Ms Emily Waters
Associate Editor
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July 09, 2014

Dear Ms Emily Waters;

I am pleased to submit the manuscript entitled "Mass Spectrometric Approaches to Study Protein Structure and Interactions in Lyophilized Powders" to *Journal of Visualized Experiments*. The manuscript is co-authored by Balakrishnan S. Moorthy, Lavanya K. Iyer and myself. The submission summarizes original research by authors that has not been published previously and is not under consideration for publication in any other journal.

This work demonstrates the detailed protocol for hydrogen deuterium exchange (ssHDX-MS) and photolytic labeling (ssPL-MS) methods for studying protein conformations in lyophilized powders. Though several high resolution methods are available to study protein structure in solution, the methods for characterizing protein in amorphous solid are more limited. Our group has recently developed ssHDX-MS and ssPL-MS methods to assess protein conformations and molecular interactions in lyophilized powders with high resolution. In this study, a model protein (myoglobin, Mb) was co-lyophilized with different excipients and the conformation of Mb in lyophilized samples were assessed using ssHDX-MS and ssPL-MS at both the intact protein and peptide level. Our results show that the methods are capable of distinguishing formulations with different excipients. This study supports the use of ssHDX-MS and ssPL-MS as formulation screening tools for developing protein therapeutics. I believe that this work is suitable for publishing in JoVE's unique multimedia format, as it would provide readers with detailed protocols and video support for performing these measurements.

Thank you for considering our manuscript. Please feel free to contact me if you have any questions about this submission.

Sincerely,

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