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Ishta Kaul

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"Docking studies: *In silico* analysis of polystyrene degrading bacterial enzymes"

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DISSERTATION

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IN
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Submitted by
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CERTIFICATE

I hereby certify that the project dissertation titled '**Docking studies: *In silico* analysis of polystyrene degrading bacterial enzymes'** which is submitted by Ishta Kaul, 2K20/IB/05, Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge this work has not been submitted in part or full for any degree or diploma to this university or elsewhere.

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CANDIDATE'S DECLARATION

I, Ishta Kaul, 2K20/IBT/05, student of M.Tech (Industrial Biotechnology), hereby declare that the project dissertation titled **'Docking studies: *In silico* analysis of polystyrene degrading bacterial enzymes'** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of the degree of Master of Technology, is original and not copied from any source without proper citation. This work has not previously formed the basis for the award of any degree, diploma associateship, fellowship or other similar title or recognition.

Place: Delhi
Date – 31-05-2022

ISHTA KAUL
(2K20/IBT/05)

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ABSTRACT

Since its popularization, there has been a rapid increase in usage of plastic products over the years. Pollution caused by plastic has become a global concern in today's era. Plastic pollution is caused by human, industrial and domestic activities. The discharge of plastic from various sources is expected to negatively affect the water quality of marine systems as well as soil ecosystems. Most of the plastics that get dumped in the ocean are produced and used on land. Plastic remains in the environment for a long time and does not get degraded easily. The rate of natural removal of plastic is on the scale of decades to centuries. By the disintegration of plastics, microplastics & nano-plastics are produced and accumulated in large quantities in the environment. Management of plastic is the need of the hour and a global monitoring system is required to control this global issue. Microbial communities colonizing plastics have gained significant attention in recent years. Bacterial enzymes have the capability to degrade plastics in an eco-friendly and cost-effective way. In this study, different bacterial enzymes were molecularly docked with polystyrene to check the binding energy and ligand efficiency. The results indicated that MHETase had the strongest binding energy with polystyrene which denotes that it has the highest plastic degrading capacity.

Keywords- Plastic, Pollution, Degrade, Environment, Enzymes, Polystyrene, Docking

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LIST OF ABBREVIATIONS

PP- Polypropylene

PUR – Polyurethane

PE – Polyethylene

PS- Polystyrene

PET- Poly(ethylene terephthalate)

CO2 – Carbon dioxide

H2O – Water

CH4 – Methane

EPS- Expanded polystyrene

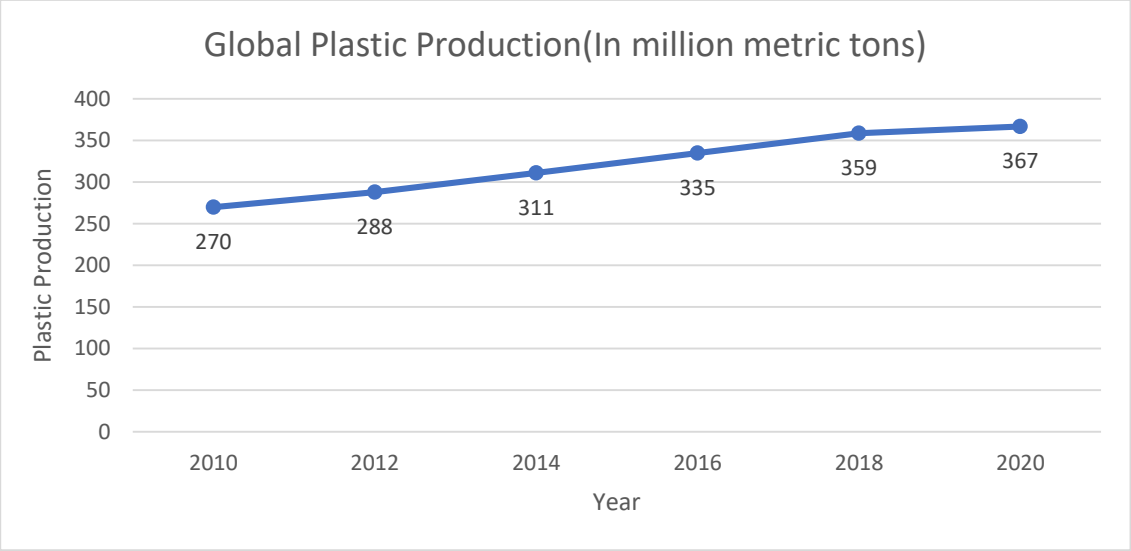
GPPS- General purpose polystyrene

XPS- Extruded polystyrene

HIPS- High impact polystyrene

CHAPTER 1 – INTRODUCTION

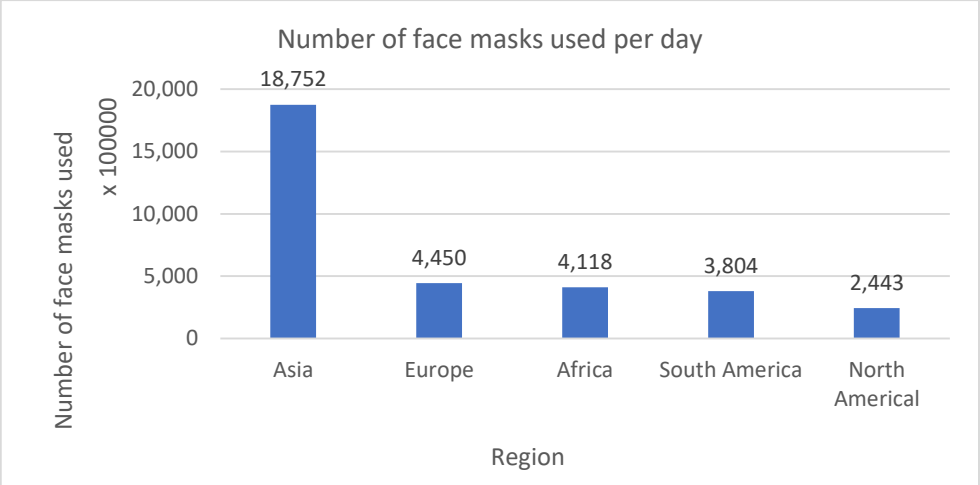
Plastic pollution is found globally from mountains to ocean, deserts to farms, in tropical landfills and Arctic snow as well. Plastic emissions are rising and will continue to rise in the future as well. The global production of plastics increases every year. World plastic manufacturing increased from 359 million metric tons in 2018 to 367 million metric tons in 2020 as shown in figure 1 [1].



Graph 1: Plastic production from 2010 to 2020 in million metric tons

The world is seeing an epidemic of mismanaged plastic waste because plastic is very resilient to degradation. Due to excessive usage and incorrect disposal, plastics enter the aquatic ecosystem and remain persistent for a long period of time [2]. Currently, 85% of aquatic litter is mostly plastic. The amount of plastic in oceans will nearly triple by 2040. Amount of waste added in the ocean is nearly 23-37 million metric tons yearly. By 2050, greenhouse gas emissions from plastics is projected to increase to approximately 6.5 gigatons [3]. Moreover, scientists estimate that the amount of plastic waste in the ocean will be greater than the number of fishes by 2050. In addition to this, up to 10% of plastic debris produced will enter the aquatic system by 2050 [4]. There are many deleterious effects of plastic on the environment. Plastics

can destroy habitats and marine life[5] and can facilitate the transfer of invasive chemicals across habitats[6]. There can be physical and chemical reactions of plastic on aquatic life if plastic is being consumed by them. Plastic often gets entangled with the marine animals. Apart from entanglement, they can suffer from digestive diseases as well like blockages in the digestive tract [7]. Plastic gets deposited in sediments that harms the animals that live and forage in the benthos [8]. Plastic gets disintegrated into smaller fragments of size less than 5mm, referred as microplastics [9]. According to various studies, plastics does not degrade rather it gets disintegrated [10]. Prolonged exposure to UV light and physical abrasion can cause larger plastic to disintegrate into smaller fragments[11]. Use of plastics increased in the year 2020 due to the onset of COVID-19 pandemic. There was a rapid rise in the usage of masks and gloves without any proper system for its decomposition. According to a study, plastic waste generated in COVID-19 was more than eight million tons globally and more than 25,000 tons entered the global sea [12]. A face mask was found in the stomach of a Magellanic penguin that resulted in its death. Polymers including polyethylene, polypropylene, polystyrene, polyacrylonitrile and polyester is used to produce disposable face masks. As per WHO, each month approximately 89 million medical masks were needed in Covid-19 [13].



Graph 2: Number of face masks used by each region per day[14]

Polypropylene (PP), polyurethane (PUR), polyethylene (PE), nylons, polystyrene (PS),

poly(butylene terephthalate) (PBT), poly(ethylene terephthalate) (PET), polyvinyl chloride (PVC) are the most widely used polymer materials. There are currently, 5300 grades of plastic produced with a wide range of chemical additives likes pigments, surfactants, plasticizers, stabilizers, and inorganic fillers.

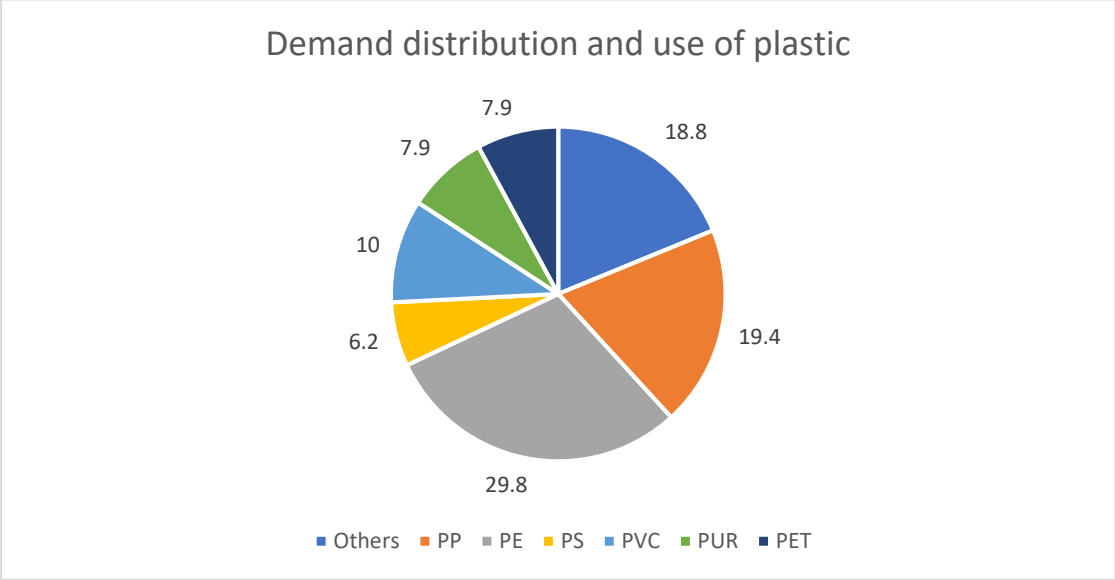


Fig 1: Demand distribution and use of plastic

For degradation of plastics, bacterial enzymes have gained significant attention over the years. Researchers all over the world are working towards reducing the impact of plastic on the environment by different methods and ways. One of them is to use microorganisms that are competent of degradation of synthetic and natural polymers. This method is called biodegradation of plastics[15]. The most widely used microbial agents for degradation are *Micrococcus, Pseudomonas, Corynebacterium, Arthrobacter and Streptomyces* [16]. The bacterial enzymes mostly responsible for degradation of plastics include hydrolase, esterase, laccase, protease, urease and cutinase[17]. Different microorganism have different properties, hence, degradation varies from one microorganism to other. The properties of organism, type of polymer and type of treatment are different factors that are responsible for biodegradation of plastics[18]. There are different indications that suggest that the plastic is getting degraded like cracking, discoloration, delamination, phase separation and erosion. These changes take

place due to synthesis of new functional groups, transformation due to chemicals and breakage of bonds[19]. Figure 2 shows the mechanism of biodegradation of plastic using microorganisms. In the present work, molecular docking was carried out on polystyrene using bacterial enzymes laccase, esterase, cutinase and MHETase. The binding energy and were calculated for each case to find out which enzymes is most capable for plastic biodegradation.

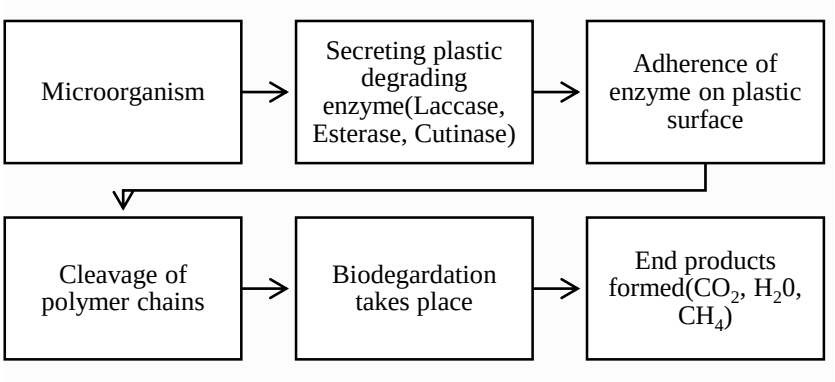


Fig 2: Mechanism of plastic degradation by plastic

CHAPTER 2 – REVIEW OF LITERATURE

2.1 Polystyrene – a threat to the environment

Polystyrene is one of the six majorly produced and consumed polymers[20]. PS is made from styrene monomer and is an amorphous thermoplastic. It has low shrinkage, low specific weight, absence of color, high transparency and brilliance, is chemically inert and has an easy production procedure[21]. Industrial production of PS started in 1930[22]. Different varieties of PS is available in market for example, EPS, GPPS, XPS and HIPS. PS has a variety of application like it is used for packing of food/non-food material items, it is used in automobile and electronic industries, it is used as a building insulator and it can be used for the manufacture of household items as well[23]. There are many additives that are incorporated into PS such as, antioxidants, processing lubes, antistats, UV stabilizers, and flame retardants (FRs). These additives can leach into the environment and cause ill effects. Not just additive, synthetic polymer analogues may leach into the natural environment as well. The degradation of styrene-based polymeric materials for instance, rubbers, PS and resins lead to the formation of styrene oligomers (SOs)[24]. In 2019, the production capacity of PS globally amounted to be 15.61 million metric tons. It is expected to grow to 15.68 million metric tons by 2024[25]. Most of the PS produced ends up in the aquatic system. It poses serious threat to wildlife, marine life, ecosystem and eventually human health. In an experiment, it was demonstrated that red blood cells were affected due to smaller PS particles with diameters of 460 nm and 1 μm [26]. Figure 2 shows the structure of polystyrene.

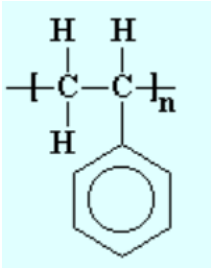


Fig 3: Structure of polystyrene[27]

2.2 Bacterial enzymes

2.2.1 Laccase

Laccases is an oxidoreductase enzyme that catalyse the oxidation of various non-phenolic and phenolic compounds. Bacterial laccase are stable at high temperature and pH. This enzyme is being produced by bacteria either by intracellular or extracellular ways and they can perform in a wide range of pH and temperature. Bacterial enzymes are used in a variety of application like bioremediation, biosensors, biobleaching and pollutant degradation[28]. Many studies have been done on the plastic degrading properties of bacterial enzymes. According to Santo et al. (2013), PE-degrading bacterium, *Rhodococcus ruber* C208 secrets an extracellular laccase and that has the potential to oxidize PE films. [29].

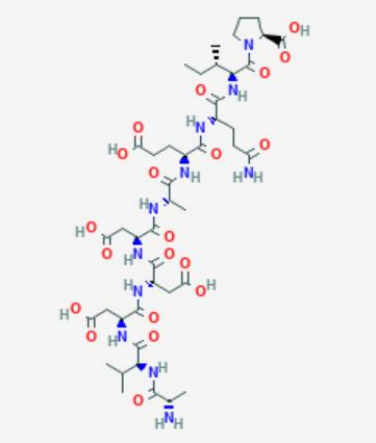


Fig 4: Structure of laccase[30]

2.2.2 Esterase

An enzyme that catalyze the hydrolysis of an ester group from various substrate is called esterase. An esterified acid is released after the hydrolysis. Lipase is the major group of esterase that is mostly used in industries. Lipases are used in detergents and in degreasing of leather [31]. Esterase and lipase hold a major proportion of enzymes that have the potential to degrade biodegradable plastics. Lipase and esterase both belong to α/β hydrolases. Esterases are inactive towards water-insoluble long chain triacylglycerols and favorably break the ester bonds of water-soluble shorter chain fatty acids[32]. Plastic is hydrolyzed by esterases and

lipases by breaking the ester bond in the carbon chain & are mainly functional on aliphatic polyesters[33]. According to various reports, bacterial esterase has potential to degrade plastic. For instance, aromatic polyesterase synthesized by *Ideonella sakaiensis* has shown high PET degradation efficiency[34]. There are studies that demonstrate the ability of bacterial esterase to degrade polyurethanes[35].

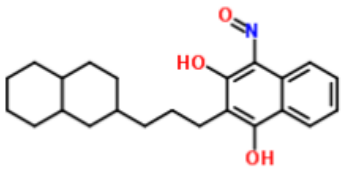


Fig 5: Chemical structure of esterase[36]

2.2.3 Cutinase

Cutinases catalyzes the cleavage of ester bond of cutin. Cutin is a dense biopolymer comprised of hydroxy & epoxy fatty acids that makes the structural component of higher plants cuticle. These enzymes have similar catalytic properties like lipases and esters, and present the unique characteristic of activity regardless of an oil-water interface, making them useful as biocatalysts in various industrial processes like hydrolysis, esterification reactions and esterification. Cutinases have high stability in ionic liquids and organic solvents, both microencapsulated and free in reverse micelles. These properties allow enzymes to be applied in various fields such as the cosmetic and food industry, fine chemicals, pesticide and insecticide degradation, textile fiber processing and washing, and polymer chemistry[37]. Phyllo-spheric fluorescent *Pseudomonas putida, Corynebacterium sp*. and *Pseudomonas mendocina have been used to* isolated and characterize various bacterial cutinases[38]. According to a study, for getting carbon and energy, fusarium secreted polycaprolactone(PCL) depolymerase, identified as cutinase, hydrolyzed the insoluble polyester [39]. Many reports suggest that synthetic polyester, PET, PCL, PBS and phthalate plasticizers can be degraded by cutinases[40].

2.2.4 MHETase

Lately, a unique strain of bacteria called *Ideonella sakaiensis* 201-F6 was discovered that produces some rare enzymes, polyethylene terephthalate hydrolase and mono(2-hydroxyethyl)terephthalic acid hydrolase (MHETase), which allows the bacteria to utilize PET as the sole source of carbon.[41]. MHETase belongs to the esterase/tannase family, which comprise of fungal and bacterial ferulic acid esterase, fungal and bacterial tannases, and different bacterial homologs of unknown function[42]. When degrading PET plastics, *Ideonella sakaiensis* attaches to the substrate with tendrils and produces two enzymes, MHETase and PETase. PET plastic gets hydrolyzed into MHET and a small fraction of bis(2-hydroxyethyl) terephthalic acid (BHET) and TPA with the help of PETase. MHETase hydrolyzes MHET & BHET to TPA and EG[43].

2.3 Interaction between enzyme and plastic

The plastic polymer is being consumed and degraded into simple monomeric units by the microorganism. These monomeric units are easily adapted by the environment and the microorganisms accumulate them as their carbon source. These monomeric units are further broken down into metabolic products like water, carbon dioxide, methane and nitrogen [44]. There are many indications that suggest that plastic is being degraded by microorganisms(Fig 6). Microorganisms colonizing the plastic surface first cause the polymer to reduce in size, breaking it down into monomers that can be taken up by microbial cells, and then these monomers are activated within their cells by enzymatic degradation, using the monomers as a carbon source for growth. If the plastic is processed before microbial attack for breaking down the polymer into monomers through physical/chemical methods like chemical decomposition, heating, freezing, cooling, thawing, the process of plastic degradation can be modified. Following enzymatic degradation, mineralization of the monomers occur and the end products that are delivered include carbon, water, methane, nitrogen and many more metabolic products.

Further, use of these end products can be quite beneficial in completely removing harmful plastics from the environment. Methane is considered a biogas that is used as a fuel for the production of heat & light and is also used as an ingredient in the manufacture of certain typed of organic acids[45]. The plastic found in the environment by nature are hydrophobic. Through hydrophobic interactions, extracellular enzymes mainly produced by wide range of microorganisms adheres to the plastic surface. In most hydrolases, a hydrophobic cleft is present near the enzyme's active site. This cleft can accommodate the hydrophobic groups present near the enzyme's active site. The hydrophobic groups present which is present in the polymer is being accommodated by the cleft that leads to improved enzyme accessibility of the polymer[46]. The enzyme's active site is involved in the hydrolysis of long polymer chains into smaller monomers or dimers, which can be accumulated and consumed by the microbial organism as a source of carbon[47].

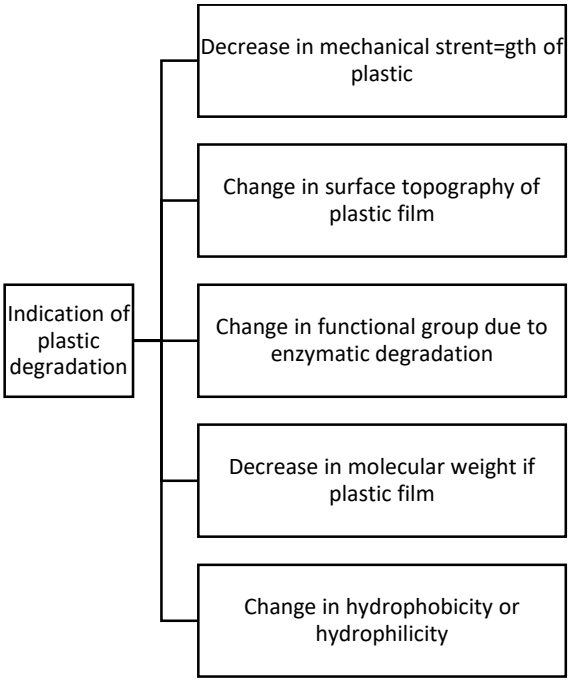


Fig 6: Indications suggesting plastic degradation

2.4 Molecular docking

Molecular docking was first developed in 1980s. Since then, molecular docking has been successfully employed to assess the mechanism of pollutants. Molecular docking is a very useful and low-cost technique that helps us understand the reaction mechanism of enzymes with ligands with a high precision. Though molecular docking, the orientation that is more preferred of one or more molecules in the protein's active site can be detected [48]. Binding affinity, molecular recognition and binding modes can be predicted and understood with the help of molecular docking software. Ligand-protein docking is the molecular docking performed between a small molecule and a target macromolecule. Molecular docking has a wide range application in drug discovery[49]. Molecular docking is widely used as a tool in drug discovery. Earlier, the docking methods was based on the lock-and-key model. It stated that the receptor and ligand should be treated as rigid structures[50]. After few years, the "induced-fit" theory came into light which said that the ligand and receptor should be considered as flexible structures during docking [51]. For commercial and academic use, more than 60 docking tools and programs have been developed for example, AutoDock, AutoDock Vina, Glide, LigandFit and many more [52].

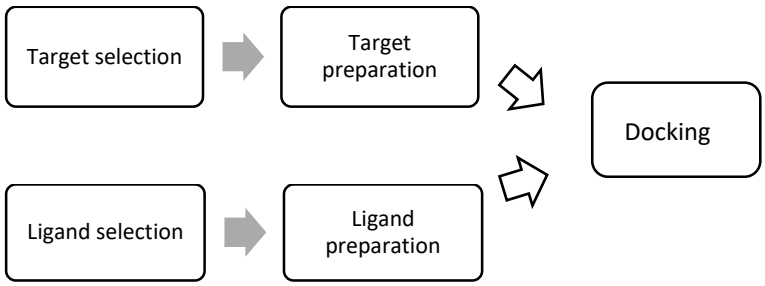


Fig 7: Basic steps of docking

CHAPTER 3 – MATERIAL AND METHOD

3.1 Enzymes used in study

Crystal structures of laccase(PDB id: 1GSK), esterase(PDB id: 1QLW), cutinase(PDB id: 6AID) and MHETase(PDB id: 6QZ4) were taken from protein data bank.

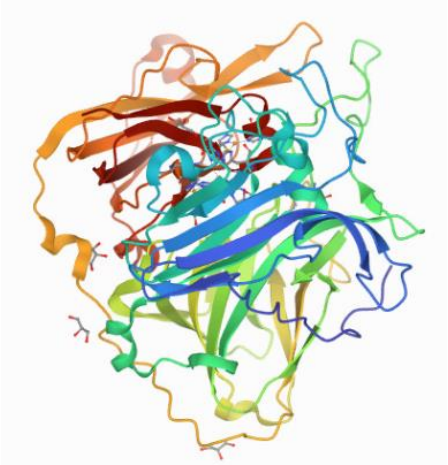


Fig 8: Crystal structure of laccase(1GSK)

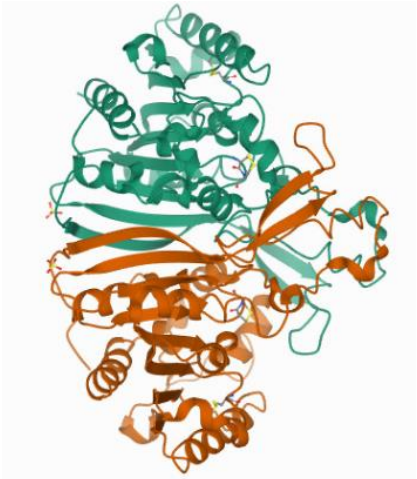


Fig 9: Crystal structure of esterase(1QLW)

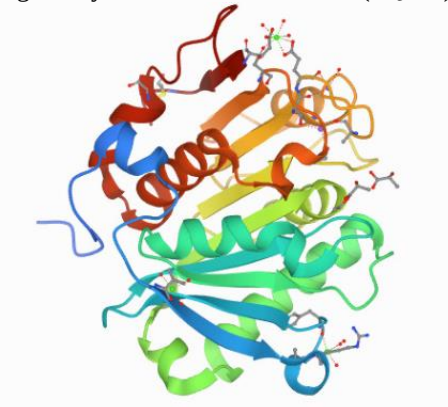


Fig 10: Crystal structure of cutinase(6AID)

Fig 11: Crystal structure of MHETase(6QZ4)

3.2 Molecular docking

Molecular docking was performed to determine the molecular interactions of the ligands with the substrate-binding moieties. This study is done first to see the difference in quality of the interaction of the enzymes with the ligand. In this study I focused on the coupling of the four enzymes laccase, esterase, cutinase and MHETase with the polystyrene ligand. The structure of the ligand was obtained from pubchem. Using the BABEL program (version 2.3.1) the SDF file format of the ligands was transformed to the PDB format. To predict the active of the four enzymes AutoDock version 4.2.6 and AutoTools 1.5.6 software were used. Water molecules, polar hydrogen and non-polar hydrogen were removed from the proteins. Further, the total Kollman and Gasteigher charges were allotted, individually. The same method was performed on the ligand to ensure that torques for rotation were perfectly adopted during docking. The grid box parameters were set as coordinate spacing of ±1,000 Å, keeping sizes 48, 48, 50, and 50 in the X, Y, and Z dimensions, respectively. The grid parameters were set with size 44 for the axes X, Y and Z. Later, the protein-ligand complex was achieved in PDBQT format. On each ligand, a small number of independent docking runs were implemented. Finally, the PDBQT file that was generated was studied by selecting AutoDock to classify the binding energy. To study protein-ligand interactions, the PDBQT format was then converted to a PDB file for visualization by LigPlot and PyMOL.

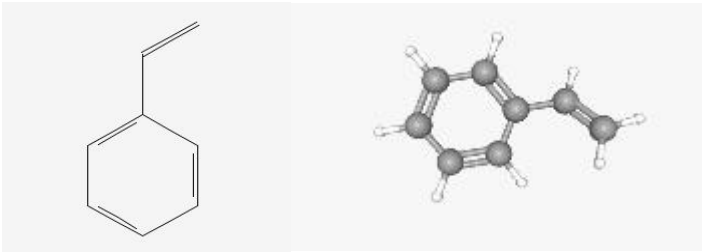


Fig 12: Structure of styrene

CHAPTER 4- RESULTS AND OBSERVATIONS

Docking studies were completed by the use of Autodock vina. According to docking analysis, if a molecule has lesser binding energy, it proves that the compound has higher activity and the ligand has most stable complex interaction with the enzyme.

Binding energy of each compound was calculated using this formula,

Binding energy = A + B + C - D

Where, A = final intermolecular energy + van der Walls energy (vdW) + hydrogen bonds + desolvation energy + electrostatic energy (kcal/mol),

B = final total internal energy (kcal/mol),

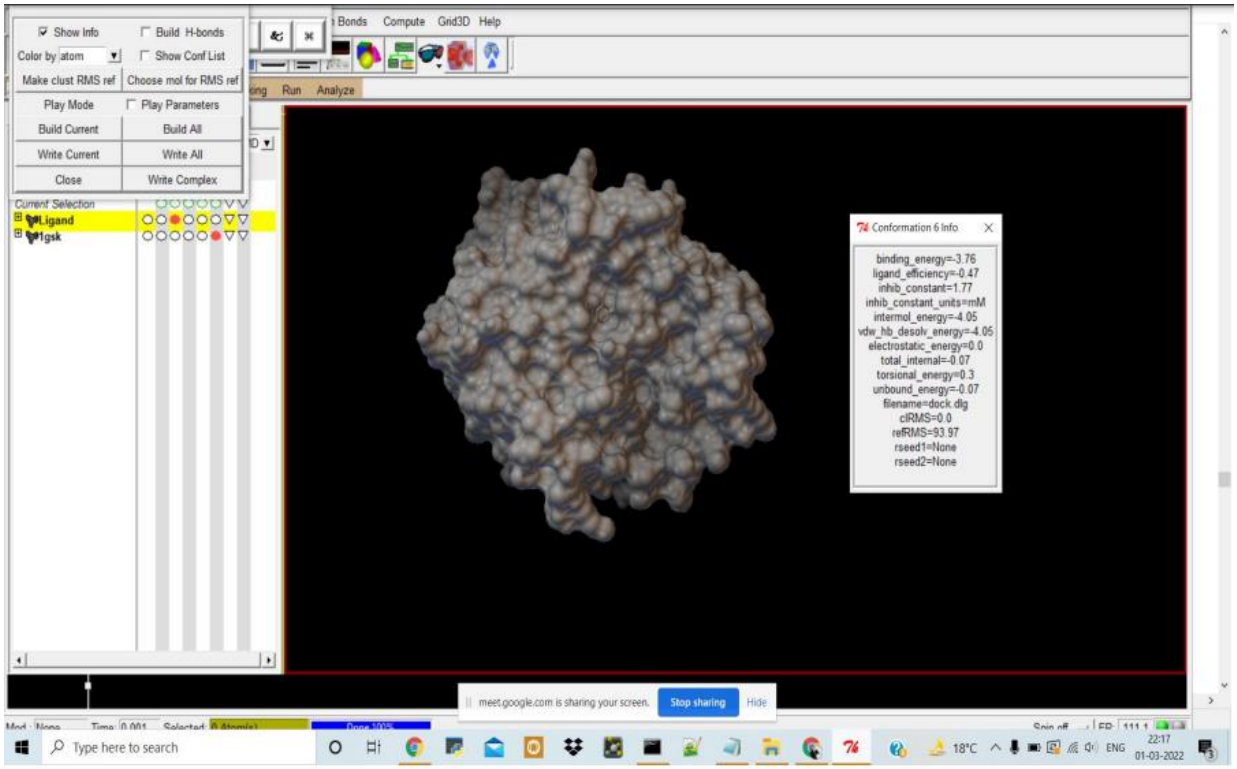
C = torsional free energy (kcal/mol),

D = unbound system's energy (kcal/mol).

It was observed that the best binding energy was of MHETase. This suggest that MHETase has the strongest binding with polystyrene and is best suited for plastic degradation. The more negative the binding energy, stronger is the bond between the receptor and ligand. The probable binding sites of enzymes was, LYS 178, LEU 175, ALA 155, ASN 7, ILE 156, ASN 8, GLU 145, VAL 148, VAL 153, LYS 176, LYS 154, TYR 177. After docking the results showed that MHETase had better binding energy(-5.0 kcal/mol). This proves that MHETase has better binding with polystyrene & us better degrading enzymes among others. Furthermore, two more parameters like intermolecular energy and inhibition constant (Ki) were also established. Inhibition constant is directly proportional to binding energy. Theoretical inhibition constant was determined with the help of AutoDock 4.2. MHETase had the highest inhibition constant of 1.46 µM. This implies that MHETase were found to be higher activity against polystyrene. Intermolecular energy is also directly proportional to binding energy. MHETase had better intermolecular energy (-5.3 kcal/mol). This outcome also proved that MHETase consist of better polystyrene inhibitory activity. Table 1 shows the results.

Parameters	Laccase	Esterase	Cutinase	MHETase
Binding energy	-3.76	-4.33	-3.87	-5.0
Ligand efficiency	-0.47	-0.54	-0.48	-0.63
Inhibition constant	1.77	699.01	216.71	1.46
Intermol energy	-4.50	-4.6	-4.17	-5.3

Table 1: Comparison table of enzymes



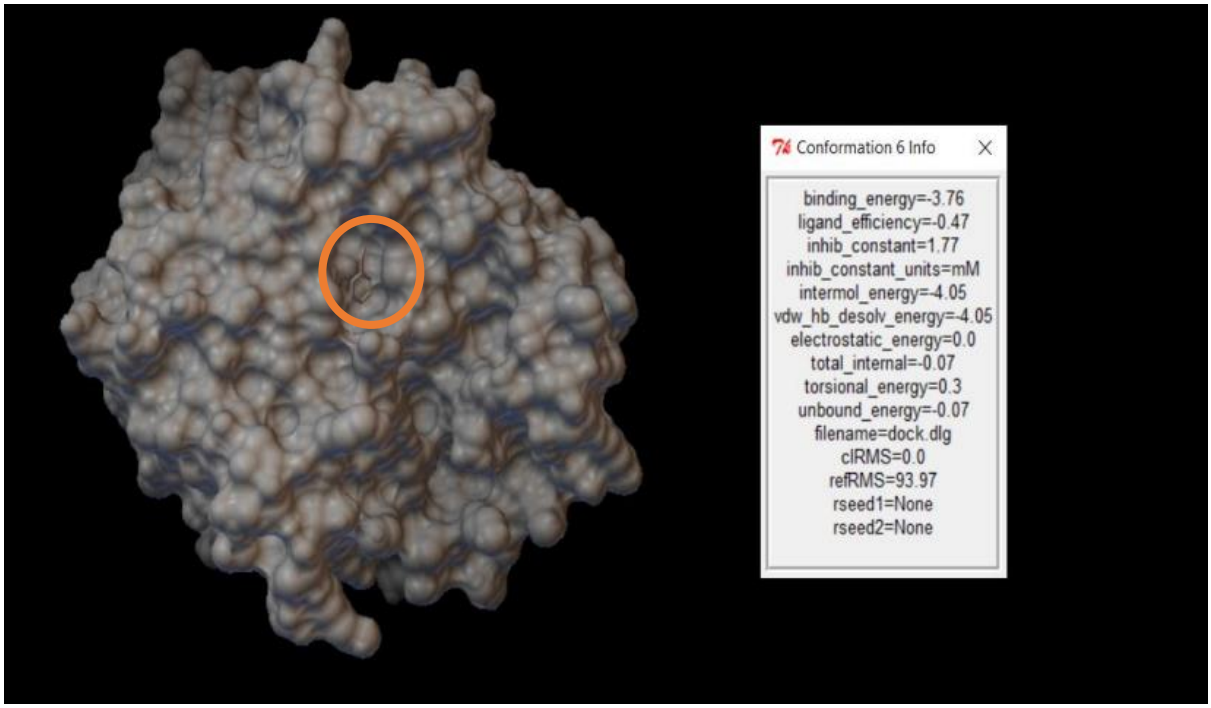


Fig 13: Molecular docking of laccase(1GSK)

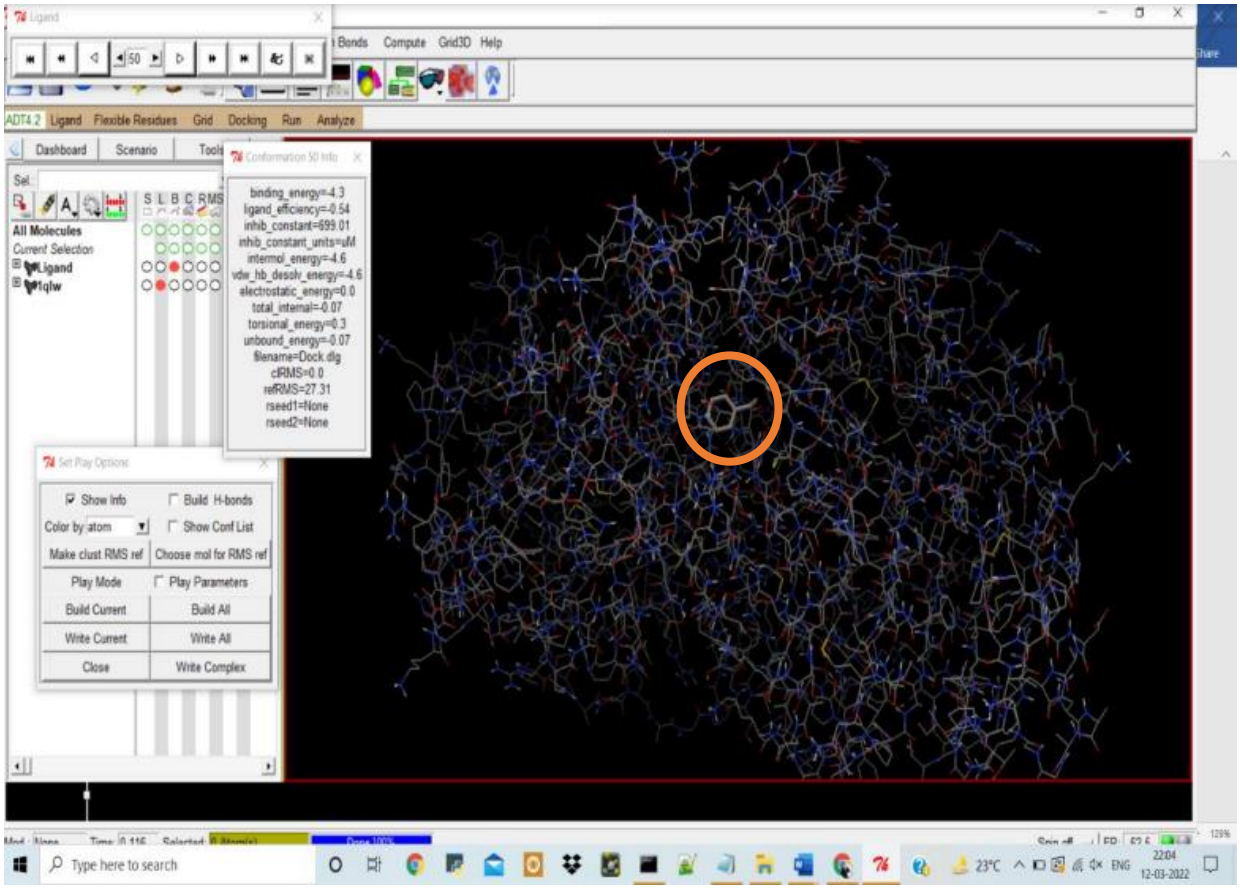
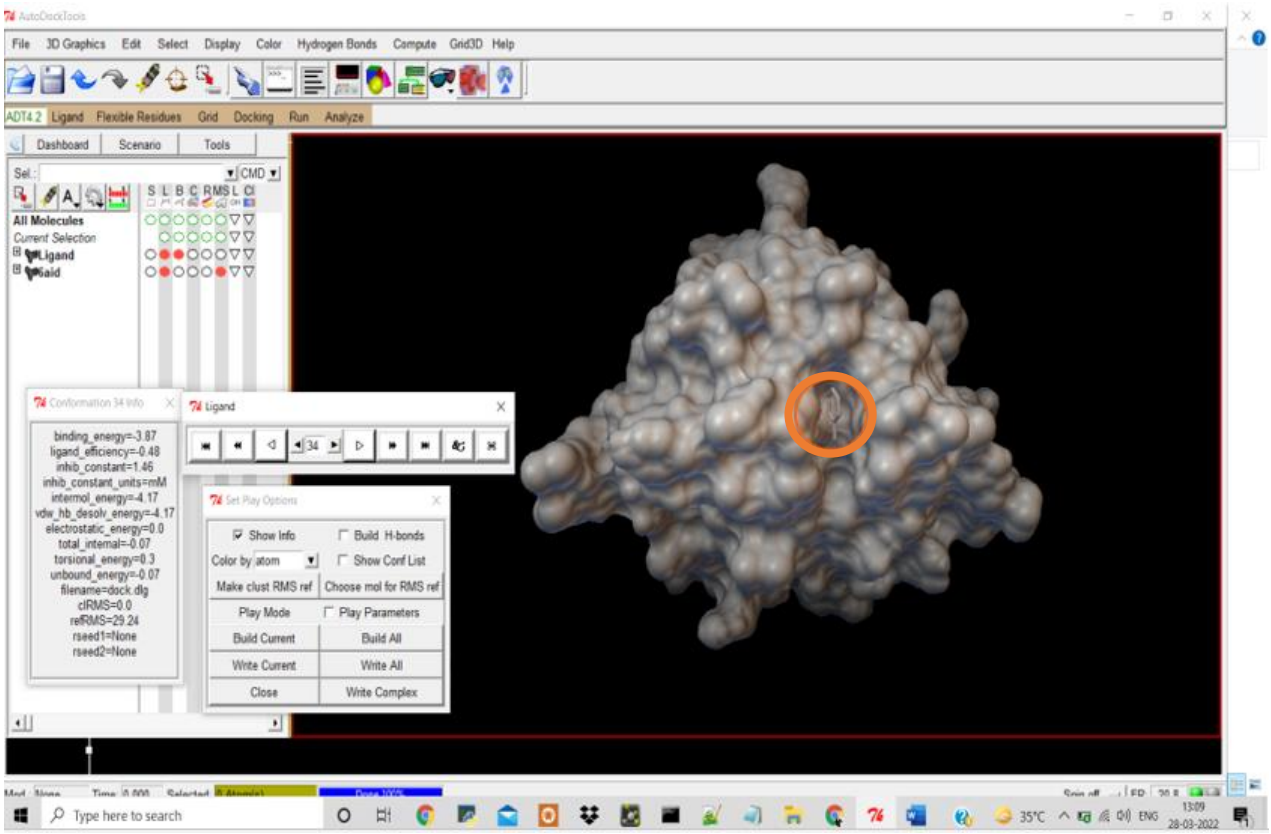


Fig 14: Molecular docking of esterase(1QLW)



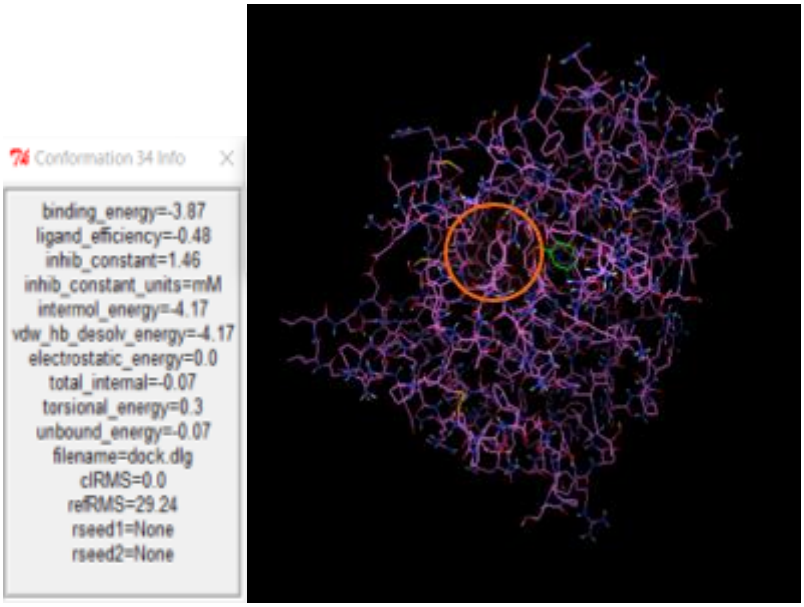
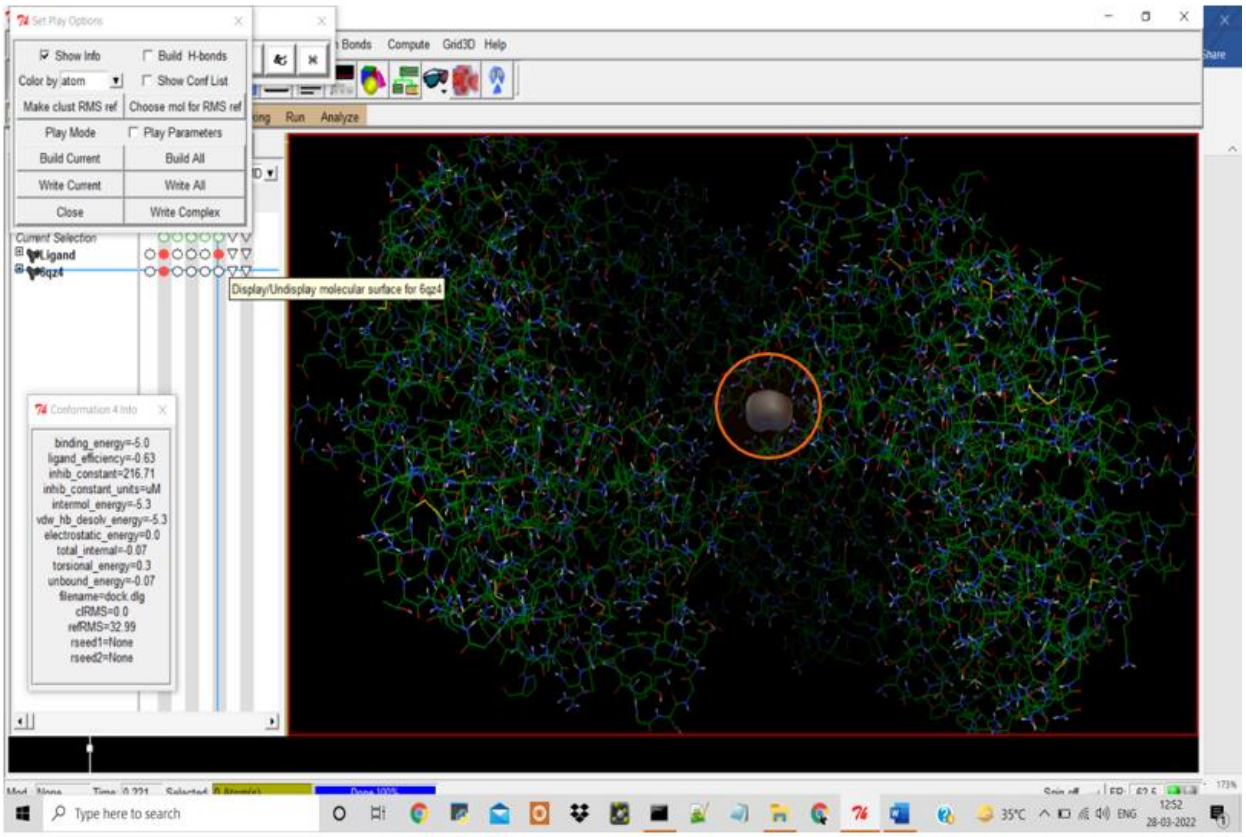


Fig 15: Molecular docking of cutinase(6AID)



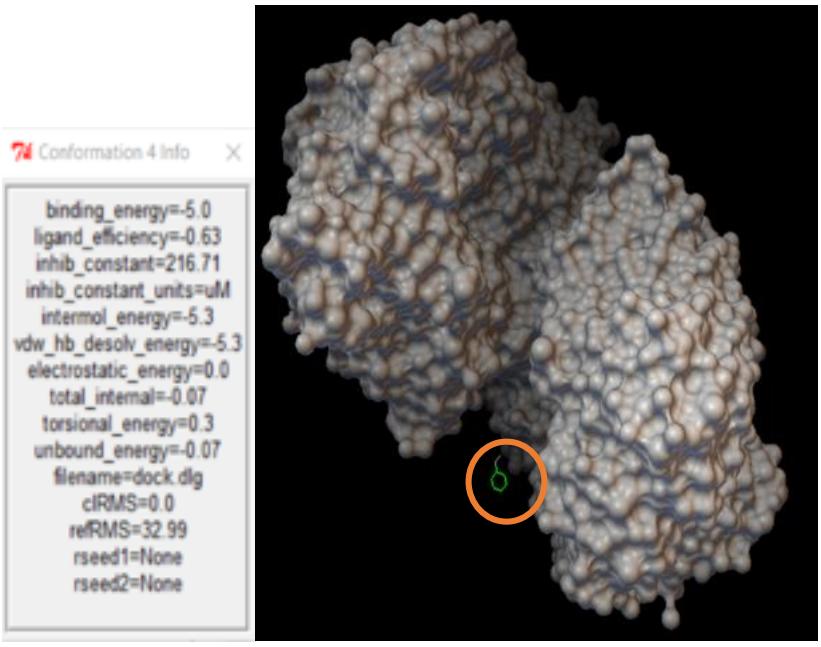


Fig 16: Molecular docking of MHETase(6QZ4)

CHAPTER 5- CONCLUSION

Synthetic plastics play a central role in our current lifestyle and therefore their accumulation is a major problem for the environment and human health. Plastic pollution is increasing day by day and is becoming a major concern. Scientists from all around the world and trying to find solution for this problem. Recently enzymatic degradation of plastics has gained attention. I performed docking on four enzymes namely esterase, laccase, cutinase and MHETase with polystyrene on Autodock Vina to find out the best suited enzyme for polystyrene degradation and according to the docking results, MHETase has the highest binding energy which means it has the highest potential to degrade polystyrene and forms the most stable ligand receptor complex. This approach can help in identifying enzymes that can be used to degrade other plastic as well apart from polystyrene. This understanding can help in development of strategies for controlling polystyrene pollution by using MHETase as a degrading agent.

CHAPTER 6 – REFERENCES

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