Response to Christina Klass Co-Editor-in-Chief ms BG-2020-253

Dear Dr Klass

First, we acknowledge that your comments are fully justified and have encouraged us to modify the data presentation accordingly. You will find below a step by step response to your comments/recommendations.

the written text requires quite some editing

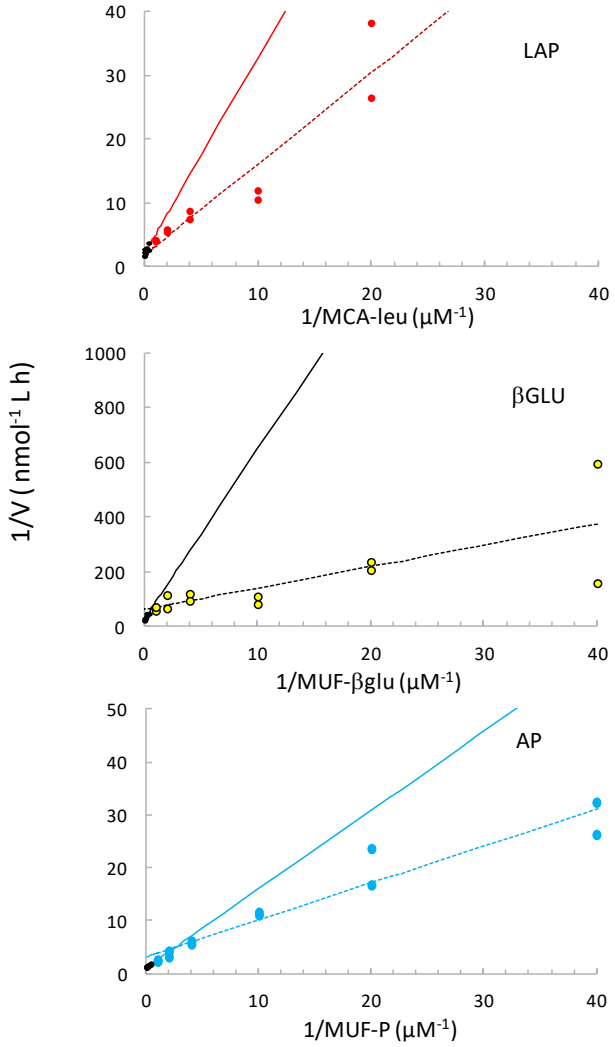
You made a big effort to improve from page 1 to 9 a ms which was only the first version of the ms. We apologize for this. Indeed, the BGD process did not allow to submit the corrected version in the first round of review (which had been edited in English as well), only the answers to the 2 reviewers have been posted. We have now improved this revised version with your suggestion, please find the attached document. All typos have been corrected, we underlined in yellow the main modifications made in the ms.

nowhere in the data and analysis it presents robust evidence of biphasic kinetics.

You are right, this was not sufficiently detailed. We added new paragraphs in the methods and in the results section and 2 additional figures (Figure 4, Figure S2) and one table (Table S2). For assessing the presence of biphasic kinetics statistically, we used the F test presented in Tholosan et al. (1999). The lines 287 - 303 in the method section describe the statistical tool, and the results of statistics are now presented on lines 362 - 411 and on table S2. We added also a new paragraph at the beginning of the discussion (lines 518 - 526).

This is in contrast to previously cited work such as Tholosan et al. (1999; with the use of Lineweaver-Burk plots)

Note that Tholosan et al. (1999) used the Lineweaver-Burk plots to illustrate evidence of biphasic kinetic but they used non linear regression fits from the Michaelis-Menten kinetic to make their statistics. We plotted you below the Lineweaver-Burk plots fitted to the data set presented in Figure 3 (full lines Lineweaver-Burk fit for the 2.5-50 µM concentration set; dotted lines fit for the 0.025-1 µM concentration set). We found that this representation, in the frame of our concentrations ranges (the inverse of a 0.025-1 µM concentration set to be compared to the inverse of a 2.5-50 µM set) is not easy to visualize, and therefore choose not to present such plots in the revised ms.



the standard errors presented (which in the case of your manuscript are also too large to corroborate the existence of two enzymatic systems).

The % variation of the standard errors of the Km and Vm values are listed on Table S2 and standard errors are visible on Figure 4 in which plots are not in log scale. With all these new information and tables, we prove the existence of a biphasic system in 60% of the cases.

Further, if enzymes operate at different range of concentrations, it is contradictory to use the whole data range for the estimates of the low affinity system.

In the first part of the results section, we present now the 3 series of data set. Note that the Km_{50} and Vm_{50} terms in this revised version corresponds to kinetic parameters derived for a 2.5-50 µM concentration range whereas the terms Km_{all} and Vm_{all} are now describing the kinetic parameters derived from a 0.025-50 µM, i.e whole concentration range. We demonstrate that, linked to the uncertainty in the distribution of the data points used to describe Michaelis-Menten kinetics, the kinetic parameters obtained by using the global model or the model 50 were not so different, and at least still very different than those obtained by using the model 1. We also showed on Figure S2 that a series of Michaelis-Menten kinetics can be obtained by addition of successive increasing concentration in the data

set. Finally, we decided not to focus the discussion *sensus stricto* on biphasic kinetics, but rather on the consequences of using a concentration set restricted to low concentration (up to 1 µM) in comparison to most published studies that use a concentration set reaching much higher concentrations (see the first part of the discussion), and for this we considered that the global model was more representative. The title of the ms was also modified accordingly.