20th March, 2019

Dear editor,

Thanks very much for your letter and the reviewers’ professional comments concerning our manuscript entitled "A single mutation increases the thermostability and activity of Aspergillus terreus amine transaminase" (Manuscript ID: molecules-465920). The reviewers’ comments were valuable and very helpful for clarifying and improving this manuscript. We have studied the comments carefully, and the manuscript has been revised thoroughly and carefully to correct language deficiencies. Revised portion are marked in red in the manuscript. In the following pages are our point-by-point responses to each comment of the reviewers. We hope that the corrections in the manuscript and our accompanying responses can meet with your approval and the manuscript suitable for publication in Molecules.

We are looking forward to hearing from you at your earliest convenience.

Yours sincerely,

Prof. Le-He Mei
Reviewer #1:

1. The authors should consider the possibility to extend the simulation time scale and the analysis on the trajectory structure at least up to 150 ns. If not, they should clearly mention this limitation and justify it. My major concern relates to the convergence of the 100ns MD simulation that indeed has not been thoroughly assessed. In fact, there is no evidence that a sort of equilibrium has been reached in the trajectory. The achievement of an adequate convergence in the MD simulations should be checked by calculating the root mean square inner product (RMSIP), i.e. for a 150 ns simulation between the two halves of the equilibrated trajectories (50-100 ns and 100-150 ns) considering the motion of Ca atoms along the first 10 eigenvectors [see Amadei, A., Ceruso, M. A. & Di Nola, A. On the convergence of the conformational coordinates basis set obtained by the essential dynamics analysis of proteins' molecular dynamics simulations. *Proteins* 36, 419-424 (1999)].

**Response 1:** We thank the reviewer for raising this critical issue. As pointed out, the current molecular dynamics simulation results are indeed insufficient for this article. Considering the accuracy and completeness of this article, these uncertain contents has been removed in our revised manuscript, but this does not affect the novelty of the total paper. We would like to express our sincere appreciation for your careful reading and helpful comments.

2. The authors are kindly requested to specify which is the reference sequence that has been provided as input to the MISTIC server.

**Response 2:** The reference sequence is A3XII7_LEEBM/39-275.

3. What is the meaning of “intensive interaction residues”. Please specify.

**Response 3:** The “intensive interaction residues” means that “residues which are associated with multiple other residues in a coevolutionary network”.
4. In Figure S5, the error bars have been omitted.

**Response 4:** Thank the reviewer for pointing out the questions. The error bars have been added in the revised manuscript, as shown in Figure S5.

![Figure S5](image)

Figure S5. Thermal unfolding of wild-type AT-ATA and its mutants were monitored by DSF.

5. Overall, the written English is rather poor and the authors are kindly advice for a substantial editing carried out by a native English speaker.

**Response 5:** Thanks for your suggestions. We feel very sorry for our poor writings. According to the reviewer’s comments, we have made the manuscript be edited by a native English-speaking editor. All changes were marked in red in the revised manuscript. And we hope that the revised manuscript could meet with your approval. The certificate of editing is as follows:
6. The following few sentences just represents typical examples:

Abstract, lines 28 and 29:

“Furthermore, molecular dynamic (MD) simulation demonstrated that the mutant L118T lowered the overall root mean square deviation (RMSD) and consequently increased protein rigidity.”

Response 6: Special thanks for the pertinent comments. In the revised manuscript, this inaccurate sentence of “Furthermore, molecular dynamic (MD) simulation demonstrated that the mutant L118T lowered the overall root mean square deviation (RMSD) and consequently increased protein rigidity.” has been removed.

7. Now, MD simulations DO NOT DEMONSTRATE anything. MD simulations may instead unveil a structural feature such as flexibility, etc.

“... lowered the overall RMSD”

RMSD of which parameter: the Ca position? Please specify.

Response 7: Thank the reviewer for pointing out the inaccurate statement. We have deleted the section “molecular dynamics simulation”.

8. Main text line 45

“...such as combination of B-factor coupling with folding free energy calculations...” should read ...such as combination of the B-factor profile and folding free energy...
Response 8: We are grateful to the reviewer for correcting our inaccuracies. In the revised manuscript, this sentence has been revised to “To enhance the thermal stability of an (R)-selective amine transaminase from Aspergillus terreus (AT-ATA), rational strategies, such as combination of the B-factor profile and folding free energy calculations ($\Delta \Delta G_{\text{fold}}$), the introduction of disulfide bridges, and consensus mutagenesis, were investigated”.

9. Main text lines 165-166 It is hard to believe that MD simulations make it possible to obtain insights into the molecular mechanisms. Moreover, the associated Reference 24 seems to be not appropriate.

Response 9: We thank the reviewer for the valuable comments. In the revised manuscript, the inaccurate sentence “MD simulation makes it possible to investigate the conformation of proteins and obtain insights into the molecular mechanisms [24]” has been removed.