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

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Potential competing interests: No potential competing interests to declare.

We consider the manuscript interesting and pertinent.

The study focuses on kinetics studies of the as-prepared BSA-gold nanozymes using 3,3',5,5'-tetramethylbenzidine (TMB), and 3,3'-diaminobenzidine (DAB) as substrates.

Dear authors, please define the abbreviations (TMB, DAB) in the abstract.

The objectives of this work are clearly stated **but not clearly justified** ***and the limitations of the study are lacking.***

The introduction integrates the theme's main aspects.

The references used in the introduction are recent.

This article must be rewritten mainly in the discussion. The authors almost

The core experimental design of the chemistry part of the manuscript seems carefully elaborated and meticulously presented. However, regarding the methodology of enzyme assays, **serious concerns were raised.**

The graphs in Figure 2 **do not seem to be Michaelis Menten, that is, the curve of a rectangular hyperbole** Please dear authors, what were the **enzyme concentrations (the nanoenzyme used seems lacking preliminary studies of temperature, pH ,….) , substrate concentrations and how did you ensure the maximum reaction rate**?

Another concern raised was the validity of Lineweaver-Burk plots. Regarding the methodology in the kinetic modeling (***graphical determination*** of parameters by linearization of the MM hyperbolic curve) we***have serious concerns to announce***. These linearizations have been heavily criticized by enzymologists for decades, to name a few:

I)"Improper use of the double-reciprocal plot" in Kinetic Mechanisms of Enzyme Inhibition and Activation: Tutorials, examples, mechanism validation, and advice for publishing by Professor Antonio Baici. (taken from his page https://www.enzyme-modifier.ch/constructive-criticism-new/.)

The most popular method to analyze steady-state data enzyme-catalyzed reactions, graph results and calculate kinetic constants is still the double-reciprocal plot. The statistical problems of this method have been discussed by the authors

themselves (1), even before describing its applications in more detail (2).

***Lineweaver, Burk and Deming recommended**:*

The estimated limits of experimental error … , based on a weighting according to the reciprocals of the squares of the assigned deviations, … [(1), p. 227].

*Other authors analyzed in detail the problems of the double-reciprocal plot, starting with **Dowd and Riggs**, who came to the conclusion:*

… the Lineweaver-Burk transformation tends to give a deceptively "good" fit, even with unreliable points. The marked inferiority of the Lineweaver-Burk plot strongly suggests that it should be abandoned as a method for estimating Km and V from unweighted points … [(3), p. 869]

***II) "...KM estimates made using double-reciprocal plots and the MM equation were consistently inferior to estimates made with nonlinear least-square fitting methods"** [Schnell, S. and Maini, P.K. (2003) A Century of enzyme Kinetics: Reliability of the KM and Vmax Estimates. Comments on Theoretical Biology, 8, 169-187. http://dx.doi.org/10.1080/08948550302453].*

***II) "Today, there is no reason for fitting data using either the linear transformation of the Michaelis–Menten equation in analyzing the concentration dependence of the initial velocity"** in [Johnson 2013 A century of enzyme kinetic analysis, 1913 to 2013, FEBS Lett. 2013 Sep 2;587(17):2753-66. doi: 10.1016/j.febslet.2013.07.012.]*