# Reviewer 1:

*The manuscript by Sieg et al. reports the determination of folding thermodynamic parameters of RNA double helices using the MeltR software which is an open-source package in R programming language. The authors applied the software to fitting melting curves of RNA, and they compared thermodynamic parameters calculated using MeltR to those using the comparable software MeltWin. MeltR provides three analyses of RNA melting curves, and the analyses using MeltR are shown to be as accurate as MeltWin. More importantly, MeltR provides global-fitting analysis, not provided by MeltWin. The authors demonstrate the global-fitting analysis can produce thermodynamic parameters with minimal user specifications. This manuscript concludes that accurate determination of folding thermodynamic parameters can be made by MeltR. I think the study is important to the fields of biophysics. I recommend the publication of this manuscript after the authors consider the following comments.*

We thank the reviewer for the fast and positive review.

# Comments:

*(1) I understand the program MeltR may apply to diverse biopolymers other than nucleic acids. However, the manuscript exclusively studies melting curve-fitting of RNA. I suggest the title of this manuscript (facile determination "biopolymer" thermodynamics) is changed to more specific to nucleic acids.*

We made the requested change to the title.

(2) All RNA helices that the authors used for the comparison with MeltWin (Table S1) were relatively short (< 10 bp). Did the authors compare thermodynamic parameters of longer RNA helices calculated using MeltR to those using MeltWin? The additional study would demonstrate the advantage and wide application of MeltR.

*(3) I am very interested in the assessment of RNA melting curves that the upper or lower baseline is not clear in the experimental temperature range. Does MeltR work well for RNA helices with very high and low Tm, in comparison to MeltWin? The choices of baselines are particularly important in analyzing such RNA helices.*

We did not include RNA melting curves where the upper or lower baseline is not clear because, ideally, the experiment should be designed to avoid this situation. However, we understand that this is not always possible. MeltR can work for helices with very high and low melting temperatures, both the *meltR.A* and the *BLTrimmer* can fit data in these cases. For example,

The limiting case for *MeltWin* is when the data is so truncated that it does not resemble a melting curve and there is no information to fit. This is the same limit as *MeltWin*.

*(4) It is mentioned that MeltR provides "Monomolecular.2State" for self-structured strands. However, the manuscript does not contain the data of self-structured strands like hairpins. Can the authors add the data of monomolecular RNA folding?*

We included data modeled assuming monomolecular folding RNA and determined that MeltR

(5) To make the manuscript more easy reading, I recommend enlarging the letters in Supplementary Figures (the letters "Frequency" in the vertical axis of Figure S2 are hard to read, as an example).