January 8, 2023

Dear Professor Enderlein,

Here we wish to submit our manuscript “*MeltR: A Software Package that Provides Facile Determination of Biopolymer Thermodynamics. Application to RNA UV-Absorbance Melting Data*” as an original research paper to *Biophysical Reports*. Thermodenaturation (melting) curves provide thermodynamic insight into macromolecular structure. In the nucleic acids field, melting curves have provided the ubiquitous nearest neighbor model for calculating the energy of secondary structures. The melting curve fitting software *MeltWin,* introduced in 1996, provided a consistent and facile melting curve analysis platform used in a generation of studies. Unfortunately, MeltWin software is not maintained and relies on idiosyncratic choices of baselines by the user. Lastly, improvements to melting curve analysis and applications to nucleic acid are areas of active investigation in *Biophysical Society* journals.1,2

Our present manuscript introduces the reader to the melting curve-fitting functions in our *MeltR* software package for analysis of macromolecular thermodynamics data. *MeltR* serves as a replacement and improvement to *MeltWin*. We introduce the reader to *MeltR* functions, demonstrate their application, and benchmark their accuracy. In particular, we describe new autobaseline trimming capabilities, which deal with an important but poorly understood aspect of extracting thermodynamic parameters from melting curves. *MeltR* is a needed software for the nucleic acids community and has applications for analyzing non-nucleic acid melting data. *MeltR* is also open source and freely available to be used, modified, and redistributed, providing a platform for the community to improve. As such, we expect this work to have broad appeal to the readership served by *Biophysical Reports*.

*MeltR* can be installed and copied from this website: https://github.com/JPSieg/MeltR

We recommend Professor Rebecca Berlow as an associate editor and Professors Doug Turner, John SantaLucia, and Nakano Sugimoto as reviewers.

This manuscript is not under consideration for publication and has not been published elsewhere.

Thank you very much for handling our manuscript.

Yours Sincerely,



Philip C. Bevilacqua

Distinguished Professor of Chemistry and of Biochemistry & Molecular Biology

(1) Majikes, J. M.; Zwolak, M.; Liddle, J. A. Best Practice for Improved Accuracy: A Critical Reassessment of van’t Hoff Analysis of Melt Curves. Biophys. J. **2022**, 121 (11), 1986–2001. https://doi.org/10.1016/j.bpj.2022.05.008.

(2) Arteaga, S. J.; Adams, M. S.; Meyer, N. L.; Richardson, K. E.; Hoener, S.; Znosko, B. M. Thermodynamic Determination of RNA Duplex Stability in Magnesium Solutions. Biophysical Journal 2022, S0006349522039352. https://doi.org/10.1016/j.bpj.2022.12.025.

Cover letter

Please include a cover letter describing the content and significance of the article. If applicable, please include the title and manuscript tracking number of any related submissions and any additional information the editor may need to be aware of.

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