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Molecular docking studies and in vitro degradation of four aflatoxins (AFB 1 , AFB 2 , AFG 1 , and AFG 2) by a recombinant laccase from *Saccharomyces cerevisiae*

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24 **ABSTRACT:** Aflatoxins are widely distributed mycotoxins with high concentration in
25 food and feed. They may have negative impacts on human health, animal productivity
26 and the economy, these being accompanied by removal difficulties. Enzymatic
27 degradation of aflatoxins is recently becoming an efficient strategy to ensure food and
28 feed safety . Here, molecular docking was used to predict and compare interactions
29 between laccase and four aflatoxins (AFB₁, AFB₂, AFG₁ and AFG₂) as well as their
30 degradation at a molecular level. Docking simulation studies indicated that aflatoxins
31 may interact near the T1 copper center through H-bonds and hydrophobic interactions
32 with amino acid residues His481 and Asn288, His481, Asn288 and Asp230, His481 and
33 Asn288. Removal tests were performed in vitro in the presence of a recombinant fungal
34 laccase. Degradation increased as incubation time increased from 12h to 60h and the
35 maximum degradation obtained for AFB₁, AFB₂, AFG₁ and AFG₂ was 90.33%, 74.23%,
36 85.24% and 87.58%, respectively. Maximum degradation of aflatoxins was obtained
37 with a laccase total activity 4U at 30 °C in 0.1M phosphate buffer, pH 5.7 after 48h
38 incubation. The experimental results are consistent with that of docking calculation on
39 the removal of four aflatoxins by laccase.

40
41 **Key Words: Laccases; degradability; aflatoxins.**

42

43 INTRODUCTION

44 Mycotoxins are low-molecular weight molecules produced as secondary metabolites
45 by several species of fungi that contaminate various agricultural products, such as
46 cereals, maize, oilseeds and nuts, either before or under post-harvest conditions[1, 2].
47 Among various types of mycotoxins, aflatoxins produced by *Aspergillus flavus*, *A.*
48 *parasiticus* and *A. nomius*, pose serious threat to public health and livestock
49 productivity, even at relatively low concentration. They constitute a group of closely
50 related compounds leading to highly toxic, mutagenic and carcinogenic[3, 4]. Therefore,
51 it is necessary to remove them timely from foodstuffs.

52 Among the 20 well-known aflatoxins, aflatoxinB₁ (AFB₁), aflatoxinB₂ (AFB₂),
53 aflatoxinG₁ (AFG₁), aflatoxinG₂ (AFG₂) are the most toxic ones (toxicity of AFB₁ >
54 AFG₁ > AFB₂ > AFG₂). They are both frequently found in many human dairy
55 foodstuffs and animal feeds and difficult to remove. AFB₁ is the most potent naturally
56 occurring hepatotoxic, immunotoxin and teratogenic properties reported to date
57 (classified as Group 1 carcinogen by International Agency for Research on Cancer).
58 Although it affects primarily the liver, AFB₁ is pluripotent acting on multiple organs
59 and multiple systems [5], and is causing both acute and chronic toxicity [6]. The main
60 toxic structures are the coumarin lactone ring, the double bond in the furan ring and the
61 cyclopentenone ring.

62 Currently, many researchs are focused on aflatoxins degradation or removal, and
63 various strategies have been reported to reduce aflatoxins levels. The most common
64 way to remove aflatoxin is by physical treatment, such as heating at high temperatures,
65 plasma degradation [7], extrusion or electromagnetic radiation treatment[8]. In addition,
66 chemical treatments such as ammoniation, nixtamalization, ozonation [9], acids and
67 bases treatments are also effective. However, these methods are so far not leading to
68 practical applications because of consecutive nutritional quality losses and because they
69 hardly comply with security, cost and productivity requirements for commercialization,
70 as well as they may cause environmental damages. Biological processes, including
71 degradation by plant extracts[10], microorganisms and enzymes, or the direct
72 adsorption to the cell surface of microorganisms like *Saccharomyces cerevisiae* [11]
73 and *Lactobacillus casei*[12], which are gentle, low cost and effective with less or no
74 intermediates harmful to humans and animals, have been identified as promising routes.

75 Laccases (EC1.10.3.2) belong to the family of the blue multicopper oxidases. They
76 contain four copper ions, a type 1 copper acting as a primary electron acceptor from
77 reductant species and a trinuclear cluster (TNC) involved in dioxygen reduction.
78 Widely distributed in plants, fungi, bacteria, and insects, these enzymes have the
79 catalytic ability to oxidize a wide range of aromatic substrates with a concomitant
80 reduction of dioxygen to water[13]. Globally, fungal laccase have better pH/or
81 temperature stability, higher metal tolerance and are able to oxidize a wider range of
82 substrates as enzymes from other origin[14]. At present, they are broad application
83 prospects of these enzymes in environment, food, medicine, cosmetics and other fields.
84 Considering the structural characteristics of aflatoxins, laccases may have a great
85 potential in AFs' biodegradation applications.

86 The use of laccases may result in rapid and significant recalcitrant substrate
87 degradation[15]. With the maturity of computer simulation technology and the deep
88 understanding of the three-dimensional structure of laccase, it becomes easier and faster
89 to study the relationship between laccase and substrate through molecular docking.
90 Laccase is taken as redox enzyme while molecular docking is used to conduct
91 simulation studies focusing on substrates binding abilities and binding modes. Catalytic
92 reactions then occur only if substrates get close enough to the T1 copper center of the
93 enzyme. Molecular docking has been used in a good deal of researches to study the
94 interaction of laccase with substrates. They agree that molecular docking is an effective
95 analytical tool to assess the enzyme-substrate interaction. E.D. Morales-Alvarez[16] has
96 used molecular docking to evaluate interactions between the recombinant laccases
97 GILCC1 and POXA 1B enzymes with Crystal Violet (CV) or Malachite Green (MG)
98 dyes. L. Dellafiora[17] have investigated the enzyme-substrate interaction for various
99 enzyme isoforms through 3D molecular docking techniques. D. Mo[18] has employed
100 molecular docking to study the interaction mechanism between nonylphenol and
101 octylphenol isomers and a laccase from *Trametes versicolor*. Y. Zhang[13] has used
102 molecular docking method to analyze the interactions between laccase produced by
103 *Trametes versicolor* and Triton X-100. Comparing three-dimensional models of
104 laccases A.K.S. Kameshwar[19] has performed molecular docking studies using lignin
105 model compounds to understand the structural and functional properties of laccase. K.K.
106 Sharma[20] has used molecular docking to study the interaction of different
107 biomolecules with laccases from *C. neoformans* and different enteropathogenic bacteria
108 to understand their probable role in the oxidation of cellular metabolites and the
109 formation of the reactive intermediates.

110 In this work, we investigated the enzymatic degradation of aflatoxins by a laccase.
111 An homology model of the enzyme was first obtained then molecular docking analysis
112 was used to evaluate interactions between the laccase and four aflatoxins (AFB₁, AFB₂,
113 AFG₁ and AFG₂). This analysis was used to better understand the basis of the enzyme-
114 substrates interaction at a molecular level, highlighting interaction areas around the
115 catalytic site and specific differences among the aflatoxins in terms of enzyme surface-
116 ligand recognition. Results were compared to degradation tests performed alongside.
117 The comparison of theoretical and experimental data help to broaden our knowledge on
118 the degradation of aflatoxins and provide a basis for later molecular modifications of
119 laccase.

120 MATERIALS AND METHODS

121 Chemical and Reagents

122 LAC3 from *Trametes versicolor* produced heterologously in *Saccharomyces*
123 *cerevisiae* was gift from Dr. Thierry Tron's lab. Aflatoxins (purity >99%) was obtained
124 from J&K. ABTS was purchased from sigma. Methanol and formic acid were of high
125 performance liquid chromatography grade. Britton-Robinson buffer consisting of
126 0.04M phosphoric, 0.04M acetic acid, 0.04M boric acid was used during the
127 experiments. Ultrapure water was used throughout all of the experiments.

128 **Preparation of Aflatoxins Working Solution**

129 Solid aflatoxins were dissolved into 10mL of methanol to obtain 0.1 mg/mL standard
130 aflatoxins solutions and subsequently stored at -20°C.

131 **Preparation of Laccase**

132 Laccase secreting *Saccharomyces cerevisiae* cells were cultivated at 30°C for 4 days
133 in S-Gal medium on a rotary shaker incubator at 160 rpm. Cells were pelleted by
134 centrifugation (8000rpm for 30min). The recombinant laccase was found in the
135 supernatant. Purification was carried out as previously described[21]

136 **Laccase Activity**

137 Laccase assay was based on 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonate)
138 (ABTS) oxidation in 0.04M Britton-Robison buffer (pH 4.0) at 30°C. Oxidation of
139 ABTS was monitored spectroscopically by absorbance measurements at 420nm ($\epsilon =$
140 $3.6 \times 10^4 \text{M}^{-1} \text{cm}^{-1}$). One unit of laccase oxidizes 1 μ mol of substrate per minute[22].

141 **Extraction of residual Aflatoxins and analysis by HPLC-MS**

142 Aflatoxins were extracted three times from samples with chloroform (1 :1, v/v) as
143 described by Teniola et al[23]. Then chloroform fractions were pooled and evaporated
144 under nitrogen to dry.

145 The samples were dissolved in methanol, filtered (0.22 μ m) and separated on an
146 Inertsil ODS-3 C18 column,(4.6 \times 150mm, 3 μ m) coupled to a 1260 HPLC system
147 equipped with 6240 Triple Quad LC/MS (Agilent, United States) detector. LC-MS
148 conditions for Aflatoxins analysis were the followings: for regular analysis, 5 μ L of
149 sample or standard solutions were injected; the mobile phase, pumped at a flow rate of
150 0.2mL/min, consisted in an isocratic mixture of aqueous solution of 0.1% formic
151 acid/methanol (3:7, v/v); column temperature was maintained at 30°C; the total
152 operation time was 30 min; compounds were analyzed in positive mode. MS conditions
153 were as follows: capillary temperature 300°C; sheath gas flow and auxiliary gas flow
154 (11L/min and 3L/min, respectively) were adjusted to get stable spray; Fragmentor
155 Voltage, Collision Energy, and Cell Accelerator Voltage were 135V, 30eV, 4KV,
156 respectively. Data were collected in MRM Scan Type with the precursor ions of AFB₁,
157 AFB₂, AFG₁, AFG₂ measured at m/z 313, 314, 328, 330 and product ions measured at
158 m/z 285, 287, 243 and 245, respectively.

159 **Aflatoxins Degradation by Laccase**

160 Aflatoxins degradation experiments (methanol solution) were carried out in 10mL
161 tubes. Laccase was incubated with toxins (0.1mg/mL) at intervals of enzyme activity,
162 incubation periods and temperature. The control was prepared with addition of 0.1 M
163 PBS pH 5.7 instead of laccase. Samples were placed in a shaker-incubator and
164 incubated under constant agitation (200rpm/min) at 30°C. Afterward, the toxins content
165 in the reaction mixture was extracted and determined as described above. Experiments
166 were performed independently for each aflatoxin.

167 The effect of a variation of the enzyme concentration on degradation rates was

168 conducted with enzyme loads corresponding to 1-5U. The effect of the incubation time
169 on degradation rates was investigated sampling the reactions every 12h for 60h. The
170 effect of temperature on degradation rates was investigated in the range of 25-45°C.

171 All experiments were performed in triplicate and the percentage (%) of degradation
172 was calculated with the following equation:

$$173 \quad C(\%) = [(A - B) / A] * 100$$

174 Where, A corresponds to the initial mass of the aflatoxins and B is the residual mass
175 of the aflatoxins, C is the degradation rate of laccase for aflatoxins.

176 **Homology Modeling of the laccase LAC3**

177 The target sequence LAC3 was retrieved from Uniprot (Uniprot ID Q6TH77).
178 Template crystal structures were screened through a BLAST search; the structure with
179 the highest homology score was downloaded from the RCSB Protein Data Bank (PDB
180 ID: 3KW7). Homology modeling was conducted in MOE v2014.0901 (Chemical
181 Computing Group Inc, 1010 Sherbooke St. West, Suite#910, Montreal, QC, Canada,
182 H3A 2R7. 2014). The protonation state of the protein and the orientation of the
183 hydrogen atoms were optimized by LigX at a pH of 7 and a temperature of 300 K. First,
184 the target sequence was aligned to the template sequence, and ten independent
185 intermediate models were built. These different homology models were the result of the
186 permutational selection of different loop candidates and side chain rotamers. Then, the
187 intermediate model with the highest score according to the GB/VI scoring function was
188 chosen as final model, and then subjected to further energy minimization using the
189 AMBER12/EHT force field.

190 **Modeling of Aflatoxins**

191 AFB₁、 AFB₂、 AFG₁、 AFG₂ were used to perform docking analysis with the model
192 constructed for LAC3 from *Trametes sp.* C30. 2D structures of the AFB₁(CAS: 1162-
193 65-8), AFB₂(CAS: 7220-81-7), AFG₁(CAS: 1165-39-5) and (AFG₂, CAS: 7241-98-7)
194 were drawn in ChemBio Draw 2014 and converted to 3D in MOE v2014.0901 through
195 energy minimization. 2D structure of ligands are shown in Table 1.

196 **Molecular Docking Experiments**

197 MOE Dock in MOE v2014.0901 was used for molecular docking simulations of the
198 aflatoxins and prediction of their “binding affinity” to the homology model of LAC3.
199 The 3D structure of the LAC3 was predicted through homology modeling. And then,
200 the protonation state of the enzyme and the orientation of the hydrogens were optimized
201 by LigX, at the PH of 7 and temperature of 300 K. Prior to docking, the force field of
202 AMBER12: EHT and the implicit solvation model of Reaction Field (R-field) were
203 selected. The docking workflow followed the “induced fit” protocol, in which the side
204 chains of the receptor pocket were allowed to move according to ligand conformations,
205 with a constraint on their positions. The weight used for tethering side chain atoms to
206 their original positions was 10. All docked poses of aflatoxins molecules were ranked
207 by London dG scoring first, then a force field refinement was carried out on the top 30
208 poses followed by a rescoring of GBVI/WSA dG.

209 **Statistical Analysis**

210 All the experiments were performed in triplicate. All the data are graphically presented
211 as means \pm standard deviation of triplicates($n=3$), and the data were analyzed using
212 single factor analysis of variance(ANOVA). ANOVA was performed using SPSS
213 software(Version 8.5). Difference among the means were considered for significance at
214 $P < 0.05$ using Duncan's multiple range test.

215 **RESULTS**

216 **Interaction of Aflatoxins with the laccase**

217 In the absence of an available 3D structure of LAC3, the investigation of the binding
218 mode of aflatoxins with this enzyme required first the construction of an homology
219 model. Results are displayed in Figure 1 and Table 1. Analysis of the Ramachandran
220 plot revealed that ninety-nine percent of LAC3 residues map in allowed regions which
221 underlines that our homology model of LAC3 conforms to stereo chemical rules (Figure
222 2). Therefore, we docked aflatoxins AFB₁, AFB₂, AFG₁ and AFG₂ on the LAC3
223 homology model.

224 Docking scores of interactions between aflatoxins and LAC3 are reported in Table 3.
225 The minor differences observed among the values obtained for each aflatoxins may be
226 related to their chemical similarities. The different binding models between ligands and
227 laccase may result from different binding ability. The computational result indicates
228 that each of the aflatoxins tested can interact with laccase with a binding ability of
229 AFB₁>AFG₂>AFG₁>AFB₂.

230 The lowest energy configurations of aflatoxins and their binding mode at the surface
231 of LAC3 are illustrated in Figure 3. We assume that these predictions correspond to the
232 most likely configurations. In our models, the oxygen atom of the methoxy group of
233 AFB₁, regarded as hydrogen bond acceptor, forms one hydrogen bond with the side
234 chain of His481 which is coordinated to the nearby copper ion (i.e. T1 Cu^{II}) [24]
235 whereas the oxygen atom of the terminal furan ring, regarded as hydrogen bond
236 acceptor, forms one hydrogen bond with the side chain of Asn288. Similarly, a carbon
237 atom of the benzene ring in AFB₂, regarded as hydrogen bond acceptor, forms one
238 hydrogen bond with the sidechain of His481. Two carbon atoms of the terminal furan
239 ring of AFG₁, regarded as hydrogen bond acceptors, form two hydrogen bonds with the
240 sidechain of Asn288 whereas the oxygen atom of 5-membered heterocyclic ring of
241 AFG₁, regarded as hydrogen bond acceptors, form one hydrogen bond with the
242 sidechain of Asp230. The carbon and oxygen atoms of the terminal furan ring of AFG₂,
243 regarded as hydrogen bond acceptors, forms two hydrogen bonds with the side chain
244 of Asn288 in laccase. Eventually, the carbon atom of methoxy group of AFG₂, regarded
245 as hydrogen bond acceptor, forms one hydrogen bond with the side chain of His481.

246 **Degradation of Aflatoxins using the Recombinant Laccase LAC3 Produced in** 247 ***Saccharomyces cerevisiae***

248 The aflatoxins(B₁, B₂, G₁ and G₂) detoxifying efficacy of LAC3 was tested at

249 different incubation times, enzyme activities and temperatures. Generally speaking, the
250 degradation of aflatoxins by the laccase was a rapid process.

251 The kinetic of degradation of aflatoxins was investigated at an enzyme activity of 3U
252 and at 30°C. As it can be seen from Figure 4(A), degradation rate increased first from
253 12 to 48h and then no significant difference was observed from 48 to 60h. In these
254 conditions, the maximum degradation of AFB₁, AFG₁ and AFG₂, respectively 90.33%,
255 85.24%, 87.58%, was observed after 48h of incubation time, while for AFB₂ the
256 maximum of degradation was reached after 60h of incubation. This may be explained
257 that the activity sites of laccase were more and the concentration of aflatoxins were
258 higher during the beginning of the reaction, the declining activity sites limited the
259 reaction.

260 Degradation experiments with laccase were tested at different enzyme activity (i. e.
261 different enzyme loads). Experiments were performed at 30°C for 12h and results are
262 presented in Figure 4(B). Expectedly, degradation increased with an increase in the
263 enzyme load. Starting to plateau at 4U of laccase, the maximum degradation of AFB₁,
264 AFB₂, AFG₁, AFG₂, respectively 74.87%, 62.36%, 66.94%, 70.88%, was obtained at
265 5U of laccase. It is more likely to predict that the substrate-binding sites may have
266 reached to the saturation point as the activity of laccase was above 4U.

267 The effect of temperature on degradation rate of aflatoxins was conducted at an
268 enzyme activity of 3U for 12h and the results are shown in Figure 4(C). The percentage
269 of degradation of aflatoxins by laccase increased slightly from 25 to 40°C and then
270 decreased with an increase of temperature from 40 to 45°C. The maximum degradation
271 of AFB₁, AFB₂, AFG₁, AFG₂ respectively, i.e., 77.45%, 51.84%, 56.06%, 68.33%, was
272 observed at 30°C. LAC3, like most of fungal laccase, has been described as a fairly
273 temperature tolerant enzyme. However, temperature tolerance is function of the
274 exposure time. Therefore, the decrease in activity observed when the reaction is
275 performed at a temperature over 40°C is probably a consequence of the long exposure
276 (12h) of the enzyme at this temperature. Hence, the optimal temperature was set at 30°C
277 in future studies.

278 All together the experimental are in agreement with the theoretical study that
279 predict aflatoxins “binding affinity” for LAC3, in particular that of AFB₂ that was found
280 minimum among the aflatoxins tested.

281 **DISCUSSION AND CONCLUSION**

282 It is important to reduce the mycotoxin contents in food and feed for public health
283 and globe trade. A wide number of effective strategies are already used to control toxin
284 accumulation levels at pre- or post-harvest. Comparing with cultivation techniques,
285 conventional breeding, genetic engineering and other physical or chemical methods,
286 biological treatments have good application potential due to their ability to transform
287 toxins to non - or less harmful compounds during food processing [25]. Among these,
288 the degradation by means of enzymes is considered as the strategy with the most highest
289 potential for the mitigation of toxins content. So far, J.F. Alberts et al. have tried to
290 degrade AFB₁ by laccase from white rot fungi *Peniophora*, *Pleurotus ostreatus* and
291 *Trametes versicolor* achieving degradation of 40.45%, 35.90% and 87.34%,

292 respectively[26]. Zeinvand-Lorestani H et al. also applied a commercial laccase
293 preparation from *Trametes versicolor* to AFB₁ with 67% of the toxic substrate
294 removal[27]. Loi M et al. have shown that AFB₁ and AFM₁ degradation by a pure
295 enzyme form *Pleurotus pulmonarius* is greatly enhanced from 23% to more than 90%
296 in the presence of redox mediators [28]. However, screening diversity to find out proper
297 active enzymes is challenging and time-consuming, and more importantly does not
298 allow to study an interaction mechanism between ligands and the target enzyme.
299 Investigation of potential enzyme-substrate interactions by in silico methods could be
300 used as screening tool as it provides increasingly reliable and informative insights [17].
301 While taken alone there may be still some deviations due to computer simulation,
302 performing alongside molecular docking analysis and in vitro experiment allows a
303 benchmarking of methodologies. The consistency between in vitro experimental results
304 and that of docking calculation will contribute to find out a faster, more precise and
305 effective way in aflatoxins removal.

306 Here, we employed molecular docking to study the interaction of four aflatoxins with
307 the surface of a typical fungal laccase aiming to highlight possible differences in ligand-
308 enzyme interactions. According to our models, relevant differences exist among
309 aflatoxins in terms of interaction with the enzyme surface. AFB₁ is likely interacting
310 the most favorably with laccase among all the aflatoxins. Docking simulation studies
311 indicate that the residue His481 present in the coordination sphere of the T1 copper
312 may interact with aflatoxins and therefore could mediate the electron transfer during
313 oxidation[29]. Different hydrogen bonding patterns arise from calculation: in addition
314 to His481, AFB₁ may interact with Asn288, AFB₂ may interact with His481, AFG₁
315 may interact with both Asn288 and Asp230, AFG₂ may interact with both His481 and
316 Asn288. Amino acid residues involved in H-bonds are considered as key residues for
317 the interaction of laccase with a given ligand[18]. H-bonds details as shown in Table 4
318 suggest different interactions between the laccase and the ligands which may
319 correspond to differences in binding ability with AFB₁>AFG₂>AFG₁>AFB₂, an order
320 consistent with that drawn from experimental results on the biodegradation of
321 aflatoxins by LAC3 by , and the of aflatoxins at the same time is
322 AFB₁>AFG₂>AFG₁>AFB₂.

323 The binding affinity is related to the efficiency of enzyme-ligand interactions which
324 is affected by structural characteristics and distorting extent of ligands and the shape
325 complementation between the ligand and the enzyme surface[30, 31]. With the higher
326 binding affinity, the ligands have stronger capability to reach the pocket-like structure
327 of enzyme. Differences in toxin degradation appears to be related primarily to group
328 contacts established with the laccase surface. The stronger the aflatoxin-laccase
329 interaction is the more efficient is the oxidation of the toxin.

330 In silico analysis provides insight into the interaction of aflatoxins with laccase and
331 allows to point structures interactions relevant for the degradation. An in-depth
332 mapping of enzyme-ligand potential interactions will provide a theoretical basis for
333 molecular modification strategies of laccase set to improve degradation or for screening
334 for natural laccase with high toxins degrading capabilities from a variety of
335 microorganisms. In both cases, molecular docking will be advantageous to improve the

336 biodegradation techniques.

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444

445 Table Legends

446 Table 1: Homology modeling of LAC3 (*Trametes* sp. C30)

447 Table 2: 2D structure of ligands

448 Table 3: Docking score of molecules binding to Laccase

449 Table 4: H-bonding distances between laccase and aflatoxins

450 Figure Legends

451 Fig. 1: Homology models of laccase protein sequences (*Trametes* sp. C30)

452

453 Fig. 2: Ramachandran Plot: dark green dots represent the residues in most favored
454 regions; yellow dots represent the residues in additional allowed regions; Small red
455 cross represents the residues in not-allowed regions.

456

457 Fig. 3: Models of AFB₁, AFB₂, AFG₁ and AFG₂ interacting with the laccase LAC3. A.
458 The binding model of AFB₁ on molecular surface of the laccase. B. The interaction
459 model of AFB₂ with the laccase. C. The binding model of AFB₂ on molecular surface
460 of the laccase. D. The interaction model of AFB₂ with the laccase. E. The binding model
461 of AFG₁ on molecular surface of the laccase. F. The interaction model of AFG₁ with
462 the laccase. G. The binding model of AFG₂ on molecular surface of the laccase. H. The
463 interaction model of AFG₂ with the laccase. The ligands are colored in cyan, the T1
464 copper ion is colored in pink and the surrounding residues in the binding pockets are
465 colored in orange. The backbone of the receptor is depicted as lightblue ribbon.

466

467 Fig. 4: Effect of incubation time (A), enzyme activity (B) and temperature (C) on the
468 degradation of aflatoxins by the laccase LAC3. Values are means of three replicates and
469 their standard errors. Means with different letters representing differences within the
470 group are significantly different according to Duncan's test($P \leq 0.05$).

Table Legends

Table 1:

Protein	Template	Query Cover	Identity
Laccase	3KW7 , A	93%	77%

Table 2 :

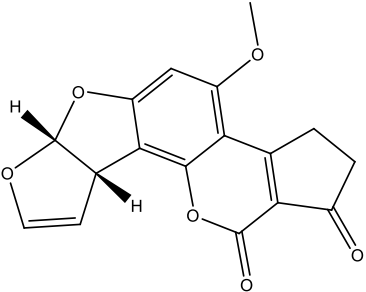
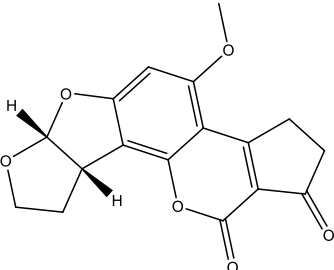
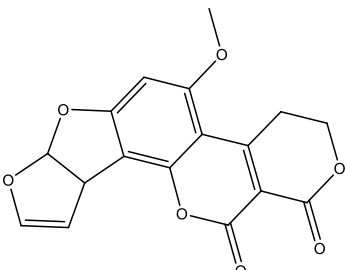
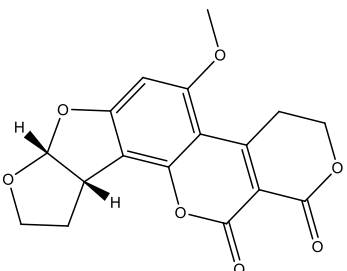
Name	Structure
AFB ₁	 <p>The chemical structure of AFB₁ (Aflatoxin B₁) consists of a difuran ring system fused to a coumarin ring system, which is further fused to a cyclopentenone ring. A methoxy group (-OCH₃) is attached to the coumarin ring at the 10-position. Stereochemistry is indicated with wedged bonds for the hydrogens at the 2 and 3 positions of the difuran ring.</p>
AFB ₂	 <p>The chemical structure of AFB₂ (Aflatoxin B₂) is similar to AFB₁ but features a different ring fusion pattern between the difuran and coumarin systems. It also has a methoxy group at the 10-position of the coumarin ring. Stereochemistry is indicated with wedged bonds for the hydrogens at the 2 and 3 positions of the difuran ring.</p>
AFG ₁	 <p>The chemical structure of AFG₁ (Aflatoxin G₁) is similar to AFB₁ but has a different ring fusion pattern between the difuran and coumarin systems. It also has a methoxy group at the 10-position of the coumarin ring. Stereochemistry is indicated with wedged bonds for the hydrogens at the 2 and 3 positions of the difuran ring.</p>
AFG ₂	 <p>The chemical structure of AFG₂ (Aflatoxin G₂) is similar to AFB₁ but has a different ring fusion pattern between the difuran and coumarin systems. It also has a methoxy group at the 10-position of the coumarin ring. Stereochemistry is indicated with wedged bonds for the hydrogens at the 2 and 3 positions of the difuran ring.</p>

Table 3:

Ligands	Receptor	Docking score (kcal/mol)
AFB₁	laccase	-6.4532
AFB₂	laccase	-5.5306
AFG₁	laccase	-5.9708
AFG₂	laccase	-6.3328

Table 4 :

Enzyme-ligand	H-bonds	Enzyme residues	Ligand atom	H-bond length(Å)
LAC-AFB₁	2	His481	O	3.16
		Asn288	O	3.2
LAC-AFB₂	1	His481	C	3.14
LAC-AFG₁	3	Asn288	C	3.17
		Asn288	C	3.16
		Asp230	O	2.76
LAC-AFG₂	3	His481	C	2.98
		Asn288	C	3.19
		Asn288	O	2.93

Figure Legends

Fig. 1



Fig. 2

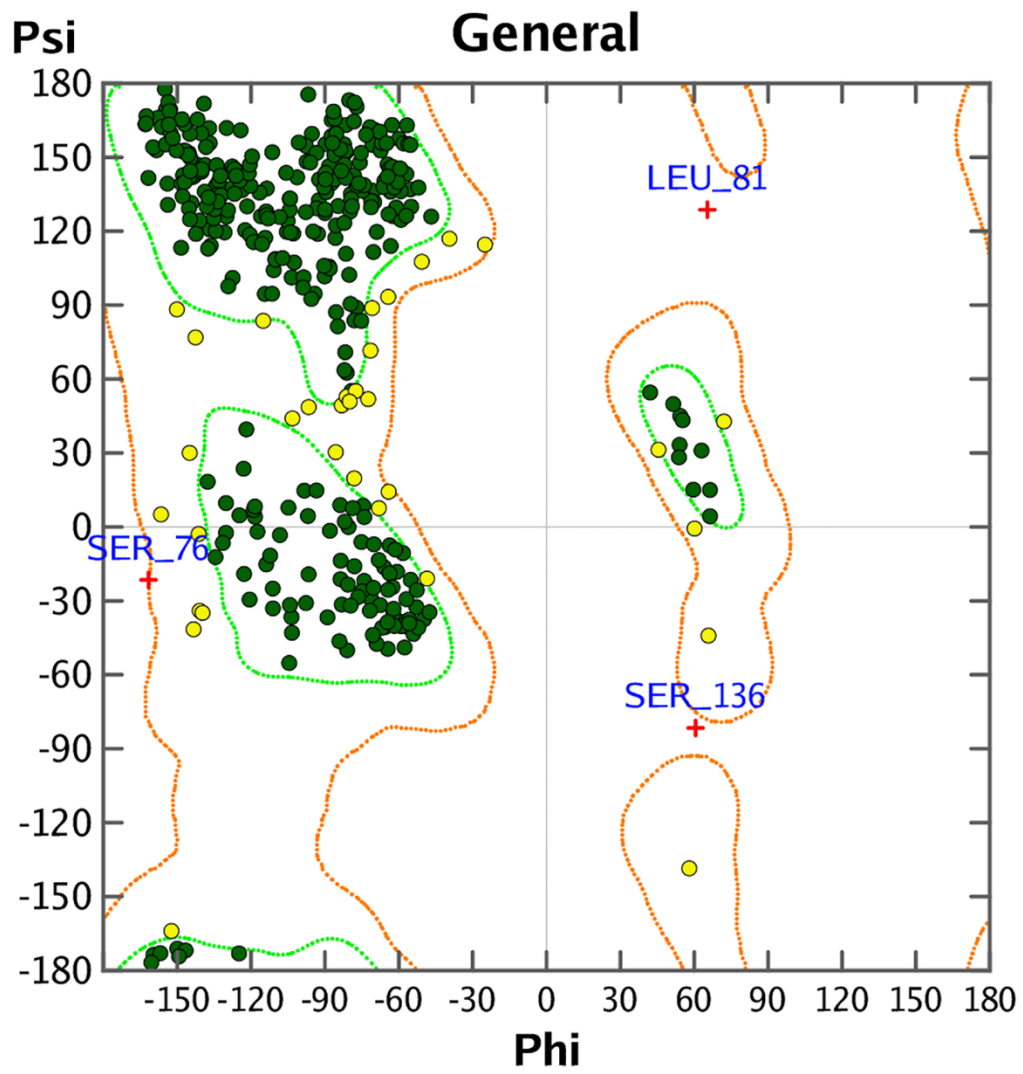


Fig. 4

