Review of: "Enhancement of protein thermostability by three consecutive mutations using loop-walking method and machine learning"

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In this article, Yoshida et al. presented a "loop-walking method" to improve the thermostability of the Burkholderia cepacia lipase, with both experimental and computational methods. The procedures are detailed, and the resulted data are solid. However, I do not think that there is great originality in the "loop-walking method", as the machine learning has been a routine approach to explore the structure-activity relationships of proteins. Furthermore, I have some other concerns toward this article as follows:

1.The activity assay method seems to be incomplete to account for the thermostable property of the enzyme. Although the residual activity can indicate the intrinsically catalytic function of the heat-treated enzymes, the Circular Dichroism (CD) and solubility of the heat-treated enzymes are also important parameters for interpretation of protein's thermostability. The authors could compare these results with the residual activities in their article to get a a full understanding of the enzyme's thermostable property.

2. The lipase activity and thermostability has been a widely-studied topic in research. The authors should discuss and compare the previously published results in this area with their findings, to help the readers to evaluate the values of their research.

3.The machine learning procedure is not in detail and is unclear to non-professional readers. For instance, data set, feature description and model.

4.The structural investigations seem to be weak. Specifically, homology modeling of the mutated enzyme is not in detail, and a solid molecular dynamics simulation is needed to elucidate the structural mechanism of both the wild-type and mutated enzymes. For instance, what is the difference of B-factors of wild-type and mutated enzymes as well as their key residues? This finding is significant to understand the structural basis of thermostability of the enzyme.

5.Overall, the paper is well-written and the results are meaningful for the protein thermostability engineering or other properties. We suggest the authors to supply some new results toward the above concerns in the future studies.