



## BAISHIDENG PUBLISHING GROUP INC

8226 Regency Drive, Pleasanton, CA 94588, USA

Telephone: +1-925-223-8242 Fax: +1-925-223-8243

E-mail: bpgoffice@wjgnet.com <http://www.wjgnet.com>

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### Answering reviewers

Thank you for the favorable consideration of our manuscript entitled "Disruption of NAD<sup>+</sup> binding site in GAPDH affects its intranuclear interactions", and the invitation to make the revisions of our paper.

We are grateful to the reviewers for thoughtful comments and interesting suggestions aimed to improve the quality of our manuscript. In line with reviewers' suggestions, we now present our data in a clearer form, and revised discussion of our results. We believe our revised manuscript is now suitable for publishing in your Journal.

Below you will find a point-by-point summary of how our manuscript has been revised. We have paraphrased each reviewer's comment in italics, and then indicated the nature and location of changes made in the manuscript.

#### Reviewer 1 comments:

1. *"Abstract: Please be consistent with amino acid abbreviations".*

Now we used single letter code for amino acids throughout the text.

2. *"Material and methods: In Transfection with pEGFP-GAPDH and FRAP experiments in page 8, in the equation  $D=0.88*w^2/(4t1/2)$  for calculating diffusion coefficient, it seems to be  $\tau(\text{tau})1/2$ , instead of  $t1/2$ . Please check again".*

To calculate diffusion coefficient  $D$  (page 8), we used the equation presented in the application letter by Kappel and Eils “Fluorescence recovery after photobleaching with the Leica TCS SP2”. Confocal application letter August 2004 #18. The authors used  $t_{1/2}$  to designate the recovery half time, which is related to the characteristic relaxation time  $\tau$  by the equation  $t_{1/2} = \tau \ln 2$ . To keep the equation consistent with the references, we chose to designate the recovery half-time as  $t_{1/2}$ .

3. *“Results: The interpretation that GAPDH T99I mutant variant has affinity for glyceraldehyde 3-phosphate close to that of wild type enzyme may explain further, since the curve for T99I mutant in figure 4A has low saturation, even though the  $K_m$  of T99I is calculated as similar to that of wild type in Table 2. There might be some description about it”.*

This is an important notion, and we now have re-phrased the description of our results on page 13 to reflect it: “T99I variant had the lowest affinity to  $\text{NAD}^+$ : its  $K_m(\text{NAD}^+)$  was increased by more than an order of magnitude compared with wild type GAPDH ( $741 \pm 257$  vs.  $57 \pm 11.1 \mu\text{M}$ ). This low binding of  $\text{NAD}^+$  explains why T99I variant has low saturation under conditions of experiment (i.e., at  $\text{NAD}^+$  concentration  $260 \mu\text{M}$ ), as depicted on Figure 4A.”

4. *“Discussion: The functions of GAPDH isoforms may be described in detail in the first sentence in discussion.*

Now we added more text on page 15 paragraph 1, to address the current knowledge about GAPDH isoforms.

5. *“References: Please carefully check references again”.*

References have been re-checked and formatted according to the instructions to the authors.

6. *Tables and figures: The legends for open or closed symbols may be added in figure 6.”*

Figure 6 has been corrected

## **Reviewer 2 Comments**

1. *“What happens will be the substitution of T99D?”*

The mutation T99D could bring important insights, because D is often used as an analog of a phosphorylated amino acid. We plan to generate and study this variant GAPDH in our future studies.

2. *"Fig. 7 panel D. suggests to exhibit E97 position."*

Positions E97 and T99, along with the hypothetical hydrogen bond are indicated on Fig. 7C. We highlighted the hydrogen bond with yellow color, to facilitate interpretation of the image.

### **Reviewer 3 Comments**

1. *"Part of these results appear to have been presented in preliminary form to some FASEB conference. The authors should mention this in the manuscript"*.

The reference was added to the discussion (page 15 paragraph 1 reference 26).

2. *"The manuscript lacks statistical analysis"*.

Statistical analysis has been performed as described in Materials and Methods section (page 9), and added to the text and figure legends.

3. *"Figs. 5 and 6 scale bars are missing"*

Scale bars were added to Figures 5 and 6.

We believe that the revised manuscript now presents the objectives, strategy, and results of our study in a more clear and comprehensive way. We believe that our novel findings will be of interest to the broad audience of your Journal.

We look forward to hearing from you, regarding the status of our manuscript.

Sincerely,

Evgeny Krynetskiy, PhD, DSc  
Temple University School of Pharmacy  
Philadelphia, PA 19140  
Phone: 215-707-4257  
Email: ekrynets@temple.edu