Supplemental Material

Cloning of a Novel Nicotine Oxidase Gene from *Pseudomonas* sp. Strain HZN6 that Non-enantioselectively Degrades Nicotine to Pseudooxynicotine

Jiguo Qiu,[a,*] Yun Ma,[b,*] Jing Zhang,[a] Yuezhong Wen,[a] and Weiping Liu[a,**]

Running title: A Novel Nicotine Oxidase Gene

[a] *Key Laboratory of Environmental Remediation and Ecosystem Health, Ministry of Education, College of Environmental and Resource Sciences, Zhejiang University, Hangzhou 310058, China;*

[b] *Research Center of Environmental Science, College of Biological and Environmental Engineering, Zhejiang University of Technology, Hangzhou 310014, China*

∗ These authors contributed equally to this article.

∗∗ Corresponding author: Weiping Liu; E-mail: wliu@zju.edu.cn

Tel: (86)-571-88982740 Fax: (86)-571-88982341

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PREDICTED METHODS

Construction of structural model of NOX

To construct the tertiary structure of the NOX, the homology modeling was performed by the iterative threading assembly refinement (I-TASSER) server (http://zhanglab.ccmb.med.umich.edu/I-TASSER/) (1). The initial template was retrieved from the PDB library and identified by the LOMETS, a locally installed meta-threading approach. The following structure assembly, model selection, refinement, and structure-based functional annotation were performed with the reported protocols (2).

Molecular Docking

To predict the binding modes of *R*- and *S*-nicotine isomers to NOX, the program Molegro Virtual Docker (MVD, version 4.2.0) were used. The binding pocket was defined as a sphere with a user-defined origin and a radius of 15 Å. Twenty times of the MolDock SE algorithm runs were carried out, and the energetic evaluation of the complexes was performed with the MolDock Score. Ten poses of each molecular docking process were finally generated.

PREDICTED RESULTS AND DISCUSSION

The tertiary structure for the NOX protein was generated by the I-TASSER software (Fig. S1). The predicted structure showed most structural similarity to the 6-hydroxy-L-nicotine oxidase of *Arthrobacter nicotinovorans*. As shown in Fig. S1, the NOX consists of three distinct domains: the FD (putative FAD-binding domain), the S1 (putative substrate-binding domain 1), and the S2 (putative substrate-binding domain 2). The conserved FAD-binding motif GxGxxG

existing in many flavoproteins was observed in the FD domain of NOX (GGGFAG), which suggested a similar mechanism of cofactor binding. Furthermore, two putative substrate-binding cavities were identified (Fig. S2). One cavity (C1) was proposed for the binding of FAD, and the other (C2) was for nicotine.

To improve the understanding of the interaction between the NOX and the nicotine isomers, both *R*- and *S*-nicotine were separately docked into the C2 cavity. As shown in Fig S3, it is important to note that both enantiomers shared the same binding mode with the NOX, and located in almost mirror symmetry. The N1 of both nicotine enantiomers formed the hydrogen bond with the His456. The results agreed with the experimental findings that the *Pseudomonas* sp. strain HZN6 degrades the racemic mixture of nicotine. The docking results of both *R*- and *S*-nicotine in the predicted tertiary structure of the NOX protein might provide a reasonable clue of the the non-enantioselective degradation. The FAD was proposed to be a cofactor of the NOX. The conserved FAD-binding GxGxxG motif was found in the NOX sequence and the model docking results also showed that the FAD has formed strong hydrogen bond with this motif (Fig. S4).

However, the homology of NOX to known proteins (including those whose structures have been solved) is rather low (about 30% identity). The reliability of homology model is also low. To obtain the actual structure and the mechanism of the nicotine degradation by the NOX, the protein should be overexpressed in a suitable host strain and subsequently purified and crystallized.

Table S1. EFs changes during the degradation of *RS*-nicotine by *Pseudomonas* sp. strain HZN6 and transconjugant KT-nox. A, at the beginning of the reaction, no substrate was degraded. B and C, at the points when approximately 60 and 85% of the substrate were degraded, respectively.

EFs of nicotine		
Time	HZN6	KT-nox
A	0.496±0.004	0.495±0.002
B	0.502±0.003	0.493±0.004
C	0.499±0.003	0.502±0.004

Fig. S1 Tertiary structure of NOX generated by homology modeling. FD, putative FAD-binding domain (yellow); S1, putative substrate-binding domain 1 (violet) and S2 putative substrate-binding domain 2 (cyan); the conserved GGGFAG motif (red). N, N-termination; C, C-termination.

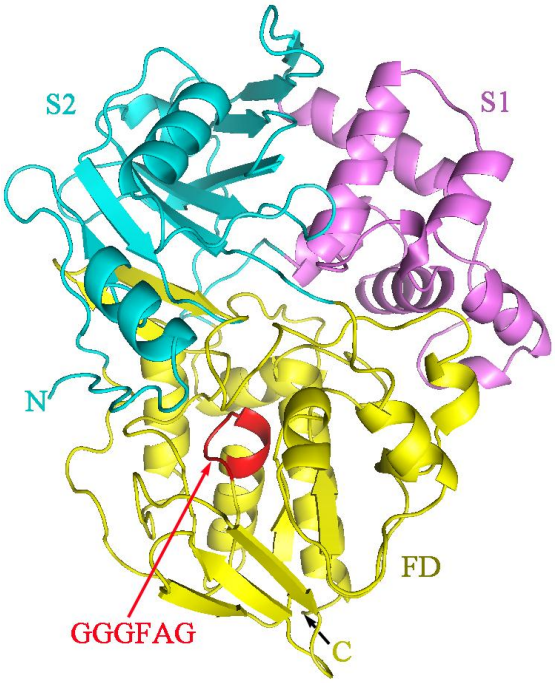


Fig. S2 Tertiary structure of NOX generated by homology modelling and two putative substrate-binding cavities C1 and C2 (green). FD, putative FAD-bonding domain; S1, putative substrate-bonding domain 1; S2 putative substrate-bonding domain 2. The Ŭ-helix, ɓ-sheet and loop were shown in red, blue and white color, respectively.

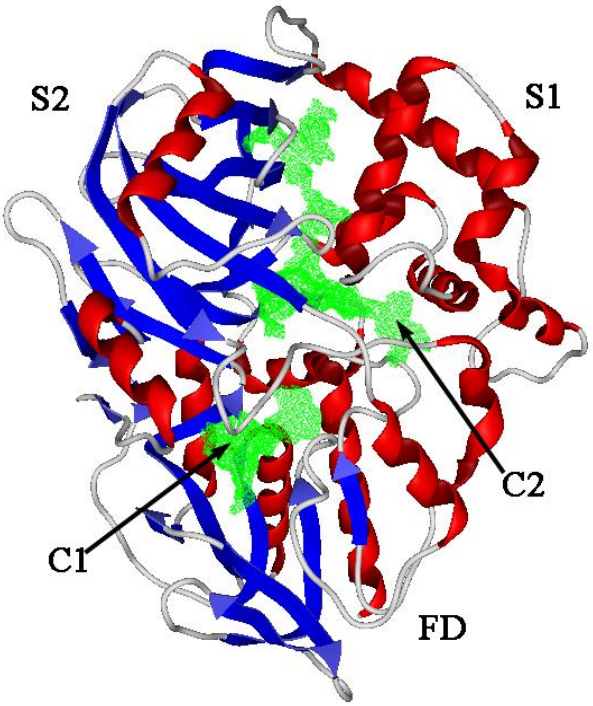


Fig. S3 The best docked conformation of *R*-nicotine (yellow) and *S*-nicotine (green) in NOX. The hydrogen bond was showed by cyan broken line.

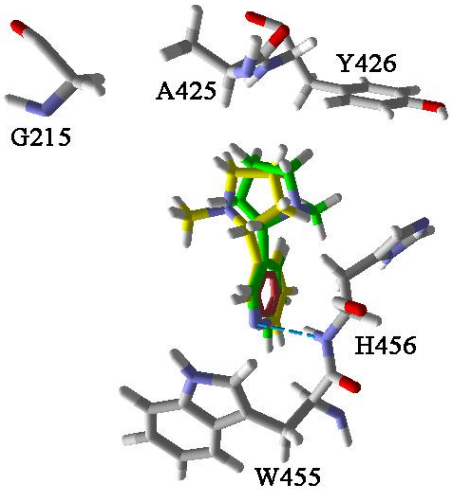
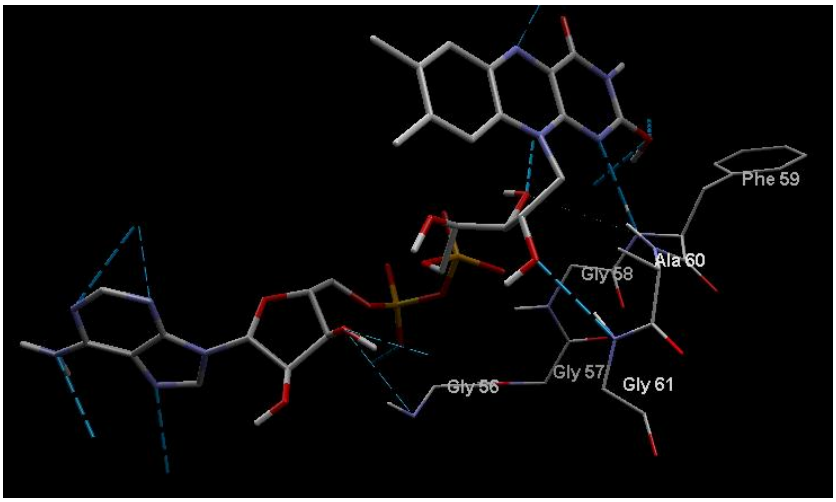
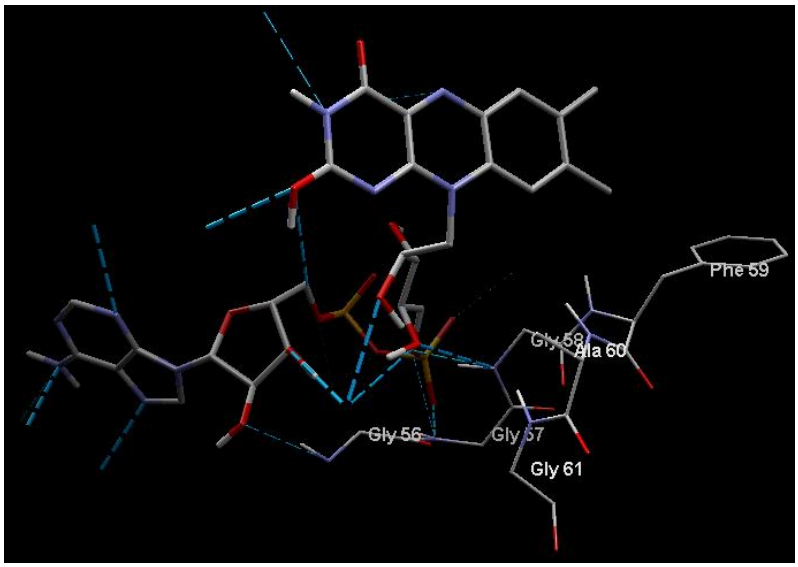


Fig. S4 Two configurations of FAD docked in NOX C1 cavity and hydrogen bonds with the motif GGGFAG. The hydrogen bonds were showed by cyan broken line.





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