August 12, 2022

Dear Dr. Erick Carreira,

Here we wish to submit our contribution “*The Metabolome Weakens RNA Helix Stability and Increases RNA Chemical Stability*” as an original research paper to the *Journal of the American Chemical Society*. The development of experimental techniques that study RNA in the cell has uncovered a chasm in knowledge between RNA folding and function *in vivo* and the insight provided by simple systems *in vitro.* *In-vivo*-like systems that simulate multiple elements of the complex cellular environment thus provides a gateway to understanding RNA. To date, the development of *in vivo*-like conditions to study RNA has focused on simplified systems, usually non-biological crowders, such as polyethylene glycol (PEG), or a few biologically relevant small molecules. These systems have been published in JACS in recent years.1–5

Our manuscript, in contrast, describes development of “Eco80”, an artificial cytoplasm that contains 80% of *E. coli* metabolites, as a manageable surrogate for studying RNA *in vivo*. We demonstrate that Eco80 metabolites have a number of RNA-affecting molecular attributes: it buffers the free Mg2+ concentration, destabilizes RNA helices, protects RNA from degradation, and supports RNA catalysis. The RNA-affecting molecular attributes of metabolites support an RNA-friendly environment *in* vivo, where the is less Mg2+-concentration noise, metastable RNA misfolds destabilized, and RNA is protected from degradation. Moreover, Eco80 is likely the most biologically relevant and chemically accessible artificial cytoplasm available. Eco80 is an environment for characterization of a wide range of systems, including RNA, proteins, and membranes. We expect this work to have broad appeal, and it is thus appropriate for *JACS*.

We recommend Professor Mathew Disney as an associate editor and Professors Gary Pielak, Danny Incarnato, Martin Gruebele, Dan Herschlag, Jean-Denis Beaudoin, and Silvie Rouskin, 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

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