Application of Enzyme in Pharmaceutical Engineering

Ouyang Tu
YK PAO School, Shanghai, 200333, China

Keywords: Biocatalyst, Enzyme, Pharmaceutical Engineering, Drugs, Lipase.

Abstract: Enzymes have found massive applications as every industry is becoming more environmentally friendly. In recent decades, the pharmaceutical industry has successfully applied enzymes in drug manufacturing as catalysts and as a part of API. Biocatalytic progress is mild and green compared to the chemical catalytic process. This paper discovers the new pharmaceutical engineering progress from three aspects: the development of biocatalysts, potential risks of biocatalytic process, and a significant biocatalyst-lipase. This paper also explores the process through recent experiments and achievements, discusses the advantages and disadvantages of enzyme-catalyzed processes through comparisons with the original chemical catalysts. The research on biocatalysts helps more scientists and students to learn about the latest techniques and achievements in pharmaceutical engineering. It's possible to solve problems associated with complex molecules and pollutions created by chemical catalyzed processes. The application of enzyme is a crucial improvement in developing drugs.

1 INTRODUCTION

The enzyme has become an important part of the food, feed, chemical, biological and pharmaceutical industries. The application and development of recombinant DNA and bioengineering using various enzymes in the past decades (Goutam, 2016), such as using genetically engineered bacteria to make human insulin, made huge progress in manufacturing drugs. As enzymes are environmental-friendly, biodegradable, and sustainable, biocatalysts are established as a better alternative to original chemical catalysts (Liang et al. 2016). Therefore, enzymes are not only applied in API, multiple kinds of enzymes are useful in the chemical synthesis of complex molecules and the improvement of drug qualities. The conditions of producing enzyme are mild temperature, normal pressure, and neutral pH level, which saves cost, energy, and improves the stability and safety of the production comparing to the chemical catalytic process. The catalytic efficiency is 100 times higher at least (Andrew et al. 2016). With these advantages, many biocatalytic and enzymes have been used for commercial benefits. It's one of the reasons for the fast development of biopharmaceutics. However, it's still a niche tool in the whole pharmaceutical engineering. This paper focused on three aspects of recent achievements and risks in utilize biocatalysts and enzymes, including one of the most special hydrolytic enzymes, lipase. The importance of lipases exceeded the status of proteases and amylases as the complexity of API continuously increases, according to the enzyme's advantage in chemical synthesis (Saxena et al. 1999).

Different types of medicine required specific biocatalytic as they have unique chemical functions of API. Sophisticated compounds can be achieved by late-stage modification, which requires biocatalysts. Discovering the suitable biocatalyst through the process of biocatalytic retrosynthesis followed by engineering is the basic route. The theory of how to form multi-functional compounds while ensuring their reactivity is important (Elvira et al. 2021). Even though it seems like a time-consuming and expensive process, it's still much quicker and cheaper than finding a new chemical synthesis for the catalytic process (Andrew et al. 2016).

In the recent development, biocatalysts are usually used during producing small molecule intermediates and APIs. Biocatalysts are efficient in the entire process of developing new medicines, especially with complex drug targets. Despite the benefits, there are several risks to be considered. Investigating potential hazards in the process of API synthesis using biocatalysts is crucial, as the quality of drugs should be ensured. There will be some

Tu, O.
Application of Enzyme in Pharmaceutical Engineering.
DOI: 10.5220/0011196900003443
In *Proceedings of the 4th International Conference on Biomedical Engineering and Bioinformatics (ICBEB 2022)*, pages 231-237
ISBN: 978-989-758-595-1

residual enzymes in the drug, and these might be harmful to certain patients. It's essential to develop appropriate residual enzyme control strategies when the drug enters clinical trial to assure the quality by raising the purity of API (Andrew et al. 2016).

Lastly, lipase is one of the most outstanding micro-origin enzymes that is known for its massive contributions and wide application as a biocatalyst (Rohit et al. 2013). The reason behind this is its unique attributes. Microbial lipases can be biocatalytic in both aqueous and non-aqueous reactions, which differentiates lipases from proteases and amylases. Its huge potential in organic synthesis determines its remarkable importance. A magnificent usage of lipases in the production of chiral drugs, anticholesterol, and anti-Alzheimer's are two examples (Ramesh et al. 2001). As lipase is a micro origin enzyme, it can be easily extracted from bacteria with unlimited supplies at any time. Also, it's relatively cheap. Lipases are suitable for commercial applications (Rohit et al. 2013).

The job content of developing medicines with biocatalysts is using biocatalytic retrosynthesis to identify suitable synthons, then design the corresponding biocatalysts. This process needs to be repeated for every enzyme because each one of them has unique and distinct composition (Elvira et al. 2021). The details of the process will be discussed in the next section. The application of biocatalysts is significant in two perspectives: sustainable development and medical development. Firstly, the enzyme-catalyzed process saves an enormous amount of energy and costs comparing to chemical catalytic progress. Most enzymes are fairly cheap to purchase, and the reaction only requires mils conditions (Andrew et al. 2016). Enzymes are also biodegradable, so zero pollution is released from the production. Recently, scientists discovered that when enzymes are conjugated to stimuli-responsive polymers, they can protect enzymes by changing their structures and manage enzymes' activities when facing external stimuli. Therefore, enzymes can be extracted from the reaction mixture effortlessly and be reused in later reactions (Truppo, 2017). Secondly, as enzymes are basically proteins from the human body, they usually have better therapeutical effects. Protein, hormone, and polypeptide's molecular weight is over 10000u, which exceeds the most advanced techniques of organic chemistry. Biocatalysts are exquisitely selectivity in protein engineering, which lead to the success in manufacturing new drugs with complicated formulas and solving the bottlenecks in organic chemistry (Truppo, 2017). By decreasing the price of drugs, more people can afford the treatment. Overall, the health condition of the whole population will increase.

2 LITERATURE REVIEW

2.1 Development and Advantages of Biocatalyst and LSMs

In organic chemistry, the C-H bond is the fundamental of all compounds. Before enzymes emerge, the selective modification of the C-H bond is the top challenge. In early-stage functionalization, the modification starts from a nonfunctionalized compound. After multiple steps, the compound is attached to the same functional group (Figure 1). There's no diversification. In late-stage functionalization with biocatalyst, targeted modification of C−H and C−heteroatom bonds becomes realistic. The site-specific transformations with functional groups on the compound offer diversification at the final synthetic step. With various possibilities of multi-functional compounds, many new drugs are discovered. However, the main drawbacks of this method are cross-reactivity and incompatibility due to the different chemical properties of different atoms (Elvira et al. 2021).

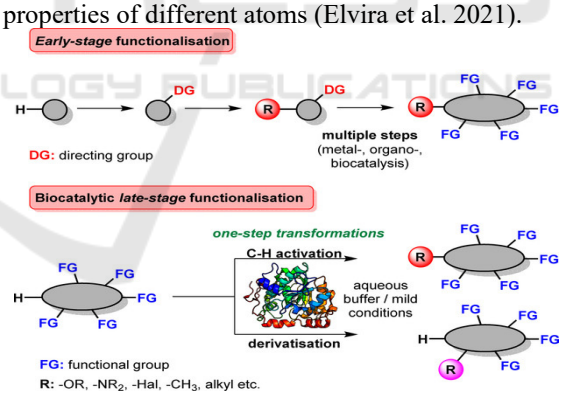

Figure 1: Progress of Early-stage functionalization VS Late-stage functionalization

Another advantage of applying enzymes is that enzymes are functional for the synthesis of complex metabolites in aqueous media with no requirements of extra costs on protection. An example of metal catalysis is catalytic metallodrugs, which contain artificial nucleases and artificial proteases. With a catalytic metal center and a targeting domain, catalytic metallodrugs can overcome the limitation of normal drugs binding reversibly to their targets (Joyner et al. 2013). This development can

significantly improve the effectiveness and reduce the toxicity of drugs (Robinson et al. 2004).

Before the usage of biocatalysts, every substance requires specified biocatalysts. Using biocatalytic retrosynthesis on target molecules, which is disconnecting chemical bonds to identify reasonable synthons for biocatalysis. The results can be verified by computer-aided synthesis planning. There are four ways in the pyramid to screen, design, discover or engineer the suitable enzyme in order to satisfy the tailored biocatalytic manufacture (Figure 2). Genetic engineering, rational mutagenesis, and detect unseen biocatalysts from nature are three common, potent methods to increase biocatalyst diversity. Ancestral sequence reconstruction is a new method. The artificial ancestors of known enzymes are likely endued with higher robustness and can accept extended types of substrates (Elvira et al. 2021).

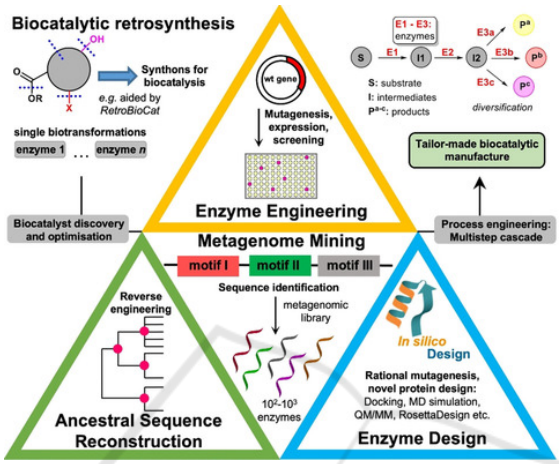

Figure 2: Process of developing tailored biocatalysts.

Even though the bond energy of C-H does not always have a positive correlation with the reactivity of the compound, the bond strength still decides the selectivity of activation in most of the reactions. The C-H bond is directed functionalization is affected by a metal ion. Comparing to catalyst-controlled functionalization, biocatalyst protects the C-H bonds by using a site-specific modification (Figure 3). Therefore, in this example, the reactivity of the hydrocarbon compounds manufactured by directed functionalization is probably lower than hydrocarbon compounds manufactured by catalyst-controlled functionalization (Elvira et al. 2021).

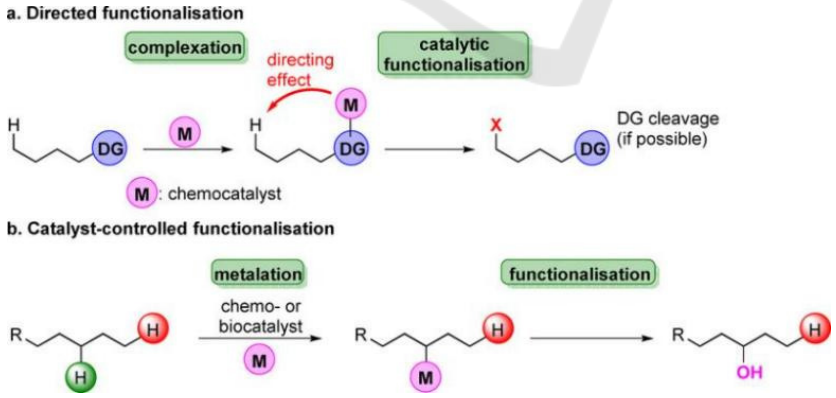

Figure 3: Directed functionalization and catalyst-controlled functionalization.

The development of enzyme makes the manufacture of complex compounds with high yields possible. In 2011, a survey shows that amide couplings are applied to 16% of all reactions in medicinal chemistry. As one of the most commonly used motif, the improvement in biocatakysts are crucial. The biosynthesis of tabtoxin discovers a miscellaneous ATP-grasp enzyme, TabS. It is able to constitute variety of dipeptides from unprotected amino acids. The suitable dipeptides including 136

types of amino acid combinations is a supreme challenge for chemical engineering. The original method to produce 244 is by the usage of different biocatalytic approaches on prochiral ketone precursor (242) or the racemic amine (rac-244). The application of AspRedAm, which produce 244 from 242 and propargylamine (243), has the conversion of 97% in Figure 4 (Elvira et al. 2021).

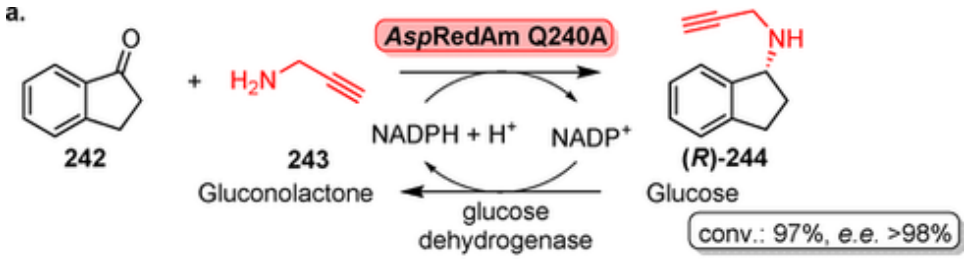

Figure 4: Biocatalytic approaches for (R)-rasagiline (244) synthesis.

2.2 Quality of Drug Manufactured by Biocatalyst

For the procedure of selecting appropriate enzymes, biocatalytic retrosynthesis and a reliable database can be helpful. Publishing detailed rules and guidelines of biocatalytic retrosynthesis can require chemists to have a deeper understanding of the structures and properties of the molecule as they need to investigate the potential transformations and applicable intermediates for the biocatalytic process. The support of computer-aided synthesis planning can ensure the enzymes are viable. A database containing information on identified and practiced biocatalytic reactions are also extremely conducive to chemists. If they can check the safety, scalability, substrate scope, conversions, and productivity of a range of suitable biocatalysts before the real experiment, so time and money can be saved. As engineering, a workable biocatalyst is already more cost-effective and less time-consuming than chemical catalysts without the database, the positive effect on the whole manufacturing process' efficiency, sustainability, and safety will assure the drug quality (Andrew et al. 2013).

After the usage of biocatalysts, some residue enzymes are possibly left in API. The property and safety data of the protein residues should be considered in science-based risk assessment. For example, amino acids and peptides are not toxic. Furthermore, ease patients should fill an individual risk assessment according to their condition to clarify their endurance to protein residues (Andrew et al. 2013) (Figure 5).

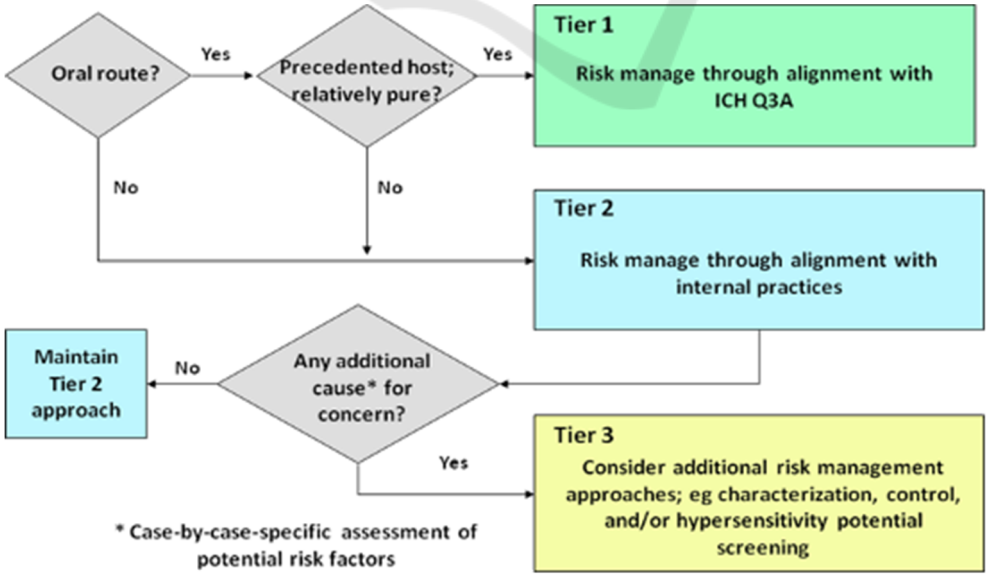

Figure 5: Basic tiered risk assessment.

Table 1: Enzyme Fate Across Three API Projects.

	Example 1: pregabalin	Example 2: atorvastatin	Example 3: API 3
dosing route/risk management	oral tier 1	oral tier 1	oral tier 1
enzymatic step	post RSM ester hydrolysis	post RSM, ketone reduction	pre RSM, C−C bond formation
product phase	launched	launched	launched
regulatory approval	yes	yes	yes
stage in synthesis. analysis carried out on the intermediate stage	several steps before API	several steps before API	several steps before API
enzyme type	lipolase (liquid formulation of enzyme produced in *A. oryzae* fermentation)	KRED (liquid formulation of enzyme produced in *E. coli* fermentation)	enzyme (Cells from *E. coli* fermentation)
control strategy	test for total proteins, amino acids, and endotoxins; demonstrate fate and purge; no enzyme residue specifications for API	test for total proteins; demonstrate fate and purge; no enzyme residue specifications for API	test for total proteins, DNA, endotoxins and microbiological residues; demonstrate fate and purge; no enzyme residue specifications for API

During the manufacture of API, continuous operations are enabled to remove residue enzymes. E.g., filtration, distillation, and pH adjustment. None of the residue proteins from enzyme preparations are expected to pass through the operations with the API. Therefore, the anticipation is the absence of residue enzymes in intermediates and APIs in Table 1. The production of atorvastatin is a piece of supportive evidence. A biocatalytic process is carried out for the synthesis of Acetonide 7, which is a crucial intermediate in the formation of atorvastatin as can be seen from Figure 6. The procedure was implemented by adding acetone to recombinant E, an organic layer containing diol 6, and an aqueous phase containing the enzyme are formed. The analysis of crude 6 samples and isolated intermediate 7 both from 4 lots have the result of no perceivable protein (Bradford protein assay, LOD < 0.01%). This data demonstrates that separating aqueous and organic phases can practically remove residue enzymes, which proves the absence of residue enzymes in atorvastatin. All agencies responsible for drug registration approved the strategies of not assaying residue enzymes in APIs (Andrew et al. 2016).

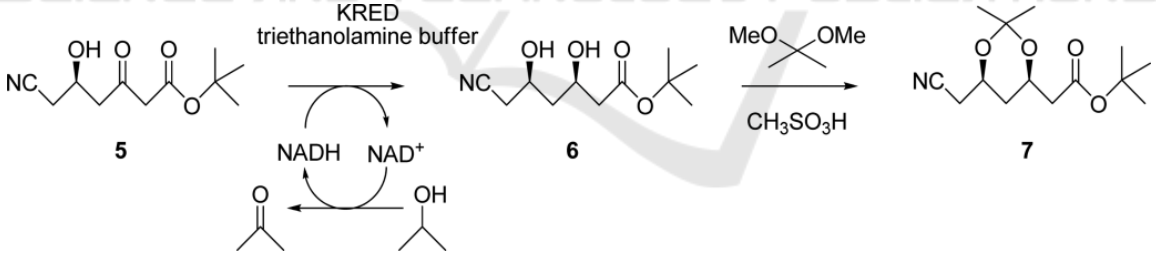

Figure 6: Chemoenzymatic synthesis of pregabalin.

Pregabalin, which is the API in Lyrica, is also manucfactured through a chemoenzymatic process. as can be seen from Table 1. 3 samples form 10 lots are tested with Bradford protein assay, and 20 lots are tested with the Micro BCA assay for total proteins. The result is no residue protein are perceived with LOD 0.04% w/w and LOD < 0.1). After derivatization with EZfaast, 24 commercial lots of pregabalina are also analyzed with LC/MS/MS. None of the samples have amino acidsabove the LOD (0.05%) (Andrew et al. 2016).

2.3 Contributions of Lipase

Lipases have become the top choice in enzymes for organic chemists, pharmacists, and other professors because of their unique properties. The activity of lipases can be easily controlled as it only works at the oil-water interface in Figure 7. Fats are only hydrolyzed in this certain condition. By adding an emulsifier, followed by stirring, the interface area will increase constantly until its limitation (Saxena et al. 1999). Therefore, the efficiency of API manufacturing and the efficacy of drugs containing lipases can reach the optimum.

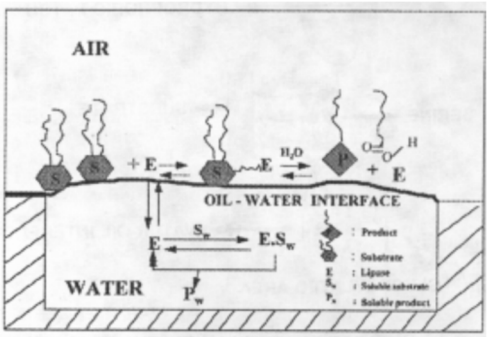

Figure 7: lipolytic reaction at the oil-water interface.

According to the properties mentioned above, lipases are very popular in organic synthesis. Lipases can be applied in the discovery or development of drugs using the method of organic synthesis. Chemists can manage the speed of the reactions easily. Additionally, catalysts are always expensive, so lipases enabled more chemists to implement more researches without worrying about costs by the technique of crude enzyme preparation. The regiospecificity and strong tolerance to a variety of organic substances make lipases even more suitable and capable for the catalyst of organic synthesis as most of the enzymes do not have these two properties (Saxena et al. 1999).

Lipases also have magnificent contributions to chiral drugs due to their enantioselective and regioselective nature. The effectiveness and efficacy of many drugs rely on chirality. With the ability of selective reaction at functional group and the preference of forming enantiomers, preparing chiral drugs intermediates using biocatalysts is well recognized (Rohit et al. 2013). For example, one of the key intermediates in the synthesis of an antihypertensive drug, Diltiazem, is successfully produced after lipases solve the hydrolysis of epoxy ester alcohols (Saxena et al. 1999).

Most of the biocatalysts have disadvantages, such as being easily affected by heat, poor stability, require a neutral pH level and room temperature. It is simple for enzymes to become denatured. Although enzymes are relatively cheap, they should be employed for at least 3 months or 30 batches. Any accidental denature that happened is considered a loss. Nowadays, scientists are trying to discover thermostable biocatalysts as thermo stabilization is a necessary step to improve the robustness of enzymes (Shakya et al. 2018). Robustness of enzymes is especially vital because designing biocatalysts requires enzymes to constantly expose in the probably unstable environment of organic reactions, which increases the chance of denaturing and inactivation. Normally, thermo stabilization is achieved by accumulating numbers of mutations in directed evolution. However, the potential risk is to sacrifice the catalytic function (Shakya et al. 2018). Thermophilic lipases have been discovered in recent decades. It can endure harsh conditions while keeping the former advantages. Also, it is extracted from various microorganisms, for example, escherichia coli (Rohit et al. 2013). Microbial origin lipases then become one of the best choices in enzymes.

3 CONCLUSION

The emerging, developssment and application of biocatalysts in pharmaceutical industries have effectively made the industry more advanced and more reliable. LSF is the fundamental support of successful drug developments. The achievement of complex scaffolds, which stimulates the diversification of compounds, determines the huge progress made in modern synthetic organic chemistry. Comparing to chemical catalytic progress, biocatalysis is more sustainable, cost-effective, and environmentally friendly. Testing in the API specification is not necessary, because the basic chemical operations can purge residue proteins. Therefore, the possible risks of residue enzymes don't really exist. Lipases are one of the most important biocatalysts. The special properties of lipases solved many crucial problems and produced many complex intermediates that have no resolution in the chemical industry now. It is not just applicable in various industries, but also the best catalyst to many pharmaceutics. Despite the significant achievements of lipases, more researches are needed to completely understand it.

Biocatalysts will probably be more important and widespread in pharmaceutical engineering by assessing the potential commercial benefits that enzymes can create. The whole process of engineering biocatalysts will have huge improvements and become significant on drug discovery. The most crucial part is to apply computer-aided synthesis planning in recognizing possible synthons and choose the most efficient process to design the suitable biocatalysts (Elvira et al. 2021). With the tremendous efforts of some drug companies inventing drugs with enzyme-catalyzed processes, the environment will be better when we dispose of less toxic gases and chemical products. More complex diseases can be treated and more people will ve cured. The application of biocatalysts offers something more beneficial than engineering tanglesome molecules or

solving problems in organic chemistry. The healthcare and life expectancy in the world will slowly increase. Scientists and pharmaceutics have only learned the tip of the iceberg, there are so much to explore, expand and reinforce.

ACKNOWLEDGMENTS

Writing a paper about new techniques can be very challenging for a high school student. I want to appreciate Professor Axel for teaching me basic knowledge of pharmaceutical engineering, so I have the chance to explore this topic. My TA, Ben, also explains and expands professor's contents, which enables me to understand and write about the niche topic. Ms. Wang helps me to state and paraphrase my ideas into a paper, so I should be thankful to her, too. Lastly, my parents are always being supportive and helpful. Without them, I might not have the courage and capacity to write this paper. Once again, I would like to express my heartfelt thanks to everyone who helped me.

REFERENCES

Andrew S. Wells, John W. Wong, Peter C. Michels, David A. Entwistle, Keith Fandrick, Gregory L. Finch, Animesh Goswami, Heewon Lee, Stefan Mix, Thomas S. Moody, Long Pang, Robert K. Sato, Nicholas J. Turner, and Timothy J. Watson. (2016). Case Studies Illustrating a Science and Risk-Based Approach to Ensuring Drug Quality When Using Enzymes in the Manufacture of Active Pharmaceuticals Ingredients for Oral Dosage Form, pp.594-600.

Elvira Romero, Bethan S. Jones, Bethany N. Hogg, Arnau Rué Casamajo, Prof. Martin A. Hayes, Prof. Sabine L. Flitsch, Prof. Nicholas J. Turner, Dr. Christian Schnepel. (2021). Enzymatic Late-Stage Modifications: Better Late Than Never.

Eric J. Moore, Dmitri Zorine, William A. Hansen, Sagar D. Khare and Rudi Fasan. (2017). Enzyme Stabilization via Computationally Guided Protein Stapling, National Academy of Sciences, Vol. 114, No. 47, pp. 12472–12477.

Goutam Brahmachari. (2016). Biotechnology of Microbial Enzymes, Academic Press, pp.6.

Joyner J. C., Cowan J. A. (2013). Target-directed catalytic metallodrugs, Brizillian Journal of Medical and Biological Research, Vol.46, No. 6.

Ramesh N. Patel. (2001). Enzymatic Synthesis of Chiral Intermediates for Drug Development.

Robinson, Mark A., Stuart T. Charlton, Philippe Garnier, Xiang-tao Wang, Stanley S. Davis, Alan C. Perkins, Malcolm Frier, Ruth Duncan, Tony J. Savage, David A. Wyatt, Susan A. Watson, Benjamin G. Davis, Robert Langer. (2004). "LEAPT: Lectin-Directed Enzyme-Activated Prodrug Therapy, National Academy of Sciences, Vol. 101, No. 40, pp. 14527–14532.

Rohit Sharma, Vishal Thakur, Monika Sharma, Nils-Kåre Birkeland. (2013). Biocatalysis Through Thermostable Lipases: Adding Flavor to Chemistry, Thermophilic Microbes in Environmental and Industrial Biotechnology, Springer, Dordrecht, pp.905-927.

Saxena, R., Ghosh, P., Gupta, R., Davidson, W., Bradoo, S., Gulati, R. (1999). Microbial lipases: Potential biocatalysts for the future industry, Current Science, Vol. 77, No. 1, pp. 101–115.

Shakya, Akhilesh Kumar, Kutty Selva Nandakumar. (2018). An update on smart biocatalysts for industrial and biomedical applications, Journal of the Royal Society, Vol. 15, No. 139.

Truppo, Matthew D. (2017). Biocatalysis in the Pharmaceutical Industry: The Need for Speed, ACS medicinal chemistry letters, Vol. 8, No. 5, pp. 476-480.

Youyun Liang, Mingzi M. Zhang, Ee Lui Ang, Huimin Zhao. (2016). Biocatalysis for Drug Discovery and Development.