


Communication

Identification of New Dioxygenases Able to Recognize Polycyclic Aromatic Hydrocarbons with High Aromaticity

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Abstract: Polycyclic aromatic hydrocarbons (PAHs), products from the incomplete combustion of crude oil, are pollutants present in nature. Ring hydroxylating dioxygenase enzymes are able to catalyze polycyclic aromatic hydrocarbons in the biodegradation process with a high degree of stereo-, regio-, and enantiospecificity. In this work, we present the first approximation of the binding modes of 9 PAHs with high aromaticity in the catalytic sites of biphenyl or naphthalene dioxygenases from four microorganisms usually used in bio-remediation processes: *Sphingobium yanoikuyae*, *Rhodococcus jostii* RHA1, *Pseudomonas* sp. C18, and *Paraburkholderia xenovorans*. Molecular modeling studies of two biphenyl dioxygenases from *Sphingobium yanoikuyae* and *Paraburkholderia xenovorans* showed good binding affinity for PAHs with 2–4 benzene rings (fluoranthene, pyrene, and chrysene), and both enzymes had a similar amount of substrate binding. Molecular docking studies using naphthalene dioxygenase from *Pseudomonas* sp. C18 showed that the enzyme is able to accommodate PAHs with high aromaticity (benzo(a)pyrene, indeno(1,2,3-cd)pyrene), with good docking scores. This study provides important insight into the utility of naphthalene dioxygenases in the degradation of HAPs with high aromaticity.

Keywords: biodegradation; dioxygenase; molecular modeling; polycyclic aromatic hydrocarbons



Citation: Rodríguez, A.; Zárate, S.G.; Bastida, A. Identification of New Dioxygenases Able to Recognize Polycyclic Aromatic Hydrocarbons with High Aromaticity. *Catalysts* **2022**, *12*, 279. <https://doi.org/10.3390/catal12030279>

Academic Editors: Keith Hohn, Kotohiro Nomura, Evangelos Topakas, Vincenzo Baglio, C. Heath Turner, Leonarda Francesca Liotta, Jean-François Lamonnier and Maria A. Goula

Received: 14 February 2022

Accepted: 1 March 2022

Published: 2 March 2022

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), products from the incomplete combustion of crude oil, are pollutants present in nature [1,2]. There are more than 100 PAHs; their hydrophobicity increases and solubility decreases with an increased number of benzene rings. These compounds are of environmental concern because of their toxic, mutagenic, and carcinogenic properties [3]. Bioremediation is a highly efficient and cost-effective process that mainly uses microorganisms or enzymes (dioxygenases) to degrade contaminants in the environment (Figures S1 and S2) [4–6]. Communities of microorganisms with the capacity to degrade low-molecular-weight polycyclic aromatic hydrocarbons have been isolated and characterized, but those microorganisms were much less able to degrade high-molecular-weight PAHs (>4 benzene rings) [7–12]. Naphthalene is biodegraded by *Sphingomonas* (96%) [13], *Sphingobium*, *Actinobacteria*, and *Rhodococcus* (100%). Anthracene is decomposed by *Actinobacteria* (93%), *Sphingobium* (28%), *Mycobacterium* (50–70%), *Rhodococcus* (90%), *Nocardia*, and *Paracoccus* [14]. Pyrene, fluoranthene, and chrysene are decomposed by *Sphingomonas* (80–90%), *Rhodococcus* (90%), *Dyadobacter*, *Mycobacterium* (50–70%), *Sphingobium* (20–60%), *Pseudomonas aeruginosa* (60–70%), and *Dyadobacter koreensis* (80%) (Table S1) [8]. The concentration of PAHs with 2–3 benzene rings in nature decreases faster than the concentration of PAHs with 4–6 rings, which may be due to a recalcitrant effect or a lack of microorganisms capable of degrading these compounds [15].

PAH degradation by bacteria proceeds through dioxygenase attacks on a PAH to form a *cis*-dihydrodiol aromatic compound using two atoms of O₂ (Figure 1) [16,17]. These proteins are also able to perform oxidation with a high degree of stereo-, regio-, and enantiospecificity at the catalytic mononuclear iron site [18]. Dioxygenases are metalloenzymes present in a wide variety of microorganisms that are able to oxidize PAHs using oxygen molecules and two electrons from a reductase to give *cis*-arene diols (Figure 1) [19,20]. A wide range of 3D dioxygenase structures have been determined, giving insight into substrate recognition and regioselectivity [19,21]. The structure of most dioxygenases is an $\alpha_3\beta_3$ hexamer molecule, with each alpha subunit containing a Rieske [2Fe-2S] cluster and another alpha subunit containing a mononuclear iron center, where the catalytic reaction takes place [22,23]. The catalytic domain of these enzymes is antiparallel sheets, and the active site contains an Fe³⁺ ion usually coordinated by 2 His and 1 Asp amino acid [16]. In the Rieske center, Fe-1 is coordinated by 2 Cys and the second Fe by 2 His being conserved in this family of enzymes (Figure 1) [22].

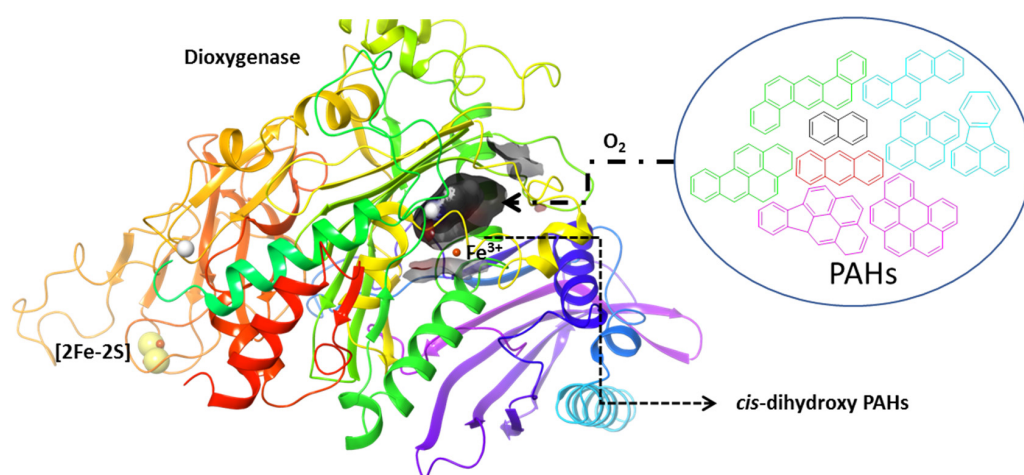


Figure 1. Schematic presentation of dioxygenase from *Rhodococcus jostii* RHA1 (Protein Data Bank ID 1ulj). PAHs and oxygen molecule are substrates in binding sites of dioxygenase (Fe³⁺) to produce *cis*-hydroxylated compounds.

Dioxygenases from different organisms have been studied for the deoxygenation of low PAHs (1–3 benzene rings). In particular, dioxygenase from *Sphingomonas* CHY-1 has been described as capable of recognizing different PAHs using molecular modeling [13,16,24]. Computational tools can be very useful for finding dioxygenases capable of degrading highly condensed PAHs, allowing these enzymes to be used in biocatalytic processes. In this paper, we carried out molecular docking studies of dioxygenases from different microorganisms involved in the biodegradation of highly condensed PAHs that are present in nature.

2. Results and Discussion

Genes responsible for the degradation of PAHs were studied for the first time in strains of *Pseudomonas putida* isolated in culture media with naphthalene or phenanthrene as the only carbon source (Figure S2b). Bacteria generally present high metabolic versatility and are capable of utilizing a broad spectrum of PAHs. To carry out the molecular docking studies, we selected naphthalene and biphenyl dioxygenases with 3D structure solved (PDB ID 2gbx, 1ulj, 4hjl, and 2xsh) from *Sphingomonas*, *Rhodococcus*, *Pseudomonas*, and *Paraburkholderia* (Table 1).

Table 1. Protein Data Bank ID codes of dioxygenases from different microorganisms.

Code (PDB)	Organism	Dioxygenase/ Uniprot No.	Ions/Ligand
2ckf	<i>Sphingomonas</i> sp. CHY-1	Naphthalene 1,2-	Fe ₂ S ₂ , Fe ³⁺
2gbw	(Gram+)	Q65AT1	—
2gbx	<i>Sphingobium yanoikuyae</i>	Biphenyl 2,3-	Biphenyl
2i7f	(Gram+)	/A2TC87	
2b1x	<i>Rhodococcus</i> sp. NCIMB 12038 (Gram-)	Naphthalene	Fe ₂ S ₂ , Fe ³⁺
2b24		Q9 × 3r9	Indol
1ulj	<i>Rhodococcus jostii</i> RHA1	Biphenyl 2,3	Fe ₂ S ₂ , Fe ³⁺
1uli	(Gram-)		Biphenyl
1eg9	<i>Pseudomonas putida</i>	Naphthalene 1,2	Fe ₂ S ₂ , Fe ³⁺ , SO ₄ [−]
1ndo	(Gram-)	P0A110	Indole
1o7n		Toluene 2,3-/A5W4F2	Toluene
1o7p			—
3en1	<i>Pseudomonas</i> sp. C18	Naphthalene 1,2-/P0A111	Fe ₂ S ₂ , Fe ³⁺ , SO ₄ [−]
3eqq	(Gram-)		Naphthalene
4hjl +			Benzamide
4hkv			
2xsh	<i>Paraburkholderia xenovorans</i> LB400	Biphenyl/	Fe ₂ S ₂ , Fe ³⁺ , SO ₄ [−]
2xrx	(Gram-)	P37,334	Biphenyl
2xso			—
2xsh			

+ To be published. Underlined pdb numbers correspond to proteins that have been used to study binding modes with PAHs by molecular docking.

The PAHs used in this study were anthracene, naphthalene, pyrene, chrysene, fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, indeno(1,2,3-cd)pyrene, and benzo(ghi)perylene (Figure 2). We chose these PAHs because they are listed by the United States Environmental Protection Agency (EPA) as priority pollutants.

The oxidation of these aromatic compounds by dioxygenases from different microorganisms is described in Table S1. Most dioxygenases are able to recognize up to three benzene rings (biphenyl, naphthalene, and anthracene); few enzymes are able to degrade compounds with more benzene rings [25].

Sphingobium yanoikuyae strain B1 was able to oxidize low-molecular-weight PAHs with four benzene rings (chrysene, pyrene, and fluorene) in in vitro assays [25]. Molecular modeling studies of PAHs into biphenyl 2,3-dioxygenase from *Sphingobium yanoikuyae* (PDB ID 2gbx) have shown that naphthalene, anthracene, fluoranthene, pyrene, and chrysene interact with nearby hydrophobic amino acid (F350, F201, L305, F404, V208, and T308) (Figure 3a). The experimental data support this enzyme as a good degrader of 2–4 benzene rings. PAHs were bound in the same hydrophobic internal pocket that the biphenyl substrate presents in the 3D structure of the protein. The chrysen–dioxygenase complex presents three Pi-Pi stacking interactions with two His (H207 and H293) of the enzyme in the binding site, giving a good docking score (−7.6 kcal/mol) (Table S3, Figure 3b). The surface area of the catalytic pocket of this enzyme is 361.44 Å², so it is not surprising that it would not be able to accommodate larger PAHs (Table S2).

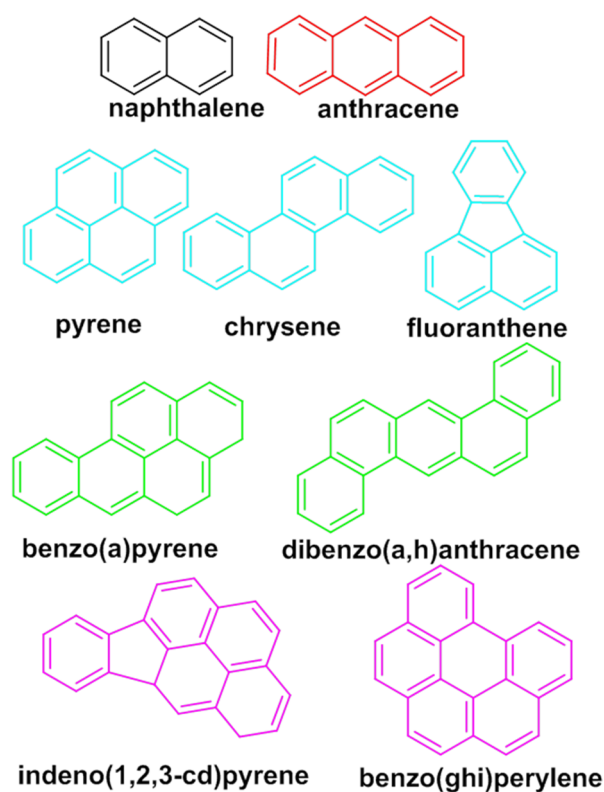


Figure 2. Structure of polycyclic aromatic hydrocarbons (PAHs) present in the environment as pollutants. Solubility (mg/mL): naphthalene, 31.7; anthracene, 0.07; pyrene, 0.14; chrysene, 0.0006; fluoranthene, 0.26; benzo(a)pyrene, 0.003; dibenzo(a,h)anthracene, 0.0005; indeno(1,2,3-cd)pyrene, 0.062; and benzo(ghi)perylene, 0.062.

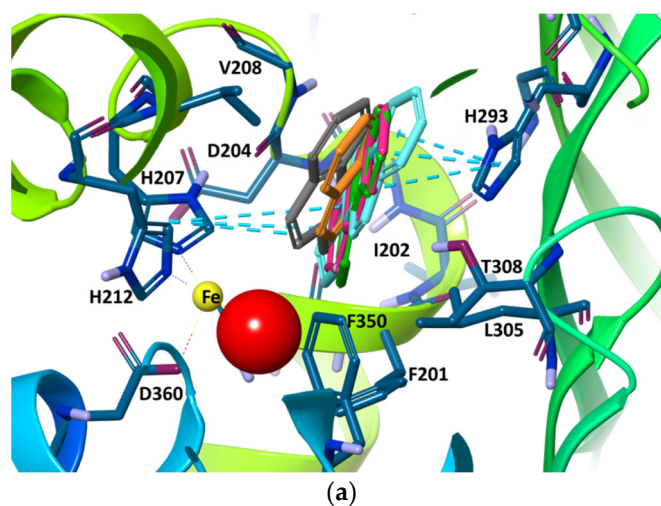


Figure 3. Cont.

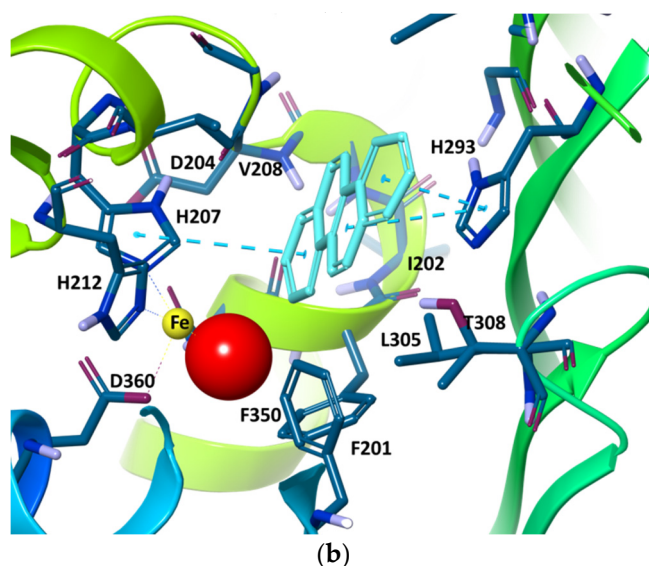


Figure 3. (a) Model of naphthalene (orange), anthracene (pink), fluoranthene (green), pyrene (gray), and chrysene (cyan) docked into binding site of biphenyl dioxygenase from *Sphingobium yanoikuyae* (ID 2gbx), with docking scores of -5.1 , -7.6 , -6.9 , -7.1 , and -7.6 kcal/mol, respectively (Table S3). Red ball is an oxygen molecule, and dashed cyan lines indicate aromatic staking interaction in the catalytic pocket. (b) Model of chrysene docked into substrate pocket.

The same study was carried out with another biphenyl dioxygenase from *Paraburkholderia xenovorans* (also known as *Burkholderia xenovorans* PDB ID 2xsh), which has a similar molecular surface area in the binding pocket of biphenyl dioxygenase from *Sphingobium yanoikuyae* (421.550 \AA^2) (see Supporting Materials). In addition, the amino acids that form the catalytic pocket are very similar in both enzymes (phenylalanines and histidines). The enzyme co-metabolizes many polychlorinated biphenyl compounds and has become a model system for the bacterial breakdown of very persistent environmental contaminants [26]. Figure 4 shows the binding of naphthalene, anthracene, fluoranthene, pyrene, and chrysene in the catalytic pocket, where their positions and conformations lie in very similar positions. The docking score results and structural features were very similar between these two biphenyl 2,3-enzymes. These studies demonstrate that the PAH binds in the enzyme with the atoms that would be oxidized closest to the Fe ion. Then, the enzymes can control the substrate orientation, as in product stereoselectivity/regioselectivity.

Rhodococcus wratislaviensis IFP 2016 and *Rhodococcus aetherivorans* IFP 2017 were able to degrade 15 petroleum compounds (low-molecular-weight PAHs or aliphatic compounds: xylene, toluene, octane, and so on) (Table S2) [27]. The molecular docking of 9 PAHs into biphenyl dioxygenase from *Rhodococcus jostii* RHA1 (PDB ID 1ulj) revealed that only naphthalene and anthracene could be accommodated in the binding site, with good docking scores of -6.7 and -7.3 kcal/mol, respectively (Table S3) (Figure 5), although the enzyme presents a large catalytic pocket (surface area of 1360.404 \AA^2). Naphthalene and anthracene share similar special occupancies with two aromatic staking interactions in the catalytic pocket (F368 residue for naphthalene and H224 for anthracene) (Figure 5). This result may be due to the ability of the enzyme's catalytic pocket to recognize low-molecular-weight PAHs.

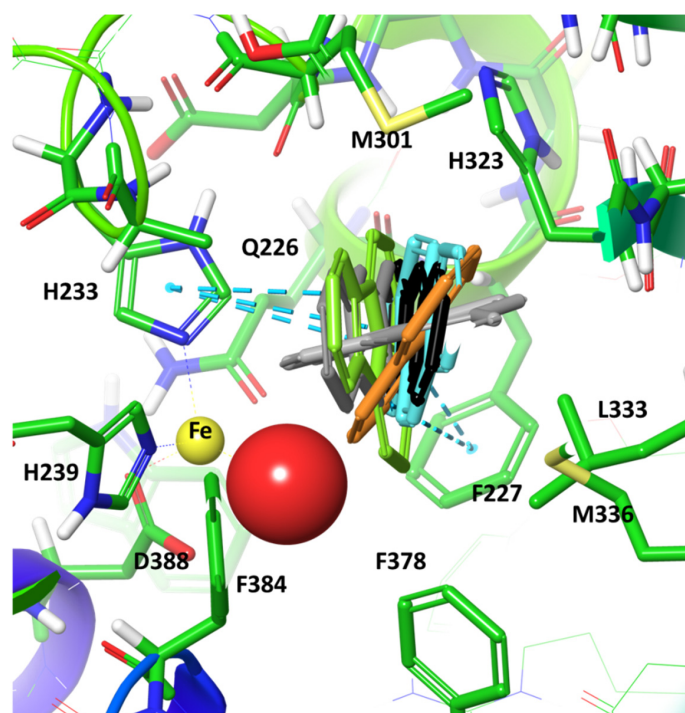


Figure 4. Molecular docking of naphthalene (orange), anthracene (black), fluoranthene (green), pyrene (gray), and chrysene (cyan) in catalytic pocket of dioxygenase from *Paraburkholderia xenovorans* LB400 (ID 2xsh), with docking scores of -5.9 , -8.3 , -6.5 , -7.9 , and -8.4 , respectively. Red ball represents oxygen molecule, and Fe ion interacts with His233, Asp388, and His239.

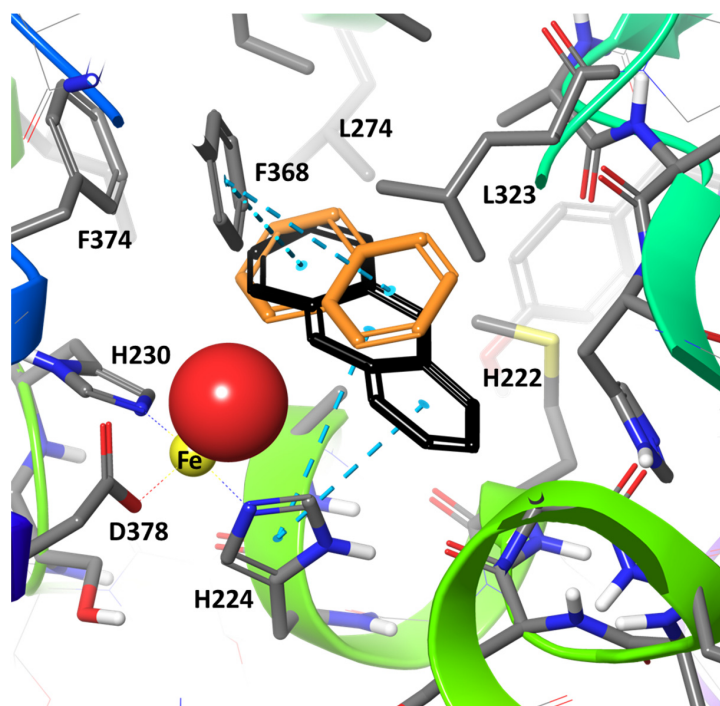


Figure 5. Three-dimensional conformation and contact of anthracene (orange) and naphthalene (black) in binding site of biphenyl dioxygenase from *Rhodococcus jostii* RHA1 (ID 1ulj), with docking scores of -6.7 and -7.3 kcal/mol, respectively. Remaining binding site residues not shown to clarify the picture.

The biodegradation of PAHs in a culture made up of a consortium of five microorganisms, including *Pseudomonas aeruginosa*, was able to completely eliminate benzo(k)fluoranthene, indeno(1,2,3-cd)pyrene, and dibenzo(a,h)anthracene [28]. Docking studies with PAHs inside the substrate pocket of naphthalene dioxygenase from *Pseudomonas* sp. C18 (ID 4hjl) (Figure 6 Table S3) reveal that seven PAHs interacted with hydrophobic amino acids (F202, A206, V260, V209, L207, F224, I408, and L253) (Figure 6) by Pi-Pi staking interactions. The docking scores with benzo(a)pyrene (five benzene rings) and indeno(1,2,3-cd)pyrene (six rings) into the naphthalene enzyme (-9.9 and -9.0 kcal/mol, respectively) were analyzed (Figure 6).

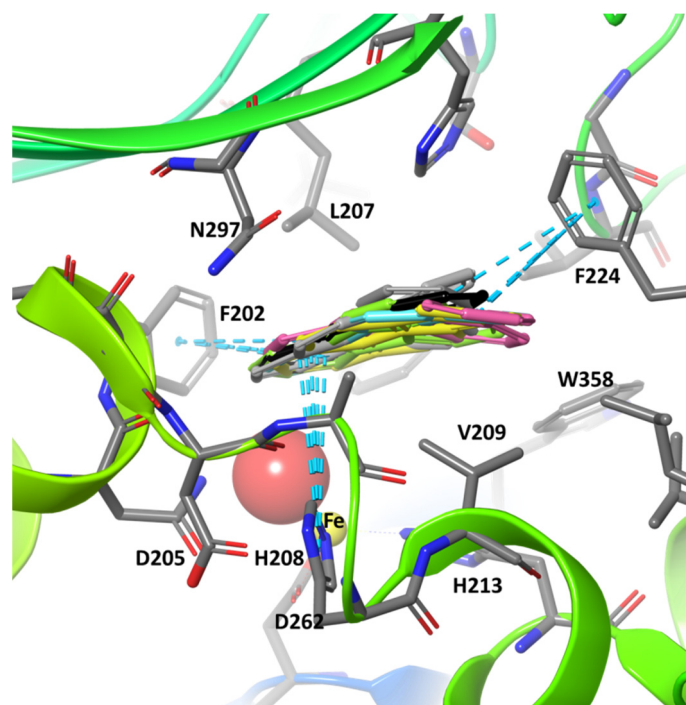


Figure 6. Model of naphthalene (orange), anthracene (pink), fluoranthene (green), pyrene (gray), chrysene (cyan), benzo(a)pyrene (yellow), and indeno(1,2,3-cd)pyrene (salmon) docked into binding site of naphthalene dioxygenase from *Pseudomonas* sp. C18 (ID 4hjl), with docking scores of -6.1 , -7.6 , -8.1 , -8.1 , -8.9 , -9.9 , and 9.0 (purpura) kcal/mol, respectively. Remaining binding site residues not shown to clarify the picture.

Residues F202, F224, and H208 are expected to affect the topology of the binding pocket and consequently select the shape of allowed PAHs (Figure 7). So, the protein could oxidize a wide range of PAHs because its binding site is large enough to accommodate up to six rings.

Dibenzo(a,h)anthracene and benzo(ghi)perylene (five or six fused aromatic rings) did not interact within any dioxygenases used in this study. Molecular modeling studies provide important insights into the utility of naphthalene or biphenyl dioxygenases in the degradation of HAPs with high aromaticity.

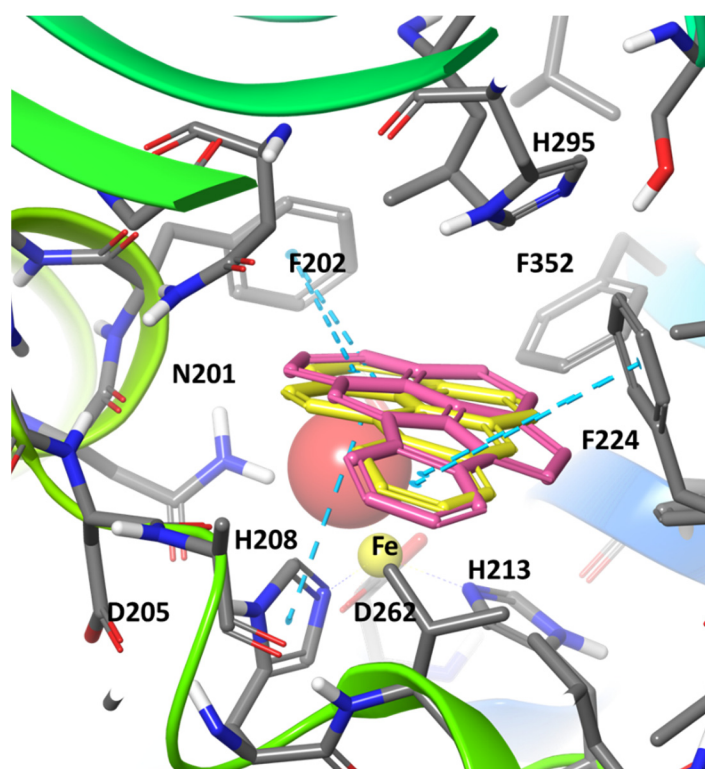


Figure 7. Molecular docking of benzo(a)pyrene (yellow) and indeno(1,2,3-cd)pyrene (salmon) into naphthalene dioxygenase from *Pseudomonas* sp. C18 (ID 4hjl), with docking scores of -9.9 and -9.0 kcal/mol, respectively. Dashed cyan lines indicate aromatic staking interaction in catalytic pocket (Phe224, Phe202, and H208). Red ball represents oxygen molecule. Fe interacts with His213, Asp362, and His208. Remaining binding site residues not shown to clarify the picture.

3. Materials and Methods

The docking evaluation was carried out for four proteins (Table 1). The crystal structure of the dioxygenases used in this evaluation was retrieved from the Protein Data Bank (PDB). The catalytic α -subunit was selected, and all water molecules were removed. An oxygen molecule was placed near the Fe ion [29], and a 3D structure model of the proteins was optimized using the Protein Preparation Wizard tool integrated in Maestro (Schrödinger, New York, NY, USA) [30]. Missing hydrogen atoms were added, assuming the standard protonation state of titratable residues. The grid of the enzymes was using the receptor grid generation tools in Glide. The size of the docked molecules was set to within 20 \AA . The molecular surface of the proteins and ligands was carried out using the binding surface area analysis tool integrated in Maestro.

Three-dimensional structures of the PAHs were retrieved from PubChem with extension. sdf and CID: anthracene 8418, naphthalene 931, fluoranthene 9154, pyrene 31423, chrysene 9171, benzo(a)pyrene 2336, dibenzo(a,h)anthracene 5889, indeno(1,2,3-cd)pyrene 9131, and benzo(ghi)perylene 115240. These compounds were prepared using the LigPrep module in the Schrödinger package. All polycyclic aromatic hydrocarbons were minimized using the OPLS3e force field implemented in Maestro. Molecular dynamic simulations of protein–PAH complexes were carried out using the Schrödinger bioinformatics suite. To achieve this, molecular docking was performed using the Glide-dock XP module [31,32]. Ligand poses generated in this way were run through a series of hierarchical filters to evaluate ligand interactions with the receptor. Docking score, glide e-model, ionization penalty, glide gscore, and TPSA were used to select the docking poses.

4. Conclusions

Molecular modeling was performed in an attempt to identify novel dioxygenases from microorganisms able to degrade PAHs with high aromaticity. Biphenyl dioxygenase from *Rhodococcus jostii* RHA1 only showed binding of naphthalene and anthracene (smallest PAHs). The other two biphenyl dioxygenases, from *Sphingobium yanoikuyae* and *Paraburkholderia xenovorans*, showed good binding affinity for 5 of the 9 PAHs studied (particularly fluoranthene, pyrene, and chrysene), and both enzymes had very similar volumes of substrate binding (421.550 and 361.44 Å³, respectively). Molecular docking of 9 PAHs into naphthalene dioxygenase from *Pseudomonas* sp. C18 showed the good accommodation of seven PAHs (5–6 fused aromatic rings) that presented large, accessible surface areas (1500.585 Å²). This study shows that structural features are essential for determining the binding mode of PAHs. This study provides important insight into the utility of naphthalene dioxygenases in the degradation of PAHs with high aromaticity.

Supplementary Materials: The following supporting information can be downloaded at <https://www.mdpi.com/article/10.3390/catal12030279/s1>: Figure S1. Main principles of aerobic degradation of PAHs; Figure S2. (a) Bioremediation of PAHs by microorganism and (b) pathways for degradation of naphthalene by *Pseudomonas putida*; Table S1. PAH biodegradation by different microorganisms; Table S2. Molecular surface area of dioxygenases and PAHs; Table S3. Docking scores of biodegraded PAHs by dioxygenases from different microorganisms.

Author Contributions: Conceptualization, A.B. and A.R.; methodology, S.G.Z.; software, S.G.Z.; formal analysis, A.R.; investigation, A.B. and A.R.; resources, S.G.Z. and A.R.; writing—original draft preparation, A.B.; writing—review and editing, A.R. and S.G.Z.; supervision, A.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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