Responds to Reviewer 3:

Thank you very much for your beneficial suggestion. We highly appreciate your carefulness review and the broad knowledge on the relevant research fields. We are sorry for making some mistakes in the former manuscript. The suggestion are very helpful for writing the revised manuscript. The following are the responses, which we hope to meet with your approval. Thank you.

1. **In abstract, value of optimal pH should be placed.**

Response: Thank you very much. We have added the optimal pH in line **4-5**.

The revised manuscript:

Est_{BAS}ΔSP exhibited the highest activity towards p-nitrophenyl hexanoate at the optimum temperature and pH (50 ℃, pH 8.0).

2. **In introduction, short explanation is necessary, why obtaining of chloramphenicol palmitate is important.**

Response: Thank you for giving us this beneficial suggestion. We have added the importance of chloramphenicol palmitate (line **39-41**).

The revised manuscript:

Chloramphenicol palmitate is an important chloramphenicol derivatization, which avoids the bitterness of chloramphenicol and can be rapidly hydrolyzed in vivo into the biologically active drug.

3. **In text is necessary explanation, why did the authors optimize the synthesis of**

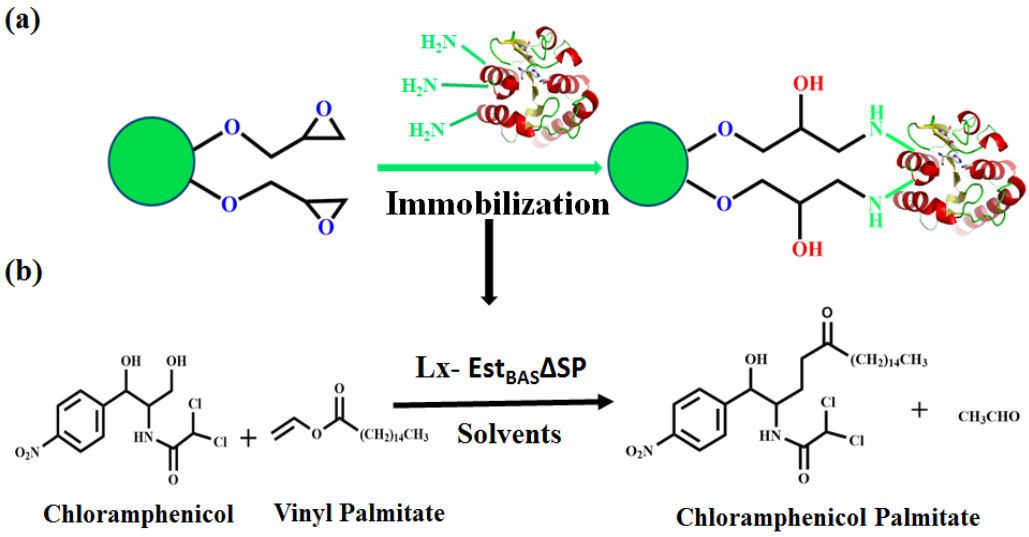
p-nitrophenyl hexanoate and not chloramphenicol palmitate.

Response: Thank you for giving us this beneficial suggestion. P-nitrophenyl hexanoate was used for the characterization of enzyme activity, chloramphenicol palmitate is the embodiment of enzyme application. In order to study the substrate specificity of $Est_{BAS}\Delta SP$, a series of substrate was used to obtain the optimum substrate. In text, our aim is mainly to compare the difference in the conversion rate between the free enzyme and immobilized enzyme under the same conditions, so we did not optimize the synthesis of chloramphenicol palmitate.

4. Publication would gain on readability if it has contained a reaction scheme.

Response: Thank you for giving us this beneficial suggestion. We have added the reaction scheme in the manuscript (scheme 1).

The revised manuscript:



Scheme 1. Preparation of $Est_{BAS}\Delta SP$ immobilization onto epoxy resin Lx-105s and the application for the regioselective synthesis of chloramphenicol palmitate.

5. **In manuscript should be placed discussion of obtained result with literature date about other lipases. Short explanation is necessary, why the best activity of enzyme was in case of p-nitrophenyl hexanoate, why only Mg^{2+} activated enzyme and why addition of all detergents inhibited enzyme.**

Response: Thank you very much. We had added a discussion with literature date about other lipases in line **255-259, 330-337**.

The revised manuscript:

(1)Line **255-259:** The reason was that the formed covalent bond with the support limited the conformational changes of Est_{BAS}ΔSP, resulting in a more rigid structure of Lx-Est_{BAS}ΔSP. However, the loss of enzyme activity was due to some specific interaction of the detergents with the enzyme surface.

Line **330-337:** Li et al. also observed that SMG1-F278N immobilized on epoxy resin ECR8285 retained 98% of its initial activity after 7 cycles. The above results proved that enzyme interacted with epoxy resin by covalent binding exhibited the advantage of strong and functional groups of support, which made the enzyme a high retention of the catalytic activities. However, the decrease of activity was due to the bound water molecules were stripped from the enzyme by hydrophilic organic solvents in the reaction system, the increased hydrophilic solvent content resulted in reduction of enzyme activity.

(2) Esterase is a kind of enzyme that show the highest activity toward water-soluble or emulsified esters with relatively short fatty acid chains (carbon chain length <10). Est_{BAS}ΔSP showed the best activity towards p-nitrophenyl hexanoate (*p*-NPH), which

indicated that Est_{BAS}ΔSP is a “true” esterase that preferentially hydrolyzes short acyl chain substrates.

(3) line **242-246:** The activity of Est_{BAS}ΔSP was decreased in presence of many metal ions. This results indicated that Est_{BAS}ΔSP activity did not require the presence of those metal ions, and Zn^{2+} was the most significant inhibition factor. On the other hand, the activity of Est_{BAS}ΔSP was slightly increased, similarly, Mishra et al. also observed this activation function by Mg^{2+}. He observed that Lecitase® Ultra phospholipase activity had a 1.5-fold activation in the presence of 10 mM Mg^{2+}. He concluded that Mg^{2+} could activate the enzyme in a lesser extent, and is also able to function without other metal ions (Reference: Mishra. M. K.; Kumaraguru, T.; Sheelu. G., Lipase activity of Lecitase® Ultra: Characterization and applications in enantioselective reactions. *Tetrahedron Asymmetry*. **2009,** *20,* 2854-2860.).

(4) Line **257-259**: The esterase activity was decreased by addition of all detergents. The reason was that the esterase activity could slightly be stimulated by some detergents in low concentration (0.5%)， but some detergents with a higher concentration could inhibit the esterase activity. In the article, the concentration of some detergents are too high for Est_{BAS}ΔSP.

6. **Figure 9 is illegible. Each NMR spectrum should be in separate figure. In my opinion NMR spectrum should be in Supplementary Material but in Materials and Method description of NMR spectrums should be placed. Description should contain multiplicity of signals.**

Response: Thank you for giving us this beneficial suggestion. We have transferred NMR spectrum into the supplementary material and each NMR spectrum in a separate figure. Multiple signals of chloramphenicol and chloramphenicol palmitate are accordingly marked in the spectrum of ^{1}H-NMR and ^{13}C-NMR (**Supplementary Figure S2**).

7. **In Materials and Methods conversion and yield of chloramphenicol palmitate should be provided.**

Response: Thank you for giving us this beneficial suggestion. We have added yield of chloramphenicol palmitate in materials and methods (line **482-487**).

The revised manuscript:

The conversion ratio of chloramphenicol is represented by the reduction rate of chloramphenicol in the HPLC chromatograms:

conversion ratio = $1-A_t/A_0$

Where A_0 is the area chloramphenicol at time 0 and A_t is the area chloramphenicol at time t.

8. **All used shortcut (e.g. p-NP) must be explained in text of manuscript.**

Response: Thank you very much. We have explained all used shortcut in in text of manuscript and in the part of abbreviations used, which was marked in yellow.

9. **Whole text of manuscript and tables must be written in the same font size**

Response: Thank you very much. The whole text of manuscript and tables were written in the same font size.