Molecular docking simulation on the interactions of laccase from *Trametes versicolor* with nonylphenol and octylphenol isomers

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Abstract

The biodegradation of nonylphenol (NP) and octylphenol (OP) isomers by laccase has attracted increasing concerns. However, the interaction mechanism between these isomers and laccase remains unclear, especially for fungal laccase. In this work, molecular docking was employed to study this issue. The results indicated that the structural characteristic of alkyl chain (position and branching degree) affected the interactions between *Trametes versicolor* (*T. versicolor*) laccase and isomers. The binding affinity between them was closely related to the position and branching degree of alkyl chain in isomers. The binding affinities between linear isomers and *T. versicolor* laccase were *para*-position < *meta*-position < *ortho*-position. For selected branched 4-NP, the isomers with bulky α-substituent in alkyl chain had higher binding affinities. Additionally, hydrophobic contacts between *T. versicolor* laccase and NP or OP isomers were necessary, while H-bonds were optional. The isomers with similar structure may have more common residues involved in hydrophobic contacts. The H-bonds of selected NPs and OPs were all connected with phenolic hydroxyl. These findings provide an insight into detailed interaction mechanism between *T. versicolor* laccase and isomers of NP and OP. It is helpful to broaden the knowledge of degradation technology of NPs and OPs and provide theoretical basis on biological remediation of these contaminants.

Keywords

Nonylphenol; Octylphenol; Isomer; Laccase; Molecular docking; interaction

Introduction

Emerging organic contaminants (EOCs), including a wide class of chemical compounds such as endocrine disrupter chemicals (EDCs), personal care products and pesticides, have recently received growing attentions [1-6]. EDCs are defined as exogenous substances that can alter the normal function of endocrine system, and lead to harmful health effects in living organisms or their progenies and populations in consequence [7, 8]. Alkylphenols (APs) are typical EDCs and exist in the environment ubiquitously. APs have the capability to disrupt the normal endocrine system and hormonal regulation. Therefore, APs, as anthropogenic EDCs, possess a serious risk for living organisms. Typical APs such as nonylphenol (NP) and octylphenol (OP), especially 4-octylphenols and 4-nonylphenols have been defined as priority pollutants, which are concluded in Directives of Priority Substances in Water Policy amending by European Parliament and Council [9].

NP and OP, principally occur in the production of alkylphenol ethoxylates (APEs), which are used as non-ionic surfactants in many industrial applications [10]. The hydrophilic ethylene oxide chains of APEs are broken down into NP and OP in nature readily. As long-chain alkylphenol (AP), NP and OP are the most commercially universal members of AP family with NP representing approached 80%, while OP occupying approximately 20% of the whole AP market. 4-NP is a technical mixture of 211 structures owing sorts of branches of alkyl chain theoretically on account of complex industrial production process [11]. The alkyl chain of isomers may locate at *para*-positon, *ortho*-positon and *meta*-positon of the phenol ring [11]. The *para*-isomers of AP are in the majority, while *ortho*-isomers and *meta*-isomers are in the minority [12, 13]. It has been illustrated that the attachment of the ring and the feature of alkyl chain may impact the biodegradation of AP isomers by laccase [14-16]. Moreover, the quaternary α-carbon of alkyl chain is a structural characteristic that may influences degradation pathways of short-chain APs [14]. In addition, nonyl chain of NP was proved to be attacked by fungi in the metabolic routes of degradation [14].

NP and OP isomers have similar structures and physical properties. But their estrogenic effects are diverse. In this study, the linear isomers (NP and OP) and the typical branched 4-NP and 4-OP isomers with strong estrogenic activity were selected to study their interactions with *Trametes versicolor* (*T. versicolor*) laccase.

Laccase as one of the major enzymes used in EDCs biodegradation is glycosylated multicopper oxidase with four copper ions [17-19]. Laccase, widely existing in plants, fungi, bacteria and insects, has the catalysis ability to oxidize and reduce electron of a broad range of substrates [20]. This class of enzyme is monomeric, dimeric or tetrameric dioxygen oxidoreductases with three copper-binding types located at the catalytic site [21]. Fungal laccase has some advantages compared with bacterial laccase for its high-concentrated contaminant degrading ability and low nutrient need. *T. versicolor* laccase is one of the fungal laccases that considered as green biocatalyst. Therefore, *T. versicolor* laccase was selected as typical fungal laccase in the present work due to its wide application and great degradation ability. Previous study compared the half-life of 4-n-NP with branched 4-NPs (branched alkyl chain in *para*-position) in *T. versicolor* laccase culture [22]. Another relevant study demonstrated that *T. versicolor* laccase was able to catalyze the oxidation process in the conversion of NP into carbon dioxide; laccases purified from *T. versicolor* culture catalyzed degradation of NP into oligomerization in oxidation process [22]. Moreover, Catapane et al. compared the removal rates of OP and NP at the same concentration by *T. versicolor* laccase catalytic activity [23]. Additionally, a study that 4-tert-Octylphenol (4tOP) remedied with *T. versicolor* laccase has found that 4tOP completely disappeared after 5 days [24]. Nevertheless, the study on degradation of NPs and OPs by laccase is still incomplete since just few products as oligomers and polymers have been reported until now [25]. Therefore, molecular docking approach was performed to research the interactions between laccase and several NP and OP isomers as representative models.

The effects of the chemical structure on biological degradation mechanism between laccase and APs lead to estrogenic variation [26]. It is necessary to understand how laccase interacts with APs and expands the molecular mechanisms knowledge

118 referred to metabolic pathways. However, there is little research about the effect of
119 NPs and OPs structure on their interaction with *T. versicolor* laccase at molecular
120 level. Furthermore, experiment studies are often time-consuming and costly [27].
121 Molecular docking used algorithm to predict the optimum binding mode between
122 ligand and receptor rapidly and reliably which might explain the experimental results
123 [27]. As the above advantages, molecular docking is increasingly employed to reveal
124 rational binding of ligand and receptor in molecular mechanism to shorten studying
125 time and save research funds. This method was originally applied in medicine and has
126 been extensively used to study environmental problems nowadays [28-30]. For
127 example, combination of experiment and molecular docking was applied to analyze
128 the effect of surfactant on degradation of phenol catalyzed by laccase [31]. Thus,
129 molecular docking was selected to study the interactions between NP and OP isomers
130 and *T. versicolor* laccase at the level of molecules in this work.
131 In this study, Molegro Virtual Docker [32] (MVD) was applied to analyze the
132 enzyme-catalyzed process of isomers (11 NP isomers and 5 OP isomers) and *T.*
133 *versicolor* laccase at molecular level by molecular docking in order to find out the
134 influences of interaction.

135 ## Materials and methods

136 ### Structural model of laccase

137 The 3D structure of *T. versicolor* laccase was taken from PDB (Protein Data Bank)
138 database with the code 1GYC at 1.90Å resolution [33].

139 ### Preparation of NP and OP isomers structures

140 The amount of 16 isomers was selected as NP and OP models in this research. The
141 structure, molecule name, abbreviation, formula, α substitution, β substitution, γ
142 substitution and main alkyl chain length of analyzed isomers were presented in Table1.
143 The abbreviations of NP isomers in Table 1 were obtained by Guenther et al [34]. All
144 the ligand structures were optimized by energy minimization using Austin Model 1
145 [35].

Molecular Docking

Molecular docking provides all effective conformation and orientation of the binding models in order to have an insight into the interaction of ligand and enzyme. General conceptual framework of this study was depicted in Fig. 1. MVD was utilized for the docking of refined structures of APs isomers and 1GYC. The software of MVD first confirmed the binding site in laccase by a high-accuracy grid-based cavity prediction algorithm [36-37]. Afterwards, ligand models were docked into the certain cavities to analyze the interaction situation. Subsequently, the top poses were returned and ranked by the MolDock scoring function (MolDock score). MolDock SE algorithm operated 10 times to avoid stochastic mistakes. Finally, the re-docking protocol was used to verify the docking accuracy [38].

In this study, all selected isomer structures were performed docking with *T. versicolor* laccase. The best pose was selected for further investigation.

Binding affinity and interaction analysis

The binding affinity analysis of the ligands was carried out via the empirical correlation formula which was trained to predict binding affinity by MVD. Multiple linear regressions were employed to obtain the coefficients of binding affinity formula. This formula calibrated by sorts of complexes was used to examine the complex robustness combined with the best pose [39-40]. The software of LigPlot^{+} was used to explore the binding patterns between laccase and isomer compounds [41].

Identification of hydrophobic contacts similarity

The similarity of amino acid residues involved in hydrophobic contacts is used to compare the hydrophobic interactions in various isomers with laccase. It was analyzed by the software R 3.3.3 (The R Project for Statistical Computing) (https://www.r-project.org/). The method of Jaccard distance was used to measure the samples [42]. Unweighted Pair Group Method with Arithmetic Mean (UPGMA) was employed as clustering method [43].

Results and discussion

Validation of docking accuracy

Re-docking protocol was carried out on the best docking pose as reference ligand. The best pose estimated by the lowest MolDock score was used to inspect accuracy via re-docking. The binding affinities of docking complexes were simultaneously revealed in Table 2. RMSD (Root-mean-square Deviation) values resulted from re-docking between enzyme and ligands were less than 1.2 Å. Since all the re-docking RMSD data were lower than 2.00 Å, the binding patterns and the activity sites of ligand docking into the laccase cavities were regarded as accurate and acceptable [36]. The MolDock score and Re-Rank score for the best docking complex were listed in detail (Table 2). For NP and OP isomers with linear alkyl chain, *para*-isomer had minimum MolDock score, while *ortho*-isomer had relatively higher MolDock score than *meta*-isomer. The pocket volume shown in Table 2 reflected the size of cavity. The pockets were flexible to fit the interaction of enzyme.

Steric energy

The steric energy [32] of the docking complex can be seen in Table 2. It represents steric interaction energy between protein and ligand. For the docking results, steric energy in LAC-2NP, LAC-3NP and LAC-4NP were -98.649 kJ mol^{-1}, -102.738 kJ mol^{-1} and -104.672 kJ mol^{-1}; LAC-2OP, LAC-3OP and LAC-4OP were -91.919 kJ mol^{-1}; -98.944 kJ mol^{-1} and -107.230 kJ mol^{-1}, respectively. It can be found that linear isomers with alkyl chain in *para*-position had minimum energy. The difference in steric energy may be due to steric effect, electronic effect and shape matching of enzyme and ligand cause by the different location of alkyl chain. When compared with the complexes of LAC- NP_2 and LAC-NP_9, LAC-NP_{30} and LAC-NP_{65}, LAC-NP_{167} and LAC-NP_{165}, isomers with more bulky α-substitution had higher steric energy. It is worth noting that the minimum steric energy was -115.373 kJ mol^{-1} of LAC-NP_{30} among the selected NPs, which may be due to its ethyl substitution effect in α-carbon. Compared with LAC-4dOP and LAC-4tOP, 4tOP with more bulky alkyl chain had higher steric energy. These findings were due to the structural difference of alkyl chain. There were some reasons may explain these results. On one hand, alkyl

chain was an electron-supplying group and larger branches structure could bring about conjugate effect of molecule [14, 26, 44]. On the other hand, the bulky alkyl structures may bring about steric hindrance [45]. Prior research claimed that different α-carbon substituents and branching patterns of side chain in NP isomers might affect degradation [46, 47].

Binding affinity

The binding affinity data of enzyme-ligand complexes of NPs and OPs were shown in Table 2. The binding affinity of 2NP (*ortho*-position), 3NP (*meta*-position) and 4NP (*para*-positon) were -17.556 kJ mol^{-1}, -16.872 kJ mol^{-1}, -16.341 kJ mol^{-1}, respectively. The trend shown that for the complexes of linear NPs binding with laccase, the binding affinities were *para*-positon < *meta*-position < *ortho*-position. The similar trend was also observed in 2OP (*ortho*-position), 3OP (*meta*-position) and 4OP (*para*-positon) with binding affinity value of -17.208 kJ mol^{-1}, -16.741 kJ mol^{-1} and -16.597 kJ mol^{-1}, respectively. When turning to 4-NPs with bulky α-substitution in alkyl chain, the binding affinities were estimated to be higher. For instance, the binding affinity of LAC-NP_2 and LAC-NP_9, LAC-NP_{30} and LAC-NP_{65}, LAC-NP_{167} and LAC-NP_{165}, LAC-NP_{172} and LAC-NP_{169} were -16.433 kJ mol^{-1} and -17.521 kJ mol^{-1}, -16.693 kJ mol^{-1} and -17.738 kJ mol^{-1}, -18.129 kJ mol^{-1} and -19.371 kJ mol^{-1}, -18.083 kJ mol^{-1} and -19.843 kJ mol^{-1}, respectively, indicating that ligand structure with smaller α-substitution may be related to minor binding affinities. The binding affinity of LAC-4dOP and LAC-4tOP were -17.607 kJ mol^{-1} and -18.427 kJ mol^{-1}, showing OPs ligand with more branched alkyl chain may lead to higher binding affinity.

This research associated the structural feature of isomers with binding affinity, especially focus on the difference of binding affinity between linear isomers and branched isomers of 4-NPs and 4-OPs. By comparison of NP and OP isomers with linear alkyl chain in different positions, the docking results indicated binding affinity as follows: *para*-position < *meta*-position < *ortho*-position. The linear isomers with alkyl chain in *para*-position received the minimum affinity were influenced by steric

energy. Interestingly, isomers with linear alkyl chain in *meta*-positon and *para*-position had tiny difference in binding affinity as well as binding orientation and hydrophobic effect, implying similar modes and mechanisms of interaction.

There are some experiments can verify the results of binding affinity. For example, the binding affinity of LAC-4NP and LAC-4OP were -16.341 kJ mol^{-1} and -16.597 kJ mol^{-1} which may indicate less binding tightness in LAC-4NP. The experimental research on the removal of 4OP and 4NP by *T. versicolor* laccase has found that the complete removal time of 4OP was shorter than 4NP to show less biodegradation efficiency [23]. The results might imply that binding affinities of linear isomer 4NP with longer alkyl chain was minor than linear isomer 4OP with shorter alkyl chain. For another example, the experimental study of NP degradation by *T. versicolor* laccase showed that the half-life of 4NP was about a day, while the branched 4-NPs was less than one day [22]. The experimental results may accord with our study that the binding affinity of 4NP was minimum among the selected NPs. The correlation of ligand structure and binding affinity may depend not only on hydrophobicity, but also on steric energy and electronegative interactions in enzyme-ligand binding pocket.

In general, binding affinity was affected by interaction profile, such as distorting extent of ligand and the shape complementation of ligand and cavity. With the higher binding affinity, the ligands have stronger capability to reach the pocket-like structure of enzyme [48]. The binding affinity may be related to interaction efficiency of enzyme-ligand and imply the tightness of the binding complex. However, the binding affinity could not play decisive role in catalytic activity of enzyme [49].

In a word, binding affinity may be affected by structural characteristics of isomer, e.g. the relative position of phenolic hydroxyl and alkyl chain, the substituent group of alkyl chain. The isomers with more branching structure may have higher degradation efficiency. For analyzed laccase-nonylphenol complexes, the effect of α-substitution in alkyl chain may be more important than γ-substitution and β-substitution for binding affinities.

The characteristic of binding orientations

In Fig. 2 and Fig. S1, the binding orientations and the poses in the cavities of laccase and AP isomer complexes were shown to explore the profile of complementation and interaction. The detailed views of NP and OP isomers in binding sites of *T. versicolor* laccase were shown in Fig. 3. It is generally believed that the orientation of ligand plays a significant role in enzyme binding effect [49]. Binding modes presented in Fig. 2 and Fig. S1 indicated diverse ligand binding sites of laccase. It is noteworthy that 2NP, NP_{65}, NP_{167}, NP_{165} and 2OP had similar docking site.

According to laccase-ligand binding pocket images, some ligands embedded in the pocket and joined the laccase, such as Lac-4NP complex. Some ligands located on the surface of laccase, such as Lac-NP_{167} complex.

Binding interaction contacts

LigPlot^{+} was carried out to study the binding role of hydrophobic contacts and H-bonds in the best pocket (Fig. 4 and Fig. S2). The mechanisms of *T. versicolor* laccase degrading NP and OP isomers were different. These differences may be partly due to different binding interaction function [27], such as hydrophobic contacts and H-bonds. It can be observed that hydrophobic contact was much more vital than H-bond. The amino acid residues involved in hydrophobic contacts and H-bonds were considered as key residues for enzyme-ligand interaction.

Because of the hydrophobic nature of NP and OP, the dominate reaction were hydrophobic interaction. The residues involved in the hydrophobic contacts in common were likely to play vital roles in the binding between *T. versicolor* laccase and ligands. For amino acid residues involved in hydrophobic effects (Table 3), both of the NP and OP isomers with linear alkyl chain in *meta*-position and *para*-position exhibited similar hydrophobic contacts, while differ from the ones with alkyl chain in *ortho*-position. The common residues of Leu459, Phe450, Pro346, Ser113, Leu112, Glu460, Phe81 and Ala80 participated in the formation of hydrophobic bonds were crucial for the complexes of LAC-3NP (*meta*-position) and LAC-4NP (*para*-position). Similarly, the common amino acid residues of Leu459, Pro346, Ser113, Leu112,

290 Phe344 and His111 were presented as hydrophobic interactions in LAC-3OP
291 (*meta*-position) and LAC-4OP (*para*-position). Compared with Lac-NP_2 and Lac-NP_9,
292 the residues of Leu459, Pro346, Ser113, Leu112, Glu460, Phe81, Phe344, Ala80 and
293 Ipa1514 formed hydrophobic contacts were in common. However, for residues
294 involved in hydrophobic contacts, Lac-NP_{30} bored no resemblance to Lac-NP_{65} may
295 due to the difference of α-substitution. When it came to more branched NP isomers,
296 Lac-NP_{167} and Lac-NP_{165} had a certain extent similar in hydrophobic binding with the
297 same residues of Leu300, Phe239, Gln237, Ile301, Gln242, Ala410 and Glu302. The
298 residues of Leu459, Phe450, Pro346, Ser113, Glu460, Phe344, Ala80, Ipa1514,
299 His111 and Thr354 involved in hydrophobic contact were the same for Lac-NP_{172} and
300 Lac-NP_{169}. For selected NP complexes, the most common residue Leu459, Pro346,
301 Ser113, Glu460 were the most essential. In addition, for Lac-4dOP and Lac-4tOP, the
302 common residues took part in hydrophobic effects were Leu459, Phe450, Pro346,
303 Ser113, Leu112, Glu460, Phe81, Ala80, His111 and Gly462. The residues of Leu459,
304 Pro346, Ser113, Leu112 and His111 were all involved in the hydrophobic contact of
305 Lac-3OP, Lac-4OP, Lac-4dOP and Lac-4tOP.

306 To analyze the relevance of hydrophobic function of different laccase-isomer
307 complexes, UPGMA method was used to research the residues involved in
308 hydrophobic contact. The analytical results were depicted in dendrogram (Fig. 5).
309 From the diagram, it could be illustrated that all the complexes were classified into
310 three groups. The first main cluster included LAC-NP_{30}, LAC-4OP, LAC-NP_{169},
311 LAC-NP_2, LAC-NP_{172}, LAC-4NP, LAC-4tOP, LAC-NP_9, LAC-4dOP, LAC-3NP and
312 LAC-3OP. The second cluster contained LAC-NP_{165}, LAC-NP_{65} and LAC-NP_{167}. The
313 third cluster was consisted of LAC-2NP and LAC-2OP.

314 The structure feature and the binding orientations of NPs and OPs may be
315 connected with hydrophobic function. The relationship between them can be analyzed
316 comparatively. The complexes with similar ligand structure may have similar binding
317 orientation and more common residues involved in hydrophobic contacts. According
318 to Fig. 2 and Fig. 5, LAC-2NP and LAC-2OP with similar ligand structure and
319 binding orientations constituted one main cluster. In another instance, the complexes

of LAC-3NP and LAC-3OP formed the smallest sub-cluster. LAC-3NP, LAC-3OP, LAC-4NP and LAC-4OP were classified into the same main cluster. The binding complexes (e.g. LAC-2NP, LAC-NP_{65}, LAC-NP_{167}, LAC-NP_{165} and LAC-2OP) which had similar docking sites and orientations were included into the same main cluster. In addition, some branched isomers with similar alkyl chain were in identical smallest cluster, such as LAC-NP_{172} and LAC-NP_{169}, which indicated that 4-NP isomers with similar alkyl chain were likely to have high similarity in amino acid residues involved in hydrophobic contacts.

H-bond details could be seen in Table 4 and appeared to be different in the interaction of laccase and ligands. Linear NPs and OPs were both connected with laccase via H-bonds. The isomers with linear alkyl chain in *ortho*-position formed more H-bond contacts than the ones with linear alkyl chain in *meta*-positon or *para*-position. For isomers with linear alkyl chain in *ortho*-position, 2NP related to laccase via Gln242, Glu302 and Ser427 by three H-bonds, while 2OP connect with Leu300 by two H-bonds. Nevertheless, H-bond may be selective in the complexes of branched NP and OP isomers with *T. versicolor* laccase. Lac-NP_{65}, Lac-NP_{165} and Lac-4tOP formed H-bond via phenolic hydroxyl and residents of Tyr244, Arg423 and Phe344 severally. It is worth mention that the formation of H-bond always connects with oxygen atom of phenolic hydroxyl.

From the results above, enzyme-ligand interactions were mainly hydrophobic function which may be due to the aliphatic chain in NP and OP, while H-bond was a kind of selective function. The hydrophobic contacts may be impacted by ligand structure. Moreover, it is supposed that H-bonding sites will not be used completely in general and may be adverse to H-bond formation by topological restriction [50].

Conclusion

In this work, we investigated the binding orientations, interaction contacts, steric energy and binding affinities between *T. versicolor* laccase and a variety of isomers by molecular docking. The study results indicated that hydrophobic contacts were necessary and H-bonds were optional in interactions. For selected docking complexes,

the ligands with similar structure have more common residues involved in hydrophobic contacts. Moreover, hydrogen bonds of enzyme-ligand complexes were connected with oxygen atoms of phenolic hydroxyl groups. For the complexes of linear NPs and OPs with enzyme, hydrogen bonds were necessary. For the complexes of branched NPs and OPs with enzyme, hydrogen bonds may be weaker. Therefore, the interactions of linear NPs and OPs depend on both hydrogen bonds and hydrophobic contacts while branched NPs and OPs may mainly rely on hydrophobic contacts.

Furthermore, the binding affinities of linear NPs and OPs with alkyl chain in different positions were as follows: *para*-position < *meta*-position < *ortho*-position. Moreover, the ligands with more branched alkyl chain, especially bulky α-substitution may lead to higher binding affinities of the docking complexes, which may indicate their higher degradation efficiency.

These findings will provide an insight into the interaction of typical NPs and OPs with *T. versicolor* laccase and try to find the relevance of structure-interaction for environmental remediation. The in-depth understanding will contribute to provide important theoretical basis for intracellular interaction of *T. versicolor* laccase with isomers in related experiments. Moreover, full understanding of interaction contacts may help to improve biodegradable environment in order to promote biodegradation. The scientific basis on the formulation of enzyme-ligand complexes contribute to screen laccase with highly degrading capability of estrogenic and resistant contaminants from various kinds of microorganisms. The extension of knowledge in the enzyme-ligand interaction rationally promotes the application of molecular simulation on bioprocesses and the development of APs-biodegrading techniques.

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Compliance with ethical standards

Conflict of interest

The authors declare that they have no conflict of interest.

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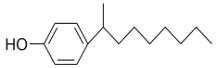
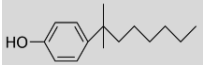
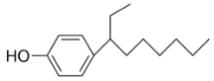
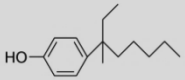
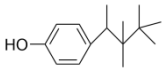
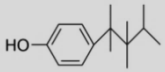
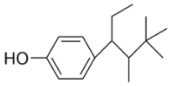
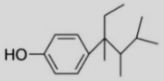
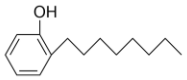
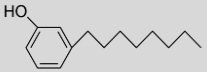
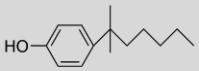
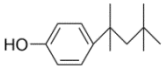
Table 1 Structure, molecule name, abbreviation, formula, α substitution, β substitution, γ substitution and main alkyl chain length of analyzed NP and OP isomers.

Structure	Molecule Name	abbreviation	Formula	α substitution	β substitution	γ substitution	Main alkyl chain length
	2-Nonylphenol	2NP	$C_{15}H_{24}O$	None	None	None	9
	3-Nonylphenol	3NP	$C_{15}H_{24}O$	None	None	None	9
	4-Nonylphenol	4NP	$C_{15}H_{24}O$	None	None	None	9
	4-(1-methyloctyl)phenol	NP_2	$C_{15}H_{24}O$	Methyl	None	None	8
	4-(1,1-dimethylheptyl)phenol	NP_3	$C_{15}H_{24}O$	Methyl, Methyl	None	None	7
	4-(1-ethylheptyl)phenol	NP_{30}	$C_{15}H_{24}O$	Ethyl	None	None	7

	4-(1-ethyl-1-methylhexyl)phenol	NP_{65}	$C_{15}H_{24}O$	Methyl, Ethyl	None	None	6
	4-(1,2,2,3,3-pentamethylbutyl)phenol	NP_{167}	$C_{15}H_{24}O$	Methyl	Methyl, Methyl	Methyl, Methyl	4
	4-(1,1,2,2,3-pentamethylbutyl)phenol	NP_{165}	$C_{15}H_{24}O$	Methyl, Methyl	Methyl, Methyl	Methyl	4
	4-(1-ethyl-2,3,3-trimethylbutyl)phenol	NP_{172}	$C_{15}H_{24}O$	Ethyl	Methyl	Methyl, Methyl	4
	4-(1-ethyl-1,2,3-trimethylbutyl)phenol	NP_{169}	$C_{15}H_{24}O$	Methyl, Ethyl	Methyl	Methyl	4
	2-Octylphenol	2OP	$C_{14}H_{22}O$	None	None	None	8
	3-Octylphenol	3OP	$C_{14}H_{22}O$	None	None	None	8
	4-Octylphenol	4OP	$C_{14}H_{22}O$	None	None	None	8

	4-(1,1-dimethylhexyl)phenol	4dOP	$C_{14}H_{22}O$	Methyl, Methyl	None	None	6
	4-(1,1,3,3-tetramethylbutyl)phenol	4tOP	$C_{14}H_{22}O$	Methyl, Methyl	None	Methyl, Methyl	4

533

534 **Table 2** Profile of pocket volume, MolDock score, Re-Rank score, Re-docking
535 RMSD, steric energy and binding affinity for the best docking complex of laccase and
536 ligands.

Laccase-ligand complex [a]	Pocket volume ($Å^3$)	MolDock score	Re-Rank score	Re-docking RMSD(Å)	Steric energy (kJ mol^{-1})	Binding affinity (kJ mol^{-1})
LAC-2NP	43.52	-93.999	-71.013	1.046	-98.649	-17.556
LAC-3NP	54.784	-106.034	-76.047	1.544	-102.738	-16.872
LAC-4NP	63.488	-108.984	-82.765	1.088	-104.672	-16.341
LAC-NP_2	56.320	-104.906	-79.809	1.046	-107.471	-16.433
LAC-NP_9	73.728	-100.282	-80.249	0.872	-106.737	-17.521
LAC-NP_{30}	59.392	-112.058	-83.902	0.904	-115.373	-16.693
LAC-NP_{65}	44.544	-91.621	-63.798	0.478	-99.913	-17.738
LAC-NP_{167}	41.472	-70.445	-57.060	0.212	-89.316	-18.129
LAC-NP_{165}	46.080	-66.933	-57.365	0.136	-82.459	-19.371
LAC-NP_{172}	64.512	-88.889	-56.278	0.209	-99.465	-18.083
LAC-NP_{169}	52.224	-90.925	-60.486	0.116	-104.921	-19.843
LAC-2OP	45.568	-85.240	-70.382	0.797	-91.919	-17.208
LAC-3OP	61.952	-100.698	-78.291	0.708	-98.944	-16.741
LAC-4OP	57.344	-102.482	-71.380	1.055	-107.230	-16.597
LAC-4dOP	58.880	-99.843	-78.150	0.725	-105.182	-17.607
LAC-4tOP	60.416	-96.510	-71.319	0.110	-99.220	-18.427

537 [a] Refers to the best docking complex.
538

539 **Table 3** Amino acid residues involved in hydrophobic contacts in the binding of laccase to NP and OP isomers.

Enzyme-ligand complex[a]	Amino acid residues involved in hydrophobic contacts														
LAC-2NP	Leu300	Phe239	Gln237	Val426	Tyr244	Pro299	Ala410	Thr210	Ser235						
LAC-3NP	Leu459	Phe450	Pro346	Ser113	Leu112	Glu460	Phe81	Phe344	Ala80	Pro79					
LAC-4NP	Leu459	Phe450	Pro346	Ser113	Leu112	Glu460	Phe81		Ala80	Ipa1514	His111				
LAC-NP_2	Leu459		Pro346	Ser113	Leu112	Glu460	Phe81	Phe344	Ala80	Ipa1514	His111	Thr345			
LAC-NP_9	Leu459	Phe450	Pro346	Ser113	Leu112	Glu460	Phe81	Phe344	Ala80	Ipa1514	Gly462				
LAC-NP_{30}	Leu459	Phe450	Pro346	Ser113	Leu112	Glu460	His109	Phe344	Ala80	Ipa1514	His111	Thr345	Tyr116	Asp456	Ser110
LAC-NP_{65}	Leu300	Phe239	Gln237	Ile301	Ser427	Gln242	Ala410	Glu302	Arg423	Gly411	Thr303	Ser409	Ser412		
LAC-NP_{167}	Leu300	Phe239	Gln237	Ile301	Tyr244	Gln242	Ala410	Glu302	Arg423	Gly411					
LAC-NP_{165}	Leu300	Phe239	Gln237	Ile301	Ser427	Gln242	Ala410	Glu302							
LAC-NP_{172}	Leu459	Phe450	Pro346	Ser113		Glu460		Phe344	Ala80	Ipa1514	His111	Thr345			
LAC-NP_{169}	Leu459	Phe450	Pro346	Ser113	Leu112	Glu460	Pro79	Phe344	Ala80	Ipa1514	His111	Thr345	Val82		
LAC-2OP	Ile236	Thr210	Pro299	Ser235	Tyr244	Gln237	Phe239	Glu302	Ala410	Ser427					
LAC-3OP	Leu459	Phe450	Pro346	Ser113	Leu112		Phe81	Phe344	Ala80		His111				
LAC-4OP	Leu459	Ser110	Pro346	Ser113	Leu112	His452	His109	Phe344	Asp456	Ipa1514	His111	Thr345	Tyr116		

LAC-4dOP	Leu459	Phe450	Pro346	Ser113	Leu112	Glu460	Phe81	Phe344	Ala80		His111	Gly462
LAC-4tOP	Leu459	Phe450	Pro346	Ser113	Leu112	Glu460	Phe81		Ala80	Ipa1514	His111	Gly462

[a] Refers to the best docking complex.

542 **Table 4** Hydrogen bonds in the binding of laccase to NP and OP isomers.

Enzyme-ligand complex[a]	Number of H-bonds	Enzyme residues atom	Ligand atom	H-bond Length (Å)
LAC-2NP	3	Gln242 N	O	2.66
		Glu302 O	O	2.87
		Ser427 N	O	3.14
LAC-3NP	2	His111 N	O	3.30
		His111 O	O	2.90
LAC-4NP	1	Phe344 O	O	2.97
LAC-NP_{65}	1	Tyr244 O	O	2.68
LAC-NP_{165}	2	Tyr244 O	O	2.35
		Arg423 N	O	2.86
LAC-2OP	2	Leu300 N	O	2.87
		Leu300 O	O	2.81
LAC-3OP	1	Glu460 O	O	3.16
LAC-4OP	1	Glu460 O	O	3.10
LAC-4tOP	1	Phe344 O	O	2.79

543 [a] Refers to the best docking complex.

Figure captions:

Fig. 1 General conceptual framework of this study**.**

Fig. 2 Binding orientations and binding pockets of NP and OP isomers in *T. versicolor* laccase. (A) LAC-2NP; (B) LAC-3NP; (C) LAC-4NP; (D) LAC-2OP; (E) LAC-3OP; (F) LAC-4OP. The elements of oxygen and carbon are represented as red and gray spheres, respectively. The binding pockets of complexes are drawn as green grids.

Fig. 3 The detailed views of NP and OP isomers in active sites of *T. versicolor* laccase. (A) LAC-2NP; (B) LAC-3NP; (C) LAC-4NP; (D) LAC-NP_2; (E) LAC-NP_9; (F) LAC-NP_{30}; (G) LAC-NP_{65}; (H) LAC-NP_{167}; (I) LAC-NP_{165}; (J) LAC-NP_{172}; (K) LAC-NP_{169}; (L) LAC-2OP; (M) LAC-3OP; (N) LAC-4OP; (O) LAC-4dOP; (P) LAC-4tOP. The *T. versicolor* laccase are shown in molecular surface form created by electrostatic properties. The red sections represent electropositive and the blue sections represent electronegative. NP and OP isomers are shown in gray stick models.

Fig. 4 Detailed interaction between *T. versicolor* laccase and NP (or OP) isomer complexes. (A) LAC-2NP; (B) LAC-3NP; (C) LAC-4NP; (D) LAC-2OP; (E) LAC-3OP; (F) LAC-4OP. The atoms of oxygen, carbon and nitrogen are colored by red, black and blue. The purple solid lines stand for NP isomers, while orange solid lines stand for amino acid residues belonged to laccase and the green letters and numbers beside them are shown as the type of residues. The imaginary lines are shown as hydrogen bonds and the numbers on the line indicate hydrogen bond length (Å). The models in red solid wires are denoted as hydrophobic contacts in the binding of laccase to NP isomers. The black letters and numbers beside the red models are shown as the type of amino acid residues involved in hydrophobic contacts.

Fig. 5 Dendrogram of amino acid residues involved in hydrophobic contacts in laccase-isomer complexes using UPGMA method. Vertical axis stands for analyzed complexes as samples. Horizontal axis stands for average distance between different clusters.

Fig. S1 Binding orientations and binding pockets of NP and OP isomers in *T.*

versicolor laccase. (A) LAC-NP_2; (B) LAC-NP_9; (C) LAC-NP_{30}; (D) LAC-NP_{65}; (E) LAC-NP_{167}; (F) LAC-NP_{165}; (G) LAC-NP_{172}; (H) LAC-$NP_{169;}$ (I) LAC-4dOP; (J) LAC-4tOP. The elements of oxygen and carbon are represented as red and gray spheres, respectively. The binding pockets of complexes are drawn as green grids.

Fig. S2 Detailed interaction between *T. versicolor* laccase and NP (or OP) isomer complexes. (A) LAC-NP_2; (B) LAC-NP_9; (C) LAC-NP_{30}; (D) LAC-NP_{65}; (E) LAC-NP_{167}; (F) LAC-NP_{165}; (G) LAC-NP_{172}; (H) LAC-$NP_{169;}$ (I) LAC-4dOP; (J) LAC-4tOP. The atoms of oxygen, carbon and nitrogen are colored by red, black and blue. The purple solid lines stand for NP isomers, while orange solid lines stand for amino acid residues belonged to laccase and the green letters and numbers beside them are shown as the type of residues. The imaginary lines are shown as hydrogen bonds and the numbers on the line indicate hydrogen bond length (Å). The models in red solid wires are denoted as hydrophobic contacts in the binding of laccase to NP isomers. The black letters and numbers beside the red models are shown as the type of amino acid residues involved in hydrophobic contacts.