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IN SILICO MOLECULAR DOCKING ANALYSIS OF *PSEUDOMONAS PUTIDA* CATECHOL 2, 3-DIOXYGENASE FOR POLYCYCLIC AROMATIC HYDROCARBON (PAH) DEGRADATION

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Abstract

Polycyclic Aromatic Hydrocarbons (PAHs) are persistent environmental pollutants known for their carcinogenic and mutagenic effects, presenting serious threats to human health and ecological balance. Despite the established role of catechol 2,3-dioxygenase (C23O) in PAH degradation, its binding interactions with high-molecular-weight PAHs remain inadequately understood. This study aims to computationally analyze the structural and functional interactions between catechol 2,3-dioxygenase from Pseudomonas putida and several toxic PAHs to explore its bioremediation potential. The enzyme structure was predicted using AlphaFold 3 and validated by Ramachandran plot analysis, confirming a stable conformation. Pollutant structures were retrieved from PubChem, and molecular docking was conducted using CB-dock2. Among the tested pollutants, indeno[1,2,3-cd]pyrene exhibited the strongest binding affinity (–7.8 kcal/mol), followed by benzo[b]fluoranthene (–7.7 kcal/mol) and dibenz[a,h]anthracene (–7.6 kcal/mol). Key residues involved in binding interaction of indeno[1,2,3-cd]pyrene and enzyme included Phe218, Ala198, Arg150, Lys197, Asp152, Phe267, His199, Gly270, Asp271, Gly269, and Tyr271. The interactions were primarily stabilized by hydrogen bonding, van der Waals forces, π–π stacking, and π-alkyl interactions. Toxicological analysis confirmed the high environmental and health risks associated with these PAHs. These findings highlight catechol 2,3-dioxygenase as a promising enzymatic candidate for the biodegradation of high-molecular-weight PAHs. However, in vitro and in vivo validations are essential to confirm its functional performance under real environmental conditions.

Keywords: *AlphaFold, Bioremediation, Catechol 2,3-dioxygenase, Environmental pollution, Molecular docking, Pseudomonas putida, Polycyclic aromatic hydrocarbons (PAHs)*

INTRODUCTION

Polycyclic Aromatic Hydrocarbons (PAHs) are a widespread group of organic contaminants that pose serious environmental and health risks due to their toxic, mutagenic, and carcinogenic properties (1). These compounds are commonly introduced into the environment through the burning of fossil fuels, industrial emissions, vehicle exhaust, and even natural events like forest fires. Because of their stable chemical structure and hydrophobic nature, PAHs tend to persist in soil and aquatic systems, where they can accumulate and enter the food chain. The Environmental Protection Agency (EPA) in the United States has identified 16 priority PAHs that are frequently monitored due to their hazardous effects (2).

While several chemical and physical methods exist to remediate PAH-contaminated environments, they often come with high costs and potential risks of generating secondary pollutants (3). As a result, there has been growing interest in bioremediation using microorganisms to naturally break down pollutants into less harmful products (4, 5). Among the bacterial species known for their biodegradation capabilities, *Pseudomonas putida* stands out for its adaptability and ability to metabolize a wide variety of harmful

compounds. Catechol 2,3-dioxygenase is a crucial enzyme that plays a central role in the degradation of PAHs by *Pseudomonas putida* (6). This enzyme plays an essential role in the microbial breakdown of aromatic rings through what's known as the meta-cleavage pathway (7). It acts on catechol, a central intermediate in PAH degradation, transforming it into smaller, more manageable compounds that can be further metabolized. The gene encoding this enzyme, nahH, is part of the well-studied NAH7 plasmid, which contributes to the bacterium's efficiency in degrading complex hydrocarbons (8).

Although catechol 2,3-dioxygenase has been explored in several studies, most existing data focus on general degradation mechanisms rather than detailed interactions with high-molecular-weight PAHs. There remains a significant gap in understanding how this enzyme binds and interacts with specific, structurally complex PAHs (9). This limits the ability to fully evaluate its practical effectiveness in diverse environmental conditions and restricts the development of targeted bioremediation strategies.

This study was designed to explore how catechol 2,3-dioxygenase from *Pseudomonas putida* interacts with environmentally persistent PAHs, using a computational approach. By predicting the enzyme's structure and its interactions with various toxic PAHs, we aim to better understand its potential role in bioremediation strategies. This finding may serve as a preliminary step for future laboratory and field-based studies, offering insights into how this enzyme can be harnessed to address local and global pollution challenges.

MATERIALS AND METHODS

RETRIEVAL OF TARGET ENZYME SEQUENCE AND FUNCTIONAL ANNOTATIONS

FASTA sequence of target protein relevant to catechol 2,3-dioxygenase from *Pseudomonas putida* was retrieved using NCBI's protein database https://www.ncbi.nlm.nih.gov/ (10). Functional annotation was performed using UniProt-GO https://geneontology.org/docs/go-enrichment-analysis to categorize functional protein domains and biological roles associated with environmental biodegradation pathways.

PHYSICOCHEMICAL ANALYSIS

Physicochemical characterization of catechol 2,3-dioxygenase was performed using the Expasy ProtParam tool https://web.expasy.org/protparam/ . Parameters analyzed included molecular weight, theoretical isoelectric point (pI), amino acid composition, instability index, aliphatic index, and GRAVY score. These parameters helped determine the enzyme's overall stability, hydro-philicity, and suitability for environmental applications (11).

STRUCTURE PREDICTION AND VALIDATION

Secondary structure was predicted by employing PSIPRED tool (12). The 3D structure of catechol 2,3-dioxygenase was modeled using AlphaFold 3 (https://alphafold.ebi.ac.uk/ (13) and validated using the MolProbity server http://molprobity.biochem.duke.edu/ . Ramachandran plot analysis was used to determine stereo chemical quality, with a majority of residues observed in favored regions and no significant outliers (14).

RETRIEVAL OF PAHS

Tertiary structures of PAHs were retrieved from PubChem database (https://pubchem.ncbi.nlm.nih.gov/). The selected compounds include naphthalene, anthracene, fluoranthene, pyrene, benzo[a]pyrene, chrysene, benzo[b]fluoranthene, indeno[1,2,3-cd]pyrene, and dibenz[a,h]anthracene. Their chemical properties, molecular structures, and compound IDs were carefully documented. These PAHs were chosen due to their significant environmental relevance as common and persistent organic pollutants (15).

VIRTUAL SCREENING AND MOLECULAR DOCKING ANALYSIS

PyRx software was utilized for virtual screening of all compounds, while CB Dock2 (https://cadd.labshare.cn/cb-dock2/index.php) was utilized for cavity detection and pocket-based docking

prediction. Protein-ligand binding interactions were visualized using Discovery Studio to analyze different binding interaction such as hydrogen bonds, hydrophobic interactions, and van der Waals forces (16).

TOXICITY ANALYSIS

ProTox-III (https://tox.charite.de/protox3/) is an online tool that predicts the toxicity of chemical compounds by analyzing their structures, specifically using ligand SMILES input. It employs advanced machine learning models to estimate a range of toxicity endpoints such as acute toxicity, hepatotoxicity, carcinogenicity, mutagenicity, and cytotoxicity. This allows researchers to efficiently assess the potential harmful effects of compounds on different body organs and overall safety (17).

MOLECULAR DYNAMIC SIMULATION

I-MODS server (https://imods.iqf.csic.es/) was employed to conduct molecular dynamics simulations of the protein-ligand complexes. This web tool predicts the stability and flexibility of the protein structure. It also identifies the key interaction sites where the ligand binds to the protein. By analyzing these interactions, insights into binding affinity and complex stability were obtained (18).

RESULTS

SEQUENCE RETRIEVAL AND FUNCTIONAL ANNOTATION OF CATECHOL 2,3-DIOXYGENASE AND ASSOCIATED POLYCYCLIC AROMATIC HYDROCARBONS (PAHS)

Catechol 2,3-dioxygenase enzyme of *Pseudomonas putida* was retrieved from NCBI database with accession number BAJ06512 and annotated using UniProt and Gene Ontology databases for functional characterization. Functional annotation confirmed the role of this enzyme in aromatic hydrocarbon degradation, specifically in the cleavage of catechol via the meta-cleavage pathway into less toxic intermediates. Table I depicts the pollutant, its source, toxicity, and other commonly associated PAHs subjected to biodegradation studies using *Pseudomonas putida.*

Table I. Functional Annotation of Catechol 2,3-Dioxygenase and Associated Polycyclic Aromatic Hydrocarbons (PAHs)

Pollutant	Source/Use	Toxicity/Concern
Phenanthrene	Derived from PAHs	Persistent, carcinogenic, mutagenic
Catechol 2,3-dioxygenase	Enzyme from Pseudomonas putida	Breaks down PAHs into less harmful compounds
Gene	nahH (on plasmid NAH7)	Codes for catechol 2,3-dioxygenase
Accession Number	Available on NCBI Gene	Complete coding sequence (cds)
Naphthalene	Mothballs, tobacco smoke, vehicle exhaust	Toxic, air pollutant
Anthracene	Dye production	Toxic to aquatic life
Fluoranthene	Incomplete combustion of fuels	Possible mutagen
Pyrene	Grilled food, coal tar	Moderate carcinogen
Benzo[a]pyrene	Cigarette smoke, charred meat	Highly carcinogenic
Chrysene	Fossil fuels, wood smoke	Suspected carcinogen
Benzo[b]fluoranthene	Burning coal, oil, gasoline	Hazardous air pollutant
Indeno[1,2,3-cd]pyrene	Diesel exhaust	Persistent organic pollutant
Dibenz[a,h]anthracene	Soot, tobacco smoke	Very toxic, persistent in the environment

SECONDARY STRUCTURE PREDICTION

The secondary structure of the catechol 2,3-dioxygenase enzyme, predicted using PSIPRED, indicates a well-organized arrangement consisting of 55 alpha-helices (pink), 111 beta-strands (yellow), and 141 coils (grey) out of a total of 307 amino acids. This balanced distribution of structural elements suggests a stable and functionally active conformation, essential for the enzyme's role in the degradation of polycyclic aromatic hydrocarbons (PAHs).

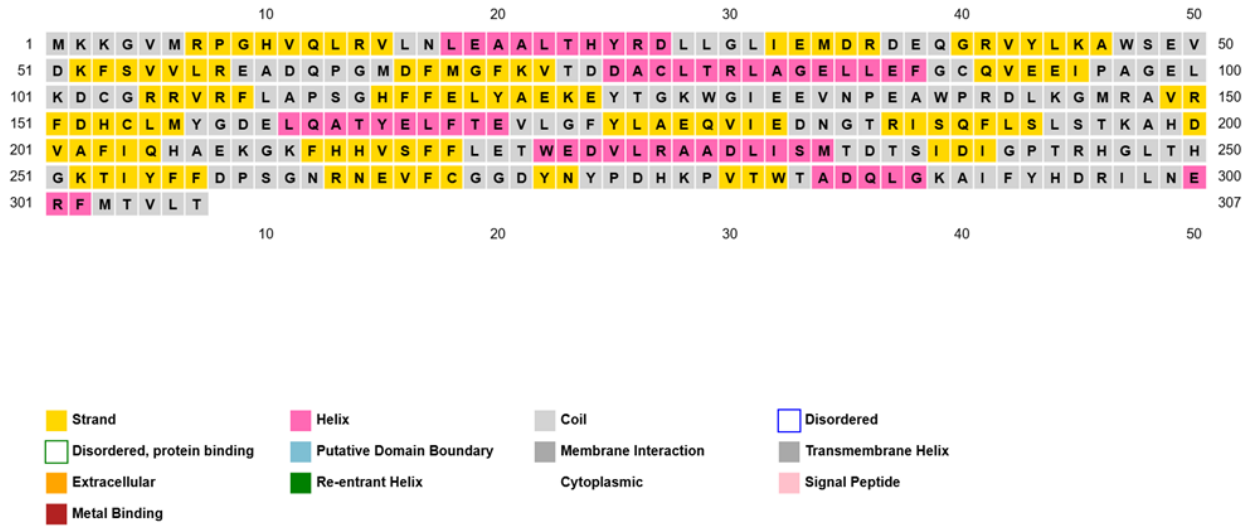


Fig. 1. Secondary structure of catechol 2,3-dioxygenase enzyme

TERTIARY STRUCTURE PREDICTION AND VALIDATION

The tertiary structure of the catechol 2,3-dioxygenase enzyme was predicted using AlphaFold3, resulting in a high-quality three-dimensional model as shown in Fig. 2a. To ensure the accuracy and reliability of this predicted structure, its stereochemical properties were assessed using the MolProbity Ramachandran plot viewer (Fig. 2b). The validation results were very promising, with over 98% of amino acid residues falling within the favored regions of the plot. Importantly, no outlier residues were detected, which strongly supports the overall structural integrity of the model. These findings indicate that the predicted structure is both accurate and dependable, making it well-suited for detailed structural analyses and functional characterization in future studies. This validated model can serve as a solid foundation for understanding enzyme mechanisms or for designing targeted experiments such as molecular docking and molecular dynamic simulation.

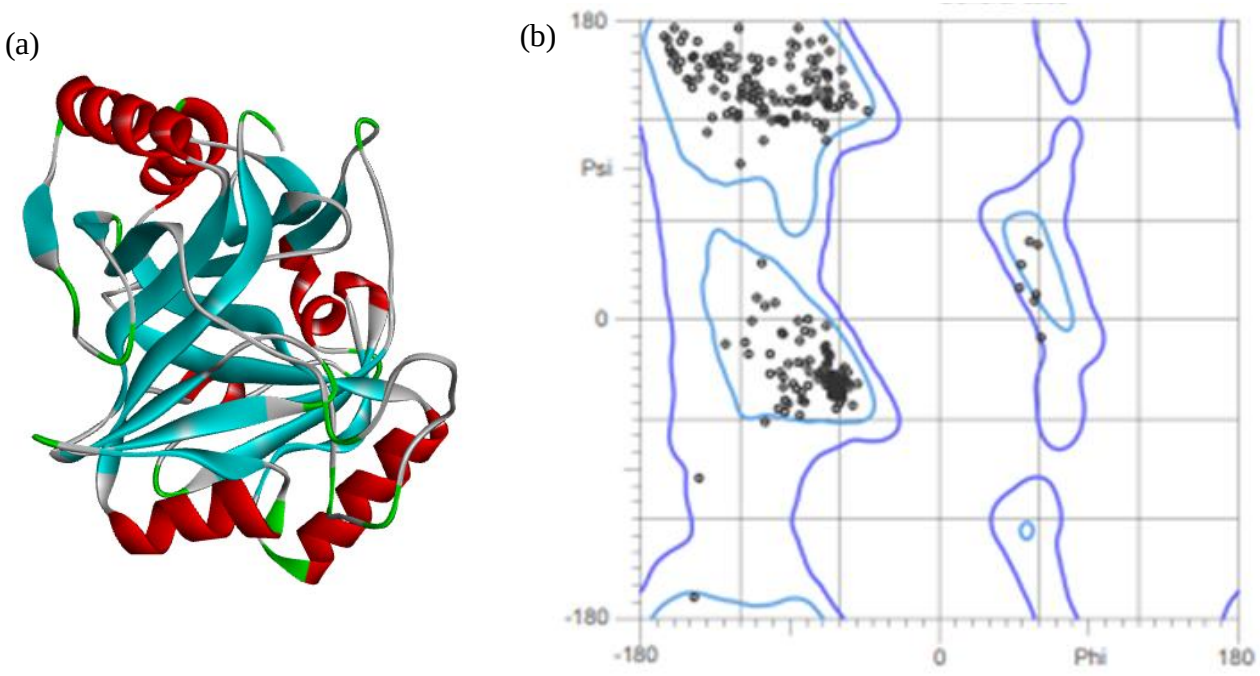
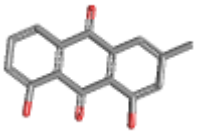
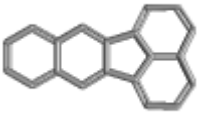
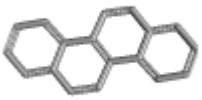
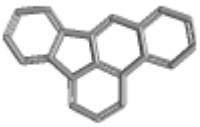
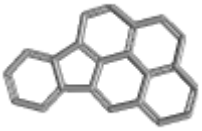
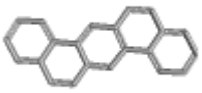


Fig. 2 (a). Tertiary structure of catechol 2, 3-dioxygenase enzyme; (**b).** Ramachandran plot predicted by molprobity

RETRIEVAL OF POLLUTANTS FROM PUBCHEM

Tertiary structures of different Poly Aromatic Hydrocarbons were retrieved from the PubChem database in SDF file format for in silico analysis. Table II lists the selected pollutants along with their Compound IDs, molecular weights (MW), and availability of 3D structures.

Table II. Retrieval of pollutants from PubChem database

Serial No.	Pollutants	Compound ID	MW	3D structure
1	Naphthalene	931	128.169 g/mol	
2	Anthracene	10208	254.24 g/mol	
3	Fluoranthene	9158	252.3 g/mol	
4	Pyrene	31423	202.25 g/mol	
5	Benzo[a]pyrene	2336	252.3 g/mol	
6	Chrysene	9171	228.3 g/mol	
7	Benzo[b]fluoranthene	9153	252.3 g/mol	
8	Indeno[1,2,3-cd]pyrene	9131	276.3 g/mol	
9	Dibenz[a,h]anthracene	5889	278.3 g/mol	

PHYSICOCHEMICAL PROPERTIES ANALYSIS OF ENZYME

The catechol 2, 3-dioxygenase enzyme consists of 307 amino acids and possesses a molecular weight of approximately 34.6 kDa. It has 49 negatively charged (Asp + Glu) and 34 positively charged (Arg +

Lys) residues. The instability index was below 40 (27.12), suggesting the protein is stable, If the value exceeds 40, it indicates that the protein is unstable. The aliphatic index of the protein was calculated to be 80.03. This relatively high value suggests that the protein is likely to be stable over a wide temperature range. The protein exhibited a GRAVY value of -0.319, indicating it is hydrophilic and likely soluble in aqueous environments. In contrast, proteins with positive GRAVY values tend to be more hydrophobic, which suggests reduced water solubility and a preference for interacting with lipid membranes or nonpolar environments.

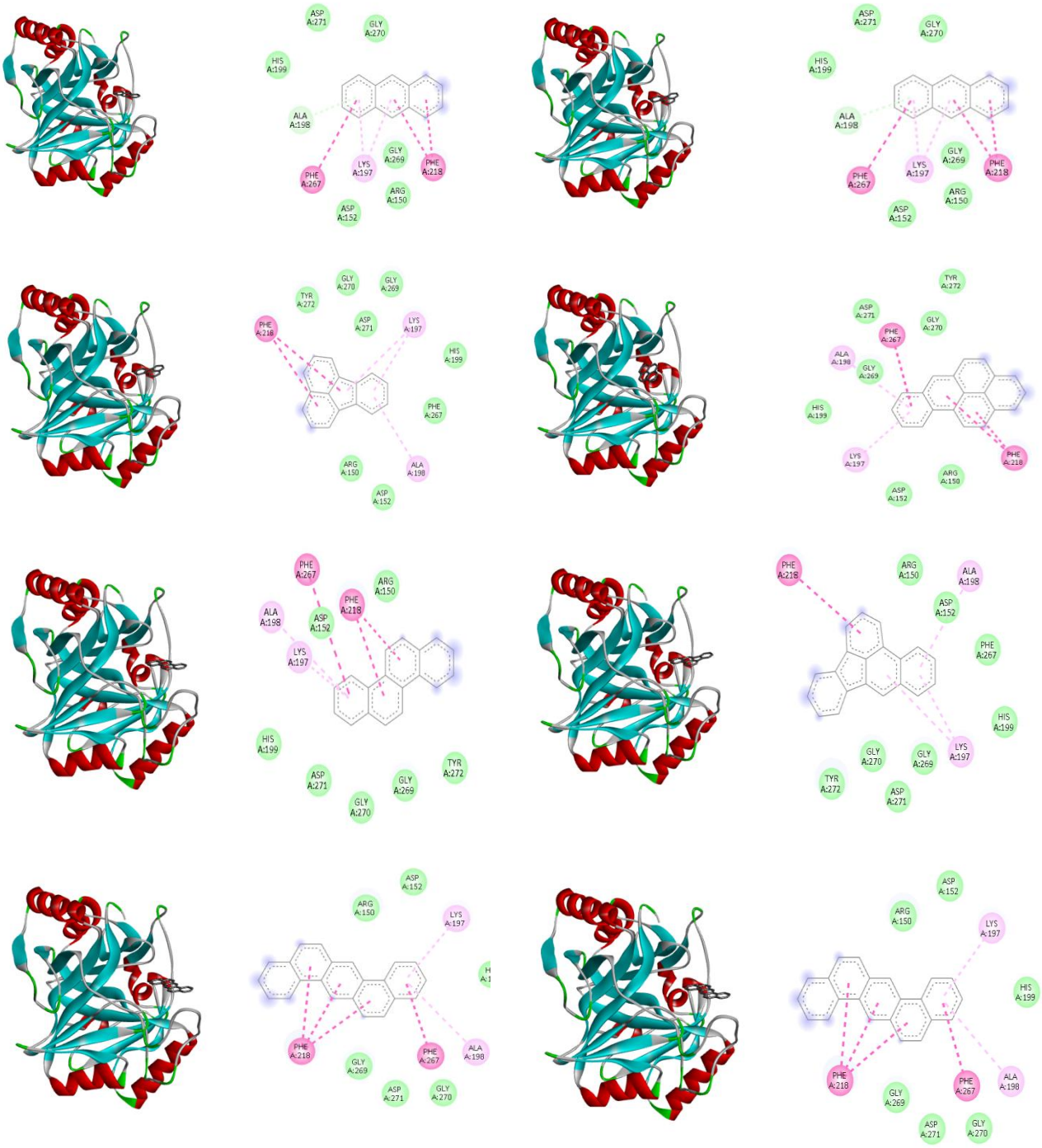


Fig. 4 (a). Dock Complex and 2D interaction of Naphthalene; **(b).** Dock Complex and 2D interaction of Anthracene; **(c).** Dock Complex and 2D interaction of Fluoranthene; **(d).** Dock Complex and 2D interaction of Benzo [a]pyrene; **(e).** Dock Complex and 2D interaction of Chrysene; **(f).** Dock Complex and 2D interaction of Benzo[b]fluoranthene; **(g).** Dock Complex and 2D interaction of Indeno[1,2,3-cd]pyrene; **(h).** Dock Complex and 2D interaction of Dibenz [a,h]anthracene

MOLECULAR DOCKING AND INTERACTION ANALYSIS

CB-dock 2 was utilized to perform molecular docking between catechol 2, 3-dioxygenase and a set of selected polycyclic aromatic hydrocarbons (PAHs). Among the tested compounds, indeno [1,2,3-

cd]pyrene exhibited the strongest binding affinity, with a docking score of -7.8 kcal/mol. Analysis of the binding interactions revealed that several key active site residues of enzymes were involved in stabilizing the complex, including PHE218, ALA198, ARG150, LYS197, ASP152, PHE267, HIS199, GLY270, ASP271, GLY269, and TYR271. The enzyme and pollutant engaged in multiple types of interactions such as van der Waals forces, Pi-Pi stacking, Pi-Alkyl interactions, and hydrogen bonding, all contributing to a stable and specific binding within the enzyme's active site. These interactions highlight the important roles of aromatic and charged residues in facilitating strong ligand binding, which could be critical for the enzyme's function in degrading PAHs. The results of the docking analysis, including binding energies, interacting residues, and types of bond interactions depicting in Fig. 4. Table Supplementary I has been showing the binding energies of all pollutants.

TOXICITY PREDICTION

Toxicological profiles of selected PAHs were predicted using the ProTox-II online tool. The tool estimates organ-specific toxicities, including hepatotoxicity, nephrotoxicity, neurotoxicity, and carcinogenic potential based on chemical structure and known toxicophore markers (Table III).

Table III. Toxicity prediction of pollutants

Pollutants	Hepatotoxicity	Neurotoxicity	Nephrotoxicity	Respiratory toxicity	Cardiotoxicity
Naphthalene	Active	Active	Inactive	Active	Inactive
Anthracene	Active	Active	Inactive	Active	Inactive
Fluoranthene	Active	Active	Inactive	Active	Inactive
Pyrene	Active	Active	Inactive	Active	Inactive
Benzo[a] pyrene	Active	Active	Inactive	Active	Inactive
Chrysene	Active	Active	Inactive	Active	Inactive
Benzo[b] fluoranthene	Active	Active	Inactive	Active	Inactive
Indeno[1,2,3-cd] pyrene	Active	Active	Inactive	Active	Inactive
Dibenz[a,h] anthracene	Active	Active	Inactive	Active	Inactive

MOLECULAR DYNAMICS (MD) SIMULATION

Molecular dynamics (MD) simulation was conducted to evaluate the structural stability and dynamic behavior of the catechol 2,3-dioxygenase–pollutant complex. The simulation helped in determining the flexibility, stability, and interaction persistence of the protein-ligand system (Fig. 5a). The main-chain deformability analysis revealed high flexibility at specific residue positions, indicating potential hinge regions that may play a role in molecular motion and structural transitions (Fig. 5b). Experimental and theoretical B-factors were analyzed, with the theoretical values derived from Normal Mode Analysis (NMA) (Fig. 5c) using the equation $B = (8\pi^2) \times$ mobility. These results showed a consistent pattern of atomic displacement comparable to experimental observations, validating the model's dynamic behavior. The eigenvalue (Fig. 5d) associated with the first few normal modes was found to be low, suggesting that minimal energy is required for structural deformation, thereby highlighting the intrinsic flexibility of the protein-ligand complex. Correspondingly, the variance (Fig. 5e) analysis showed a high degree of movement in the lower modes, with red bars indicating individual variances and green bars depicting cumulative motion contributions, reflecting the dominant role of these modes in the overall dynamics. Further insight into residue interactions was obtained through the covariance map (Fig. 5f), which displayed

a mix of correlated (red), uncorrelated (white), and anti-correlated (blue) residue pair motions, indicating coordinated structural rearrangements throughout the simulation. The elastic network model (Fig. 5g) helped identify inter-residue connections, with stiffness values visually represented by grayscale dots—darker shades denoting stiffer connections. This model confirmed a dense and rigid inter-residue connectivity around the protein core, with relatively flexible loops and terminal regions. Together, these simulation outputs confirm that the protein maintains a dynamic yet stable conformation in the presence of the bound pollutant, with critical residues exhibiting stable interactions while allowing necessary flexibility for potential functional transitions.

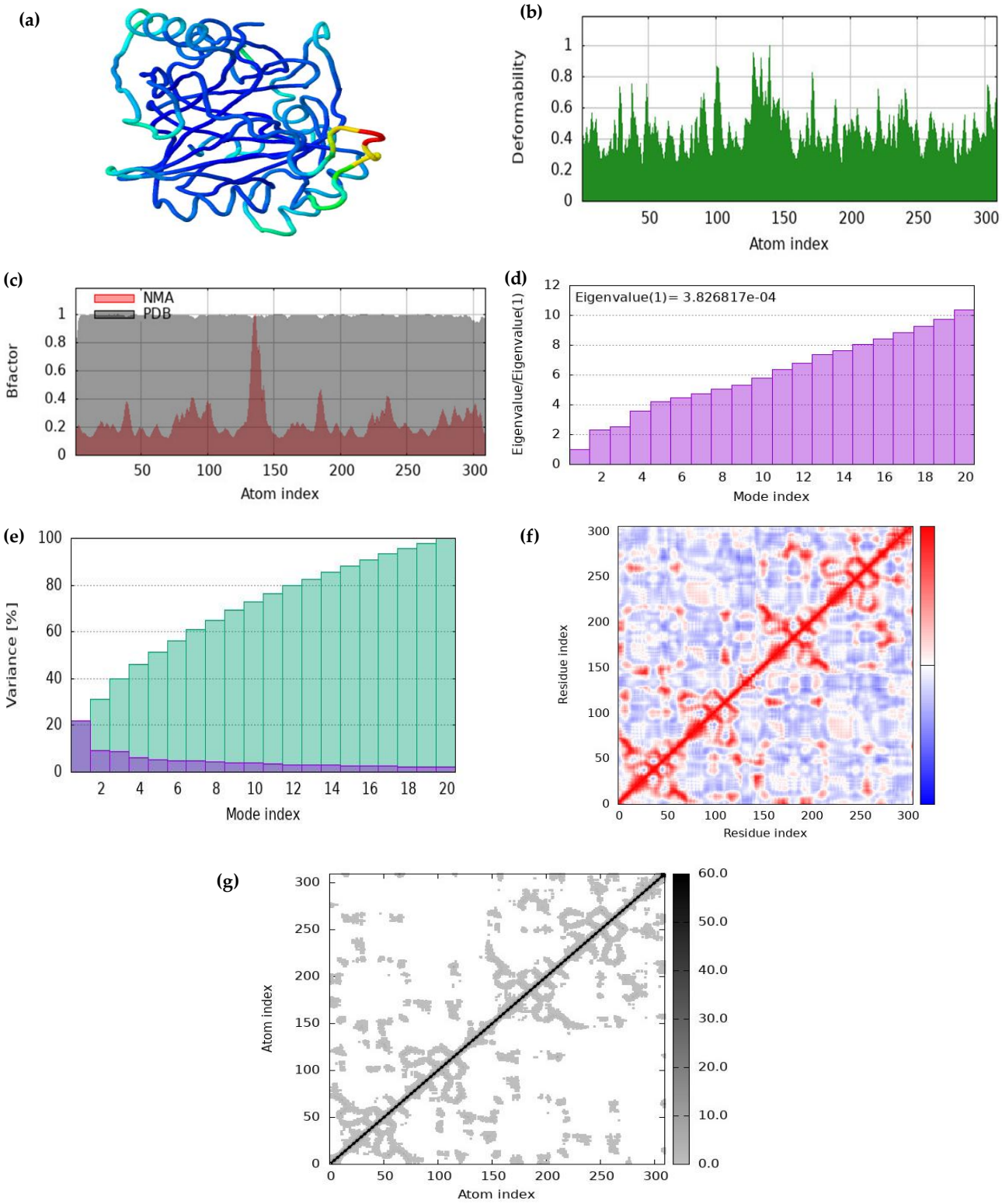


Fig. 5 (a). Ribbon diagram of the protein structure generated using iMODS, illustrating the dynamic flexibility of the molecule. Color coding represents mobility, with blue indicating rigid regions and red highlighting the most flexible areas; **(b).** Deformability graph; **(c).** Normal Mode Analysis (NMA); **(d).** Eigenvalue graph; **(e).** Variance graph; **(f).** Covariance map; **(g).** Elastic network

DISCUSSION

This study explored the molecular interactions between *Pseudomonas putida* catechol 2,3-dioxygenase and a set of environmentally persistent polycyclic aromatic hydrocarbons (PAHs) using an in silico approach (19). The enzyme demonstrated favorable physicochemical properties, including thermal stability, hydrophilicity, and structural integrity. Among all pollutants tested, indeno[1,2,3-cd]pyrene showed the strongest binding affinity (–7.8 kcal/mol), with significant interactions involving key active site residues such as Phe218, Arg150, Gly270, and His199.

Previous studies have extensively documented the role of *Pseudomonas putida* in the biodegradation of PAHs due to its metabolic versatility and the presence of catabolic plasmids like NAH7. For instance, *P. putida* has been reported to efficiently degrade naphthalene, phenanthrene, and pyrene via the meta-cleavage pathway involving catechol 2,3-dioxygenase (20). In addition, other dioxygenase enzymes such as biphenyl dioxygenase and toluene dioxygenase have shown activity against structurally diverse pollutants including polychlorinated biphenyls (PCBs) and benzene derivatives (21). These enzymes facilitate the initial oxidative attack on aromatic rings, making them crucial in microbial degradation pathways. A study by Das et al. (2023) also highlighted the structural adaptation of dioxygenases to accommodate bulky substrates, which is essential for degrading high-molecular-weight PAHs (22).

The strong binding of high-risk PAHs with the enzyme suggests that *P. putida*'s catechol 2,3-dioxygenase could be strategically employed in bioremediation systems aimed at detoxifying highly polluted environments such as industrial wastewater, oil spills, and contaminated soils. Since these PAHs pose significant health risks including hepatotoxicity and neurotoxicity the ability to biologically degrade them could substantially reduce public exposure and long-term ecological damage.

Docking analysis revealed a network of stabilizing interactions, including van der Waals forces, hydrogen bonding, and π–π stacking. The frequent involvement of aromatic residues like Phe218 and Phe267 emphasizes the enzyme's structural compatibility with planar, hydrophobic compounds. Furthermore, molecular dynamics simulations showed that the protein-ligand complexes maintain structural stability with low eigenvalues and flexible hinge regions, indicating that the enzyme can accommodate molecular shifts while retaining active site conformation. Despite these promising results, the current study is entirely computational. While molecular docking and dynamics provide valuable theoretical insights, they cannot fully replicate enzymatic behavior under real-world conditions. Variables such as enzymatic turnover rates, substrate specificity under competitive conditions, and environmental factors (pH, temperature, pollutants) were not evaluated experimentally.

Future research should include experimental validation through enzyme assays and kinetic studies to confirm the actual degradation rates of the most strongly bound PAHs. Additionally, site-directed mutagenesis could be used to enhance enzyme specificity or binding strength. Environmental simulation studies or bioreactor-based field trials would further strengthen the case for using *P. putida* enzymes in large-scale remediation efforts.

CONCLUSION

This in silico investigation underscores the promising biotechnological role of catechol 2,3-dioxygenase from *Pseudomonas putida* in the degradation of hazardous polycyclic aromatic hydrocarbons (PAHs). The enzyme demonstrates stable structural and physicochemical properties, making it a suitable candidate for environmental applications. Notably, the PAHs selected for this study exhibit considerable toxicity, reinforcing the urgent need for effective biodegradation strategies. As a future direction, molecular docking and dynamic simulation studies are underway to evaluate the enzyme's binding efficiency and specificity toward a broader range of PAHs. These insights are expected to guide protein engineering approaches aimed at enhancing catalytic efficiency under environmental conditions. Overall, this work introduces a computational framework that can accelerate the discovery and optimization of microbial enzymes for tackling industrial pollution, offering a sustainable and targeted approach to environmental remediation.

Authors' contribution:

RS Conceptualization and research experiments; SM, MTH Methodology; FB, AN, UR Critical analysis and experimentation; MF, SA Editing and review; AF, ER, WA, SZ computational simulation & RA, RN Comparative analysis.

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