Response letter

Comment from the Handling Editor

One the two former referees has reviewed the revised version of your manuscript. He found that despite your detailed responses you have not really modified the content of the manuscript to fit with the main message it seems to convey: the theoretical model you developed is better than the other models. I must agree that this statement lacks of empirical (statistical) demonstration.

I propose you to re-submit a revised version of your manuscript where you present your theory as an alternative approach to model the temperature response of enzyme activities. You can say that this theory can solve some limitations of the MMRT approaches but further works are required to verify whether this alternative approach provide reproduce observed patterns than the MMRT approaches. After submission, this revised version will be sent to a new reviewer specialist in enzyme kinetics.

Of course, you can decide to not re-orientate the message of your manuscript and submit it elsewhere. Please inform me of your decision

Regards,

Sébastien Fontaine

Response: Dear Prof. Fontaine, many thanks for your patience in handling our manuscript. Based on you suggestion, we have rewritten our manuscript that specifically focuses on the chemical kinetics theory, while minimizing criticisms on MMRT. Since the manuscript has changed so significantly, we did not submit a track-change version. For the same reason, we only responded to questions from the last reviewer that are sufficient generic and potentially of interest to the new reviewer of this rewritten manuscript.

Comment 3. Previous comment about a bulge in the data Figure 4 authors response "However, it was also caused by the bug in the plotting script. The corrected Figure 3d is smoother." The bulge is still very evident in the resubmitted version and is derived from their equilibrium equation. Do the authors believe this is reasonable fit? it does not look like enzyme data to me.

Response: Figure 3d is a theoretical prediction based on inferred parameters from Figure 2k, where the model fitting is found very smooth and accurate.

Comment 5. No errors are given for the parameters derived from the fitting of the equilibrium model are given and the authors state that this is because they could not get errors of the data that they extracted. I would have though the error of parameters that they were interested in not in the variability of individual points but rather the error associated with fitting their 4-parameter equation to data. There is a reasonable number of enzyme temperature response data that can be collected from the literature or indeed by the authors to formally test their ideas. But if we accept where they have reached this does not allow the authors to make assertions about a better model from a fit perspective if they don't have errors or AIC comparisons.

Response: We explained in the revised manuscript that because "fminsearch" always obtained the same values for the best-fitting parameters even by starting from different initial guesses, it made it difficult to quantify the uncertainty by running the model fitting multiple times. For other reasons explained in section 2.3, we were not able to obtain meaningful uncertainty by Monte-

Carlo method, the finite difference method, or the bootstrapping method. In addition, we note that the Ratkowsky model, a special case of the chemical kinetic theory, has been proven very successful at fitting hundreds of published datasets (e.g., Ratkowsky et al., 2005; Corkrey et al., 2012; Ghosh et al., 2016), which testifies the merit of our theory here. More importantly, in the revised manuscript, we stressed that the major merit of the chemical kinetics theory is that it provides us with more mechanistic insights by its consideration of one well-known observation that thermally revisable enzyme denaturation is ensured by the ceaseless thermal motions of molecules and ions in the enzyme solution, and three well-established chemical reaction theories: (1) the law of mass action; (2) diffusion-limited reaction theory; and (3) transition state theory.

Comment 6. The assertion that the equilibrium model can explain why when adding substrate can lead to a lower Topt (ln 285) and an explanation for the lower Topt reported in e.g., Numa et al when glucose is added needs a fair comparison. The reported lower Topt predicted using the equilibrium model and lowering the activation energy in the resubmitted paper was from a Topt of 57° to 55°C (that is 2°C shift for a large change inactivation energy). This is not reasonable to compare this to Numa et al where the Topt of respiration from glucose (and other sample substrates including a mixture of yeast extract) amendment samples was about 35°C and without glucose was greater than the highest incubation temperature of ~52°C a downward shift of 17°C.
Response: In the revised manuscript, we made it clear that the actual interpretation of the shift of optimal temperature requires a much more comprehensive modeling framework that is beyond this study. However, for the sing-substrate-single-enzyme reaction, we find that the substrate availability can shift the optimal temperature as much as 35 K (i.e., Figure 3c). Therefore, it is reasonable to hypothesize that our approach when included into a comprehensive model may be able to explain the optimal temperature change as observed in those experiments. We will evaluate this hypothesis elsewhere.