Appl Biochem Biotechnol (2013) 170:1614–1623
DOI 10.1007/s12010-013-0276-1

Enzyme–Substrate Binding Landscapes in the Process of Nitrile Biodegradation Mediated by Nitrile Hydratase and Amidase

Yu Zhang · Zhuotong Zeng · Guangming Zeng · Xuanming Liu · Ming Chen · Lifeng Liu · Zhifeng Liu · Gengxin Xie

Received: 23 January 2013 / Accepted: 29 April 2013 /
Published online: 29 May 2013

Abstract The continuing discharge of nitriles in various industrial processes has caused serious environmental consequences of nitrile pollution. Microorganisms possess several nitrile-degrading pathways by direct interactions of nitriles with nitrile-degrading enzymes. However, these interactions are largely unknown and difficult to experimentally determine but important for interpretation of nitrile metabolisms and design of nitrile-degrading enzymes with better nitrile-converting activity. Here, we undertook a molecular modeling study of enzyme–substrate binding modes in the bi-enzyme pathway for degradation of nitrile to acid. Docking results showed that the top substrates having favorable interactions with nitrile hydratase from *Rhodococcus erythropolis* AJ270 (ReNHase), nitrile hydratase from *Pseudonocardia thermophila* JCM 3095 (PtNHase), and amidase from *Rhodococcus* sp. N-771 (RhAmidase) were benzonitrile, 3-cyanopyridine, and L-methioninamide, respectively. We further analyzed the interactional profiles of these top poses with corresponding enzymes, showing that specific residues within the enzyme's binding pockets formed diverse contacts with substrates. This information on binding landscapes and interactional profiles is of great importance for the design of nitrile-degrading enzyme mutants with better oxidation activity toward nitriles or amides in the process of pollutant treatments.

Electronic supplementary material The online version of this article (doi:10.1007/s12010-013-0276-1) contains supplementary material, which is available to authorized users.

Y. Zhang · G. Zeng · M. Chen · L. Liu · Z. Liu · G. Xie
College of Environmental Science and Engineering, Hunan University, Changsha 410082, People's Republic of China

Y. Zhang · G. Zeng (✉) · X. Liu · M. Chen · L. Liu · Z. Liu · G. Xie
Key Laboratory of Environmental Biology and Pollution Control (Hunan University), Ministry of Education, Changsha 410082, People's Republic of China
e-mail: zhangyu19432@163.com

Z. Zeng
Xiangya School of Medicine, Central South University, Changsha 410013, People's Republic of China

Keywords Nitrile biodegradation · Nitrile hydratase · Amidase · Molecular docking · Molecular modeling

Introduction

Nitriles are widespread in the environment as a result of the discharge of industrial wastewater, which mainly contains acrylonitrile, fumaronitrile, and succinonitrile [1]. Such compounds are highly toxic and resistant to chemical degradation [2]. Traditional physical and chemical methods failed to convert nitriles in a "green" manner, whereas microbial degradation is more advantageous due to their convenience and environmental friendly nature [2, 3]. Nitrilase, nitrile hydratase (NHase), and amidase are main enzymes for nitrile degradation in microbes. Nitrile degradation can occur by three pathways: hydrolysis, oxidation, and reduction [4]. Hydrolysis mediated by nitrilase, NHase, and amidase is found to be the most common way for nitrile degradation [2]. NHases are divided into cobalt NHase (Co-type NHase) and ferric NHase (Fe-type NHase), due to that cobalt or ferric is in the catalytic center [5]. NHases show a wide range of substrate specificities and cannot only degrade aromatic nitriles but also are capable of catalyzing the conversion of aliphatic nitriles to corresponding amides [6]. Large quantities of microorganisms have been observed to contain amidase activity [7].

Several studies have successfully adopted microbial degradation as a cost-effective method of removing nitrile pollutants [1]. However, efforts to develop microbial remediation technologies have been hampered by the fact that the key features of interaction profiles between nitriles and nitrile-degrading enzymes are not well understood. Binding of nitriles to corresponding enzymes is crucial for nitrile degradation. Comprehensive characterization of the nitrile binding specificities to nitrile-degrading enzymes is experimentally challenging, in part due to the requirement of expensive research and lengthy experiments [8]. Therefore, it is necessary to introduce novel strategies for exporting their binding landscapes. Alternatively, bioinformatics methods are a good option [9]. We have analyzed simple sequence repeats in many environmental microbial genomes by bioinformatics methods [10–12]. Recently, several studies have reported attempts to analyze the interactions between enzymes and their substrates by molecular docking or molecular simulation in the environmental field [9, 13]. Molecular docking is a bioinformatics technology which is able to detect the optimal binding conformation of ligand to the active site of a receptor [14–16]. Technologies such as X-ray diffraction and nuclear magnetic resonance, bringing many 3D structures of nitriles and nitrile-degrading enzymes, are now providing the opportunity to comprehensively evaluate the binding landscapes in the process of nitrile biodegradation mediated by NHase and amidase.

In the present study, we reported the use of molecular docking to map nitrile/amide binding landscapes for a variety of collections of nitrile-degrading enzymes including Fe-type NHase, Co-type NHase, and amidase. The specific aim of the present study was to provide a basis for the interpretation of nitrile metabolisms and design of nitrile-degrading enzymes with better nitrile-converting activity.

Materials and Methods

As a case study, one of the available 3D structures for each type of nitrile-degrading enzyme was downloaded from the Protein Data Bank (PDB) [17] (http://www.pdb.org/pdb/home/home.do): 2QDY (Fe-type NHase from organism *Rhodococcus erythropolis*

Table 1 List of analyzed nitrile-degrading enzymes and corresponding substrates

Nitrile-degrading enzyme	Organism	PDB ID	Chains	*R*	Selected substrates
Fe-type NHase	*Rhodococcus erythropolis* AJ270	2QDY	A, B	1.30	2,2-Dimethylcyclopropanecarbonitrile, 2-hydroxy-4-phenylbutanenitrile, 3-cyanopyridine, 4-chloro-3-hydroxybutanenitrile,acetonitrile, acrylonitrile, benzonitrile, butyronitrile, isobutyronitrile, methacrylonitrile, and propionitrile
Co-type NHase	*Pseudonocardia thermophila* JCM 3095	1IRE	A, B	1.80	3-Cyanopyridine, acrylonitrile, benzonitrile, methacrylonitrile, and *tert*-butylisonitrile
Amidase	*Rhodococcus* sp. N-771	3A1K	A	2.17	2,2-Dimethylcyclopropanecarboxamide, acetamide, acrylamide, benzamide, butyramide, formamide, hexamide, isobutyramide, L-alaninamide, L-leucinamide, L-methioninamide, L-prolinamid, L-threoninamide, methacrylamide, nicotinamide, and propanamide

AJ270, ReNHase) [5], 1IRE (Co-type NHase from organism *Pseudonocardia thermophila* JCM 3095, PtNHase) [18], and 3A1K (amidase from *Rhodococcus* sp. N-771, RhAmidase) [7]. Their substrate specificities have been described and can be obtained from BRENDA (http://www.brenda-enzymes.org/index.php4), which is a comprehensive database of enzyme information [19]. The chemical 2D structure and 3D conformation of the selected substrates were retrieved from ChemSpider (http://www.chemspider.com/) [20] and is summarized in Table 1. The number of independent docking runs for each modeled structure was 10. Water molecules, cofactors, and bound ligands of each selected enzyme were removed before docking. Various substrates docking to nitrile-degrading enzymes were carried out with the Molegro Virtual Docker (MVD) program using MolDock Score [GRID] function and MolDock SE

Table 2 Molecular docking pose scores between ReNHase and nitriles

Name of nitriles	ID	Rotated bond	MolDock score	Re-rank score
2,2-Dimethylcyclopropanecarbonitrile	228831	1	−58.6892	−48.3675
2-Hydroxy-4-phenylbutanenitrile	9085126	4	−39.3875	6.88525
3-Cyanopyridine	78	1	−64.2838	−56.1564
4-Chloro-3-hydroxybutanenitrile	83714	3	−66.0146	−53.3895
Acetonitrile	6102	1	−35.8553	−29.9185
Acrylonitrile	7567	1	−44.4021	−37.5019
Benzonitrile	7224	1	−64.8815	−56.6631
Butyronitrile	7717	2	−51.7784	−43.4884
Isobutyronitrile	6311	1	−48.5356	−40.9661
Methacrylonitrile	29101	1	−48.3002	−40.7617
Propionitrile	7566	1	−42.0651	−35.3587

Table 3 Interactional profile of ReNHase with benzonitrile

Benzonitrile atom	ReNHase atom	Distance (Å)	Type of contacts
N1	OG1 THR126 A	3.0	Hb
N1	N THR126 A	3.0	Hb
N1	O ILE24 B	3.6	Hb
N1	NE2 HIS29 B	3.9	Hb
C1	CA PRO125 A	3.6	Ph
C3	CD1 ILE24 B	3.1	Ph
C3	CG1 ILE24 B	3.7	Ph
C3	CA PRO125 A	4.2	Ph
C4	CB LEU123 A	3.6	Ph
C4	CG2 THR27 B	3.6	Ph
C5	CD1 ILE24 B	3.6	Ph
C5	CG1 ILE24 B	4.4	Ph
C6	CG2 THR27 B	3.5	Ph
C6	CB LEU123 A	4.0	Ph
C7	CA PRO125 A	3.5	Ph
C7	CB PRO125 A	4.2	Ph
C7	CD1 ILE24 B	4.4	Ph
C2	CE2 TYR73 B	5.2	Ar
N1	CB ILE24 B	4.4	HH
N1	CA PRO125 A	3.8	HH
N1	CB PRO125 A	4.3	HH

Hb hydrogen bonding, *Ph* hydrophobic, *Ar* aromatic–aromatic, *HH* hydrophilic–hydrophobic

algorithm [14]. The binding pockets or cavities in enzymes were detected, in order to provide potential sites for the binding of substrates. All docking experiments were finished using the following default parameters: 1,500 for max iterations, 50 for max population size, 100.00 for energy threshold, 300 for max step, 1.00 for neighbor distance factor, and 5 for max number of poses returned. The conformations with the lowest re-rank scores were selected as the best binding poses. Consistent with our previous study [9], the LPC/CSU server was used to analyze various enzyme–substrate interactions [21].

Results and Discussion

Fe-Type NHase

Fe-type NHase selected in the present study was from *R. erythropolis* AJ270 (ReNHase). Nitriles were selected as 2,2-dimethylcyclopropanecarbonitrile, 2-hydroxy-4-phenylbutanenitrile, 3-cyanopyridine, 4-chloro-3-hydroxybutanenitrile, acetonitrile, acrylonitrile, benzonitrile, butyronitrile, isobutyronitrile, methacrylonitrile, and propionitrile (Tables 1 and 2). Up to date, there is no report on binding modes between these nitriles and ReNHase, although it has been well demonstrated that ReNHase is capable of degrading these nitriles [22–27]. Here, we presented the first binding analysis between them by MVD (Fig. S1). The entrance of ReNHase is very

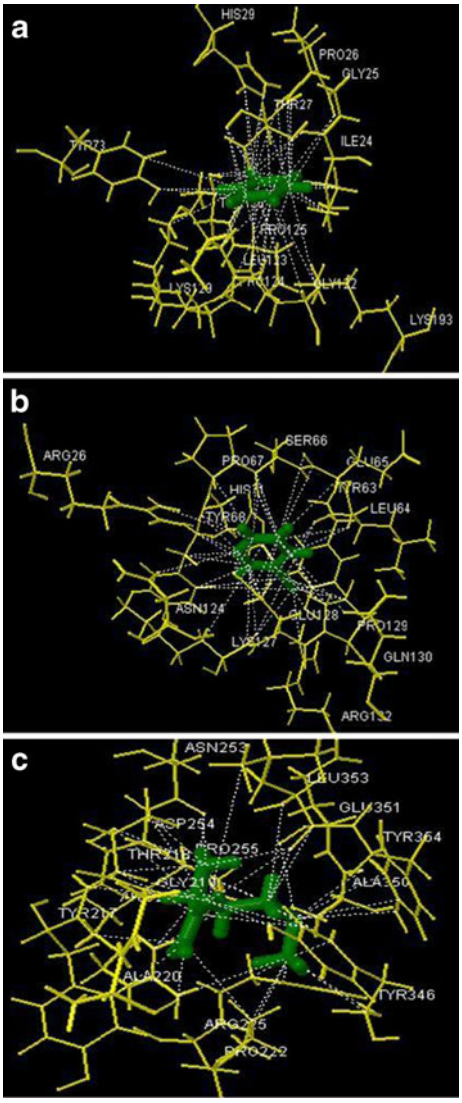

Fig. 1 Interactions of nitrile-degrading enzyme/substrate complexes. **a** ReNHase/benzonitrile complex. **b** PtNHase/3-cyanopyridine complex. **c** RhAmidase/L-methioninamide complex. The 3D structures of ReNHase, PtNHase, and RhAmidase are shown in *yellow stick models*; the 3D structures of benzonitrile, 3-cyanopyridine and L-methioninamide are represented in *green stick models*. Interactions are shown in *dashed lines*

narrow and thus is very suitable for residence of small nitriles. The pose with the lowest re-rank score was chosen as the preferred solution. Table 2 listed the MolDock scores and re-rank scores of the best docked poses generated by MVD. A previous study showed the NHase of *R. erythropolis* strain (N′4) owned a high specific activity toward methacrylonitrile and butyronitrile, but was found to have a low activity toward benzonitrile [25]. However, docking results showed that benzonitrile had the

Table 4 Molecular docking pose scores between PtNHase and nitriles

Name of nitriles	ID	Rotated bond	MolDock score	Re-rank score
3-Cyanopyridine	78	1	−63.9557	−56.056
Acrylonitrile	7567	1	−41.2224	−34.521
Benzonitrile	7224	1	−63.7441	−55.6398
Methacrylonitrile	29101	1	−49.6155	−41.8706
tert-Butylisonitrile	22045	1	−46.5442	−38.9895

most favorable interactions with NHase than methacrylonitrile and butyronitrile. This may be because the selected *R. erythropolis* strains in these two studies were different (N′4 vs. AJ270). Among the microbial degradation of nitrile mediated by ReNHase, ReNHase interacted with nitrile and further cleaved the nitrile to the corresponding amide [5]. Our study focused on the interactive details between nitriles and ReNHase which are unclear until now. The LPC/CSU server, a program for automatically analyzing the enzyme–substrate interaction, was used to explore the interactional profile in ReNHase/benzonitrile complex which was found to have the lowest re-

Table 5 Interactional profile of PtNHase with 3-cyanopyridine

3-Cyanopyridine atom	PtNHase atom	Distance (Å)	Type of contacts
N1	N TYR68 B	3.1	Hb
N1	OE2 GLU128 A	3.7	Hb
N1	OD1 ASN124 A	4.7	Hb
N2	NH1 ARG132 A	3.2	Hb
N2	OH TYR63 B	3.5	Hb
N2	O LYS127 A	3.5	Hb
C1	CD PRO129 A	3.6	Ph
C1	CG GLU128 A	3.7	Ph
C2	CD PRO129 A	3.4	Ph
C2	CG GLU128 A	3.7	Ph
C3	CG GLU128 A	3.5	Ph
C3	CD PRO129 A	4.4	Ph
C4	CG GLU128 A	3.6	Ph
C4	CB TYR68 B	3.7	Ph
C4	CB LYS127 A	4.9	Ph
C5	CA PRO67 B	4.2	Ph
C6	CD PRO129 A	3.4	Ph
C6	CG PRO129 A	3.7	Ph
C4	CE1 HIS71 B	3.8	Ar
C4	CD1 TYR68 B	4.1	Ar
C1	CE1 HIS71 B	3.6	Ar
N1	CB TYR68 B	3.5	HH
N1	CG GLU128 A	3.4	HH
N2	CG PRO129 A	3.5	HH

Hb hydrogen bonding, *Ph* hydrophobic, *Ar* aromatic–aromatic, *HH* hydrophilic–hydrophobic

Table 6 Molecular docking pose scores between RhAmidase and amides

Name of nitriles	ID	Rotated bond	MolDock score	Re-rank score
2,2-Dimethylcyclopropanecarboxamide	499714	1	−72.2052	−61.2639
Acetamide	173	0	−46.5134	−39.3331
Acrylamide	6331	1	−54.968	−47.8015
Benzamide	2241	1	−78.5198	−69.6819
Butyramide	10464	2	−62.1936	−52.5859
Formamide	693	0	−35.711	−30.1548
Hexamide	11827	4	−82.7027	−69.4855
Isobutyramide	61707	1	−58.4459	−52.0186
L-Alaninamide	392718	1	−60.0299	−53.4233
L-Leucinamide	62841	3	−86.2464	−73.1773
L-Methioninamide	129201	4	−86.7419	−76.0224
L-Prolinamid	99870	1	−75.071	−65.0817
L-Threoninamide	392091	2	−70.0342	−63.809
Methacrylamide	6346	1	−59.2672	−52.8855
Nicotinamide	911	1	−76.4217	−68.5355
Propanamide	6330	1	−53.8982	−47.1523

rank score and was selected as representative to determine the interactional details between nitrile and ReNHase. Our results showed that the THR126 A and ILE24 B were essential for benzonitrile binding by hydrogen bonding (Hb) interactions (Table 3). HIS29 B bound to benzonitrile also use Hb interactions. Benzonitrile made hydrophobic (Ph) contacts with residues PRO125 A, ILE24 B, LEU123 A, and THR27 B. ReNHase exhibited no acceptor–acceptor (AA) contacts with benzonitrile. The C2 atom of benzonitrile formed aromatic–aromatic (Ar) contact with TYR73 B. ILE24 B and PRO125 A from ReNHase were involved in benzonitrile binding in the form of hydrophilic–hydrophobic (HH) contacts. Various interactions found between ReNHase and benzonitrile might be responsible for the stabilization of the complex ReNHase/benzonitrile during ReNHase catalysis (Fig. 1a).

Co-Type NHase

In the present study, we have determined the PtNHase–nitrile bindings for the first time by MVD (Fig. S2). The MolDock score and re-rank score of the top poses for all docked substrates are given in Table 4. PtNHase showed the lowest re-rank score for 3-cyanopyridine among all surveyed nitriles. NHase has been extensively used in the industry [18]. The best example is related to the production of acrylamide by NHase [2]. However, the binding landscape between NHase and nitrile has not been examined until now. We investigated here the interactions of PtNHase with the best pose (3-cyanopyridine) using the LPC/CSU server. TYR68 B, GLU128 A, ASN124 A, ARG132 A, TYR63 B, and LYS127 A made Hb contacts with 3-cyanopyridine (Table 5). In the complex PtNHase/3-cyanopyridine, PRO129 A, GLU128 A, TYR68 B, PRO67 B, and LYS127 A formed Ph contacts with the ligand 3-cyanopyridine. We have identified a set of PtNHase residues that formed HH contacts with 3-cyanopyridine, including TYR68 B, GLU128 A, and PRO129 A. HIS71 B and TYR68 B from PtNHase adhered to 3-

Table 7 Interactional profile of RhAmidase with L-methioninamide

L-Methioninamide atom	RhAmidase atom	Distance (Å)	Type of contacts
O1	N GLY219 A	2.8	Hb
O1	OH TYR346 A	3.1	Hb
N1	O LEU353 A	3.2	Hb
N1	O ASN253 A	3.2	Hb
N1	OG1THR218 A	3.4	Hb
N2	O TYR217 A	3.2	Hb
N2	O ALA220 A	3.2	Hb
S1	CD PRO255 A	4.5	Ph
S1	CB ALA350 A	4.6	Ph
S1	CG PRO255 A	4.4	Ph
C1	CD PRO255 A	3.7	Ph
C3	CE2 TYR346 A	3.5	Ph
C3	CD2 TYR346 A	3.5	Ph
C3	C LEU353 A	4.2	Ph
C3	CD PRO255 A	4.4	Ph
C5	CD2 TYR346 A	3.8	Ph
C5	CA TYR346 A	4.2	Ph
C5	CG PRO222 A	4.3	Ph
C5	CD ARG225 A	4.4	Ph
C5	CB PRO222 A	4.7	Ph
C1	NH1 ARG256 A	3.4	HH
C3	N LEU353 A	4.5	HH
C5	O TYR346 A	3.3	HH
C5	NE ARG225 A	3.5	HH
N1	CG2 THR218 A	3.5	HH
C3	O LEU353 A	3.2	HH
O1	CD1 LEU353 A	5.7	HH
C5	NH2 ARG256 A	4.4	HH
O1	CG2 THR218 A	4.0	HH
N2	CG PRO222 A	4.0	HH
O1	O LEU353 A	3.7	AA

Hb hydrogen bonding, *Ph* hydrophobic, *HH* hydrophilic–hydrophobic, *Ar* aromatic–aromatic

cyanopyridine in the form of Ar contact. PtNHase did not interact with 3-cyanopyridine via AA contacts. The contact network between PtNHase and 3-cyanopyridine likely played a key role in forming the complex PtNHase/3-cyanopyridine (Fig. 1b).

Amidase

Binding of substrate to amidase was surveyed by molecular docking (Fig. S3). Weak hydrolysis activity of RhAmidase for acrylamide was observed in another study [7], likely partly due to a weak interaction between RhAmidase and acrylamide (Table 6). High amide binding may prolong the duration of amidase's action. Amidase selected in this study belonged to amidase signature (AS) family in which the Ser-*cis*Ser-Lys catalytic triad is conserved [7]. However, these three types of amino acids were not observed to play an

important role in binding of L-methioninamide (Table 7). Key residues involved in the binding of L-methioninamide to RhAmidase did not come from the same domain. Instead, a large domain and a small domain participated in the interactions of RhAmidase with L-methioninamide, and these interactions contributed to the formation of the RhAmidase/L-methioninamide complex (Fig. 1c). The Hb contacts between RhAmidase and L-methioninamide were formed by residues GLY219 A, TYR346 A, LEU353 A, ASN253 A, THR218 A, TYR217 A, and ALA220 A. PRO255 A, ALA350 A, TYR346 A, LEU353 A, ARG225 A, and PRO222 A participated in the Ph contacts with the ligand L-methioninamide. The Ph contacts were most abundant between RhAmidase and L-methioninamide and might be the main driving force for the generation of the RhAmidase/L-methioninamide complex. Six residues ARG256 A, LEU353 A, TYR346 A, ARG225 A, THR218 A, and PRO222 A were important in the binding of RhAmidase to L-methioninamide by HH contacts. Unlike ReNHase and PtNHase, RhAmidase had an AA contact with its substrate, but no Ar contacts were observed.

Conclusions

Here, we have reported the binding landscape between three types of nitrile-degrading enzymes and their substrates and successfully identified the interactional profiles of three complexes (ReNHase/benzonitrile, PtNHase/3-cyanopyridine, and RhAmidase/L-methioninamide complexes). In spite of substrate difference, ReNHase/benzonitrile and PtNHase/3-cyanopyridine complexes exhibited comparable re-rank scores, whereas the RhAmidase/L-methioninamide complex had a lower re-rank score. Interestingly, these three identified nitrile-degrading enzyme/substrate complexes exhibited very similar interactive needs: Hb, Ph, and HH contacts were found in each complex for the binding of selected substrates to corresponding nitrile-degrading enzymes. However, it must be noted that the abundance of various interactions was different between these complexes. The difference of interactional profiles between nitrile-degrading enzyme/substrate complexes may be partly due to the difference of chemical characters between interacted substrates or nitrile-degrading enzymes. These data are very useful for the design of enzyme mutants with better oxidation activity toward nitriles or amides. However, the theoretical hypothesis should be proven with experimental data before application and more in-depth investigation is needed.

Acknowledgments The authors sincerely thank the editor and anonymous reviewers for suggestions on improving the paper. The study was financially supported by the National Natural Science Foundation of China (nos. 50608029, 50978088, 50808073, 51039001), the Hunan Provincial Natural Science Foundation of China (10JJ7005), the Hunan Key Scientific Research Project (2009FJ1010), the Hunan Provincial Innovation Foundation For Postgraduate (CX2009B078, CX2010B157), the Program for New Century Excellent Talents in University (NCET-08-0181), and the Research Fund for the Doctoral Program of Higher Education of China (20100161110012).

References

1. Wyatt, J. M., & Knowles, C. J. (1995). *International Biodeterioration & Biodegradation, 35*, 227–248.
2. Banerjee, A., Sharma, R., & Banerjee, U. C. (2002). *Applied Microbiology and Biotechnology, 60*, 33–44.

3. Baxter, J., & Cummings, S. P. (2006). *Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology, 90*, 1–17.
4. Gupta, N., Balomajumder, C., & Agarwal, V. K. (2010). *Journal of Hazardous Materials, 176*, 1–13.
5. Song, L., Wang, M., Shi, J., Xue, Z., Wang, M. X., & Qian, S. (2007). *Biochemical and Biophysical Research Communications, 362*, 319–324.
6. Prasad, S., & Bhalla, T. C. (2010). *Biotechnology Advances, 28*, 725–741.
7. Ohtaki, A., Murata, K., Sato, Y., Noguchi, K., Miyatake, H., Dohmae, N., et al. (2010). *Biochimica et Biophysica Acta, 1804*, 184–192.
8. Yanover, C., & Bradley, P. (2011). *Proceedings of the National Academy of Sciences of the United States of America, 108*, 6981–6986.
9. Chen, M., Zeng, G., Tan, Z., Jiang, M., Li, H., Liu, L., et al. (2011). *PLoS One, 6*, e25647.
10. Chen, M., Tan, Z., Zeng, G., & Peng, J. (2010). *Molecular Biology and Evolution, 27*, 2227–2232.
11. Chen, M., Tan, Z., Jiang, J., Li, M., Chen, H., Shen, G., et al. (2009). *FEBS Letters, 583*, 2959–2963.
12. Chen, M., Zeng, G., Tan, Z., Jiang, M., Zhang, J., Zhang, C., et al. (2011). *FEBS Letters, 585*, 1072–1076.
13. Aristilde, L., Marichal, C., Miéhé-Brendlé, J., Lanson, B., & Charlet, L. (2010). *Environmental Science and Technology, 44*, 7839–7845.
14. Thomsen, R., & Christensen, M. H. (2006). *Journal of Medicinal Chemistry, 49*, 3315–3321.
15. Araújo, J. Q., Lima, J. A., Pinto, A. C., de Alencastro, R. B., & Albuquerque, M. G. (2011). *Journal of Molecular Modeling, 17*, 1401–1412.
16. Cassidy, C. E., & Setzer, W. N. (2010). *Journal of Molecular Modeling, 16*, 311–326.
17. Rose, P. W., Beran, B., Bi, C., Bluhm, W. F., Dimitropoulos, D., Goodsell, D. S., et al. (2011). *Nucleic Acids Research, 39*, 392–401.
18. Miyanaga, A., Fushinobu, S., Ito, K., & Wakagi, T. (2001). *Biochemical and Biophysical Research Communications, 288*, 1169–1174.
19. Scheer, M., Grote, A., Chang, A., Schomburg, I., Munaretto, C., Rother, M., et al. (2011). *Nucleic Acids Research, 39*, D670–676.
20. Pence, H. E., & Williams, A. (2010). *Journal of Chemical Education, 87*, 1123–1124.
21. Sobolev, V., Sorokine, A., Prilusky, J., Abola, E. E., & Edelman, M. (1999). *Bioinformatics, 15*, 327–332.
22. Yeom, S. J., Kim, H. J., & Oh, D. K. (2007). *Enzyme and Microbial Technology, 41*, 842–848.
23. Reisinger, C., Osprian, I., Glieder, A., Schoemaker, H. E., Griengl, H., & Schwab, H. (2004). *Biotechnology Letters, 26*, 1675–1680.
24. Kamble, A., & Banerjee, U. C. (2008). *Applied Biochemistry and Biotechnology, 151*, 143–150.
25. Choi, Y. H., Uhm, K. N., & Kim, H. K. (2008). *Journal of Molecular Catalysis B: Enzymatic, 55*, 157–163.
26. Misty, L. K., Salette, M., Natalie, G., Uwe, B., Dali, L., & Richard, C. H. (2012). *Biochemical and Biophysical Research Communications, 424*, 365–370.
27. Kathrin, M., Giang-Son, N., Frank, H., & Ulf, H. (2013). *Enzyme Microb Tech, 52*, 129–133.