

Reviewer: 2

In this paper, EcR/USP of *Plutella xylostella* were obtained, the physical size, zeta potential, and molecular weight of the heterodimer were characterized as well. The affinity of the heterodimeric protein to PonA and tebufenozide was determined and significantly enhanced compared with the results of previous in vitro transcriptional translation protein tests and other insect-derived yeast and insect cell line transcriptional activation assays, indicating the high sensitivity of the target protein applied to the radioligand method in this study. Furthermore, this work provides a rapid and sensitive method for screening ecdysone agonists for ecdysone receptor studies at the in vitro level. In general, the article is well organized and the data are detailed. I suggest this manuscript can be accepted after minor revision. The comments are as follows:

Response: We sincerely thank you for your valuable feedback. According the comments, we have modified our manuscript. We have invited native English scientist from AJE to edit our revised manuscript again. All changes of our manuscript were labelled with blue-colored text. The point to point responds to the comments are listed as following.

1. In section 2.6, hydrogen bond interaction was important, so the distance of hydrogen bond should be calculated and added.

Response: We are grateful for your advice, and the distance of hydrogen bond have been calculated and added in section 2.6. Please see line 257、 Figure 4C and Figure 4D in the revised manuscript.

2. In section 3, some units failed to unified, e.g. mM or mmol·L⁻¹.

Response: Thank you for your careful review. We have unified the unit of this section as “ mmol·L⁻¹”. Please see lines 333 – 335 in the revised manuscript.

3. In section 3.2, 3.3 and 3.5, some related references need be added.

Response: We appreciate your advice, References 40, 41, and 44 have been added to sections 3.2, 3.3 and 3.5 of the revised manuscript. Please see lines 287, 308 and 328 in the revised manuscript.

4. Some proper nouns should be added the full name when it first appearance, e.g. EcR/USP.

Response: Thank you for your suggestion. We are very sorry for our miswriting and they have been corrected. Please see line 9 in the revised manuscript.

Thank you for your careful review. We really appreciate your efforts in reviewing our manuscript during this unprecedented and challenging time. We wish good health to you, and your family. Your careful review has helped to make our study clearer and more comprehensive.