

# Review of: "Determining kinetics parameters of bovine serum albumin-protected gold nanozymes toward different substrates"

Pedro Valencia<sup>1</sup>

<sup>1</sup> Universidad Técnica "Federico Santa María"

Potential competing interests: No potential competing interests to declare.

The research concerns the kinetic characterization of an albumin-gold nanozyme for the oxidation of two different substrates. The article quality is very low due to the scarce input on scientific knowledge. Besides this issue, the manuscript requires major revision on the design of experiments and writing.

An important methodological issue was detected. The experimental methodology did not consider the control experiments. It means that the reaction rate in the absence of the catalyst was not quantified. The research results do not allow to discriminate between the non-catalyzed and catalyzed reaction between hydrogen peroxide and the corresponding substrate. For this reason, the kinetic parameters were not properly determined, and the research lacks scientific validity. The experimental design must also include the reaction rate at zero substrate concentration. In this case the zero-rate experiment must be rigorously designed due to the involvement of two reactants in the reaction.

The discussion must be expanded beyond the observation that kinetic constants for both substrates are different due to the reactivity and mechanism. Discussion must provide an explanation for those differences.

Some other important issues are listed below:

- The abstract requires improvement. Please add more than just results. A brief description of the interest and motivation of the research, objectives, methodology, discussion, and conclusion must be provided.
- The author self-cited several articles. In some cases, it is appropriate when referring to specific commentaries about previous research. Nevertheless, some cites refer to general aspects of enzyme immobilization. In these cases, the author's articles seem not adequate for such a purpose. I suggest substituting the general citing for review articles in the topic.
- The antecedents must include information regarding the interest on this catalyst, some information about nanozymes and the mentioned protection exerted by albumin on the particles.
- The lower performance of immobilized regards free enzymes was explained by conformational changes caused by the immobilization. This is correct; however, a very significant effect can be caused also by the diffusional restrictions. The authors must mention this factor, even considering the possibility that a low impact could be observed with nano sized

particles.

- Each acronym must be defined the first time it appears in the text.
- The comment: “a wide variety of the introduced nanomaterials reveal excellent enzyme-like activity”. There are at least two possible interpretations on this comment:
  - Such materials exhibit enzyme-like activity.
  - Such materials are excellent supports for enzyme immobilization.
- Please explain which is the case (maybe both) and modify the comment to express exactly what the author means. In the case of enzyme-like activity, please specify the exact attributes associated with this comparison (rate, selectivity, specificity, enantioselectivity, etc.).
- Please provide the definition of nanozyme in the introduction.
- The hypothesis underlying the use of BSA-gold nanozymes must be declared to understand the reason to evaluate this type of catalyst. Some arguments were done about the use of gold nanozymes. However, BSA-gold nanozymes lack of fundamentals. The potential of this catalyst for the industry must also be mentioned.
- The methodology must mention the method used to empirically estimate the kinetic constants. The author mentioned he based on Michaelis-Menten equation and the Lineweaver-Burk method. The Michaelis-Menten equation is just that, an equation, not a method. The Lineweaver-Burk method is a graphical and regression approach. The kinetic method was not mentioned. It could correspond to the initial rates method, based on the use of different initial substrate concentrations to quantify the respective initial reaction rates. So, the method is based on the determination of two variables: the initial reaction rate (dependent variable) and the initial substrate concentration (independent variable; parameter). Some concerns about this method must be clarified:
  - i) Is the reaction progress linear during the measurements? This is a requirement to properly estimate the reaction rate. In the case it was not linear, a proper method must be described.
  - ii) Is the reaction rate dependent on just the substrate concentration? The reaction described proceed in the presence of two reagents. Indicate that reaction was carried out under a constant concentration of hydrogen peroxide.
  - iii) Are the kinetic constants dependent on the hydrogen peroxide concentrations? If the answer is yes, all kinetic constants estimated from these experiments are apparent kinetic constants.
- A lot of grammar errors were detected. Punctuation, misspelling, verb conjugation, etc. must be corrected.