1.13 References

1. Bornscheuer, U. T., Huisman, G. W., Kazlauskas, R. J., Lutz, S., Moore, J. C., & Robins, K. (2012). Engineering the third wave of biocatalysis. Nature, 485(7397), 185.
2. Sheldon, R. A., & Pereira, P. C. (2017). Biocatalysis engineering: the big picture. Chemical Society Reviews, 46(10), 2678-2691.
3. Reetz, M. T. (2013). Biocatalysis in organic chemistry and biotechnology: past, present, and future. Journal of the American Chemical Society, 135(34), 12480-12496.
4. Rosenthaler, L. (1908). Durch enzyme bewirkte asymmetrische synthesen. Biochem. Z, 14, 238-253.
5. Förster, S., Roos, J., Effenberger, F., Wajant, H., & Sprauer, A. (1996). The first recombinant hydroxynitrile lyase and its application in the synthesis of (*S*)-cyanohydrins. Angewandte Chemie International Edition in English, 35(4), 437-439.
6. Dadashipour, M., & Asano, Y. (2011). Hydroxynitrile lyases: insights into biochemistry, discovery, and engineering. ACS Catalysis, 1(9), 1121-1149.
7. Glick, B. R., & Pasternak, J. J. (1998). Principles and applications of recombinant DNA. ASM, Washington DC, 683.
8. Estell, D. A., Graycar, T. P., & Wells, J. A. (1985). Engineering an enzyme by site-directed mutagenesis to be resistant to chemical oxidation. Journal of Biological Chemistry, 60(11), 6518-6521.
9. Jensen, V. J., & Rugh, S. (1987). Industrial-scale production and application of immobilized glucose isomerase. Methods in Enzymology 136, 356-370.
10. Bruggink, A., Roos, E. C., & de Vroom, E. (1998). Penicillin acylase in the industrial production of β-lactam antibiotics. Organic Process Research & Development, 2(2), 128-133.
11. Sheldon, R. A., & van Pelt, S. (2013). Enzyme immobilisation in biocatalysis: why, what and how. Chemical Society Reviews, 42(15), 6223-6235.
12. Zhang, Y. H. P., Sun, J., & Ma, Y. (2017). Biomanufacturing: history and perspective. Journal of Industrial Microbiology & Biotechnology, 44(4-5), 773-784.
13. Tao, J. A., Lin, G. Q., & Liese, A. (2009). Biocatalysis for the pharmaceutical industry: discovery, development, and manufacturing. John Wiley & Sons.
14. Liese, A., Seelbach, K., & Wandrey, C. (Eds.). (2006). Industrial biotransformations. John Wiley & Sons.
15. Enzymes market size expected to reach $17.50 Billion by 2024. https://www.grandviewresearch.com/press-release/global-enzymes-market.
16. Hulshof, L. A., & Roskan, J. H. (1989). Phenylglycidate stereoisomers, conversion products thereof with eg 2-nitrothiophenol and preparation of diltiazem. European Patent Application, 43714.
17. Martinez, C. A., Hu, S., Dumond, Y., Tao, J., Kelleher, P., & Tully, L. (2008). Development of a chemoenzymatic manufacturing process for pregabalin. Organic Process Research & Development, 12(3), 392-398.
18. Griengl, H., Schwab, H., & Fechter, M. (2000). The synthesis of chiral cyanohydrins by oxynitrilases. Trends in biotechnology, 18(6), 252-256.

19. Nagasawa, T., Nakamura, T., & Yamada, H. (1990). Production of acrylic acid and methacrylic acid using *Rhodococcus rhodochrous* J1 nitrilase. Applied Microbiology and Biotechnology, 34(3), 322-324.
20. Bilal, M., Iqbal, H. M., Shuqi, G., Hu, H., Wang, W., & Zhang, X. (2017). State-of-the-art protein engineering approaches using biological macromolecules: a review from immobilization to implementation view point. International Journal of Biological Macromolecules, 108, 893-901.
21. Röthlisberger, D., Khersonsky, O., Wollacott, A. M., Jiang, L., DeChancie, J., Betker, J., & Albeck, S. (2008). Kemp elimination catalysts by computational enzyme design. Nature, 453(7192), 190.
22. Siegel, J. B., Zanghellini, A., Lovick, H. M., Kiss, G., Lambert, A. R., Clair, J. L. S., & Houk, K. N. (2010). Computational design of an enzyme catalyst for a stereoselective bimolecular Diels-Alder reaction. Science, 329(5989), 309-313.
23. Turner, N. J. (2004). Enzyme catalysed deracemisation and dynamic kinetic resolution reactions. Current Opinion in Chemical Biology, 8(2), 114-119.
24. Schmid, A., Dordick, J. S., Hauer, B., Kiener, A., Wubbolts, M., & Witholt, B. (2001). Industrial biocatalysis today and tomorrow. Nature, 409(6817), 258.
25. Smith, M. R., Khera, E., & Wen, F. (2015). Engineering novel and improved biocatalysts by cell surface display. Industrial & Engineering Chemistry Research, 54(16), 4021-4032.
26. Burton, S. G., Cowan, D. A., & Woodley, J. M. (2002). The search for the ideal biocatalyst. Nature Biotechnology, 20(1), 37.
27. Van Beilen, J. B., & Li, Z. (2002). Enzyme technology: an overview. Current Opinion in Biotechnology, 13(4), 338-344.
28. Keller, K., Friedmann, T., & Boxman, A. (2001). The bioseparation needs for tomorrow. Trends in Biotechnology, 19(11), 438-441.
29. Sheldon, R. A., & Woodley, J. M. (2017). Role of biocatalysis in sustainable chemistry. Chemical reviews,118(2), 801-838.
30. Lilly, M. D., & Woodley, J. M. (1996). A structured approach to design and operation of biotransformation processes. Journal of Industrial Microbiology, 17(1), 24-29.
31. Woodley, J. M. (2006). Choice of biocatalyst form for scalable processes. Biochemical Society Transactions, 34(2) 301-303.
32. Carvalho, C. C. (2017). Whole cell biocatalysts: essential workers from Nature to the industry. Microbial Biotechnology, 10(2), 250-263.
33. McAuliffe, J. C. (2012). Industrial enzymes and biocatalysis. Handbook of Industrial Chemistry and Biotechnology, 1183-1227. Springer.
34. Lin, B., & Tao, Y. (2017). Whole-cell biocatalysts by design. Microbial cell factories, 16(1), 106.
35. Atsumi, S., Hanai, T., & Liao, J. C. (2008). Non-fermentative pathways for synthesis of branched-chain higher alcohols as biofuels. Nature, 451(7174), 86.
36. Galanie, S., Thodey, K., Trenchard, I. J., Interrante, M. F., & Smolke, C. D. (2015). Complete biosynthesis of opioids in yeast. Science, 349(6252), 1095-1100.
37. Paddon, C. J., & Keasling, J. D. (2014). Semi-synthetic artemisinin: a model for the use of synthetic biology in pharmaceutical development. Nature Reviews Microbiology, 12(5), 355.
38. Zhao, H., & Van Der Donk, W. A. (2003). Regeneration of cofactors for use in biocatalysis. Current Opinion in Biotechnology, 14(6), 583-589.

39. Wachtmeister, J., & Rother, D. (2016). Recent advances in whole cell biocatalysis techniques bridging from investigative to industrial scale. Current Opinion in Biotechnology, 42, 169-177.
40. Ladkau, N., Schmid, A., & Bühler, B. (2014). The microbial cell-functional unit for energy dependent multistep biocatalysis. Current Opinion in Biotechnology, 30, 178-189.
41. Jakoblinnert, A., Mladenov, R., Paul, A., Sibilla, F., Schwaneberg, U., Ansorge-Schumacher, M. B., & de María, P. D. (2011). Asymmetric reduction of ketones with recombinant *E. coli* whole cells in neat substrates. Chemical Communications, 47(44), 12230-12232.
42. Müller, C. A., Dennig, A., Welters, T., Winkler, T., Ruff, A. J., Hummel, W., & Schwaneberg, U. (2014). Whole-cell double oxidation of n-heptane. Journal of Biotechnology, 191, 196-204.
43. Wilding, K. M., Schinn, S. M., Long, E. A., & Bundy, B. C. (2018). The emerging impact of cell-free chemical biosynthesis. Current Opinion in Biotechnology, 53, 115-121.
44. You, C., & Zhang, Y. H. P. (2017). Biomanufacturing by *in vitro* biosystems containing complex enzyme mixtures. Process Biochemistry, 52, 106-114.
45. Hold, C., & Panke, S. (2009). Towards the engineering of *in vitro* systems. Journal of the Royal Society Interface, 6(Suppl. 4), S507-S521.
46. Honda, K., Kimura, K., Ninh, P. H., Taniguchi, H., Okano, K., & Ohtake, H. (2017). *In vitro* bioconversion of chitin to pyruvate with thermophilic enzymes. Journal of Bioscience and Bioengineering, 124(3), 296-301.
47. You, C., Shi, T., Li, Y., Han, P., Zhou, X., & Zhang, Y. H. P. (2017). An *in vitro* synthetic biology platform for the industrial biomanufacturing of myo-inositol from starch. Biotechnology and Bioengineering, 114(8), 1855-1864.
48. Zhang, X., Wu, H., Huang, B., Li, Z., & Ye, Q. (2017). One-pot synthesis of glutathione by a two-enzyme cascade using a thermophilic ATP regeneration system. Journal of Biotechnology, 241, 163-169.
49. Morgado, G., Gerngross, D., Roberts, T. M., & Panke, S. (2016). Synthetic biology for cell-free biosynthesis: fundamentals of designing novel *in vitro* multi-enzyme reaction networks. Synthetic Biology-Metabolic Engineering. Springer, 117-146 .
50. Choi, J. M., Han, S. S., & Kim, H. S. (2015). Industrial applications of enzyme biocatalysis: current status and future aspects. Biotechnology Advances, 33(7), 1443-1454.
51. Pilone, M. S., & Pollegioni, L. (2002). D-amino acid oxidase as an industrial biocatalyst. Biocatalysis and Biotransformation, 20(3), 145-159.
52. Pollegioni, L., & Molla, G. (2011). New biotech applications from evolved D-amino acid oxidases. Trends in Biotechnology, 29(6), 276-283.
53. Volpato, G., C Rodrigues, R., & Fernandez-Lafuente, R. (2010). Use of enzymes in the production of semi-synthetic penicillins and cephalosporins: drawbacks and perspectives. Current Medicinal Chemistry, 17(32), 3855-3873.
54. Vera, C., Guerrero, C., Conejeros, R., & Illanes, A. (2012). Synthesis of galacto-oligosaccharides by β-galactosidase from *Aspergillus oryzae* using partially dissolved and supersaturated solution of lactose. Enzyme and Microbial Technology, 50(3), 188-194.
55. Rodriguez-Colinas, B., de Abreu, M. A., Fernandez-Arrojo, L., de Beer, R., Poveda, A., Jimenez-Barbero, J., & Plou, F. J. (2011). Production of galacto-

oligosaccharides by the β-galactosidase from *Kluyveromyces lactis*: Comparative analysis of permeabilized cells versus soluble enzyme. Journal of Agricultural and Food Chemistry, 59(19), 10477-10484.
56. Schallmey A, Domínguez de María P, Bracco P. Biocatalytic Asymmetric Oxidations in Stereoselective Synthesis. Stereoselective Synthesis of Drugs and Natural Products. Hoboken, John Wiley & Sons.
57. Nestl, B. M., Nebel, B. A., & Hauer, B. (2011). Recent progress in industrial biocatalysis. Current Opinion in Chemical Biology, 15(2), 187-193.
58. Bommarius, A. S., Blum, J. K., & Abrahamson, M. J. (2011). Status of protein engineering for biocatalysts: how to design an industrially useful biocatalyst. Current Opinion in Chemical Biology, 15(2), 194-200.
59. Arnold, F. H., & Volkov, A. A. (1999). Directed evolution of biocatalysts. Current Opinion in Chemical Biology, 3(1), 54-59.
60. Aharoni, A., Amitai, G., Bernath, K., Magdassi, S., & Tawfik, D. S. (2005). High-throughput screening of enzyme libraries: thiolactonases evolved by fluorescence-activated sorting of single cells in emulsion compartments. Chemistry & Biology, 12(12), 1281-1289.
61. Morley, K. L., & Kazlauskas, R. J. (2005). Improving enzyme properties: when are closer mutations better?. Trends in Biotechnology, 23(5), 231-237.
62. Huang, P. S., Boyken, S. E., & Baker, D. (2016). The coming of age of *de novo* protein design. Nature, 537(7620), 320.
63. Kiss, G., Çelebi-Ölçüm, N., Moretti, R., Baker, D., & Houk, K. N. (2013). Computational enzyme design. Angewandte Chemie International Edition, 52(22), 5700-5725.
64. Kuhlman, B., Dantas, G., Ireton, G. C., Varani, G., Stoddard, B. L., & Baker, D. (2003). Design of a novel globular protein fold with atomic-level accuracy. Science, 302(5649), 1364-1368.
65. Steiner, K., & Schwab, H. (2012). Recent advances in rational approaches for enzyme engineering. Computational and Structural Biotechnology Journal, 2(3), e201209010.
66. Hutchison, C. A., Phillips, S., Edgell, M. H., Gillam, S., Jahnke, P., & Smith, M. (1978). Mutagenesis at a specific position in a DNA sequence. Journal of Biological Chemistry, 253(18), 6551-6560.
67. Cedrone, F., Ménez, A., & Quéméneur, E. (2000). Tailoring new enzyme functions by rational redesign. Current Opinion in Structural Biology, 10(4), 405-410.
68. Hult, K., & Berglund, P. (2003). Engineered enzymes for improved organic synthesis. Current Opinion in Biotechnology, 14(4), 395-400.
69. Strohmeier, G. A., Pichler, H., May, O., & Gruber-Khadjawi, M. (2011). Application of designed enzymes in organic synthesis. Chemical Reviews, 111(7), 4141-4164.
70. Lutz, S. (2010). Beyond directed evolution semi-rational protein engineering and design. Current Opinion in Biotechnology, 21(6), 734-743.
71. Lehtovaara, P. M., Koivula, A. K., Bamford, J., & Knowles, J. K. C. (1988). A new method for random mutagenesis of complete genes: enzymatic generation of mutant libraries *in vitro*. Protein Engineering, Design and Selection, 2(1), 63-68.
72. Stemmer, W. P. (1994). Rapid evolution of a protein *in vitro* by DNA shuffling. Nature, 370(6488), 389.
73. Zhao, H., Giver, L., Shao, Z., Affholter, J. A., & Arnold, F. H. (1998). Molecular

evolution by staggered extension process (StEP) *in vitro* recombination. Nature Biotechnology, 16(3), 258.
74. Ostermeier, M., Shim, J. H., & Benkovic, S. J. (1999). A combinatorial approach to hybrid enzymes independent of DNA homology. Nature Biotechnology, 17(12), 1205.
75. Chen, K., & Arnold, F. H. (1993). Tuning the activity of an enzyme for unusual environments: sequential random mutagenesis of subtilisin E for catalysis in dimethylformamide. Proceedings of the National Academy of Sciences, 90(12), 5618-5622.
76. Reetz, M. T., Zonta, A., Schimossek, K., Jaeger, K. E., & Liebeton, K. (1997). Creation of enantioselective biocatalysts for organic chemistry by *in vitro* evolution. Angewandte Chemie International Edition, 36(24), 2830-2832.
77. Wong, T. S., Roccatano, D., Zacharias, M., & Schwaneberg, U. (2006). A statistical analysis of random mutagenesis methods used for directed protein evolution. Journal of Molecular Biology, 355(4), 858-871.
78. Kumar, A., & Singh, S. (2013). Directed evolution: tailoring biocatalysts for industrial applications. Critical Reviews in Biotechnology, 33(4), 365-378.
79. Wijma, H. J., Floor, R. J., & Janssen, D. B. (2013). Structure-and sequence-analysis inspired engineering of proteins for enhanced thermostability. Current Opinion in Structural Biology, 23(4), 588-594.
80. Porebski, B. T., & Buckle, A. M. (2016). Consensus protein design. Protein Engineering, Design and Selection, 29(7), 245-251.
81. Kretz, K. A., Richardson, T. H., Gray, K. A., Robertson, D. E., Tan, X., & Short, J. M. (2004). Gene site saturation mutagenesis: a comprehensive mutagenesis approach. Methods in Enzymology. Academic Press, 388 3-11.
82. Reetz, M. T., & Carballeira, J. D. (2007). Iterative saturation mutagenesis (ISM) for rapid directed evolution of functional enzymes. Nature Protocols, 2(4), 891.
83. Reetz, M. T., Bocola, M., Carballeira, J. D., Zha, D., & Vogel, A. (2005). Expanding the range of substrate acceptance of enzymes: combinatorial active-site saturation test. Angewandte Chemie, 117(27), 4264-4268.
84. Reetz, M. T., Kahakeaw, D., & Lohmer, R. (2008). Addressing the numbers problem in directed evolution. ChemBioChem, 9(11), 1797-1804.
85. Reetz, M. T., Wilensek, S., Zha, D., & Jaeger, K. E. (2001). Directed evolution of an enantioselective enzyme through combinatorial multiple-cassette mutagenesis. Angewandte Chemie International Edition, 40(19), 3589-3591.
86. Reetz, M. T., Carballeira, J. D., & Vogel, A. (2006). Iterative saturation mutagenesis on the basis of B factors as a strategy for increasing protein thermostability. Angewandte Chemie International Edition, 45(46), 7745-7751.
87. Reetz, M. T., Wang, L. W., & Bocola, M. (2006). Directed evolution of enantioselective enzymes: iterative cycles of CASTing for probing protein-sequence space. Angewandte Chemie, 118(8), 1258-1263.
88. Reetz, M. T. (2011). Laboratory evolution of stereoselective enzymes: a prolific source of catalysts for asymmetric reactions. Angewandte Chemie International Edition, 50(1), 138-174.
89. Landau, M., Mayrose, I., Rosenberg, Y., Glaser, F., Martz, E., Pupko, T., & Ben-Tal, N. (2005). ConSurf 2005: the projection of evolutionary conservation scores of residues on protein structures. Nucleic Acids Research, 33(Suppl. 2), W299-W302.
90. Vázquez-Figueroa, E., Chaparro-Riggers, J., & Bommarius, A. S. (2007).

Development of a thermostable Glucose dehydrogenase by a structure-guided consensus concept. ChemBioChem, 8(18), 2295-2301.
91. Polizzi, K. M., Chaparro-Riggers, J. F., Vazquez-Figueroa, E., & Bommarius, A. S. (2006). Structure-guided consensus approach to create a more thermostable penicillin G acylase. Biotechnology journal, 1(5), 531-536.
92. Conti, G., Pollegioni, L., Molla, G., & Rosini, E. (2014). Strategic manipulation of an industrial biocatalyst evolution of a cephalosporin C acylase. The FEBS Journal, 281(10), 2443-2455.
93. Agranat, I., Wainschtein, S. R., & Zusman, E. Z. (2012). The predicated demise of racemic new molecular entities is an exaggeration. Nature Reviews Drug Discovery, 11(12), 972.
94. Albarrán-Velo, J., González-Martínez, D., & Gotor-Fernández, V. (2018). Stereoselective biocatalysis: A mature technology for the asymmetric synthesis of pharmaceutical building blocks. Biocatalysis and Biotransformation, 36(2), 102-130.
95. Drauz, K., Gröger, H., & May, O. (2012). Enzyme catalysis in organic synthesis (Vol. 1). John Wiley & Sons.
96. Wohlgemuth, R. (2010). Asymmetric biocatalysis with microbial enzymes and cells. Current Opinion in Microbiology, 13(3), 283-292.
97. Batwal, R. U., & Argade, N. P. (2015). Chemoenzymatic collective synthesis of optically active hydroxyl (methyl) tetrahydronaphthalene-based bioactive terpenoids. Organic & Biomolecular Chemistry, 13(46), 11331-11340.
98. Batwal, R. U., & Argade, N. P. (2016). Chemoenzymatic access to (+)-Artabotriol and its application in collective synthesis of (+)-Grandiamide D,(–)-Tulipalin B,(+)-Spirathundiol, and (+)-Artabotriolcaffeate. Synthesis, 48(13), 2130-2136.
99. Servi, S., Tessaro, D., & Pedrocchi-Fantoni, G. (2008). Chemo-enzymatic deracemization methods for the preparation of enantiopure non-natural α-amino acids. Coordination Chemistry Reviews, 252(5-7), 715-726.
100. Caligiuri, A., D'Arrigo, P., Gefflaut, T., Molla, G., Pollegioni, L., Rosini, E., & Servi, S. (2006). Multistep enzyme catalysed deracemisation of 2-naphthyl alanine. Biocatalysis and Biotransformation, 24(6), 409-413.
101. Ghosh, A. K., & Sarkar, A. (2016). Enantioselective syntheses of (–)-alloyohimbane and (–)-yohimbane by an efficient enzymatic desymmetrization process. European Journal of Organic Chemistry, (36), 6001-6009.
102. Gao, X., Ma, Q., & Zhu, H. (2015). Distribution, industrial applications, and enzymatic synthesis of D-amino acids. Applied Microbiology and Biotechnology, 99(8), 3341-3349.
103. Song, Y., Li, J., Shin, H. D., Liu, L., Du, G., & Chen, J. (2016). Biotechnological production of alpha-keto acids: Current status and perspectives. Bioresource Technology, 219, 716-724.
104. Zhang, J., Xu, Y., & Li, R. (2013). Preparation method of aspoxicillin. China Patent. A, 103333180.
105. Veine, D. M., Yao, H., Stafford, D. R., Fay, K. S., & Livant, D. L. (2014). A D-amino acid containing peptide as a potent, noncovalent inhibitor of α5β1 integrin in human prostate cancer invasion and lung colonization. Clinical & Experimental Metastasis, 31(4), 379-393.
106. Sanchez, C. J., Akers, K. S., Romano, D. R., Woodbury, R. L., Hardy, S. K., Murray, C. K., & Wenke, J. C. (2014). D-amino acids enhance the activity of antimicrobials against biofilms of clinical wound isolates of *Staphylococcus*

aureus and *Pseudomonas aeruginosa*. Antimicrobial Agents and Chemotherapy, 58(8), 4353-4361.
107. Molla, G., Melis, R., & Pollegioni, L. (2017). Breaking the mirror: L-Amino acid deaminase, a novel stereoselective biocatalyst. Biotechnology Advances, 35(6), 657-668.
108. Mou, S., Li, J., Yu, Z., Wang, Q., & Ni, Z. (2013). Keto acid-supplemented low protein diet for treatment of adult patients with hepatitis B virus infection and chronic glomerulonephritis. Journal of International Medical Research, 41(1), 129-137.
109. Pantaleone, D. P., Geller, A. M., & Taylor, P. P. (2001). Purification and characterization of an L-amino acid deaminase used to prepare unnatural amino acids. Journal of Molecular Catalysis B: Enzymatic, 11(4-6), 795-803.
110. Bommarius, A. S., Schwarm, M., & Drauz, K. (1998). Biocatalysis to amino acid-based chiral pharmaceuticals examples and perspectives. Journal of Molecular Catalysis B: Enzymatic, 5(1-4), 1-11.
111. Wakayama, M., & Moriguchi, M. (2001). Comparative biochemistry of bacterial N-acyl-D-amino acid amidohydrolase. Journal of Molecular Catalysis B: Enzymatic, 12(1-6), 15-25.
112. Kobayashi, J., Shimizu, Y., Mutaguchi, Y., Doi, K., & Ohshima, T. (2013). Characterization of D-amino acid aminotransferase from *Lactobacillus salivarius*. Journal of Molecular Catalysis B: Enzymatic, 94, 15-22.
113. Pollegioni, L., Motta, P., & Molla, G. (2013). L-Amino acid oxidase as biocatalyst: a dream too far?. Applied Microbiology and Biotechnology, 97(21), 9323-9341.
114. Isobe, K., Tamauchi, H., Fuhshuku, K. I., Nagasawa, S., & Asano, Y. (2010). A simple enzymatic method for production of a wide variety of d-amino acids using L-amino acid oxidase from *Rhodococcus* sp. AIU Z-35-1. Enzyme Research, 2010.
115. Findrik, Z., Geueke, B., Hummel, W., & Vasić-Rački, Đ. (2006). Modelling of L-DOPA enzymatic oxidation catalyzed by L-amino acid oxidases from *Crotalus adamanteus* and *Rhodococcus opacus*. Biochemical Engineering Journal, 27(3), 275-286.
116. Hanson, R. L., Bembenek, K. S., Patel, R. N., & Szarka, L. J. (1992). Transformation of N-ε-CBZ-L-lysine to CBZ-L-oxylysine using L-amino acid oxidase from *Providencia alcalifaciens* and l-2-hydroxy-isocaproate dehydrogenase from *Lactobacillus confusus*. Applied Microbiology and Biotechnology, 37(5), 599-603.
117. Pollegioni, L., Molla, G., Sacchi, S., Rosini, E., Verga, R., & Pilone, M. S. (2008). Properties and applications of microbial D-amino acid oxidases: current state and perspectives. Applied Microbiology and Biotechnology, 78(1), 1-16.
118. Campillo-Brocal, J. C., Lucas-Elío, P., & Sanchez-Amat, A. (2015). Distribution in different organisms of amino acid oxidases with FAD or a quinone as cofactor and their role as antimicrobial proteins in marine bacteria. Marine Drugs, 13(12), 7403-7418.
119. Zeller EA, Maritz A. Über eine neue l -Aminosäure-oxydase. (1944). Helv Chim Acta, 27(1) 1888-1902
120. Mesecar, A. D., & Koshland Jr, D. E. (2000). Structural biology: a new model for protein stereospecificity. Nature, 403(6770), 614.
121. Singh, S. (2014). L-Amino acid oxidases-microbial and snake venom. Jornal of

Microbial and Biochemical Technology, 6, 128-134.
122. Lukasheva, E. V., Efremova, A. A., Treshalina, E. M., Arinbasarova, A. Y., Medentzev, A. G., & Berezov, T. T. (2011). L-Amino acid oxidases: properties and molecular mechanisms of action. Biochemistry (Moscow) Supplement Series B: Biomedical Chemistry, 5(4), 337-345.
123. Yang, C. A., Cheng, C. H., Lo, C. T., Liu, S. Y., Lee, J. W., & Peng, K. C. (2011). A novel L-amino acid oxidase from *Trichoderma harzianum* ETS 323 associated with antagonism of *Rhizoctonia solani*. Journal of Agricultural and Food Chemistry, 59(9), 4519-4526.
124. Macheroux, P., Seth, O., Bollschweiler, C., Schwarz, M., Kurfürst, M., Au, L. C., & Ghisla, S. (2001). L-Amino-acid oxidase from the Malayan pit viper *Calloselasma rhodostoma*. The FEBS Journal, 268(6), 1679-1686.
125. Moustafa, I. M., Foster, S., Lyubimov, A. Y., & Vrielink, A. (2006). Crystal structure of LAAO from Calloselasma rhodostoma with an L-phenylalanine substrate: insights into structure and mechanism. Journal of Molecular Biology, 364(5), 991-1002.
126. Lee, Y. C., & Huh, M. H. (1999). Development of a biosensor with immobilized L-amino acid oxidase for determination of L-amino acids. Journal of Food Biochemistry, 23(2), 173-185.
127. Hossain, G. S., Li, J., Shin, H. D., Du, G., Liu, L., & Chen, J. (2014). L-amino acid oxidases from microbial sources: types, properties, functions, and applications. Applied Microbiology and Biotechnology, 98(4), 1507-1515.
128. Guo, C., Liu, S., Yao, Y., Zhang, Q., & Sun, M. Z. (2012). Past decade study of snake venom L-amino acid oxidase. Toxicon, 60(3), 302-311.
129. Motta, P., Molla, G., Pollegioni, L., & Nardini, M. (2016). Structure-function relationships in L-amino acid deaminase, a flavoprotein belonging to a novel class of biotechnologically relevant enzymes. Journal of Biological Chemistry, 291(20), 10457-10475.
130. Massad, G., Zhao, H., & Mobley, H. L. (1995). *Proteus mirabilis* amino acid deaminase: cloning, nucleotide sequence, and characterization of *aad*. Journal of Bacteriology, 177(20), 5878-5883.
131. Baek, J. O., Seo, J. W., Kwon, O., Seong, S. I., Kim, I. H., & Kim, C. H. (2011). Expression and characterization of a second L-amino acid deaminase isolated from *Proteus mirabilis* in *Escherichia coli*. Journal of Basic Microbiology, 51(2), 129-135.
132. Ju, Y., Tong, S., Gao, Y., Zhao, W., Liu, Q., Gu, Q., & Zhou, H. (2016). Crystal structure of a membrane-bound L-amino acid deaminase from *Proteus vulgaris*. Journal of Structural Biology, 195(3), 306-315.
133. Liu, L., Hossain, G. S., Shin, H. D., Li, J., Du, G., & Chen, J. (2013). One-step production of α-ketoglutaric acid from glutamic acid with an engineered L-amino acid deaminase from *Proteus mirabilis*. Journal of Biotechnology, 164(1), 97-104.
134. Hou, Y., Hossain, G. S., Li, J., Shin, H. D., Liu, L., Du, G., & Chen, J. (2016). Two-step production of phenylpyruvic acid from L-phenylalanine by growing and resting cells of engineered *Escherichia coli*: process optimization and kinetics modeling. PloS one, 11(11), e0166457.
135. Rosini, E., Melis, R., Molla, G., Tessaro, D., & Pollegioni, L. (2017). Deracemization and stereoinversion of α-amino acids by L-Amino acid deaminase. Advanced Synthesis & Catalysis, 359(21), 3773-3781.

2. Aim of the work

The increasing demand of optically pure chiral compounds from pharmaceutical and agrochemical industry requires the development of new green processes for the synthesis of high value-added compounds.

The use of enzymes, which are versatile biocatalysts for stereoselective biocatalysis, turned out to be a competitive approach both in terms of yield and cost in comparison to the classical organic asymmetric synthesis. However, enzymes have evolved under the drive of the evolutionary pressure to enhance their "fitness" related to their natural role. Therefore, usually, enzymes are not readily suitable for biotechnological applications and, in their wild-type form, rarely fulfill the manifold requirements of industrial biocatalysis. For this reason, enzymes are optimized by protein engineering methods for biotechnological applications.

L-Amino acid oxidases (LAAOs) and deaminases (LAADs) are enantioselective flavoenzymes that catalyze the oxidation of L-amino acids to produce the corresponding α-keto acids and ammonia. At the difference of LAAOs, the reduced cofactor of LAADs is not reoxidized by molecular oxygen, but electrons are transferred to a membrane electron acceptor. As a consequence, LAADs do not produce hydrogen peroxide during catalysis.

L-Amino acid deaminase from *Proteus myxofaciens* (PmaLAAD) has a broad substrate specificity, being active on aromatic or aliphatic L-amino acids and on several unnatural amino acids of biotechnological relevance such as L-DOPA and substituted alanines. This enzyme can be employed in biotransformation reactions to convert D,L-amino acid mixtures into pure D-amino acids, and for the production of α-keto acids from the correspondent L-amino acids, which are used as raw materials or intermediates in the production of pharmaceuticals, agrochemicals, and food additives.

The purpose of this PhD project is the development of a novel stereoselective biocatalytic process exploiting PmaLAAD in a pure form for the production of

pure D-amino acids and/or α-keto acids of biotechnological or pharmaceutical relevance.

The project is splitted into two main stages. In the first part of the project, wild-type PmaLAAD will be investigated as a biocatalyst for several enantioselective biotransformations on natural or synthetic L-amino acids of biotechnological importance. This part of the project takes advantage from the broad substrate scope of PmaLAAD and from the possibility to produce the enzyme in a recombinant form. In the second part of the project, the knowledge of the 3D structure of PmaLAAD that was recently solved by the hosting research group will be exploited to design different variants to wider and/or altered substrate specificity for industrial applications. To this purpose, a semi-rational directed evolution approach supported by a comprehensive computational analysis will be performed. This multidisciplinary approach will take advantages of the production of "smaller and smarter" variant libraries, which will increase the chance to identify PmaLAAD variants with improved catalytic properties.

3. Results

3.1 Deracemization and stereoinversion of α-amino acids by L-amino acid deaminase

Rosini, E., **Melis, R**., Molla, G., Tessaro, D., & Pollegioni, L. (2017). *Advanced Synthesis & Catalysis*, *359*(21), 3773-3781.

3.1.1 Supplementary materials

3.2 In vitro evolution of an L-amino acid deaminase active on L-1-naphthylalanine

Melis, R., Rosini E., Pirillo,V., Pollegioni, L., & Molla G.(2018) (Manuscript Submitted)

3.2.1 Supplementary materials

3.2.2 Screening of PmaLAAD variants (obtained by site-saturation mutagenesis) on different substrate of biotechnological interest

3.3 Alternative strategies to improve PmaLAAD substrate specificity

(Unpublished results)

3.3.1 Conversion of the type-I PmaLAAD into a type-II L-amino acid deaminase

3.3.2 PmaLAAD ancestral active site

3.3.3 Rational design of a PmaLAAD variant active on L-penicillamine