sepsis, where phosphorylation leads to strong COX inhibition followed by energy depletion, and ischemia/reperfusion injury, where hyperactive ETC complexes generate pathologically high mitochondrial membrane potentials, leading to excessive ROS production. Although operating at opposite poles of the ETC activity spectrum, both conditions can lead to cell death through energy deprivation or ROS-triggered apoptosis. This article is part of a Special Issue entitled: "Respiratory Oxidases".

2.3-COX1 ROLE IN ELECTRON TRANSPORT

Clearly, an ability of membrane protein to modulate the local structures of the lipid matrix would provide a new perspective on lipid-protein interactions and their functional consequences. For example, in addition to its well defined role in electron transport, this highly basic protein is known to bind electrostatically to negatively charged phospholipids to penetrate (partially) lipid bilayers (Shechter *et al.*)and monolayers (Morse *et al.*), to enhance the cation permeability of lipid bilayers (Deamer *et al.*), to decrease the transition temperatures and enthalpy of phospholipid gel-liquid crystalline transitions and can induce lateral phase separation in mixed lipid systems(Birell *et al.*, Brown *et al.*).

Because of cytochrome c oxidase implications for electron transport and programmed cell death, the interactions of cyt*c* with phospholipids, fatty acids, detergents, and other related molecules have been studied for half a century. The structures of cardiolipin (CL) thought to be the main lipid binding partner of cyt*c* – and other relevant lipid molecules.

Notably, the formation, stability, and function of individual respiratory chain complexes and of respiratory chain supercomplexes strongly relies on the presence of CLs in the membrane (Pfeiffer K. *et al.* Gohil V. *et al.,*). CLs bound to individual complexes have been reported by several structural studies on various organisms(Robinson N. C., Zborowski J. and Talbert L. H.,Arnarez C., Mazat J.-P., Elezgaray J., Marrink S.-J. and Periole X.), leading to hypotheses on their possible involvement in a proton uptake pathway and/or in assuring the structural integrity of individual complexes and supercomplexes (Arnarez C., Mazat J.-P., Elezgaray J., Marrink S.-J. and Periole X.)

Vijayasarathy *et al.* reported that CcO activity varies in heart, brain, kidney, and liver and in different heart compartments depending on the oxidative capacity and workload of the tissue.

2.4-CARDIOLIPIN ACTIVITIES

CL is required for full activity of cyt c oxidase and it has been suggested that cyt c-CL interactions may potentially recruit cyt c to this redox enzyme (Vik SB, *et al.*).The presence of CL is critical for maintaining mitochondrial function, structure, and membrane fluidity. Perturbation of CL synthesis alters mitochondrial bioenergetics, resulting in reduced membrane potential, inefficient coupling of respiration, and decreased ATP synthesis.

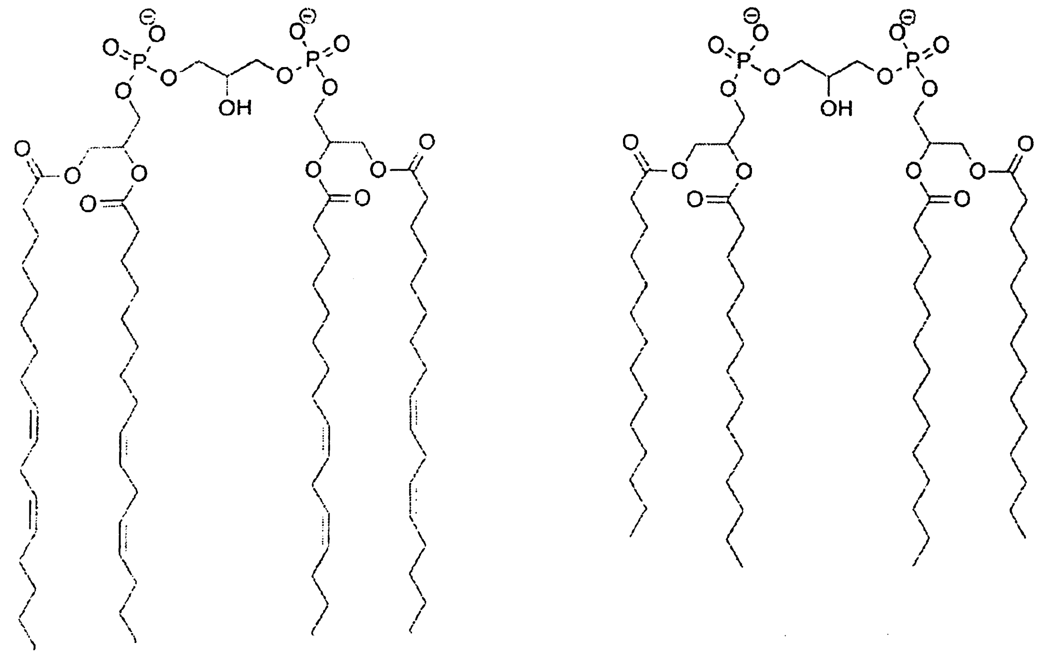


Fig 4: Shows Structure of cardiolipin (CL)

In the inner membrane, CL is tightly associated with several proteins in respiratory complexes I, III, and IV. CL is essential for the stability of respiratory chain supercomplexes that, in yeast, are composed of dimericubiquinol-cytochrome c oxidoreductase (complex III) and one or two complexes of cytochrome c oxidase (complex IV). Perturbation of CL synthesis due to mutations in the CL remodeling enzyme tafazzin causes the severe human genetic disorder. Although perturbation of CL synthesis due to loss of tafazzin leads to cardio- and skeletal myopathy, neutropenia, and growth retardation in BTHS, the clinical presentation of this disorder is highly variable, ranging from neonatal death to lack of clinical symptoms.These findings clearly point to a central role for cardiolipin homeostasis in programmed cell death.

Recent work has suggested that a DNA-based identification system, founded on the mitochondrial gene, cytochrome c oxidase subunit 1 (COI), can aid the resolution of this diversity. While past work has validated the ability of COI sequences to diagnose species in certain taxonomic groups, the present study extends these analyses across the animal kingdom. The results indicate that sequence divergences at COI regularly enable the discrimination of closely allied species in all animal phyla except the Cnidaria. This success in species diagnosis

reflects both the high rates of sequence change at COI in most animal groups and constraints on intraspecific mitochondrial DNA divergence arising, at least in part, through selective sweeps mediated via interactions with the nuclear genome.cox1 is often used as a DNA barcode to identify animal species. MT-CO1 gene sequence is suitable for this role because its mutation rate is often fast enough to distinguish closely related species and also because its sequence is conserved among conspecifics. Contrary to the primary objection raised by skeptics that MT-CO1 sequence differences are too small to be detected between closely related species, more than 2% sequence divergence has been detected between such organisms, proving the barcode effective.

MATERIALS AND METHODS

MATERIALS AND METHODOLOGY

3.1 MATERIALS

The following materials have been used to achieve the objectives

- NCBI
- Expasy/ProtParam
- Protein DataBank
- BLAST
- Modeller-9.12
- ChemSpider
- Autodock 4.2
- AutodockVina
- Pymol
- SOPMA
- ProtPararm
- ModRefiner
- LigPlot

3.1.1-NCBI

The National Center for Biotechnology Information (NCBI) is a part of the United States National Library of Medicine (NLM), a branch of the National Institutes of Health. The NCBI is located in Maryland Coordinates was founded in 1988 through legislation sponsored by Senator Claude Pepper. The NCBI houses genome sequencing data in GenBank and an index of biomedical research articles in PubMed Central and PubMed, as well as other information relevant to biotechnology. All these databases are available online through the Entrez search engine. The NCBI is directed by David Lipman, one of the original authors of the BLAST sequence alignment program and a widely respected figure in Bioinformatics. He also leads an intramural research program, including groups led by Stephen Altschul (another BLAST co-

author), David Landsman, and Eugene Koonin (a prolific author on comparative genomics). This is available at www.ncbi.nlm.nih.gov/.

3.1.2-PROTEIN DATA BANK (PDB)

Protein databank is a repository of three dimensional structures of biological macromolecules like DNA, RNA, Proteins and Protein-Protein complexes. Till 3rd June 2014100547structures are available. Any three dimensional structure can be downloaded from PDB using a PDB ID which is a four letter code of alpha numeric. This is available at http://www.pdb.org/.

3.1.3-BLAST

In Bioinformatics, Basic Local Alignment Search Tool, or BLAST is an algorithm for computing primary biological sequence information, such as the amino-acid sequences of different proteins or the nucleotides of DNA sequences. A BLAST search enables a researcher to compare a query sequence with a library or database of sequences, and identify library sequences that resemble the query sequence above a certain threshold. The BLAST program was designed by Eugene Myers, Stephen Altschul, Warren Gish, David J. Lipmen and Webb Miller at the NIH & is available at http://blast.ncbi.nlm.nih.gov/Blast.cgi.

3.1.4-MODELLER

3.1.4.1-MODELLER 9.12

Modeller is a computer program that models three-dimensional structures of proteins and their assemblies by satisfaction of spatial restraints. Modeller is most frequently used for homology or comparative protein structure modeling. The user provides an alignment of a sequence to be modeled with known related structures and Modeller will automatically calculate a model with all non-hydrogen atoms. It is available at http://salilab.org/modeller/.

Modeller implements comparative protein structure modeling by satisfaction of spatial restraints and can perform many additional tasks, including de novo modeling of loops in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc. Modeller can

also perform multiple comparison of protein sequences and/or structures, clustering of proteins, and searching of sequence databases. The program is used with a scripting language and does not include any graphics.

3.1.5-CHEMSPIDER

ChemSpider is a free chemical structure database providing fast access to over 30 million structures, properties and associated information. By integrating and linking compounds from more than 470 data sources, ChemSpider enables researchers to discover the most comprehensive view of freely available chemical data from a single online search. It is owned by the Royal Society of Chemistry. It is available at http://www.chemspider.com/.

ChemSpider builds on the collected sources by adding additional properties, related information and links back to original data sources. ChemSpider offers text and structure searching to find compounds of interest and provides unique services to improve this data by curation and annotation and to integrate it with users' applications.

3.1.6-AUTODOCK 4.2

AutoDock is a free suite of automated docking tools. It is designed to predict how small molecules, such as substrates or drug candidates, bind to a receptor of known 3D structure. AutoDock is an automated procedure for predicting the interaction of ligands with biomacromolecular targets. The motivation for this work arises from problems in the design of bioactive compounds, and in particular the field of computer-aided drug design. AutoDock uses Monte Carlo simulated annealing and Lamarckian genetic algorithm to create a set of possible conformations. LGA is used as a globaloptimizer and energy minimization as a local search method. Possible orientations are evaluated with AMBER force field model in conjunction with free energy scoring functions and a large set of protein-ligand complexes with known protein-ligand constants. AutoDock 4.2 actually consists of two main programs: auto dock performs the docking of the ligand to a set of grids describing the target protein; autogrid pre-calculates these grids. It is available athttp://autodock.scripps.edu/.

3.1.7-AUTO DOCK VINA

AutoDock Vina is a new open-source program for drug discovery, molecular docking and virtual screening, offering multi-core capability, high performance and enhanced accuracy and ease of use. AutoDock Vina significantly improves the average accuracy of the binding mode predictions compared to AutoDock 4, judging by our tests on the training set used in AutoDock 4 development. AutoDock Vina has been tested against a virtual screening benchmark called the Directory of Useful Decoys by the Watowich group, and was found to be "a strong competitor against the other programs, and at the top of the pack in many cases". It should be noted that all six of the other docking programs, to which it was compared, are distributed commercially. It is available at http://www.softpedia.com/get/Science-CAD/AutoDock-Vina.shtml.

3.1.8-PYMOL

PyMOL is an open-source, user-sponsored, molecular visualization system created by Warren Lyford DeLano and commercialized by DeLano Scientific LLC, which is a private software company dedicated to creating useful tools that become universally accessible to scientific and educational communities. It can produce high quality 3D images of small molecules and biological macromolecules, such as proteins. It is available at http://www.pymol.org/.

3.1.9-SOPMA(Self-Optimized Prediction Method with Alignment)

SOPMA is an improvement of SOPM method. These methods are based on the homologue method of Levin et al. The improvement takes place in the fact that SOPMA takes into account information from an alignment of sequences belonging to the same family. If there are no homologous sequences the SOPMA prediction is the SOPM one new method called the self-optimized prediction method (SOPM) has been described to improve the success rate in the prediction of the secondary structure of proteins. It predicts all the sequences of a set of aligned proteins belonging to the same family. This improved SOPM method (SOPMA) correctly predicts 69.5% of amino acids for a three-state description of the secondary structure (alpha-helix, beta-sheet and coil) in a whole database containing 126 chains of non-homologous (less than 25% identity) proteins.

3.1.10-PSIPRED

PSIPRED (Psi-blast based secondary structure prediction) is a technique used to investigate protein structure. PSIPRED employs neural network, machine learning methods in its algorithm. It is a server-side program, featuring a website serving as a front-end interface, which can predict a protein's secondary structure (beta sheets, alpha helices and coils) from the primary sequence.

3.1.11- LIGPLOT

LIGPLOT is a computer program that generates schematic 2-D representations of protein-ligand complexes from standard Protein Data Bank file input. The output is a colour, or black-and-white, PostScript file giving a simple and informative representation of the intermolecular interactions and their strengths, including hydrogen bonds, hydrophobic interactions and atom accessibilities. The program is completely general for any ligand and can also be used to show other types of interaction in proteins and nucleic acids.

3.1.12-PROTPARAM

It is a tool which allows the computation of various physical and chemical parameters for a given protein stored in Swiss-Prot or TrEMBL or for a user entered sequence. The computed parameters include the molecular weight, theoretical pI, amino acid composition, atomic composition, extinction coefficient, estimated half-life, instability index, aliphatic index and grand average of hydropathicity (GRAVY).

3.1.13-ENERGY MINIMIZATION

3..1.13.1-MODREFINER

ModRefiner is an algorithm for atomic-level, high-resolution protein structure refinement, which can start from either C-alpha trace, main-chain model or full-atomic model. Both side-chain and backbone atoms are completely flexible during structure refinement simulations, where conformational search is guided by a composite of physics- and knowledge-based force field. ModRefiner has an option to allow for the assignment of a second structure which will be used as a reference to which the refinement simulations are driven. One aim of ModRefiner is to draw the initial starting models closer to their native state, in terms of hydrogen bonds, backbone topology and side-chain positioning. It also generates significant improvement in

physical quality of local structures. The stand alone program also supports ab initio full-atomic relaxation, where the refined model is not restrainted by the initial model or the reference model.

3.2-METHODOLOGY

- Primary sequence of cytochrome c oxidase extracted was from NCBI.
- Computation of physiochemical properties of target protein using PROTPARAM tool.
- Searching of homologous structure using BLAST algorithm.
- Best template choosen on the basis of e-value and identity score.
- Prediction of three dimensional structure of target protein(cytochrome c oxidase subunit 1) using modeller9.12.
- Structure evaluation of model by SAVes server through Ramachandran Plot.
- Best predicted model selection by superimposition by using pymol tool.
- The ligand compound was extracted from ChemSpider.
- The ligand molecule was prepared for Docking using Modeller 9.12
- Both target and template was imported to Autodock 4.2.
- Target and ligand was docked using AutodockVina.
- The best interaction between the protein and ligand was visualized in using Pymol.

3.2.1HOMOLOGOUS STRUCTURE PREDICTION

3.2.1.1-MODELLER9.12

Latest version of modeller was downloaded from the URL: http://salilab.org/modeller/download_installation.html.

MODELLER is used for homology or comparative modeling of protein three-dimensional structures .The user provides an alignment of a sequence to be modelled with known related structures and MODELLER automatically calculates a model containing all non-hydrogen atoms. MODELLER implements comparative protein structure modeling by satisfaction of spatial restraints and can perform many additional tasks, including de novo modeling of loops

in protein structures, optimization of various models of protein structure with respect to a flexibly defined objective function, multiple alignment of protein sequences and/or structures, clustering, searching of sequence databases, comparison of protein structures, etc.

MODELLER is written in Fortran-90 and is run on UNIX, Windows, Linux and Apple systems. It is available free of charge to academic non-profit institutions from the MODELLER website (http://salilab.org/modeller/).

3.2.2-GRAPHICAL INTERFACE:

MODELLER runs via scripts written in the Python language, and does not provide any graphical interface. A graphical interface to MODELLER is available as part of Accelrys' Insight II, and Discovery Studio interactive molecular modeling programs, which also contain many other tools for protein modeling and structural analysis. These programs facilitate preparation of input files for MODELLER (e.g. alignment file) as well as an analysis of the results (e.g., an evaluation of the models).

For the required purpose latest version of modeller-modeller9.10 was downloaded for windows operating system by clicking on the Download & Installation option on the homepage of the Modeller.

Then, there was a link register for license on the web page was clicked and on that resulting page several required information like name, email id, etc are given to have a license. The team, maintaining the modeller online sent the required password to the given e-mail id.

The modeller icon was clicked after agreement of required declaration the provided password was given for the modeler installation. After successful installation of the Modeller, it works on a command mode from the start menu.

3.2.3-HOMOLOGY/COMPARATIVE MODELLING METHOD

3.2.3.1-Homology modelling

All homology-modeling methods consist of the following four steps:

(i) Template selection;

(ii) Target template alignment;

(iii) Model building;

(iv) Evaluation.

These steps can be iteratively repeated, until a satisfying model structure is achieved.

Before going for template selection one has to build .ali file of the target sequence which is accepted by the modeller. The format for the .ali file is like

```
>P1;cytoc

LVFGAWAGMVGTALSLLIRAELSQPGSLLGDDQIYNVIVTAHAFVMIFFMVMPILIGGF
GNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEAGAGTGWTVYPPLAGL
AHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKPPAISQYQTPLFVWSVLVTAVLLLL
SLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHL*
```

The first line contains the sequence code, in the format ">P1; code". The second line with ten fields separated by colons generally contains information about the structure file, if applicable. Only two of these fields are used for sequences, "sequence"(indicating that the file contains a sequence without known structure) and "TARGET" (the model file name). The rest of the file contains the sequence of TARGET, with "*" marking its end. The standard one -letter amino acid codes are used.

Template selection

This step involves in searching of a template or template by any similarity search program.

A BLASTP search with default parameters were performed against the Brook Heaven Protein Data Bank (PDB) to find suitable templates for homology modeling of growth hormone protein of L. rohita. From the BLASTP result page a .pdb file (3hhr.pdb) with more than 80% query coverage with the query sequence was selected and further downloaded from Protein Data Bank (the text pdb file of the 3D structure) and put in a same folder of execution.

The required file format is:-

```
From modeller import *
env = environ()
aln = alignment(env)
```

```
mdl = model(env, file='1occ', model_segment=('FIRST:A','LAST:A'))
aln.append_model(mdl, align_codes='1occA', atom_files='1occ.pdb')
aln.append(file='cytoc.ali', align_codes='cytoc')
aln.align2d()
aln.write(file='cytoc-1occ.ali', alignment_format='PIR')
aln.write(file='cytoc-1occ.pap', alignment_format='PAP')
```

To run this file, the needed command is:-

>mod9.12 align2d.py

3 files were generated (one PAP file, one Text file and one ALI file)

Model building

To generate the core of the model, the backbone atom positions of the template structure are averaged. The templates are thereby weighted by their sequence similarity to the target sequence, while significantly deviating atom positions are excluded. The template coordinates cannot be used to model regions of insertions or deletions in the target-template alignment. To generate those parts, an ensemble of fragments compatible with the neighboring stems is constructed using constraint space programming (CSP). The best loop is selected using a scoring scheme, which accounts for force field energy, steric hindrance and favorable interactions like hydrogen bond formation. If no suitable loop can be identified, the flanking residues are included to the rebuilt fragment to allow for more flexibility. In cases where CSP does not give a satisfying solution and for loops above 10 residues, a loop library derived from experimental structures is searched to find compatible loop fragments.

The file is model_single.py

The required file format is :- 18

```
from modeller import *
from modeller.automodel import *
env = environ()
a = automodel(env, alnfile='cytoc-1occ.ali',
knowns='1occA', sequence='cytoc',
```

```
assess_methods=(assess.DOPE, assess.GA341))
a.starting_model = 1
a.ending_model = 5
a.make()
```

To run this file, the command was > mod9.12 model_single.py

36 files were generated including 5 PDB files , and one text file. In the bottom of the text file the best model could be predicted from the pdb file having the lowest DOPE score.

```
>> Summary of successfully produced models:
Filename                     molpdf       DOPE score     GA341 score
----------------------------------------------------------------------------------------------------
cytoc.B99990001.pdb       1396.75598   -23637.42578     1.00000
cytoc.B99990002.pdb       1324.00586   -23543.67773     1.00000
cytoc.B99990003.pdb       1331.18347   -23252.16602     1.00000
cytoc.B99990004.pdb       1413.17212   -23373.54492     1.00000
cytoc.B99990005.pdb       1399.28882   -23739.39648     1.00000
```

From there above list the best structure was predicted from the cytoc.B9990005.pdb(due to lowest DOPE score).

3.2.4-MULTI TEMPLATE MODELING

By use of use of multiple templates we can build precise and accurate models.To run these following files we need templates(1occ,1v54,1qle) files(PDB files) in one folder with these python files.

Salign.py file

The reads in all of the sequences from PDB files (using the append_model command), and then uses salign multiple times, to generate an initial rough alignment and then improve upon it by using more information. The alignment is then written out in both PIR and PAP formats, and a quality score is calculated by calling salign one more time.

```
from modeller import *
log.verbose()
env = environ()
env.io.atom_files_directory = './:../atom_files/'
aln = alignment(env)
for (code, chain) in (('1occ', 'A'), ('1qle', 'A'), ('1v54', 'A')):
mdl = model(env, file=code, model_segment=('FIRST:'+chain, 'LAST:'+chain))
aln.append_model(mdl, atom_files=code, align_codes=code+chain)
for (weights, write_fit, whole) in (((1., 0., 0., 0., 1., 0.), False, True),
                                    ((1., 0.5, 1., 1., 1., 0.), False, True),
                                    ((1., 1., 1., 1., 1., 0.), True, False)):
aln.salign(rms_cutoff=3.5, normalize_pp_scores=False,
rr_file='$(LIB)/as1.sim.mat', overhang=30,
           gap_penalties_1d=(-450, -50),
           gap_penalties_3d=(0, 3), gap_gap_score=0, gap_residue_score=0,
dendrogram_file='fm00495.tree',
alignment_type='tree', # If 'progresive', the tree is not
                                    # computed and all structues will be 20
                                    # aligned sequentially to the first
feature_weights=weights, # For a multiple sequence alignment only
                                    # the first feature needs to be non-zero
improve_alignment=True, fit=True, write_fit=write_fit,
write_whole_pdb=whole, output='ALIGNMENT QUALITY')
aln.write(file='fm00495.pap', alignment_format='PAP')
aln.write(file='fm00495.ali', alignment_format='PIR')
```

```
aln.salign(rms_cutoff=1.0, normalize_pp_scores=False,
rr_file='$(LIB)/as1.sim.mat', overhang=30,
        gap_penalties_1d=(-450, -50), gap_penalties_3d=(0, 3),
gap_gap_score=0, gap_residue_score=0, dendrogram_file='1is3A.tree',
alignment_type='progressive', feature_weights=[0]*6,
improve_alignment=False, fit=False, write_fit=True,
write_whole_pdb=False, output='QUALITY')
```

Align2d.mult file

Now we need to align our query sequence to the template structures. For that task we again use the salign() command (file `align2d_mult.py').

```
from modeller import *
log.verbose()
env = environ()
env.libs.topology.read(file='$(LIB)/top_heav.lib')
# Read aligned structure(s):
aln = alignment(env)
aln.append(file='fm00495.ali', align_codes='all')
aln_block = len(aln) 21
# Read aligned sequence(s):
aln.append(file='cytoo.ali', align_codes='cytoo')
# Structure sensitive variable gap penalty sequence-sequence alignment:
aln.salign(output='', max_gap_length=20,
gap_function=True,  # to use structure-dependent gap penalty
alignment_type='PAIRWISE', align_block=aln_block,
feature_weights=(1., 0., 0., 0., 0., 0.), overhang=0,
```

```
        gap_penalties_1d=(-450, 0),
        gap_penalties_2d=(0.35, 1.2, 0.9, 1.2, 0.6, 8.6, 1.2, 0., 0.),
similarity_flag=True)
aln.write(file='cytoo-mult.ali', alignment_format='PIR')
aln.write(file='cytoo-mult.pap', alignment_format='PAP')
```

Model_mult file

Next, we build the new model for the TvLDH target sequence based on the alignment against the multiple templates using the `model_mult.py' file:

```
from modeller import *
from modeller.automodel import *
env = environ()
a = automodel(env, alnfile='cytoo-mult.ali',
knowns=('1occA','1qleA','1v54A'), sequence='cytoo')
a.starting_model = 1
a.ending_model = 5
a.make()
```

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Evaluate model file

Finally, we use the DOPE potential to evaluate the new model coordinates using the `evaluate_model.py' file:

```
from modeller import *
from modeller.scripts import complete_pdb
log.verbose()    # request verbose output
env = environ()
env.libs.topology.read(file='$(LIB)/top_heav.lib') # read topology
env.libs.parameters.read(file='$(LIB)/par.lib') # read parameters
# read model file
mdl = complete_pdb(env, 'cytoo.B99990001.pdb')
```

```
# Assess all atoms with DOPE:
s = selection(mdl)
s.assess_dope(output='ENERGY_PROFILE NO_REPORT', file='cytoo.profile',
normalize_profile=True, smoothing_window=15)
```

AutoDock Vina

AutoDock Vina is a new generation of docking software from the Molecular Graphics Lab. It achieves significant improvements in the average accuracy of the binding mode predictions, while also being up to two orders of magnitude faster than AutoDock 4. Because the scoring functions used by AutoDock 4.2 and AutoDock Vina are different and inexact, on any given problem, either program may provide a better result.

For its input and output, Vina uses the same PDBQT molecular structure file format used by AutoDock. PDBQT files can be generated (interactively or in batch mode) and viewed using MGLTools. Other files, such as the AutoDock and AutoGrid parameter files (GPF, DPF) and grid map files are not needed.

Vina avoids imposing artificial restrictions, such as the number of atoms in the input, the number of torsions, the size of the search space, the exhaustiveness of thesearch,etc.The invariance of the covalent bond lengths is automatically verified in the output structures.

Docking

Docking includes following steps :

1. Protein Preparation
2. Ligand Preparation
3. Grid Formation

Protein Preparation

File – Read molecule

This loads a Molecule named 'protein' into ADT. The bonds between bonded atoms are represented as lines; while non bonded atoms, such as metal ions and oxygen atoms of water

molecules, are shown as small squares. The non-bonded atoms here in 'protein' are the oxygen atoms of waters that were present in the crystal structure. These waters will be removed later.

Edit – Hydrogen – Add

Choose to add Polar Only using Method no Bond Order with yes to renumbering. Click OK to add the polar hydrogens. 330hydrogen atoms are added to protein.

File – save –write pdb

Choose Sort Nodes but leave all the other check-buttons off so that no CONECT records are written. Click on OK to write the file.

Ligand file preparation for Autodock :

Ligand – Input – Open

Ligand – Torsion tree – Detect Root

Ligand – Tortion tree – Choose Torsions

Ligand – Torsion tree – Set no of Torsions

Ligand – Output – Save as PDBQT

Opens a file browser allowing you to enter a name. Type in 'ind.out.pdbq' and click Save.

Then a configuration file was prepared for Grid Box setting around the receptor molecule named as conf.txt.

The contain of the configuration file is as follows:

receptor = protein.pdbqt

ligand = ligand.pdbqt

out = out.pdbqt

center_x = 40.755

center_y = 40.705

center_z = 40.671

size_x = 40

size_y = 40

size_z = 40

exhaustiveness = 8

RESULTS
AND
DISCUSSION

CHAPTER-IV

RESULTS AND DISCUSSION

Table 1-:The cytochrome c oxdase sequence taken from NCBI.

>gi|194173367|gb|ACF34406.1| cytochrome c oxidase subunit 1 [*Labeo rohita*]
LVFGAWAGMVGTALSLLIRAELSQPGSLLGDDQIYNVIVTAHAFVMIFFMVMPILIGGF
GNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEAGAGTGWTVYPPLAGN
LAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKPPAISQYQTPLFVWSVLVTAVLLL
LSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHL

Table2: Shows the hits of Blast P for Cytochrome c oxidase sequence

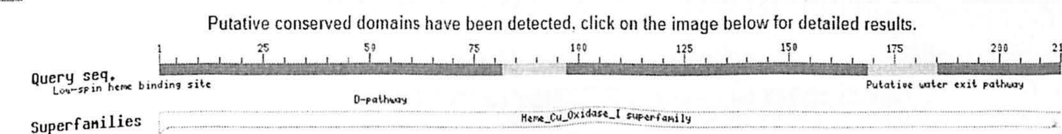
Acc. No.	Description	Identity	Query Coverage	E-value
1OCC A	Chain A, Structure Of Bovine Heart Cytochrome C Oxidase At The Fully Oxidized State [Bostaurus]	95%	100%	8e-123
1V54 A	Chain A, Bovine Heart Cytochrome C Oxidase At The Fully Oxidized State [Bostaurus]	95%	100%	le-122
1QLE A	Chain A, Cryo-structure Of The Paracoccus Denitrificans Four-subunit Cytochrome C Oxidase In The Completely Oxidized State Complexed With An Antibody Fv Fragment	50%	96%	3e-67
1ARI A	Chain A, Structure At 2.7 Angstrom Resolution of The Paracoccus Denitrificans Two-Subunit Cytochrome C Oxidase Complexed With An Antibody Fv Fragment	50%	96%	5e-67
3OM3 A	Chain A, Catalytic Core Subunits (I And Ii) of Cytochrome C Oxidase from Rhodobacter Sphaeroides With K362m Mutation	47%	98%	3e-66

Score	Expect	Method	Identities	Positives	Gaps
362 bits(928)	8e-123	Compositional matrix adjust.	205/215(95%)	212/215(98%)	0/215(0%)

```
Query  1    LVFGAWAGMVGTALSLLIRAELSQPGSLLGDDQIYNVIVTAHAFVMIFFMVMPILIGGFG  60
            L+FGAWAGMVGTALSLLIRAEL QPG+LLGDDQIYNV+VTAHAFVMIFFMVMPI+IGGFG
Sbjct  20   LLFGAWAGMVGTALSLLIRAELGQPGTLLGDDQIYNVVVTAHAFVMIFFMVMPIMIGGFG  79

Query  61   NWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEAGAGTGWTVYPPLAGNLAHA  120
            NWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASS VEAGAGTGWTVYPPLAGNLAHA
Sbjct  80   NWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSMVEAGAGTGWTVYPPLAGNLAHA  139

Query  121  GASVDLTIFSLHLAGVSSILGAINFITTTINMKPPAISQYQTPLFVWSVLVTAVLLLLSL  180
            GASVDLTIFSLHLAGVSSILGAINFITT INMKPPA+SQYQTPLFVWSV++TAVLLLLSL
Sbjct  140  GASVDLTIFSLHLAGVSSILGAINFITTIINMKPPAMSQYQTPLFVWSVMITAVLLLLSL  199

Query  181  PVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHL  215
            PVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHL
Sbjct  200  PVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHL  234
```

Fig 5: BLAST alignment between Query and Template sequence

Show Conserved Domains



Distribution of 11 Blast Hits on the Query Sequence

Mouse over to see the defline, click to show alignments

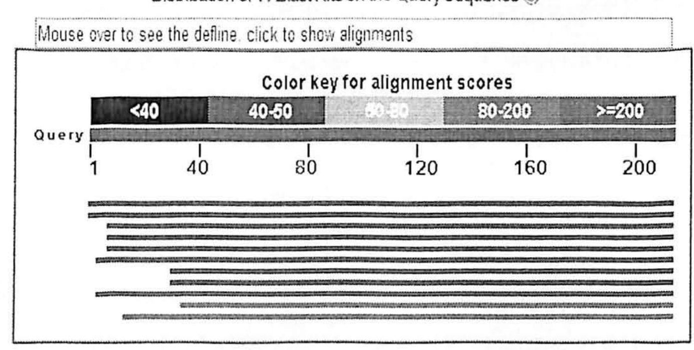


Fig 6: BLAST Result Page

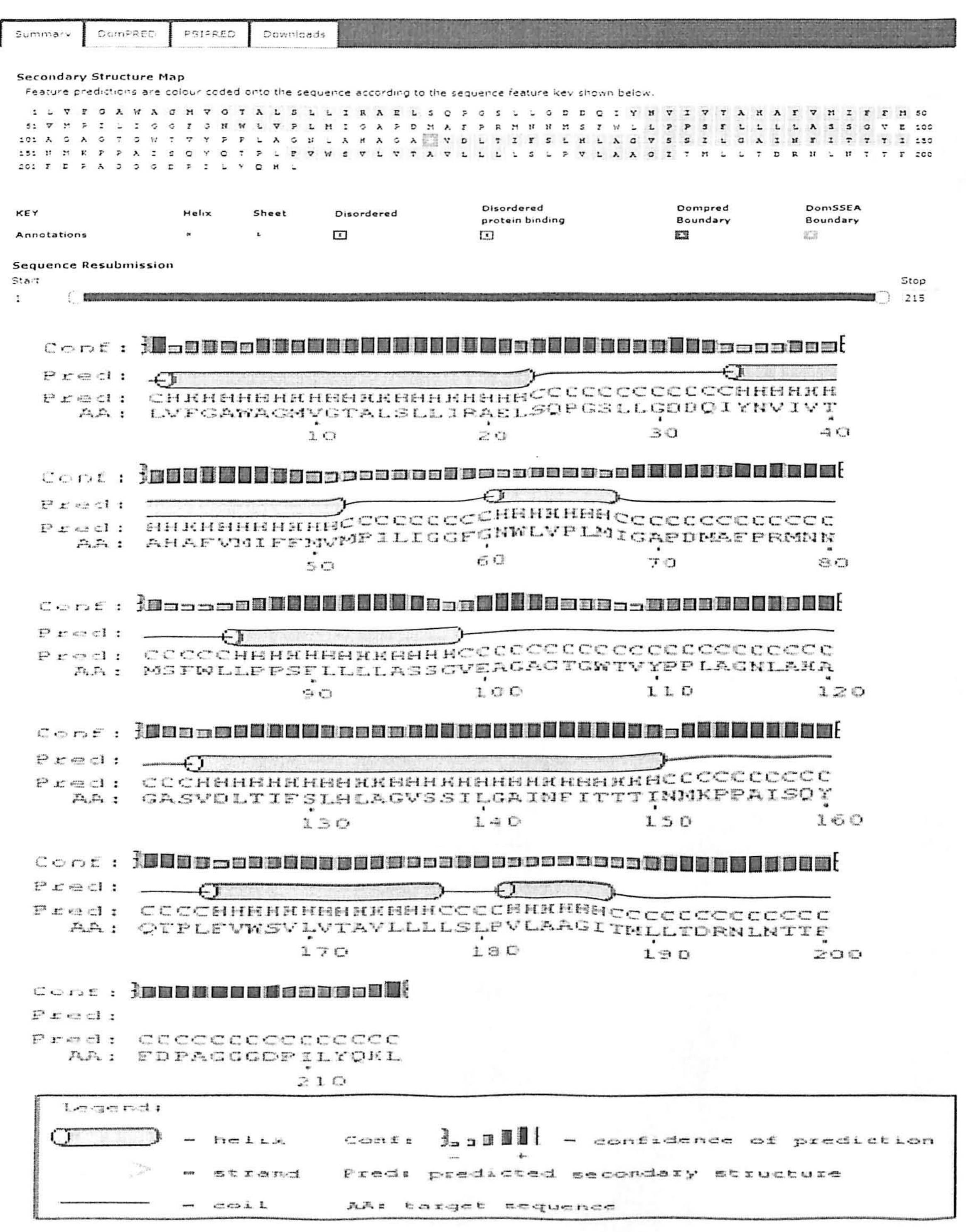


Fig 7 :PSIPRED result page

ClustalW2

Input form | Web services | Help & Documentation

Tools > Multiple Sequence Alignment > ClustalW2

Results for job clustalw2-I20140605-072334-0830-16237254-pg

Alignments | Result Summary | Guide Tree | Phylogenetic Tree | Submission Details

Download Alignment File | Show Colors | Send to ClustalW2_Phylogeny

```
CLUSTAL 2.1 multiple sequence alignment


gi1      -------------------LVFGANAGMVGTALSLLIRAELSQPGSLLGDDQIYNVIVTA 41
gi2      MFINRWLFSTNHKDIGTLYLLFGANAGMVGTALSLLIRAELGQPGTLLGDDQIYNVVVTA 60
                            *:********************.***:**********:***

gi1      HAFVMIFFMVMPILIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSGVEA 101
gi2      HAFVMIFFMVMPIMIGGFGNWLVPLMIGAPDMAFPRMNNMSFWLLPPSFLLLLASSMVEA 120
         *************:****************************************** ***

gi1      GAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAINFITTTINMKPPAISQYQ 161
gi2      GAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGVSSILGAINFITTIINMKPPAMSQYQ 180
         *********************************************** *******:****

gi1      TPLFVWSVLVIAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPILYQHL------ 215
gi2      TPLFVWSVMITAVLLLLSLPVLAAGITMLLTDRNLNITFFDPAGGGDPILYQHLFWFFGH 240
         ********::********************************************

gi1      ------------------------------------------------------------
gi2      PEVYILILPGFGMISHIVTYYSGKKEPFGYMGMVWAMMSIGFLGFIVWAHHMFTVGMDVD 300

gi1      ------------------------------------------------------------
gi2      TRAYFTSATMIIAIPTGVKVFSWLATLHGGNIKWSPAMMWALGFIFLFTVGGLTGIVLAN 360

gi1      ------------------------------------------------------------
gi2      SSLDIVLHDTYYVVAHFHYVLSMGAVFAIMGGFVHWFPLFSGYTLNDTWAKIHFAIMFVG 420

gi1      ------------------------------------------------------------
gi2      VNMTFFPQHFLGLSGMPRRYSDYPDAYTMWNTISSMGSFISLTAVMLMVFIIWEAFASKR 480

gi1      ----------------------------------
gi2      EVLTVDLTTTNLEWLNGCPPPYHTFEEPTYVNLK 514
```

Fig8: ClustalW Result Page

```
         10        20        30        40        50        60        70
          |         |         |         |         |         |         |
LVFGAWAGMVGTALSLLIRAELSQPGSLLGDDQIYNVIVTAHAFVMIFFMVMPILIGGFGNWLVPLMIGA
eeehhhhhhhhhhhhhhhhhhh  tt      thhhhheehhtthheeeeeehhhhhhhhhhhhe hheet
PDMAFPRMNNMSFWLLPPSFLLLLASSGVEAGAGTGWTVYPPLAGNLAHAGASVDLTIFSLHLAGVSSIL
      hhhhhheeee thhheeehhhh          eee           tt   eeeeeehhhhhhhhh
GAINFITTTINMKPPAISQYQTPLFVWSVLVTAVLLLLSLPVLAAGITMLLTDRNLNTTFFDPAGGGDPI
hhhhhhhhhh   tt        eehhhhhhhhhheee   hhhhhhheeeh tt   eee tttt    e
LYQHL
eeehh

Sequence length :    215

SOPMA :
   Alpha helix          (Hh) :    96 is   44.65%
   3_10 helix           (Gg) :     0 is    0.00%
   Pi helix             (Ii) :     0 is    0.00%
   Beta bridge          (Bb) :     0 is    0.00%
   Extended strand      (Ee) :    44 is   20.47%
   Beta turn            (Tt) :    17 is    7.91%
   Bend region          (  ) :     0 is    0.00%
   Random coil          (  ) :    58 is   26.98%
   Ambigous states      (?)  :     0 is    0.00%
   Other states              :     0 is    0.00%
```

50 100 150

50 100 150

```
Parameters :
   Window width           :  17
   Similarity threshold   :  8
   Number of states       :  4
```

Fig 9: SOPMA result for cytochrome c oxidase subunit 1, partial (mitochondrion) (Labeo rohita)

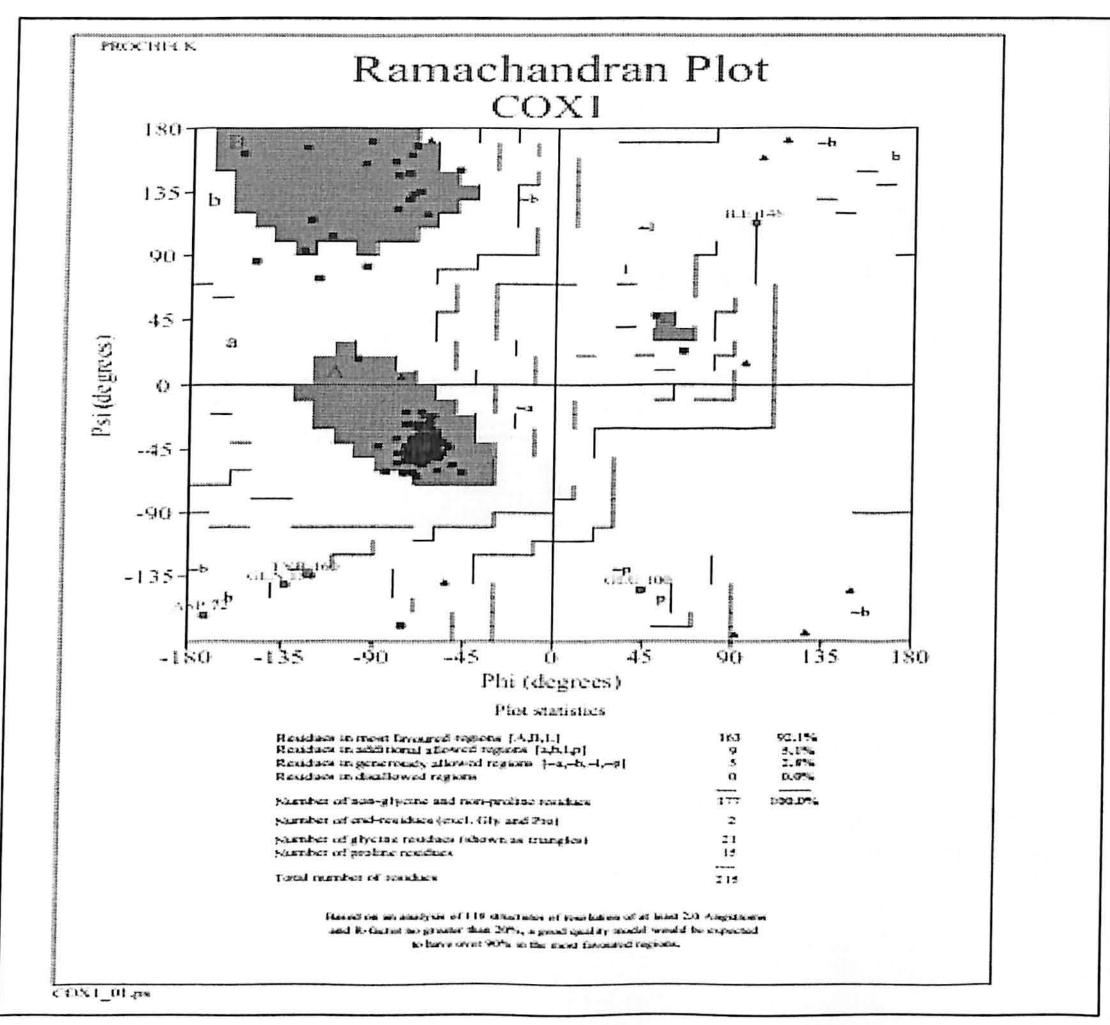


Fig.10: Shows Ramachandran Plot for predicted Cytochrome c oxidase Subunit 1 Labeo rohita structure.

Table 3 :Ramachandran Plot Statistics details

Sl No.	**Plot Statistics**	*No. of Residues*	*Percentage(%) of Residues*
1.	Most favoured regions[ABL]	*163*	*92.1%*
2.	Additional allowed regions[a,b,l,p]	*9*	*5.1%*
3.	Generously allowed regions[~a,~b,~l,~p]	*5*	*2.8%*
4.	Disallowed regions	*0*	*0.0%*

Table 4: Shows the Energy Scores of cytochrome c oxidase subunit 1(COX1) sequence

Filename	Molpdf	DOPE SCORE	GA341 SCORE
cytoc.B99990001.pdb	1396.75598	-23637.42578	1.00000
cytoc.B99990002.pdb	1324.00586	-23543.67773	1.00000
cytoc.B99990003.pdb	1331.18347	-23252.16602	1.00000
cytoc.B99990004.pdb	1413.17212	-23373.54492	1.00000
cytoc.B99990005.pdb	**1399.28882**	**-23739.39648**	1.00000

PROTPARAM RESULT

Number of amino acids: 215

Molecular weight: 22800.9

Theoretical pI: 4.94

Amino acid composition

Ala (A) 22 10.2%
Arg (R) 3 1.4%
Asn (N) 9 4.2%
Asp (D) 7 3.3%
Cys (C) 0 0.0%
Gln (Q) 5 2.3%
Glu (E) 2 0.9%
Gly (G) 21 9.8%
His (H) 4 1.9%
Ile (I) 15 7.0%
Leu (L) 35 16.3%
Lys (K) 1 0.5%
Met (M) 10 4.7%
Phe (F) 13 6.0%
Pro (P) 15 7.0%
Ser (S) 14 6.5%
Thr (T) 14 6.5%
Trp (W) 5 2.3%
Tyr (Y) 4 1.9%
Val (V) 16 7.4%
Pyl (O) 0 0.0%
Sec (U) 0 0.0%
(B) 0 0.0%
(Z) 0 0.0%
(X) 0 0.0%

Total number of negatively charged residues (Asp + Glu): 9

Total number of positively charged residues (Arg + Lys): 4

Instability index

The instability index (II) is computed to be 25.78

This classifies the protein as stable.

Aliphatic index: 122.51

Grand average of hydropathicity (GRAVY): 0.879

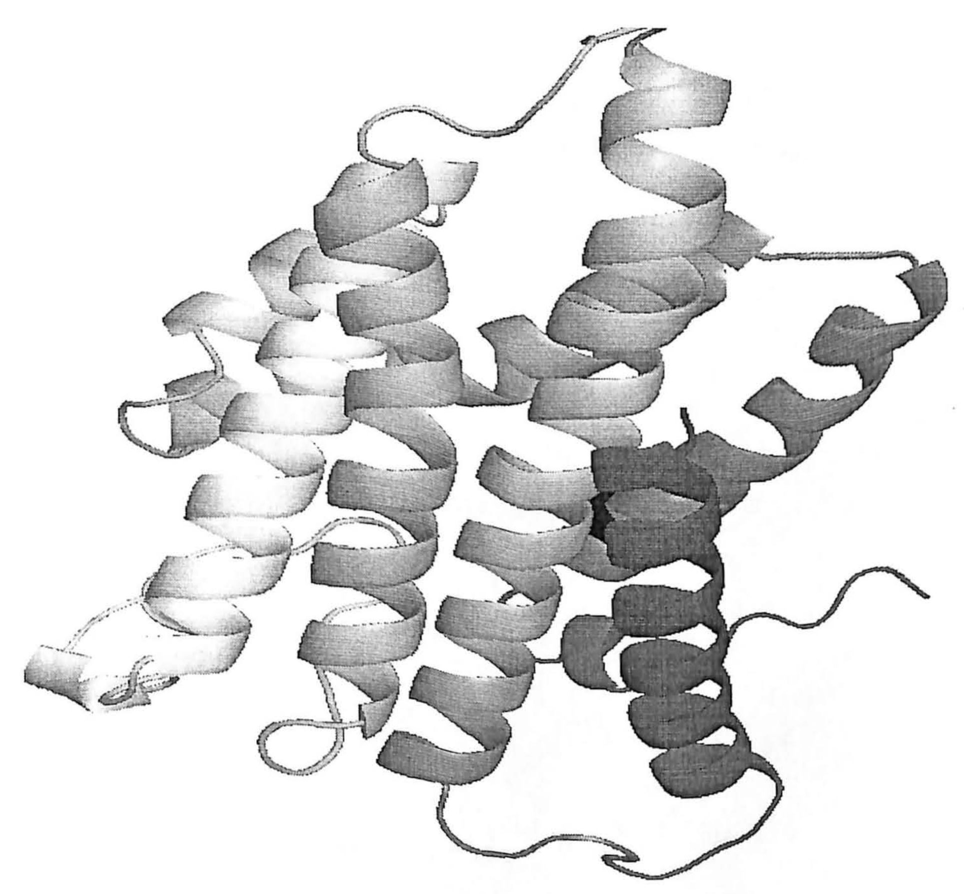


Fig 11 : Shows the best model structure (cytoc.B99990005.pdb) resulted using modeller9.12on the basis of DOPE score in Pymol

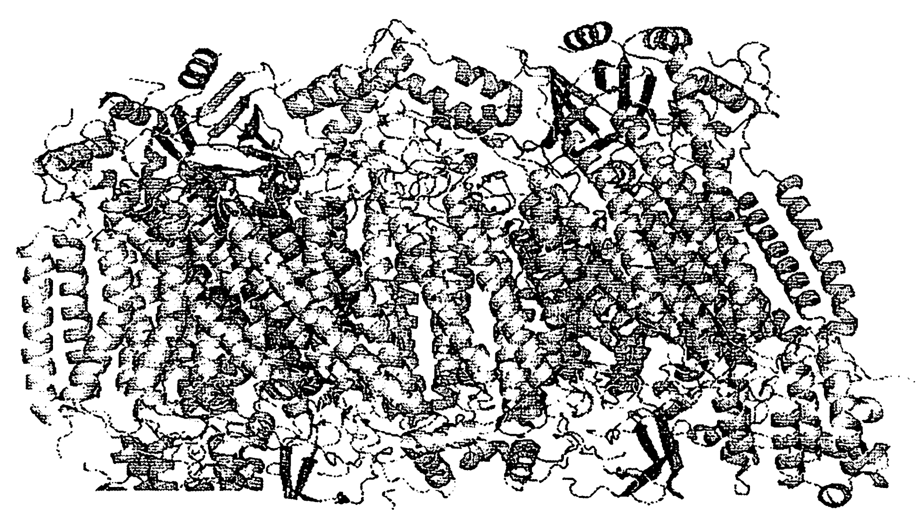


Fig12: Shows the superimposition of best model structure of COX1 shown in red color(cytoc.B99990005.pdb) with template (1OCC in blue color) in Pymol

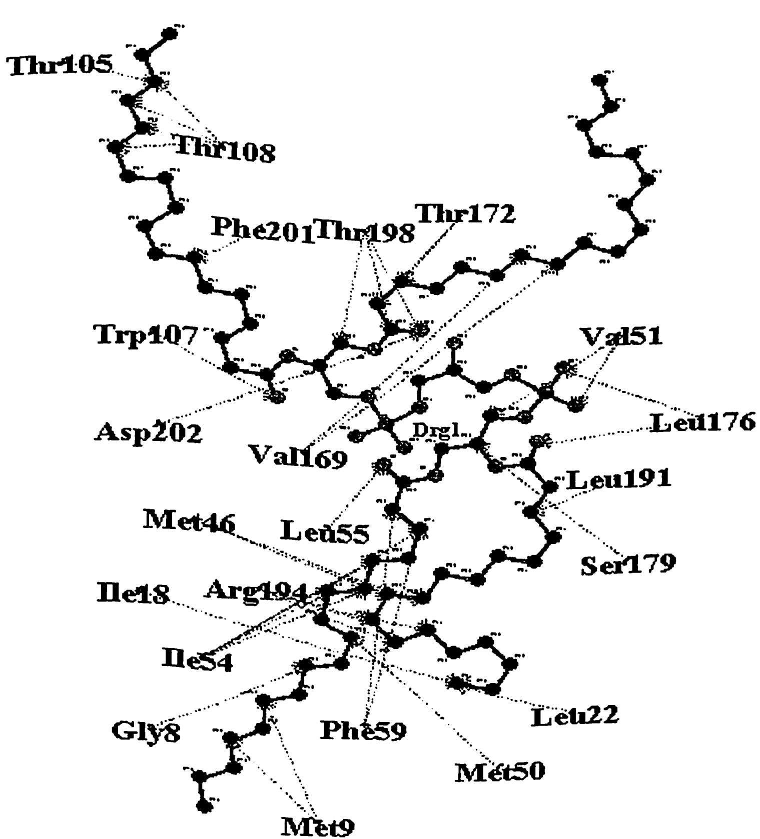


Fig 13: 2D ligand-protein interaction in LigPlot

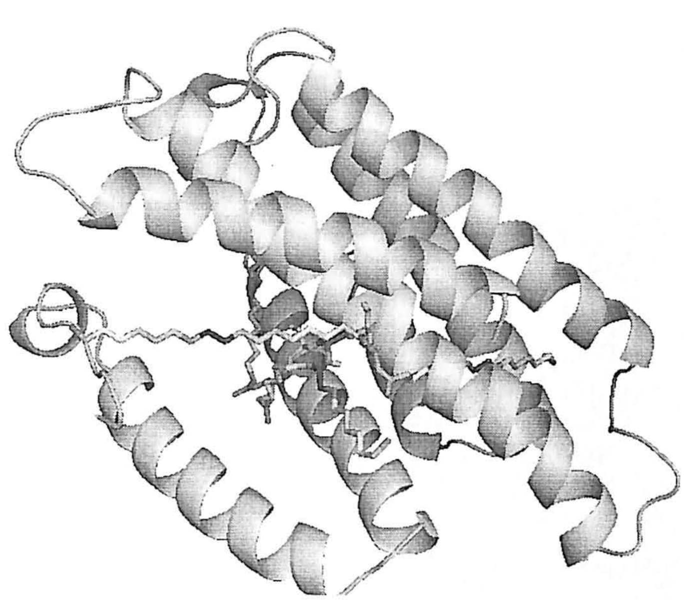


Fig14: Shows Docking interaction result between COX 1&cardiolipin in Pymol

```
C:\Vina>vina --config conf.txt --log log.txt
#################################################################
# If you used AutoDock Vina in your work, please cite:          #
#                                                               #
# O. Trott, A. J. Olson,                                        #
# AutoDock Vina: improving the speed and accuracy of docking    #
# with a new scoring function, efficient optimization and       #
# multithreading, Journal of Computational Chemistry 31 (2010)  #
# 455-461                                                       #
#                                                               #
# DOI 10.1002/jcc.21334                                         #
#                                                               #
# Please see http://vina.scripps.edu for more information.      #
#################################################################

WARNING: The search space volume > 27000 Angstrom^3 (See FAQ)
Detected 4 CPUs
Reading input ... done.
Setting up the scoring function ... done.
Analyzing the binding site ... done.
Using random seed: -1625580800
Performing search ...
0%   10   20   30   40   50   60   70   80   90   100%
|----|----|----|----|----|----|----|----|----|----|
***************************************************
done.
Refining results ... done.

mode |   affinity | dist from best mode
     | (kcal/mol) | rmsd l.b.| rmsd u.b.
-----+------------+----------+----------
   1        -12.0      0.000      0.000
   2        -11.6      2.493      3.619
   3        -11.1      4.279      5.703
   4        -11.0      3.059     15.496
   5        -10.9      4.004     15.760
   6        -10.9      2.826     16.618
   7        -10.8      3.080     15.207
   8        -10.8      2.880     16.181
   9        -10.7      4.504     17.645
Writing output ... done.

C:\Vina>vina_split --input out.pdbqt
Prefix for ligands will be out_ligand_
Prefix for flexible side chains will be out_flex_

C:\Vina>_
```

Fig.15: Shows results of docking between protein and ligand (cardiolipin) using AutoDock Vina.

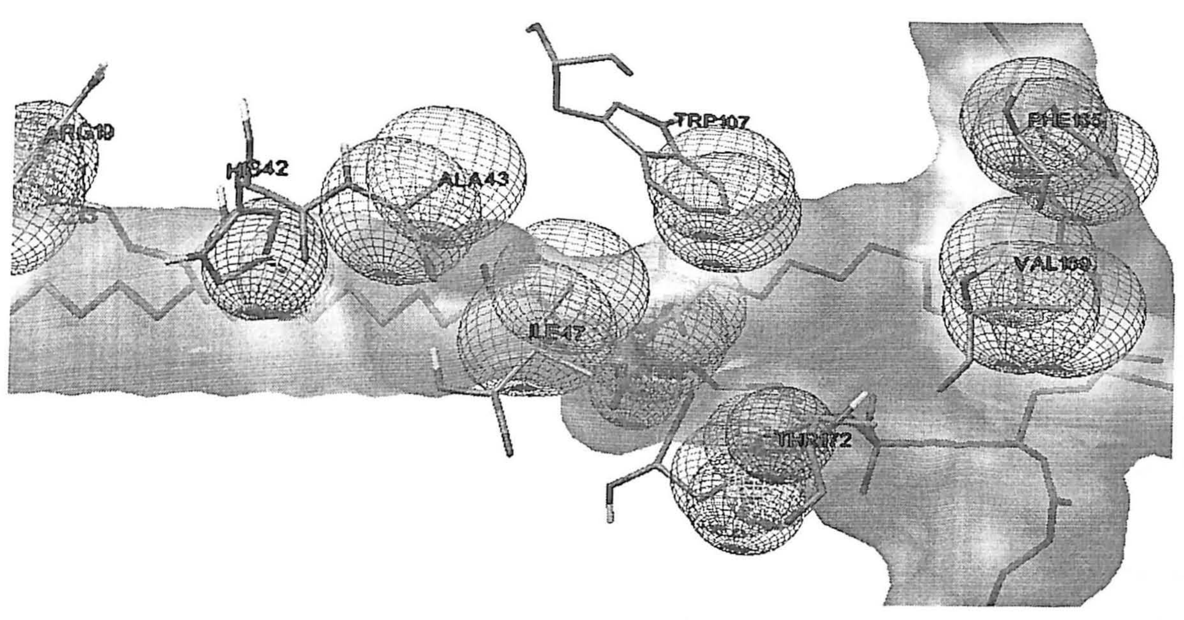


Fig 16: *Shows Best conformation of docking result between COX 1(along with interacting amino acid residues) and Cardiolipin using AutoDock Vina*

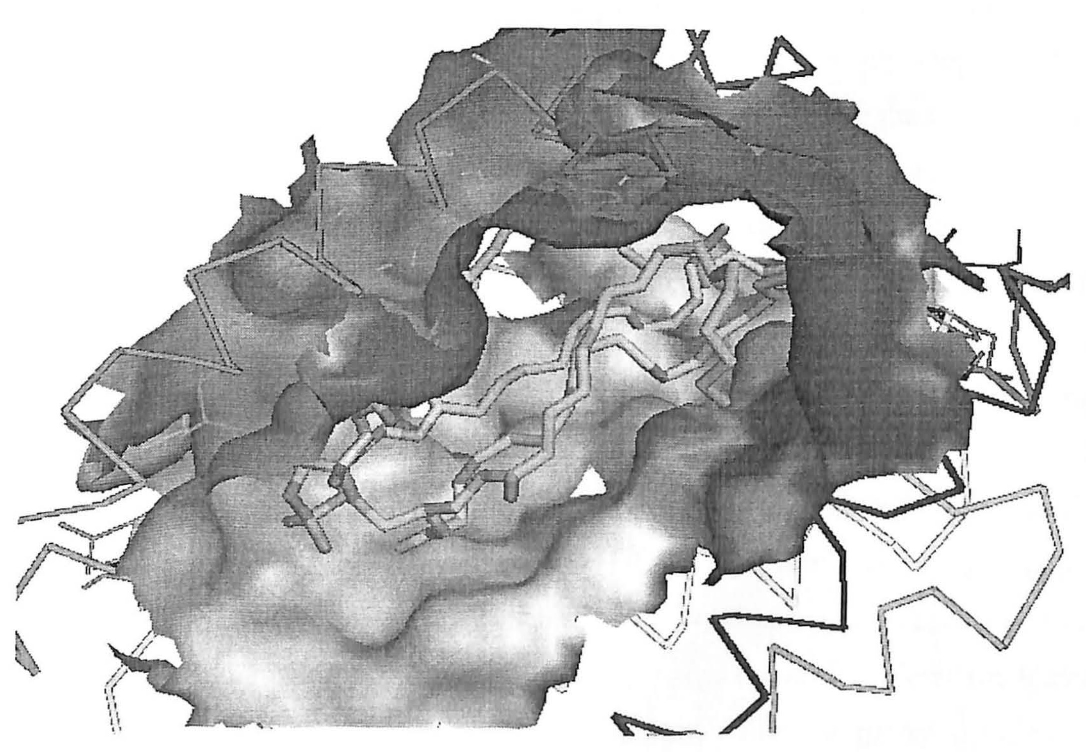


Fig 17: *Best conformation of docking using PatchDock in Pymol*

DISCUSSION

Table 1 shows the cytochrome c oxidase of *Labeo rohita* and then cytochrome c oxidase subunit 1 sequence (Target) was taken for homology search using blast algorithm of NCBI web server from all the hits resulted from blast search. It was observed the crystal structure of Bovine Heart Cytochrome c oxidase show best match with our target sequence reported in table 3 and it was taken as the template(1OCC).As our target sequence is a cytochrome c oxidase subunit 1 therefore the template selected from BLAST was supported by existing literature report. The structure of template was imported to Modeller 9.12and cleaned the chain configuration and bond length. The target and template alignment were perform in ClustalW and the alignment are given in the figure 6.The alignment of target and template are the secondary structure prediction are also perform by using Sopma given in the figure 8. The 3D structure of target was predicted using Modeller 9.12 the model score before and after loop modelling were predicted given in table 4.The resulted Ramachandran plot reported in fig.9 supported the correctness of predicted structure as approximately 92.01% amino acid residues were found in favourable regions and 0% residue in disallowed region. Structural superimposition were done using Pymol the target protein before and after loop modelling given in fig.11.From the superimposition result it is observed that the loop refinement of predicted structure was properly done was given in Pymol. Furthermore the predicted structure was docked with Cardiolipin using AutoDock. The water molecule and hetatm were removed from the structure for docking purpose. As we know the cardiolipin is working as a binding ligand (phospholipid), it's structure was downloaded from Chemspider database in sdf format then it was converted to pdb using pymol. Both the target and structure (cytochrome c oxidase subunit 1and cardiolipin) were imported to AutoDock 4.2 for Docking purpose then hydrogen atom are added to the target structure. Then the target protein format was converted to PDB to PDBQT using in Auto dock 4.2.The Ligand was then imported to AutoDock tool where it was converted to pdbqt format for docking purpose. The Ligand PDB file is modified by addition of set of charges namely Gasteiger charges then the Ligand molecule was prepared for detection of rotatable and non-rotatable bonds. From the result it was found that there was 6 rotatable bonds present in cardiolipin structure. Then a configuration file was prepared for Grid Box setting around the receptor molecule named as conf.txt. It contains configuration file as follows:

Receptor =protein.pdbqt

Ligand = ligand.pdbqt

Out = out.pdbqt

Size_X = 40

Size_Y = 40

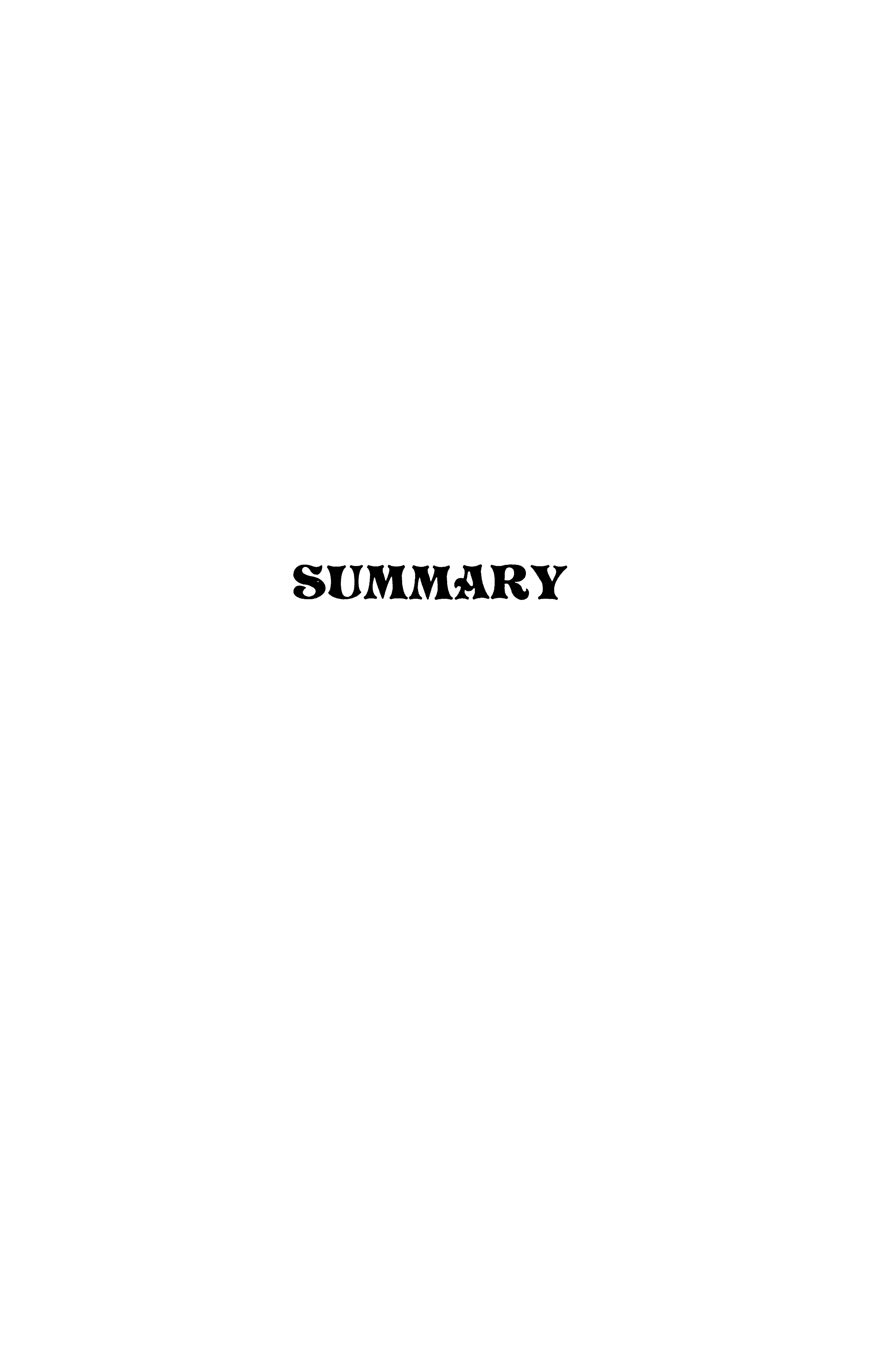
Size_Z = 40

Centre_X = 40.755

Centre_Y = 40.705

Centre_Z = 40.671

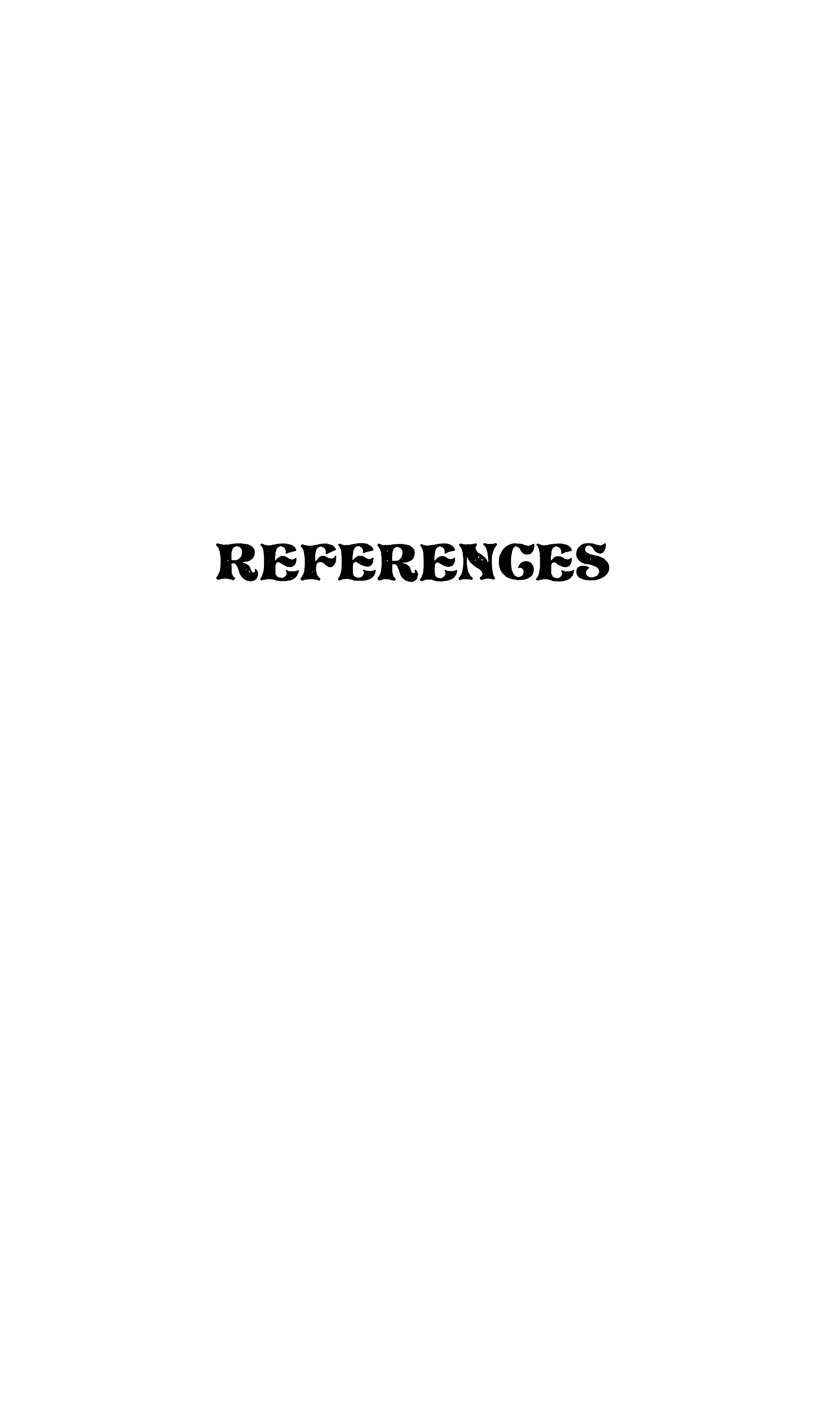
A grid parameter file was created as grid.gpf and a docking parameter file was created as named dock.dpf. The docking search parameter was selected as Genetic Algorithm docking output in the form of Lamarckian GA 4.2 .In the docking the protein or receptor molecule was chosen as rigid molecule. The ligand and receptor was dock using AutoDock Vina of the Scripp's research Institute as shown in figure 13. From the docking score it was observed the second mode of binding is the best among all interaction. From fig 15 it was observed the amino acid residues namely MET (46,50), THR(172), PHE (201 &165) , VAL(51), TRP (107) and ARG(194)are interacting with cardiolipin compound. In Fig.11 2D ligand-protein interactions were shown in LigPlot. Best conformation of Docking was shown in Fig.16 in solid surface by using PatchDock in Pymol.



SUMMARY

SUMMARY

Cytochrome c oxidase subunit I(COX1) is the main subunit of the cytochrome c oxidase complex. Cytochrome c oxidase is a key enzyme in aerobic metabolism. Interaction between cytochrome c oxidase subunit 1 and cardiolipin are important for both electron transfer and apoptopic function of *Labeo rohita.* Interactions of cytc oxidase with the mitochondrial phospholipid cardiolipin (CL) play an important role in mediating or activating caspases in apoptosis(Programmed Cell Death) role of conformational dynamics for the protein peroxidase function& also required for its(COX1) full activity. Hence an attempt has been made in the current study to predict the three dimensional structure of cytochrome c oxidase of *Labeo rohita* having GenBank Accession Number ACF34406.1 by implicating comparative modelling approach. The structural evaluation and quality test results using Ramachandran plot analysis and Verify 3D Plot proved the correctness of the predicted structure of cytochrome c oxidase. The structural superimposition result supported the fact that the predicted structure of cytochrome c oxidase of *Labeo rohita* is mostly similar with the Bovine Heart which was taken as template resulted from BLASTP. Furthermore molecular docking technique was imposed by taking cardiolipin template as ligand with the predicted cytochrome c oxidase of *Labeo rohita.* The molecular Docking result shown quite similar types of interaction between the cytochrome c oxidase of *Labeo rohita* and cardiolipin factor taken from ChemSpider which emphasis on the fact that a similar biological activity also might be possible in case of *Labeo rohita.*The 3D structure of COX1 was prepared in Modeller 9.12 and out of the five structures the best structure was choosen on the basis of lowest DOPE scores then the structure was analysed under SAVES server. The SAVES result showed that about 92.1% residues are present in the most favoured region in Ramachandran plot and 5.1% residues are present in additional allowed region which conforms the good quality of the structure. The best model was visualized in Pymol. The Docking study was done in AutoDock, AutoDock Vina, PatchDock and refined showed that the COX 1 was mostly active at residues between VAL51 & LEU 55. The work can be extended further to predict possible mechanism for the control of electron flow to the oxidase and other expects. In future this study can be intensively carried out to understand cytochrome c oxidase of Indian Major Carp *Labeo rohita* and cardiolipin interaction by MD simulation approach.



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