Investigating the Enzymatic Mechanism in Polystyrene Degradation: The Computational Modeling Approach

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ABSTRACT

Polystyrene, commonly known as Styrofoam, poses an urgent problem in today's environmental context. The material resists degradation, allowing it to accumulate in aquatic environments and indirectly enter the food chain as microplastics. While current research has focused on the merits of biodegrading this material, and several microorganisms have been identified to have this capability, the specific enzymatic mechanism through which degradation occurs has not been identified. This research aims to identify a potential mechanism between an identified enzyme (alkane-1-monooxygenase (AlkB) from Acinetobacter johnsonii JNU01) and the substrate, polystyrene. A robust understanding of this mechanism could lead to potential development of a widespread solution that can mitigate polystyrene pollution. This paper will review the problems posed by polystyrene, benefits of biodegradation, current research into plausible enzymes, the specific characteristics of the JNU01 AlkB enzyme, and conclude with a computational docking experiment that demonstrates the interaction between the JN01 AlkB enzyme and polystyrene molecule. This study concluded that the most likely mechanism by which polystyrene is degraded enzymatically is through backbone cleavage, in which the enzyme hydrolyzes the carbon-carbon backbone in order to depolymerize the molecule, and allowing for subsequent styrene monomer degradation.

KEYWORDS Styrofoam, Enzymatic degradation, Computational modeling, Biodegradation

INTRODUCTION

Polystyrene pollution remains a prevalent problem in today's ecological and climate landscape. As a recalcitrant material that takes approximately 500 years to degrade, polystyrene resists degradation, resulting in microparticles known as microplastics (26). These particles can be easily swept by the wind and end up in run-off matter, leading to accumulation in landfills, oceans, and other natural environments (17). Marine ecosystems are particularly impacted, with almost 27 million tons of microparticles floating in the North Atlantic ocean itself, as polystyrene particles can adsorb persistent organic pollutants, leading to bioaccumulation in aquatic species and potential harm to human health through the food chain (20). These materials are toxic to ingest, and can cause artery blockage resulting in immune system harm both to humans and animals (1). Even with proper water filtration systems, the removal of these particles from water sources results in sludgelike waste that returns to water sources. This sludge harms surrounding plant growth and can continue to be ingested by marine life. In addition, polystyrene production contributes to greenhouse gas emissions and ozone depletion, further exacerbating climate change and global environmental degradation (49).

As a result, addressing the challenges posed by polystyrene pollution is crucial for promoting environmental sustainability and safeguarding ecosystems. The detrimental impact of this material raises the question of how we can prevent the pollution of the material.

LITERATURE REVIEW

History and Use of Polystyrene

Polystyrene, also commonly known as styrofoam, has a history that dates back to 1839. Eduard Simon, from a German apothecary, isolated styrol, from Turkish sweetgum tree resin, which thickened over a few days, which he thought was associated with oxidation (18). In the following years, chemists John Buddle Blyth and August Wilhelm von Hofmann confirmed Simon's findings and demonstrated that styrol could be transformed without oxygen and could be polymerized to form a new substance, which they called "meta styrol" (5). In 1922, Hermann Staudinger conducted research on polymers, during which he determined that heating chains of the styrol molecules created a plastic material with rubber-like properties, which is now called polystyrene (18). In the 1930's, German chemical firm I.G. Farben developed the commercial production process for polystyrene, with BASF and Dow Chemical soon introducing the material to the market. Dow Chemical brought a trademarked form of polystyrene foam insulation, originally patented by Carl Munters, Styrofoam, to the US market during World War 2 to be used for rubber products and military application in construction, packaging, food services, and insulation (18). Polystyrene has since become a versatile material used in a variety of industries.

Polystyrene is a synthetic polymer made from the polymerization of styrene, a liquid hydrocarbon with the chemical formula $C_6H_5CH=CH_2$. Styrene, a colorless and oily liquid, is derived from petroleum or natural gas byproducts, such as ethylbenzene or benzene (38). The polymerization process involves the conversion of the double bond in styrene monomers to a single bond, linking the individual monomers together to form long chains known as polystyrene.

Polystyrene is commonly used in the consumer goods sector for protective packaging materials such as packing peanuts, CD and DVD cases, and egg cartons, and also functions in insulating buildings and developing models, prototypes, and 3D printed objects (10) (35). However, despite its numerous applications, concerns regarding polystyrene's environmental impact have led to increased research into biodegradable alternatives and more efficient recycling methods. Nonetheless, polystyrene remains an important material in today's world, with ongoing innovations aimed at improving its sustainability and reducing its ecological footprint.

Current Degradation Methods

Several methods are currently employed for polystyrene degradation, including thermal, chemical, and photodegradation processes. Each method has its own set of limitations.

Thermal degradation involves the use of high temperatures to break down molecular structure and properties (19). This process is achieved through incinerators and combustion techniques. Polystyrene consists of hydrocarbon chains and aromatic rings, thus, the hydrogen can easily dissociate from the main skeleton of the substance through pressurized gas and compressors within the calorimeter. However, the carbons are double bonded to one another and contain an attachment to aromatic rings making them harder to dissociate. The result of this partial dissociation is char production. Char production is the solid residue present after thermal combustion takes place, and can significantly alter the remaining combustion of the material by

blocking certain chemical pathways (2). Thermal degradation can lead to the release of harmful gasses and emissions, such as carbon monoxide, carbon dioxide, and styrene monomers. Additionally, incomplete combustion can result in the formation of toxic byproducts, including dioxins and furans, contributing to air pollution (30).

Chemical degradation is another method that uses strong acids, solvents, or other reactive chemicals to break down polystyrene (29). This process can be conducted using organic solvents like acetone or acids such as sulfuric acid. While it can partially degrade polystyrene, chemical degradation poses risks to human health and the environment due to the potential release of toxic compounds. A study by Huang et al. reports on a light-driven, acid-catalyzed method of polystyrene degradation. Using one bar of oxygen, the method hyperoxidizes the molecule and cracks the C-C bonds. However, it yields low amounts of useful chemical products, which can be potentially harmful to environments the process is conducted in, such as benzoic acid, phenol, formic acid, acetic acid, and styrene. Additionally, the process is sensitive to impurities and requires visible light irradiation, making it impractical for large-scale applications and waste streams (16).

Photodegradation is the process by which polymers are exposed to light sources, and thus deteriorate (51). Polystyrene, when exposed to ultraviolet radiation, undergoes embrittlement as the polymer chains begin to break down. However, this process is slow and can take hundreds of years for polystyrene to degrade in the environment. Moreover, it leads to the formation of microplastics, which can harm aquatic ecosystems and marine life (51). The release of these free radicals further exacerbates the problem of polystyrene pollution, rather than minimizing it.

Biodegradation

These traditional methods of polystyrene degradation face challenges such as energy-intensive processes, toxic emissions, slow degradation rates, and potential environmental hazards. Therefore, researchers are exploring alternative degradation methods like biodegradation, which uses enzymes within microorganisms to break down polystyrene (40). Enhancing recycling processes and developing biodegradable polymers are also potential strategies to address polystyrene waste and its environmental impact. Biodegradation is the breakdown of organic matter by means of microorganisms (36). Materials that are biodegradable have a more positive effect on the environment as their potential for recycling and conversion to other materials results in less pollution and matter build-up in ecosystems. The products created from this process are typically non-toxic, simpler compounds that do not harm the environment in which they are released (14). Additionally, materials that are biodegradable produce less greenhouse gas emissions, further emphasizing their minimal impact on the environment, and making these materials more preferable to non-biodegradable materials.

Utilizing enzymes to degrade polystyrene offers several advantages. Enzymes do not produce harmful byproducts or require harsh chemicals. Additionally, enzymes are highly selective and efficient, targeting specific components of the polymer structure and breaking it down (26). Moreover, enzymatic degradation can be conducted under mild conditions, eliminating the intense energy requirements associated with thermal or chemical degradation (23). Enzymatic degradation has the potential for optimization through engineering and directed evolution, as

exemplified by the production of insulin (22). By modifying the enzymes responsible for polystyrene degradation, researchers can improve their efficiency and specificity, further increasing the effectiveness of this degradation method. Enzymatic degradation can be incorporated into waste treatment facilities or on-site to degrade polystyrene pollution in the environment.

Method	Advantages	Challenges
Thermal degradation	Easy dissociation of hydrogens from the main skeleton.	Char production and incomplete combustion that results in the creation of dioxin and furans. Additionally, the emission of CO, CO2 and styrene monomers.
Chemical Degradation	Cracking of carbon-carbon bonds using one bar of oxygen in light-driven, acid-catalyzed reaction.	Partial degradation using toxic materials and release of toxic byproducts such as benzoic acid, phenol, formic acid, acetic acid, and styrene. Sensitive to impurities and requires visible light irradiation. Impractical for large-scale applications and waste streams.
Photodegradation	Breakdown of polymer chains.	Extremely long process that also leads to the formation of microparticles.
Biodegradation	Production of non-toxic by-products, no greenhouse gases released, substrate specific, minimal external energy input.	Unclear degradation mechanisms.

Figure 1. Comparing Degradation Methods

Proposed Enzymatic Functions

Several studies have investigated the potential of enzymes in degrading polystyrene, particularly focusing on the role of cutinases and lipases in breaking down polystyrene's ester bonds. Additionally, these studies highlight the effectiveness of various enzymes, from both microbes and fungi, in degrading polystyrene, with fungi belonging to the genera *Aspergillus, Penicillium, and Fusarium* being the most studied for their degrading abilities. The degradation of polystyrene is speculated to be conducted in two phases, oxidation, utilizing laccases,

manganese peroxidases, and lignin peroxidases to remove electrons from the molecules, and hydrolysis, utilizing esterases, cutinases, lipases, and proteases along with water to break down the chemical bonds (42).

Cutinase enzymes produced by *Humicola insolens* have been found to degrade polyethylene terephthalate (PET), another thermoplastic polymer akin to polystyrene (42). In addition, esterases were found to degrade PET as well. After identification, research on esterases focused on effectively and efficiently engineering the enzymes on an industrial level for commercial use in order to degrade the wide spread material (54). These studies demonstrate the potential of enzymatic degradation as a promising solution for overall plastic waste management and environmental remediation.

While research has concluded that microorganisms have been able to degrade polystyrene, the official process has not been confirmed, as the enzyme involved in the depolymerization of polystyrene has not been identified. Depolymerization is the breaking down of polymers into their simple building block units, monomers (4).

Depolymerization of Polystyrene and Styrene Monomers

Polystyrene is made up of styrene monomers. Once depolymerized, enzymes such as styrene monooxygenase (SMO), styrene oxide isomerase (SOI), phenylacetaldehyde dehydrogenase (PAD), and styrene dioxygenase (StyAB) act on the styrene monomers to degrade it (32). Studies show that styrene monomers are entirely biodegradable within naturally occurring catabolic pathways such as the citric acid cycle and other unknown degradation pathways.

The initial oxidation of styrene is conducted by SMO, which converts it into styrene oxide. SMO is a two-component flavoprotein enzyme system consisting of a monooxygenase component (StyA) and a reductase component (StyB) (12). Styrene oxide isomerase (SOI) then catalyzes the isomerization of styrene oxide, transforming it into phenylacetaldehyde through a reaction called the Meinwald rearrangement (21). PAD is an enzyme that oxidizes phenylacetaldehyde, converting it into phenylacetic acid (6). This leads to the subsequent metabolism of styrene-derived compounds.

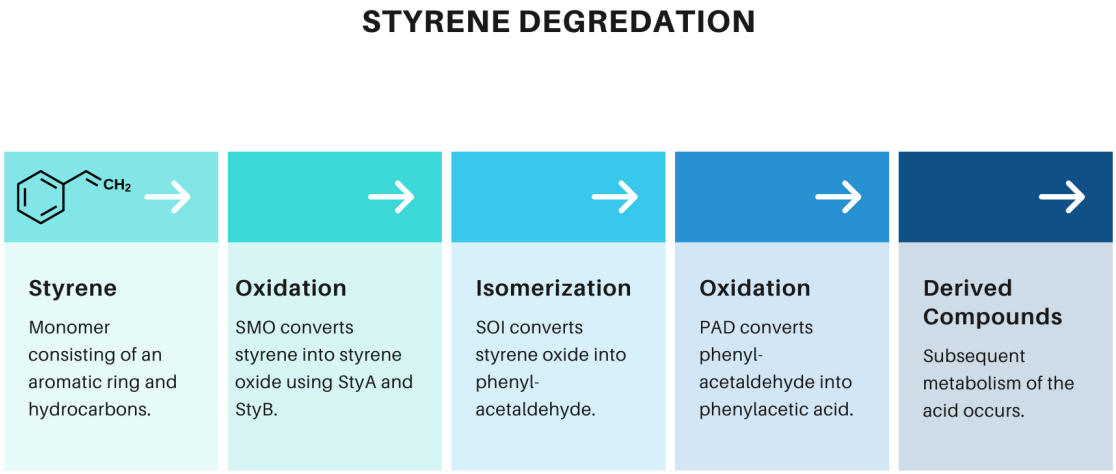


Figure 2. Styrene Degradation (created by author).

Some bacterial species possess a two-component dioxygenase enzyme system, StyAB, which can degrade styrene directly (34). StyAB begins the aerobic degradation of styrene, leading to the formation of metabolites that can be further degraded by subsequent enzymes.

Although styrene itself is catabolically degradable, polystyrene is generally considered non-biodegradable due to the difficulty in pulling apart the polymer chain into monomers. Depolymerizing polystyrene would result in the products of simple styrene monomers that can be naturally degraded. This paper aims to build support for an enzymatic mechanism involved in the degradation of polystyrene.

Enzyme families found in microorganisms that demonstrate a high potential for depolymerizing and degrading polystyrene are: Cytochrome P450, Monooxygenases, and Aromatic ring Hydroxylases (15). These families fall under the enzyme class of Oxidoreductases, which are enzymes that catalyze the transfer of electrons from donors to acceptors by incorporating electrons (11). These enzymes function with the support of cofactors such as NAD+ and NADP+ and have the potential to cleave the backbone of polystyrene, thereby supporting depolymerization (15).

a. **Cytochrome p450:** Cytochrome P450 (CYP) enzymes play a crucial role in the biodegradation of various organic compounds, including polystyrene. The CYP enzyme family contains many heme-containing enzymes that catalyze oxidation reactions, including hydroxylation and epoxidation (50). For example, alongside the presence of hydrogen peroxide, Cytochrome P450 CPY152A1 and cytochrome P450 CPY152B1 can catalyze the epoxidation of styrene. Hou & Majumder hypothesize that Cytochrome P450s, due to their ability to convert long alkane chains to alcohols and engage in sub-oxidation steps, can possibly break the C-C bonds in polystyrene, and degrade the remaining monomers. A recent proposal suggests engineering synthetic bacteria with CYP enzymes to drive a cascade enzymatic pathway for polyethylene that can likely be made applicable to polystyrene (50).

b. **Monooxygenases:** Monooxygenases play a significant role in the biodegradation of various xenobiotic compounds, such as polystyrene. These enzymes incorporate one atom of oxygen into a substrate, leading to the formation of an alcohol or epoxide (46). Monooxygenases are particularly relevant in polystyrene degradation because they are theorized to act on the β-carbon of the carbon chain and cleave the carbon backbone of polystyrene. This would result in styrene monomers that can be degraded through oxidation (15). Monooxygenases could potentially break down the carbon-carbon bonds in the polymer chain, enabling its transformation into less harmful compounds.
c. **Aromatic ring hydroxylases:** Aromatic ring hydroxylases are another enzyme family theorized to be involved in polystyrene degradation. These enzymes introduce hydroxyl groups into aromatic rings, facilitating the breakdown of the compound (31). In the context of polystyrene degradation, aromatic ring hydroxylases could be involved in the initial steps of degradation, resulting in monomers that can then be further degraded by other enzymes. Hou & Majumder hypothesize that the alkane substrate enters a pocket of transmembrane helices that are hydrophobic, until a terminal methyl group is positioned to mimic the structure of an alcohol.

Enzyme Family	Function
Cytochrome p450	Theorized to catalyze the epoxidation of styrene. Can convert long alkane chains to alcohols and engage in sub-oxidation steps; possibly break the C-C bonds in polystyrene, and degrade the remaining monomers.
Monooxygenases	Theorized to act on the β-carbon of the carbon chain and cleave the carbon backbone of polystyrene by incorporating one atom of oxygen.
Aromatic ring hydroxylases	Hypothesized that the alkane substrate enters a pocket of transmembrane helices that are hydrophobic, until a terminal methyl group is positioned to mimic the structure of an alcohol.

Figure 3. Function of different enzyme families in degrading polystyrene.

Proposed Enzyme: JNU01

In a paper by Kim et al., the authors identify that alkane-1-monooxygenase (AlkB) from Acinetobacter johnsonii JNU01 to be involved in polystyrene biodegradation through transcriptional analysis and bioinformatics, that was also confirmed by the study of recombinant AlkB (25).
The alkane-1 monooxygenase from Acinetobacter johnsonaii is a homotrimeric protein with three identical subunits. Each subunit consists of a transmembrane domain and a soluble domain that contains the active site. The active site is composed of a diiron center coordinated

by histidine and glutamate residues, as well as a helix bundle that forms a hydrophobic pocket for substrate binding. This specific enzyme is involved in the hydroxylation of alkanes, which is the first step in the degradation of hydrocarbons in the environment. It catalyzes the oxidation of alkanes by activating molecular oxygen, enabling their subsequent degradation and assimilation by microorganisms (13). AlkB from Acinetobacter johnsonaii interacts with alkane substrates, which are linear or branched hydrocarbons. The enzyme's active site contains a hydrophobic pocket that recognizes and binds the alkane substrate. This interaction positions the alkane molecule in close proximity to the diiron center, allowing for the activation of molecular oxygen and subsequent hydroxylation of the alkane. The hydroxylation reaction leads to the formation of an alcohol, which is then released from the enzyme's active site.

Research Aim

This research paper aims to answer the question: What is the interaction between the JNU01 AlkB enzyme and polystyrene, and what specific factors make this mechanism favorable? An enzyme kinetics simulation will be conducted utilizing the Alkane Monooxygenase B enzyme from Acineobacter Johnsonaii, in relation with the substrate, polystyrene, and will focus on the docking complex between substrate and protein. By identifying the enzymatic mechanism of polystyrene degradation, significant steps can be made to industrially manufacture this enzyme and increase its efficiency.

METHODS

Experimental Design

This research was conducted using a convergent parallel design. The simultaneous use of both qualitative and quantitative analysis allows for a docking simulation to provide insight on the energy intensity (a quantitative model of how favorable an interaction between enzyme and substrate is based on atom chemistry) of the complex, and the supporting literature allows for the feasibility of this interaction to be proposed. Data obtained from literature was used to develop the necessary components for the experiment, and qualitative research was used to propose several hypotheses and questions to be answered by the experiment. As the experiment took place, the results and output of the docking were cross-referenced with literature to check their reliability and draw final interpretations of the mechanism between the enzyme and substrate.

In order to collect data on the enzymatic interaction between polystyrene and JNU01 AlkB, AutoDock was used. AutoDock Vina is used for molecular docking and is an open-source program designed by Dr. Oleg Trott in the Molecular Graphics Lab at The Scripps Research Institute. The computational tool is used to predict how ligands, such as drug candidates or substrates bind to a receptor with a known 3D structure. This automated docking tool plays a crucial role in enzyme studies by enabling researchers to understand and visualize the interactions between enzymes and their substrates or inhibitors at the molecular level. Using PDBQT files created with MGL Tools, AutoDock Vina generates a grid field with highly favorable docking positions between substrate and enzyme. Users can adjust docking parameters, such as the search space and scoring function, to optimize the docking performance for specific

enzyme systems. AutoDockTools (ADT), the graphical user interface for AutoDock, facilitates the preparation of input files and the analysis of docking results, making the process more accessible for researchers (47).

In order to create the 3D molecule of polystyrene, Avogadro2 was used. Avogadro2 is primarily used to edit molecules and visualise them, providing significant usefulness in fields such as materials science, computational chemistry, bioinformatics, and molecular modeling. The software utilizes VTK to access additional visualization and analysis capabilities. Using input such as SMILES (Simplified Molecular Input Line Entry System) code, which translates a chemical structure into a string. Avogadro2 is able to visualize the 3D structure of a molecule within a field and output PDBQT files that can be used for further study in other software (33).

Control Group Simulation

In order to provide a control group to test the feasibility of the docking simulation between the JNU01 AlkB enzyme and polystyrene, the docking complex was compared to a known alkane enzyme (DNA/RNA repair alkane enzyme) that docked with a nucleotide and whose function has already been studied and confirmed. This enzyme was chosen due to its structural similarity to the JNU01 AlkB enzyme, shown in Figure 4, as it could provide an accurate comparison. The DNA/RNA repair enzyme AlkB interacts with nucleic acid substrates containing alkylation damage. Alkylation damage leads to the formation of methylated bases such as 1-methyladenine and 3-methylcytosine. The enzyme's active site recognizes and binds the methylated base, positioning it in close proximity to the iron-binding motif. This interaction enables the oxidative demethylation of the damaged base, restoring it to its original form. The enzyme utilizes α-ketoglutarate as a co-substrate in the reaction, which is converted to succinate and carbon dioxide during the repair process (52).

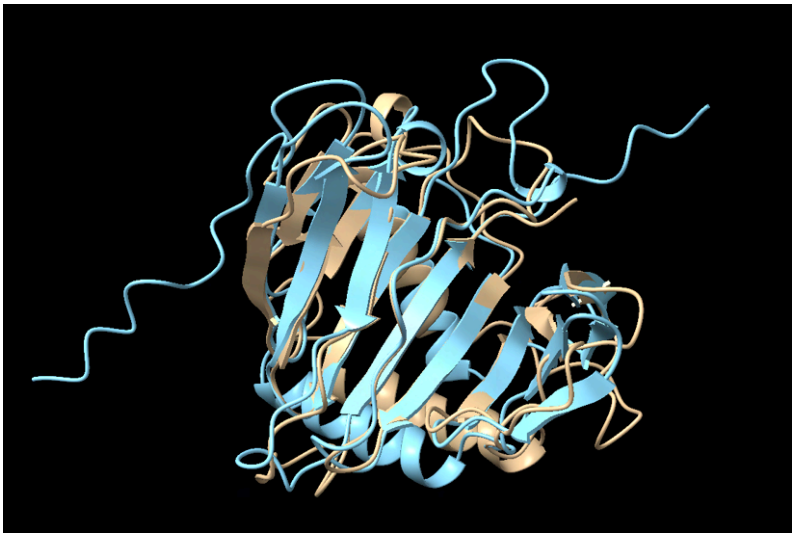


Figure 4. Aligned structures of JNU01 AlkB enzyme and DNA/RNA repair alkane enzyme.

The blue structure in Figure 4 is the JNU01 AlkB enzyme, and the beige structure in Figure 4 is the DNA/RNA repair alkane enzyme. As shown, there was apt structural similarity between the two enzymes. This figure was produced using ChimeraX.

To provide evidence of AutoDock's accuracy and compare the polystyrene complex to a known variable, the first docking procedure was conducted with AlkB in complex with Fe(II), 2-oxoglutarate, and methylated trinucleotide T-meA-T from E.Coli (PDB ID: 2FD8). The structure for the nucleotide was obtained from the RCSB file by cleaving it using ChimeraX to ensure that the sole protein structure was used, without the complex molecules, as AutoDock does not dock cofactors (7). The ligand was prepped using AutoDock. After opening the nucleotide file in the software, hydrogens were manually added and the root of the torsion tree was detected. The software added gasteiger charges, found 13 aromatic carbons, detected 16 rotatable bonds, set the TORSDOF to 16, and the molecule had a total charge error of 0.4998. The file was then saved as a PDBQT. The alkane protein was prepared accordingly and was initialized with no non-bonded atoms, 1184 non-polar hydrogens, and merged nphs. To run the single-docking experiment with AutoDock Vina, the PDBQT files saved in the preparation steps were used. Utilizing Yu et al.'s literature on the DNA/RNA protein, key amino acids (Arg 161, His 187, Arg 210, Asp 135, Trp 69, Tyr 76, and The 51) were identified for its function. Identifying their placement in the ribbon structure on ChimeraX, the GridBox was created including all critical amino acid positions. AutoDock Vina was set up and the exhaustiveness was set to 24 in the command line in order to create a more consistent docking result. After the system had run, the result was opened and exported to a PDBQT file to be opened in ChimeraX.

Simulation between JNU01 AlkB and Polystyrene

The process was repeated for the polystyrene molecule and JNU01 AlkB enzyme. The polystyrene molecule was created using Avogadro2 through the input of its chemical SMILES code ((CC(-*)C1=CC=CC=C1) and extracted the PDB file for the 3D molecule. The ligand was prepped with hydrogens and the software added gasteiger charges, found 24 aromatic carbons, detected 10 rotatable bonds, and set the TORSDOF to 10. The protein structure used was the AlphaFold prediction found on PlasticDB and was found to have no non-bonded atoms (33). The grid for the protein was set based on the location of the amino acids found in the position of the DNA/RNA alkane's place when structurally matched. These were the following: Lys 156, His 184, Arg 202, Glu 127, Tyr 76, Val 51. These amino acids shared largely the same properties of the DNA/RNA structure's amino acids. Finally, AutoDock Vina was run again with the exhaustiveness of 24 and 9 docking positions were output in a PDBQT file.

Comparison Simulations

This same process was repeated with the nucleotide and JNU01 AlkB enzyme to ensure structural consistency. The ligand was prepped the same with the exact charges and hydrogens. The grid for the protein was also likewise set. AutoDock Vina was set accordingly. Finally, the process was repeated for the polystyrene molecule and the DNA/RNA repair AlkB with the same preparation and settings.

Data Analysis

The software used for analysis was ChimeraX. UCSF ChimeraX 1.9 for macOS is a molecular visualizer software. Using input from online repositories such as Protein DataBank, Research Collaboratory for Structure Bioinformatics, and UniProt, ChimeraX can then read and write macromolecular crystallographic information files, alongside other file formats, and provide a visual of the molecule in 3D space. This includes various display styles, such as wireframe, sticks, balls and sticks, and ribbons for secondary structures. The protein can be visualized in a ribbon or atomic structure, and ChimeraX provides users with tools to study various aspects of the molecular structure such as hydrophobicity, distance between atoms, and sequence visualization. Additionally, the software provides different lighting tools, visualization styles, and coloring. ChimeraX supports docking and virtual screening tools, which can help researchers predict how enzymes and other molecules might interact. This can help identify potential inhibitors or activators of enzyme activity. The software also includes tools for analyzing protein sequences, which can be useful for understanding enzyme structure and function (48).

In order to address which amino acids were involved in the active site, ChimeraX's distance feature was utilized. Highlighting 7 amino acids involved in each ligand-protein docking, the distance from the substrate to the nearest ribbon structure was measured and recorded. In the DNA/RNA repair alkane enzyme and nucleotide complex, the amino acids identified with the closest distance to the substrate were identified as Tyr 76, Asp 133, Gln 132, Ser 129, Met 57, Leu 128, Thr 70, Gly 53, and Lys 127, which supports Yu et al.'s literature that these amino acids were crucial to protein function, and substrate activity.

Using the same feature, the amino acids in closest relation to the substrate were identified for the next two docking complexes between the nucleotide and DNA/RNA repair alkane enzyme, and the distances were recorded.

The results of the control group simulation provided support to AutoDock's ability to produce feasible docking positions. The complex with the lowest energy (most likely docking position) was compared to the identified crystalline structure from Yu et al.'s literature, and was structurally aligned. The alignment demonstrated that AutDock had placed the nucleotide in the same docking location as the crystalline structure, though the orientation of the substrate was slightly misaligned. However, the substrate remained in the same location and interacted with the same amino acids when compared to the crystalline structure, demonstrating that AutDock can produce feasible docking positions that are consistent with natural docking complexes.

RESULTS

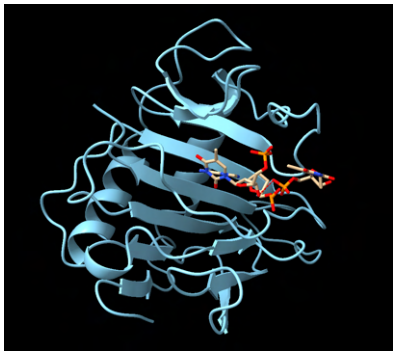
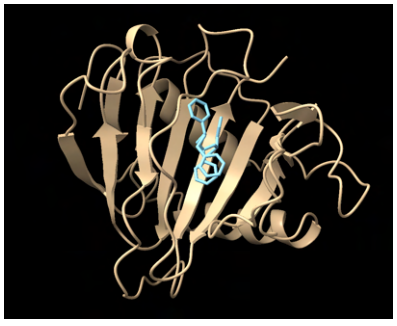
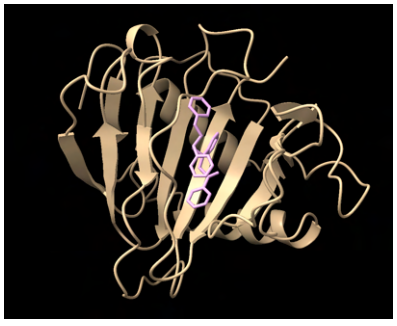
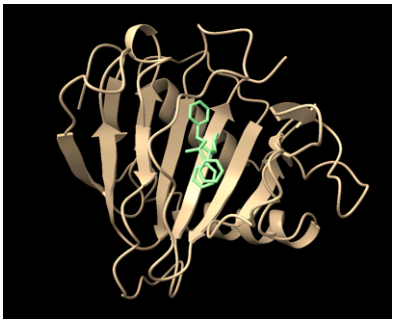
		
Energy of Conformation: -8.728 Additional Energy compared to lowest energy: 0	Energy of Conformation: -8.501 Additional Energy compared to lowest energy: 0.227	Energy of Conformation: -8.405 Additional Energy compared to lowest energy: 0.323

Figure 5. Docking complex between nucleotide and DNA/RNA alkane enzyme
The blue structure shown in Figure 5 is the 3D folding visualization of the DNA/RNA repair alkane enzyme enzyme, and the brown structure with blue and red ends is the nucleotide ligand. These images represent where in the protein the nucleotide would bind to (the active site). The nucleotide remains in the same pocket of protein ribbons, with variations only in the positions of certain molecules and orientation. The image on the far left has the lowest energy, and is therefore the most stable and feasible position. This docking complex is supported by Yu et al. 's docking of the same molecules, with only a slight variation in the orientation of the molecule.

The DNA/RNA repair alkane enzyme and nucleotide complex identified in the active site were Tyr 76, Ser 129, Asp 133, Met 57, Leu 128, Lys 127, Gln 132, Thr 70.

		
Energy of Conformation: -7.679 Additional Energy compared	Energy of Conformation: -7.397 Additional Energy compared	Energy of Conformation: -7.344 Additional Energy compared

to lowest energy: 0	to lowest energy: 0.282	to lowest energy: 0.335

Figure 6. Docking complex between polystyrene and DNA/RNA alkane enzyme.
The beige structure in Figure 6 is the DNA/RNA repair alkane enzyme protein ribbon structure. The molecule of various colors is the 3D molecule of polystyrene. The molecule remains in the same pocket throughout the different conformations, with little change in orientations. This pocket is the same pocket where the nucleotide was located in the complex with the DNA/RNA alkane. The lowest energy is -7.679, pictured in the far left picture. This is only a slight increase in energy from the complex with the nucleotide, suggesting that the docking with polystyrene remains a feasible and plausible function.

The DNA/RNA repair alkane enzyme and polystyrene complex identified in the active site were Lys 134, Asp 133, Gln 132, Leu 130, Ser 129, Leu 128, Lys 127, Gly 54, Tyr 76, and Tyr 55.

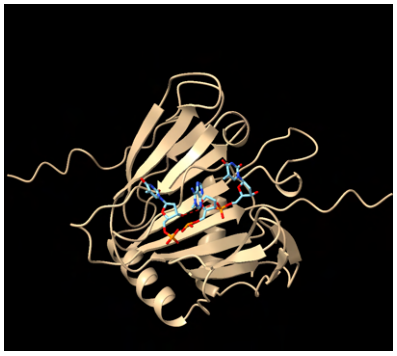
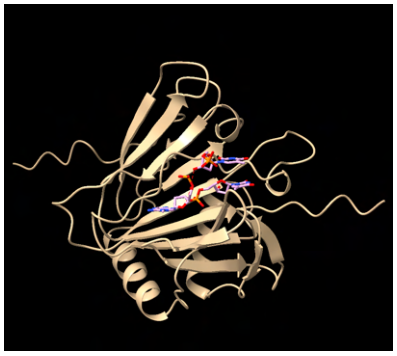
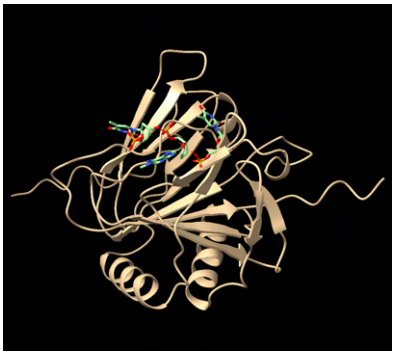
		
Energy of Conformation: -8.983 Additional Energy compared to lowest energy: 0	Energy of Conformation: -8.648 Additional Energy compared to lowest energy: 0.335	Energy of Conformation: -8.504 Additional Energy compared to lowest energy: 0.479

Figure 7. Docking complex between nucleotide and JNU01 AlkB enzyme

The beige structure in Figure 7 is the protein folding of the JNU01 AlkB enzyme, and the molecule with various colors and red and blue ends is the nucleotide. The molecule remains in the same pocket of protein ribbon with variations in orientation. The lowest energy is -8.983, which is slightly lower than the energy for the complex with the DNA/RNA repair alkane enzyme, suggesting a highly plausible function and stable position.

The JUN01 and nucleotide complex identified the amino acids in the active site to be Glu 61, Val 59, Tyr 58, Val 51, Glu 61, Val 120, Trp 122, Ser 124, Tyr 76, Asp 126, Thr 60, Leu 53, and Gly 121.

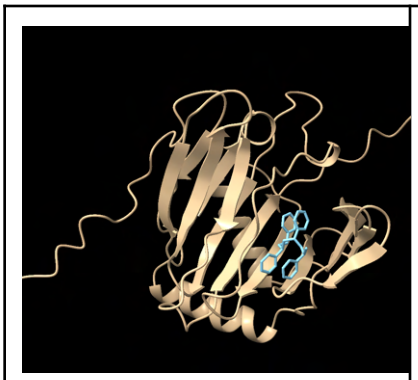
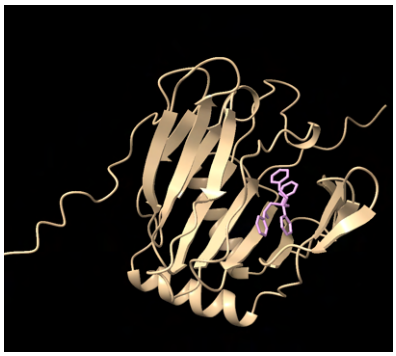
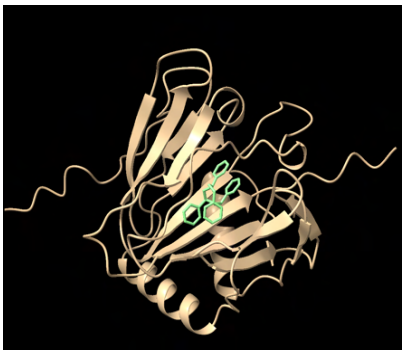
		
Energy of Conformation: -6.896 Additional Energy compared to lowest energy: 0	Energy of Conformation: -6.689 Additional Energy compared to lowest energy: 0.207	Energy of Conformation: -6.443 Additional Energy compared to lowest energy: 0.453

Figure 8. Docking complex between polystyrene and JNU01 AlkB enzyme
The beige structure in Figure 8 is the structure of the JNU01 AlkB enzyme. The molecule of various colors is the 3D structure of polystyrene. The molecule remains in the same pocket of protein ribbon, and is located in the same pocket that the nucleotide was located within this protein. There are slight changes in orientation between each structure. The lowest energy is -6.896, which is higher than the docking with the nucleotide and higher than the docking between the DNA/RNA repair alkane enzyme and polystyrene.

In the JNU10 AlkB enzyme and polystyrene complex, the amino acids identified to be in closest relation to the substrate were Ala 119, Val 120, Thr 60, Glu 50, His 123, Val 51, Tyr 58.

Distances between Amino Acids and Ligand

Analyzing the distance between particular amino acids and the ligand provides insight into which direct molecules are in activity with the substrate. By identifying the amino acids in closest relation, speculation into the mechanism can be aided by including the properties of the specific amino acids identified.

The following lists the specific amino acid within the protein, the atom in the substrate it is being compared to, and the distance between the two atoms in Angstroms. Distances less than 8 Angstroms suggest that the amino acid is active in contact. Distances less than 4 Angstroms allow for Van der waals forces to occur.

DNA/RNA repair alkane enzyme Amino Acid:	Nucleotide Atom:	Distance (in Angstroms):
Tyr 76	B DT 501 0P1	3.221

Ser 129	B DT 503 0P2	3.323
Asp 133	MA7 502 CN	3.769
Met 57	MA7 502 03’	3.809
Leu 128	B DT 503 0P1	4.124
Lys 127	B DT 503 03’	5.843
Gln 132	B DT 501 04	7.187

Figure 9. Distances between DNA/RNA repair alkane enzyme and nucleotide

DNA/RNA repair alkane enzyme Amino Acid:	Polystyrene Atom:	Distance (in Angstroms):
Ser 129	UNL 1 C	3.923
Leu 128	UNL 1 C	4.304
Tyr 76	UNL 1 C	4.373
Gln 132	UNL 1 C	4.506
Asp 133	UNL 1 C	4.639
Lys 134	UNL 1 C	5.739
Lys 127	UNL 1 C	6.580
Tyr 55	UNL 1 C	7.448

Figure 10. Distances between DNA/RNA repair alkane enzyme and polystyrene

JNU01 AlkB enzyme Amino Acid:	Nucleotide Atom:	Distance (in Angstroms):
Tyr 76	B DT 501 0P1	2.388
Thr 60	B MA7 502 0P2	3.505
Val 51	A Glu 61 0E1	3.692
Val 120	B DT 503 0P2	3.756
Thr 60	B MA7 502 03’	4.266

Ser 124	B MA7 502 0P1	4.314
Val 59	B DT 503 02	4.699
Asp 126	B DT 503 03’	5.868
Trp 122	B DT 501 02	6.086
Leu 53	B DT 503 0P2	7.564

Figure 11. Distances between JNU01 AlkB enzyme and nucleotide

JNU01 AlkB enzyme Amino Acid:	Polystyrene Atom:	Distance (in Angstroms):
Val 51	UNL 1 C	3.424
Val 120	UNL 1 C	3.573
Thr 60	UNL 1 C	4.222
Ala 119	UNL 1 C	4.993
His 123	UNL 1 C	5.399
Tyr 58	UNL 1 C	7.226

Figure 12. Distances between JNU01 AlkB enzyme and polystyrene

DISCUSSION

Analysis

Based on the similar amino acids in contact with the substrate (Figures 8 and 9), both polystyrene and the nucleotide dock in the same location within the DNA/RNA repair alkane. This supports that the two substrates share chemical and structural similarity, as they can interact similarity with the same enzyme.

In the same manner, the JNU01 AlkB enzyme had many of the same amino acids acting on polystyrene and the nucleotide. This overlap in amino acids provides evidence that the JNU01 AlkB enzyme could potentially interact with both a nucleotide and polystyrene as their chemical properties lead them to bind in similar sites.

Additionally, comparing both Figures 9 and 11, both enzymes displayed that similar amino acids interacted with the nucleotide, supporting the idea that the JNU01 AlkB enzyme could potentially function on a nucleotide. Furthermore, this suggests that the JNU01 AlkB enzyme potentially evolved from its original function of repairing DNA and RNA to degrading polystyrene as the environment surrounding the microorganism changed.

After redocking the DNA/RNA repair alkane enzyme with the nucleotide, and comparing the complex with Yu et al.'s crystalline structure, it could be determined that AutoDock provides feasible docking complexes. Furthermore, since the DNA/RNA repair alkane enzyme was able to interact with the polystyrene molecule, this supports the idea that this family of enzymes could potentially degrade polystyrene due to its structural similarity to a nucleotide as both polymers contain aromatic rings.

The amino acids (shown in Figure 9 and 10 above) largely do not fluctuate between the nucleotide and polystyrene docking, save for a few outliers, which suggests that the AlkB family of enzymes could potentially evolve into a class that can interact and degrade polystyrene.

Among the shared amino acids between both enzymes, there are three that can provide insight into a potential degradation mechanism: Tyrosine, Threonine, and Histidine.

More specifically, the Tyr found in this complex has been identified to interact with the backbone and H-bonding center within the nucleotide recognition lid in the DNA/RNA repair alkane enzyme. The presence of this amino acid also in the JNU01 AlkB enzyme suggests activity on the backbone (main) chain of polystyrene, in which the protein would directly target the breaking of the carbon-carbon backbone chain of the polymer. Although polar, the aromatic benzene ring in Tyr's side chain can engage in π-π stacking interactions with aromatic rings, and the hydroxyl group can form hydrogen bonds with polar groups. It can also participate in hydrophobic interactions with nonpolar carbon atoms (44).

Threonine (Thr) is another amino acid found to be crucial to the function of the DNA/RNA repair alkane enzyme, and was one of the amino acids identified in the polystyrene/JNU01 AlkB enzyme complex. Working on a nucleotide, Thr was identified to be involved in backbone bonding, where it was in a network with two phosphates 5' 0' to the alkylated adenine base. This further provides evidence that JNU01 AlkB enzyme likely acts on the backbone chain of the polystyrene molecule (43).

Histidine (His) is also a shared amino acid found in both proteins, that appears later down in the protein sequence of both enzymes. In the DNA/RNA repair alkane enzyme, His is identified to bind iron dioxygenase and 2OG in through the 2-keto and 1-carboxylate oxygens. This suggests that the cofactor Fe(II), 2-oxoglutarate, which is a speculated cofactor by Kim et al., is likely involved in the degradation of polystyrene.

The presence of these specific amino acids within the active site of the JNU01 AlkB enzyme suggest the mechanism of backbone cleavage, through which the protein would directly depolymerize the polystyrene molecules, by breaking the carbon-carbon chains, into styrene monomers, which are then naturally degradable through internal catabolic processes. Hou & Majumder support that alkane monooxygenases likely break the polymer chain through the formation of alcohols. They list that the alkane monooxygenase LadA from *Geobacillus thermodenitrificans* NG80-2 acts on long chain alkanes, as well as monooxygenases from *Pusillimonas* sp. strain T7-7, and from the *Acinetobacter* and *Alcanivorax* genera (15). Because the polystyrene main chain is a substituted alkane, Hou & Majumder hypothesize that alkane

monooxygenases are able to break the chain apart. Additionally, because the main chain is composed of C-C and C-H bonds, they are weaker than the C=C bonds and easier to cleave (15).

While prior research has not been conducted to confirm these results of this study, the docking of the DNA/RNA repair alkane enzyme and the nucleotide, as well as the identified amino acids in the complex support the literature and studies of its structure, function, properties, and mechanisms by Yu et al., suggesting the plausibility of this computational research.

Limitations

It is important to note that this research is completed entirely computationally. The real life applications of this study are limited by the fact that most enzymes do not work in isolation, and are usually part of enzyme complexes that work on the substrate simultaneously to completely degrade it. Thus, it is likely that there are other enzymes and proteins that are used in nature to completely degrade polystyrene, that are working alongside JNU01 AlkB enzyme to degrade it that were not identified in literature or in wet lab procedures due to complexity in isolating these individual proteins. The same would be true for cofactors, as many microbial organisms contain cofactors within their gut bacteria to aid in protein function.

This study is limited by the software's inability to dock cofactors, which are speculated to have an impact on aiding the JNU01 AlkB enzyme in degrading polystyrene by potentially catalyzing the hydroxylation of polystyrene by introducing hydroxyl groups onto the polymer chain, which could potentially lead to further degradation or modification of the polymer.

Additionally, the structure used for the JNU01 AlkB enzyme was obtained as an AlphaFold prediction, which introduces inaccuracies of the structure itself. However, when structurally matched, there was an apt similarity between JNU01 AlkB enzyme and the DNA/RNA repair alkane enzyme.

Future research

Further computational research can be conducted by performing the simulation in water, which would test the stability of the active site and demonstrate the docking in its natural environment. Additionally, studies focusing on cofactors interacting with the dynamics of the structure could also provide further insight into the exact mechanism involved.

To confirm the results of this docking procedure, as well as the speculated mechanism, in vitro and in vivo experiments could be used to provide confirmation. These experiments would aim to verify the JNU01 AlkB enzyme's polystyrene degradation capabilities in controlled laboratory settings. By isolating the enzyme and observing its interaction with polystyrene under various conditions, researchers could better understand the JNU01 AlkB enzyme's specific mechanisms and requirements for optimal activity. This would provide valuable insights into the enzyme's potential for real-world applications.

After confirming the capability of the enzyme to degrade polystyrene, enzymatic engineers could then work to improve its efficiency by further introducing strands of the amino acid sequences found in the active site more readily throughout the protein. Utilizing techniques such as adaptive laboratory evolution (ALE), site directed mutagenesis, and rational design, researchers could potentially improve the JNU01 AlkB enzyme's ability to degrade polystyrene (11, 3). This may involve altering the enzyme's structure or function to increase its stability, substrate specificity, or catalytic efficiency. A more efficient enzyme could lead to faster and more effective polystyrene degradation in environmental remediation efforts. Research could also investigate the effects of amplifying the DNA sequence identified in the active site through PCR, and introducing the sequence into other bacteria and microorganisms through transformation.

This opens the door to exploring JNU01 AlkB enzyme's capabilities, and researchers could explore the potential for employing the enzyme in bioremediation strategies to tackle plastic pollution. This could involve the use of enzyme-producing microorganisms or direct application of the enzyme in affected environments. A successful bioremediation strategy would provide a sustainable solution to mitigate the harmful impacts of plastic waste.

The identification of the mechanism through which polystyrene degrades naturally through an enzyme has far-reaching implications for waste management and sustainable materials development. It could help shift the focus from single-use plastics to biodegradable alternatives, contributing to the transition towards a circular economy. This discovery could also pave the way for more effective environmental policies and initiatives addressing plastic pollution. By identifying the mechanism of degradation, significant steps can be made in the effort to reduce plastic pollution and push progress towards the Sustainable Development Goals, specifically SDG 12 which promotes sustainable consumption.

CONCLUSION

Polystyrene, otherwise known as styrofoam, has become an integral part of consumerism because of its economic convenience. However, because the material is not biodegradable, polystyrene breaks into microplastics which build up in and contaminate environments, and inhibit bloodstreams through indirect ingestion. This paper attempted to identify the mechanism through which an identified enzyme is able to break down polystyrene into natural, organic components of carbon and hydrogen. This was conducted through a computational docking simulation between the JNU01 AlkB enzyme and a molecule of polystyrene, and comparing the results to the docking complex between the DNA/RNA repair alkane enzyme and a nucleotide. After completion of the docking, the most probable mechanism of degradation was identified to be through backbone cleavage in which the enzyme would depolymerize polystyrene by acting on the carbon-carbon main chain. With an identification of the mechanism by which the JNU01 AlkB enzyme degrades polystyrene, the enzyme could be engineered to increase its efficiency. This enzyme could then be industrially manufactured and released into the environment as a proactive solution to mitigate polystyrene pollution.

While previous research has identified the ability of JNU01 AlkB enzyme to degrade polystyrene through wet lab procedures by identifying the rate of molecular weight change, research has not identified the enzymatic mechanism of this process (25). This paper set out to bridge these

knowledge gaps by using a computational approach to identify the specific mechanism of polystyrene degradation activity by the JNU01 AlkB enzyme.

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DECLARATION OF CONFLICT OF INTERESTS

The author declares that there are no conflicts of interest regarding the publication of this article.

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